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پیام رئیس

در سال‌های اخیر رشد علم و تکنولوژی و ضرورت همگرایی صنایع، شرکت‌های دانش‌بنیان، پژوهشکده و دانشگاه‌ها جهت بهره گیری از منابع به روز و کارآمد، باعث شد تا با استعانت از ایزد منان، برگزاری سومین همایش بین المللی فناوری‌های نوین در علوم توسط دانشگاه تخصصی فناوری‌های نوین آمل و با همکاری دانشگاه‌ها و مراکز علمی مطرح ایران و جهان در **تاریخ ۲۸ اردیبهشت ماه ۱۴۰۲ در محل پردیس دانشگاه** برنامه‌ریزی گردد. طی این همایش نشست های علمی موازی، نشست‌های ارائه مقالات پژوهشی، نمایشگاه‌های تخصصی و کارگاه‌های آموزشی تخصصی ضمن سخنرانی‌های کلیدی با حضور اساتید برجسته داخلی و خارجی برای پژوهشگران، دانشگاهیان و صنعتگران در حوزه‌های فنی و مهندسی، پزشکی و سلامت، علوم زیستی، علوم دامپزشکی، فناوری گیاهان دارویی، کشاورزی و سایر رشته‌های مرتبط برگزار می‌شود. هدف از برگزاری این همایش آن است که ضمن ارائه آخرین مقالات علمی و دستاورد های پژوهشی، محققان و صنعتگران با ایراد و بیان مشکلات و چالش‌های موجود و طرح سوالات مرتبط با موضوع به صورت مقالات کوتاه و ارسال آن به دبیرخانه انجمن، از هم اندیشی و پیشنهادهای اساتید و محققین بهره مند شوند. همچنین این فضا می‌تواند نیاز صنعت به نیروی انسانی متخصص، معرفی دانشجویان و فارغ التحصیلان توانمند دانشگاهی و تعامل سازنده دانشگاه و صنعت را برآورده نماید. امید است این کنفرانس بتواند گامی موثر و استوار در عرصه احقاق اهداف چرخه تولیدات و پژوهش کشور با وحدت جامعه دانشگاهی بردارد و رشد و ترقی دانشگاه و صنعت میهن عزیزمان ایران را در پی داشته باشد. پیشاپیش از زحمات بی شائبه همراهان گرامی در حیطه‌های گوناگون نظیر گردآوردی مقالات و طرح‌ها، برگزاری کارگاه‌های آموزشی، برپایی نمایشگاه محصولات، حامیان محترم همایش، مسئولین اجرایی، روابط عمومی و تبلیغات تشکر نموده و از درگاه باری تعالی برای همگان سلامتی، بهروزی و توفیق روزافزون را مسئلت می‌نمایم.

دکتر اکبر حاجی زاده مقدم

پیام دبیرعلمی

سومین همایش بین المللی فناوری‌های نوین در علوم ۲۸ اردیبهشت ۱۴۰۲ در دانشگاه تخصصی فناوری‌های نوین آمل با پشتوانه‌ی برگزاری دو همایش قبلی در طول بازه سالیان گذشته، برگزار خواهد شد. هدف این است که شرکت کنندگان در این همایش تجربیات علمی، آموزشی، پژوهشی و فنی خود و موسساتی که به آن وابسته اند، را به اشتراک بگذارند. بنابراین یکی از دستاوردهای مهم این پژوهش، شناخت نقاط قوت و ضعف این افراد و موسسات و لذا برقراری پیوند بین آن‌ها جهت یاری رساندن به یکدیگر است. برقراری این پیوند وقتی اهمیت پیدا می کند که دانش آموختگان رشته‌های حوزه پزشکی و سلامت، فنی و مهندسی، دامپزشکی و همچنین زیست فناوری و گیاهان دارویی در این همایش با همکاران بالقوه‌ی خود در سرتاسر جهان آشنا شده و در آینده ای نه چندان دور منجر به همکاری‌های گسترده‌ای خواهد شد. اعضای کمیته‌ی علمی همایش از دانشگاه‌ها و موسساتی دعوت شده اند که به نحوی در حوزه‌های تخصصی خود جزو بهترین‌ها بوده و در میان این عزیزان از دانشمندان مطرح داخل و خارج بهره برده شده است. سومین همایش بین المللی فناوری‌های نوین در علوم، این همایش را میعادگاه کنشگران مرزهای علم و فناوری، اساتید محترم دانشگاه، علاقمندان به مباحث پزشکی و سلامت، علوم زیستی و ژنتیک، فرآوری گیاهان دارویی، هوش مصنوعی و سایبری، شبکه‌های کامپیوتری و پردازش داده‌ها و همچنین علوم پایه می‌داند و همگان را دعوت می کند که در آن فعالانه شرکت نمایند.

دکتر فرید صمصامی خداداد

پیام دبیر اجرایی

با توجه به پیشرفت سریع علم در سال‌های اخیر و ضرورت به کارگیری منابع به روز و کار آمد در حوزه‌های مختلف علوم؛ یکی از بهترین عرصه‌هایی که می‌تواند منجر به همگرایی اثر بخش علم و عمل شود، شرکت در همایش و کنفرانس های بین المللی و معتبر دانشگاهی است، که با ایجاد فضای تعامل و هم اندیشی میان دانش پژوهان، اساتید و صنعتگران می تواند با ارائه دستاوردها و یافته‌های علمی- پژوهشی نوین همراه باشد. در این راستا با استعانت از خداوند متعال، بعد از گذشت پنج سال از برگزاری نخستین همایش بین المللی فناوری‌های نوین، مفتخریم که بار دیگر می‌توانیم برای به روز رسانی آموزه‌های علمی و تخصصی در زمینه‌های متنوع گرد هم آییم. امیدواریم میزبان خوبی برای شما همکاران و شرکت کنندگان محترم باشیم. به عنوان تیم اجرایی سومین همایش بین المللی فناوری‌های نوین در علوم که در **۲۸ام اردیبهشت ماه ۱۴۰۲در دانشگاه تخصصی فناوری‌های نوین آمل** برگزار خواهد شد، تمام تلاشمان را به کار گرفته‌ایم تا بستر مناسبی برای ارایه سخنرانی‌های اساتید گرامی و برگزاری کارگاه‌های آموزشی و نشست‌های علمی فراهم آوریم. این کنفرانس بر آن است تا با بهره‌گیری از قدرت علم، به افزایش سطح علمی و به روز رسانی دانسته‌ها در زمینه‌های مختلفی مانند علوم دامپزشکی، پزشکی و سلامت، علوم زیستی و ژنتیک، فرآوری گیاهان دارویی، حوزه‌های فنی و مهندسی و همچنین علوم پایه و .... بپردازد تا در سایه گسترش مرزهای علم و دانش به بهبود فضای کسب و کار و رونق تولید در کشور کمک کند. بدینوسیله از کلیه پژوهشگران، محققان، اساتید، دانشجویان، صنعتگران، کار آفرینان و صاحب نظران فرهیخته که نسبت به ارسال جدیدترین دستاوردها و نو آوری های علمی- پژوهشی و فناورانه خود در قالب نگارش مقالات تخصصی و ارزنده و نیز طرح‌ها و ایده‌های کارآمد اقدام نموده و ما را در برگزاری بهتر این همایش همراهی کردند، تشکر می‌نمایم.

دکتر سمیه رهایی

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Edge Detection with ResNet

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ABSTRACT
Edge detection in computer vision is essential for higher-level vision tasks such as shape matching, visual salience, image segmentation, and object recognition. The methods based on deep learning are among the methods that have been proposed to increase accuracy, which is highly popular. In this paper, a method for edge detection with one of the deep architectures that have high accuracy is proposed. The results show that the F-measure for the proposed method with ResNet architecture has improved compared to other compared methods.
Keywords: convolutional deep learning, edge detection, ResNet.

1. *INTRODUCTION*

Edge detection is one of the basic and important topics in computer vision and image processing because image edges contain very important information from the image and can be extracted using different methods [1].

Edges include essential image features such as direction, shape, changes, etc. Edge detection is used in satellite image processing, and medical image processing, such as finding the location of a gland and the location of a specific tissue, robotics, machine vision, etc [2].
Edge detection is used to detect the edges of an object among several other things. Color change and brightness change in the image lead to physical changes. Detecting the edges of an image leads to a significant reduction of data values and removes low-value information, although the important structural properties of the image are preserved. Convolutional neural network architectures have been used for edge detection. ResNet [3] architecture is one of the convolutional neural network architectures that have high accuracy and due to this vital property, accurate results can be obtained.
Some deep learning methods have also been used for edge detection [13, 14]
Cascading network (CHRNet) is proposed for edge detection that connects the sub-blocks at each stage with the output of the previous layer. After each layer, a batch normalization layer with active affine parameter is used as an erosion operation for the homogeneous region in the image [15].
A dense jump connection network (PFD-Net) with multi-scale features is proposed, which is inspired by the nonlinear mapping capability of deep learning. In this method, the information integration of high and low level features is done in multiple scales to further enhance the general and precise position of the edge [16].

2. *RELATED* WORK

Various methods have been proposed for edge detection. One edge detection method is the surround-modulation edge detection (SED) [4] method, which was modeled with nCRF by combining four elements: far, full, iso-orientation, and orthogonal-orientation surround suppression.
Edge detection with the Holistically-nested Edge Detection (HED) [5] method is proposed, which is an edge learning model that uses multiple levels and has maps to receive multi-scale side outputs. HED has two types: the side branch and the fusion branch.

Another famous edge detection method is the structured forests method (SE) [6] which uses the structure learning method for edge detection. In this method, mapping to a separate space is done and a decision tree is built based on the criterion of obtaining information in this space. This method is real-time.
The method of grouping edges in continuous detection and smooth lines is proposed with the gPb-owt-ucm method [7]. This method organizes edges into edge segments, then heuristically joins them into single-pixel continuous wide contours.
The difference between this article and previous works is:

- Using ResNet architecture in edge detection,
- Improved F-measure compared to the compared methods,
- Increasing accuracy, which is the main feature of using deep learning methods.

3. *PERPOSE* METHOD

ResNet architecture combines a set of coded models. This architecture is shown in Figure 1.

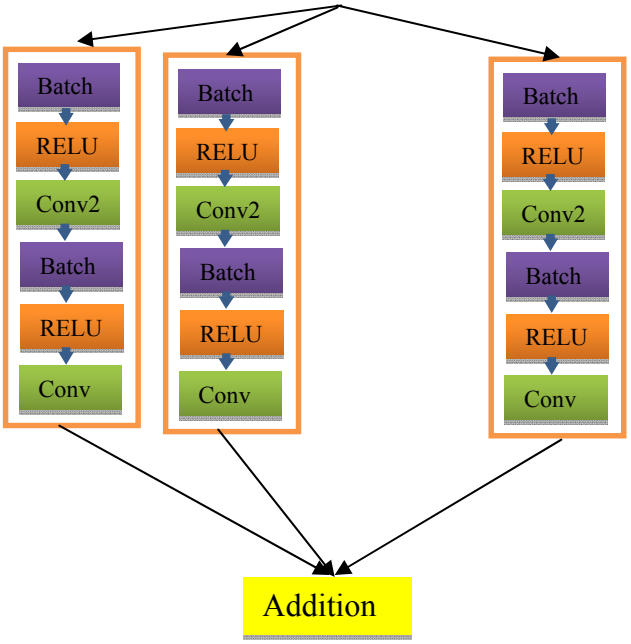
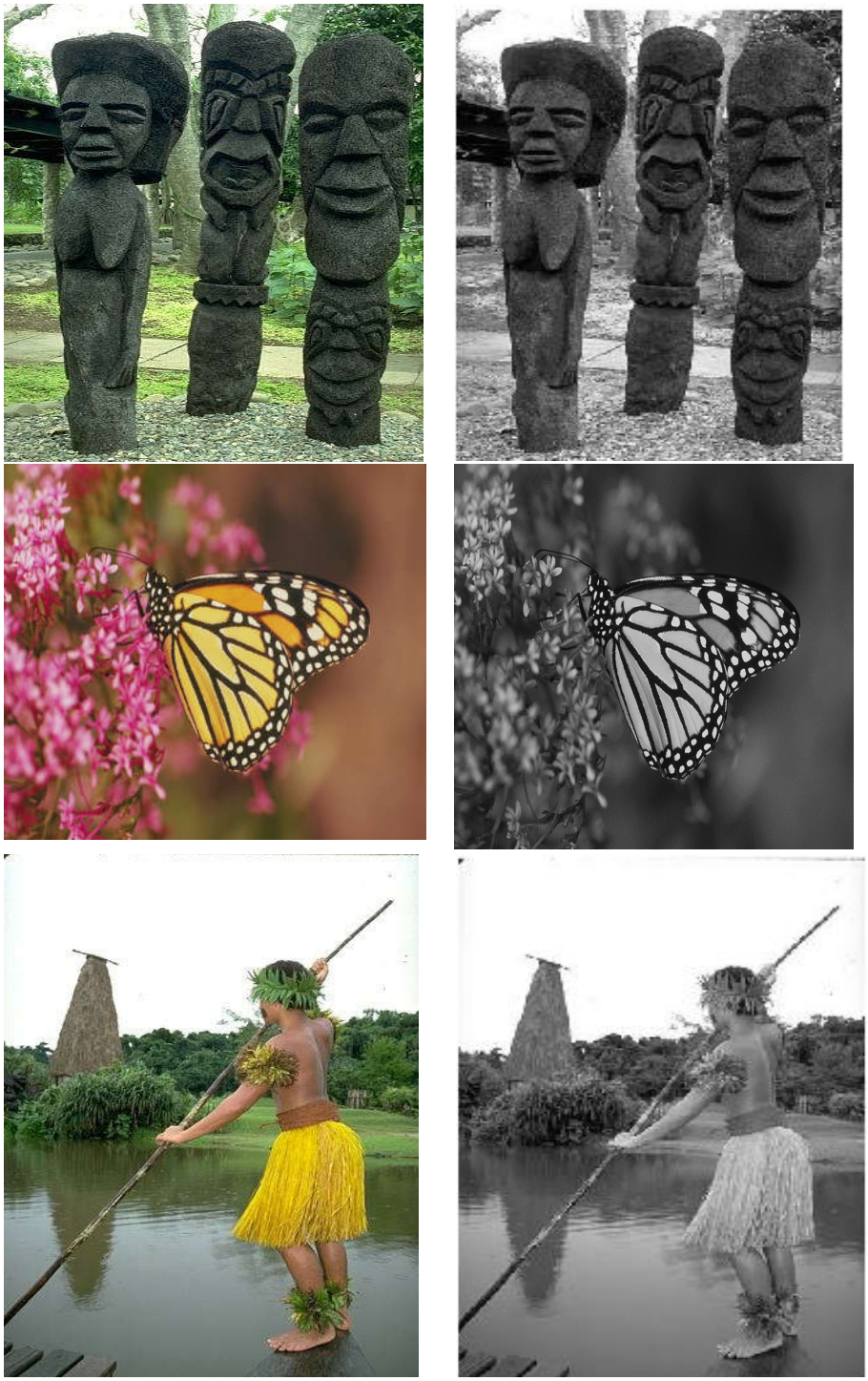


Figure 1. ResNet network.

The UNet block, that is, the same encoder-decoder, is chosen for a smooth and gradual transfer from the image to the mask segmentation. In order to achieve continuous training, the depth of the network increases, and the blocks made from the UNet architecture are replaced with the remaining modified blocks from the convolution layers. The remaining blocks were removed to largely solve the gradient fading problem that exists in deep architectures.
Shortcut connections or additional connections in this architecture are a solution that solves the problem of deepening the neural network. The difference between this architecture and the previous architectures is that the existence of a shortcut connection makes it pass through one or more layers and connects one layer to a further layer.



Original Image Grayscale Image Edge Detection

Figure 2. Edge Detection.

To have better performance in all scales, several parallel convolutions with different rates are used in each remaining block to increase the received field of each one. The logic of using these multi-scale layers is to extract the features of the object in different scales of the received field, which can improve the performance by identifying the correlation between objects in different places of the image.

In order to improve the performance of the network by including the background information, the scene decomposition integration layer has been used. In shallow architectures, where the last encoder layer has a size of fewer than 16×16 pixels, it is used in two places in the architecture: after the encoder part (i.e. in the middle of the network) and the second last layer, before creating a segmentation mask. This layer is only used close to the last output layer to deepen the architecture. In addition to the standard architecture that has a segmentation mask layer as output, there are also models in which multi-task learning is performed.

This architecture simultaneously learns four complementary tasks. The first one is the segmentation mask. The second is the common boundary between segmentation masks, which is known to improve semantic segmentation performance. The third case is the transformation of the segmentation mask distance. The fourth is the true color image; It means transforming identity from content but in a different color space.

To find the edges of the image, it is necessary to extract the information of different levels from each step to the edge pixel space. The next block is responsible for feature re-extraction and sampling, which is used for reduction after displaying the data. Therefore, the features can be mapped to the edge pixel space. The conv1_2 layer is a 1×1×1 convolution layer to reduce the dimensions and integration of features. The re-extraction block consists of three convolution layers, 1×1×32, 3×3×32, and 1×1×28. At the end of the network, a 1×1 kernel convolution layer is used to produce the final image and edge detection.

The training of neural networks is usually done based on the propagation cost function to adjust the parameters. The parameter adjustment method is to use the gradient descent algorithm to adjust the size of the parameters along the slope direction [8]. For this purpose, the entropy reduction and weighting function is used, which is based on the following equation [9].

$$C=-[t\ln(a)+(1-t)\ln(1-a)] \quad (1)$$

$$a=sigmoid(y)=1/(1+e^{-y})$$

C is the calculated loss reciprocal of a pixel and t is the target value. a is calculated with the sigmoid function. The output y of each hidden neuron is obtained from the sum of each of the weighted inputs w.x plus a bias b from the following equation [10]:

$$y=w\cdot x+b \quad (2)$$

Where y is output, x, w, and b are input, weight, and bias, respectively.

Results

Figure 2 shows the results of edge detection with the proposed method on images. In this figure, the original image, the gray image, and the edge detection results are shown.

As can be seen from the results of the figure, the proposed algorithm has demonstrated its ability to correctly identify the edges of the image. In each figure, it can be seen that the edge pixels are correctly detected.

For comparison, the F-measure is used, this is a well-known measure to quantitatively display the performance of edge detection and is given by the following relationship [11]:

$$\eta=(TN)/(TN+FP). \quad (3)$$

$$\phi=TP/(TP+FP). \quad F\text{-measure}=(2\eta\phi)/(\eta+\phi).$$

TP is true positive samples, TN is true negative samples, FP is false positive samples and FN is false negative samples.

Table 1. Comparison of edge detection with some methods

Method	***F-measure***
SED* [4]	0.707
HED [5]	0.788

Method	*F-measure*
SE [6]	0.743
gPb-owt-ucm[7]	0.729
Proposed method	**0.752**

* Available in [12]

The proposed method is more in terms of F-measure compared to the compared methods. After the proposed method, the HED method has the highest value.

4. CONCLUTION

Since deep learning and deep convolutional neural networks have been dominant in many computer vision tasks, deep learning has been widely used in edge detection. The proposed method is based on deep learning and ResNet architecture, which is used for edge detection. The results show that the edge detection accuracy has increased using this architecture. The F-measure is also improved compared to other compared methods.

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Induction of secondary metabolites in cell culture of pennyroyal (*Mentha pulegium*)

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Abstract

Plant tissue and cell culture are being used as a stable method to investigate plant secondary metabolites widely and production is being performed with high speed and low cost under in vitro conditions and independent of climatic conditions. Secondary metabolites have many applications in food industries, sanitary and pharmacy fields. According to abundant applications of pennyroyal (*Mentha pulegium*) in industries and according to the economic importance of this medicinal plant, replication and propagation of this herb has special momentous. Cell suspension culture can increase produced components and also can make new components in the plant. The aim of this research was to perform cell culture for investing secondary metabolites of *Mentha pulegium* and comparing it with the native one .

Materials and methods: The MS medium was used for suspension culture. For providing basic cellular suspension culture, the best-derived callus culture was used without agar to investigate quantitative materials, 4 levels of yeast extract elicitor (20, 40, 60 and 80 mg/l) and salicylic acid in 4 levels (2, 4, 6 and 8 mg/l) were used. Obtained extracts were analyzed by GC-MS.

Result: Statistical analysis showed that the amount of cis-pulegone oxide, terpinolene, 3-Octanol and iso-pulegol acetate was more than mentioned compounds in the natural plant as control. The secondary metabolites were increased by cell culture containing elicitors in *Mentha pulegume*.

Key words: Medicinal herbs, pennyroyal (Mentha pulegium), secondary metabolite, suspension culture.

1. Introduction

Medicinal herbs are known as an important source of producing secondary metabolites. Secondary metabolites have many applications in food industries, sanitary and pharmacy fields (1, 2).
Having limited natural habitats is the main problem for using these herbs. Low concentration of plant medicinal components, limitations in natural resources, decreasing in different species are the main points to attract plant breeders to find ways to solve problems. One of these ways is using of biotechnology methods (3-6). Cell culture, tissue culture, genetic engineering, and molecular markers are parts of biotechnology that can increase gene expression and gene production to produce the drug.

In recent years, plant tissue culture techniques have been used as powerful tools for the micro-propagation and breeding of many plant species (7).

Plant tissue culture has numerous applications in the field of medicinal plants; such as rapid and mass plant multiplication, pathogen-free plant production, enhanced performance and yield, protect endangered species, and the in vitro production of secondary metabolites. Plant culture under in vitro conditions, cause increasing in component production in comparison with growing this plant in natural conditions and also it can produce new components too. It can increase the production of components and also can make new components in comparison with the native plant (8-10).

Secondary metabolite can be produced by tissue culture methods under sterile and controlled conditions, and also purer and safer compounds are produced. Actually, the cell culture method is the best and the most economical method to produce these metabolites (11). Pennyroyal (*Mentha pulegium*) is an important medicinal plant that belongs to the Lamiaceae family and has an important position in the food industries, sanitary and pharmacy fields. The pennyroyal plant is herbaceous and the shrub height of60 cm that grows around rivers all parts of the stem have the medicinal trait. The main components of the essential oil of this plant are Alpha-Pinene, Beta-Pinene, Limonene, 3-Octyl acetate, p-Cymene, Menthone, Isomenthone, Menthol, Pulegone, Cis-pulegone oxide, Terpinolene, Iso-pulegol acetate, Caryophyllene, Lauric acid, Myristic acid, Palmitic acid, Salicyl aldehyde and etc (12-14).

Regarding tissue cultures of *M. pulegium*, few studies have been done, but there are some reports on other species of the *Mentha* genus (15). The aim of this research was to perform tissue and cell culture of *M. pulegium* for investing cis-pulegone oxide, terpinolene, 3-octanol and iso-pulegol acetate that which are important secondary metabolites of *M. pulegium* and compare it with plant in the native condition.

2. Material and methods

White and delicate callus were obtained in MS medium with 1 mg/l 2,4-D. Furthermore, this medium was used for cell culture as the liquid medium. Two grams of callus were added to 100 ml liquid media with different concentrations of elicitor. Four levels of yeast extract elicitor (20, 40, 60 and 80 mg/l) and salicylic acid in 4 levels (2, 4, 6 and 8 mg/l) were used. These elicitors were filtered to media after autoclaving. Then, they were placed on an incubator shaker with 100 rounds per minute at 25± 1°C. This study was performed in a completely randomized design (CRD) with nine treatments (two elicitors each one in four levels with one control) and three replications. After ten days, cell masses were filtered with filter paper and were dried by freeze dryer and then extracted by micro-Clevenger. Obtained extracts were analyzed by GC-MS to determine the amount of secondary metabolites in cells. Figures and tables should be included in the text and the closest place to the first project in the context. Figures and tables will be placed in the middle of the lines.

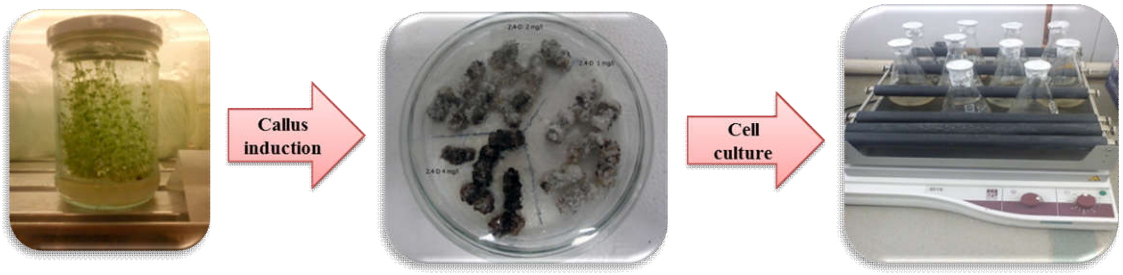

Figure 1: The schematic tissue and cell culture of pennyroyal

3. Result and Discussion

The result of this study (Figure 2 and Table 1) showed the different concentrations of elicitor in the MS media culture have a significant effect on the percentage of secondary metabolite ($P \leq 0.01$). According to Table 2, using salicylic acid and yeast extract, the amount of secondary metabolites increased significantly compared to the natural plant . The highest amount of terpinolene and cis-pulegone oxide secondary metabolites were produced in the culture media containing yeast extract elicitor (60 mg/l) . Also the amount of iso-pulegol and 3-octanol metabolites in the culture media contains salicylic acid (6 mg/l) was higher than other elicitor concentrations. By increasing the amount of both elicitors, the secondary metabolite decreases. This result is consistent with the results of other researchers.

According to the results of this research, the use of cell culture of pennyroyal is recommended to produce and increase secondary metabolites.

Table 1: Mean squares for the percentage of secondary metabolites in *Mentha pulegium*

SOV	df	terpinolen	cis-pulegone oxide	iso-pulegol acetate	3-Octanol
Elicitor	9	0.519**	0.631**	1.69**	0.382**
Error	20	0.041	0.019	0.008	0.009
CV%		8.91	6.86	2.84	5.57

**significant differences in the level of 0.01

Table 2: Mean comparison for the effect of different concentrations of elicitor on the percentage of secondary metabolites in *Mentha pulegium*

Elicitor (mg)	**Terpinolene**	**Cis-pulegone oxide**	**Iso-pulegol**	**3-Octanol**
Natural plant	1.00 b	1.49 d	1.55 g	1.46 e
Yeast 20	0.85 bc	1.92 bc	3.56 a	1.70 cd
Yeast 40	0.93 bc	2.02 ab	2.56 e	1.16 g
Yeast 60	1.57 a	2.16 a	2.38 f	1.30 f
Yeast 80	0.89 bc	1.21 e	3.04 bc	1.81 bc
SA 2	0.91 bc	2.10 ab	2.58 e	1.26 fg
SA 4	0.09 d	1.40 d	3.00 c	1.89 ab

SA 6	0.63 c	1.10 ef	3.58 a	2.02 a
SA 8	0.98 bc	1.00 f	3.15 b	1.65 d
free	1.07 b	1.80 c	2.75 d	1.40 ef

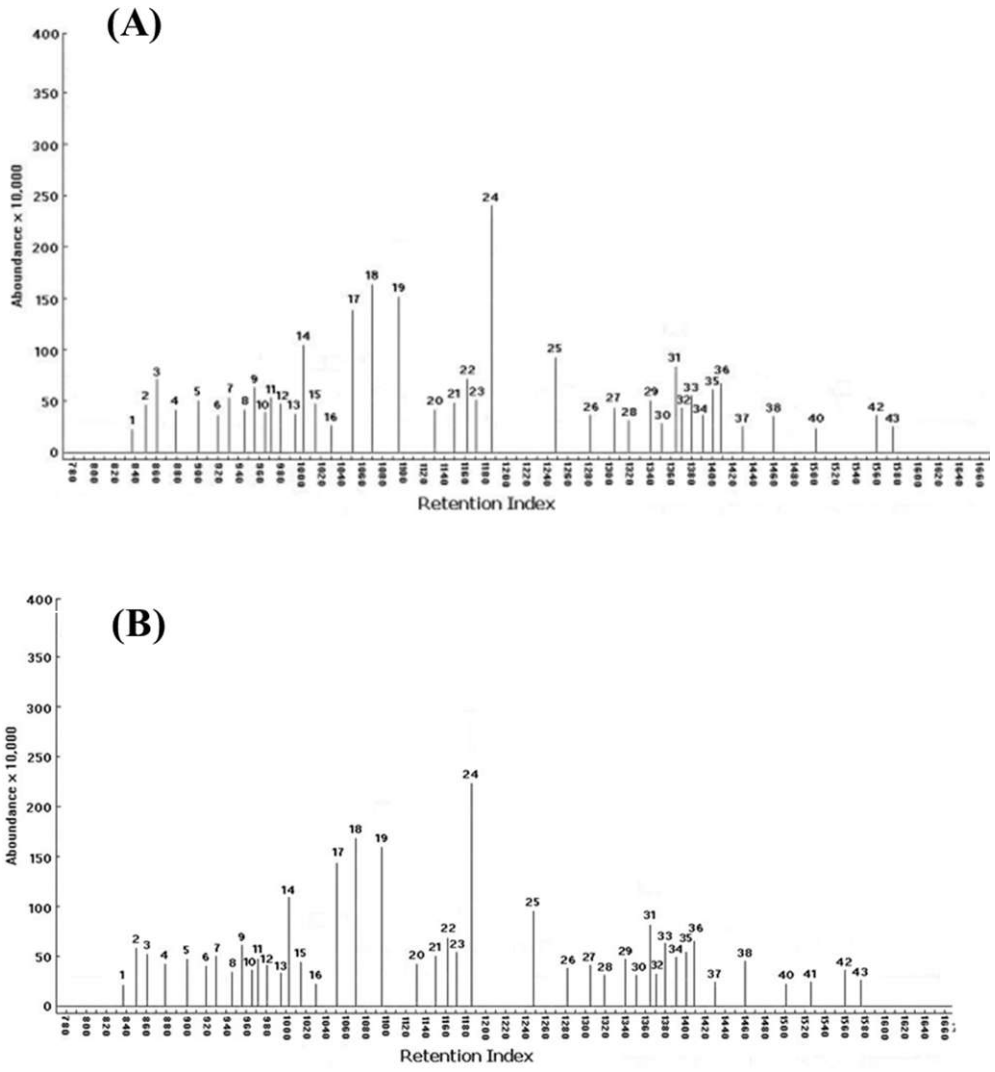

Figure 2: Investigating the effect of elicitors (A): yeast extract (B): salicylic acid on secondary metabolites in *Mentha pulegium by* GC-Mass result

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Differential cytoskeleton protein genes expression analysis between human sperm of patients with non-obstructive azoospermia and normal

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Abstract

Numerous research have recently focused on the function of cytoskeleton protein in spermatogenesis and male infertility. In the context of the etiopathogenesis of male infertility, we wanted to provide an overview of the information about the control of cytoskeleton protein gene during spermatogenesis. Overall, the results are consistent with the theory that sperm abnormalities and infertility are linked to sperm cytoskeleton protein. Differential methylation of a number of genes has been linked to poor spermatogenesis and/or reproductive failure. In order to identify the main pathways, the functional and molecular interactions of proteins were predicted using Enrich Shiny GO, STRING, and Cytoscape online assessments. This study has provided insight into the connection between cytoskeleton protein genes, including GOLGA6L6, CAPZA1, CTNND2, ACTR3B, MYO19 and KRT35. The microarray analysis of six human cases with different non-obstructive azoospermia revealed that CTNND2, ACTR3B, MYO19 and KRT35 were up-regulated, and expression of GOLGA6L6 andCAPZA1was down-regulated with the normal case. For this purpose, Enrich Shiny GO, STRING, and Cytoscape online evaluation was applied to predict proteins' functional and molecular interactions and then performed to recognize the master pathways. Enrich tool analysis revealed that the up-regulated DEGs were enriched in 3 GO terms, while down-regulated DEGs were incorporated with 3 GO terms. Enrich tool analysis revealed that the up-regulated DEGs were enriched in 3 GO terms, while down-regulated DEGs were incorporated with 3 GO terms. Functional enrichment analysis demonstrated that the biological process (BP) term “dendritic spine morphogenesis”, “peptidyl-lysine methylation” and “barbed-end actin filament capping” was significantly overexpressed in DEGs.Overrepresented molecular function (MF) terms in up-regulated DEGs included “plus-end-directed microtubule motor activity”, “myosin light chain binding” (myosin light chain binding), “microfilament motor activity” and “actin filament binding”. According to our research, these genes and the hub proteins that they interact with may contribute to understanding the pathophysiology of aberrant germ cells and infertility.

Key words: Cytoskeleton Protein, In-Silico, Microarray, Non-Obstructive Azoospermia, Gene Expression

1. Introduction

Infertility affects between 10% and 15% of adults worldwide who are of reproductive age, with male infertility making up roughly 50% of all cases. Obstructive azoospermia (OA), non-obstructive azoospermia (NOA), endocrine, varicocele, and other reproductive system infections are all intimately associated with male infertility [1]. One of the most significant causes of male infertility, NOA affects males at a rate of roughly 1% and accounts for 10-15%

of infertile men. An example of male infertility induced by spermatogenic failure of testicular tissue is non-obstructive azoospermia. Patients with NOA are either spermless or have very little sperm production [2]. Patients with NOA have irregular seminiferous tubule structure in the testis, as well as spermatogenic cell maturation blockade and spermatogenic cell meiosis arrest. Recent research has shown that spermatogenesis disorders include localized and diverse tissues [3]. The expression of this subfamily of Epigenetics genes in male germ cells has also been the subject of a few investigations. In this experimental investigation, we used microarray and in-silico analysis to demonstrate and examine the expression of Epigenetics genes in seminiferous tubules.

2.Material and Methods

Patient samples preparation

Three individuals with azoospermia caused by defective spermatogenesis were assessed, as were three healthy instances. They had an appointment for a testicular biopsy for genetic testing, followed by an intracytoplasmic oocyte injection. The egg retrieval and ICSI cycles, which happened 3-6 months later, were not planned to correspond with the testicular biopsies and sperm extraction. Testicular spermatozoa that had been frozen were found. The study was approved by the University of Heidelberg Ethics Committee. In accordance with World Health Organization recommendations, serum samples were obtained and examined. A diagnosis of azoospermia was made when no spermatozoa were found in the pellet formed from semen centrifuged at 1500 g for 10 minutes. The history of urological surgeries and gonadotoxic exposure, as well as the developmental, social, medical, and reproductive histories, were all documented. A comprehensive, systemic, and genital examination was performed on each patient. In order to determine testicular volume, a Prader orchidometer was used. Follicle-stimulating hormone (FSH) (normal value: 1-2 IU/I) and luteinizing hormone (LH) (normal value: 2-11 IU/I) plasma concentrations were measured using the enzyme linked immunoassay. Plasma testosterone was measured by radioimmunoassay (normal range: 9.4-37.0 nmol/l) (Diagnostic Products Ltd, Cardiff, Wales, UK).

Microarray analyses

R Statistical Environment 4.1.2 was used to import data from microarrays (accessed 2022-2-1). The data were compressed using the Bioconductor software affy version 3.14. To accomplish extra normalizing across samples, a multi-lowess method, a multi-dimensional variation of the lowess normalization strategy, was applied. Sample-to-sample correlations, high variance genes, preset gene sets for a Epigenetics gene from the literature, and an extended profile search were used to analyze the data. In order to evaluate gene functions and pathways, a portion of the data was entered into the IPA Ingenuity program.

Pathway enrichment analysis and gene ontology (GO) investigation

We investigated the enrichment route using KEGG (Kyoto encyclopedia of genes and genomes, https://www.genome.jp/kegg/) and Reactome (https://reactome.org/), two online tools for functional gene annotation.

Protein-protein interactions (PPI)

The functional interactions between proteins were discovered using the internet resource Search Tool for the Retrieval of Interacting Genes (STRING v.11.5). The up-regulated genes that are crucial for the development of germ cells and infertility were uploaded using the STRING tool. PPIs were highlighted, both known and prospective.

Nominate appropriate microRNAs

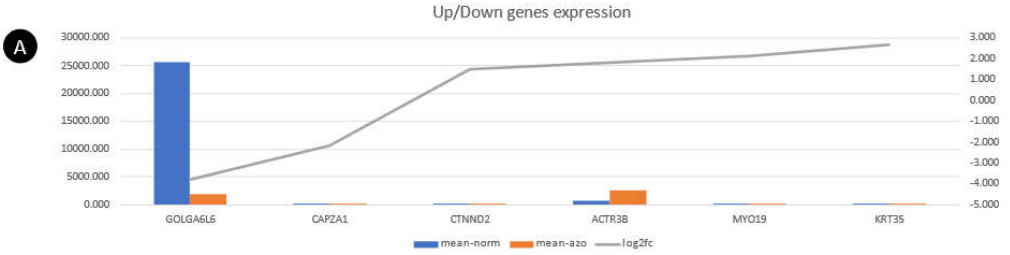
After selecting the genes and potential proteins to analyze and choose the genes-related microRNAs, we uploaded the genes to the Enrichr database in this section (https://maayanlab.cloud/Enrichr/). To target this, we made use of the Targetscan library.

3.Results

Differential gene expression analysis by microarray

We evaluated all The Epigenetics genes (185 genes) by microarray (in supplementary 1). This study has provided insight into the connection between Epigenetics genes, including *GOLGA6L6, CAPZA1, CTNND2, ACTR3B, MYO19 and KRT35.* The microarray analysis of six human cases with different non-obstructive azoospermia revealed that *CTNND2, ACTR3B, MYO19 and KRT35* were up-regulated, and expression of *GOLGA6L6 and CAPZA1* was down-regulated with the normal case.

A



B

GeneName	Description	Sperm_azoo_1	Sperm_azoo_2	Sperm_azoo_3	Sperm_normo_1	Sperm_normo_2	Sperm_normo_3	mean-norm	mean-azo	log2fc	p-value
GOLGA6L6	golgin A6 familylike 6 (GOLGA6L6)	936.410	2379.009	2252.043	13746.933	30412.519	32541.176	25566.876	1855.821	-3.784	0.050
CAPZA1	capping protein (actin filament) muscle Z-line, alpha 1 (CAPZA1)	8.458	8.269	7.747	36.150	31.220	43.155	36.842	8.158	-2.175	0.016
CTNND2	catenin (cadherinassociated protein)	41.529	35.000	40.369	18.077	18.770	4.294	13.713	38.966	1.507	0.049
ACTR3B	ARP3 actin-related protein 3 homolog B (yeast) (ACTR3B)	1951.309	3119.475	2879.497	877.695	875.340	499.868	750.967	2650.093	1.819	0.045
MYO19	myosin XIX (MYO19)	308.173	213.841	349.988	108.820	45.090	42.840	65.583	290.667	2.148	0.033
KRT35	keratin 35 (KRT35)	201.605	368.328	312.841	41.723	83.252	14.974	46.649	294.258	2.657	0.030

Figure 1. (A) Genes down/ up the expression in spermatozoa of non-obstructive azoospermia infertile men. The microarray analysis of six human cases with different non-obstructive azoospermia revealed that CTNND2, ACTR3B, MYO19 and KRT35 were up-regulated, and expression of GOLGA6L6 andCAPZA1was down-regulated with the normal case. (Fold change > 2 and P-value > 0.05).

Biological process and molecular functions of enrichment analysis:

Enrich tool analysis revealed that the up-regulated DEGs were enriched in 3 GO terms, while down-regulated DEGs were incorporated with 3 GO terms. Functional enrichment analysis demonstrated that the biological process (BP) term “dendritic spine morphogenesis” (GO:0034968) ($p < 0.002$), “peptidyl-lysine methylation” (GO:0060997) ($p < 0.0004$) and “barbed-end actin filament capping” (GO:0051016) ($p < 0.0005$) was significantly overexpressed in DEGs.

Overrepresented molecular function (MF) terms in up-regulated DEGs included “plus-end-directed microtubule motor activity” (GO:0042054) ($p < 0.0001$), “myosin light chain binding” (myosin light chain binding) ($p < 0.002$), “microfilament motor activity” (GO:0000146) ($p < 0.001$) and “GO:0000146” (GO:0051015) ($p < 0.0005$).

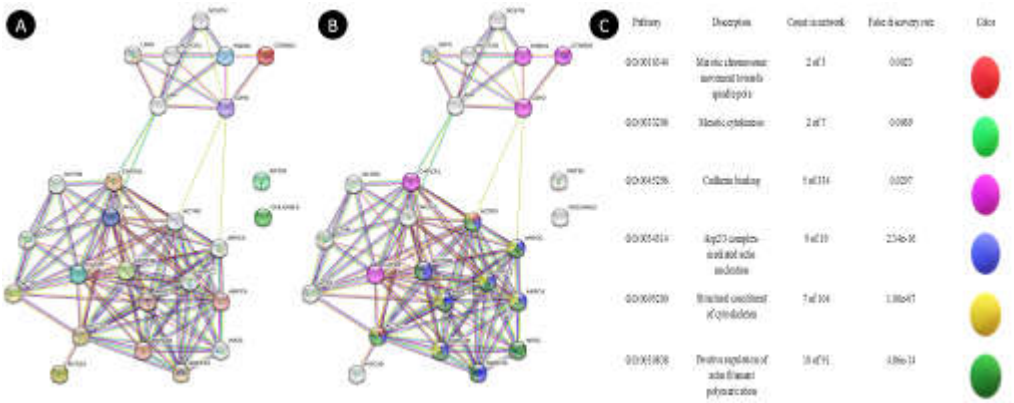


Figure 2. STRING protein-protein interaction network based on gene interaction. (A) PPI network up/down-regulation and related genes and (B) Direct linkage of genes correlated in up/down-regulation genes. (C) GO biological process, molecular function, and signaling pathways of Up/ down-regulation genes with non-obstructive azoospermia and normal cell.

Protein-protein interaction visualization of Differential gene expression

The protein-protein interaction network was visualized with 15 DEGs using the STRING database (Fig. 5). It showed a close relationship among up /down-regulated DEGs. It was shown that there was a highly positive co-expression relationship between *GOLGA6L6, CAPZA1, CTNND2, ACTR3B, MYO19 and KRT35.* Clearly, there is a strong correlation between the highlighted genes.

Identification of key Differential gene expression

Cytoscape was utilized for network analysis to identify master genes. The highlighted genes in Fig. 5 were uploaded in Cytoscape using the yfiles redial layout technique (Fig. 7 a). The network that generated classified DEGs based on their interactions. The illustration shows the 24 most correlated genes with the highest interaction. As is obvious, *GOLGA6L6* is the only and primary regulator of the most correlated genes. The Cytoscape network's other layouts revealed the interaction of less significant genes. The yfiles redial design was verified using the Cytoscape network analysis tool. The Centiscape plugin represented *CTNND2, ACTR3B, MYO19 and KRT35* as the most important hub genes based on degree and betweenness centrality.

Isolated and selected candidate microRNAs

In this section, after identifying 8 genes, *CTNND2, ACTR3B, MYO19 and KRT35*, we isolated and selected the most relevant microRNAs (Fig. 8 a). Accordingly, mmu-miR-3076-3p, hsa-miR-4536, hsa-miR-523, mmu-miR-126-3p and hsa-miR-564 were observed more clearly than other microRNAs (Fig. 8 b). These microRNAs are high-potential candidates for up and down-regulation of *CTNND2, ACTR3B and MYO19* genes.

Figure 4. (A) The picture shows that the most significant microRNAs associated with the CTNND2, ACTR3B, MYO19 and KRT35 genes were selected on the Manhattan diagram. (B) The communication network between ACTR3B, MYO19 and KRT35 are selected using the MienTurnt database. (C) P-value, q-value, and overlap gene with candidate microRNAs.

4.Discussion

According to recent research, the cadherin-catenin system is found in Sertoli-spermatid junctions. It is unclear, however, whether the cadherin-catenin system is required for the development of Sertoli-spermatid junctions [4]. The nectin-afadin system, on the other hand, is now known to be crucial for the establishment of Sertoli-spermatid junctions [5]. If this concept is extended to Sertoli-spermatid junction formation, the heterophilic trans-interaction of nectin-2 and nectin-3 would first form the cell adhesion, then recruit N-cadherin to the

nectin-based adhesion sites, and finally establish the strong adhesion undercoated with F-actin that is mediated by afadin and catenins at Sertoli-spermatid junctions. Our microarray analysis showed that THBS4 and STRC, were up-regulated, while GJB1, SIGLEC16, ITGA1, and CEACAM16 were down-regulated. These genes are necessary for the assembly of this cell polarity complex. We hypothesized that altering the expression of these genes may change the polarity protein complex formation and spermatid development.

Additionally, we speculate that throughout the processes of spermatogenesis and sperm maturation, sperm with defects in these genes are unable to distinguish and divide. Technology advancements like CRISPR are anticipated to help prove a number of posited processes by generating useful data.

5.Conclusion

The microarray analysis of six human cases with different non-obstructive azoospermia revealed that CTNND2, ACTR3B, MYO19 and KRT35 were up-regulated, and expression of GOLGA6L6 andCAPZA1was down-regulated with the normal case.

6.Acknowledgments

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Utilization of Nanotechnology to Improve the Application of Phytosterols

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Abstract

Interest in preparing foods to improve the overall human health and wellness is growing with the trending food for health concept. Among bioactive compounds, phytosterols have received much attention in managing hypercholesterolemia, or abnormally high blood cholesterol, and recently inflammation and inflammatory bowel disease, a chronic inflammatory condition of the gastrointestinal tract. Bioavailability of phytosterols is very low due to poor water solubility and high crystallinity. Decreasing the size of bioactives is known to increase the bioaccessibility and in turn the bioavailability. In recent years, there have been many efforts to decrease the size of phytosterols. This review investigates the chemical classification and bioavailability of phytosterols and also provides a description for nano-delivery systems of them to improve their therapeutic applications phytosterol nanoparticles.

Key words: *Phytosterol*, Phytochemicals, Nanoparticles, bioavailability.

1- Introduction

The consumption of fruits and vegetables is considered to be inversely related to the risk of developing many chronic diseases. As we know, vegetables and fruits contain antioxidant phytochemicals that are thought to contribute to these health benefits. Plant sterols are plant compounds with similar chemical structure and biological functions as cholesterol. Plant sterols contain an extra methyl, ethyl group or double bond. The most abundant plant sterols are sitosterol, campesterol and stigmasterol. Dietary sources include vegetable oils (especially unrefined oils), nuts, seeds and grains [1]. Absorption efficiency for plant sterols in humans is considerably less than that of cholesterol. Due to their structural similarity to cholesterol, plant sterols were first and foremost studied for their cholesterol absorption inhibition properties. In addition to their cholesterol lowering effect, plant sterols may possess anti-cancer, anti-atherosclerosis, anti-inflammation and anti-oxidation activities [2-4]. It has been reported that phytosterols have protective effects on various chronic ailments including cardiovascular diseases and diabetes. Moreover, it is suggested that diets rich in phytosterols can reduce the risk of cancer by 20% [5]. Phytosterols are easy to oxidize and can produce different phytosterol oxidation products, such as hydroxyl groups, epoxy resins, ketones and triol derivatives, especially when heated or stored for a long time. These compounds are insoluble in water and slightly soluble in oil, which limits their application in the pharmaceutical and health products industries. Drug delivery system improves the bioavailability of drugs by improving their solubility, lymphatic absorption and intestinal permeability [6].

Chemistry of phytosterols

Phytosterols belong to the family of triterpenes, being constituted by a tetracyclic ring and a side chain linked in position C-17. The structure of phytosterols is very similar to cholesterol

and up to now, more than 250 molecules were identified as belonging to phytosterols family (Fig. 1). These compounds can be classified as sterols or stanols, according to the presence or absence of a double bond at the Δ5-position. The most abundant sterol is β-sitosterol, while the most abundant stanol is sitostanol [7].

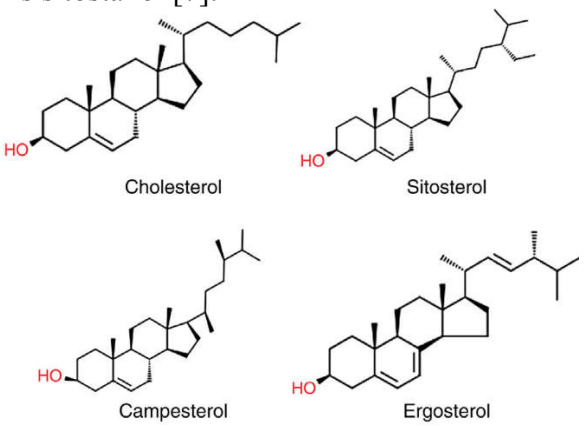

Figure 1. Chemical structures of phytosterols.

2- Health benefits of phytosterols

Phytosterols reduce the cholesterol absorption and lower the plasma LDL, as confirmed by a number of clinical studies. Absence of dietary phytosterols/stanols in the diet resulted in elevated serum LDL cholesterol . Antiatherosclerotic activity of plant sterols/stanols has been tested in numerous clinical studies. Whereas the cholesterol-lowering activity has been well-documented, other antiatherogenic properties of these substances were less evident. No beneficial effects of phytosterols on vascular function, coagulation, oxidative stress, or inflammation could be demonstrated. The relationship of phytosterols and overall cardiovascular risk remains to be elucidated. Some studies demonstrated a positive association of plasma phytosterol level and vascular disease, whereas others demonstrated an inverse or lack of relationship. In conclusion, phytosterols/stanols are currently well-recognized as cholesterol-lowering agents for primary and secondary prevention of cardiovascular diseases. The American Heart Association and the European Current Dietary Guidelines recommend phytosterols as a therapeutic option for treatment of patients with elevated blood cholesterol. The chemopreventive benefits of phytosterols have been observed in many cancers [8-9].

3- phytosterols in foods

Recently, the interest of consumers for healthy and beneficial foods is growing. One way to achieve this goal is fortifying the foods and beverages with bioactive compounds. Among bioactive ingredients, phytosterols have gained much attention due to their wellness effects on lowering both the blood cholesterol and the ratio of the low-density (LDL) to highdensity lipoprotein (HDL) bound cholesterol in serumSterols make up the largest proportion of the unsaponifiable fraction of lipids [11]. Plant fats and oils contain phytosterols as naturally occurring constituents. The most important natural sources of plant sterols in human diets are oils and margarines, although they are also found in a range of seeds, legumes, vegetables and unrefined vegetable oils. Cereal products are a significant source of plant sterols, their contents, expressed on a fresh weight basis, being higher than in vegetables. In foods, plant sterols occur as free sterols, fatty acyl esters, glycosides and fatty acyl glycosides [10]. Recent work shows that phytosterols in natural food matrices are

bioactive. The retention of phytosterols during food manufacturing and the use of foods with high phytosterol content may constitute an alternative to the use of supplements [12].

4- Nanoscale Delivery Systems for phytosterols

Most bioactive lipids are not synthesized in the human body and so they have to be obtained from food sources, such as nuts, seeds, grains, eggs, meat, fish, tomato, fruits, and vegetables. However, many people do not get enough of these bioactive compounds from their regular diets due to their low dietary levels, tendency to degrade during processing or storage, and their poor stability, solubility, and absorption characteristics within the human gut. The food industry is therefore exploring methods of fortifying foods with stable and bioavailable forms of these bioactive lipids. Nanotechnology has emerged as a powerful means of encapsulating, protecting, and delivering bioactive substances in foods, thereby improving their efficacy. These nanoscale delivery systems should be constructed entirely from food grade ingredients and should be designed to provide resistance against the elevated temperatures, light levels, and oxygen levels they may be exposed to during food processing and storage. They should also be designed to be stable within the specific food matrix that they are utilized within. Nanoparticles of phytosterols, polylactic acids, and polyethylene glycols are also being used in oils and fats. Canola Active Oil, produced by Shemen Industries has nanoencapsulated fortified phytosterols that reduces cholesterol intake by 14% [13-14]. The solubility and bioavailability of phytosterols were also enhanced by Cyclodextrins (CDs) and their characterization demonstrated the existence of intermolecular hydrogen bonds between phytosterols and hydroxypropyl β-CD which resulted in the formation of amorphous form of phytosterols into host inclusion complexes [15]. Also, chitosan NPs have been applied as carriers of γ-oryzanol as a phytosterol for improving its efficacy to decrease the blood levels of cholesterol and LDLs [16].

5- Conclusions

Food industry has recently prioritized the incorporation of bioactive compounds (e.g., phytosterols, tocopherols, carotenoids) into foods to design functional food products for improved health. Particularly, phytosterols have received great attention due to their health benefits such as LDL-cholesterol lowering effect, anticancer and antiinflammatory properties. However, the bioavailability of phytosterols is very low owing to poor water solubility and high-melting-point crystalline structure which also severely limit the potential food applications of phytosterols. Recently nano scale delivery systems for phytosterols has been a considerable interest because efficacy of phytosterols compounds are improved by increasing the solubility, bioavailability, stability and permeability of them.

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Antifungal efficacy of citric and tartaric acids and their combination against *Candida albicans* and *Malassezia furfur*

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Abstract

The present study was to evaluate the effects of citric and tartaric acids and their combination on the growth inhibition of some important pathogenic fungi *in vitro*. *Candida albicans* and *Malassezia furfur* were cultured into specific media and then, yeast cells were harvested from the medium surface and counted by hemacytometer method. The antifungal susceptibility test of citric and tartaric acids and their combination against fungi were assayed by broth macrodilution technique. The results demonstrated that citric acid had more fungistatic and fungicidal activities than those of tartaric acid against pathogenic yeasts tested. Antifungal activity of the acids combination was similar to citric acid, but higher than tartaric acid alone. Further research is needed to assess the efficacy of citric and tartaric acids as inhibitors of fungal growth in clinical trials, especially in treatment of patients with yeasts infections.

Key words: Citric acid, Tartaric acid, Antifungal activity, *Candida albicans, Malassezia furfur.*

1. Introduction

Microbiological resistance to antifungals, particularly polyenes and azoles, has been increasingly reported. Despite the increase in frequency of resistance, reports of clinical antifungal failures or single stains becoming resistant to antifungal therapies remain distinctly uncommon [1]. On the other hand, the number of serious invasive fungal infections has continued to increase due to the fact that more immunosuppressed patients are at risk for these infections. Organic acids are widely used as preservatives in foods and have been used as buffer agents in medical solutions [2]. Several studies reported the inhibitory effect of these acids such as saturated fatty acids, formic and propionic acids, lactic acid and medium-chain fatty acids against different microorganisms [3, 4]. As far as we know, there is little information about the antifungal activity of organic acids, especially citric and tartaric acids against clinically important potential yeast pathogens. The main objective of this study was to investigate the antifungal effects of two above mentioned acids against *Candida albicans* and *Malassezia furfur*.

2. Materials and Methods

2.1. Test organisms

Clinical isolates of *C. albicans* (isolated from a patient with cutaneous candidiasis) and *M. furfur* (isolated from a patient with pityriasis versicolor) were obtained from fungal collection of Faculty of Veterinary Medicine, University of Tehran, Iran.

2.2. Fungal cultivation and preparation of inocula

C. albicans was cultured onto Sabouraud dextrose agar (*Merck Co., Darmstadt, Germany*) at 32°C for 3 days and *M. furfur* was cultured onto Dixon agar (*Merck Co., Darmstadt, Germany*) at 32°C for 3 days. After incubation, *C. albicans* and *M. furfur* yeasts were flooded with sterile saline containing 0.1% Tween 80 and scraped off with the aid of a loop. The concentration of fungal cells was quantitated by counting with a hemacytometer, and adjusted at a density of 1×10^7 cell/ml for both *C. albicans* and *M. furfur*.

2.3. Preparation of citric and tartaric acids

Citric and tartaric acids (*Merck Co., Darmstadt, Germany*) were obtained as reagent-grade powders from their respective manufacturers.

2.4. Antifungal assay

The *in vitro* fungistatic and fungicidal concentrations were determined by a serial dilution method using broth macrodilution technique as previously described with modifications [5], which Sabouraud glucose broth (for *C. albicans*) and Dixon broth (for *M. furfur*) were used for test isolates. Briefly, dilutions of citric and tartaric acids were prepared from stock solutions of 50% (50 mg/ml) in deionized water. A series of concentrations of each acid and their combination, 50% (50 mg/ml), 45% (45 mg/ml), 40% (40 mg/ml), 35% (35 mg/ml), 30% (30 mg/ml), 25% (25 mg/ml), 20% (20 mg/ml), 15% (15 mg/ml), 10% (10 mg/ml), 5% (5 mg/ml), 2.5% (2.5 mg/ml), 1.25% (1.25 mg/ml) and 0.75% (0.75 mg/ml) were prepared in Dixon broth and Sabouraud glucose broth (1:1) into the tubes. Then, each tube was inoculated with 10 µl of the fungal suspensions. The tubes were incubated at 32°C for 48 h for both *C. albicans* and *M. furfur* and then scored for visible growth after vortexing the tubes gently. The minimum inhibitory concentration (MIC_{90}) was defined as the lowest concentration of the acid which produced 10% visible growth (90% inhibition). Each MIC_{90} determination was performed in triplicate. Drug-free and fungal control tubes were included for each isolate tested. The minimum fungicidal concentration (MFC) was determined in triplicate by subculturing 0.1 ml aliquots from all MIC_{90} tubes showing no visible growth on specific media.

2.5. Statistics

Student's t-test was used to assess statistical differences between the groups. Probabilities of 5% were taken to be statistically significant.

3. Results and Discussion

The antifungal activity of citric and tartaric acids and their combination against all tested fungi, as measured by agar dilution test, was presented in Table 1. All of the fungal organisms tested were affected by citric and tartaric acids and their combination when applied at concentration more than 2.5 %. Citric acid was active against all pathogenic fungi tested. Conversely, tartaric acid showed modest activity against all fungi tested but was less active than citric acid against fungi. The MIC_{90} and MFC values of the acids combination approximated the similar to those of citric acid but less than tartaric acid alone; thus, overall, neither significant synergism nor antagonism was seen from this screening assay with the

combination of citric plus tartaric acids. Totally, the mean MIC_{90} and MFC values for citric acid and their combination on different fungal isolates were 3.3 and 2.2-fold higher than those for tartaric acid. The higher activity of citric acid may be attributed to have several inhibitory mechanisms such as depression of internal pH of microbial cell by ionization of undissociated acid molecules and disruption of substrate transport by altering cell membrane permeability or reduction of proton motive force [6]. Conversely, tartaric acid, as an antimicrobial agent, is believed to act only by lowering the pH of the cell [7]. In addition to the inhibition of energy production, tartaric acid prevents the production of malic acid, which is a key intermediate in the production of glucose in the process of gluconeogenesis, the principal fuel for the cells [8]. No previous study has been carried out on antifungal activities of these acids tested, except Lee *et al.* [9] study on *C. albicans* isolate. That study showed that citrate salt was active against gram-positive species and *C. albicans* but showed little activity against gram-negative species; acetate salt showed the opposite results. Their combination did not show synergism or antagonism.

Table 1. Determination of MIC_{90} and MFC (%) of citric and tartaric acids and their combination against *Candida albicans* and *Malassezia furfur*

Acid	Fungus			
	C. albicans		*M. furfur*	
	*MIC$_{90}$	**MFC	MIC$_{90}$	MFC
Citric acid	5	2.5	10	5
Tartaric acid	20	15	15	10
Combination	5	2.5	10	5

***MIC$_{90}$:** Minimum inhibitory concentration (90% inhibition)
** **MFC:** Minimum fungicidal concentration

4. Conclusions

In summary, citric and tartaric acids are active antifungal agents *in vitro*. However, further research is required to assess the correlation between antifungal activity *in vitro* and the actions *in vivo*, and the successful results might in the future be applied in the treatment of patients with yeasts infections.

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Effect of the complex of β-glucan and *Zataria multiflora* on oxidative burst of neutrophils in BALB/c mice

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Abstract

Natural products such as β-glucan and herbal essences have been found to enhance immune functions. The aims of this study were to purify β-glucan from the cell wall of *Saccharomyces cerevisiae* and to investigate its conjugate effect with *Zataria multiflora* essence on oxidative burst in BALB/c mice. After preparing the cell walls from the yeast, β-glucan was extracted from wall by alkaline-acid treatment (glucan-S_1). This preparation contained protein and mannan, which were removed by DEAE sephacel chromatography (glucan-S_2) and con-A sepharose chromatography (glucan-S_3), respectively. Glucan-S_3, *Z. multiflora* essence, and their conjugate were injected into BALB/c mice intraperitoneally. Blood was collected at days 4 and 7 after injection and oxidative burst was assayed by chemiluminescence method. The results showed that intraperitoneally administration of glucan-S_3, *Z. multiflora* essence and their conjugate significantly increased oxidative burst of neutrophils both at days 4 and 7 when compared to control group ($P<0.05$). These findings suggest that the complex of β-glucan and *Z. multiflora* essence can be used as adjuvant agents to stimulate immune functions in immunocompromised subjects.

Key words: *Saccharomyces cerevisiae, β-glucan, Zataria multiflora essence,* oxidative burst.

1. Introduction

In respect to the high incidence of life-threatening infections among cancer patients, transplant recipients, patients with AIDS and patients receiving broad-spectrum antibiotic, corticosteroid and cytotoxic drugs, widespread efforts have been made to identify immunomodulatory agents [1]. In this case, several studies demonstrated that β-glucans isolated from the yeast exhibit antitumor, antimicrobial, and radioprotective activities [2]. *In vivo* studies revealed that β-glucan stimulated degranulation and oxidative burst of neutrophil and the secretion of IL_1, TNF-α, and IL_6 from macrophages [3]. Herbal products such as *Zataria multiflora* have been reported represented stimulatory effects on the function of innate and acquired immunity [4]. In the present study, we developed an optimal method to purify β-glucan from the yeast cell wall that was free of other components (mannoprotein and chitin) of the wall and subsequently investigated the complex of β-glucan and *Z. multiflora* essence on oxidative burst in BALB/c mice.

2. Materials and Methods

2.1. Yeast cultivation and cell wall preparation

Saccharomyces cerevisiae (ATCC 96581) was grown in YPG broth (1% Yeast extract, 2% Peptone, and 2% Glucose) and shaked (150 rpm) at 30°C for 48 hours. Yeast cells were collected by centrifugation at 4500 g for 5 minutes, and washed 3 times with sterile distilled water.

2.2. Purification of β-glucan from the cell wall

Glucan-S_1 was extracted from the yeast cell wall with alkaline extraction and acid treatment methods as described previously by Lee et al. [5]. The preparation (glucan-S_1) was loaded into a DEAE sephacel column (12×1.5 cm, Bio-Rad Laboratories, Hercules, California, USA) to remove the residual proteins. The unbound fractions containing β-glucan (glucan-S_2) were collected by eluting the column with 3 bed volumes of 0.05 M Tris-HCl (Sigma Chemical Co., Life Technologies, New York, USA) buffer at pH 8. The preparation was then applied into a con-A sepharose column (7×0.7 cm, Bio-Rad Laboratories, Hercules, California, USA) to remove mannan. The unbound fractions in the column containing water soluble β-glucan (glucan-S_3) were collected by eluting the column with 54 ml of the binding buffer (20 mM Tris-HCl, pH 7.5, containing 0.5 M NaCl, 1 mM MgCl2, 1mM MnCl2 and 1 mM CaCl2), dialyzed against double distilled water, and lyophilized.

2.3. Chemical assays

Protein concentration was measured by Bradford microassay [6]. Carbohydrate was determined by a hydrolysis procedure and subsequently total reducing sugars were assayed by ortho-toluidine method. Total glucose was enzymatically determined with a glucose oxidase kit (Ziest Chemical Co. Tehran, Iran). Glucan and mannan were identified as glucose and mannose, respectively.

2.4. Mice

Thirty and two female, 6-week old BALB/c mice were divided into four equal groups (A, B, C, and control) and fed under specific pathogen-free conditions. Mice were intraperitoneally administered with glucan-S_3 (single dose, 15 mg/ kg) in group A, *Z. multiflora* essence (single dose, 100 mg/kg) in group B, the complex of β-glucan and essence (the same doses) in group C, and distilled water in control group. At days 4 and 7, the bloods of 4 mice in each group were collected.

2.5. Oxidative burst assay

Chemiluminescence method was performed for determining oxidative burst. Briefly, 0.5 ml of heparinzed blood was diluted with dextran (Pharmacia, Uppsala, Sweden) in ratio of 2:1 (dextran: blood) and maintained at laboratory temperature for 30 minutes. Supernatant containing neutrophils was added to 0.19 ml of Ficoll (Sigma Chemical Co., St. Louis, USA), and then resuspended in 10 ml of phosphate buffer saline (PBS, pH 7.2). After centrifugation, the supernatant was harvested, brought to a final volume of 1 ml with PBS, and neutrophils were counted. 0.5 ml of PBS, 0.2 ml of luminol (Sigma Chemical Co., Deisenhofen, Germany), 0.2 ml of phorbol 12- myristate 13-acetate (PMA) solution (Sigma Chemical Co., Deisenhofen, Germany), and 0.1 ml of neutrophil suspension were added to special cuvett and its value was determined by luminometer set (BioOrbit, Finland).

2.6. Statistical analysis

Unpaired Students t-test was performed using SPSS software (version 12). *P*-values less than 0.05 were considered significant.

3. Results

3.1. Yield of alkali-soluble β-glucan (glucan-S_1)

The results showed that the total of obtained cell walls was 21.4% of the cells dry weight and glucan-S_1 included 27.5% of wall dry weight. The protein content of glucan-S_1 was 96.5-µg/ml, representing 2.4% of sample dry weight. The content of total carbohydrate, glucose and mannose of this fraction was 110.6, 32.8, and 77.8 mg/ dl, respectively. The approximate ratio of mannan to glucan-S_1 in the glucan-S_1 fraction was calculated about 70.3 to 29.7.

3.2. Yield of DEAE sephacel column fraction (glucan-S_2)

The protein content of this fraction was measured about 7.1 µg/ml, representing 0.004% of sample dry weight. The amounts of total carbohydrate, glucose, and mannose were determined 90, 21.5, and 68.5 mg/dl, respectively. Moreover, in this step, the mannan to glucan-S_2 ratio was calculated approximately 71.9 to 28.1.

3.3. Yield of con-A sepharose column fraction (glucan-S_3)

The purified β-glucan (glucan-S_3) had undetectable protein by Bradford microassay. Monosaccharide analysis of this fraction showed that the only free carbohydrate present was glucose, whereas the amount of mannose was undetectable. In respect to remove mannan completely from the sample, the ratio of mannan to glucan-S_3 was determined about zero to 100.

3.4. Oxidative burst

Statistical analysis showed a significant increase of 246% and 242% at days 4 and 7 ($P<0.05$) in group A, 165% and 235% at days 4 and 7 ($P<0.05$) in group B, and 367% and 309% at days 4 and 7 in group C when compared with control.

4. Discussion

In this study, we have applied and optimized a simple alkaline extraction followed by using sequential chromatography steps to purify β-glucan. We showed the walls from *S. cerevisiae* comprised approximately 21.4% of the cell dry weight. The yield of glucan-S_1 (27.5% of wall dry weight) was in agreement with findings of Suphantharika et al. [7]. The reasons for different contents are most likely to depend on the strain, growth and extraction conditions such as alkali concentration, alkaline extraction time, extraction temperature and its duration and also applied chemical and enzymatic methods. In this regard, the mannan to glucan-S_1 ratio of the present study (~ 70.3 to 29.7) was in agreement with the value reported by Lee et al. (70 to 30) [5]. The mannan to β-glucan ratio was calculated approximately 71.9 to 28.1 in glucan-S_2 fraction. Moreover, mannan to glucan-S_2 ratio of the present study was in agreement with the finding of Lee et al. (70 to 30) [5]. The ratio of mannan to glucan-S_3 was determined about zero to 100 in glucan-S_3 fraction. This finding was similar to those values reported by Ha et al. [8]. In our experiment, the blood collected in two stages (at days 4 and 7) after administering glucan-S_3. The results showed a significant increase in oxidative burst of neutrophils in all mice treated with glucan-S_3 at days 4 and 7 when compared to the control. There was not significant difference in oxidative burst between days 4 and 7. Ohno et al. [9] showed significant activation of neutrophils by intraperitoneally injection of beta-glucan in mice. In this study, we also evaluated the immunostimulatory effect of one of the Iranian herbal essence, *Z. multiflora*, on innate immune response. Respiratory burst mean in mice treated with herbal essence at days 4 and 7 were about 122.2 mv and 170.62 mv, indicating significantly increases (1.6 folds) and (2.3 folds) in comparison to control group, respectively. In contrast to glucan-S_3, *Z. multiflora* essence developed

enhancing respiratory burst at day 7 in comparison to day 4 (1.4 folds, $P<0.05$), indicating an increasing stimulation period. So, we demonstrated that *Z. multiflora* essence as an adjuvant in combination with glucan-S_3 was able to triggered innate immunity response in mice.

5. Conclusions

In summary, it has been proven that intraperitoneally injection of the complex of *Z. multiflora* and glucan-S_3 has more stimulatory effect on phagocytosis than the essence and glucan-S_3 when they used alone. Results presented in this paper suggested that β-glucan could be used as a prophylactic and/or therapeutic agent alone or in combination with other immunomodulatory agents such as herbal essences, *Z. multiflora*, in subjects with immune disorders at the future.

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Peptide - Au drug delivery system: Synthesis, Characterization and interaction with calf thymus DNA and *in vitro* cytotoxicity studies

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Abstract

The peptide Au nanoparticles were synthesized and characterized by UV–Vis, SEM, TEM, XRD and FTIR. The interactions between the peptide Au nanoparticles and calf thymus DNA in aqueous was studied by UV-Vis and fluorescence spectroscopy. The results indicated that the synthesized peptide Au nanoparticles can successfully insert into calf thymus DNA. The cytotoxicity of the prepared peptide Au nanoparticles was measured on the human breast cancer cells (MCF-7) and osteoblast cell lines (G292) by cell viability (MTT assay) and apoptosis (TUNEL assay). According to the MTT assay, the CC_{50} values of peptide Au nanoparticles and cisplatin are 11.3 µM and 9.7 µM, respectively in G292 which are very near to each other compared on MCF-7. Therefore, TUNEL assay was done on G292 cell lines. The results showed that apoptosis was obviously observable by 39% DNA fragmentation on G292 cell lines. Therefore, the peptide Au nanoparticles are suitable drugs which have strong potential in DNA denaturation and apoptosis especially on osteoblast cell lines (G292).

Key words: Peptide, Nanoparticle, *G292,* Self-assemble, MTT assay.

1. Introduction

Recently, new methods were developed for the preparation of nanocarriers for drug delivery provides many applications in biotechnology (1). Smart nanocarrier can carry drugs to the targets with minimum dosage and side effects. This caused to resolve the key critical matters faced with conventional medicine like the nonspecific delivery, quick clearance, uncontrollable releasing of drugs, and low bioavailability (2-4). The final result is low toxicity and/or adverse reactions. On the other hand, although there are many new methodologies, most of nanocarriers show a number of side effects causing low efficiently in medical applications. Therefore, some serious topics are highlighted in the strategy of nanocarriers for biotechnology uses, increasing from the complex media and many form interactions recognized in the particular biological environment (5-7). For using nanoparticles with effective therapeutic properties, the tumor microenvironment should be considered. The tumor cells show abnormal physiological conditions includding hypoxia and acidic extracellular pH (8). Therefore, nanoparticles could be applied for designing the modified treatment approaches (8-11).

In structure of Au nanoparticles, Au atom cores are surrounded by negatively charged groups on the surface that can be easily modified using a monolayer. For biomedical applications, Au nanoparticles are generally synthesized using the colloidal methods such as

using a metal precursor, a reductant, and a stabilizer. These methods precisely control the electrical and optical properties depending on the shapes and sizes of the synthesized Au nanoparticles (12,13). Au nanoparticles can be easily modified through ionic, covalent bonding or physical absorption using different biomolecules such as drug molecules, proteins, antibiotics, genes, and various ligands. Current studies showed nontoxicity, biocompatibility and biodegradability of Au nanoparticles in some human cells *in vivo* (14, 15). Au nanocarriers are mainly interesting because of the surface plasmon resonance (SPR) bands (16).

Based on bio-mineralization procedures, peptides are able to act as reducing and capping agents in synthesis of Au nanoparticles (17, 18). This ability allows that the in situ nucleation and growth of Au nanoparticles carry out in the mild - conditions (19). Moreover, peptides are capable with specific self-assembly properties which widely used to produce nanocarriers (20). The combination of bio-mineralization, molecular recognition, and self-assembly make peptides as an efficient compound to synthesize Au nanoparticles. This assembles them into custom architectures with sequence-specific properties and functionalities (21).

In this study, we will consider peptide ability to self-assemble and potential applications of peptide based nanocarriers for nanomedicine. In addition, we will provide a suitable platform for the non-covalant interactions of calcitonin with Au-nanoparticles. The synthesized peptide Au nanoparticles were characterized using UV-Vis, FTIR, XRD, SEM and TEM. *In vitro* cytotoxic activity of calcitonin capped Au nanoparticles and cisplatin was studied on osteoblast cancer G292 cells and breast cancer MCF-7 cells. The apoptotic effect of peptide Au nanoparticles in the cell lines was tested using TUNEL assay. Moreover, the binding interactions with CT-DNA have been assessed using UV-Vis and fluorescence studies.

2. Materials and Methods

Chemical: Poly (ethylene glycol), Tetrachloroauric (III) acid, Ansouline (polypeptide alpha), Trisodium citrate were purchased from Sigma-Aldrich (USA). Highly polymerized calf thymus DNA sodium salt (CT-DNA), Tris-HCl buffer and MTT were purchased from Merck (Germany). Ethidium bromide (EtBr) was purchased from Fluka (Switzerland). MCF-7 and Osteoblast G292 cell lines were purchased from of Institute Pasteur of Iran. Triton X-100, paraformaldehyde, phosphate buffered saline and sodium citrate were purchased from sigma (USA). TdT-mediated dUTP Nick-End Labeling (TUNEL) kit was obtained from Roche Applied Science (Germany).

2-1 Synthesis of peptide - Au nanoparticles

To synthesize the peptide -Au nanoparticles, 12 mL of PEG (0.2 g, 1wt%), 6 mL of 0.0625 M $HAuCl_4$, 0.1 g of $Na_3C_6H_5O_7.2H_2O$ and 0.9 mL ansoline were used as follows: 50 µL of $HAuCl_4$ and 100 µL of PEG (as stabilizer) were added in the flask at intervals of 60 seconds until 6 mL of $HAuCl_4$ and 12 mL of PEG were used. Then 0.1 g of $Na_3C_6H_5O_7.2H_2O$ was dissolved in 20 mL distilled water and was added to the previous solution. The resulting solution was refluxed at 60°C. The color of the solution changed from yellow to red. Then 0.8 mL of ansouline (1%) was added to the primary solution while stirring slowly for 3 h at 37° C.

The obtained solution was centrifuged at 17800 rpm for 20 min and washing several times with distilled water. The resulting peptide - Au nanoparticles were stored at 4°C.

2-2 Measurement instruments

Electronic absorption spectra of peptide Au nanoparticles were determined on a JASCO UV-Vis 7850 (Germany). The fluorescence spectra were determined on a Cary Eclipse spectrofluorimeter (Varian, USA). Circular dichroism (CD) spectra were recorded on an Aviv Spectropolarimeter model 215 (USA). The XRD analysis of the calcitonin-capped Au nanoparticles were determined on a Bruker D8 ADVANCE X-ray diffractometer (Germany). Morphologies and size of peptide Au nanoparticles were obtained on a HITACHI S-570 and Philips EM208 scanning and transmission electron microscopy (SEM and TEM). Treated cells were determined using monochrome camera (Olympus, Germany).

2-3 Interaction with DNA

UV- Vis spectrophotometry and fluorescence methods were used for interaction studies of peptide Au nanoparticles. The stock solutions of 1.5 mM ansouline Au nanoparticles and 4 mg/mL CT-DNA were prepared in 20 mM Tris-HCl buffer (pH 7.0). The absorption titration measurements were carried out at λ_{max}=360 nm and calcitonin-capped Au nanoparticles-DNA were incubated for 1h at 300 K and 310 K. For fluorescence study, the excitation and emission wavelength at 471 nm and 500-700 nm were recorded. CT-DNA concentrations were determined using ε_{260}=6600 $M^{-1}cm^{-1}$ (22, 23).

2-4 Cell culture and in vitro cytotoxicity analysis: MTT assay

Osteoblast G292 cells and breast cancer MCF-7 cells were cultured similar to the method used (24, 25). Cytotoxicity assay in both G292 and MCF-7 cells was done. The cultured cells were treated with different concentrations from 10 to 250 µM of calcitonin-capped Au nanoparticles and cisplatin as an anti-cancer drug and were incubated for 24, 48, and 72 h. It placed to MTT. 150 mL of media and 50 mL MTT solutions were added to each well and incubated for 4 h at 37°C in a CO_2 incubator. Then the formed formazan crystals with DMSO were dissolved. Finally the absorbance at 570 nm was recorded.

2-5 Apoptotic study by TUNEL assay

Osteoblast G292 cells with 15 µM of peptide Au nanoparticles for 72 h, got cured. Cells were disinfected with PBS and then were fixed in paraformaldehyde (4%) mixed with PBS for 1h. Then a solution including; 0.1 g sodium citrate, 0.1 g Triton X-100 and 100 mL H_2O, was added to the cells. PBS-washed cell were incubated with TUNEL reaction mixture for 1 h at 37°C in the dark and were rinsed with PBS three times. The cells using fluorescent microscope were imaged. 200-400 cells were counted for quantification of apoptotic cells.

3. Results and Discussion

3-1 UV-Visible Spectroscopy

The UV–Vis spectrum of peptide Au nanoparticles, figure۱ including: a peak at 330 nm and a band at 574 nm; that were probably because, the ligand to metal charge transfer and the surface plasmon resonance (SPR) effect respectively (26). After binding of calcitonin to Au nanoparticles, a shift in the maximum wavelength of about 2.7 and 1.9 nm was observed. This was due to the asymmetric environment of the ansoline attached to the Au nanoparticles .

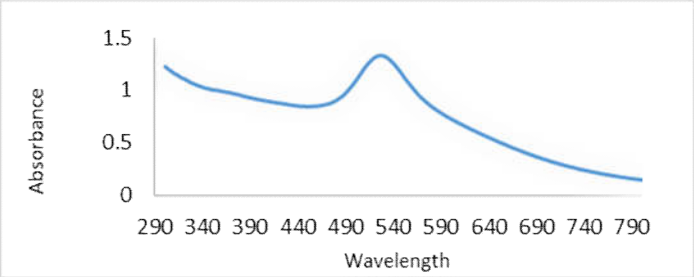

Figure۲ : UV–Vis absorption spectrum of peptide Au nanoparticles

3-2 FTIR analysis

In this spectrum, the vibrational frequency changes of thiol peptide were determined after it was attached to the Au nanoparticles. The stretching bands of the asymmetric and symmetric at 1654 cm^{-1} and 1389 cm^{-1} corresponds to COO^{-}. The stretching band in 1527 cm^{-1} is attributed to NH bend. The stretching band of NH_3^{+} was observed in the 3000–3500 cm^{-1} range. The SH bend of cysteine was observed at about 2580 cm^{-1} region (27). However, when cysteine binds on the Au nanoparticles surface with high electron density, changes in the vibrational frequency spectra were detected. For the dipole moment, band stretching of COO^{-} and NH_3^{+} were shifted. Which can confirm the capping of calcitonin to Au nanoparticles.

3-3 XRD analysis

The XRD of the peptide Au nanoparticles is shown in figure 2. The peaks at 2θ equals to (111), (200), (220), and (311) are compatible with the Au° metal. This specification corresponding to the face-centered cubic structure (28). Using Scherrer equation, $D_c = K\lambda/\beta Cos\theta$, where the width of the observed diffraction peak at its half maximum intensity is β, the shape factor is K (about 0.9), and the X ray wavelength is λ (Cu Kα radiation, equals to 0.154 nm) was about 2.307 nm.

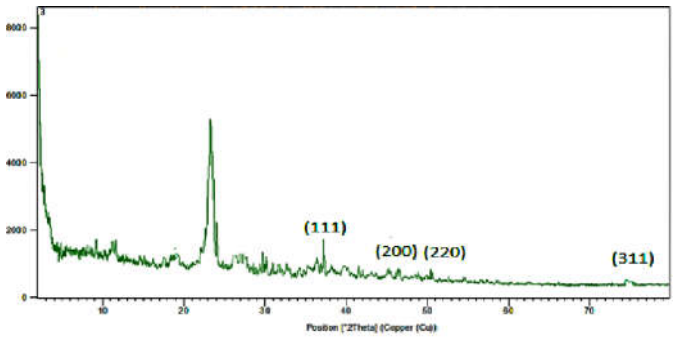

Figure 2: XRD pattern of the peptide Au nanoparticles.

3-4 SEM/TEM images

Figure 3, the SEM images of peptide Au nanoparticles are shown. Based on this, the regular morphology and size of nanoparticles was determined to be about 2-20 nm.

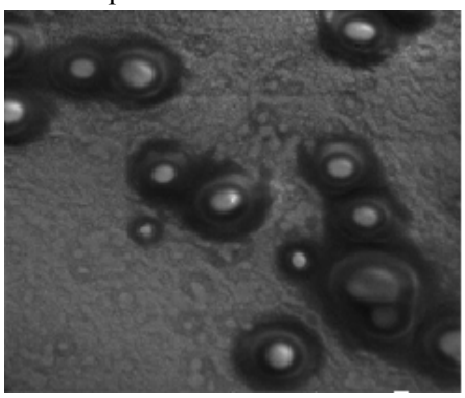

Figure 3: SEM images of self-assembly peptide Au nanoparticles.

Transmission electron microscopy (TEM), was used to obtain a more accurate particle diameter of peptide Au nanoparticles (Figure 4). Which indicates the average size of the peptide Au nanoparticles of about 2-20 nm.

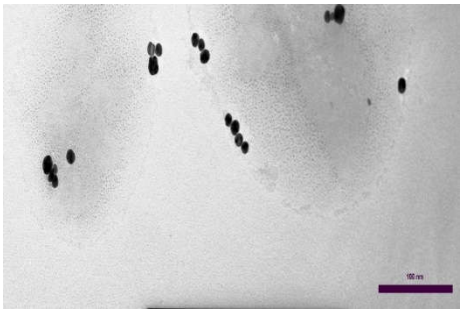

Figure 4: TEM image of peptide Au nanoparticles.

3-5 DNA denatoration

To study of DNA denaturation with peptide Au nanoparticles the similar method was used in our previously reported articles (29, 30). Figure 5, the denaturation changes of CT-DNA interaction by calcitonin-capped Au nanoparticles at 300K and 310K are shown. According to this diagram, the amount of absorption increases with increasing the amount of peptide Au nanoparticles to CT- DNA. This is because of with the denaturation of CT- DNA, the purine and pyrimidine bases of CT-DNA are more exposed to light.

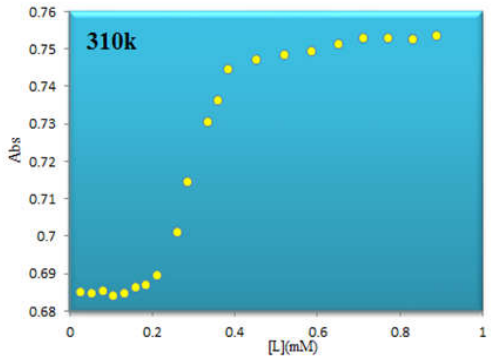

Figure 5: The changes in UV absorbance for CT-DNA at 310 K.

3-6 Absorption titration measurements

In the next experiment, a fixed value of CT-DNA with different values of the peptide Au nanoparticles at 300 K and 310 K was titrated. Figure 6, the Scatchard plots for the interaction of the peptide Au nanoparticles with CT- DNA are shown. In this diagram, $\nu/[L]_f$ is drawn relative to ν, where ν is the ratio of the concentration of bound calcitonin-capped Au nanoparticles to total CT-DNA concentration and $[L]_f$ is the concentration of free peptide Au nanoparticles(31). The positive slope is observed in the diagram, cooperative binding properties were explained (32).

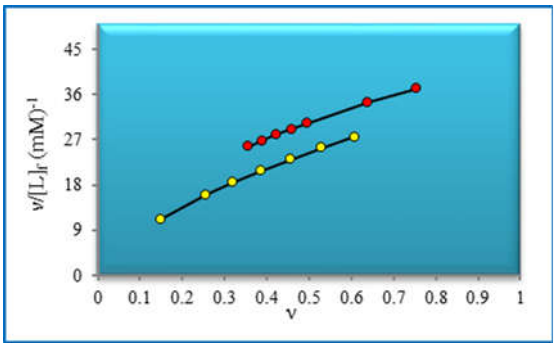

Figure 6: Scatchard plots for binding of peptide Au nanoparticles to CT-DNA.

3-7 Fluorescence spectroscopic studies

Due to high sensitivity of fluorescence spectroscopy for study the interaction of complexes with CT-DNA, this method is used. The DNA binding of the peptide Au nanoparticles was tested by ethidium bromide. For strong intercalation between the DNA base pairs and EtBr, the fluorescence intensity of EtBr is significantly increased (33). Addition of peptide Au nanoparticles to the EtBr-DNA system, fluorescence intensity resulted decrease. Where due to replacement of the EtBr molecule by the peptide Au nanoparticles (34). Figure 7, the emission spectra of EtBr bound to CT-DNA in the absence and the presence of the peptide Au nanoparticles are shown. Using the emission curve, indicates that from the EtBr and calcitonin-capped Au nanoparticles, intercalation affinity of the calcitonin-capped Au nanoparticles is more than that of EtBr.

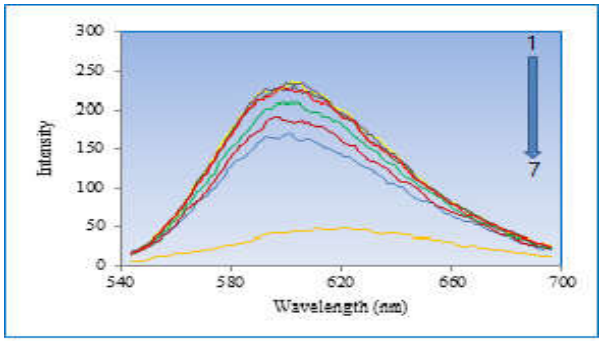

Figure 7: Florescence emission spectra of EtBr-DNA in the absence (1) and presence of different concentration of the peptide Au nanoparticles.

In order to determine the number of EtBr molecules connected to CT-DNA in the presence of different concentrations of the peptide Au nanoparticles, scatchard analysis were used (31). In this test, a certain amount of peptide Au nanoparticles was added to the solution of CT-DNA-EtBr. Then, the fluorescence spectra measurement of the solution were taken after 2 hours of incubation. Figure 8 is shown the fluorescence scatchard plots for CT-DNA-EtBr solution. That is shows decreases the value of apparent association constant with increasing the concentration of peptide Au nanoparticles. This type behaviour of DNA binding (type-A) specifies that the peptide Au nanoparticles inhibits the EtBr binding to CT-DNA.

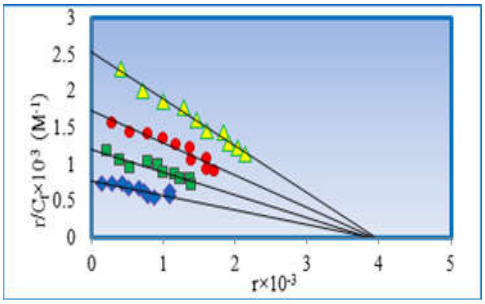

Figure 8: Scatchard plot. In curves nos. 1(Δ: CT-DNA= 60 µM alone), 2 (●), 3 (■) and 4 (◆) respectively, 40, 80 and 120 µM of peptide Au nanoparticles.

3-8 Apoptosis assay

TUNEL assays were performed to express that whether the cell death was attributable to calcitonin-capped Au nanoparticles induced apoptosis (with CC_{50} = 11.6 µM peptide-capped Au nanoparticles). Figure 9, a level of apoptosis was evident in G292 cells after treatment with prepared peptide Au nanoparticles are shown.

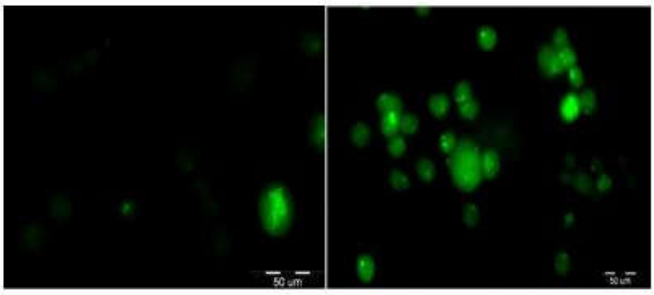

Figure 9: TUNEL assay in osteoblast cancer G292 cells.

4 - Conclusions

Since the main purpose of smart drugs is to achieve a specific goal and reduce toxicity therefore in this work smart peptide-based nanocarrier for the smart controlled of drug molecules were synthesized. The peptide Au nanoparticles were characterized by spectral techniques. The TEM images proven that the calcitonin-capped Au nanoparticles by about 2-20 nm in size were obtained. The SEM images accepted that smart peptide-based nanocarrier of peptide Au nanoparticle has a good and suitable morphology for pharmaceutical application. UV–Vis, fluorescence method and CD spectroscopy technique were used to study the interaction peptide Au nanoparticles with CT-DNA (DNA binding). These results showed that the peptide Au nanoparticles binds to CT-DNA through the intercalation. The peptide Au nanoparticles at low concentration was given a conformation change in CT-DNA.

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Determining the antioxidant capacity of the liver of rainbow trout exposed to yersiniosis vaccine

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Abstract

Objective: To date, there are no studies about the antioxidant capacity of liver in fish exposed to yersiniosis vaccine. The aim of the study was to compare the antioxidant capacity of the liver of rainbow trout exposed to the yersiniosis vaccine and non-exposed fish. Methods: The amounts of carotenoids, cupric assay, and lipid peroxidation levels were determined. The samples were normalized by Bradford test. Results: Walnut brown seed coat at 1% was effective as BHT in inhibition of lipid peroxidation. This extract presented the highest Vitamin E content (1.4± 0.042 mg/g). DPPH assay showed the extract have high radical scavenging ability. Conclusions: The results of this study indicate walnut brown seed coat possesses strong antioxidative properties in vitro.

Key words: Yersiniosis vaccine, Antioxidant activity, Cartenoids, Liver

1. Introduction

The cause of yersiniosis or red mouth disease is Yersinia rocker. This complication is one of the serious diseases with high economic losses in the rainbow trout industry. Yersinia rocker belongs to the family Enterobacteriaceae, rod-free, without spores, negative oxidase and positive and mobile catalase, grows at 37 degrees, and does not lose its motility. The yersiniosis vaccine, which is a killed bacterium in an oil emulsion, is made to prevent disease and used in salmon farms. It can be given by bath or injection. Safety studies show that the use of the vaccine does not cause side effects in fish. However, the antioxidant capacity of fish has not been studied. The aim of this study was to evaluate the antioxidant capacity of rainbow trout liver exposed to yersiniosis vaccine and compare it with fish without vaccination.

2. Materials and Methods

2.1. Procedure of Animal Experiments

The fish were splited into two groups and all conditions equalized except injecting the vaccine.

Homogenization of liver tissue: Homogenization of liver samples will take place on ice so that the tissue is not heated and proteins are not broken down.

2.2. Measurement of protein content

The results should be normalized with the amount of protein. Bradford test is used for normalization. In this method, bovine serum albumin protein is used as a standard. In Bradford method, Kumasi dye reacts with protein in ionizable groups and changes color. The color change is proportional to the amount of protein.

2.3. Determination of Carotenoids

The solution absorbance was read on spectrophotometer at 470 nm. Its carotenoids content was calculated on the standard curve of B carotene. Initially, 0.01 of the standard carotenoid weight will be increased to 10 cc with distilled water. From this stock solution, dilutions of 0.001, 0.0001 and 0.0001 will be prepared. Dilute 2 g of the sample with 20 cc of distilled water, and then make one cc of the obtained solution with 10 cc of water due to the increase in the concentration of the solution and its non-reading by spectrophotometry. The adsorption of standard solution concentrations and the sample obtained will be read by a spectrophotometer with a wavelength of 470 nm. The line curve is drawn using standard concentrations.

2.4 Determination of Cupric Ion Reducing Assay (Cupric)

This method measures the cupric reducing capacity. The samples were mixed with solutions of CuCl2 and neocuproine reagent in ammonium acetate buffer. The absorbance of solutions was read at 450 nm after incubation at 50 degrees C for 20 min.

2.5. Measurement of lipid peroxidation in liver tissue by TBARS method

Briefly, the samples were mixed with 20% trichloroacetic acid and the mixture centrifuged. Then, thiobarbituric acid was added to the supernatant and heated. The absorbance of the supernatant was read at 532 nm. The values were expressed in μ moles of malodialdehyde(MDA).

2.6. Statistical analysis

Statistical analyzes were performed by SPSS software.The data represent the mean± standard deviation for samples. Kolomogorov-smirnov test was used to determine the parametric or nonparametric of the data. Carotenoid and lipid peroxidation data were normal. T test was used to determine the significance. Total antioxidant was not normal; So Mann-whitney test was used.

3. Results

The results are shown in Tables 1. In order to eliminate variances from the samples, results of individual samples were normalized to the protein content. The concentration of carotenoid pigments in the extracts was calculated using the standard curve obtained by a commercial β-carotene reagent. The formula used for the calculation was as follows;R^2, y=8.3303x+0.0031. The values presented in Table 1 show that, the level of lipid peroxidation changed significantly (P=0.05). The changes in other parameters; carotenoids (p=0.26) and cupric assay (p=0.84) were not significant.

Table 1: The antioxidant capacity of vaccinated and non-vaccinated fish

	Vaccinated fish	Non- Vaccinated fish
Lipid peroxidation	0.074±0.06*	0.11±0.11*
Carotenoids	0.1±0.03	0.089±0.01
Cupric assay	0.24±0.05	0.34±0.24

*P=0.05, *Each value represents the Mean ± SD per group. Lipid peroxidation data were significantly*

4. Discussion

In this study the total carotenoids, lipid peroxidation, cupric assay were measured and compared in two groups. To date, there are no studies about the antioxidant capacity of liver in vaccinated fish.

This assay is necessary because the vaccine and its exposure doses may have side effects in addition to preventing yersiniosis. The oxidative stability was assayed by measuring content of malondialdehyde. In present study the level of malondialdehyde was lower than vaccinated fish. The lipid oxidation in rainbow trout due to its relatively high unsaturated fatty acid profile can be an important factor in spoilage and product shelf life. The total antioxidant capacity are usuallys investigated by two different methods: ferric reducing antioxidant power (FRAP) and cupric ion reducing capacity assay (Cupric assay). The cause of yersiniosis, which is the same as septicemia-yersinia or enterobacterial red mouth disease, is Yersinia rocker. This complication is one of the serious diseases with high economic losses in the rainbow trout breeding industry (Tobback et al. 2009). Yersinia rocker belongs to the family Enterobacteriaceae, rod-free, without spores, negative oxidase and positive and mobile catalase, and grows at 37 degrees but does not lose its motility (Bestor et al. 2010). In recent years, biotype 2 species have played a significant role in the prevalence and mortality of yersiniosis in vaccinated fields, as in Chile, the United States, Spain, the United Kingdom, and a number of other European countries. Commercial vaccines are often made against biotype 1.

Therefore, considering that no similar study has been done in this regard before, its evaluation can bring practical results for decision makers and breeders in fish farms. Collectively, the use of the vaccine, in addition to increasing resistance to yersiniosis, also could prevent the lipid oxidation of fish liver.

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Numerical Thermo-optical properties of Metal Nanoparticles

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Abstract

This paper presents a numerical study of the colloidal metal nanoparticles under the irradiation of a continuous Gaussian laser beam. The effect of the concentration of the NPs on thermo-optical properties of the colloids is also investigated. It is shown that increasing the concentration of the NPs enhances the nonlinear refractive index of the colloids using low laser power at wavelength of 532 nm. The numerical data show that the liquids with more viscosity and less thermal expansion coefficient, lead to more concentric far-field diffraction patterns.

Keywords: Thermo-Optical Nonlinearity, Far-Field Intensity, Diffraction Pattern, Colloidal Metal Nanoparticles, Photonic Devices

1. Introduction

Many applications in different fields such as photonics devices, chemical and biomolecular sensing, photo-thermal therapy is presented based on the interesting properties of metal [1-5]. Great attention has been paid to study the thermal nonlinear properties of the colloidal nanoparticles under exposure to CW laser light at wavelength 532 nm [6-9]. It has been found that thermal third-order nonlinear refraction originated from temperature gradient induced by linear absorption of laser energy is the main mechanism responsible for observed nonlinearity in the colloids. The nonlinear thermo-optical material can be a good candidate for various optical devices, including optical power limiters that protect eyes and sensors from intense light radiation. Different refractive indexes originated by thermal third-order nonlinear refraction result in a phase shift of light wave that creates an interference pattern at a far-field distance. Recently, the far-field intensity distribution of the continuous Gaussian laser beam passing through the liquids has been presented experimentally and theoretically for optical photonic devices [6-10].
In this work, the far-field intensity distribution of the CW laser beam passing through the NP colloids as a thermal nonlinear medium, is numerically analyzed. The influence of the physical properties of the host medium on intensity profile at a far distance is predicted.

2. Theory

The absorbed energy originated by exposure of laser light creates a spatial temperature distribution in the sample, followed by the process of heat diffusion in the medium. The created temperature gradient acts as a lens, and a phase difference in the wave occurs which produces a diffraction pattern on the far-field plane [10]. The intensity of the passed laser beam through the sample can be obtained, as:

$$I(x',y',t) \propto \left| \int_{-\infty}^{\infty} dx \int_{-\infty}^{\infty} dy\, f(x,y;x',y') \exp\left(-i\left[k\frac{r^2}{2R} - \Delta\phi(x,y,t)\right]\right)\right|^2$$

(1)

where $f(x,y;x',y')$ contains all the radially intensity variations, $k = 2\pi/\lambda$ is the wavenumber, λ is the laser beam wavelength in vacuum, $\Delta\phi(x,y,t)$ is the created phase shift and R is the radius of curvature of the beam's wavefront. To study the thermal nonlinear effect on the laser- material interaction, it is required to determine the thermo-optical phase shift due to heat transfer in the sample. The temperature distribution can be obtained in terms of the absorbed energy of the laser at each point x and y after the time t [9]:

$$\Delta T(x,y,t) = \frac{\alpha P}{\pi C_p \rho}\left\{\int_0^t \frac{d\tau}{8D\tau+\omega^2} exp\left(-\frac{2[(x-v_x\tau)^2+y^2]}{8D\tau+\omega^2}\right)\right\}$$

(2)

where $D = K/C_p\rho$ is the heat diffusion coefficient, C_p is the specific heat capacity, ρ is the density, K is the thermal conductivity coefficient and ω is the beam radius, α is the linear absorption coefficient, P is the incident laser power and $v_x = \frac{\beta g \Delta T_m \pi \omega_0^2}{16\mu}$ is the convective velocity due to the equilibrium between the fluid viscous forces and the heat buoyancy force [10], β is the thermal expansion coefficient, ΔT_m is the maximum temperature, g is the gravitational acceleration, ω_0 is the laser beam waist, and μ is the viscosity of the host medium. The induced temperature gradient changes the refractive index of the material according to relation $n(x,y,t) = n_0 + \frac{dn}{dT}\Delta T(x,y,t)$, where dn/dT is the thermo-optical coefficient of the sample. Created phase shift of the light electric field due to heat transfer in the sample including conduction and convection phenomena, is obtained by [10]:

$$\Delta\phi(x,y,t) = 2\frac{dn}{dT}\alpha PL/\lambda K \; D\int_0^t \frac{d\tau}{8D\tau+\omega^2}\left\{exp\left(-\frac{2[(x-v_x\tau)^2+y^2]}{8D\tau+\omega^2}\right) - exp\left(-\frac{2(v_x\tau)^2}{8D\tau+\omega^2}\right)\right\}$$

(3)

3. Numerical results

The simulation of the Gaussian laser beam behavior passing through the NPs colloids with different viscosities and thermal expansion coefficients are shown in figures 1. For this simulation, the fixed values of the parameters are listed in table 1.

Table 1 Physical parameters used for simulation of the laser beam behavior.

Physical parameters	Value
$D(s^{-1}m^2)$	0.757×10^{-7}
$K\ (Wm^{-1}K^{-1})$	0.17
$\beta\ (K^{-1})$	750×10^{-6}
$\mu\ (m^2S^{-1})$	1.36×10^{-6}
$\alpha 2\ (m^{-1})$	50
dn/dt (K^{-1})	4×10^{-4}

As is clearly shown in figure 1, by increasing the viscosity of the host medium, which causes the convection current to decrease, the diffraction rings pattern and normalized intensity profile become more symmetrical. The observed asymmetric nature of the far-field intensity distribution in the medium with the highest viscosity, suggests that the convection heat flow is one of the main mechanisms of the heat distribution process in the colloids [11].

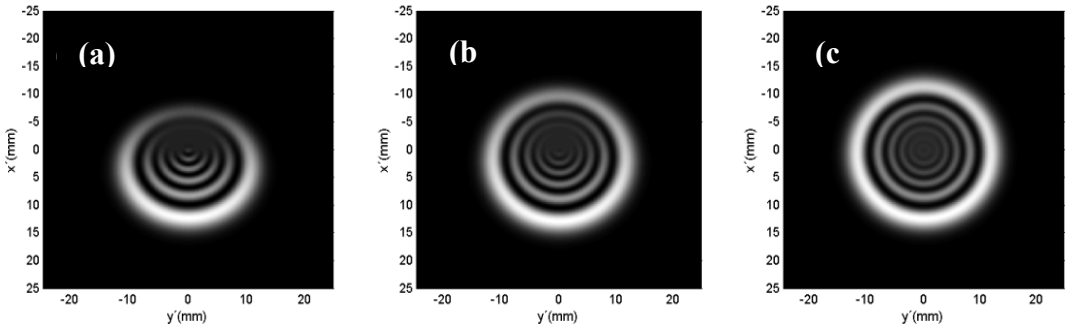

Figure 1 The simulated behavior of the laser beam propagating through the colloidal nanoparticles with the host medium viscosities of a) $\mu = \mu_1$; b) $\mu = 2\mu_1$; c) $\mu = 6\mu_1$ (for all viscosities, $\beta = \beta_1$) and with thermal expansion coefficients of a) $\beta = \beta_1$; b) $\beta = \beta_1/2$; c) $\beta = \beta_1/6$ (for all thermal expansion coefficients, $\mu = \mu_1$)

In fact, the higher the thermal expansion coefficient, the more particles collide with each other, and the more convection occurs. Thus, the change in the volume expansion coefficient is expected to affect the symmetry of the circular rings. Figure 1 shows that by decreasing the thermal expansion coefficient of the host medium, the diffraction rings and normalized intensity profile become more symmetrical due to the decrease in the convection current. Moreover, as it can be seen in figure 1, there exists no significant difference among the obtained beam divergence and number of the rings with various amount of viscosity and thermal expansion coefficient of the liquid. It is worth noting that the increasing μ and decreasing β show the same results.

The influence of the NPs concentration on intensity profile at a far distance is investigated numerically. Figure 2 illustrates that by increasing the concentration of the NPs, the beam divergence and the number of circular rings increased leading to significant enhancement of thermal nonlinear effect.

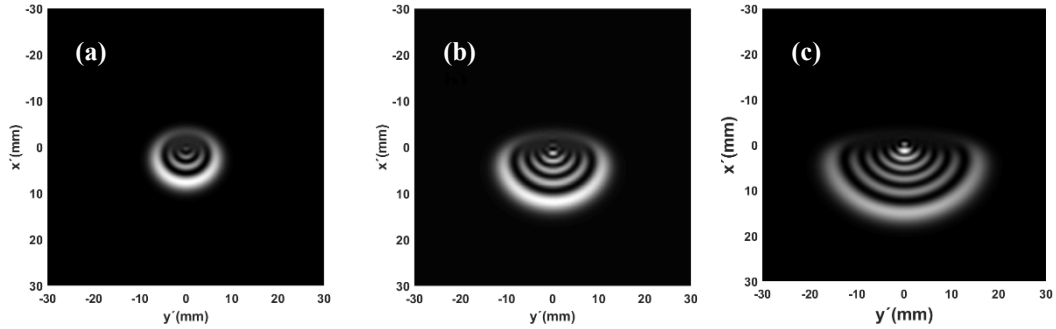

Figure 2 The simulated behavior of the laser beam propagating through the colloids with linear absorption coefficients of a) $\alpha = \alpha_1 = 0.25\ cm^{-1}$, b) $\alpha = 2\alpha_1$ and, c) $\alpha = 4\alpha_1$

4. Conclusion

We report a systematic investigation on the thermo-optics nonlinearity of metal colloids under irradiation of a continuous Gaussian laser beam. Our results show that thermo-optical effect of the colloids enhances with an increase in the concentration of metal NPs in the liquid. By increasing the viscosity of the solution, the most symmetrical diffraction rings were observed, because of the lowest convection current. It is noted that effect of increasing the viscosity and decreasing the thermal expansion coefficient show the same results.

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The Effect of Micro Needle Patch Advanced Approach on the Prevention Rate of Influenza Disease

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Abstract

Health literacy is a flourishing research and practice field that relates to people's potential to meet complex health demands throughout life that considers how to find, understand, evaluate, use and communicate health information, health literacy is very important in improving prevention and control of infectious diseases. Therefore, improving community health literacy regarding infectious diseases can act as an essential factor in controlling infectious disease epidemics around the world. Micro needling is a developing method for transferring pharmaceutical compounds through the skin. All types of microneedles penetrate the layer of the skin to create a microchannel through which pharmaceutical compounds are transmitted. The aim of this study was to determine the effect of micro needle patch advanced approach on the prevention of influenza disease. This interventional study was conducted with narrative review approach of the type of a review study in 2022 using searching for keywords such as microneedle, influenza and drug delivery system in valid databases such as Science Direct, Scopus, Web of Science, PubMed, according to the results of the articles, that prevention of the pandemic relies on the coverage of vaccination caster to create maximum immunity. But conventional vaccines do not last long. Microneedle adhesives can be placed outside the cold chain for longer than conventional flu vaccines due to formulations containing arginine and heptagloconate. The components used in these adhesives can maintain efficiency at room temperature for three months. These adhesives are prominent due to the painless distribution of the drug and prevent damage to the blood vessel because they are used in deep layers of the skin.

Key words: Microneedle Adhesive, Microchannel, Technological Approach, Influenza

1. Introduction

Influenza, an infectious viral disease that affects the upper respiratory tract, circulates annually, causing significant illness and mortality, mainly among adult's ≥ 65 years old and children < 5 years old. Approximately 3 to 5 million cases of severe illness and about 650,000-290,000 respiratory deaths are reported worldwide due to influenza infection each year, the majority of influenza-related deaths (more than 85% of deaths and Mir) occurs in adults older than 65 years of age; And treatment can improve people's knowledge of contagious diseases and thus promote the development of appropriate preventive behaviors toward contagious disease. Health education

facilitates health promotion and effectively slows the spread of infectious diseases, the effect of drug delivery through micro needling mechanisms is expanding dramatically. It is expected that the growth of this new technology in medicine will continue, predictions suggest that in the near future, interest in converting ordinary hollow and solid microneedles into dissolving microneedles will increase. Influenza is known as a contagious infectious disease that affects many people annually and thousands of people die. This paper reviews the hypothesis that the optimal microneedle adhesive formula can preserve the flu vaccine for longer periods outside the cold and off-refrigerator chains and stabilize it when exposed to potential stresses during production and storage. In recent decades, microneedles have been studied to deliver drugs through intra cutaneous pathways. Microneedles with micron sizes (length less than 1,000 μm) are cone or pyramid-shaped or multidimensional perforated bumps that have many advantages in injecting the drug into the body through the skin. Microneedle creates a temporary channel in the outer layer of the skin that greatly eliminates the limitations on the size of materials passable through the skin. The length of these microneedles is enough to reach the layer of the skin but does not reach the underlying nerve terminals. Therefore, the use of microneedles is basically painless. These adhesives can be easily used by the patient themselves and eliminate the need for a healthcare professional. Arya and her colleagues found in a survey in 2017 that 86 percent of participants were confident in these adhesives and 93 percent preferred these adhesives to conventional subcutaneous needles. One of the most prominent benefits of microneedle is its painlessness, which will affect the acceptance of this method by patients. Espin et al. conducted a survey in 2016 that found that diabetics were more upset with the needle and pain of their injections, which these painless adhesives are a big help to them, especially in children. In addition, the use of microneedles leads to minimal damage to the injection site. Some of the most common side effects reported in this area in human volunteers are sensitivity, erythema and pruritus at the site of use, depending on the length of microneedles. The longer the length of the bumps, the higher the level of erythema at the place of use. The therapeutic stability of microneedles is high and does not require storage in the cold chain due to the use of auxiliary materials in its formulation. The aim of this study was to determine the effect of micro needle patch technological approach on the prevention of influenza disease.

4. Figures

4-1- Figures

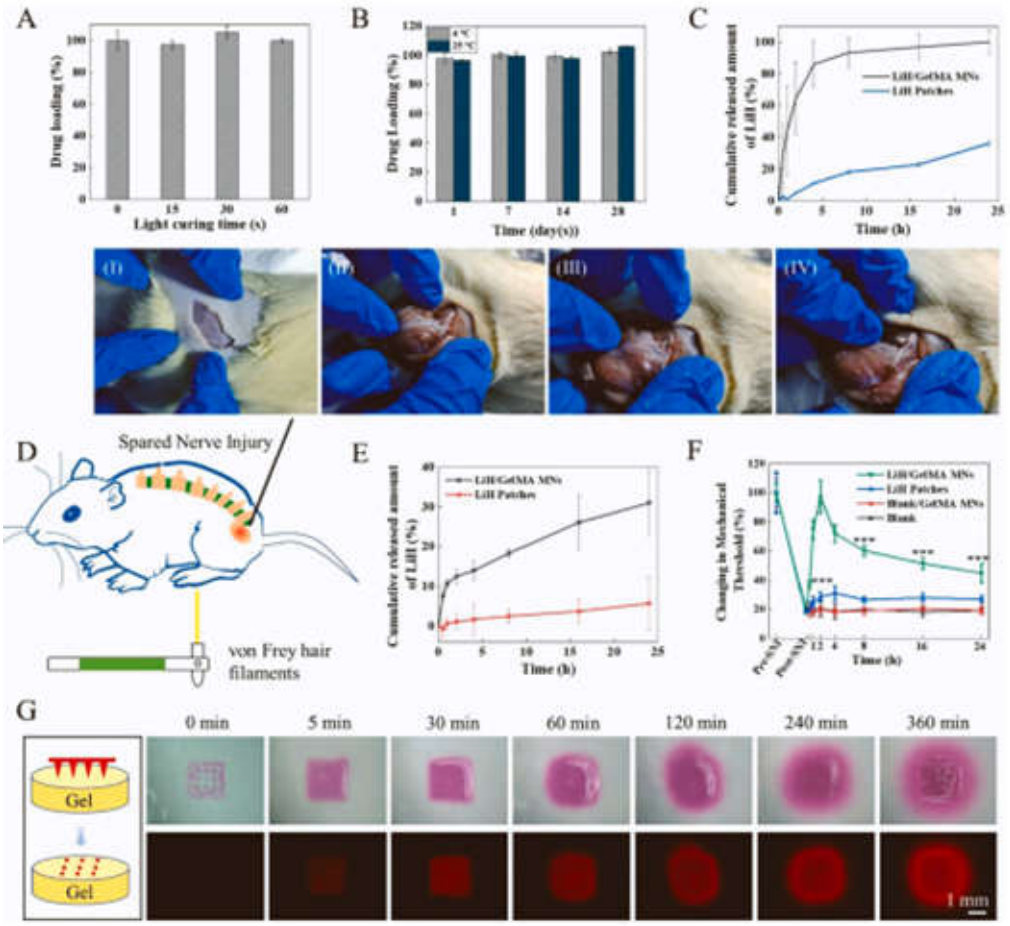

Figure۳ : Drug stability and release in GelMA MNs

(A) Drug stability under different light-curing times (n = 3). (B) Drug stability under different storage times at 4 ◦C or 25 ◦C (n = 3). (C) In vitro release of LiH from LiH/GelMA MNs or LiH patches in PBS (37 ◦C) by Franz cell diffusion setup (n = 3). (D) SNI model and mechanism of von Frey test. Cut the skin of the left leg (I) and biceps brachii muscle (II) to expose the tibial nerve, common peroneal nerve, and sural nerve; The tibial nerve and the common peroneal nerve were ligated (III) and cut off (IV) to retain the peroneal nerve. (E) In vivo release of LiH from LiH/GelMA MNs or LiH patches in the back skin of rats (n = 3). (F) Mechanical pain thresholds were tested from pre-SNI to post-SNI (0–6 h) with the treatments (Blank, Blank/GelMA MNs, LiH patches, LiH/GelMA MNs) (n = 6). One way analysis of variance, * P < 0.05, * * P < 0.01, * ** P < 0.001 (LiH/GelMA MNs vs. LiH Patches). (G) The real-time release of Rhodamine B (red color) onto the gelatin gel (0–360 mins).

5. Acknowledgments

According to the researches, microneedle adhesive is a skin adhesive with soluble micro-burning that works better than a conventional vaccine. Based on the findings of this paper, the most effective way to help increase vaccination rate is to replace it with microneedle adhesive. Also, MNs can significantly improve drug penetration by producing reversible mechanical micro pore in the skin. MNs offer painless schedules, improvements in patient access to medications, self-recalling and avoidance of the first pass effect. The production and application of solid and hollow MNs have progressed to the point where they have been allowed in clinical studies for a variety of treatments.

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Investigating the dosimetric application of nanopowders of Yttrium Iron Garnet with the substitution of bismuth and manganese cations

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Abstract

The Bi^{3+} and Mn^{4+} cations were doped in Yttrium Iron Garnet (YIG) $Y_{3-x}Bi_xFe_{5-x}Mn_xO_{12}$ (x=0, 0.1, 0.2, 0.3, and 0.5) nanoparticles by a mechanochemical method. The experimental optimum time for ball milling was 15 hours. To investigate the structure, size and shape of particles, the following techniques were used: X-ray diffraction (XRD) and transmission electron microscopy (TEM). The results of the investigation of the crystal structure show that YIG nanoparticles with an average particle size of 40 nm are formed in a single phase and with a cubic structure, and the best calcination temperature is about 1100°C for 3 hours. This temperature is lower than the desired phase formation temperature in the conventional ceramic method. The maximum thermoluminescence response to the gamma rays of the ^{60}Co source was obtained in 0.3 mol% of the impurity. A computer program based on general-order kinetics was used to determine the number of peaks in the thermoluminescence radiation curve and the kinetic parameters related to each peak. Two overlapping peaks were observed at temperatures of 398 and 422 K in the thermoluminescence curve of this nanoparticle. The results show that this nanoparticle has suitable conditions for use in dosimetry.

Key words: *mechanochemi , TL, Kinetic parameters, Yttrium Iron Garnet, Dosimetry*

1. Introduction

Dosimetry is a method for measuring, calculating and estimating the absorbed dose through ionizing particles, and the damage caused by radiation depends on energy absorption. Depending on the type of radiation, the biological effects left by the energy are different. Radiation can affect human health. Therefore, detecting radiation and finding dosimeters that have a good response is very important. On the other hand, nowadays thermoluminescence dosimetry is a popular method in the field of radiation dosimetry due to its high accuracy and ease of doing it compared to other dosimetry methods [1-2]. The amount of absorbed radiation dose is measured by the intensity of the emitted light due to the thermal stimulation of the thermoluminescent material after irradiating the sample [3]. Perhaps, the most important garnet widely studied is yttrium-iron garnet with a chemical formula of Y3Fe2Fe3O12. This compound is also known as Y3Fe5O12 and usually called YIG [4]. The YIG nanoparticles can be prepared by various chemical methods such as co-precipitation, sol-gel, and ceramic [5]. Recently, the mechanochemical process, in which a part of the activation energy of the reaction is supplied by high-energy mills, has been widely used in the production of nanometer

powders [6]. In this research, the dosimetric characteristics of $Y_{3-x}Bi_xFe_{5-x}Mn_xO_{12}$ nanostructures are investigated for (x= 0, 0.1, 0.2, 0.3, and 0.5). The mechanochemical method is found to be advantageous due to the low temperature required for the synthesis and less contamination in the final product.

2. Experimental

The precursors used for preparing YIG substituted with Bi^{3+} and Mn^{4+} cations were as follows: Yttrium-oxide (Y_2O_3), Iron oxide (Fe_2O_3), Bismuth oxide (Bi_2O_3) and Manganese oxide (MnO_2) with the minimum purity of 99% (Merck). The mixture was then milled in a planetary high-energy ball-milling system for 15 h. In order to prevent an increase in the iron-oxide stoichiometry ratio of the final mixture, the weight of iron-oxide decreased by 10% due to the grinding of steel balls. This rate was determined by a trial and error method, resulting in a single phase chemical balance compound. After milling, in order to achieve a single phase sample, the milled powders were placed into a thermal process. For this purpose, a programmable furnace was used, and the heating rate for the sample was set to 5°C/min. Moreover, the baking temperature was 1100°C for 15 h. The sample was finally allowed to cool down to room temperature. In order to characterize and examine the structure of nanoparticles and identify their phase, an X-ray diffraction (XRD) machine made by PANalytical Company, model Pert Pro MPD, x , to study the morphology and calculate the size and shape of nanoparticles, a transmission electron microscope (TEM) made by Philips, model EM 208, and a TLDreader model 4500 Harshaw was used to check the thermoluminescence properties. In this research, for reading the irradiated samples. Before irradiation, the samples were heated at a temperature of 400°C for 30 minutes using and then they were immediately cooled to room temperature.

3. Discussion and results

The X-ray diffraction spectrum obtained from yttrium-iron garnet nanoparticles substituted with bismuth and manganese elements for x=0.3 at 1000°C are shown in Fig. 1.

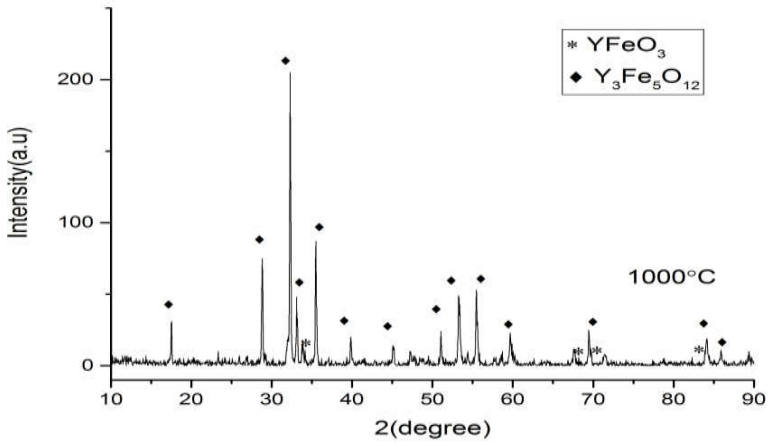
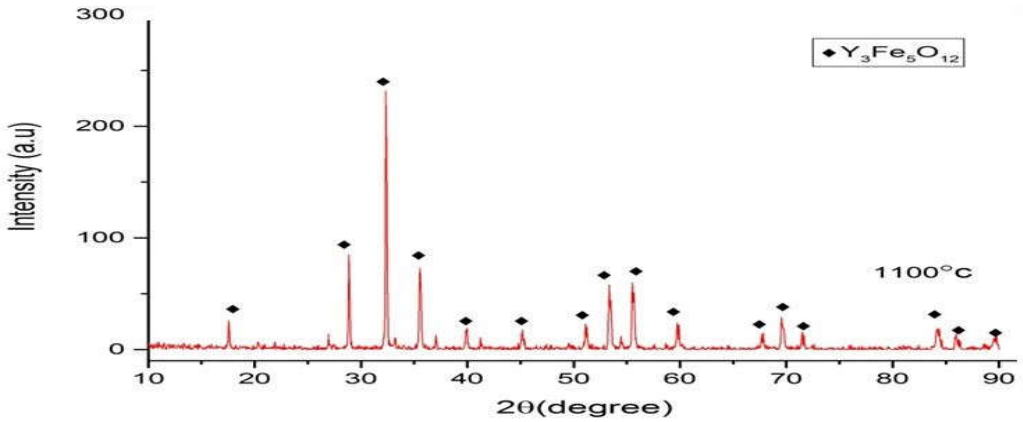
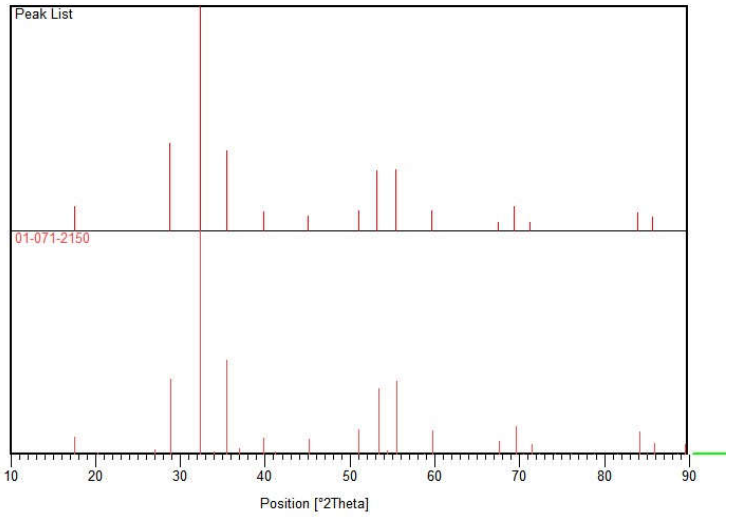


Figure۴ : X-ray diffraction spectrum of the sample YIG at the temperature 1000°C for x=0.3

Comparing the prepared sample with the existing reference showed that the sample has an impurity phase of yttrium orthoferrite with the standard card number [1345-073-01]. Of course, the presence of this phase in the formation of YIG is not only strange but also predictable. This is because the conducted studies have shown that the garnet phase is formed in two stages [7]. In the first stage, $YFeO_3$ phase is formed with cubic structure. By optimizing the temperature and milling time, the $YFeO_3$ phase is then destroyed and the garnet phase is formed gradually [8].
Fig. 2. shows the X-ray diffraction pattern of the sample prepared at a temperature of 1100°C.



(a)



(b)

Figure 2: a) X-ray diffraction spectrum of the YIG sample prepared at 1100°C for x=0.3, and b) composition (PDF card number: 01-071-2150)

As can be seen in Fig. 2, the peaks correspond to the card number [01-071-2150]. Using Scherrer's formula (equation (2)), the average particle size can be estimated as follows:

$$D = 0.9\lambda / \beta coc(\theta) \quad (1)$$

where FWHM is the width of the peak at half maximum height (in radians), θ is the Bragg angle, λ is the wavelength of X-ray, and D is the size of the crystals [9]. In this way, the size of YIG nanoparticles was found to be 40 nm.
This result is consistent with the size of YIG particles contaminated with x=0.3 in the TEM image shown in Fig. 3.

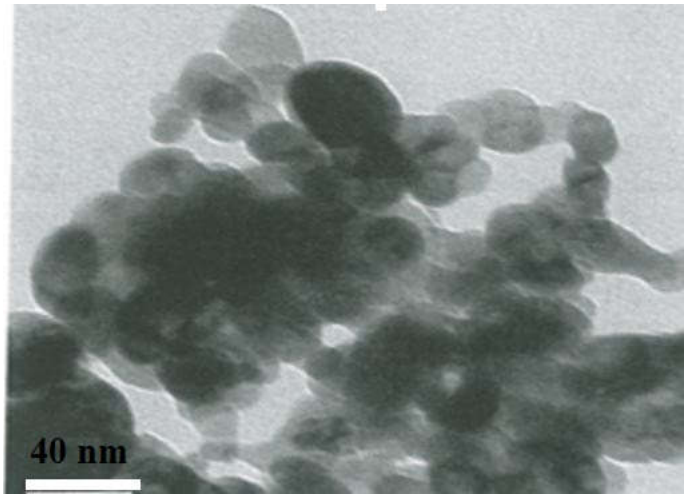


Figure 3: TEM image of the sample YIG

Fig. 4. shows deconvoluted glow curves of YIG irradiated to 50 Gy by 60Co source.

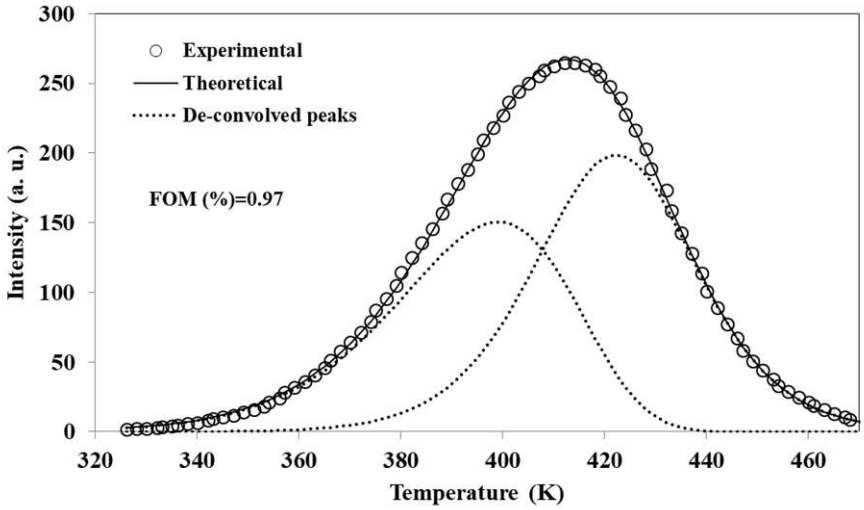


Figure (4): fitted thermoluminescence curve of garnet sample YIG with manganese and bismuth impurity

According to Fig. 4, the TL glow curve of the YIG nanoparticles is formed from two peaks at temperatures 398 and 422 K. Amount FOM it is equal to 0.97 which is a sign it gives a good match between the theoretical and experimental curves [10]. Table (1) shows the results of kinetic parameters obtained from fitting TL glow curve of synthesized sample.

Table 1: Kinetics parameters of YIG.

peak	b	E (eV)	T_m (K)	I_m (a.u)
1	1.00	0.75	398	150
2	1.70	1.25	422	198

4. conclusion

In this research, YIG nanoparticles substituted with different amounts of bismuth and manganese impurities were prepared using a mechanochemical method. It was found the substituted nanoparticles had lower temperature required for the single phase compared to the sample prepared by a ceramic method. The correctness of the formation of nanoparticles was confirmed using X-ray diffraction (XRD) analysis. Transmission electron Microscopy (TEM) analysis showed the formation of particles with a cubic structure. In the investigations carried out, it was found that the samples with 0.3 mole percentage of TL impurity have the highest thermoluminescence response to gamma rays. The irradiated nanoparticles consist of a broad curve that consists of overlapping two peaks at temperatures of 398 and 422 K. The radiation curve of this crystal consists of a suitable temperature for dosimetry purposes. Therefore, this synthesized thermoluminescence sample can be considered a suitable choice for dosimetry.

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Application of alumina nanoparticles in thermoluminescence dosimetry

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Abstract

In this research, alumina nanoparticles were prepared by a sol-gel method, and characterized via X-ray diffraction (XRD) and scanning electron microscope (SEM) to understand the structure, size, and shape of particles. In addition, the thermoluminescence radiation curve of these nanoparticles, irradiated with gamma rays from the ^{60}Co source, was fitted with a computer program and their trapping parameters were calculated numerically. Two overlapping peaks at temperatures of 443 and 461 K were observed in the thermoluminescence curve of the nanoparticles. The obtained results show that the alumina nanoparticles are suitable for use in dosimetry.

Key words: Nanoparticles, sol-gel, TL, kinetic parameters, Al_2O_3

1. Introduction

Today, it has become important to measure the dose that a person is exposed to in the work environment [1-2]. Considering the importance of this issue and as a result of dosimetry and radiation protection, thermoluminescence is one of the most effective practical methods for dosimetry applications . Thermoluminescence is a luminescence phenomenon, and luminescence excitations in materials can be created by various types of sources such as ultraviolet rays, electric fields (electroluminescence), cathode rays (cathodeluminescence), etc. [3]. When the materials are irradiated for the thermoluminescence phenomenon, the energy of the beam is excited by the electrons of the valence layer of the charge carriers, and they are separated from their alignment, thus beginning to move freely throughout the crystal lattice to finally reach the trap points caused by lattice imperfections or impurities. In fact, they are created in the host substance and get trapped. Accordantly, the energy of the radiated rays is stored in the material, so that the trapped charge carriers are excited and released from the dopant centers by heat [4]. The transition of electrons and holes from the trapping levels to their ground state leads to photon emission. These photons have the extra energies released by charge carriers. On the other hand, materials in nanodimensions show behavior and characteristics that will induce different properties compared with the bulk state [5]. In other words, nanotechnology has created a clear perspective for us in this field, and today the study of the properties of nanomaterials has become one of the research topics [6]. Alumina structure with heat resistance is used to make flame retardant coatings in fire extinguishers. At the nanoscale, alumina nanoparticles are used as an electrical insulator and as a very high thermal conductor due to their hardness. Alumina nanoparticles are also employed as one of the biomaterials in the medical and

health industries to replace hip joints [7]. In this research, alumina nanoparticles are synthesized by a sol-gel method, and their dosimetric properties are studied.

2. Experimental details

Alumina nanoparticles were synthesized using a sol-gel method. The precursor materials used were aluminum nitrate [Al $(NO_3)_3$] and oleic acid ($C_{18}H_{34}O_2$), and obtained from Merck, Germany with high purity. Initially, 0.5 g of aluminum nitrate was mixed with 10 ml of water for half an hour under magnetic stirring, followed by adding oleic acid. The remaining solution was then kept at 180°C for 3 h until the water completely evaporated, thereby forming a black gel. We calcined the gel at different temperatures, and heated the sample to temperatures of 650°C and 1200°C for 20 min and 3 h, respectively. Finally, a transparent white precipitate was obtained, containing alumina nanoparticles. An X-ray diffraction (XRD) device (model: Rigaku D/Max diffractometer) was used to investigate the structure and to confirm the formation of Al_2O_3 nanoparticles. A scanning electron microscope (SEM; Philips XL30) was employed to observe the shape and size of the nanoparticles. In this research, a TLD reader (model: 4500 Harshaw) was used to read the irradiated samples. All the samples were irradiated under the same conditions using a ^{60}Co source in the range from 50 °C to 250 °C with a temperature rate of 2 °C/s.

3. Results and discussion

The XRD spectrum of alumina nanoparticles is shown in Fig. (1).

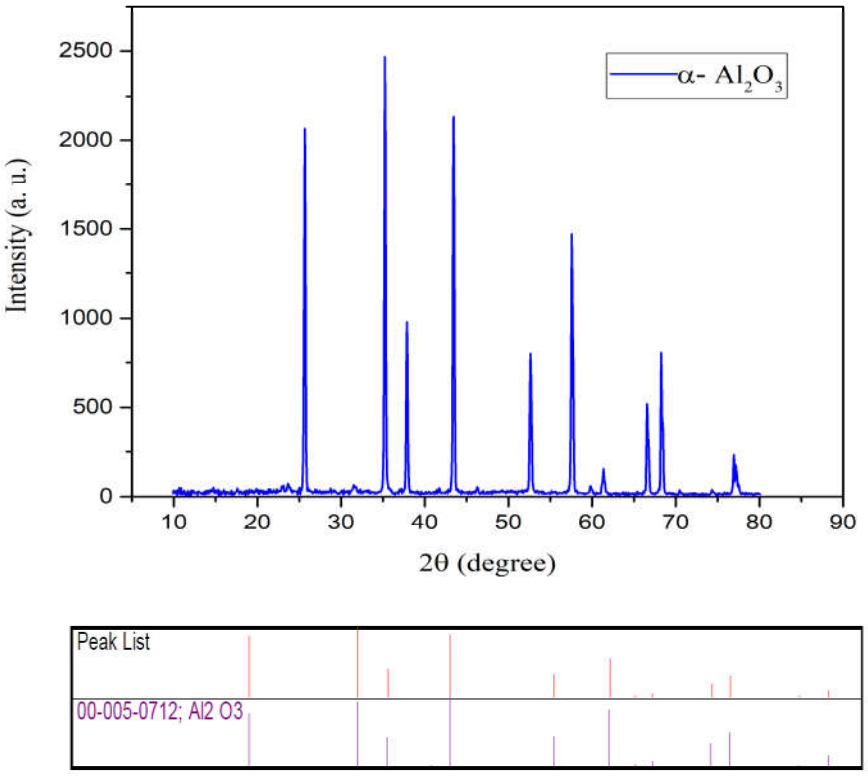

Figure 1: XRD spectrum of alumina nanoparticles synthesized at 1200°C.

According to this figure, peaks at 2θ=25.58°, 35.13°, 37.78°, 43.61°, 52.78°, 57.59°, 66.43°, 68.51° and 77.25° are attributed to (012), (104), (110), (113), (024), (116), (214), (300), and (1010) planes, respectively. These planes are in complete agreement with the alumina reference sample with the standard card number [0712-005-00], indicating the formation of Al_2O_3 crystals with a rhombohedral structure.

Using the Scherrer formula (equation (1), the average size of the crystal can be estimated as follows:

$$D = \frac{0.9\lambda}{\beta Cos(\theta)} \quad (1)$$

where β is the full width at half maximum (FWHM; in terms of radians), θ is the angle Bragg peak diffraction the to related, and λ is the X-ray wavelength [8]. crystal the of size The was obtained to be 55 nm. The result of SEM analysis of the alumina nanoparticles is shown in Fig. 2. The nanoparticles are observed to have good aggregation and dispersion. Also, the size of the nanoparticles is in agreement with the crystal size obtained from the Scherrer formula.

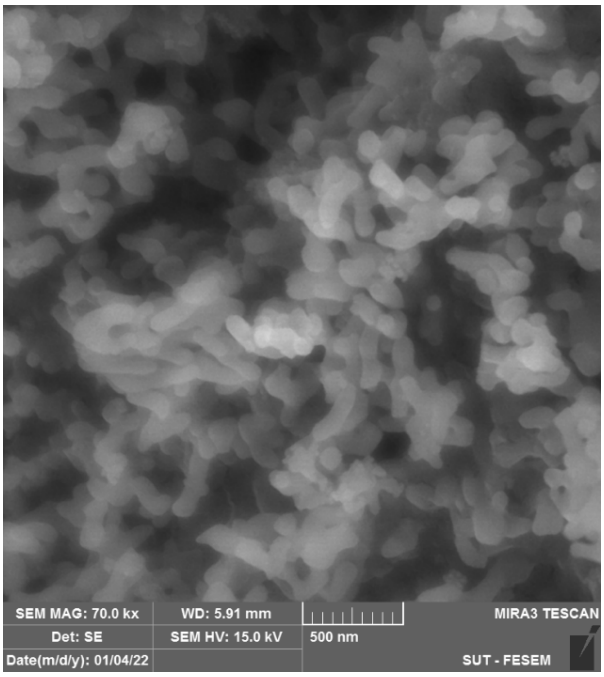

Figure (2): SEM image of Al_2O_3 nanoparticles at a magnification of 500 nm.

According to the literature, the general order kinetic model was used and curve fitting was performed by software based on the Levenberg-Marquardt algorithm. The following relationship was used to calculate the kinetic parameters E and b [9]:

$$I(T) = I_m b^{\frac{b}{b-1}} exp\left(\frac{E(T-T_m)}{KTT_m}\right) \times \left\{\frac{T^2}{T_m^2}(b-1)\left(1-\frac{2KT}{E}\right)exp\left(\frac{E(T-T_m)}{KTT_m}\right)+1+(b-1)\frac{2KT_m}{E}\right\}^{\frac{-b}{b-1}} \quad (2)$$

where b is the kinetic parameter (ranging between 1–2), E is the activation energy, T is the temperature (in Kelvin), T_m is the maximum temperature, and k is the Boltzmann constant. According to Fig. 3, the glow curve of the nanoparticles is formed from two peaks at temperatures of 443 and 461 K. Equation (3) was used to determine the degree of conformity between theoretical and experimental thermoluminescence curves:

$$FOM = \frac{\Sigma |y_i - f_i|}{\Sigma y_i} \times 100 \qquad (3)$$

where y_i denotes to the original values or experimental data, and f_i is the best value that can be obtained through this fit [10]. The FOM amount is found to be 2.17, indicating a good fit between the theoretical and experimental curves. Table (1) shows the results of kinetic parameters obtained from the fitted curve.

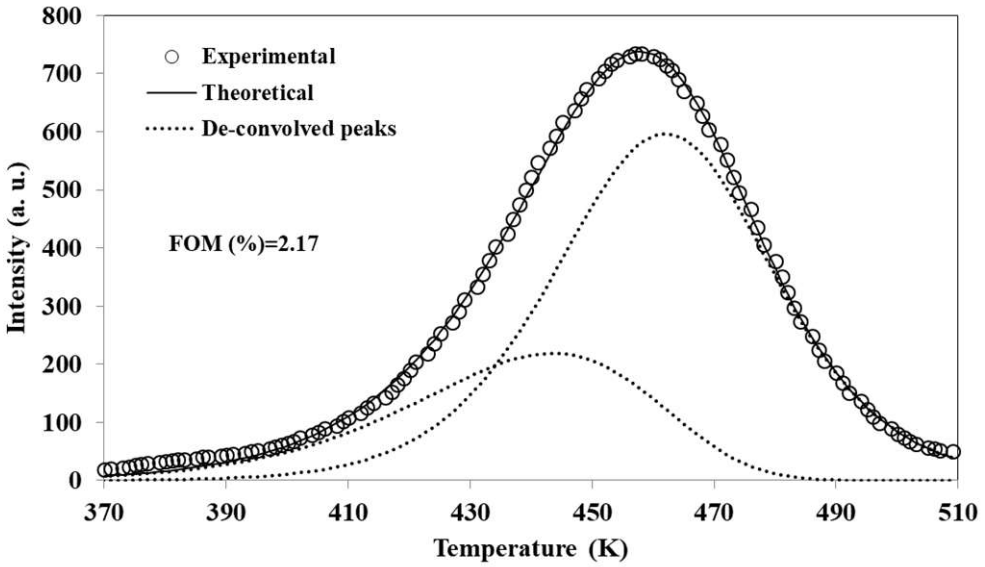

Figure (3): Fitted thermoluminescence curve of Al_2O_3 nanoparticles

Table 1: Kinetics parameters of Al_2O_3 nanoparticles.

Peak	b	E (eV)	T_m (K)	I_m (a.u)
1	1.00	0.82	443	218
2	1.70	1.33	461	596

4. Conclusion

In this research, alumina nanoparticles were synthesized by a sol-gel method in order to study their dosimetric properties. Among the notable points regarding the sol-gel method, one can mention about its cost-effectiveness. Also, the method used was advantageous for the synthesis of the nanoparticles, because the formed particles were uniform in size and the resulting structure was in complete agreement with the Al_2O_3 crystal. The formation of the nanoparticles was confirmed by XRD analysis. SEM analysis showed the formation of rhombohedral particles. The irradiated nanoparticles consisted of a broad curve, indicating the overlapping of two peaks at temperatures of 443 and 461 K. This provided a suitable temperature for dosimetry purposes. Therefore, the synthesized thermoluminescence sample can be considered a good candidate for dosimetry.

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The Amalgamation of Nanoparticles from Plant -Intermediary

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Abstract
The key pathways for synthesizing nanoparticles are physical and chemical, usually expensive and possibly hazardous to the environment .Nanoparticles and its green synthesis with plants have become an important field of nanoscience due it is great benefits provided to humanity through its cost effective, least harm to humans and the environment also .Green nanotechnology using plant extract opens up new possibilities for the synthesis of novel nanoparticles with the desirable characteristics required for developing biosensors, biomedicine, cosmetics and nano-biotechnology, and in electrochemical, catalytic, antibacterial, electronics, sensing and other applications.
Keywords :Green Synthesis,Green Chemistry,Bio Synthesis,Nano Particles

1. Introduction

Creation, exploitation and synthesis are nanotechnology concepts that typically consider materials smaller than 1 mm in dimension [1]. Many different methods, such as physical, chemical and green (biological) techniques, have been used to synthesize nanoparticles [2, 3].Nanoparticles with a high surface area ratio exhibit unique physical, chemical, and optical properties [4].Another interfering of this important can be indicated at Poorly soluble drugs that are made into particles by using nanoparticle technology, thereby enhancing their solubility due to a decrease in their particle size from micron to nanometer scale[5].Also the increasing of stability and biocompatibility of nanomedicines are suitable for biomedical and environmental applications which can be involved in restraining such diseases like coronavirus pandemic (COVID-19) [6] .Therefore, nanoparticles are used in a variety of applications such as electronics, optoelectronics, optics, electrochemistry, biomedical, food, textiles, catalysts, sensors, energy, and the environment [6].

2. Mechanism of Nanoparticles Synthesis

NPs are synthesized using various physical, chemical, and biological techniques, which results in different shapes and sizes for use in numerous applications [7,8].These physicochemical characteristics of nanoparticles are critical in influencing the nanoparticles' properties to be utilized in specific applications [12].The synthesized nanoparticle's general characterization is usually carried out through scanning electron microscopy (SEM), transmission electron microscopy (TEM), energydispersive X-ray spectroscopy (EDX), ultraviolet–visible spectroscopy (UV–Vis), Fourier-transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD).

2.1. Plant Extraction

Plants contains wide range of bioactive compounds which includes alkaloids, flavonoids, terpenoids, steroids, etc which act as a reducing agent in the synthesis of nanoparticles. Plants including Acalypha indica, Ficus benghalensis, Zingiber officinale, Plumbago zeylanica, Centella asictica, Parthenium hysterophorus, Sapindus rarak, Passiflora foetida, etc have recently been used to synthesise various types of nanoparticles .Plant extracts have greater benefits than microorganisms for synthesis of green nanoparticles because it's one-steps process, nonpathogenic and cost-effective process [9].

2.2. Affecting Factors of Nanoparticles Synthesis

Toxicity is an important factor in considering when using nanoparticles for biomedical applications to ensure that they are safe and effective [9]. In certain studies, metal nanoparticles have been shown to be dangerous to people. Metal nanoparticles' toxicity is governed by their size and surface load. It is mandatory to recognize potential health hazards related to nanoparticles. It is critical to investigate small cellular changes in DNA injury and oxidative stress in human tissues in order to rule out probable genotoxicity [9].

3. Antioxidants

Antioxidants are compounds that scavenge free radicals and plant extract has the power of doing it, therefore it is used for the NPs synthesis[10]. Traditional medicines are now being revalued across the globe due to substantial study on many plant species and their medicinal properties. Studies have shown experimental evidence to support the theory that reactive oxygen species(ROS) and free radicals have a role in a wide range of illness [10].

4. Discussion

A recent study has shown that green approaches can be used for synthesizing ZnONPs by using leaf extract of P. austroarabica (Yemeni mistletoe)[Figure2] as an reducer,stabilizer and capping agent witch is considered that they have high antioxidant activity compared to ascorbic acid and even P. austroarabica extract itself witch indicates that the green synthesized ZnONPs from P. austroarabica extract could be a promising material as bio-environmental probe to detect and remove the hazardous pollutants in various fields [11].Another research has shown that it is possible to use sargassum horneri(marine-alga)[Figure3]extract as capping and reducer agent in achieving green(biological)synthesis of AgNPs and AuNPs. marine-algae nanoparticles can effectively decompose a variety of harmful dyes and exhibit excellent activity and reactivity as catalysts. The synthesized nanoparticles have the potential to be used to prevent water pollution and to treat wastewater in a variety of industries that use dyes.Also a green synthesis of silver nanoparticles was successfully carried out using Oxalis griffithii[Figure1] methanolic leaf extract. The biosynthesized AgNPs in the current study are shown antibacterial activity against E. coli and Bacillus subtilis, which suggested that they may be useful in developing new drugs [12].

Figure1:Oxalis-griffithii

Figure2:P.austroarabica

Figure3:sargassum horneri

5. Compendium

It is indicated that these days,resent studies in nanoparticle amalgamations(synthesizing) via green(biological) approaches are driven into plant-intermediary(mediated) extractions that are covered by such technics that provides promising materials that has desirable characteristics required for developing biosensors, biomedicine, cosmetics and nano-biotechnology, and in electrochemical, catalytic, antibacterial, electronics, sensing and other applications. Even though biological approaches also possess limitations, such as instability and low yield of nanoparticles, they have exhibited reassuring results in several biomedical applications, including antiviral efficacy against disease-causing viruses(COVID-19).

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Application of nanobiosensors in detection of SARS-CoV-2

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Abstract

Viruses of widespread notoriety have emerged in recent years and gained international prominence was covid -19. Biosensors, which have many other medical applications, including diagnosis, therapy, and prevention, were also proposed as a viable option for diagnosing covid. When nanoparticles are incorporated into biosensors, the resulting devices are referred to as nanobiosensors. These nanobiosensors are sensing devices that utilize elements with electromagnetic, chemical, acoustic, thermoelectric, or optoelectronic properties to detect and measure biological phenomena. This article provides an overview of the strategy for using these ligand-based biological nanosensors to detect Covid-19.

Key words: nanosensors, *sars -cov-2*, diagnosis, virus infection

1. Introduction

Corona, also known as covid-19, is one of the most well-known viruses that have recently afflicted the globe. In the wake of this illness, the WHO declared an epidemic on March 11, 2020. Researchers have shown that the coronavirus may transmit vertically (via the air) and horizontally (by contact with an infected person or animal) [2]. Based on comparing its sequence to other known viruses and a phylogenetic tree, SARS-CoV-2 has been assigned to the β-CoVs family. CoVs come from various natural environments and are a class of positive-sense, single-stranded, enclosed RNA viruses. Symptoms of these viruses include respiratory illness, diarrhea, liver failure, and even brain dysfunction. Based on their genotypes and the antibodies they elicit, coronaviruses are classified into four distinct subfamilies α ،β ،γ and δ-CoVs. Both α- and β-are capable of infecting humans[1]. The World Health Organization (WHO) suggests using reverse transcriptase polymerase chain reaction to identify SARS-CoV-2 viral RNA to diagnose coronary disease (RT-PCR). Although RT-PCR is considered the gold standard, collecting the daily samples needed to stop its spread takes time. It requires specialized testing facilities staffed by highly competent workers[9]. Using powerful

nanobiosensors, which can detect viral infections at various wavelengths, is one of these ways to catch Covid-19[3]. The nanosensor incorporates a sensitive layer through a covalent bond to a transducer[4]. There are several different types of biosensors, each employing a different detection principle. These include resonance biosensors (coupled sound waves), optical biosensors (light), thermal biosensors (heat), ion-sensitive biosensors (change in surface potential), electrochemical biosensors (change in electrical properties of the medium), and amperometric or potentiometric biosensors[5]. Electrochemical and optical sensors are potential methods for detecting the coronavirus. The quicker a virus is detected, the more effective it may be treated. Since nanobiosensors are low-cost, portable, and rapid, so they considerably aid in this process step. Wishing that the therapeutic steps might be taken sooner. Metal oxides, quantum dots, carbon nanotubes, graphene nanotubes, and polymeric nanomaterials are only some of the nanoparticles employed for virus detection [7]. These nanoparticles typically include biomolecules from the infection deposited on their surface [8].
This article discusses using nano biosensors based on diagnosis components in detecting corona virus illness. These elements include whole-cell biosensors, peptide-based, DNA aptamer-based, enzyme-based, and immunological sensors [10].

2. Immunobiosensor

12.1. Paper-based nanosensors

According to the results of a study, the paper-based biosensor was tested using nasopharyngeal swabs from COVID-19 patients, and the fluorescence signal was observed in a matter of minutes. Nasopharyngeal swabs and saliva samples were cooked in a lysis buffer at 60 degrees Celsius. Then, synthetic SARS-CoV-2 genomes were placed in a reaction tube for a lateral flow readout time of 2 minutes using a commercial lateral flow test strip. The viral genome may be detected at a concentration of only 100 copies per milliliter. Joung, et al[12] [11].

2.2. FET-based nanosensor

In a field-effect transistor (FET), the three electrodes—source, drain, and gate—connect the metal oxide channel and semiconductor outside the device. One-dimensional (1D) nanostructured materials like silicon nanowires (SiNWs) and carbon nanotubes (CNTs) and two-dimensional (2D) nanostructured materials like graphenetransition metal dichalcogenides (TMDCs) and black phosphorus are commonly used in bio-FET sensors [13]. In order to identify SARS-CoV-2 in clinical samples, researchers have created a biosensor based on field-effect transistors (FETs). This empathetic sensing technique is carried out by graphene in this gadget. To create a biosensor, particular antibodies were attached to the graphene layers of the FET. Humans with infections had nasopharyngeal swabs obtained for analysis. The FET biosensor detected the SARS-CoV-2 spike protein in the growth media. There was no cross-reactivity with MERS-CoV antigen, and the device was susceptible, allowing for the rapid detection of even a trace quantity of target. Seo et al[15][14].

2.3. Plasmonic nanosensors

When an antigen binds to an antibody-functionalized surface, the reflection angle changes, which measures the resonance conditions. This is how plasmonic biosensors operate. Surface plasmon resonance (SPR) sensors, localized SPR (LSPR) sensors, and Surface-enhanced Raman scattering (SERS) sensors have all been used to monitor environmental biological molecules, ensure food safety, and track the spread of illness [16].Using these biosensors, scientists have been able to identify viruses for quite some time. Within 15 minutes of sample/sensor interaction, a nanomolar concentration of

viral antibodies in human serum could be detected using an SPR sensor covered with a peptide monolayer and functionalized with recombinant SARS-CoV-2 nucleocapsid protein. It was designed to find covid-19 and makes use of the synergistic effects of photothermal plasmonics and LSPR. Hybridization with target nucleic acids allows complementary DNA receptors coupled with 2D gold nanoarrays (AuNIs) to identify specific SARS-CoV-2 sequences. As a means of further improving detecting abilities, plasmonic heating was induced on the AuNIs surface. Qiu et al [17][14].

3. DNA aptamer nanosensors

Aptamers are sequences of oligonucleotides that, are between 25 and 80 bases in length, like monoclonal antibodies . They undergo a series of transformations that result in a wide variety of three -dimensional structures that are then affixed to designated targets [18]. MSA1 and MSA5 are two DNA aptamers discovered via a series of selection procedures in the lab and are currently employed in aptamer-based research. The S1 subunit of the trimeric spike protein is bound with high affinity (multi-nM range) by this family of selective aptamers in both the wild-type and wild-type B alleles. It has been revealed that two aptamers, CoV2-RBD-1C and CoV2RBD-4C, which were found using machine learning approaches, specifically target the receptor binding domain (RBD) of the SARS-CoV-2 spike protein. The resonance shifts caused by the binding of specific analytes form the basis of the surface-enhanced Raman spectroscopy (SERS) approach, which may be used in conjunction with nanostructures to detect SARSCoV-2. Silver nanoparticles and the surface-enhanced Raman scattering concept are used by aptamer-based nanosensors to identify SARS-CoV-2. This single-step technique revealed a strong SERS signal at 587 cm $^{(-1)}$and silver nanoparticle agglomerated after the tagged aptamer interaction with a cultured virus. Stanborough et al [19] [20]. The G-quadruplex is an atypical nucleic acid structure comprising four stacked guanine tetrads[21]. Since it does not need nucleic acid extraction, it is being looked at as a potential replacement for the aptamer in detecting SARS-CoV[22].

4. Peptide-based nanosensor

Peptides have the potential to replace proteins as bioreceptors in (bio) recognition and bioassay events because of their improved stability against denaturation, smaller sizes, lower cost, and more chemical diversity. An enzyme-linked immunoassay (EIS) was used to identify and quantify the interaction between Spike protein and a synthetic thiolated peptide bioreceptor adsorbed on a screen-printed gold working electrode (SPAuE). The final device had a linear dynamic range of clinical significance and a low limit of detection (LOD). Also, the Spike protein from COVID-19-positive patients could be identified in clinical samples using the peptide-based biosensor, demonstrating its specificity for SARS-CoV-2. The results of using this peptide in this platform, which was previously utilized to assess SARS-CoV-2, demonstrate significant analytical performance.Soto et al [23].

5. Whole cell nanosensor

Typically, whole-cell biosensors detect the target analyte from inside the cell. Because of the constraints of the cell membrane, such designs can only detect analytes that can passively diffuse or be actively transferred over the membrane[24]. As part of an ideal cell design, membrane engineering was employed to build cellular components that better bound proteins. Antibodies against the SARSCoV2 S1 spike protein were electrochemically injected. The results showed that the electrical measurements of the damaged cell membranes were modified by the biomolecules associated with the released antibodies. Electrical behavior features have changed noticeably, as seen by the data. Biosensors that have already shown their worth in other contexts are helpful for medical diagnosis, surveillance, and viral control in many ways. A simple and compact reader may read this biosensor's surface antigens for SARSCoV2. This sensor has proven beneficial for real-time estimation of the number of patients infected with the virus. Mavrikou et al [25].

6. Enzyme nanosensor

Antibodies that have been enzyme-labeled are crucial to detecting SARS-CoV-2 because they generate and amplify the detection signal. Each substrate is unique to the antibody-labeled enzyme, and several detection methods may be used to identify the result of its catalysis. Examples include the recent publication of the electrochemical finding of the SARSCoV2spike (S) protein by catalyzing one naphthyl phosphate substrate on the surface of a printed carbon electrode with a secondary tagged alkaline phosphatase enzyme (SPCE). However, this nanosensor has received less attention since enzymes are unstable and may easily be influenced by their surroundings. Enzymes are often used as universal sensing elements due to their excellent substrate specificity, sensing resistance, and reduced sample volume requirements. Fabiani et al [26] [27].

7. Discussion

Today, nanosensors are undoubtedly used more and more in various fields of medicine, and disease diagnosis is one of the broad fields of their use. Of course, nanosensors have not yet been widely placed in the clinical phase. Still, they have shown a high potential for detecting viral diseases, especially corona, which can be used in the future. most of all, paper-based nanosensors based on the test Point of care are more recommended than others for clinical use due to their speed and cost-effectiveness.

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Quercetin loaded albumin nanoparticles: Synthesis and release kinetic study

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Abstract

One of the ways to improve release behavior and efficiency of hydrophobic pharmaceutical compounds in the aqueous environment of the body is loading of these drugs in delivery systems. In this regard, nanoparticles-based drug delivery systems (DDSs) have shown a great potential. In this study, albumin nanoparticles were prepared as a delivery system for hydrophobic quercetin compound. According to the FESEM imaging, prepared nanoparticles showed mean particle size of 116.58 ± 2.65 nm with spherical and uniform morphology. Also, the results of the in vitro release study showed that the release of quercetin from albumin nanoparticles occurs in a controlled and sustained manner. Finally, the fitting of the release data on Zero-order, First-order and Higuchi kinetic models showed that the release mechanism mainly follows the Higuchi model.

Key words: Albumin, Quercetin, Drug Delivery, Release Mechanism

1. Introduction

Drug release refers to the process by which a pharmaceutical dosage migrates from its initial position in a DDS to the outside surface and is exposed to absorption, distribution, metabolism and excretion to achieve pharmacological activity (1). Controlled release is one of the ways of releasing drugs from DDS that has been greatly improved in recent years. In controlled release DDSs, the drug incorporated in a special structure is released for a long-time by predefined rates with minimal side effects (2). On the other hand, the bioavailability and efficacy of hydrophobic drugs can be improved under physiological conditions by loading in DDSs. In this regard, albumin nanoparticles have been widely used in various researches as a potent DDS due to their unique properties including biocompatibility, biodegradability, non toxicity and non-immunogenicity (3). Furthermore, quercetin (3,5,7,3′,4′ pentahydroxyflavone) is a natural flavonoid, found in most fruits,vegetables and nuts. This active ingredient of the human diet, apart from strong antioxidant property, has shown pro-apoptotic effect on tumor cells and inhibitory effect of the cancer cells growth in different phases of the cell cycle (4, 5). However, instability and very low solubility of

quercetin in water and subsequently its low bioavailability limits the clinical application of this pharmaceutical compound (6). The results of various researches have shown that the utilization of DDSs can improve its stability, efficiency and bioavailability (7). Therefore, in this research, we prepared albumin nanoparticles as a DDS for quercetin. Quercetin release behavior and kinetic from albumin nanoparticles was investigated.

2. Materials and methods

1. Materials

Quercetin, Bovine serum albumin (BSA), glutaraldehyde (25% aqueous solution), phosphate buffer saline (PBS) and 12-14 kDa dialysis tube were purchased from Sigma Aldrich, Co. Ethanol 96% and hydrochloric acid (HCl) were prepared from Merck Co. HPLC grade water was used for all experiments.

2. Synthesis of drug loaded nanoparticles

Desolvation method was used for preparing of quercetin loaded albumin nanoparticles. Briefly, 0.2 g BSA was dissolved in 4 mL ultra-pure water and stirred at 500 rpm for 20 min. Then 16 mg of quercetin thoroughly was dissolved in 16 ml of ethanol solution and added to BSA solution dropwise at the rate of 2 ml/min. After adding half of the ethanolic solution, nanoparticles were formed and the suspension became turbid. Then, for crosslinking of the nanoparticles, 60 µL glutaraldehyde (8% solution in water) was added to the suspension. After stirring for a night, resulting colloidal suspension of nanoparticles was purified by centrifuge (at 10000 rpm for 10 min) and washed by ultra-pure water for three times.

3. Nanoparticles charachtrization

The morphology and size of nanoparticles was investigated by field emission scanning electron microscope (FESEM) model TESCAN MIRA 3. UV-visible spectrophotometer, model UNICO 2150-UV, was used for obtaining the wavelength of characteristic peak of quercetin and plotting the standard curve.

4. Drug release study

Drug release behavior of nanoparticles was studied under two pH of 7 and 5.5. For neutral pH condition, 1 mL of nanoparticles suspension was entrapped in a dialysis tube and placed in 15 mL of PBS-ethanol medium (7:3 v/v) at sink conditions. To achieve an environment with acidic condition, an appropriate amount of HCl was added to the medium. The samples were stirred (120 rpm) at 37 ℃. At determined time points, 5 mL of each medium was collected and equal amount of fresh medium was added. The collected samples analyzed by UV-visible spectrophotometer and using standard curve of quercetin. Finally, the curve of cumulative release percentage versus time was drawn by inserting the obtained values into the following equation:

4. Results and discussion

Synthesis of albumin nanoparticles and loading of quercetin was performed by desolvation method and Fig.1 shows the FESEM image of the nanoparticles. Also, to determine the size distribution and mean particle size, the diameter of a large number of nanoparticles were obtained using ImageJ.JS software, and the mean particle size was calculated with three repetitions. According to the results, the prepared nanoparticles have a spherical morphology and smooth surfaces with a uniform size distribution. As well, the mean particle size of 116.58 ± 2.65 nm was obtained for the nanoparticles. Studies have shown that shape and morphology play an important role in the entry of nanoparticles into cancer cells through receptor-mediated endocytosis. In general, particles with spherical morphology and sizes between 50 and 200 nm are less susceptible to phagocytosis and removal by the liver and spleen, as well as clearance by the renal system, and thus have long-term blood circulation. Therefore, the therapeutic cargo is protected from elimination and rapid metabolism (8).

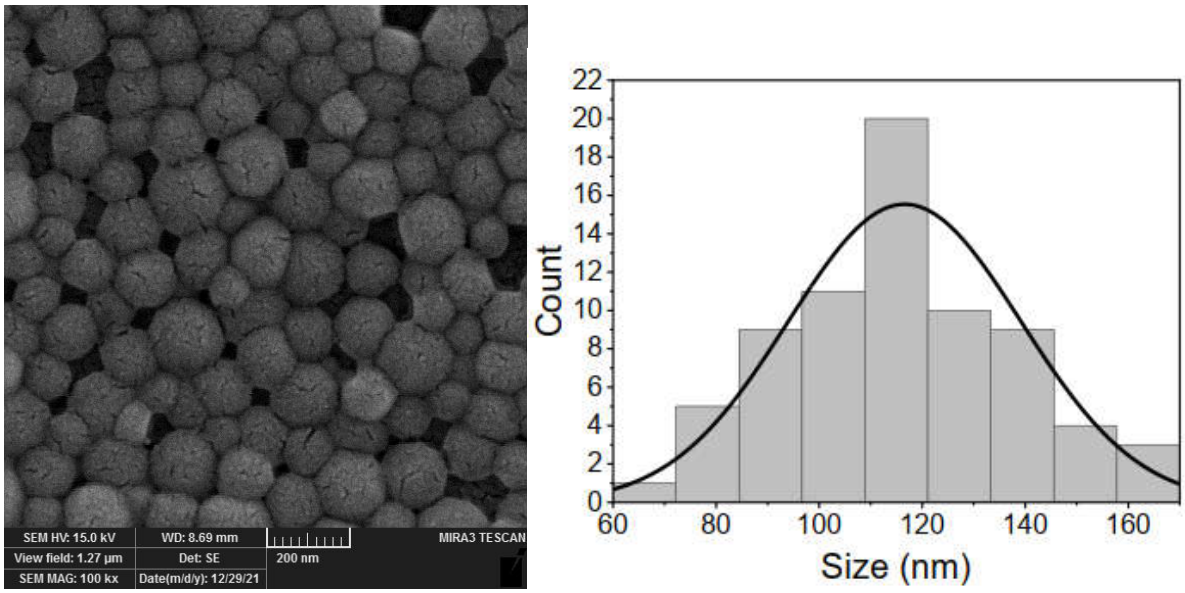

Figure 1. FESEM image and size distribution histogram of quercetin loaded albumin nanoparticles

The release of quercetin from albumin nanoparticles was investigated for 48 hours at two pH values of 7 and 5.5. Figure 2 shows the graph of cumulative release percentage versus time. At both pH of 7 and 5.5, quercetin follows a generally sustained and controlled release, with an initial release of about 12% and 19% in 6 h and following release of 8% and 19 % in 48 h, respectively. Furthermore, quercetin release is higher in acidic pH compared to neutral pH, confirming the more stability of loaded quercetin in albumin nanoparticles in the bloodstream with neutral pH, in comparison to acidicmicroenvironment of tumors and endosomes.

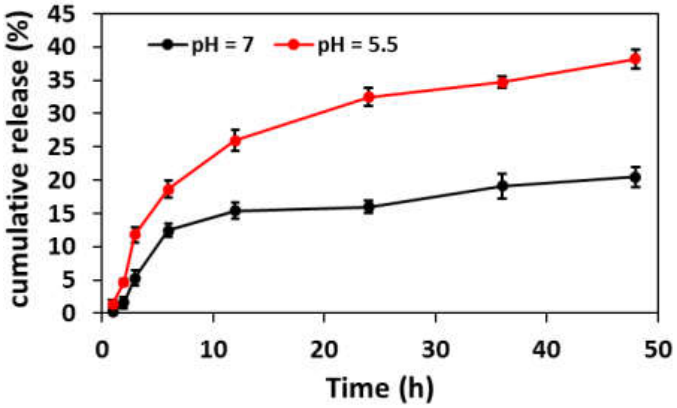

Figure 2. Quercetin release curve from albumin nanoparticles

To study drug release kinetic and mechanism, the quercetin release data were fitted with Zero order, First order and Higuchi kinetic models as follow (9, 10):

Zero order model:
In the zero order kinetic model the release is very slow and a steady state concentration profile is observed. In this model, the release rate over time is independent of the concentration, and increment or reduction of the concentration does not affect the diffusion:
$Q_t = Q_0 + K_0 t$

Q_t is the amount of drug released in time t, Q_0 is the initial amount of drug (most times, $Q_0 = 0$) and K_0 is the Zero order release constant.

First order model:
This model is based on Fick's law of diffusion and release is occurred due to difference between the concentration of drug inside and outside of the carrier. Indeed, the release rate of this model is dependent on the released concentration:
$\text{Log } Q = \text{Log } Q_0 - K_1 t/2.303$

Q is the remaining amount of drug and K_1 is the First order release constant.

Higuchi model:
This model is also based on the first law of Fick diffusion. This model assumes that: (i) initial drug concentration is higher than drug solubility, (ii) drug is diffused in one dimension, (iii) The particles of drug are smaller than system thickness, (iv) system swelling and dissolution are negligible, (v) the diffusion coefficient is constant and (vi) sink conditions are fully observed in release medium.
$Q_t = K_H \times t_{1/2}$

K_H is the Higuchi release constant.

Accordingly, the fitting curves of each model was obtained using the release data (Figure 3) and the regression coefficients (R_2) and rate constants of the models were obtained according to Table 1. both investigated pH, the Higuchi kinetic model has the highest values of R_2.

Therefore, the release of quercetin from albumin nanoparticles mainly follows the Higuchi model. According to this model release mechanism of drug involves simultaneous penetration of quercetin molecules across nanoparticles, dissolution of drug molecules and diffusion of these molecules from the nanoparticles to the medium. As maintained, the rate constant of the Higuchi model has a structural similarity with the composite Fickian diffusion coefficient. Therefore, it can be concluded that the Fickian diffusion plays a major role in drug release from nanoparticles and the driving force for release is the gradient inside and outside of the albumin nanoparticles.

Table 1: Regression coefficients of kinetic models

pH	Zero order	First order	Higuchi
	R^2	R^2	R^2
5.5	0.8062	0.8483	0.9257
7	0.7339	0.7557	0.8635

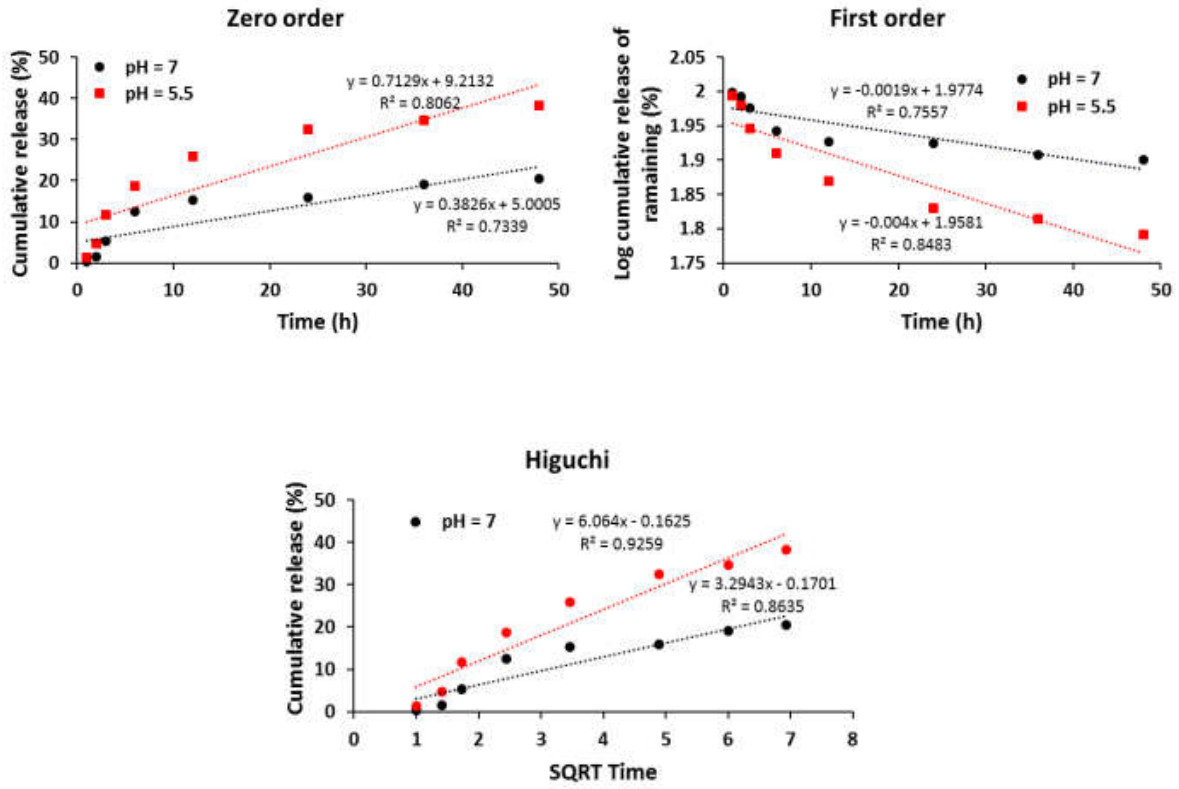

Figure 3. Zero order, First order and Higuchi release kinetic models of quercetin from albumin nanoparticles

6. Conclusion

In this investigation, quercetin loaded albumin nanoparticles were synthesized successfully via desolvation method. The prepared drug-loaded nanoparticles showed suitable particle size and for drug delivery to cancer. Also Controlled and sustained release behavior of nanoparticles makes them useful for drug delivery applications. In addition, the release mechanism of quercetin from albumin nanoparticles mainly follows the Higuchi model and diffusion has main role in release mechanism.

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Investigating Students' Misconception in High School Chemistry Education

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Abstract

Misconceptions refer to any kind of false notions that cause non-scientific beliefs, confused concepts, simple concepts and theories without scientific roots . Misconceptions cover a wide range of scientific concepts. Misconceptions not only in chemistry, but in other fields at all levels cause major problems for science educators, scientific researchers, teachers and students. If the concept in question is scientifically abstract and incomprehensible concepts, it will be much more necessary to recognize Misconceptions and deal with them. The present article, is a kind of review article in which the subject of common Misconceptions of students in teaching chemistry in the high school has been discussed. In explaining chemistry concepts, vague terms and definitions should not be used and clear and understandable explanations should be used. Teachers should refrain from providing redundant data to students. It is also a very good solution for teachers to be familiar with different types of misconceptions in different lessons.

Key words: Misconception, Chemistry education, Chemistry, Education.

1- Introduction

Learners at any age and when learning a new concept project ideas in their minds according to previous experiences and information. Those concepts that do not correspond to the accepted scientific principles and the learner uses them to describe scientific phenomena incorrectly, are called misconceptions. Considering that students have problems when learning chemistry topics about abstract concepts; to help make these abstract concepts more concrete, chemistry educators use representations such as models and simulations. Nevertheless, students still have misconceptions in a wide range of chemistry content areas (Koohifaegh).

Early experience introduction without providing proper context and developing concepts lead to memorization and prevent the development of logical thinking and abstract. Learners learn better when they solve real problems and critically discuss issues with their classmates (Robert).

it is important for teachers to detect misconceptions and to some extent they can adjust their teaching methods based on misconception of students. In fact, awareness of their mental processes will help teachers to try to make the appropriate changes in the way students learn and explore better ways and their close relationship with the real world and the goals of higher education (Margaret).

When the student enters higher levels of education, he begins to create explanations for many phenomena and events in the world around him, which often contradict the scientific point of view, and despite providing sufficient and correct explanations during teaching, he still interprets the concepts. based on his previous ideas and in an incorrect way. Most of the

students use their preconceived notions when recording the laboratory results and record the results based on their own guesses and not based on the observations they have made. The important point he mentioned is that the students' failure to record the results was not due to laziness or clumsiness. According to psychologists, a person sees not with his eyes, but with his brain, and the signals received from the eyes are only part of the information used to communicate with the environment. In other words, we see what we guess, not what is in front of our eyes. This topic is also mentioned in books related to visual errors (Taber).

It is not possible for a student to learn a subject only through its general expression; Rather, learning any subject depends on the initial beliefs and thoughts recorded in the mind, and if it is expressed incorrectly, it will definitely be much more difficult to learn that subject in the future. If a general issue is stated and not many examples are stated, the learner may find examples in his mind that are completely in conflict with the stated issue. Sometimes stating a question, using different examples, connecting the topic with different topics in the society such as mathematics, philosophical discussions, etc. helps a lot to understand the topic. For example, establishing a relationship between chemistry, mathematics, statistics and probability, especially at the microscopic level or in other words at the particle scale, will clear up the existing Misconceptions and will definitely give the student a proper understanding of the macroscopic expression of the entropy function. Sometimes asking the right questions and making the student think is better than learning by mistake or sometimes Misconception. Sometimes, using an illustrative image of a topic helps to understand it better than the explanation of even several pages (Zighomi).

In order to solve the misconceptions and improve learning, applying new ways of learning and teaching is necessary. Teaching and learning using computer software, learning simulation and instructional design approaches such as constructivism-based approaches enable new ways of learning in recent decades. In the new generation, multimedia technologies and advanced internet based learning simulators invest because creating simulation based learning is more affordable than previous methods. Kolb believes that the deep intellectual understanding can only be based on empirical findings. In other words, students learn better when they solve real problems and critically discuss issues with their classmates. The value of active learning, training simulations, problem solving and other modern educational pedagogies has been acknowledged by researchers (Kolb and Alice).

One of the important components of content knowledge of chemistry is the awareness of students' Misconceptions. Students have many Misconceptions about some chemical concept, which originates from various factors. Also, at any stage and age, students may have misconceptions about chemical bonds. Meanwhile, chemical bonding is a key concept for molecular structure and is related to the physical and chemical properties of a compound. On the other hand, these Misconceptions are very resistant to change. Therefore, as a solution, it can be mentioned to reduce the content in all educational levels so that students have enough time to build concepts. This issue is especially important for the first years when students encounter these concepts. It is suggested that teachers should make connections between different levels in Johnston's triangle (macro, micro, symbolic). Since no research work has been done in this field in Iran, it is suggested to carry out extensive research on this issue. Also, in order to reduce such Misconceptions, teachers should be regularly trained to teach concepts in the correct way (Moghayerinia and others).

Tavares, in his study to evaluate the use of computers in the learning environment in Brazil, showed that the use of computers can enhance learning environments (Tavares). Kilicman, Hassan and Sayed Husain in their research on the theme of using mathematical software in teaching and learning demonstrated the use of software to facilitate the learning

process and the achievement of learners (Kilicman). Garcia-Souza and Gamboa assessed the impact of information technology on performance and concluded that the frequency of using a computer has a positive impact on students' scores (Gamboa and Garcia). Rieber taught through simulation, in other words, he used simulation to teach the principles of acceleration and speed in physics (Mayer Rieber).

The source of Misconceptions can be the incomprehensible presentation of materials in textbooks or by teachers and even the media, as well as the heat of the entrance exam and superficial testing methods, etc. The result of this study shows that learning new concepts will not be done until the wrong concepts are removed from the students' minds. Therefore, first, correct knowledge should be restored in their minds. To do this, the teacher must identify their Misconceptions and through group discussions in the class and strengthen their reasoning power and provide examples and useful information and use active teaching methods and use conceptual questions in tests to internalize knowledge. in their minds and try to create problem solving skills in them. In the meantime, education should express the concepts clearly in the writing of textbooks and organize lesson plans in such a way that the prevailing atmosphere in schools is deep and conceptual learning, not learning superficial methods of testing and entrance exam fever (Boloori).

Constructivism is one of the theories of learning. Constructivist approach has been established by the efforts of scientists such as Piaget, Brunner and Vygotsky in the 1970s and is on the basis of the theory of cognitive psychology (Piaget and others). Constructivism is becoming a dominant paradigm and approach in the field of education and training. The essence and the cornerstone of this approach is the assumption that knowledge is built inside of the mental processes and not transferred from the outside in the minds of people. One of the important areas of education that has been greatly changed under this approach is the domain of instructional design (as one of the pillars of the teaching and learning process). Constructivism approach spent a lot of time for studying and understanding of its benefits (Seraji and Attaran). Constructivist learning environments design is very important which consists of a problem, a question or a project as subjective and interpretive center and systems. The purpose of learner is the interpretation or problem-solving or completion of the project. Items related to the issue and sources of information help to understand the problem and offer solutions. Cognitive tools help the students to interpret and work on different aspects of the problem. These tools help students to agree on the meaning of the problem and social support systems help users for using constructivist learning environment (Fardanesh and Javdani). There is a tendency in educational models such as dynamic learning, constant learning and cooperative learning (Masnavi). Constructivism insists on the fact that learners understand the meanings of the world by building on their own experiences (Fardanesh and Sheikhi Fini).

Some of the reasons for the emergence of Misconceptions can be traced to the problems in the way of using certain words, phrases and terms. Especially when introducing the concepts of materials, the particles they are made of, and the chemical symbols used to represent them. In reference to the effect of chemistry textbooks on Misconceptions in the topic of chemical bonding, it is necessary to mention the concepts of the electronic domain for the created bonds, the electrons of the capacity layer of each atom, the method of creating a dative bond, the concept of, the concept of conventional charge, etc. Each of these concepts is not mentioned in the chemistry textbooks or only a brief reference is made to them (Azemat and Khodaei).

Ross also argues that the use of terms in everyday life causes students to have a distorted understanding, and therefore, one should be careful in choosing words (Ross).

2- Acknowledgments

The widespread use of scientific expressions and terms that have been removed in the new educational system has created doubts in the minds of students and disturbed the organization of their minds. Some of these concepts are essential for students to understand. The problem here is that some of the omitted concepts are prerequisites for some other topics. But in some cases, teachers should refrain from mentioning such terms.

The additional explanations in some textbooks lead students in an undesirable direction and make their minds more involved. In most cases, the student does not communicate with additional explanations. It is interesting to note that the student cannot distinguish essential explanations from basic explanations.

Many students are not aware of their Misconceptions. This issue makes them not take steps to resolve their Misconception and their problem becomes deeper. Therefore, these Misconceptions should be resolved by expert teachers. In this matter, an expert teacher is someone who, in addition to chemistry, also benefits from educational sciences and psychology.

Some of the strategies that make students' Misconceptions to be discovered and then resolved are:

1) Increasing students' activity with frequent teacher questions and answers
2) Not being satisfied with only theoretical explanations in practical or laboratory topics.
3) Correct, principled and timely performance of all kinds of evaluations
4) not limiting chemistry to a theoretical and abstract course

The suggestion of the author of this text is that in the programs of education teacher training courses, the courses of identifying common Misconception of students in chemistry education for teachers should be included.

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Facilitated Method for Production of Chitin and Chitosan from Shrimp Shells

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Abstract

Chitin and its valuable derivative chitosan are among the most important natural polymers in the world and offer a set of unique properties: biocompatibility, biodegradability for harmless products, non-toxicity, physiological inertness, antibacterial properties, chelation of heavy metals ions. gelling properties, considerable hydrophilicity and remarkable affinity for proteins. The main resources exploited are two marine crustaceans; shrimp and crab. In the present study, our aim is to produce chitin and chitosan and compare their properties with commercial properties. Due to the possibility of industrial and mass production, pre-purification methods were used to produce standard chitin and chitosan. In this regard, after the treatment of the shrimp, their shells were further cleaned and facilitated for the processes of demineralization, deproteinization, and deacetylation. Deproteinization was done with 1 M of sodium hydroxide and demineralized with 1%, 2%, 3%, 4% and 5% of hydrochloric acid, respectively. This method showed the high purity of chitin and chitosan with less than 1% protein residue along with high molecular weight and high crystallinity. The produced chitin and chitosan were characterized by FTIR and XRD analyses. It was found that the surface morphology of chitin increases with increasing acid concentration. According to our study, the best degree of deacetylation (%DD) was 88.76 %. This modified approach has the potential for large-scale production due to ease of operation and reduced environmental issues.

Key words: Chitin, Chitosan, Shrimp Byproducts, Deproteinization, Degree of Deacetylation

1. Introduction

Chitin is the second most ubiquitous natural polysaccharide after cellulose, which is made of a linear chain consisting of poly□(1-4) N-acetyl-D-glucosamine. It is usually found on the shells of the crustaceans such as shrimp, crabs and lobsters [1,2]. Chitosan, a deacetylated product of chitin, has received increasing interest for significant applications in food technology, agriculture, wastewater treatment, cosmetics, and the textile industry due to its non-toxic, biodegradable, biocompatible and antimicrobial properties. In general, the quality of chitin and chitosan prepared from shrimp shell is controlled by three main factors, the quality of the raw materials, the effectiveness of the applied procedures and the quality of water. The best chitin/chitosan is produced from fresh shrimp shells. Chitin can be extracted in several steps. Deproteinization and demineralization processes are very important. Studies show that deproteinization should be applied before demineralization to prevent excessive foam production during the process, and increase chitin purity. Deproteinization was performed using dilute sodium hydroxide (NaOH) between concentrations of 1 and 10%

[4,5]. Demineralization is performed by treating the shrimp shell powder with hydrochloric acid (HCl) at room temperature to dissolve calcium carbonate and subsequently, release carbon dioxide as shown in Eq. (1) [6]. The concentration of HCl used for demineralization is between 1-5%.

$$CaCO_3(s) + 2HCl(aq)\ CaCl_2(aq) + CO_2(g) + H_2O \quad (1)$$

The deacetylated structure of chitin, chitosan, is obtained by treating chitin with a high concentration of NaOH. Basically, chitosan is obtained by removing the acetyl groups (CH3-CO) from the linear group of acetylglucosamine in the structure of chitin [7]. Subsequently, the acetamide group (-NHCOCH3) in chitin is converted to an amino group (-NH2) as shown in Fig. 1. Removing the acetyl group makes chitosan soluble in most dilute acid solutions such as acetic acid, formic acid and lactic acid [8]. In this study, an easy process for the pretreatment of chitin and chitosan was proposed by simply washing and soaking the shrimp shell in diluted solutions of 7% HCl for 24 hours. The pretreatment procedure removes a disproportionately large amount of waste, both mineral waste and adherent protein.

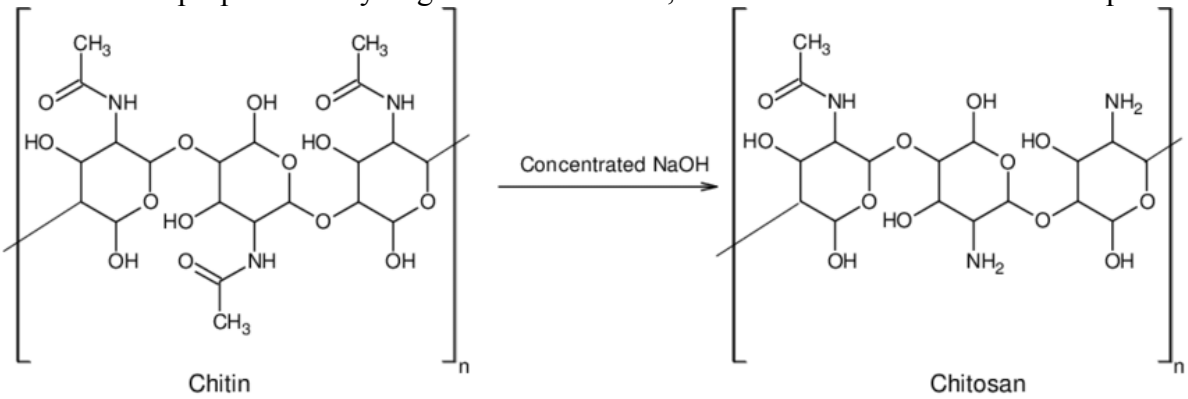

Fig. 1. Deacetylation reaction of chitin to chitosan

Deacetylation from amorphous to crystalline regions of chitin is done by removing the acetyl (C2H3O) group and forming an amino group (NH2). Deacetylation leads to copolymerization of N-acetylglucosamines and glucosamines. When the copolymer contains more than 50% N-acetylglucosamine units, it is generally called chitin. For chitin polymers, the percentage of N-acetyl-glucosamine units is called the degree of acetylation (DA) and can vary from 50% to 100%. On the other hand, when the resulting copolymer contains more than 50% glucosamine units, it is usually called chitosan. For chitosan polymers, the percentage of glucosamine units is termed the degree of deacetylation (DDA) and can range from 50% to 100%. DA and DDA are related so that DA+DDA=100%. The production of highly reactive amino groups in the chitosan polymer provides versatility in its use for food, textile, cosmetic, environmental, biomedical, and pharmaceutical applications [9].

2.Experimental

2.2. Extraction of Chitin and Chitosan

The shrimp shell was washed and dried in an oven at 50°C for two days before being ground into powder. Then, the powdered shrimp shell powder was stored in a sealed container before use. To preserve the properties of the shell, the shell powder has been stored in the freezer. Additionally, the deproteinization process, which removes protein and glucan, ensures that all the required properties are not lost [10].

2.2.1 Deproteinization and Demineralization

The shrimp shell powder weighed 50 g before alkaline treatment with 1 M sodium hydroxide with a solid - solvent ratio of 1:3 (w/v) for 20 h at ambient temperature (33 ± 2 °C). In the next step, the residue was washed and soaked in distilled water to neutralize it. After the pH of the powder reached neutral (pH= 7), the powder was filtered and dried in an oven at 60 °C before demineralization [10,11]. Fresh samples of powdered shrimps were directly soaked in different concentrations of HCl solution of 1%, 2%, 3%, 4% , and 5% in a substrate to solution ratio of 1:2 w/v at room temperature for 24 hours. Then, the residue was filtered, washed and soaked in distilled water until it reached neutral pH before being dried in an oven. The residue produced at this stage was known as chitin [12].

2.4. Preparation of Chitosan

To prepare chitosan, we used chitin, which was produced in the last step. Since increasing the concentration of HCl to 3% disperses chitin powder particles well, we used this sample to make chitosan. We soaked two grams of chitosan in 25 ml of 2 M sodium hydroxide solution to remove the acetyl group and subsequently produce chitosan. To mix chitin and sodium hydroxide as much as possible, we put it on styrene for 15 minutes. In the next step, we centrifuged the solution for 10 minutes at room temperature(37°C) at 7000 rpm to separate the alkaline phase. Next, we add 25 ml of 0.5 M sulfuric acid to the sample and then put the solution in the incubator for 16 hours. We centrifuged the solution 3 times for 10 minutes at 7000 rpm at room temperature. Then 2 M NaOH was added to the solution at a ratio of 30:1. In this step, we put the solution on styrene for 2 hours at 90°C. Acetic acid (10% wt.) was added to the solution. We put this solution in an incubator at 60 degrees Celsius for 6 hours. Next, to separate raw and pure chitin, we put the samples in a centrifuge at 6000 rpm for 15 minutes. Then, we raised the pH of the upper phase of the solution to 12 (pH= 12) using 4 M NaOH and measured it with a pH meter. Then we washed the sediments with distilled water, ethanol, and acetone. Finally, put the solution in a petri dish and put it in an oven at 150 °C for 24 hours to dry.

2.5. Characterization

2.5.1. Fourier Transform Infrared Spectroscopy (FTIR)

The chemical structure of produced chitin and chitosan was determined using the Nicolet iS50 FTIR model. FTIR spectra were recorded in the mid infrared (4000 cm^{-1} to 400 cm^{-1}) with a resolution of 2 cm^{-1}. The degree of deacetylation calculated based on Eq. (2):

$$DD = (A_{1650}/A_{3450})\tilde{A} \cdot 1.33 \times 100 \qquad (2)$$

Where the absorption band of A1658 and A3450 at 1658 and 3450 cm^{-1}, respectively [11].

2.5.2 X-Ray Powder Diffraction (XRD)

XRD analysis was used to evaluate the crystallinity of prepared chitosan. XRD measurements were performed on the powder samples using a D8 Advance Bruker apparatus with Cu radiation (40kV, 40mA, and 1,5418Å). Data were collected at a scan rate of 10°/min with a scan angle from 5 to 80 in a continuous pattern. The crystallinity index (CI) of the samples was calculated from the following Eq. (3):

$$CI = \frac{Ic}{Ic+Ia} \qquad (3)$$

where Ic is the diffraction intensity of the crystalline part and Ia is the amorphous part.

Results Discussion

3.1. Fourier-Transform Infrared Spectroscopy (FT-IR) Analysis

The FTIR spectra of chitin treated at different concentrations of HCl are shown in Fig. 2. Similar absorption bands were detected for all samples. The peaks at 1652 and 1620 cm^{-1} [13], correspond to the stretching band of the amide-I region, which indicates chitin extracted from the α-chitin isomorph of shrimp shell. Besides, the peak at 1550 cm^{-1} is assigned to the stretching vibration band of the amide II region. Meanwhile, both O-H and N-H stretching vibration were found at 3427 and 3257 cm^{-1} bands, respectively [14]. A peak at 1377 cm^{-1} was observed and identified as C-H bond [15].

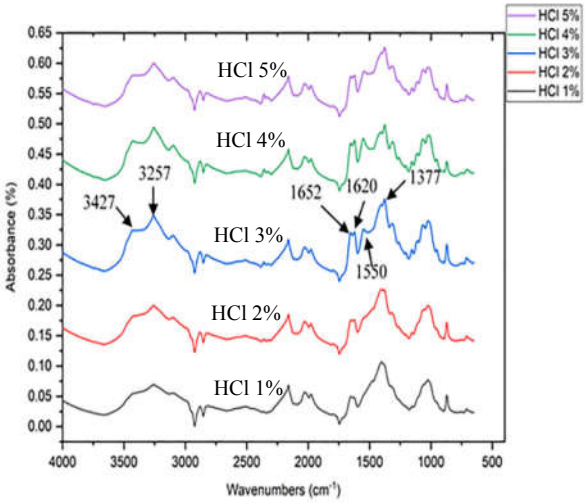

Fig. 2. FT-IR spectra of chitin treated with different concentration of HCl

FTIR measurements for chitosan were shown in Fig. 3. A broad peak at 3368 cm-1 was observed, which is identified as the (-NH) group of amines and hydrogen bonding [16]. A peak at 2940 corresponds to the symmetric stretching vibrations of -CH2 attributed to the pyranose ring [15,16]. The presence of a narrow peak at 1578 cm^{-1} indicates the –NH2 bending vibration of the amino group in chitosan. In addition, the free amino functional group (-NH2) in glucosamine was detected at 1047 cm-1 [14].

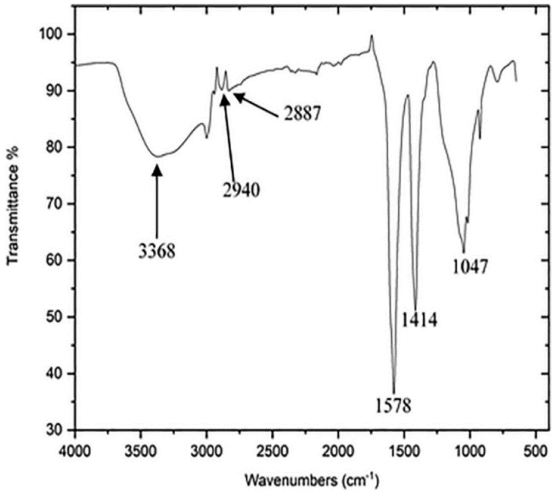

Fig. 3. FT-IR spectra of chitosan extracted from shrimp shell

3.2. X-Ray Powder Diffraction (XRD)

By comparing chitin and chitosan XRD patterns, a broad dispersion and lower intensity peaks were exhibited in chitosan The obtained XRD patterns are shown in Figure 4. The synthesized chitin showed two high peaks at 10.30° and 20.08°, and a series of several smaller peaks at

26.35°, 29.61°, and 35.88°, which is considered crystalline chitin [11,19]. For the prepared chitosan, two sharp peaks can be observed at 09.93° and 19.13°, which are considered the fingerprint of semicrystalline chitosan, in the same diffraction, three weak peaks are also observed at 23.33°, 26.38° and 39.28° [20]. In the same figure, it can be seen that commercial chitosan shows eight peaks, the peak found at 20.08° also corresponds to Crystal II in the most abundant chitosan structure; 45.87°, 56.85°, and 75.62° and 84.29° were reported for commercial chitosan at similar angles of 20°, 45°, 65° and 75° , respectively [8].

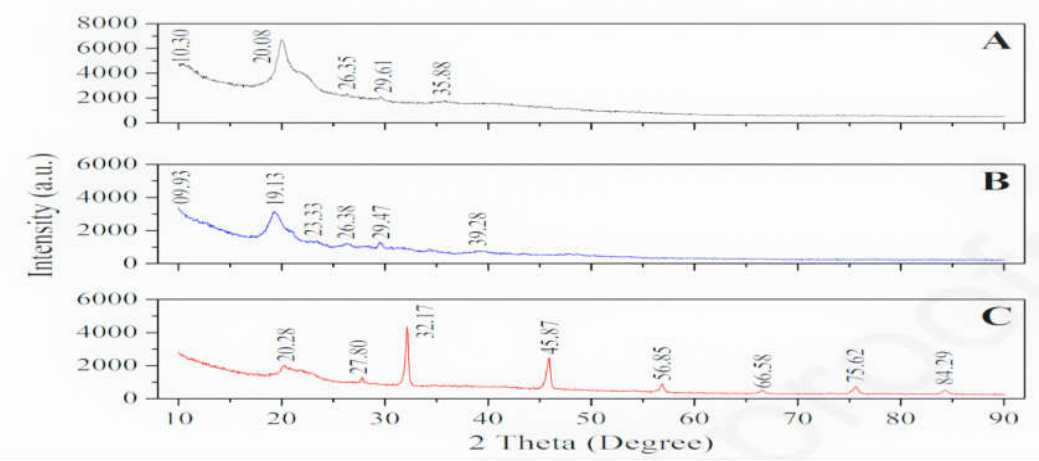

Fig.4. (A) Fabricated chitin and (B) Fabricated chitosan, and (C) Commercial chitosan

4. Conclusion

Extraction of chitin and chitosan from shrimp shell through chemical routes has been successfully carried out. To summarize, chitin extracted from virgin shrimp shell was identified as proven chitin from FTIR and XRD analysis. According to the XRD analysis, the synthesized chitin has two high peaks at 10.30° and 20.08°, which correspond to crystalline chitin. FTIR analysis shows several peaks related to O-H, N-H and C-H bands; besides, the peak at 1578 cm^{-1} indicates the $–NH_2$ bending vibration of the amino group in chitosan.

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The Effect of the Green Supply Chain Management on the Performance of Teaching Hospitals of Babol City based on the Balanced Scorecard

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Abstract

Today, one of the most important problems that people all over the world are facing is the lack of resource, ozone layer depletion, global warming, and environmental pollution. This issue resulted in the advancing of global organizations toward greening and the implementation of green supply chain management in organizations. Hospitals as one of these organizations by daily production of hazardous infectious waste have a great and undeniable role in environmental pollution are not considered as an exception. However, hospitals may encounter with this issue by applying and utilizing standards of green management, which can also help to energy saving, reduction of costs, and improvement of the process of taking care of the patient. The subject of the present research is practical regarding on matters mentioned in the hospital, and regarding its method is descriptive (survey) and correlative. To express this, two questionnaires (one of them is about green supply chain management and the other is about the performance of teaching hospitals of Babol city) were used to gather the data of this research. The reliability of questionnaires was calculated according to Cronbach's Alpha Coefficient (the questionnaire of green supply chain management equals to 0.835 and the questionnaire of the performance of teaching hospitals of Babol city equal to 0.831) also the validity of questionnaires was calculated based on content validity. Tables and figures were used to analyze the data, and regression test was use at the level of inferential statistics. The statistical population of this study includes the senior managers of hospitals that equal to 125 people, and 95 of them were selected as samples based on the Morgan Table. The results of the present research indicate that in the main hypothesis, green supply chain management does not have a significant effect over the performance of teaching hospitals of Babol city and in fact its Remarkable is weak, and also in the secondary hypotheses of the research, just teaching hospitals in the domain of green production (second secondary hypothesis) have shown a significant effect and the rest of the theories (green designing, green purchasing, green marketing, green packing, green transformation, and green recycling) does not have any significant effect on the performance of teaching hospitals of Babol.

Keywords: Green supply chain; Balanced Scorecard; Environment; Green Hospital

1- Introduction

During recent centuries, industrial development has replaced by sustainable development; environmental consequences and inadequacy of planet earth's resources have become the main concern for human. Industrial managers especially in developed countries, pursue methods to increase the performance of their organizations, along with Environmental Protection. In this regard, considering green supply is helpful. The green supply chain is a kind of supply chain in which environmental requirements are observe and products after their useful life return to chain supply, which its main goal is the reduction of environmental pollution from upstream to downstream of the supply chain. Nowadays, environmental pollution is the main problem that people is facing every day and also, emission of toxic gases

from manufacturing industries. To overcome the problem of pollution of the environment, manufacturing industries have to apply green concepts in their supply chain. Meanwhile, environmental concerns have converted into important factor in manufacturing industries (1). Nowadays, people are more alert about environmental issues like global warming, carbon emission, using toxic materials, and the lack of resources, and this is result in advancing toward greening and a lot of organizations by using their companies' (organization) green principals are trying to answer it, and there were also, environmental schedules that have been introduced by organizations voluntarily (5). Nowadays, environmental pollution is one of the main problems and if it is not being considered, has the potential to destroyed human generation on the planet earth. Air pollution is one these pollutions, which needs of urgent consideration. Hospitals are considered as the kind of organizations that by using Excessive amounts of wastes have created a disturbing situation for governments all over the world. The produced wastes by hospitals can be very hazardous and toxic and also, due to the presence of tiny harmful parasites can transfer infectious diseases via air. The pollutant from these wastes can cause undesirable effects like reduction of effects and environmental risk, and increase in ecological performance that can be effective on factors like the benefit and the goal of their portion from the market. And this indicates the integration of environmental thinking in the platform of chain management, including product design, sources of materials, the processes of production, and delivery of the final product and also managing wastes remained from the product after its useful life (4).

In this study, green supply chain management is mentioned and performance of hospital base on bsc, it is aimed to create awareness in healthcare managers in subjects of green approaches of supply chain management. In recent years, hospitality researchers and practitioners in the fields of revenue management, marketing, and hotel performance have sought to explore ways that can measure hotel performance and efficiency comprehensively and accurately for business improvement (42). One such method is a Balanced Scorecard (BSC). The BSC is more than a single measure of performance but instead considers key indicators that take into account both financial performance and key stakeholder feedback (43). A challenge of using BSC as an external benchmarking tool is the various ways data are collected and measured. To overcome this obstacle, using BSC in conjunction with a different type of methodology should be considered.

The contribution of this article is as follows.

Balanced Scorecard has been successfully implemented in hospital performance in general, and particularly in Green Supply Chain Management selection for hospital.

In general, green supply chain management for supervising reaction of environmental management schedules, is limited to more innovative methods like recycling, revival, reconstructing, environmental supplies and management, and also innovative combinations (6). Green supply chain management has converted into an important environmental action of companies to achieve profitability, and increasing market share in such a way that decreases environmental risks and increase environmental performance, which at last green supply chain management as a new environmental systematic approach that is accepted in supply chain management and applied by organizations for Providence. Given that, these environmental changes at the moment are impacting manufacturing and service actions, considering the development of environmental management and strategies for supply chain management have increased considerably. Therefore, the concept of green supply chain management as a new systematic approach has become a converted to an important factor in business. It can be truly claim that green supply chain management is consider as an environmental innovation (7). Organizations prevent themselves from ruining and destruction

through the determination of standards. By codification of standard, they are trying to establish order and harmony among their performance, they are also trying to concern about environment to improve their performance and also attract the satisfaction of customers that environmental management is in the domain of their performance of green supply chain management. Organizations can play an important role in preserving and maintenance of environment by applying standards for green management of their organizations. Dynamism and continuity of planet earth and humankind also require considering the environment. On the other hand, hospitals as one of the organizations, which produce wastes, continuously involved with environmental issues. Hospital wastes are considered as one of the main pathogenic agents that can be found all around the world, and if they are not effectively disposed, and remain on the ground, they will cause contamination of water, air pollution and overall damage to environment and impose a real threat and irrecoverable risks for the health of members of the society. However, the aim of this study is to evaluate the effect of green supply chain management over the function of teaching hospitals of Babol city based on the BSC method.

2- Research hypotheses

The main hypothesis;

- ✓ Green supply chain has a positive effect on the performance of Hospitals of Babol City.

Secondary hypothesis;

- ✓ Green design has a positive effect on the performance of Hospitals of Babol City.
- ✓ Green purchase has a positive effect on the performance of Hospitals of Babol City.
- ✓ Green Marketing has a positive effect on the performance of Iran Hospitals of Babol City.
- ✓ Green Production has a positive effect on the performance of Hospitals of Babol City.
- ✓ Green Packing has a positive effect on the performance of Hospitals of Babol City.
- ✓ Green Transportation has a positive effect on the performance Hospitals of Babol City.
- ✓ Green Recycling has a positive effect on the performance of Hospitals of Babol City.

3- Methodology

Regarding the type of the present research, it is apply, and regarding its methodology, it is considered as a descriptive (survey) and correlation type. To assess this subject matter, the data of this research is gather by two questionnaires (the questionnaire of green supply chain management and questionnaire of the performance of teaching hospitals of Babol city). The reliability of questionnaires was calculated according to Cronbach's Alpha Coefficient (the questionnaire of green supply chain management 0.785 and the questionnaire of the performance of teaching hospitals of Babol city 0.862) and also the validity of questionnaires was calculated according to the content validity. Indices like tables and figures were used to analyze the data, and regression test was used at the level of inferential statistics. The statistical population of this study includes the managers of hospitals that equal to 125 people, and 95 have been chosen as samples based on the Morgan Table.

4- Findings

In analyzing data, firstly for determining the normality of variables of test material, Kolmogorov– Smirnov Test (K – S) was used, which obtained results have verified the normality of variables (performance of hospital is equal to 0.07).In the followings, the outcome results of the regression test for the main hypothesis of the research which has been based on the effect of green supply chain management over the function of teaching hospitals of Babol city at the level $\alpha = 0/05$ of showed that it is not significant.

Table 1- The results of regression test of research's main hypothesis

Model	Non-Standard Correlation Coefficient		Standard Correlation Coefficient	t	Significance (Sig.)
	B	Standard Deviation	Beta		
(Constant)	3.44	0.52		7.19	0.000
GSCM	0.12	0.15	0.08	0.079	0.35

And also the obtained results of regression test for secondary hypotheses illustrated in the following table, which by considering outcome results of the below table at the level $\alpha = 0/05$ of just the green manufacturing variable is significant and the rest of the other variables are not significant in the study teaching hospitals.

Table 2- The results of regression test of research's secondary hypotheses

Model	Non-Standard Correlation Coefficient		Standard Correlation Coefficient	t	Significance (Sig.)
	B	Standard Deviation	Beta		
(Constant)	4.1142	0.863		4.432	0.000
Green design	0.012	0.155	0.020	0.067	0.869
Green manufacturing	0.287	0.123	0.321	2.215	0.035
Green purchase	-0.209	0.163	-0.201	-1.279	0.201
Green marketing	0.058	0.185	0.051	0.316	0.652
Green packing	-0.079	0.141	-0.114	-0.583	0.545
Green transporting	0.055	0.201	0.065	0.310	0.632
Green recycling	0.034	0.242	0.045	0.151	0.780

The results of present research indicate the fact that green supply chain does not have any significant effect over the function of teaching hospitals of Babol city, and just related teaching hospitals in the field of green manufacturing have applied a good trend toward compatibility with the environment.

5- Discussion and Conclusion

The results of present research indicate the fact that green supply chain does not have any significant effect over the function of teaching hospitals of Babol city, and just related teaching hospitals in the field of green manufacturing have applied a good trend toward compatibility with the environment.

In this regard, it is proposed that teaching hospitals in the field of green design can operate by environmental criteria like designing products to reduce the use of material. Energy, designing products for further usage, and designing products in order to prevent or decrease the usage of hazardous products; and in the area of green purchase hospitals can turn to purchase materials that are nontoxic and recyclable, materials that have a specific environmental capability like materials that have environmental label with them, and materials that have necessary standards regarding technical and environmental properties, and also materials that leave the less damage to the environment. In the area of green marketing, hospitals can do their marketing about environmental issues like emphasizing on having environmental certifications, considering a reward for those customers, who follow environmental policies, and establishing information system and marketing researches in teaching hospitals in line with assessing the needs of demands of customers. Regarding green

packing, hospitals can consider criteria such as is useful and safe for individuals and society in its entire life cycle, being made using best of the methods and technologies of clean manufacturing, its physical design for optimization of materials and energy, and is created from safe materials. In the area of green transporting, from among the proceedings compatible with the environmental criteria which teaching hospitals can do we can mention the followings, planning in all of the distribution centers, optimization of delivery paths, improvement of joint delivery, and increasing the speed of loading, which these proceedings can underlie green transportation in the mentioned teaching hospitals.

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Co-doped zinc oxide nanoparticles as eco-friendly green ceramic pigment

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Abstract

In this study, Co-doped zinc oxide nanoparticles were synthesized by combustion method and Glycine as fuel. As-prepared powders were characterized by XRD, SEM, TEM and spectrophotometer. Color properties of the synthesized samples have been investigated using the CIELAB coordinates and spectral reflectance. XRD patterns indicated that combustion reaction by glycine as fuel caused the formation of pure ZnO phase. SEM pictures showed the formation of porous spongy morphology which is related to large amount of exhaust gases from combustion reaction. The TEM images of Zn-CoO powders shows particle size of about 40 nm Colorimetric results also indicated a broad reflectance band around 540 nm (green region) for the as-prepared powders.

Key words: Chemical synthesis, Nanostructured materials, Zn-CoO, Color properties

1. Introduction

Green ceramic pigments based on chromium oxides are widely used in industry. However, Chromium oxide green can cause irritation of the skin and eyes and can cause nausea and other problems if ingested. It also can cause respiratory problems when dust is inhaled.
The health effects of chromium are primarily related to the valence state of the metal at the time of exposure. Trivalent (Cr[III]) and hexavalent (Cr[VI]) compounds are thought to be the most biologically significant. Cr(III) is an essential dietary mineral in low doses. Cr(VI) compounds are carcinogenic. Cr(VI) is generally considered 1,000 times more toxic than Cr(III) [1].
The green colored oxides of Zn-CoO system, with low Co content, have demonstrated to be similar in their physico-chemical properties with Cr(III) green pigments [2, 3].
In this work, a single step microwave combustion method was used to synthesize nano-crystalline Zn-CoO. Nano-crystalline ZnO solid solution was prepared by applying glycine as fuel under microwave radiation.

2. Experimental

Raw materials were of Analytical Grade. $Zn(NO_3)_2.6H_2O$ (Merck), $Co(NO_3)_2\ 6H_2O$ (Merck) as an oxidant and Glycine as a fuel and complexing agent were used.
Metal nitrates and glycine were dissolved in distilled water to form a solution in which Zn^{2+} and Co^{2+} concentration meet the formula of $Zn_{0.9}\ Co_{0.1}\ O$. The obtained homogeneous solutions were slowly evaporated in a water bath to form a viscous gel. Finally, the solutions were transferred into microwave oven (Samsung, Korea, 900 W, 2.45 GHz) to perform the

combustion reaction. It is noteworthy to mention that all experiments were performed in maximum power of microwave for 50 seconds.
A D-500 (Siemens, Karlsruhe, Germany) diffractometer was used for XRD analysis. The morphology of the synthesized powders was analyzed using LEO 1455VP (Oxford, UK) Scanning Electron Microscope (SEM). Ultraviolet radiation spectroscopy in the visible–ultraviolet light region was conducted with a Color Eye 7000 A spectrometer in the range between 300 and 700 nm for determination of diffuse reflectance of the obtained powders as pigments.

3. Results and Discussion

Figure 1 shows the XRD patterns of the Zn-CoO synthesized powders using different F/O ratios. The main phase characterized in different samples was hexagonal ZnO with P63mc structure (JCPDS 5-664). Moreover, the extremely broad peaks about 32 and 36° indicate nano-crystalline nature of the ZnO phase.

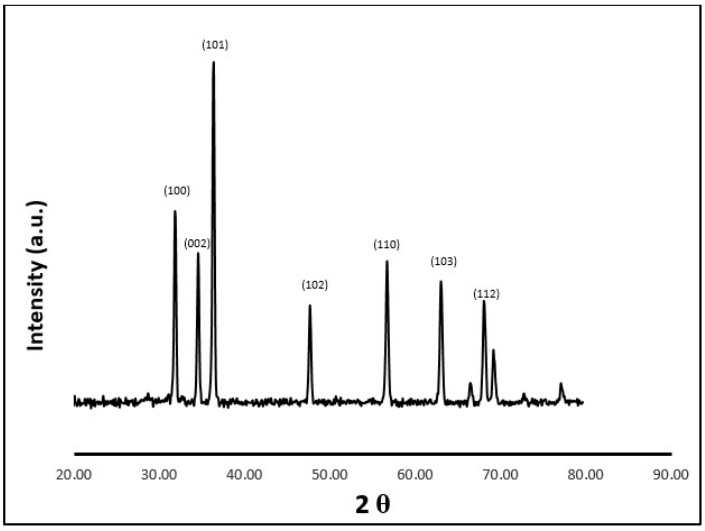

Figure 1: XRD pattern of Zn-CoO prepared by combustion synthesis

From Scherre equation the average crystallite size was calculated to 37 nm.
Figure 2 shows the SEM images of Zn-CoO powders obtained by combustion synthesis and glycine as fuel.
Wu *et.al* illustrated that by using glycine as fuel, particles with good crystallinity can be obtained [4].

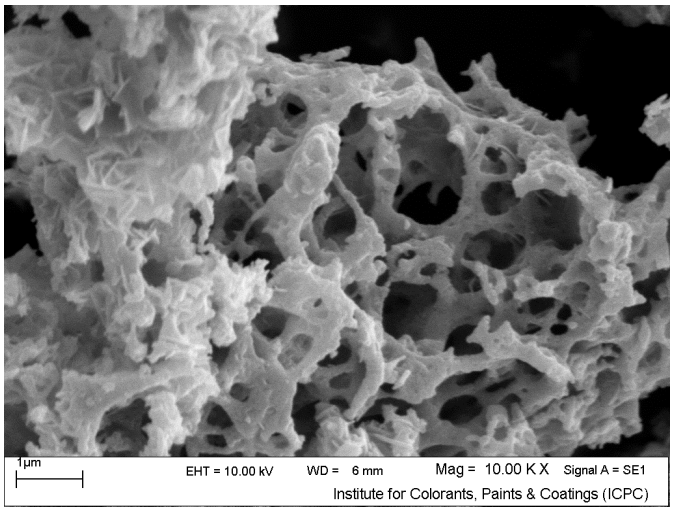

Figure 2: SEM micrographs of Zn-CoO samples prepared by combustion synthesis

As shown in figure 2 powder consists of porous spongy morphology is related to large amount of exhaust gases from combustion reaction [5]. The TEM images of Zn-CoO powders is shown in Figure 3.

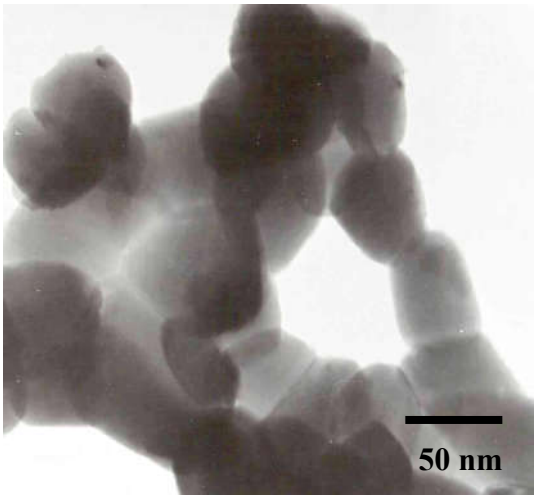

Figure: TEM micrographs of Zn-CoO samples prepared by combustion synthesis

Results show that the particle size is about 40 nm which is in accordance with the calculation from Scherre equation.
Color properties of the synthesized samples in the presence of glycine as fuel have been investigated using the CIELAB coordinates and spectral reflectance. Figure 4 shows the diffuse reflectance spectra of the as-synthesized powders.

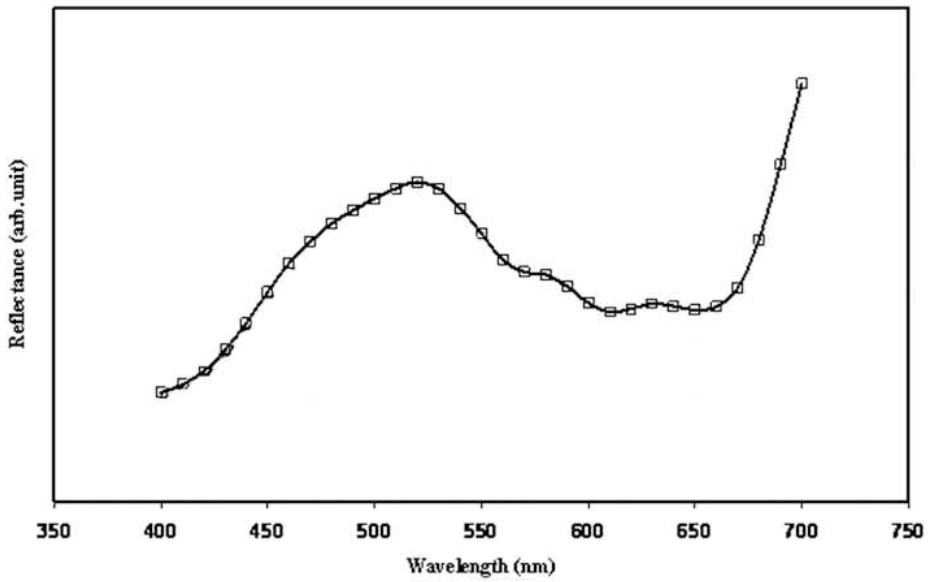

Figure 4: Diffuse reflectance spectra of the synthesized pigments

A broad reflection band around 530 nm can be observed for powder prepared by combustion synthesis being a characteristic peak for green region.

Conclusions:

Nano-crystalline Zn-CoO green pigments of crystallite size of 37 nm have been synthesized by combustion synthesis and glycine as fuel under microwave irradiation. Color properties investigation has indicated that the sample has good chromaticity and and saturated green color.

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Photodegradation of Reactive Red 222 using TiO_2 nanostructured thin films

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Abstract

In this paper, TiO_2 nanostructured thin films were prepared on glass plates by sol-gel method. TiO_2 films were characterized by X-ray diffraction (XRD) and scanning electron microscopy (SEM). The photocatalytic performance of the obtained films was investigated by the photodegradation measurements of Reactive Red 222 (RR222) in a batch reactor. Kinetic study results of this reaction represented that it obeys of was pseudo-first-order model. The degradation of RR22 was enhanced by the addition of optimum amount of hydrogen peroxide. This is due to the increasing amount of the radical hydroxyls.

Keywords: *TiO_2 thin film, Photocatalyst, RR222.*

1. Introduction

TiO_2, (in the anatase form) appears to be the most photoactive and the most practical form for widespread environmental applications such as water purification, wastewater treatment and air pollution control. These catalysts have demonstrated their efficiency in demolishing a wide variety of dyes wastewater. TiO_2 is believed to be the most beneficial species owing to its excellent photocatalytic activity, high stability and non-toxicity [1-3]. One of the upgrowing treatment technologies for the remediation of textile industry wastewater is advanced oxidation process (AOP). This process involves the generation of highly active hydroxyl radicals in order to complete mineralization the water contaminants. AOP process is consisted of the combination of oxidants and irradiation. Heterogeneous photocatalysis process by employing semiconductor and light source has been considering a promising process which leading to total destruction of nonbiodegradable pollutants [4-6].

In this paper, sol-gel method using peroxotitanic acid sol (PTA) was employed for the preparation of nanostructured TiO_2 thin film on the glass plate. The PTA is neutral and stable solution which can be easily coated onto vast variety of materials even pure metals [7].

The performance of the immobilized photocatalytic reactor was tested for the degradation of RR222 dye in aqueous steam.

2. Experimental

2.1 Preparation of nanostructured TiO_2 thin films

In the preparation of TiO_2 sol, Titanium tetra iso-propoxide ($Ti(OC_3H_7)_4$, Merck) Iso-Propanol (Merck) were mixed and then hydrolyzed with certain amount of deionized water.

After washing the precipitation to remove excess alcohol, the precipitate was dissolved in aqueous hydrogen peroxide (30%, Merck) to obtain a transparent sol of titanium peroxo complex. During dissolution certain content of distilled water was added to this solution to avoid immediate dense gel formation. Then Poly Ethylene Glycol (PEG, Merck, M_w=4000) solution was added. This titanium peroxide sol was allowed to stand 4-5 hours to form a viscous sol.

Before deposition of thin film, two glass plates (4.5× 39.5×0.2Cm) were cleaned and dried. For deposition of nanostructured TiO_2 thin films, the glass plates were dipped in the viscous titanium peroxo complex with a rate of 1mms^{-1} and pulled out with a same rate. A thin film were formed then were dried at 100^{o}C for 1 hour in electric oven. These films were calcined for 2 hours at 500°C. For three layers thin films, this deposition was repeated for 3 cycles.

A PW 1800 (philips, Germany) Diffractometer was used for X-ray diffraction (XRD) analysis. Morphology and size analysis was performed using LEO 1455VP (Oxford, UK) Scanning Electron Microscope (SEM).

The photocatalytic activity of the TiO_2 thin films was measured in a batch rectangular reactor with the capacity of 2 liter and a UV lamp (15 watt, Philips) which has been located in the center of the reactor. RR222 (RR222) was purchased from Indofix Company (Indian).

3. Results and Discussion

3.1 TiO_2 film characterization

Figure 1 shows the XRD patterns of TiO_2 and films. According to JCPDS (01-083-2243), anatase phase, were observed in the XRD patterns of the TiO_2 films. From XRD pattern, high amounts of anatase crystalline were obtained. This may be attributed to the high content of titanium dioxide with several coating cycles resulting in the obvious peak of anatase [8].

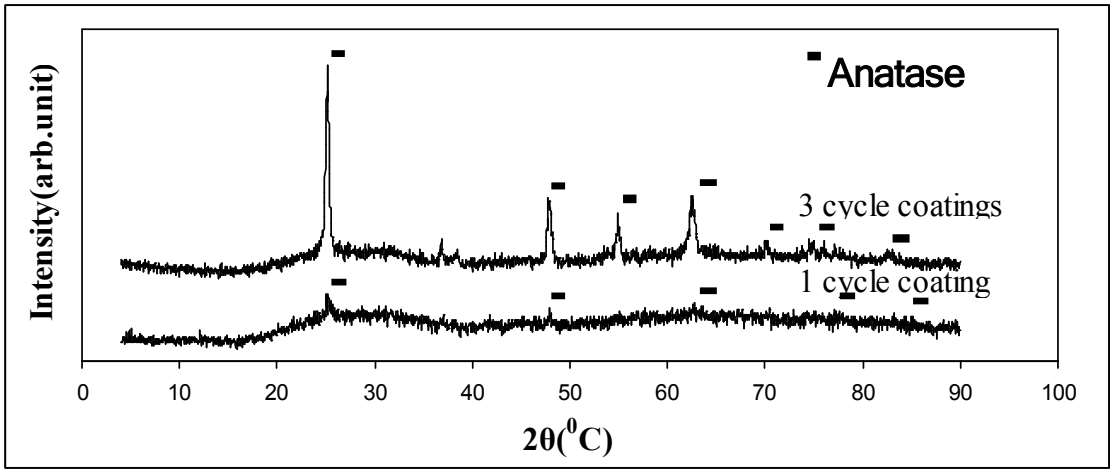

Figure 1: XRD patterns of 1 and 3 cycles coating of TiO_2 films

Figure 2 shows the SEM images of TiO_2 thin films which were prepared under different conditions, 1 dip coating without using PEG and 3 dip coating by the utilization of PEG respectively. The results show that in the absence of PEG the surface of TiO_2 thin films becomes non-uniform and cracks is created at many places, whereas the film prepared by the addition of PEG has a uniform and without cracks surface.

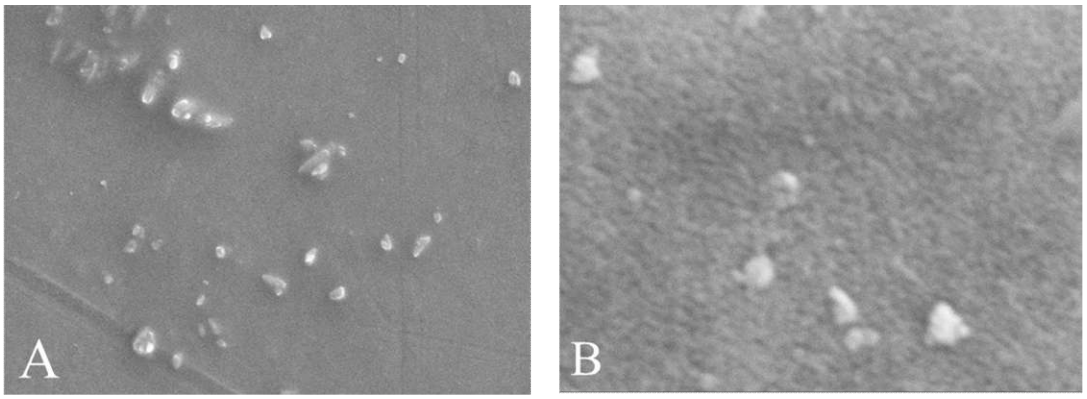

Figure 2: SEM images of the TiO_2 thin film (A) 1 cycle coating without using PEG, (B) 3 cycle coating with using PEG

3.2 photocatalytic Activity of thin films

The TiO_2 thin films were put in the reactor. The degradation of RR222 under UV light irradiation was determined by measuring absorption spectra using a Lambda25 UV-vis spectrophotometer. The degradation efficiency of TiO_2 thin films were calculated by the equation 1.

$$d(\%) = \frac{[dye]_o - [dye]}{[dye]_o} \times 100 \qquad (1)$$

Where, $[dye]_o$ and [dye] are the concentration of RR222 before and after degradation, respectively.

Figure 3 shows the photocatalytical activity of TiO_2 thin films by measuring the photodegradation efficiency of RR222 in 120 min. In the presence of TiO_2 thin film under UV irradiation 35% of RR222 was degraded, where TiO_2 film without the presence of UV irradiation showed only 14% of dye destruction. The RR222 degradation efficiency measurement was also performed under UV irradiation in the absence of TiO_2 thin film, the result was negligible (1%). The results are in well agreement with the presence of UV irradiation and TiO_2 thin films in achieving the efficient photodegradation content of RR222.

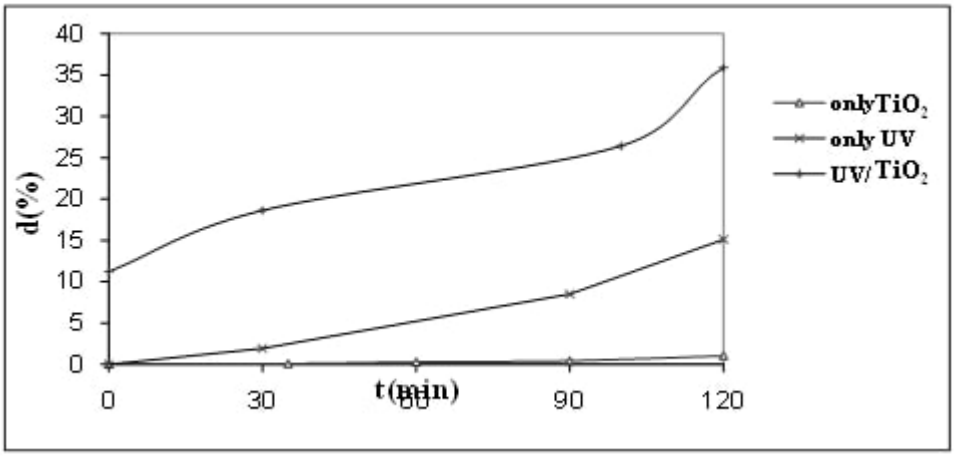

Figure 3: Effect of TiO_2 film and UV light on degradation of RR222. $[dye]_o$=20ppm, pH=8

3.3 RR222 Photodegradation Kinetics

Generally, the photodegradation rate of chemical compounds on semiconductor surfaces follows the Langmuir-Hinshelwood model.

$$r = -\frac{dC}{dt} = k_r \theta_x = \frac{k_r KC}{1 + KC} \quad (2)$$

The photocatalytic reaction rate (r) is proportional to the fraction of surface coverage by the organic substrate (θ_x), k_r is the reaction rate constant, C is the concentration of dye and K is the Langmuir adsorption constant: When the RR222 concentration is low, an apparent first-order rate constant could be expressed where $K'(min^{-1})=k_rK$ [9]:

$$-ln(\frac{C}{C_0}) = k_r Kt = K't \quad (3)$$

According to Figure 4 the plot of $-Ln (C/C_0)$ versus time for RR222 is linear. Therefore, the photodegradation reaction follows pseudo-first-order and the apparent reaction rate constant was $0.0043 min^{-1}$.

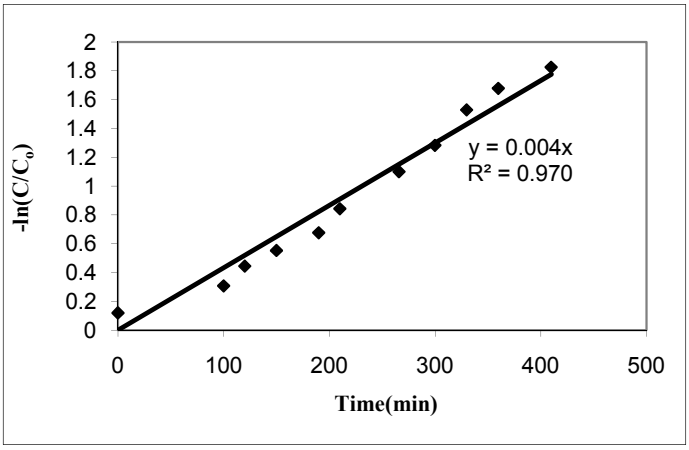

Figure 4: The kinetic data for photocatalytic degradation of RR222 in the presence of TiO_2 thin film.

4. Conclusions

This study is focused on the synthesis of TiO_2 thin film photocatalyst using sol-gel technique. on glass plates was done by using dip coating. SEM results revealed the uniform and without any cracks surface of TiO_2 thin film attached on the glass plate after 3 cycles coating and applying PEG as a surfactant. XRD analysis showed the presence of anatase crystalline phase of TiO_2 after calcination process at temperature of 500°C.

The results of RR222 degradation showed that both UV light and TiO_2 film were needed to reach to an acceptable degradation rate. The RR222 photodecomposition kinetic measurements represented that TiO_2 thin film photocatalytic activity of RR222 obeys pseudo-first-order kinetic model.

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Extraction of silk fibroin protein from *Bombyx Mori* cocoon by an optimized solvent system

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Abstract

Silk fibroin protein (SFP) has become an attractive option for biomedical applications due to its low cost, controllable biodegradability facilitates, unique mechanical properties, and high abundance. For all these applications; it needs to be extracted from its sources. In this study, we used an optimized solvent system to extract SFP from *Bombyx mori* silk cocoons, and then the optical properties and quality of the extracted protein were evaluated by UV-Vis and SDS-PAGE analysis. Based on the results obtained, the presence of a maximum absorption peak in the range of 273 nm confirmed the presence of SFP. Also, by analyzing the prominent bands formed on the SDS-PAGE polyacrylamide gel and comparing it with the protein ladder, the presence of SFP in the solution was confirmed. These results can indicate the success of the method used in the solubilization and regeneration of SFP in the present study.

Key words: Silk fibroin protein, Bombyx mori, Optimized solvent, Solubilization, SDS-PAGE

1. Introduction

Silk fibroin protein (SFP) is one of the most popular natural polymers, which is used to make biomass due to its low-cost, great biocompatibility, outstanding mechanical qualities, and high abundance (1). The silk produced by the *Bombyx mori* (*B. mori*) is composed of SFP and a glue-like glycoprotein called sericin, which in total includes about 25 to 30% of the weight of the silk cocoon (2). The SFP is a protein with 5507 amino acids, which is mainly composed of glycine (43%), alanine (30%) and serine (12%) (3). In general, this protein includes heavy-chain fibroin (350 kDa), light-chain fibroin (25 kDa), and P25 protein. The heavy chain is hydrophobic and responsible for the formation of β-sheets, the light chain is hydrophilic, which is connected to the heavy chain by a disulfide bond, and the P25 protein also causes integration in this complex (4). Sericin also has an amorphous and adhesive structure that is responsible for connecting two fibroin chains to each other (5). One of the main advantages of SFP is loading delicate drugs and biomolecules with therapeutic properties using a mild aqueous technique that provides great resistance to enzymatic degradation. Due to the unique characteristics of SFP, it can be used to design drug delivery systems such as biofilms, hydrogels, scaffolds, nanoparticles, and nanocapsules (6). Unfortunately, the presence of sericin significantly reduces the biocompatibility of SFP and limits many of its applications. Studies have shown that sericin is an allergic substance that can cause problems in the body. Therefore, a process is required to separate the sericin from the fibroin (7). Since sericin is soluble in water, it is easily separated from fibroin by using boiling water or using sodium carbonate, this process is called

degumming (8). The properties of regenerated fibroin are largely influenced by the regeneration process and the extent of sericin removal (9). However, SFP is mostly insoluble in water or weak alkaline solvents (2). The SFP dissolution is itself a complex and sometimes difficult process, depending on the source of the cocoon.

Up to now, several solvent systems like LiBr, NaOH, and $CaCl_2$/ethanol/water have been used for solubilization of SFP. However, studies have shown that choosing a suitable solvent system can greatly increase the quality of regenerated SFP. In this study, an optimized solvent system was used to extract SFP from *B. mori* cocoons, and then the quality of extracted protein was evaluated.

2. Materials and Methods

2-1. Materials

B. mori cocoons were procured from the Iran Silk Research Center. Dialysis membrane (cut-off 12 kDa), Coomassie Brilliant Blue R-250 and G-250 were purchased from Sigma-Aldrich, USA. Lithium bromide (LiBr), tetramethylethylenediamine (TEMED) and sodium dodecyl sulfate (SDS) were acquired from Merck, Germany. Formic acid, ethanol, and sodium carbonate (Na_2CO_3) were purchased from Scharlau, Spain. All reagents used were of analytical grade.

2-2. Isolation of SFP

For this purpose, about 3 g of *B. mori* cocoons were boiled for 20 min in 0.02 M Na_2CO_3 solution and then washed several times with deionized water to remove the sericin. After degumming, a mixed solvent system consisting of 2 mL formic acid and 9.3 M LiBr was prepared and silk fibers were dissolved in this optimal solvent at 60 $^{\circ}$C for 4 h. The resulting SFP solution was dialyzed using a dialysis bag (cut-off 12 kDa) for 3 days and then was stored at 4 $^{\circ}$C for further analysis.

2-3. Measurement of total protein concentration by Bradford assay

Briefly, about 100 µL of SFP solution was added to 5 mL of Bradford's reagent (containing 100 mg of Coomassie Brilliant Blue G-250, 50 mL of 95% ethanol, and 100 mL of 85% phosphoric acid in a final volume of 1 L) and after 5 min, its absorbance was read by a UV–Vis spectrophotometer at a wavelength of 595 nm. Bovine serum albumin protein (BSA) with different concentrations of 2 to 40 µg/µL was used as the standard protein and based on the standard curve, the concentration of SFP was measured (10).

2-4. UV–Vis spectroscopy

UV–visible spectra of SFP were investigated using a UV-Vis spectrophotometer (Thermo Biomate, USA) in a range of 200-800 nm.

2-5. Determination of protein molecular weights

The molecular weight of regenerated SFP was determined by SDS-PAGE according to the method reported by Laemmli (11). Briefly, a polyacrylamide gel was prepared with a 5% stacking gel and a 10% resolving gel. Then, 20 µL of SFP solution was loaded onto a polyacrylamide gel and electrophoresed at a constant current of 40 mA for 1.5 h. The molecular weight of SFP was estimated using a protein ladder (Thermo scientific prestained protein ladder) as the marker.

2-6. Statistical analysis

All the tests were performed in triplicates (n=3), and the experimental data were reported as means ± standard deviation (SD). The data analyses were performed using Image j and Origin 2019 software.

3. Results

3.1 Measurement of total protein concentration

The total protein concentration of SFP was evaluated by Bradford assay. Based on the standard curve obtained from different BSA concentrations (figure 1), the total protein concentration of the sample was determined to be 4.75 µg/µL.

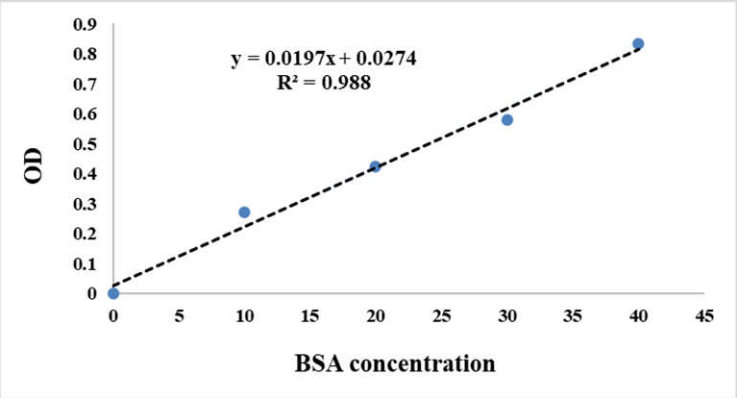

Figure 1: Standard curve of BSA

3-2. UV–Vis analysis

The optical properties of the protein were investigated by UV-Vis spectroscopy. As shown in Figure 2, the presence of a maximum absorption peak in the range of 273 nm confirmed the presence of SFP.

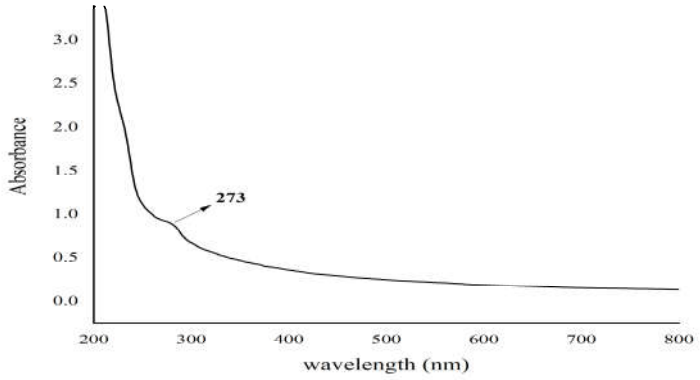

Figure 2: UV–Vis spectra of SFP

3-3. SDS-PAGE analysis

In order to measure the quality of extracted protein, the SFP solution obtained was analyzed on SDS-PAGE polyacrylamide gel. In this method, proteins are separated based on the difference in their molecular weight. As shown in Figure 3, three prominent bands with molecular weights of about 25, 30, and more than 200 kDa were detected, which correspond to light chain fibroin, p25 and heavy chain fibroin, respectively.

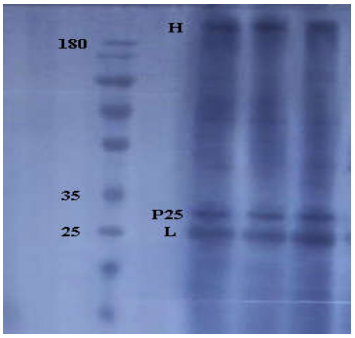

Figure 3: SDS-PAGE of SFP (H; heavy chain, L; light chain)

4. Discussion

In the present study, an optimized solvent system was used to extract SFP. As a renewable biopolymer, this protein has found potential applications in medical and therapeutic purposes (12). Briefly, after cleaning, degumming, dissolution and purification, the *B. mori* silk cocoons were converted into an aqueous solution of silk, which contained significant amounts of total protein. The results of UV-Vis analysis showed a prominent peak in the range of 273 nm. This can be due to the presence of tyrosine amino acid in the protein structure (13). This finding was in full agreement with the results described by Passi et al. (2020) (14). The SFP is a semi-crystalline protein surrounded by several layers of sericin. The outermost layer of sericin (sericin A) is easily soluble in water, while the innermost layer (sericin C) is located near fibroin and is difficult to dissolve in boiling water. A suitable solution to facilitate the degumming process is to use a common alkaline solution such as Na_2CO_3 (8). But its uncontrolled use can change the molecular structure of SFP and reduces its mechanical properties (15). Therefore, after preparing the aqueous solution of SFP, a process is necessary to check the quality of the extracted protein. SDS-PAGE analysis is one of the most basic and common experimental methods for analyzing molecular weights of protein subunits (11). However, the results of SFP electrophoresis have often been unsatisfactory. Zhang et al. (2018) considered the main reason for this to be the high molecular weight of fibroin. In addition, the method of preparing liquid SFP from silk fibers and especially the type of solvent used can be significant effective on the range of molecular mass of the regenerated SFP (16). Aznar et al. (2013) stated in a comparative study that the aqueous solubility of SFP in LiBr solvent is higher than $CaCl_2$/ethanol/ H_2O solvent (17). Kundu et al. (2014) reported that the age of silk cocoons can also affect their solubility (9). Due to the instability in the aqueous solution, the regenerated SFP easily coagulates and changes color, which has an adverse effect on the results of electrophoresis (16).

In this study, an easy and practical solution was used to solve this problem. Creating a good SDS-PAGE profile for SFP can be achieved by rapid loading of the samples as well as reducing the time and temperature when mixing the sample with the dye. The results of this test clearly showed bands within a certain range. By analyzing the created bands and comparing them with the protein ladder, the presence of SFP in the solution was confirmed. Also, the clarity of the bands indicated the quality of the extracted protein. On the other hand, the absence of bands with unknown molecular weight can indicate the purity of the SFP solution (18). These results can indicate the success of the method used in the solubilization and regeneration of SFP in the present study.

5. Conclusions

In summary, this study was based on the efficient extraction of SFP from *B. mori* silk cocoons using an optimized solvent system. UV–Vis spectroscopy confirmed the presence of SFP in the obtained aqueous solution. Next, the efficiency of the used solvent and the quality of the extracted protein were evaluated by the SDS-PAGE method. The presence of clear bands with certain molecular weights indicated the successful degumming, solubilization, and regeneration of SFP. These findings can provide a promising method to improve the extraction of SFP for various applications such as bio-integrated devices, drug delivery and tissue engineering scaffolds.

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Isolation and Characterization of *Kocuria rhizophila* as emerging opportunist pathogen in rainbow trout (*Oncorhynchus mykiss*)

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Abstract

Rainbow trout as an important commercial fish has been exposed to a phylogenetically diverse group of bacterial pathogens. A farm in Amol reported heavy mortalities of rainbow trout (*Onchorhinchus mykiss*). The current study aimed to identify the etiological agent responsible for that. The moribund and freshly dead fish were analyzed for clinical changes. Biochemical and molecular characterizations were performed to identify the etiological agents of the disease. The results of the biochemical tests and Polymerase chain reaction (PCR) assay confirmed that *Kocuria rhizophila* was responsible for the disease severity. These findings indicate that the disease outbreak in rainbow trout farm occurred as a result of *Kocuria rhizophila*. Proper understandings about the new etiological agents and the relationship of stress-related environmental component are necessary for effective management and control of diseases.

Keyword: *Iran, Kocuria rhizophila,* Rainbow Trout, Emerging Pathogen

1. Introduction

Keeping up with the global development of the aquaculture industry, fish bacterial infections have become a serious problem in fish farming throughout the world. To increase the aquaculture production, traditionally extensive fish farms have been changed to intensive ones and this intensification caused stress, which reduces the fish immunity and enhances their susceptibility to pathogenic bacteria. A disease outbreak can cause high mortality in fish that decreases their production, causing high economic loss to the fish farmers. In addition, sub-lethal diseases may affect growth rate and flesh quality and can cause undesirable visual changes. Kocuria is a Gram-positive bacteria in the family Micrococcaceae. This bacterium was first identified and described by Miroslav Kosur, a Slovakian microbiologist. Kocuria species are are commonly found in soil and water and usually considered as non-pathogenic bacteria which inhabit the normal skin and mucous membrane of human and animals (1). *Kocuria* was also isolated from various environmental and ecological niches (2).

Many of the bacterial species present in the aquatic habitat are vital to the natural aquatic environment, with no direct effect in inducing fish disease. In the world, there are various species of different families of bacteria that have been related to fish infections. Most of the bacterial pathogens responsible for induction of diseases in fish are Gram negative rods, but some are 'Gram-Positive' rods, while a few are acid-fast rods in the aquatic ecosystem.

Bacterial populations in the environment affect not only the health status of fish stocks, but also increase public health concerns because fish and their products are the potent reservoirs of human infectious bacteria. Several bacterial species have been transmitted from fish to humans by eating raw or poorly cooked food or handling the affected fish. Human infections mainly depend on the time of year, contact of patient with fish and the environment, diet and the immunity of the exposed person.

Control of infectious diseases in aquaculture is more complex than that of animal diseases of terrestrial environment due to difficulty in fish observation because fish are not close enough like terrestrial animals. The aquatic environment can favor quick disease transmission, fish catching can cause stress and disease in the fish is often difficult to identify and diagnose. There are some knowledge about the characteristics, geographical distribution, host range, and epizootiology of common fish pathogenic bacteria, but new etiological agents are being identified every year. The most common fish bacterial species that can cause diseases belong to the genera *Vibrio, Flavobacterium, Aeromonas, Yersinia, Edwardsiella, lactococcus, Streptococcus*, *Renibacterium* and *Mycobacterium*. There are flourishing indications that different infective bacterial species have broad geographic distribution and host range, causing the emergence of new bacterial pathogens. At last, the conventional and modern disease prevention methods and their control strategies are also addressed. Hence, basic knowledge of pathogen profiles and diseases, in addition to their fundamental economic background of the operational costs, is a primary requisite in the designing of strategies to control most common bacterial diseases. It is strongly recommended that all the possible limitations in control methods must be addressed critically before employing in the aquaculture sectors. Comparative pathogenomics provide important information that how similar bacterial species show different virulence, adapted to various ecological niches and new host species. The determination of main virulence factors in disease-causing strains can assist us to plan effective therapeutic and vaccines strategies to control fish diseases (3).

2. Material and methods

2-1- Sampling and bacteriological study

The affected fish demonstrate symptoms such as anorexia, melanosis, lethargy, erratic swimming, exophthalmia, and mucosal hemorrhage. Samples were collected from 20 moribund fish (100-200 gram) suspected to bacterial septicemia of a rainbow trout farm located in Amol of Iran during summer 2022. Fish kidney samples were cultured aseptically onto trypticase-soy agar (TSA) (HiMedia, India) incubated at 25°C for 48 h before Gram staining of the grown colonies. The Gram-positive isolates were first identified by phenotyping and biochemical tests including oxidase, catalase, sorbitol utilization on phenol red broth (Quelab, Canada), motility, indole, H2S production on SIM media (Merck, Germany), urease (Merck, Germany), methyl red, Voges-Proskauer tests on MR-VP broth (Merck, Germany), citrate utilization on Simmon citrate agar (Merck, Germany), and type of hemolysis on 5% sheep blood agar (Merck, Germany) (4). Finally due to the kind of presumptive isolated identified bacteria additional tests were perfoemed and Stock cultures of the bacteria were maintained at -70°C in trypticase-soy broth (TSB) (HiMedia, India) containing 15% glycerol for further identification by molecular works (5).

2-2- DNA Extraction and PCR assay

DNA samples were obtained from 10 isolates identified as presumptive *Kocuria risophyla.*

For this purpose, the sample DNA was placed in the digestion buffer containing SDS5, STE6 buffer and proteinase K enzyme. The separation process with phenol, chloroform, isoamyl alcohol (1-24/25) was done in two steps and DNA precipitation with ethanol. Finally, the DNA was dissolved in 50 microliters of sterile distilled water.

The multiplication process was carried out in a thermocycler (PeQlab, Germany). Materials used in Tables 1 are given. PCR temperature conditions include initialization of 95 °C for a period of time 5 minutes, 94 degrees for 20 seconds, 59 degrees for 40 seconds and

72 degrees. The duration is 120 seconds. Finally, enzymatic expansion was done at 72 degrees for 5 minutes. Table 2-3 is displayed. Amount of 5 µl of product on 2% agar gel in buffer. TBE11X were stained in the presence of Green fluorescent dye. PCR product by kit method. The column was washed and for sequencing by the method (6) from both sides of the primer in ABI3730xl device was sequenced. A pair of specific primers shown in Table 1 were used for detection of the 16S rDNA gene and fragment size was 1100 bp.

Table 1: Primers used in gene sequencing

Primer name	Primer sequence (5-3)	Product (bp)
27F	AGAGTTTGATCCTGGCTCAG	1500
1492R	TACGGYTACCTTGTTACGACTT	

3. Results

3-1- Phenotypic and biochemical results

Phenotypic and biochemical characterization of yellowish, round, raised colonies (Fig. 1) on isolation resulted in presumptive identification of isolates as *Kocuria risophyla*. These isolates were Gram-positive non-hemolytic cocci, arranged in pairs, and irregular clusters (Fig. 2). Oxidase positive, catalase positive, mannitol fermentation and coagulase enzyme negative, non-motile, indole negative, H2S production negative, citrate utilization negative, methyl red negative, and Voges-Proskauer negative.

Figure 1: Appearance of *Kocuria spp* on blood agar after 24 hours of aerobic incubation

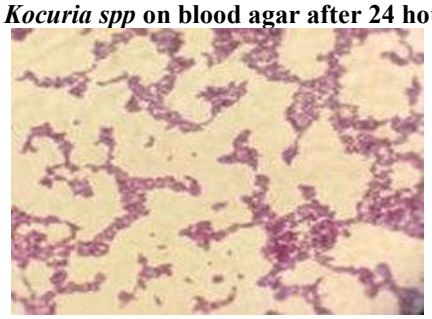

Figure 2: Gram's stain of *Kocuria* spp showing large sized cocci arranged in pairs, short chains, tetrads, clusters

In this research due to non-availability of molecular and advanced laboratory methods, *Kocuria* differentiated from *Staphylococci* and *Micrococci* by using morphological and cultural characteristics. Furthermore antimicrobial susceptibility patterns against selective antibiotics could be used to presumptively identify *Kocuria* spp. *Kocuria* spp. were sensitive to bacitracin, lysozyme and resistant to nitrofurantoin, furazolidone and lysostaphin (7, 8).

4. Conclusion

Accurate understandings about the etiological agents, biochemistry, and the relationship of stress-related environmental component are crucial for effective management and control of diseases. The

improvement in diagnosing tools can provide an opportunity to identify new infective agents. Some Gram-positive bacteria have become pathogens of particular importance in fish pathology in Iran. Some studies have noted that although Kocuria spp are commensals of humans, animals and are present in the environment, they should be considered as potential pathogens in immunocompromised individuals. The present research has highlighted the presence of Kocuria in fish infections.This bacterium has not been known until now to be pathogenic to fish. Therefore, this infection could be called an emergent pathogene (2).

Recent studies indicate that bacteria belonging to the family Micrococcaceae In addition to humans, are also pathogenic in the aquaculture industry As Micrococcus luteus was previously known as the cause of rainbow trout syndrome (RTFS2) in salmonid Fishes (9). Until 2016, the data in the gene bank showed that K. *rhizophila* had never been isolated from fish before and the isolates studied by Pekala et al. were very similar to the strains from the Food processing environments were isolated. Thus, the first report on bacterial pathogenicity K. *rhizophila* was registered in the Polish aqua culture industry in 2018 (10).

This bacterium is normally misidentified in the clinical microbiology laboratories as coagulase-negative *Staphylococci* (CoNS) based on its gram reaction, catalase positive and coagulase negative properties. The major drawback faced by many laboratories in accurately identifying this bacterium is the need for advanced techniques like 16S rRNA and Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF-MS). Although many clinical microbiology laboratories are now equipped with automated identification systems that include VITEK (BioMe´rieux Inc., Durham, NC, USA), VITEK 2 (BioMe´rieux Inc., Durham, NC, USA), API (BioMe´rieux Inc., Durham, NC, USA) and the BD Phoenix™ Automated Microbiology System (BD Diagnostic Systems, Sparks, MD) identification systems, there are studies that have noted false i- dentification of CoNS as *Kocuria* spp and its limitations to identify all the species of *Kocuria* (11).

Identification of Kocuria spp remains elusive because most clinical microbiology laboratories have limited or no access to advanced molecular techniques. Laboratory identification of *Kocuria* spp can be made conventionally only after high laboratory suspicion. An interesting observation in Gram's stained smear includes the presence of darkly stained and abnormally large clones of cocci, which are not observed in the case of *Staphylococci* and *Micrococci*.

The cause of concern is that this bacterium appears to be as a zoonotic phatogene and have a broad host range involving both immunocompromised as well as immunocompetent individuals. Reports of infection with *Kocuria* species in human have gained prominence in the late twentieth century and are showing an increased trend, signifying its pathogenic potential. Infections associated with isolation of *Kocuria* include urinary tract infections, cholecystitis, catheter-associated bacteremia, dacryocystitis, canaliculitis, keratitis, native valve endocarditis, peritonitis, descending necrotizing mediastinitis, brain abscess and meningitis (12, 13). Further studies emphasizing the determination of the virulence, pathogenic potential, predisposing factors and antimicrobial susceptibility patterns of Kocuria spp are warranted.

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Synthesis of Titanium dioxide nanoparticles using turnip extract and investigating the effect of extract concentration on the size of nanoparticles

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Abstract

Green synthesis is considered to be an eco-friendly and sustainable approach. Various plant parts and their extracts are used. The green synthesis procedure is an easy process to produce nanoparticles. The present study deals with the synthesis of titanium dioxide nanoparticles using turnip extract. Also, the effect of two different extract concentrations on the property of nanoparticles is investigated. In this research, green synthesis as an eco-friendly and cost-effective method is used. The white color dispersion shows the formation of TiO2 NPs during the chemical process. The SEM image of titanium NPs in both samples reveals the spherical-shaped NPs. The obtained results have revealed that the property of TiO2 nanoparticles was similar in both processes except for the size of the nanoparticles. More turnip extract concentration results in bigger titanium NPs. The EDS analysis shows the presence of titanium and oxygen.

Key words: Green synthesis, Nanoparticles, Turnip, TiO_2

1. Introduction

Titanium dioxide (TiO_2) is majorly consisting of two forms (crystalline and amorphous). Titanium dioxide nanoparticles (TiO_2 NPs) are used widely because of features like inexpensiveness, high chemical stability, and strong oxidizing power. The oxygen vacancies present in the lattice of TiO_2 are due to oxygen separation and emission of electrons. The main limitations of using TiO_2 NPs are the electron-hole recombination and the small surface area [1]. TiO_2 is the most used photocatalyst to degrade antibiotics and other organic pollutants. Due to its high energy efficiency, high photocatalytic activity, low cost, non-toxic, and high stability. TiO_2 is an N-type semiconductor, having an energy gap of 3.2 electron volts. Due to this reason, TiO_2 uses UV light rather than visible light [2]. Despite the drawbacks, TiO_2 NPs have a wide range of applications in cosmetics in lotions, creams, skin ointments, ultraviolet radiation, papers, food colorants, paints, and inks. It has a wide range of applications in the electronic field, such as solar cells, various types of electrodes, and photovoltaic cells. TiO_2 NPs are also used in the catalysis food industry.

Another application of TiO_2 NPs is biomedical, for example, in cancer therapy, drug delivery, cell imaging, and biosensors [3]. As the surface area of TiO_2 NPs is very small, the surface area must be increased for the electrical optical properties. The physical and chemical properties of TiO_2 NPs can be changed by particle size crystalline phase. In organic reactions, TiO_2 NPs are used as a catalyst that enhances the reaction rate [4]. Green synthesis of nanoparticles using different biological metabolites can help overcome chemical and physical methods because there is little use of chemicals and other agents during biological synthesis. Green synthesis is the most skillful, normally flexible, biologically

sound, and practical technique for the synthesis of nanoparticles. Regular concentrates from various plants are being utilized as the reducing and capping agent in green science. In the green synthesis of nanoparticles, the correlation between chemical science, biological science, and industrial engineering is used to produce nanoparticles that can be used for commercial activities [5].

2. Experimental

Fresh Turnips were well washed with the flowing water to remove the dust particles from their surface. The Turnips were further rinsed with the DI water followed by the drying process at room temperature. The chopped Turnips were kept at room temperature for 10 days and transferred to the grinder to crush into a fine powder. For the first sample, it is used 1gm of prepared Turnip powder was well mixed with 50 ml of Etanol for 15 min at 50 °C. After the color change in liquid, the extract was cooled until attaining room temperature and filtered using Whatman filtersheets. Then 20cc of obtained solution extract is added to 4cc of Titanium Isopropoxide and kept under stirring for 15min by 3rpm. For the second sample, 10 grams of prepared Turnip powder was well mixed with 50 ml of Etanol for 15 min at 50 °C. After the color change in liquid, the extract was cooled until attaining room temperature and filtered using Whatman filtersheets. Then 20cc of obtained solution extract is added to 4cc of Titanium Isopropoxide and kept under stirring for 15min by 3rpm. During the green synthesis process, the colloidal solution turns from white to yellowish-grey, which indicates the formation of titanium dioxide nanoparticles. The white color dispersion shows the formation of TiO2 NPs during the chemical process.

3. Results and discussion

3-1- Scanning Electron Microscope

The Surface morphology of TiO_2 nanoparticles was visualized using FESEM. TiO_2 nanoparticles are spherical shapes in both samples as fig 1 and 2 show. Therefore, concentration of extract does not change their shapes. But in sample1 with less concentration it can be seen that the size of TiO_2 nanoparticles are smaller than those in sample2. Meanwhile, concentration of turnip extract changes the size of TiO_2 NPs. More turnip extract concentration results bigger TiO_2 NPs.

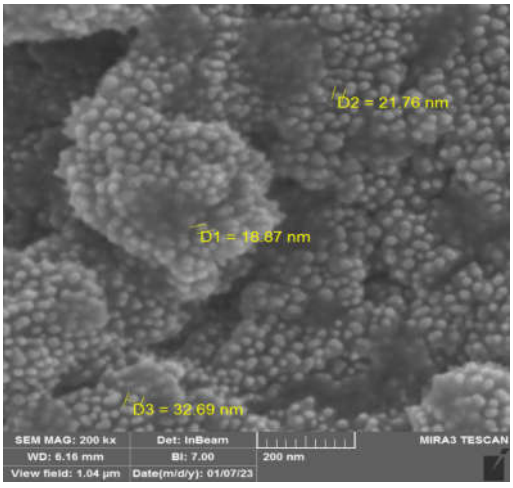

Fig. 1: FESE image of TiO_2 nanoparticles of sample 1.

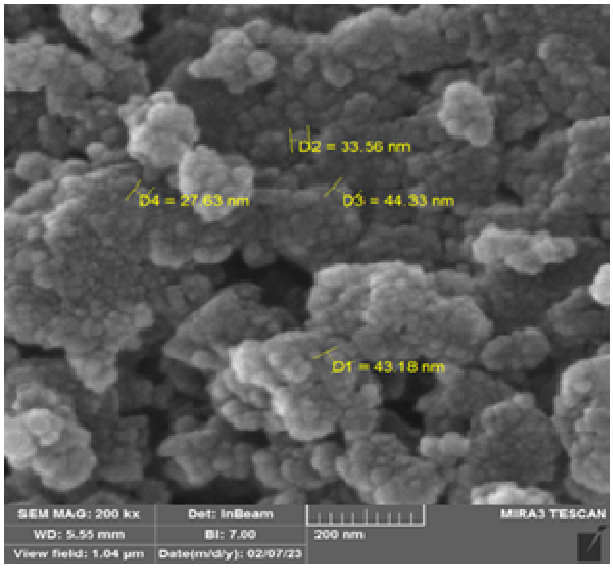

Fig. 2: FESE image of TiO_2 nanoparticles of sample2

3-2- EDS analyzing

EDS spectrum is used to determine its homogeneity and its elemental distribution of elements in the investigated compound. The elemental analysis of the chemical compounds was investigated through EDS spectra. Figures 3 and 4 show the EDS spectra of samples. The elements present in the synthesized TiO_2 NPs are Titanium (Ti), and oxygen (O). In both samples, peaks of Ti element are highly more compared to the peak of oxygen. EDS shows 40.65 for atomic weight percentage of Ti and 52.23 for atomic weight percentage of O in sample 1. But it shows 48.65 for atomic weight percentage of Ti and 50.24 for atomic weight percentage of O in sample 2. The atomic weight percentage of the Ti and O of NPs in samples 1 and 2 are tabulated in Table1 and 2.

Table 1: Weight percentage of elements in sample1

Element	W%
Ti	40.65
O	52.23

Table 2: Weight percentage of elements in sample2

Element	W%
Ti	48.65
O	50.24

The elemental analysis of the chemical compounds was investigated through EDS spectra. Figures 3 and 4 show the EDS spectra of TiO_2 NPs. The elements present in the synthesized

TiO_2 NPs are Titanium (Ti), and Oxygen (O). In the sample2, TiO_2 NPs, the composition of the titanium element is high compared to oxygen content.

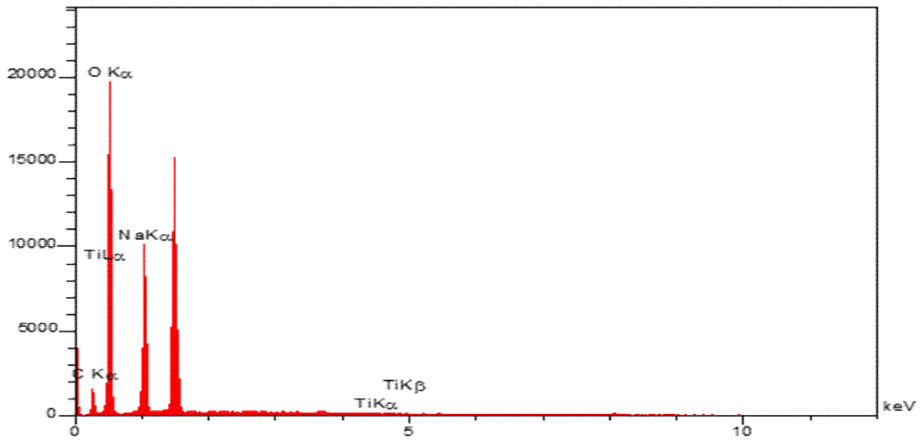

Fig. 3. EDS of sample1

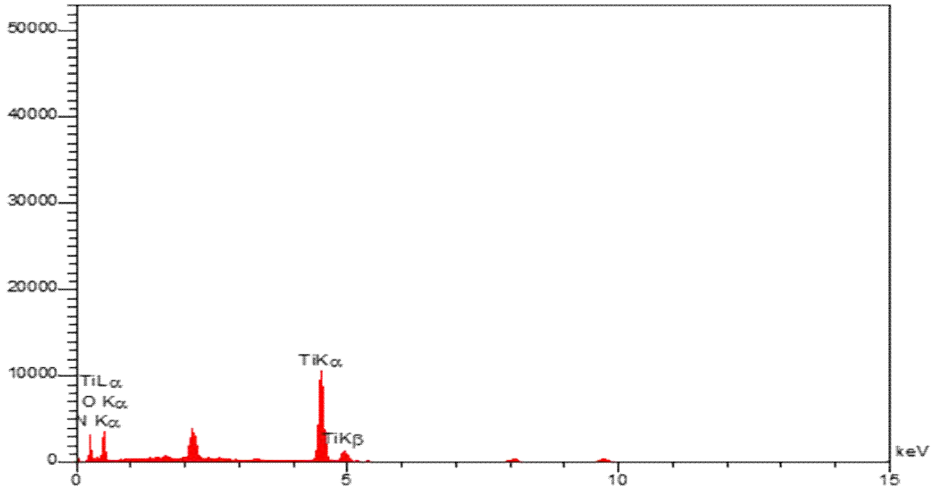

Fig. 4. EDS of sample2

4. Conclusions

In this work it was demonstrated that green synthesis procedure is an easy process to produce TiO_2 nanoparticles using turnip extract. Investigation of the effect of two different extract concentrations on the property of nanoparticles showed that more turnip extract concentration results in bigger titanium NPs. The SEM image of titanium NPs reveals the spherical-shaped NPs. In any concentration. It could be concluded that green synthesis is considered to be an eco-friendly and sustainable approach.

5. Acknowledgments
Thank Narges Bahadorifar for her support.

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Ultrasonographic And Radiographic Diagnosis Of Urolithiasis In Referred Cats To The Veterinary Hospital Of Ahvaz: A Comparative Study

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Abstract

Urolithiasis is the presence of stones in any part of the urinary tract of a cat, and can vary in its mineral composition. Ultrasonography and radiography are the most commonly used methods for detecting urinary stones in dogs and cats. The purpose of this study was to compare the findings of ultrasonography and radiography in the diagnosis of urinary stones and to determine the prevalence of urinary stones in cats referred to the veterinary hospital of Shahid Chamran University of Ahvaz over a five-year period (from 2017 to 2022). 109 cats were evaluated in this study. The findings showed that the average prevalence of urinary stones over a five-year period is 9.17% and there was no statistically significant difference between the two methods used for detecting urinary stones (P>0.05). According to the literature, the bladder is where urinary stones are most likely to form. The findings of this study demonstrated that the prevalence of urinary stones in cats in this area is comparable to that in other parts of the world, where it has been reported to range from 7 to 28%. It was anticipated that the prevalence of stones would be relatively higher in the Ahvaz region due to the hot climate, but this hypothesis does not appear to have led to an increase in urinary stones, possibly because the referred cats were kept at home and were not exposed to heat or a lack of water, both of which are significant stone-forming factors.

Key words: Cat, Urolithiasis, Radiography, Ultrasonography, *Ahvaz*

1. Introduction

Among the diseases that affect urinary tract system, urolithiasis is the second largest cause of clinical signs compatible with feline urinary tract disease. The term urolithiasis refers to the presence of uroliths in any region of the urinary tract, but it is more commonly seen in the bladder and urethra. Uroliths are classified based on the type of mineral present in their composition (1). Specific metabolic conditions (e.g. nutritional factors [a protein-rich diet], hydration [lack of water], pH [pH modification], urine volume [low urine volume], hypercalcemia, or hypophosphatemia) influence their formation (2). The most common feline urolith types are struvite (magnesium ammonium phosphate) and calcium oxalate (CaOx) (each >40% of uroliths). Purine uroliths represent about 5% of cases (3). Dietary, metabolic, genetic and infectious causes as well as factors such as breed, age, sex, age range,

obesity, sedentary lifestyle, geographic region and climate potentiate the chance of development of uroliths (1).

The clinical signs of animals with urolithiasis may vary according to the affected segment, quantity, and format of uroliths. Patients usually present signs of urinary tract disease such as stranguria, dysuria and Nonspecific signs (vomiting and anorexia) or complete absence of signs may also be observed, especially when the calculus is located within the upper urinary tract (4).

The diagnosis is based on a thorough examination of specific causes. Accordingly, urine analyses (urine strips, urinary sediment and urine culture), radiography and abdominal ultrasonography are used (5). Diagnostic imaging is usually required to determine the presence of urolithiasis. Double-contrast cystography is more accurate than survey radiography and approximately as accurate as ultrasonography (6). Most uroliths are radiopaque on survey abdominal radiographs. Radiopaque uroliths include calcium phosphate, calcium oxalate, struvite and silicate. Survey radiography is suitable for identification of these radiopaque uroliths, provided they are greater than approximately 2–3 mm in diameter. Small uroliths are detected more reliably by double-contrast cystography or ultrasonography. However, ultrasound is superior to double-contrast cystogram in experienced hands and for very small stones and sand. Ultrasonographic examination can detect radiopaque and radiolucent uroliths.Therefore, for the diagnosis of urolithiasis radiography combined with ultrasonography are preferred over contrast radiography alone (7).

According to the diagnosis of urolithiasis randomly in cats that were referred to the radiology department of the veterinary hospital for other reasons and according to the hot climate of Ahvaz region (heat above 50 degrees in summer) (8) and subsequently, dehydration in animals, it is predicted that the prevalence of uroliths will be relatively high. In Ahvaz, no consistent research has been done on urolthiasis in cats. Therefore, The objective of this study was to compare two methods of ultrasonography and radiography in the diagnosis of urolithiasis in cats showing suspicious symptoms of urolithiasis or symptoms of lower urinary tract disease referred to the veterinary hospital of the Shahid Chamran University of Ahvaz. The percentage of urinary stone prevalence in referred cats was also determined.

2. Methodology

2.1. Sample collection

Between 2017 and 2022, 109 cats with symptoms of lower urinary tract disease or suspected to urolithiasis were referred to the veterinary hospital of Shahid Chamran University in Ahvaz. The data of each cat was recorded in medical center.

2.2. Physical examination

Clinical signs help localize the problem to the lower urinary tract. Information from the animal owners helps to determine the duration and severity of symptoms. Physical examination included evaluation of body temperature, heart rate, respiratory rate, depression rate, mucous membranes color, and abdominal palpation.

2.3. Imaging investigations

Ultrasonography and radiography were used simultaneously to diagnose urinary stones and differentiate them from other diseases of the urinary system. Also the location of stones were determined. Sedation was used for ease of imaging in a limited number of cats. Abdominal radiography was performed using X-ray machine (KCD-10M-6A1T JPN). Radiographs were processed using computed radiography system. The radiographs were taken on a ventral dorsal or lateral view. In this work, ultrasonography of all cats was performed

using Landwind ultrasound machine (Mirror2) with a 5-10 MHz linear probe. The animals were positioned on lateral or dorsal recumbency, scanning area was shaved, and ultrasonic gel was applied to the skin. During the ultrasound, all parenchyma of the kidney were evaluated; the size and location of calculi were determined.

Using the data obtained from the recorded images and patient records, a comparison was made between the findings of ultrasonography and radiography, and the percentage of uroliths prevalence was also obtained. Chi-square software and logistic regression were used for comparative evaluation.

3. Results

Urolithiasis was detected in 10 out of 109 cats with lower urinary tract diseases, were referred to the veterinary hospital over a five-year period (9.17%) based on cats medical histories and ultrasonographic and radiographic examinations. moreover, out of these 10 cases of uroliths, all 10 cases were confirmed by ultrasound, whereas 8 cases were radiopaque and were confirmed by radiography, and only 2 cases were radiolucent and were diagnosed only by ultrasound, although there was a difference between ultrasonography and radiography, no significant difference was determined (P>0.05). Table 1 displays the number of urinary stone sufferers for each of the last five years.

Table 1. The frequency and occurrence of Urolithiasis in cats in Ahvaz over the course of 5 years are displayed.

year	Number of referred cats	Number of cat with uroliths	Prevalence rate (%)
2017-2018	21	2	9.52
2018-2019	19	2	10.53
2019-2020	15	1	6.67
2020-2021	18	1	5.55
2021-2022	36	4	11.11
total	109	10	9.17

Uroliths were only found in the urinary bladder in 60% (n = 6) of cases, and only in the kidneys in 20% of cases (n = 2). Additionally, uroliths have been occasionally seen in the kidney and bladder at the same time. (20%; n=2). Radiographic and ultrasound images of some patients with Uroliths are shown in pictures 1 and 2.

Less than 5% of referred cats with lower urinary tract disease were spayed, which was insufficient for statistical analysis. In addition, all referred cases were cats which were kept indoors and were not exposed to a warm outdoor environment.

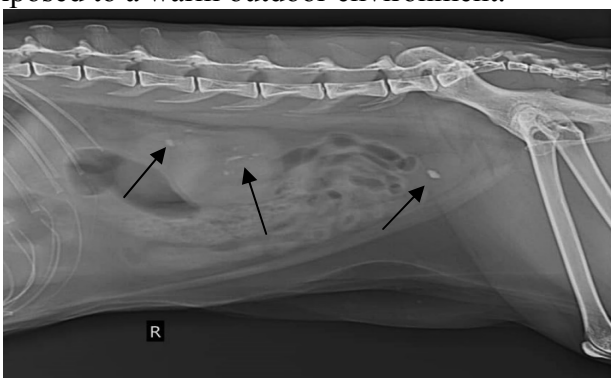

Figure 1: X-ray image of a cat with several stones in both kidneys and urinary bladder from the lateral view

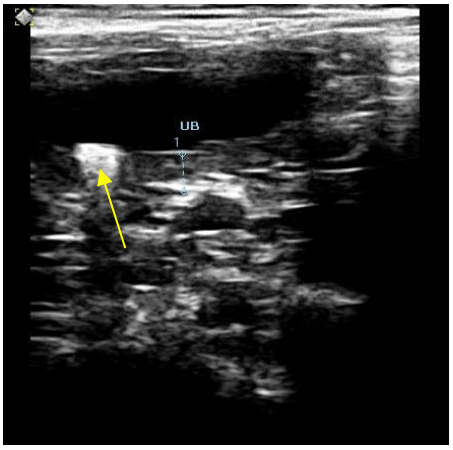

Figure 2- Ultrasound image of a cat with a stone in the bladder

4. Discussion

Until today, many studies have been conducted on lower urinary tract diseases and uroliths analysis, and many researchers have presented prevalence rate of urolithiasis. Some researchers analyzed the types of uroliths through surgery and stone removal, however in the present study it was not possible to analyze and check the types of stones.

On average Urolithiasis accounted for 9.17% of the cases presented to veterinary hospital in Ahvaz University. Recent studies have reported that 7-28% of cats presenting for lower urinary tract signs were diagnosed with urolithiasis (3). Hribova et al.(9) stated a 5% prevalence of uroliths, in a study on the diseases of the lower urinary tract of cats in the Czech Republic. The method and the number of referred cats in mentioned study were almost similar to the present study. This difference in percentage can be due to the different geographical area and probably the condition of their maintenance or the type of feeding. Kochan and Simsek (5) reported that the dietary regimens of the patients, especially drinking tap water, may account for the high incidence of urolithiasis (28.9%) in their research compared to present study. According to Nururrozi et al (10), the prevalence of uroliths was reported as 13 percent and the formation of crystal struvite and oxalates was observed in most urolithiasis cases. In the present study, it was not possible to analyze stones. Castration is a predisposing factor, with a description of 85% of analyzed uroliths coming from castrated cats, meaning a risk of 8.3 times greater than non castrated animals (1). Castrated males had an increased risk of urolithiasis compared to spayed females. Castration and spaying are considered risk factors associated with the inhibition of urethral growth, induction of weight gain, and a sedentary lifestyle (10). In the present study, less than 5% of cats had been spayed, and this could be a factor in the lower prevalence percentage compared to the higher prevalence percentage in some studies. It seems that the difference in the prevalence rate of uroliths in studies is due to different factors such as the type and amount of food and water rations, number of referred cats, the rate of castration and different geographical regions.

In 2021, a similar study was conducted on dogs in Ahvaz region, and the prevalence of uroliths was 4.65% (11). It seems that the prevalence of uroliths is higher in cats than in dogs.

The sensitivity of survey abdominal radiography for the diagnosis of ureterolithiasis in cats is 81%. abdominal ultrasonography has a sensitivity of 77%. A combination of survey radiography and ultrasonography has a sensitivity of 90% for the diagnosis of ureterolithiasis, so it is the preferred approach (12). In the present study, radiography and ultrasound were used to diagnose urinary problems in all referred cats which is like the method used by many researchers to diagnose urinary disorders (5, 9, 10). According to the research of Hřibová et

al. (9) abdominal ultrasound was performed on almost all cats, and radiography was performed on a small number of them, while in present study, radiography was performed first (to determine the location of stones in most cats with uroliths) and then ultrasound was performed. In present study Lithiasis was identified ultrasonographically by their hyperechogenic aspect, with the presence of rear acoustical shadow but there wasn't a significant difference between the results of the two techniques used.

In 2019, Mendóza-López et al. (13) reported the most common site of stone formation in the bladder (75.3%), the second most common location was the urethra (9.9%) and only a few were located in the kidney and urinary bladder simultaneously (6.2%). Burggraaf et al. (14) in a study on 3497 cats with uroliths reported that In 85.5% of cats, stones were removed from the bladder. Gomes et al. (4) stated that by comparing the anatomical location where urinary stones were removed, those found in the lower urinary tract were more common than those in the upper tract. In the present study, By ultrasonography, In 60% (n = 6) of cases, uroliths were located in the urinary bladder and in 20%, the stone was present only in the kidney or kidneys, and in the remaining 20%, the uroliths were located in both the kidney and the bladder at the same time. It seems that the urinary bladder is the most prone place for the formation of Uroliths.

Dehydration is related to abdominal discomfort and stress, resulting in lower water intake (4). It seems dehydration in the cats was not an issue in the present study, because all the referred cases were indoor cats and had enough drinking water at their disposal.

5. Conclusion

The present study demonstrated that the average prevalence of uroliths in cats in Ahvaz region was 9.17%, which was in line with recent studies that reported the prevalence of uroliths in cats. Also there was no statistically significant difference between radiography and ultrasonography in the diagnosis of urolithiasis. The site of formation of most uroliths was the urinary bladder. Considering the hot climate of Ahvaz region, it was expected that the prevalence of uroliths would be relatively higher. However, this theory does not seem to have led in an increase in urinary stones which can be due to keeping the cats at home and subsequently not exposing the referred cats to heat or lack of water

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A Bioinformatic Approach to scRNAseq Analysis Uncovers New Aspects of Neurophysiology

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Abstract

Our understanding of cellular diversity and nervous system function has been revolutionized by advances in single-cell RNA sequencing (scRNAseq) technology. In this reappraisal essay, we delve into the exercises of scRNAseq in neurophysiology, involving the identification of new cell types, the analysis of cell signaling pathways, and the study of gene statement dynamics. We also argue the bioinformatic difficulties and opportunities in scRNAseq analysis, similar to the want for grade controller and standardization forms. Likewise, we point to the integration of scRNAseq in different ways, similar to spatial transcriptomics and electrophysiology Eventually, we argue the coming directions of scRNAseq in neurophysiology, involving the eventuality for the identification of new cure marks and the evolution of individualized medicine. This review highlights the import of scRNAseq technology in advancing our understanding of the nervous system and its implicit to chip into the evolution of new remedial programs for neurological disorders.

1- Introduction

Recent improvements in single-cell RNA sequencing (RNA-Seq) technology have handed an unknown occasion to research the transcriptome of individual cells and identify gene expression patterns specific to cell types [1]. In neurophysiology, scRNAseq technology is particularly useful for analyzing the molecular basis of neuronal diversity, identifying new cell types, and understanding the pathophysiology of neurodegenerative diseases [2] [٣]. In addition, integrating scRNAseq data with different omics data can build complex regulatory networks that reveal complex interactions between genes and regulatory elements in the nervous system [2].

This review essay aims to give an inclusive overview of the applications of scRNAseq technology in neurophysiology, with a specific focus on the bioinformatic protests and openings consociated with assaying scRNAseq data. We'll research how scRNAseq technology can be used to study gene formulation in diverse types of cells in the nervous system, involving immune cells, neurons, and glia [2][٤]. We will also discuss how scRNAseq technology has been used to investigate the pathophysiology of neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and Huntington's disease [2]. Finally, we address the bioinformatics challenges associated with scRNAseq data analysis, including data normalization, dimensionality reduction, and cell type identification, and highlight potential future directions for scRNAseq technology in neurophysiology [1][٥].

2- Background

The nervous system is made up of a complex variety of cell types, including neurons, glial cells, and immune cells, each with different functions and patterns of gene expression [6].

Conventionally, bulk RNA sequencing (RNAseq) has been utilized to ponder gene expression in the nervous system [٧]. However, a limitation of bulk RNAseq big data is that it provides an average gene expression profile for all cells in a sample [٨], masking potential cell-to-cell heterogeneity [9].

Recently, scRNAseq technology has overcome these limitations and has become a powerful tool to study gene expression at the individual cell level [10]. scRNAseq innovation empowers the distinguishing proof and characterization of cell sorts and subpopulations [١١], as well as the location of uncommon cell sorts that will be missed utilizing bulk RNAseq [12]. Additionally, scRNAseq technology allows the exploration of the nonsupervisory networks that control gene expression in unalike cell types [١٣][١٤], delivering insight into the essential molecular mechanisms that govern cellular variety in the nervous system [6]. As shown in Figure 1 (adapted from Svensson et al., 2018) [١٥], the field of single-cell RNA sequencing has undergone significant evolution over the past decade, with increasingly sophisticated and accurate analysis techniques being developed.

The application of scRNAseq innovation in neurophysiology has provided insight into the cellular diversity in the nervous system and the convoluted molecular interactions that uphold normal and pathological conditions [16]. For example, scRNAseq technology has been utilized to recognize new neuronal subtypes [١٨], determine the molecular properties of glial cells, and study the immune responses in the nervous system [19][20]. Additionally, scRNAseq technology has been used to study the molecular mechanisms of neurodegenerative maladies such as Alzheimer's and Parkinson's, and Huntington's diseases [21]. By permitting gene expression studies at the cellular level, scRNAseq technology could revolutionize our knowledge of the nervous system and its diseases [٢٢].

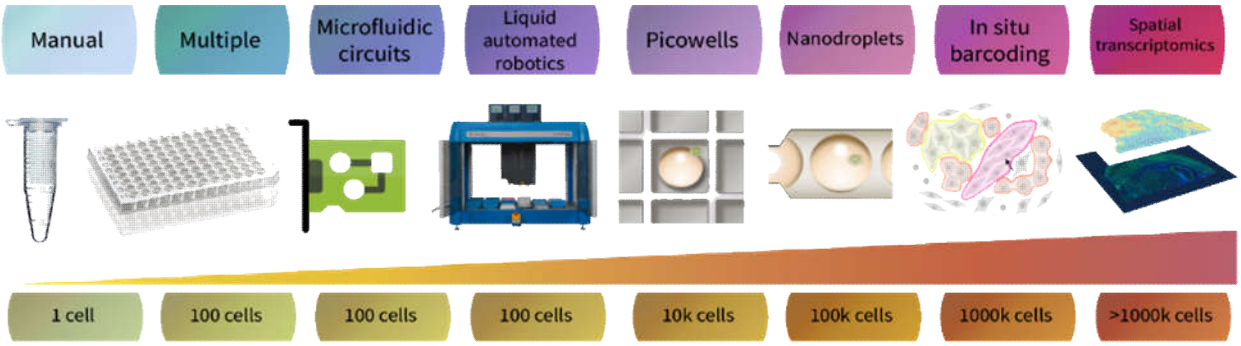

Figure 1 Over the past decade, advancements in single-cell RNA sequencing technology have led to the development of more sophisticated, accurate, and high-throughput analysis method

1. Figure adapted from Svensson et al., 2018 with refinement

3- Applications of scRNAseq in Neurophysiology

In neurophysiology, scRNAseq technology enables the identification of new cell types, characterization of cellular heterogeneity, and study of gene expression patterns in different neurological disorders. We then underline some of the most important applications of scRNAseq technology in neurophysiology.

- **Cell Type Identification and Characterization**

scRNAseq technology helps to identify and characterize new types of cells in the nervous system [٢٣]. For illustration, mouse cortical scRNAseq assays revealed a previously unknown type of inhibitory neuron that expresses the gene Vip [٢٤], which is required to maintain the balance between excitation and inhibition in the brain [6]. In another study, scRNAseq examination of the retina recognized a new cell type, bipolar rod cells, not already depicted in humans [9].

In addition to recognizing new cell sorts, scRNA seq technology has moreover been utilized to characterize the diversity of cell populations in the nervous system. For case [٢٥], the mouse hypothalamus scRNAseq assay revealed the existence of different subpopulations of neurons that

are affected in the checking of feeding treatment and energy homeostasis [10]. Similarly, human brain scRNAseq analysis identified a subpopulation of oligodendrocytes involved in myelination [12].

- **Neurological Disorders**

The scRNAseq technique has also been used to study gene expression patterns in different neurological disorders [١٧]. For instance, scRNAseq assay of the postmortem brain tissue of individuals with Alzheimer's disorder (announcement) has uncovered fluctuations in gene expression in astrocytes[26], microglia, and oligodendrocytes, supplying novel discernment into the pathogenesis of AD [16]. So also, scRNAseq investigation of the mouse brain has uncovered changes in gene expression in special cell sorts in reaction to traumatic brain affliction (TBI) [19].

- **Cell Signaling Pathways**

The scRNAseq technique was used to study cellular signaling pathways involved in neurophysiological operations [20]. For case, scRNAseq examination of the mouse retina has uncovered the different expression of genes affected in the Wnt signaling pathway in various cell types, indicating a part of this path in retinal evolution and function [21]. Also, scRNAseq examination of the rat hypothalamus revealed a different subpopulation of neurons affected in the melanocortin signaling path that controls energy homeostasis [27].

- **Developmental Biology**

scRNAseq technology was used to look for gene expression patterns during neuronal evolution [28]. The rats' embryonic cortex scRNAseq assay reveals sequential activation of transcription factors that are vital for the discreteness of cortical neurons [29]. Also, scRNAseq analysis of the evolving human brain revealed expression patterns of cell-type specific genes involved in the formation of functional circuits in the cerebra [30].

Generally, scRNAseq technology has given profitable bits of knowledge into the cellular heterogeneity and gene expression designs within the nervous system [31][32], permitting a profound understanding of neurophysiological operations and neurological disorders.

4- Integration of scRNAseq with other techniques

This study aimed to integrate scRNAseq with other techniques to obtain comprehensive information about cellular processes [33]. For illustration, the combination of scRNAseq and single-cell epigenetic investigation gives unused experiences in gene regulation and cell differentiation [34]. The use of scRNAseq and single-cell protein measurements can provide valuable insights into the regulation of gene expression and protein levels in individual cells [35].

Spatial transcriptomics allows the mapping of gene expression to specific locations within a tissue or organ (e.g.between scRNAseq and spatial transcriptomic [36], allowing for mapping of gene expression to specific locations within a tissue or organ) [37]. This integration makes it as easy to identify new cell sorts and subpopulations as simple as the characterization of tissue spatial diversity and cellular interactions [38]. A recent study combined scRNAseq and imaging mass cytometry to reveal the spatial organization of various types of immune cells in the tumor microenvironment [39].

Likewise, the integration of scRNAseq with ancestry tracking ways can give perception into cellular isolation pathways [40]. A recent study reveals the developmental trajectory of cortical interneurons in mice using scRNAseq in combination with different lineages [41]. The ability to integrate scRNAseq data with CRISPR/Cas9 genome editing will allow scientists to investigate the function of specific genes and pathways in cancer [42]. A study,

by identifying the regulator, could help to prevent or reduce the number of T cells that are exhausted by cancer by using scRNAseq and CRISPR/Cas9 [43].

Overall, scRNAseq can be used to monitor cellular function and interaction in a wide range of tissues and provide information on how different cellular components interact with one another [۱۷]. This information can be used to understand the complexity of cellular processes and to develop treatments or interventions for specific diseases.

5- Conclusion

In summary, scRNAseq technology has revolutionized the area of neurophysiology, by empowering analysts to study the transcriptomic prospect of individual brain cells. This has driven the identification of novel cell sorts, cell signaling paths, and genes that were already obscure. applications of scRNAseq in neurophysiology are different and include the study of neurodevelopment, neurodegenerative diseases, and psychiatric disorders.

Integrating scRNAseq with different strategies, like electrophysiology, imaging, and epigenetics, has resulted in a more complete understanding of the complicated neural circuitry and fundamental activities of brain function. Nevertheless, testing of scRNAseq information presents bioinformatic difficulties, such as information normalization, batch impacts, and identification of exquisite cell populations.

Unborn headings of scRNAseq in neurophysiology involve the study of cell-cell relations, the progress of spatial transcriptomics, and the use of machine knowledge calculations for information investigation. Furthermore, scRNAseq holds good promise for the progress of individualized pharmaceutical approaches within the range of neurology and psychiatry.

In conclusion, scRNAseq innovation has incredibly made strides in our understanding of the molecular and cellular instruments' principal brain function and dysfunction. It could transform the diagnosis and treatment of neurological and psychiatric disorders and quicken the development of new therapies.

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Misconception of Students in Teaching Thermodynamics

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Abstract

The studies conducted on the nature and environment of learning show that the researches conducted in this field are mostly focused on external factors affecting learning such as teaching methods, strategies, teachers' qualifications, textbooks, educational contents and classroom environment. One of the reasons for creating misconceptions in students is the existence of similar misconceptions in teachers, so it is necessary to identify and correct the misconceptions of students and teachers. Heat and energy are two main concepts in thermodynamics. This review article showed that the use of incorrect words and textbooks are factors that cause misconception. The general expression of the entropy function macroscopically without addressing the molecular structure and molecular theories also causes misconceptions and a wrong understanding of it. It is not possible for a student to learn a subject only through its general expression, but the learning of any subject depends on the initial beliefs and thoughts recorded in the mind, and if it is stated incorrectly, it will definitely be much more difficult to learn that subject in the future.

Key words: Thermodynamics, Misconception, Physical Chemistry, Education.

1- Introduction

Undoubtedly, one of the concerns of mankind throughout history has been learning. All human progress and achievements are the product of learning. Learning is a continuous process throughout human life. From the complex process of speaking at the beginning of life, which seems to be done with ease, to learning complex scientific theories and solving difficult problems, everything is based on the learning process. Skinner 1 believes that an effective and real educational system cannot be created unless the two processes or actions of learning and teaching are fully known and understood (Shariatmadari, 1990).

According to Piaget, learning is the learner's work to absorb and accommodate mental construction (Piaget, 1993). Early experience introduction without providing proper context and developing concepts lead to memorization and prevent the development of logical thinking and abstract. Learners learn better when they solve real problems and critically discuss issues with their classmates. Accordingly, teaching is based on experimental method and class discussion. This method makes learners active and lasting effect on their minds. thinking (Robert, 2006). However, teaching and learning, is always accompanied with misconception in the performance of the students and can cause confusion and failure in solving problems. So, it is essential to analyze the roots of mistakes in basic science. Falsification of concepts may cause misconception. Some researchers, instead of misconception, proposed the term "raw or simple theory" and some other so-called imagination, but the greatest emphasis is on the word misconceptions or misconception. So, understanding depends on proper ideas and new connections (Wen and John, 2001). When the students understand the causes of misconceptions and actively cope with the guidance of the teacher as fix and correct it, they will lead

to the growth of thinking and insight (Patrick and Svafvrd, 2010). Also for dealing with abuse misconception not only necessary that teachers be aware of their students' ways of thinking but also it is essential to develop strategies that focus on meaningful learning. Common example of basic science in high schools is power and energy topics. For example, learners believe that power is a property of a material (Barrows, 1986), while noting that the troops should be dependent and related to the materials and are not their features. They do not yet understand that the power is constantly intrinsic. Because learners' mistakes take the time of the class, finding suitable solutions to analyze and correct training will have great benefits (Barker, 2004; Smith et al., 1993)

The studies conducted on the nature and environment of learning show that the researches conducted in this field are mostly focused on external factors affecting learning such as teaching methods, strategies, teachers' qualifications, textbooks, educational contents and classroom environment. However, it should be noted that learners are not separate from the learning process and their brains cannot be considered as empty vessels that are filled by the teacher. Students have a series of assumptions and preconceived notions that help them understand the world around them; But these assumptions are not correct from a scientific point of view. Education researchers have called these misconceptions as misconceptions (Mirzaei et al., 2015).

A misconception occurs when a person believes in a concept that is reasonably false. It is assumed that due to the mental nature of man, every person has some types of misconception. In this matter, no one has complete knowledge and does not have a correct mental representation of the world. Distortion of a concept is not a misconception; But it may cause misconception. Each person may choose only one set of information to present in the transfer of a concept, the receiver can imagine other concepts about the presented concept that may be incorrect (Shah Mohammadi Ardabili, 2009).

Of course, it should be noted that misconception is not the only obstacle to students' learning, and other obstacles such as absenteeism, environmental conditions, distraction, motivation, lack of understanding of the teacher's meaning, etc. are also in the way of his learning. Of course, it should be noted that misconception is one of the main obstacles and not paying attention to it can cause a deep split in the act of learning (Taber, 2002).

Misconceptions can be classified into categories such as preconceived notions, scientific opinions, perceptual misconceptions, native misconceptions, and factual misconceptions (Mirzaei et al., 2015).

Some of the reasons for students' misconception are:

1 (Students' learning does not always progress according to the speed of presentation of concepts in textbooks and many educational items designed by the teacher:

2 (The language used by teachers and textbooks may confuse some students.

3 (There is often an unknown conflict between students' daily experiences and what is presented in textbooks or classes.

4 (Students are usually expected to understand the material before they have the opportunity to study the material. In fact, instead of giving students an opportunity to discover and understand ideas or models; They impose ideas on them.

5 (Pictures, diagrams and two-dimensional models in textbooks can be misleading and cause misconception.

6 (Removing theories can cause more problems, especially for good university students (Khaki et al., 2021.(

Thermal or thermodynamic chemistry deals with the study of heat released or absorbed by physical and chemical processes (Mortimer, 2014).

Thermodynamics, which is one of the topics of chemistry, is based on four laws, each of which is one of the important laws of this topic:

The zeroth law of thermodynamics: This law is proposed to express the isothermal state, and based on it, if two objects are in thermal or thermal equilibrium separately with the third object, then they will also be in thermal or thermal equilibrium together.

The first law of thermodynamics: according to this law, energy is conserved during the conversion of different energies into each other.

The second law of thermodynamics: according to this law, during a spontaneous change or process, while preserving energy, the level of order and concentration of energy decreases.

The third law of thermodynamics: according to this law, the temperature cannot be brought to absolute zero from a series of limited and consecutive coolers (Zighomi, 2013).

Some of the definitions related to the topic of thermodynamics are as follows. The macroscopic part of the world that is studied thermodynamically is called a system. The parts of the world that can interact with the system are called the environment (Levine, 2018).

The heat released or absorbed by reactions that are carried out at constant Far can be attributed to a property called enthalpy, which is represented by the symbol H. The heat of reaction is the difference between the reactant H and the products H, so it is denoted by the symbol ΔH (Mortimer, 2014).

If the enthalpy of the products of a reaction is greater than the enthalpy of the reactants, then the reaction will involve heat absorption. These types of reactions are called endothermic and their enthalpy change is positive. If the enthalpy of the products of a reaction is lower than the enthalpy of the reactants, the reaction will be accompanied by the release of heat. A reaction of this type is called exothermic and the enthalpy of this type of reaction is negative (Mortimer, 2014).

In our daily life, we encounter many physical and chemical changes that happen naturally without the help of external factors; Such processes are called spontaneous developments. But some physical transformations in certain conditions are done only with the help of external factors; Such phenomena are called non-spontaneous processes. It is obvious that if a phenomenon occurs spontaneously in certain conditions, its reverse phenomenon in the same conditions is non-spontaneous (Mortimer, 2014).

Enthalpy is one of the basic thermodynamic ideas in chemistry. These concepts are presented in chapter 2 (pursuing healthy food) in the 11th chemistry book. The ideas and misconceptions of students about enthalpy have received very little attention from educators and science researchers, and only a few researches have been done to discover students' and students' awareness of enthalpy change or energy changes in a chemical reaction and bond energy (Sozbilir, 2001; Carson and Watson, 1999; Boo, 1998). If chemistry teachers are aware of misconceptions, they can have better planning for educational activities and prevent new misconceptions (Sozbilir, 2004).

Thermodynamic concepts such as enthalpy, entropy and free energy were studied by Johnstone, MacDonald and Webb (1977). They designed a thermodynamic approach test to investigate students' conceptual problems and tested 98 students from ten different schools with it. The results showed that almost one out of every six students has a misconception about the concept of "exothermic reactions cannot be spontaneous". They attributed this finding to the universal law of "situations tending spontaneously to a lower energy state" and also noted that this misconception was not a new discovery and famous chemists, such as Berthelot and Thomson in 1878, believed that "Reactions must be self-heating to be spontaneous" have pointed out. By teaching in a small group of students, they realized that this misconception can be overcome and that a lecture method is not a suitable way to present thermodynamic concepts in a chemistry class (Khaki et al., 2021).

In another research, this concept was conducted on university students and the results showed that there is a misconception in 75% of the students who participated in a chemistry and physics course. 60% of students thought that no heat transfer occurs in isothermal conditions (Thoms, 1997).

In a study conducted by Carson and Watson (1999) on the understanding of "enthalpy change" in first-year undergraduate chemistry students, they found that students consider enthalpy as "a type of energy". In addition, none of them were able to understand the connection between "work" and chemical reactions and the concept of "work, volume and pressure". Finally, it was found that 9 out of 16 students are not able to provide a precise definition of it and their definitions were limited to a specific type of reactions such as neutralization (Khaki et al., 2021).

Ross (1993) found in his research that many students, contrary to the opinion of chemists, think that energy is released as a result of breaking chemical bonds. Ross considers the reason for this misconception to be the connection between burning and the energy created as an obstacle to learning concepts. He believes that students' misconception is due to the use of these terms, so words should be chosen carefully in teaching concepts (Khaki et al., 2021).

Among the scientific laws of thermodynamics, the second law of thermodynamics is one of the most significant known scientific laws. It gradually moves away from this state and goes towards destruction or in some way it goes towards the maximum entropy and some people have also

considered it rejected. Some have also mentioned entropy as the gradual death of order. The second law of thermodynamics expresses the concept that a process only proceeds in a certain direction and cannot be done in the opposite direction. There are different expressions of the second law of thermodynamics, but the most common expression of the second law of thermodynamics expresses the concept that a process only proceeds in a certain direction and cannot be done in the opposite direction. The main basis of the second law of thermodynamics is a function called the entropy function; which increases in spontaneous events and remains constant during equilibrium or reversible events (Zighomi, 2013).

Unfortunately, what is presented in the textbooks is such that the student has misconceptions that are sometimes in conflict with the phenomena in nature. When talking about the second law of thermodynamics, it is mostly expressed or understood in this way that entropy is a measure of disorder and entropy in a system or in a process is generally increasing and unfortunately The real concept and various expressions or examples of it are not discussed, and in general, its relationship with temperature, volume, energy, time, probability, etc. is ignored or less attention is paid. What happens in different situations and conditions, what interactions and changes occur in the system, or what internal and external factors affect the system are not discussed, which causes many misconceptions and Misconception of the second law of thermodynamics and, as a result, a wrong understanding of the entropy function (Zighomi, 2013).

Using the wrong words causes misconceptions in this field, so it is appropriate to try to use more appropriate words and replace inappropriate words with appropriate words. The use of the word entropy in literature can be the same as entropy (change and transformation), but when this word is used in sciences such as thermodynamics, it is definitely not a suitable word because it is not only a change and transformation, but with temperature, volume, time, and energy. It is related to probability and, in other words, it is subject to these factors, in addition to this, many external and internal factors are involved in it. Because entropy depends on temperature, volume, state of a substance, type and amount of substance. Therefore, the word (entropy function) is a suitable word, not the word (entropy), when it is said that the entropy function actually indicates its function and its compliance with the conditions that arise for it. To express the entropy function of Eddington's famous sentence, it is enough to say (entropy is the arrow of time). In fact, using the arrow of time shows both its function (time function) and a special direction in the spontaneous process. Maybe this difference between the two words is not very noticeable, but over time and with the establishment of the correct word in the mind, the student's dynamic mind will have a stronger understanding of the second law of thermodynamics and the entropy function, and as a result, the ambiguities will be resolved. In this context, it will be easier in the mind of the student (Zighomi, 2013).

The general expression of the entropy function macroscopically without addressing the molecular structure and molecular theories also causes misconceptions and a wrong understanding of it. In fact, the macroscopic expression of the entropy function is not a suitable expression in the first step, and the microscopic expression and dealing with the molecular structures of the system and molecular theories will be of great help in understanding it (macroscopic expression). The entropy of a system is closely related to the quantum states or the number of microscopic states of the system; In fact, the more positions there are, the higher the entropy of the system. Unfortunately, most of the students avoid the communication between chemistry and mathematics, especially on a microscopic scale, and for this reason, sometimes, despite having the ability to solve the related problems, they are unable to truly understand the subject and understand the macroscopic expression of the subject. (Zighomi, 2013).

It is not possible for a student to learn a subject only through its general expression, but the learning of any subject depends on the initial beliefs and thoughts recorded in the mind, and if it is stated incorrectly, it will definitely be much more difficult to learn that subject in the future. If a general topic is stated and many examples are not stated, the learner may find examples in his mind that are completely in conflict with the topic. Sometimes stating a question, using different examples, connecting the topic with different topics in the society such as mathematics, philosophical discussions, etc. helps a lot in understanding the topic. Establishing a relationship between chemistry, mathematics, statistics and probability, especially at the microscopic level, or in other words at the particle scale, will clear up existing misconceptions and will definitely give students a proper

understanding of the macroscopic expression of the entropy function. sometimes raising appropriate questions and making the student think is better than learning by mistake or sometimes misconception; Sometimes the use of an illustrative picture of a subject makes it better understood than explanations of even several pages (Zeighemi, 2013).

2-Acknowledgments

Some secondary school students who have learned the concept of thermochemistry in the 11th grade chemistry book have some misconceptions in some concepts, which are:

1. Arrangement of energy levels in gases, liquids and solids
2. Identifying the formula of enthalpy change and lag and lead of product and reactant
3. Endothermic or exothermic reaction of dinitrogen tetraoxide decomposition to nitrogen dioxide
4. Endothermic or exothermic reaction of dissolving dry calcium chloride in water
5. purity of bond enthalpy and reaction enthalpy
6. endothermic or exothermic of bond enthalpy
7. Endothermic or exothermicity of burning enthalpy
8. Intensity and quantity properties
9. ependence of thermal energy on the amount of matter (Khaki et al., 2021).

In addition to the explanatory explanations of thermodynamics, students also have a misconception in the mentioned facts, and on the other hand, due to the importance of thermodynamics and its long-term relationship with other chemistry topics, its fundamental and accurate learning should be at the focal point of educational content development. Therefore, by expanding and objectifying these concepts and making this topic the focus, it is possible to remove the misconceptions that have arisen, and it is suggested to chemistry teachers to have a deep understanding of this topic by allocating appropriate time and generalizing the definitions to each other and adopting a suitable teaching method. Students should pay (Khaki et al., 2021).

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Mixed convection analysis and Thermal management in a lid-driven enclosure via FEM

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Abstract

This article discusses the thermal behavior of a nanofluid contained within a rectangular cavity with a split lid and an circular obstacle. Forced and natural convection occur in sequence to create a combination of lid movement and heat transfer from the heating wall to the cooling wall. The temperature of the bottom wall remains constant, while the side walls are cold. Energy is conveyed through the top wall of the cavity and propelled by the split lids that move at a constant speed. The finite element method is employed to manage the dimensionless system of partial differential equations for velocity, temperature, and flow. The local Nusselt number along the heating surface is used to calculate the heat transfer rate caused by forced convection. The results shows displacement of the lid walls to a certain extent has a significant effect on the distribution of temperature within a system. When the inner path of the lid walls is opposite, the central area of the system tends to have the most efficient flow patterns.

Key words: *Mixed convection, Partial lid driven, Finite element method, Heat Transfer*

1. Introduction

The theory of heat transfer pertains to how energy is transferred and how temperature is distributed throughout a thermal system. The three modes of heat transfer are conduction, convection, and radiation. In this particular experiment, convective heat transfer is being studied, which involves the principle of convection. Convection is the transfer of energy between a solid surface and a moving liquid, regardless of changes in temperature. External sources such as pumps, fans, or suction tools are often used to generate fluid motion, which is an effective way to transfer heat. Forced convection is frequently used in various applications, including cooling channels, automatic control cooling, automobile industry cooling, nuclear power, petrochemicals, battery system cooling, ventilation, and cooling. Ducts such as air conditioning and petrochemical plants are also prevalent examples of forced convection. This study focuses on the performance of laminar forced convective heat transfer within a cavity for the laminar flow region. The main objectives of the research related to heat transfer characteristics are to increase thermal conductivity, improve operating processes of accessible devices. A numerical simulation on a moving door cavity with a warm corrugated wall to analyze the mixed convection was carried out by Azizul et al. [1]. They found that oscillating the corrugated wall with a low Ri number is enough to achieve the optimal heat transfer inside the cavity. Shulepova et al. [2] investigated a lid-driven cavity containing nanofluid for mixed convection analysis. Thermal radiation is applied to the cavity, and a time-dependent solid heat source is located inside the cavity. They discovered that increasing the number of

nanoparticles decreases the heater temperature and convection flow rate. Forced convection in a cavity with an elliptic-shaped obstacle was studied by Shah et al. [3]. A constant velocity is maintained by the lid-driven that controls the top of the cavity's wall. Their findings revealed that the Lewis number has a dominant effect on the isotherm and concentration. Also, the maximum heat transfer rate is obtained with smaller values of the buoyancy ratio. Fedotov et al. [4] displayed a study on the free convection in a thin cylindrical layer, focusing on a particular range of Ra numbers. The study showed that the 2D problem can provide a reasonably accurate prediction rate of heat transfer for low Ra numbers. This text discusses the popularity and efficiency of air cooling for electronic devices and computer systems. It highlights the need for designing an efficient cooling system with low power demand. The mechanism for air cooling involves a mixed convection heat transfer that combines forced convection with shear-driven flow and natural convection with buoyancy-driven flow. The study focuses on forced convection heat transfer in a partly lid rectangular cavity with concentration present, and the numerical investigation is carried out using FEM for different governing parameters.

2. Problem model and formulation

Figure 1 illustrates a rectangular cavity with an inner circular obstacle, where the inclined walls are cold. The top wall is partially split and moves at different velocities from left to right and right to left. The inner circular obstacle is assumed to be cold throughout the numerical investigation. The thermo-physical properties of the fluid are assumed to be constant, except for the density fluctuations in the buoyancy conditions, if the Boussinesq approximation is applied.

For the steady, laminar, 2-dimensional lid-driven heat and mass flow, the established equations of mass, momentum, energy and species concentrations follows;

$$\nabla . V = 0$$

(1)

$$u^* \frac{\partial u^*}{\partial x^*} + v^* \frac{\partial u^*}{\partial y^*} = \frac{-1}{\rho} \frac{\partial p^*}{\partial x^*} + \nu \nabla^2 u^*,$$

(2)

$$u^* \frac{\partial v^*}{\partial x^*} + v \frac{\partial v^*}{\partial y^*} = \frac{-1}{\rho} \frac{\partial p^*}{\partial y^*} + \nu \nabla^2 v^* + g[\beta_{T^*}(T^* - T_c^*) + \beta_{c^*}(c^* - c_1^*)],$$

(3)

$$u^* \frac{\partial T^*}{\partial x^*} + v^* \frac{\partial T^*}{\partial y^*} = \alpha \nabla^2 T^* \quad ,$$

(4)

$$u^* \frac{\partial c^*}{\partial x^*} + v^* \frac{\partial c^*}{\partial y^*} = D\nabla^2 c^* \quad ,$$

(5)

The equation shown above involves the velocity field and nabla operator used to describe two-dimensional fluid flow. The fluid's density and kinematic viscosity are represented by ρ and ν, respectively. Additionally, T* represents temperature, pressure represents pressure, concentration represents concentration, g represents gravitational acceleration, α represents thermal diffusivity, and D represents mass diffusivity. To convert the equation into dimensionless form, the primary parameters can be replaced with their corresponding dimensionless variables, which are listed below. This modification can be applied to equations (2-5) of the model.

$$(XY) = \left(\frac{x^*}{L}\frac{y^*}{L}\right), (UV) = \left(\frac{u^*}{u_o}\frac{v^*}{u_o}\right), T^* = T_c^* + (T_h^* - T_c^*)\theta, P^* = \frac{p^*}{\rho u_o}, c^* = c_1^* + (c_h^* - c_1^*)C$$

(6)

$$\frac{\partial U}{\partial X} + \frac{\partial V}{\partial Y} = 0$$

(7)

$$\vec{V}.\vec{\nabla}V = -\frac{\partial P^*}{\partial X} + \frac{1}{\mathrm{Re}}\nabla^2 U,$$

(8)

$$\vec{V}.\vec{\nabla}V = -\frac{\partial P^*}{\partial Y} + \frac{1}{\mathrm{Re}}\nabla^2 U + Ri(\theta + BrC),$$

(9)

$$\vec{V}.\vec{\nabla}\theta = \left(\frac{1}{RePr}\right)\nabla^2\theta,$$

(10)

$$\vec{V}.\vec{\nabla}C = \left(\frac{1}{\mathrm{Re}\,\mathrm{Pr}\,Le}\right)\nabla^2 C,$$

(11)

where the evolving physical parameters are, Reynolds number (Re), Richardson number (Ri), Lewis number (Le), Prandtl number (Pr) and the buoyancy ratio (Br) are defined as follows:

$$Re = \frac{u_o L}{\nu}, Ri = \frac{g\beta_{T^*}(T_h^* - T_c^*)L^3}{\nu^2 Re^2}, Le = \frac{\alpha}{D}, \Pr = \frac{\nu}{\alpha}, Br = \frac{\beta_{c^*}(c_h^* - c_1)}{\beta_{T^*}(T_h^* - T_c^*)},$$

(12)

The dimensionless boundary conditions to the corresponding eq. (8–12) are as follows;

At the surface of inner obstacle:

(U, V) = (0, 0), θ = C = 0 and $\Psi = 0$

(13)

At top right part of wall:

(U, V) = (-1, 0), θ =1, C = 1 and $\Psi = 0$

(14)

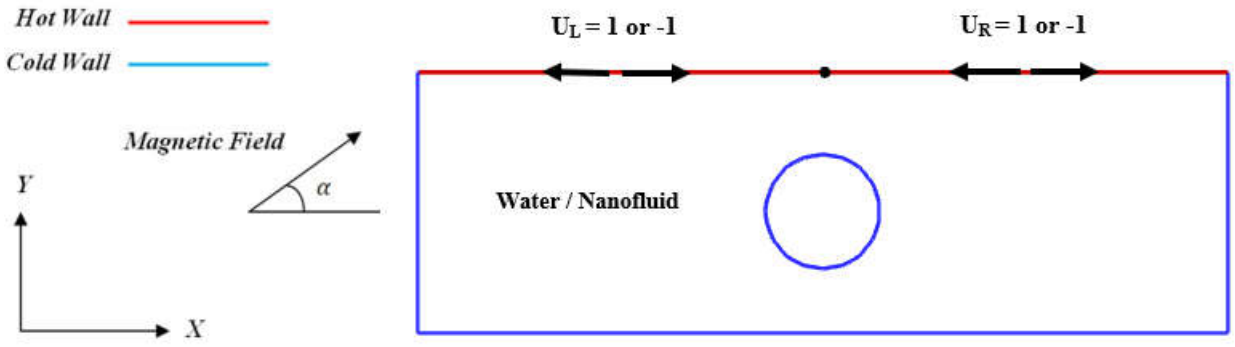
At top left part of wall:

(U, V) = (1, 0), θ =1, C = 1 and $\Psi = 0$

The local Nusselt number is determined using the above equations for the heat transfer rate calculation.

$$Nu = -\left(\frac{\partial \theta}{\partial Y}\right)_{Y=0}$$

(15)



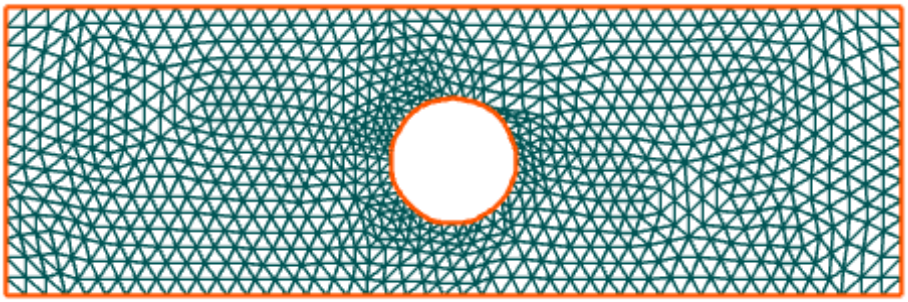


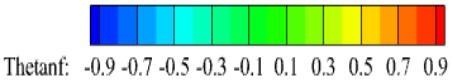
Fig1. Geometry, boundary conditions and adaptive mesh refinement

3. Numerical Solution Procedure

GFEM (Galerkin Finite Element Method) is becoming increasingly useful for analytical modeling of engineering problems due to its adaptability and ability to provide trustworthy results. The grid of computation can be triangular or multilateral arrays, and linear or cubic modes are used to specify the value of each node. Open-source code compiler FlexPDE is a quick solution with a user-friendly interface and a dynamic mesh domain that refines or coarsens local areas based on solution convergence. Additionally, CVFEM (Combined Finite Volume and Finite Element Method) is used for improved and reliable calculations due to its incorporation of finite volume with finite element.

5. Results and Disscusion

This article presents a numerical analysis of the flow structure and temperature profile in a partially lid-driven rectangular cavity, with different values of the direction of partial lid walls. The numerical analysis was carried out with a fixed Prandtl number (0.71) and a circular obstacle kept at a cold temperature.Figure 2 illustrates the changes in streamlines and isotherms as the partial lid-wall moves in different directions. The streamlines increase as the lid-wall moves to the left or right. Heat behaves differently depending on the direction of the lid-wall movement. When both partial lid-walls move outward, the maximum heat transfer occurs in the corner lid walls, while when they move inward, the maximum heat transfer is observed in the center of the lid-walls. Figure 3 displays the local Nusselt number's heat rate flow behavior against the directions of the lid-walls. When the lid-walls move to the right, heat transfer increases, and it decreases when they move to the left. Inward movement of the lid-walls results in the maximum heat transfer in the middle of the cavity in Fig.3, whereas outward movement results in the minimum heat transfer at the middle of the cavity.



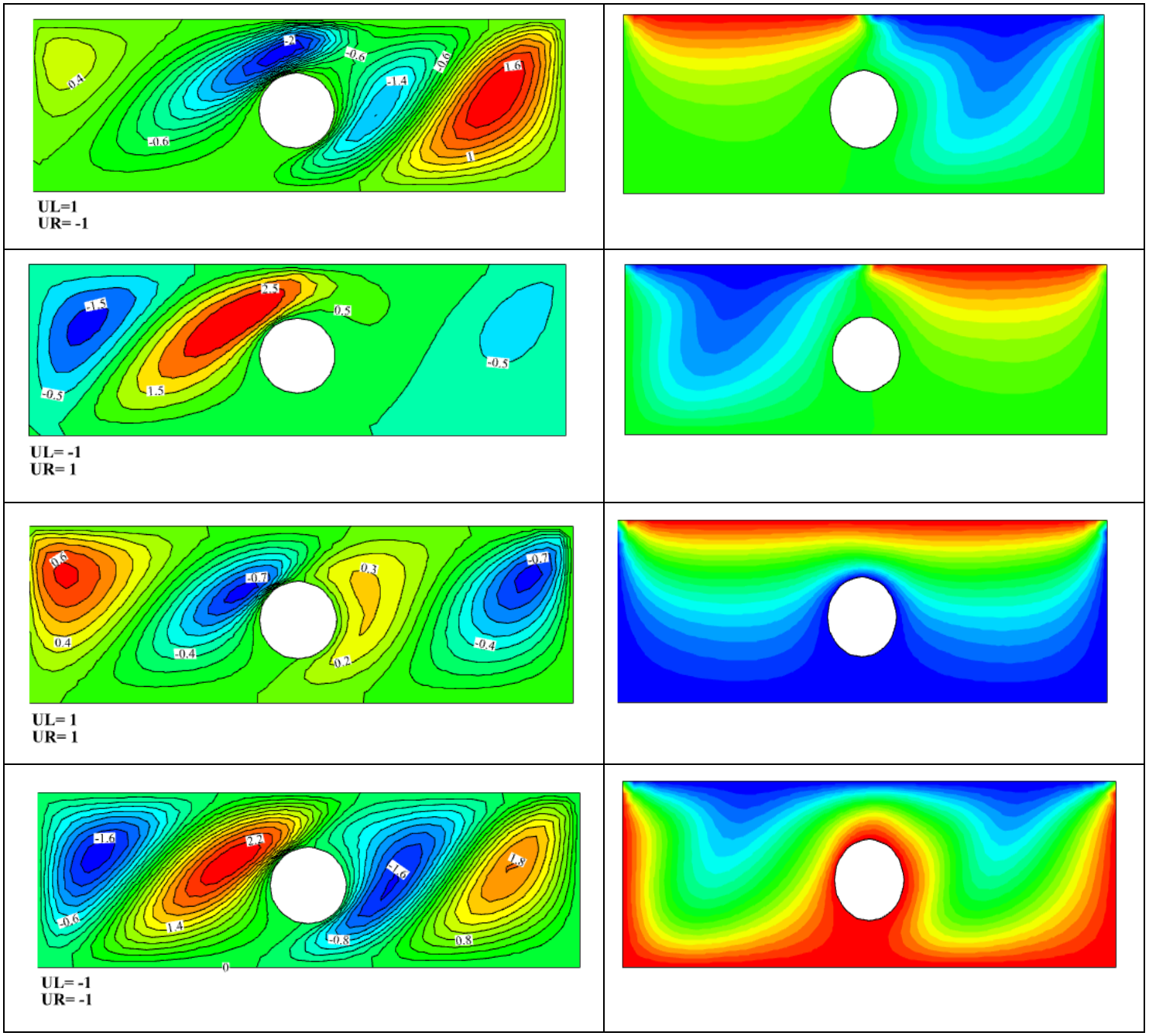


Fig2. Variation of streamlines and isotherms with respect to various directional velocity of lid-walls when Ri = 0.1, Re = 300, Le = 0.5, Br = 4 for cold elliptic obstacle.

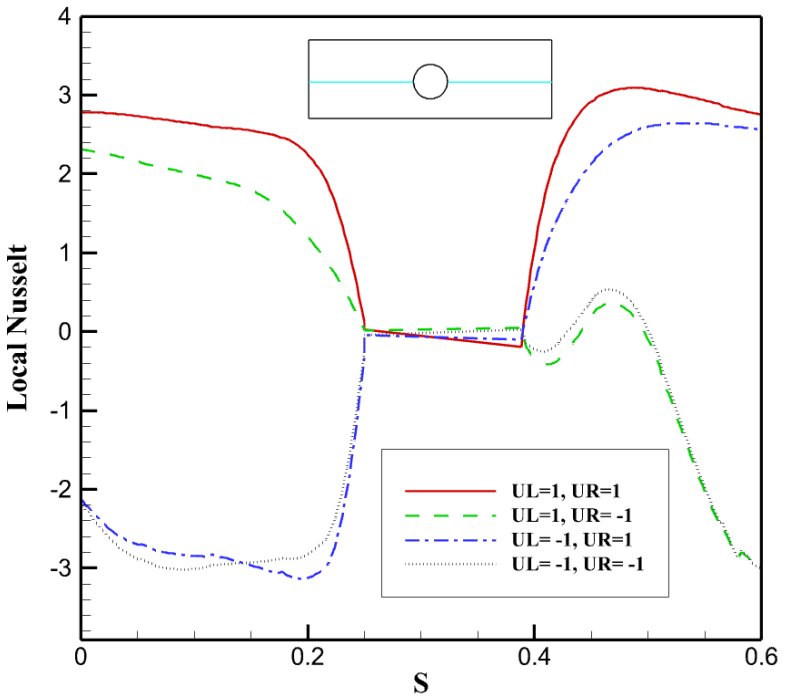


Fig3. Variation of Local Nusselt number with respect to various directional velocity of lid-walls for cold elliptic obstacle.

6. Conclusion

A two-dimensional, partially lid-driven rectangular cavity with circular cold obstacle concentration employs forced convective heat transfer. The partial lid wall's various directional velocities account for the impact on streamlines and isotherms activity. The effect of moving partial lid walls on isotherms is greater. When the inner path of the lid walls is on the opposite side, the core of the cavity typically has the best streamlines. The velocity direction of the partly closed walls has significantly increased the rate of mass and heat transfer. Thus, the movable wall of the partial lid can serve as effective control parameters for managing thermal conditions within a system.

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Healing Beyond Nature's Limits: A Look into the Fascinating World of Tissue Engineering

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Abstract

Tissue engineering (TM) is a field of research that aims to create functional biological tissues using a combination of cells, biomaterials, and growth factors. This technology has the potential to revolutionize healthcare by providing new solutions for treating injuries, diseases, and congenital defects. Tissue engineering can be used to create replacement tissues and organs, as well as in vitro models for drug testing and disease research. It involves a range of techniques, including cell culture, biomaterial synthesis, and scaffold fabrication, and requires a deep understanding of the biology of the target tissue. While tissue engineering is still in its early stages, there have already been significant advances in the field, particularly in the development of skin, bone, cartilage, and heart muscle tissue. The potential applications of tissue engineering are vast, and include the development of new treatments for diseases such as diabetes, heart disease, and cancer, as well as the creation of artificial organs for transplantation. With further research, tissue engineering has the potential to transform the way we approach medical treatments and improve outcomes for patients around the world.

Key words: tissue engineering, Biomaterials, Scaffold, Cell culture, Regenerative medicine

1. Introduction

Tissue engineering (TM) is a new combination of science and engineering tools with biological knowledge. One of the most important tissue engineering approaches is the replacement of healthy tissue with damaged tissue in a patient with an injury or organ defect. To execute tissue engineering or regenerative medicine strategies, it is necessary to incorporate suitable physical and cellular signals that facilitate interaction and integration with tissues and cells [1]. The primary aim of tissue engineering research is to create innovative clinical technologies that can effectively treat diseases that conventional methods have been unable to cure [2].

As of now, performing tissue engineering procedures remains a complex task that involves multiple challenges including the preparation of cells and appropriate scaffolds for implantation into the patient's body. However, with ongoing research and development, it is optimistic that these challenges will be addressed in due course.

2. History of TM

The idea of generating new tissues and restoring body parts or organs has been present in human imagination since the beginning of recorded history. In ancient Egypt, for instance, revitalization in the Afterlife was believed to require reuniting and reassembling the body through the restoration of body parts - a belief that was reflected in the spells known as the "Pyramid Texts" (2375 BC) [3].
According to Nerlich and colleagues, they have documented an ancient Egyptian prosthetic false toe dating back to 950-710 BC, which is considered to be the earliest known limb prosthesis (figure 1a) [4]. In 278 AD, Saints Cosmas and Damian carried out a leg transplant procedure by transplanting a leg from a deceased donor onto a patient who had previously undergone an amputation (figure 1b) [5]. In a more recent incident in 2013, doctors in China successfully saved a man's severed hand by first grafting it to his ankle and then reattaching it to his arm (figure 1c) [6].

figure 1: Evolution of Tissue Engineering: Examples of Developments Over Time [7].
(a) ancient Egyptian prosthetic false toe (950-710 BC)
(b) The leg transplant procedure by transplanting a leg from a deceased donor and attaching it to a patient who had an amputation in the past (278 AD).
(c) In China, doctors were able to save a man's severed hand by attaching it to his ankle first and then reattaching it to his arm. The procedure was successful (2013).

In the mid-1980s, Joseph Vacanti and Robert Langer developed scaffolds that were appropriate for delivering cells rather than just seeding them on a matrix. This was a turning point for TM as an emerging technology. As a result of their collaboration, Tissue Engineering was published.
by the mid-1990s, when the BBC broadcast featuring auriculosaurus, the mouse with a human ear, TM gained public attention [8, 9] (figure 2).

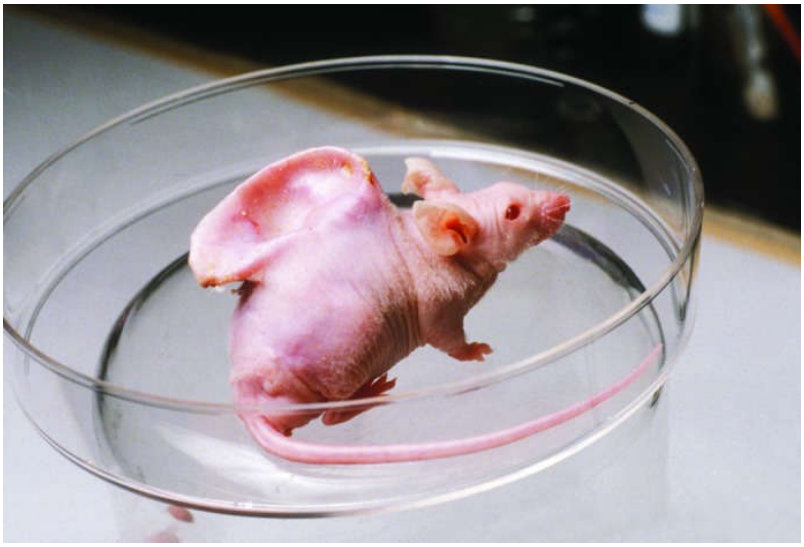

Figure 2: The auriculosaurus, a mouse developed by the Vacanti laboratory, is known for its unique feature of bearing human-like ears. This creation has come to symbolize the remarkable progress made in the field of Tissue Engineering [10].

3. The three fundamental components of tissue engineering

Tissue engineering applications rely on three key components: cells, signals, and scaffolds, which are collectively referred to as the "triad of tissue engineering" (as presented in Figure 3). Although certain tissue engineering applications may not involve all three pillars, the combination of cells, signals, and scaffolds is generally deemed essential for success. Recent advancements in tissue engineering have focused on improving each pillar of the triad [7]. In Figure 4, different cell sources has been shown.

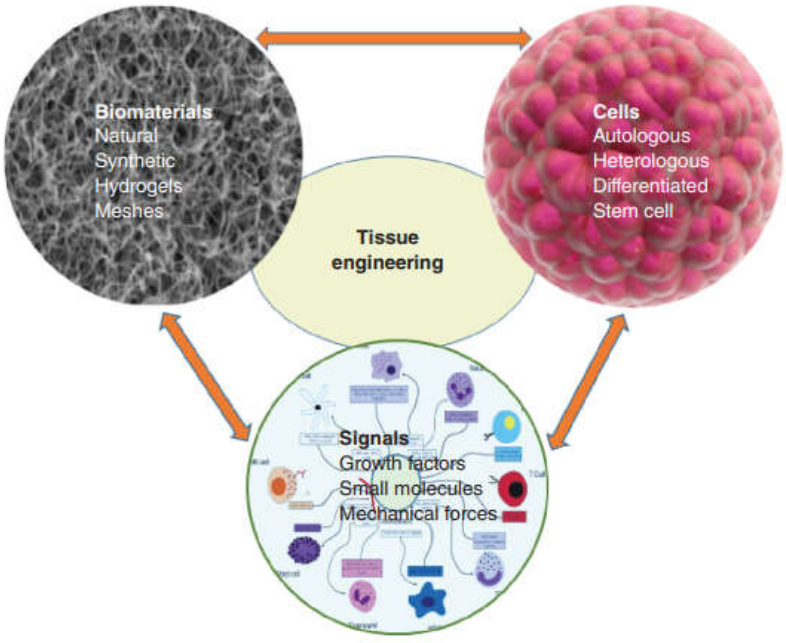

Figure 3: three key components of TM: cells, signals, and scaffolds [7]

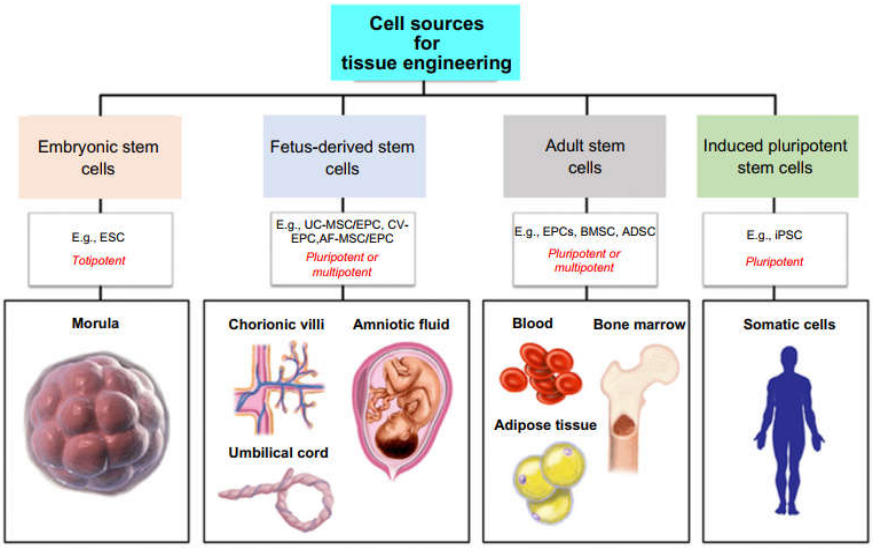

Figure 4: Various cell sources utilized in tissue engineering [11]. (Some portions of the original artwork have been modified. see the original figure in [12])

4. Applications of TM

1.Restoring or replacing damaged bodily tissues
2.Utilizing as educational and research models
3.Assessing the effectiveness of new drugs (using engineered tissues to test their performance)
4.Generating food products (producing engineered meats that resemble conventional meats) [7, 13]

5. Challenges and future

Despite many current Tm products, limitations are mainly due to difficulties in achieving biological functions of cellular constructs and host compatibility. However, advances in fields such as stem cells and bioengineering are expected to improve the critical challenges of engineering tissues in clinical settings (figure 5) [14].

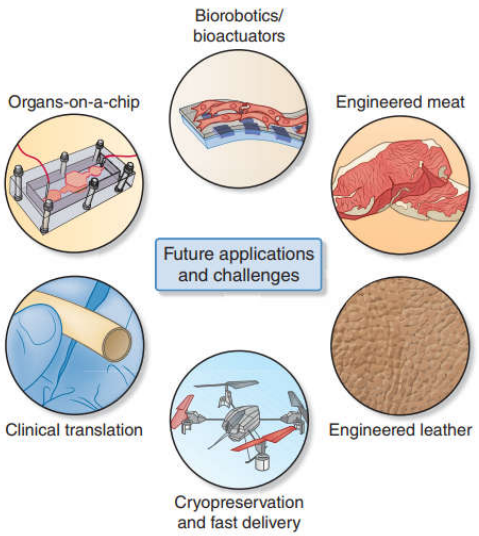

Figure 5: Future and challenges of TM [14]

6.Conclusion

In conclusion, tissue engineering is a promising field that has the potential to revolutionize medicine by providing new solutions for replacing damaged or diseased tissues and organs. Through the use of biocompatible materials, growth factors, and stem cells, researchers are developing innovative approaches to engineer functional tissues that can integrate with the body's natural systems. While there are still challenges to overcome, such as achieving proper vascularization and avoiding immune rejection, the progress made in tissue engineering thus far has been significant and provides hope for the future of regenerative medicine [15-18].

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A Protection Method for Solar Microgrids Based on Harmonic Analysis

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Abstract

The trend toward solar Microgrids (MGs) is significantly increasing by employing photovoltaic Distributed Generators (DGs) which leads to new challenges, especially in the fault detection. This paper proposes an algorithm based on the Total Harmonic Distortion (THD) of the grid voltages to detect the events of faults in solar MGs. The algorithm uses the THD together with the estimate amplitude voltages and the zero-sequence component for the detection and identification of the faults. The performance is evaluated by using MATLAB/Simulink simulations to validate the capability for detecting different fault types in the least possible time.

Key words: Solar Microgrids, Power System Faults, Protection Method, Total Harmonics Distortion.

1. Introduction

Energy demand is rising due to economic growth driving the electrical power system to expand. However, this expansion is constrained by financial aspects considering the construction of extensive infrastructure. Depending on distributed generation (DG) units appears to be applicable concept to satisfy the growing energy demand without extensive investments in infrastructure. DGs are small-scaled electric power generation units that vary between 1 kW to 50 MW and generally installed at the distribution level near the loads. They can be connected at customer level or at the distribution feeder, which support independence, facilitate sustainability, and deliver effective cost-saving solutions in the long term [1]–[4].

A microgrid (MG) is a low-voltage power network with some distributed generations (DGs) and a cluster of loads that can run in parallel with the utility grid or independently. MGs are a means to increase the distributed penetration of renewable energy, such as photovoltaic and wind systems into the electrical grid. MGs have a positive impact on the environment and the economy by supplying power locally, eliminating losses in the lines, and offering continuous energy supply with improved reliability and efficiency [5]. MGs have several technical challenges related to its operational modes and characteristics, being the protection system a major issue, since should be protected from different kind of faults [6]. The magnitude and direction of fault currents in a microgrid change depending on the system configuration, due to the bi-directional power flow from the loads and the generators passing through the protective devices (PDs) [7]. The grid is the source of the majority of faults during grid-connected mode of operation, which results in very large fault currents. However, for islanding mode operation, the faulted currents are much smaller, due to the power limitations of semiconductor

devices, which could be not enough to trigger a breaker [8]. Under such circumstances, the traditional PDs are unable to acquire enough information about the fault to detect the problems caused, which could lead to equipment instability and damage [9]. In recent years, several fault detection methods have been proposed for protecting microgrids, which can be grouped into differential, voltage, adaptive, and harmonics methods, each of them having advantages and weak points. Differential based methods are relatively simple [10], giving a high speed and sensitive fault detection response, and being unaffected by changes in the current's flow direction and magnitude. However, they have problems due to the rely on communication channels and because of imbalances and transients. Voltage based methods [11] have a good ability for preventing blackout in the system. But, they have inadequate sensitivity in grid-connected mode, and the voltage drops induced by faults can create errors. Adaptive based methods [12-14] are those in which the relay settings are automatically readjusted to be compatible with the power system conditions. However, they require a fault analysis and computation for determining the relay settings, as well as prior knowledge for network upgrades. Harmonic based methods [15-17] use the harmonic content of voltage and current for the fault protection. In [15], the Fourier transform (FFT) is used for achieving the required harmonics and, therefore, compute the THD and define the protection algorithm. However, the FFT implementation supposes a high computational burden for a digital processor. In [16], a costeffective solution is introduced for microgrid protection using a new relay to detect and isolate the fault by injecting harmonic signals. Therefore, it acts like a directional relay with no need for any voltage transformer. Another interesting approach is presented in [17], which involves introducing a certain amount of a fifth harmonic to the fault current so that the protection device can identify the fault based on the low harmonics extracted using a digital relay with a FFT. This paper presents a microgrid fault detection algorithm based on the measured THD levels of the grid voltages, the estimated amplitude voltages, and the zero-sequence components to detect, and identify, the faults that could happen in different locations of a MG [18]. This paper is submitted as a part of the Ph.D. of Electrical Engineering work at Polytechnic University of Catalonia. The rest of the paper is structured as follows: Section II presents the proposed detection algorithm in detail. Section III presents a MG as a case study to test the approach. Simulations are carried out to validate the performance of the proposed algorithm in section IV. Finally, the conclusion is provided in section V.

2.Protection Method

Faults might occur in one or more phases of the grid to the ground, or between phases only. Then, in this paper, an algorithm is defined using three stages for fault detection. Fig. 1 depicts the block diagram of the algorithm.

1-2- Pre-processing part

In this part, the three-phase voltage signals, v_{abc}, are sensed in time domain at the solar MG to measure the THD, named as THD_{abc}, obtain an estimate of the voltage amplitudes, defined as $\tilde{A}_{abc}$, and the zero-sequence components which defined as V_{abc0}, for fault detection and identification. The THD is computed for each phase of the grid, THD_{abc}, according to the method reported in [19] which is obtained according the standard definition of [20, 21] as: $THD = \frac{\sqrt{\sum_h |A_h|^2}}{A_1} \times 100\%$. where h is the harmonic order and Ah is the amplitude of the h-th harmonic component, with h≠1, and A1 is the

amplitude of the fundamental component. Fig. 2. Depicts the block diagram of the THD method, composed by few blocks: second order generalized integrator (SOGI) grid monitoring system [22], a LPF and few math operations. The SOGI is used to provide an estimate of A1 and the rest of harmonic components contained in the voltage signal, named as e(t). The zero-sequence voltages are used to identify between 2PH and 2PH-G, as both have the same conditions at the fault starting. The zero-sequence voltage calculates as $V_{abc0} = (v_a + {}_b + v_c)/3$.

2-2- Detection part

The fault detection is made, at the middle of Fig. 1, by using a threshold comparison with the $THDabc$ voltages that had been measured in the previous stage. For the fault identification $\tilde{A}\ abc$ and $Vabc0$ are also used. The algorithm is tested in a 0.42kV MG. The IEEE standard 519-2014 provides recommended values to limit the voltage harmonic distortion to 5% [23]. And according to the technical requirements in the Spanish grid code for reliable energy integration, the acceptable grid voltage drop range at the same level is set to 7.5 % [24]. Therefore, in this paper two thresholds are defined to help in fault detection and identification:
- α to detect the fault when the $THDabc$ surpass a 5%.
- Δv to detect the fault when the estimate of the voltage amplitude $\tilde{A}\ abc$ drops more than 7.5%.

3-2- Decision part

In this stage, at the lower part of Fig. 1, the detection is done based on the behavior of $\tilde{A}\ abc$, $THDabc$, and $Vabc0$ as follows, and depending on the fault case. The faults had been classified into eleven categories numbered from 0 to 10 as shown in Table 1.

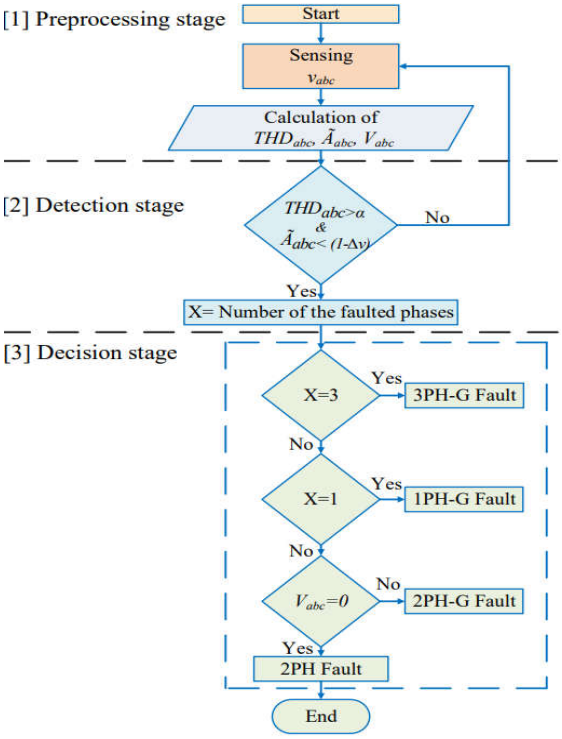

Figure۵ : Flowchart of the protection algorithm

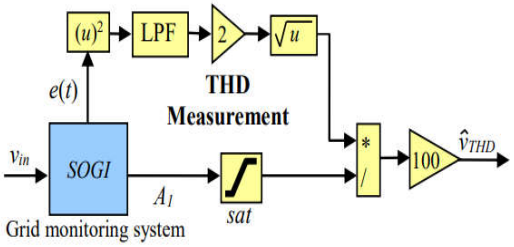

Figure 2: THD measuring block diagram

Table۱ : FAULT CLASSIFICATION

Fault Case	AG	BG	CG	ABG	ACG	BCG	ABC	AB	AC	BC
Code	0	1	2	3	4	5	6	7	8	9

1) Symmetrical faults

These faults affect the three phases equally. The faults can happen between the three phases to ground (3PH-G) or between the three phases (3PH). In both cases, there are an abrupt increase in $THDabc$ and, at the same time, a sudden decrease in$\tilde{A}\ abc$ to 0 pu.

2) Unsymmetrical faults

Unsymmetrical faults cause an imbalance between the phases, which regarding the ground can be classified into:

a) Phase-to-ground faults: These faults can occur in two of the phases to ground (2PH-G), or in only one of the phases to ground (1PH-G). In this case, there is an abrupt increase in the measured THDs and, at the same time, a sudden drop in the estimated amplitudes to 0 pu that happen at the phases affected by a fault.

b) Two phase faults: These faults can happen between two of the phases (2PH), between a-b, a-c, or b-c phases. Then, an abrupt increase in the THD and a sudden drop in the estimated amplitudes of two of the grid phases is produced. However, in these cases, unlike the other grounded fault cases (i.e 2PH-G), and due to the absence of the ground connection and the low impendence between the faulted phases the estimated amplitude voltages go down just to 0.5 pu. As there are no zero-sequence sources in the 2PH faults [25], then if $V_{abc0} = 0$ the fault consists in a 2PH and to 2PH-G if $V_{abc0} \neq 0$.

3. CASE STUDY

The electric system used for testing the behavior of the algorithm is shown in Fig. 3 and the parameters are listed in Table II. The system is composed by a 11kV grid with a Distribution Line (DL1), passing through a step-down transformer connected to a MG. The DL has the breakers (CB3 and CB4) that allows the disconnection in a fault event. A Solar DG (DG1) and local load (Load1) are connected to form a MG in Zone 1, that has its own relay and breakers (Relay1, CB1 and CB2). The algorithm is defined inside the relay for fault detection.

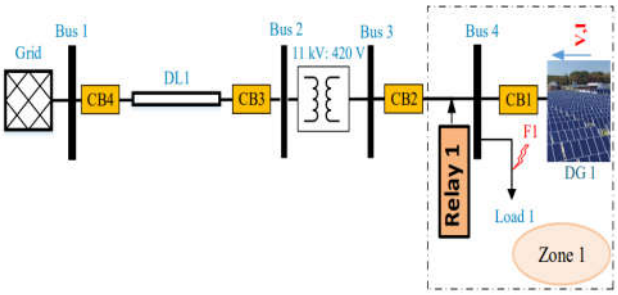

Figure 3: the test solar microgrid

Table 2: test network data

Main Grid	20 KV	
Line	R=0.16Ω/km	L=0.109H/km
	C=0.31μF/km	Length=4km
Transformer	20/0.4kV	500kVA
Load1	400kW	0.4kV
DG1	80kW	0.4kV

5. RESULTS

Simulations had been performed using MATLAB/Simulink at steady state to validate the performance of the detection algorithm under fault events. In this section, the fault cases are carried out in F1 location of Fig. 3 at 0.2s.

A. Three phase fault (3PH-G) Fig. 4 shows the detection algorithm behavior during the fault. In the healthy condition, when the system operates normally before the faults, the voltages have no harmonics, so the waveforms are sinusoidal. The measured THD is 0 and the estimated amplitude voltages are 1 pu. In this condition, the algorithm is waiting for an event of fault, therefore no detection action is performed. At 0.2s, the THD_{abc} increase abruptly, and when the condition THD_{abc} > 5% is meet the fault is detected. At the same time, $\tilde{A}_{abc}$ drops towards 0 pu and when $\tilde{A}_{abc}$ < 0.926 pu, at this moment and due to the achievement of the two conditions, the fault is identified. Notice that $THDabc$ have a behavior that creates a peak and after a short time exponentially decays to zero. The detection process has been measured and it takes 7ms as shown in Fig. 5.

B. Tow phase fault (2PH) A fault between phases b and c, BC-fault, is considered in this case. Fig. 6 depicts the algorithm behavior during the fault. As in the previous case, at 0.2s THD_{bc} increase abruptly, which makes the fault to be detected when THD_{bc} > 5%, while THD_a<5%. Meanwhile, $\tilde{A}_{bc}$ drops towards 0.5 pu, due to the absence of the ground connection and to the impedance between the phases ($Zf = 1m\Omega$), while $\tilde{A}\ a$ remains unaffected (1 pu). Then, when $\tilde{A}_{bc}$ < 0.925 pu and V_{abc0} is checked to be zero, the fault is identified. Similar to the previous case, the THD_{bc} peak behaviour exponentially decays to zero after a short time. The detection process has been measured and it consists in 7.5ms as shown in Fig. 7.

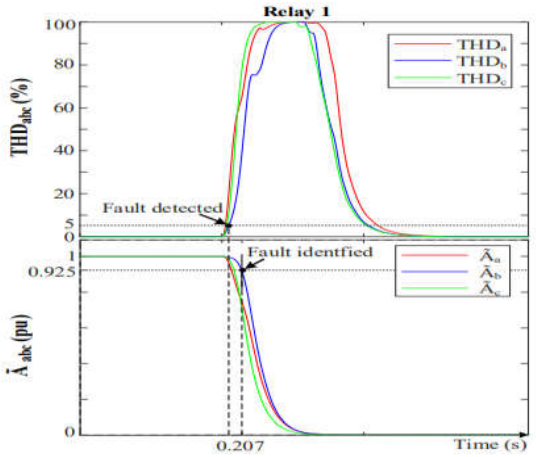

Figure 4: THDs and $\tilde{A}$ during the 3PH-G fault at F1.

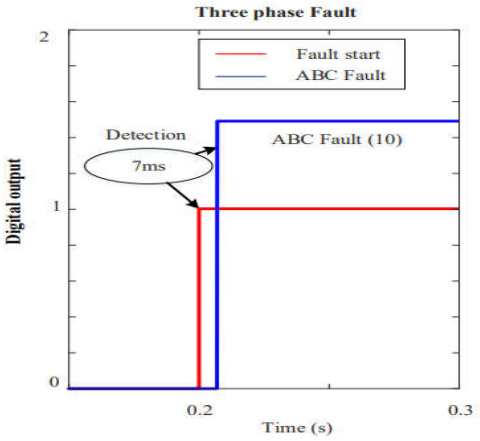

Figure 5: Digital outputs of the algorithm in case of 3PH fault

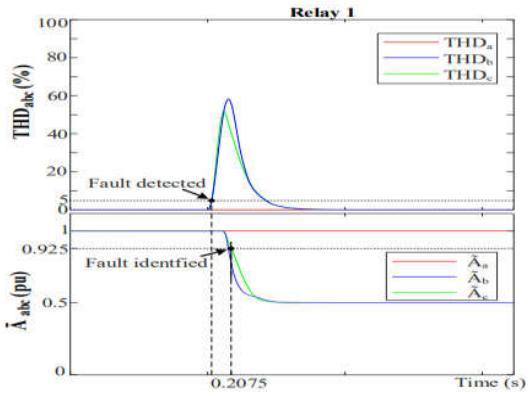

Figure 6: THDs and $\bar{A}$ during the 2PH fault at F1.

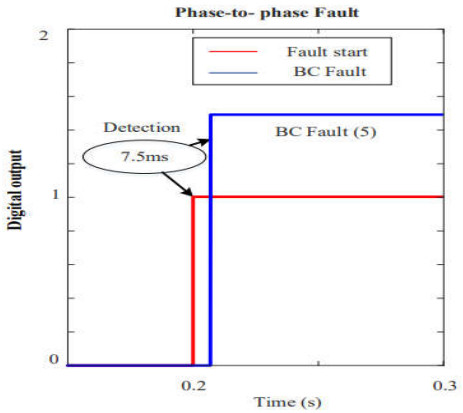

Figure 7: outputs of the algorithm in case of 3PH fault.

5. CONCLUSION

This paper presents a fault detection algorithm for MGs that is based on the total harmonic behavior of the grid voltages. The THD levels of the grid voltages, the estimated amplitude voltages, and the zero-sequence components have been used to design the algorithm. Each phase in the system has its own measurement block to provide the necessary data to be mentored in the algorithm. The MATLAB/Simulink simulations results show that the algorithm has the capability to detect and identify different types of faults that might occur in the electric system in the least possible time. The detection time in all the fault cases is less than 10 ms. The method used for obtaining the THD supposes a low computational burden for being implemented in a digital signal processor.

6. Acknowledgments

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Outbreak of Koi herpesvirus (KHV) disease in Shahmirzad, Semnan

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Abstract

The aim of present study was to determine the cause of mass mortality in koi during June 2022 in Shahmirzad in Semnan province. A total of 32 fish out of 450 were referred to Aquatic animal Diseases laboratory, Faculty of Veterinary Medicine at Semnan University. Gill necrosis, enophthalmia and notched nose were the most prominent clinical signs in reffered fish. Specimens of Gill and kidney from the infected koi were taken for identification using PCR. In this study, PCR products were amplicons with size of 409bp that indicated the presence of *Cyprinid Herpesvirus -3*(CyHV-3) in all of the samples which were taken .It is the first report of KHV in Semnan Province.

Key words: KHV, Koi, PCR, Ornamental fish

1. Introduction

Last few years, ornamental fish industry has considerable growth (1). Koi (*Cyprinus rubrofuscus*) is a type of ornamental carp that are highly prized for their beauty and elegance. They are native to Japan, but have been introduced to many other countries around the world. Koi fish come in a wide range of colors and patterns, including white, black, red, orange, yellow, blue and green. They are often kept outdoor ponds or indoor aquariums and require a carefully balanced environment to thrive (2). Koi fish are known for longevity, with some individuals living for more than 50 years. They are also considered symbols of good luck and prosperity in many cultures.

Intensive culture resulting in poor welfare and lead to increasing disease prevalence and death of this beautiful fishes (3). Major emerging viral diseases of ornamental fish can be divided into distinct families, including Alloherpesviridae, Iridoviridae, and Nodaviridae (4).

KHV or Koi herpesvirus is a highly contagious viral disease that affects common carp (*Cyprinus carpio*) and its varieties including mirror, leather, ghost, koi etc (5). KHV or CyHV-3 belongs to the family of Alloherpesviridae (6). It can cause significant mortality rates in infected populations. The virus primarily targets the gills, skin and internal organs of fish, leading to respiratory distress, skin lesion and oragan failure (7). Incubation period of KHV can range from 3 to 14 days, during which infected fish may not show any symptoms. Once symptoms appear, infected fish may become lethargic, lose their appetite,

and develop white or gray patches on their skin (8). Infected fish may also exhibit rapid breathing, flashing and excess mucus production. KHV is highly contagious and can spread through direct contact between infected and healthy fish as well as through contaminated water and equipment. Lowering the water temperature can result in reduced, or even no, mortalities, but the fish can become persistently infected and a major source of concern for the spread of the virus. It's important for koi owners to take preventive measures, such as quarantining new fish and regularly testing their water quality, to reduce risk of KHV outbreaks in their ponds or aquariums. There is no known treatment for this disease same as other viral disease. The aim of this study was to investigate the main cause of mass mortality in koi which was kept and breed in garden pond in Shahmirzad, Semnan province.

2-Materials and Methods

Following sudden death of koi, 32 live fish with clinical sign was transferred to Aquatic animal Diseases laboratory, Faculty of Veterinary Medicine at Semnan University. In history taking, owner stated that fish had erratic swimming, were inappetence. In macroscopic examinations, enophthalmos and notched nose were evident and gill necrosis. First, wet smears were prepared from skin and gills, and examined under a light microscope for parasitological examination. Then, fish were euthanized, necropsied, and internal organs were examined separately. Bacterial culture from kidney tissues was performed on blood agar and incubated at 25 °C for up to 72 h. Histological analysis and PCR assay were also performed using samples obtained from kidney and gill tissues.

2-1-DNA extraction

The tissue samples were homogenized and total DNA was extracted using Sina Pure ^{TM} Kit (DNA Extraction) according to the manufacturer's instructions. Qualitative and quantitative assessment of the extracted DNA was conducted by measurement of absorbance using the Smart Nano (Canada).

2-2-Molecular identification

Virus DNA detection and quantification were performed using real-time qPCR according Matras et al.(2019). Briefly, qPCR was performed as follows: the forward primer sequence was 5GGG-TTA-CCT-GTA-CGA-G -3′; the reverse primer was 5′- CAC-CCA-GTA-GAT-TAT-GC -3″. Cycling conditions were 1 cycle at 95°C for 15 min and 40 cycles at 94°C for 15 sec and 60°C for 60 sec.

3-Results

Infected fish typically have white, necrotic patches on the gill filaments (Fig.1), sunken eyes (Fig.2), notched nose(Fig.3), haemorrhages on the body surface, and excessive mucus production with rough pale patches on the skin. Histopatological examination revealed degenerative and necrotic changes in gill epithelium. There was hyperplasia and fusion of the secondary gill lamellae were found in prepared slides. Intranuclear inclusion bodies were observed in Kidney, spleen and liver. In this study, PCR products were an amplicons with size of 409bp that indicated presence of koi herpes virus 3 in all of the specimens which were collected from 32 koi and examined using chain reaction (PCR) assay.

Fig.1: Necrotic patches on the gill fillaments

Fig.2: Endophthalmi and notched nose in infected koi with KHV

4-Discussion

Over the last years, KHV is an important emerging infectious disease that affect carp and Koi and should be reported to the OIE. This disease were reported from many countries, but it is more commonly in Asia, Europe and North America. The virus was first identified in Israel in the 1990s and has since spread to other parts of the world (10). First time Rahmati Holasoo et al. reported KHV from koi in Iran (11). A factor having an important impact on the rate and extent of spread of KHV has been unrestricted trade in koi. Besides the possibility of horizontal transmission of the virus directly from fish to fish, the ability for the virus to spread by vectors like water, should also be taken into consideration. However, hypothetically, animate vectors, *e.g.* other fish species, parasitic invertebrates, and piscivorous birds and mammals can also be involved in transmission (12). Temperature is a critical factor in the pathogenesis of KHV infection. The virus is persistent, and this is related to a cycle of persistent infection and reactivation in hosts given that the virus was found to be present not only in water and pond sediment (13), but also in tank standpipes up to 6 days after removing fish (Tolo et al.,2021). Effective disinfection is crucial to reduce the spread of the disease.

Detection and identification of KHV in Koi fish samples is important for several reasons:

1- Early detection and control of outbreak: identifying KHV in Koi fish can help in early detection and control of outbreaks, thereby preventing the spread of the virus to other fish population

2- Understanding genetic diversity and evolution of the virus which can help in the development of effective vaccines and treatments.
3- Prevention of economic losses: Koi fish are valuable commodity in the ornamental fish industry and outbreaks of KHV can result in significant economic losses. Early detection and identification can prevent such losses.
4- Protection of wild fish populations: KHV can also infect wild carp populations which can have ecological implications. Identifying KHV in Koi fish help in monitoring and protecting wild fish populations from the virus.

Several emerging infectious diseases have serious implications for the trade and husbandry of ornamental fish. Although many of these diseases have been well studied and described in certain species, there are still many diseases that are not well understood and more investigation is needed.

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Investigating the effect of CD33 and TREM2 genes on the pathogenesis of Alzheimer's disease and their therapeutic targets

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Abstract

Alzheimer's disease (AD) is the leading cause of dementia worldwide, which presents great challenges for policy makers, health care professionals, and family members of AD patients. Symptoms of AD include progressive memory loss, impaired executive function, and inability to perform daily life activities. AD is histologically characterized by intracellular neurofibrillary tangles (NFTs) composed of hyperphosphorylated tau protein in neurons and accumulation of extracellular plaques composed of amyloid beta peptide (Aβ) in the brain. These protein clumps are associated with the loss of synapses and neurons. In genome-wide association studies (GWAS), many Alzheimer's disease risk genes have been identified to be associated with the immune system response, particularly microglia, including the phagocytic receptors CD33 and TREM2. Impaired microglial phagocytosis leads to amyloid beta (Aβ) accumulation. This leads to neuroinflammation, which is the main cause of neurodegeneration. CD33 and TREM2 modulate Alzheimer's disease pathogenesis and neuroinflammation and have emerged as therapeutic targets in Alzheimer's disease. TREM2 is essential for microglia recognition and response to signs of neurodegeneration, and TREM2 expression correlates with phagocytosis rate. The CD33 receptor, also called Siglec-3, inhibits TREM2 receptor-induced phagocytic activity of microglia. Targeting neuroinflammation through CD33 inhibition or TREM2 activation with gene therapy, small molecules, or immunotherapy may have important implications for neurodegeneration in Alzheimer's disease and may be complementary to anti-Aβ monoclonal antibody therapies that target plaques which removes plaques without reducing neuroinflammation. Advances have also been made in inhibiting CD33 by gene therapy, small molecules or immunotherapy, and increasing TREM2 activity by immunotherapy, which may reduce neuroinflammation in the brains of Alzheimer's patients.

Key words: Alzheimer's disease, AD, Gene Expression, CD33, TREM2.

1. Introduction

Alzheimer's disease (AD) is a progressive neurological disorder characterized by cognitive and functional impairment and memory loss (1). According to recent reports, it is estimated that as the population ages, the number of dementia cases in the world will rise to 152 million by 2050, which is currently the most common form of dementia. Most AD cases are sporadic and with late onset (LOAD) that occurs at age 65 or older (2). The disease leads to a wide range of social and financial burdens. Therefore, there is an urgent need to understand the cause and pathogenesis of disease and early diagnosis. For decades, the amyloid cascade hypothesis was the main pathogenic theory. Improper processing of amyloid beta precursor protein or dysfunctional clearance of beta-amyloid peptide (Aβ) leads to accumulation of Aβ plaque followed by deposition of nerve fibrillar screws (NFTs), ultimately causing disruption and loss. However, the failure of numerous therapeutic trials targeting Aβ clearing suggests that there should be other pathogenic mechanisms such as inflammation. More recently, some genome-wide association studies (GWAS) have found links between AD and key microglial genes, including the CD33 and TREM2 genes that are involved in innate immunity, suggesting that mediated action is a key factor in the development of the gene pool (1).

2. Structure and function of CD33

In terms of the gene map CD33 is located on chromosome 19q13.33 in humans. CD33 or Siglec-3 encodes a transmembrane glycoprotein of 67 kDa and is a member of the Sialic acid-binding immunoglobulin-type lectins (Siglecs). In mammals, CD33 is expressed on hematopoietic and phagocytic cells, including hematopoietic precursors, myelomonocytic precursors, macrophages, monocytes, dendritic cells and microglial cells. The CD33 gene contains seven encoding exons, which can be described from extracellular to cytosol as follows: Exon 1 encodes an 18-amino acid signaling peptide (SP, aa1–18), followed by a 31-amino acid peptide and an early stop codon. CD33 translation of ATG begins at Exon 1. Exon 2 encodes the Ig-like V-type (IgV) (aa19-135), which is the binding site of sialic acid ligands and is considered the functional domain of CD33. Exons 3 and 4 encode a C2-type Ig repeat structural domain (C2-Ig domain, aa145–228). CD33 contains only one C2-Ig domain and is the shortest member of the Siglec family. This means that CD33 is more likely to bind ligands (glycans or glycolipids) on the surface of the same cell (cis ligands or cis activation) and prevent CD33 from binding to other cells or foreign objects (trans ligands). Exon 5 encodes a transmembrane domain (aa260–282). Exons 6 and 7 encode two cytosolic immune tyrosine inhibitory motifs (ITIMs): a membrane-proximal ITIM (aa338–343) and a membrane-distal ITIM-like motif (aa356–361) (Figure 1). ITIM is the main domain for transmitting inhibitory signal in the cell. An ITIM-like motif has been preserved between humans and mice and possibly maintains regulatory function related to endocytosis. The ITIM-like motif may also play a role in tyrosine immune receptor-based inhibitory signaling. Due to the specific expression of CD33 in immune cells, one of the important roles of CD33 is its ability to modulate immune cell function (3). CD33 plays a role in phagocytosis and several other processes such as cellular adhesion, endocytosis, immune cell growth and inhibition of cytokine release by monocytes (4).

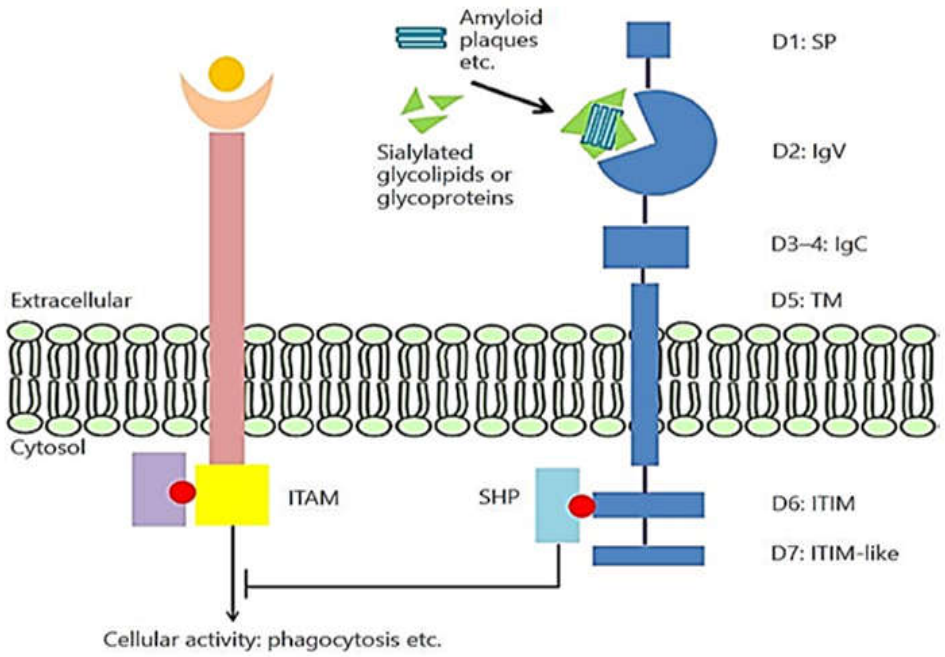

Figure 1. Schematic image of CD33 exon structure and its deterrence mechanism (3).

3. Structure and function of TREM2

The TREM2 gene is located on chromosome 6p21.1 in humans and chromosome 17 in mice. The TREM2 gene encodes a 693 base pair complementary DNA that can be translated into a 230-amino acid polypeptide. TREM2 is a cell surface transmembrane glycoprotein with a V-immunoglobulin extracellular domain and a cytosolic tail. TREM2 also has a short intracellular domain without signaling motifs. The extracellular domain of TREM2 can bind to lipopolysaccharides (LPs), lipoteichoic acids (LTAs), high-density and low-density lipoproteins (HDLs and LDLs), and several apolipoproteins. Among these apolipoproteins, APOE is a more well-documented ligand for TREM2. In addition, Aβ was recently known as the TREM2 ligand because it binds directly to TREM2 and activates TREM2 signaling. The TREM2 transmembrane region is associated with DAP12 intracellular adapter protein. TREM2 signaling via DAP12 leads to phosphorylation of the immune tyrosine activation motif (ITAM) via the recruitment of SYK tyrosine kinase. TREM2 can also operate via DAP10 adapter. DAP10 promotes the recruitment of phosphatidylinositol 3-kinase (PI3K). TREM2-DAP12-DAP10 signaling causes a cascade of signaling and subsequent immune response, leading to calcium mobilization, mammalian target of rapamycin (mTOR), mitogen-activated protein kinase (MAPK) signaling and the activation of energy metabolism (Figure 2). TREM2 is essential for detecting microglia and responding to signs of neurodegeneration. The expression rate of TREM2 is associated with phagocytosis rate (5). TREM2 activates immune responses in macrophages and dendritic cells (6). In dendritic cells, it mediates the regulation of CCR7 chemokine receptor and their maturation and survival (4). It plays a role in the positive regulation of osteoclast differentiation (7). In vitro, when TREM2 expression is increased, the phagocytosis rate of apoptotic neurons, cellular debris and bacteria or bacterial products is also increased. The loss of TREM2 can also lead to decreased phagocytosis rate (5).

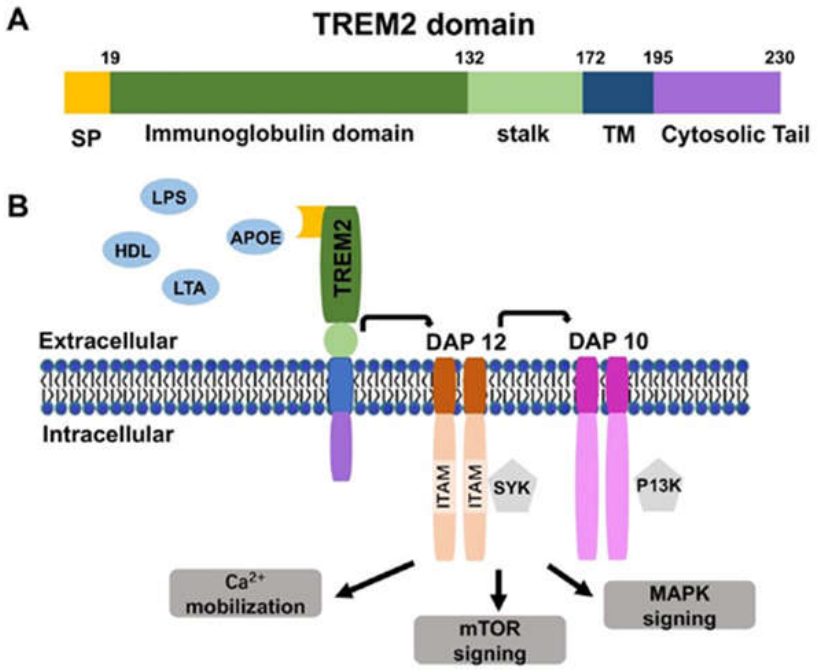

Fig 2. TREM2 domain, structure, ligands, signaling and function. SP = signaling peptide, Tm = transmembrane (5).

4. Effect of CD33 and TREM2 genes on Alzheimer's disease

Genomic-wide association studies (GWAS) have implicated innate immune genes, including CD33 and TREM2, in Alzheimer's disease. These two genes interact with DAP12 either directly (TREM2) or through common intracellular signaling factors (CD33). Thus, DAP12 and cross-signaling molecules, e.g., SHP1/2, SYK, and PI3K are probably key interfering factors between CD33 and TREM2. Interestingly, CD33 controls TREM2-induced signaling in mouse microglia. For example, the knockout of the CD33 receptor in 5xFAD genetically modified mice reduced AD pathology, while the TREM2 knockout intensified Aβ pathology and neurodegeneration. It was also shown that deletion of CD33 weakened the pathology of amyloid beta (Aβ) and improved cognition in 5XFAD mice, both of which were cancelled by additional removal of TREM2. But TREM2 knockout leads to neurodegeneration in 5xFAD mice, which is not saved by an additional knockout of CD33 (8).

In immune cells, cellular processes (e.g. phagocytosis, cytokine release, apoptosis, etc.) are characterized by the tyrosine-receptor-based activation motif (ITAM) that contain activating receptors and inhibitory receptors containing ITIM. CD33 inhibitory receptor can be activated by glycoproteins and glycolipids containing sialic acid, which are constantly being made in amyloid plaques in the brains of Alzheimer's patients. When binding to ligands, tyrosine is phosphorylated in ITIM found in CD33 and acts as a binding site of phosphatases containing the SH2 domain (SHPs) (3). It seems that the SHP-1 activated by CD33 inhibits downstream signaling by TREM2, particularly the phagocytosis caused. It is known that SHP-1 inhibits PI3K signaling and so it can prevent phagocytosis (9). The TREM2 receptor has several ligands of its own that can activate microglia and induce phagocytosis. The activation of CD33 receptor inhibits TREM2-induced phagocytosis in microglia. The sialic acids of glycocalyx act as ligands and activate the inhibitory CD33 receptor in microglia. The CD33 receptor activates SHP1, SHP2 protein phosphatase, which inhibits TREM2/DAP12-induced signaling via the SYK/PI3K pathway (10).

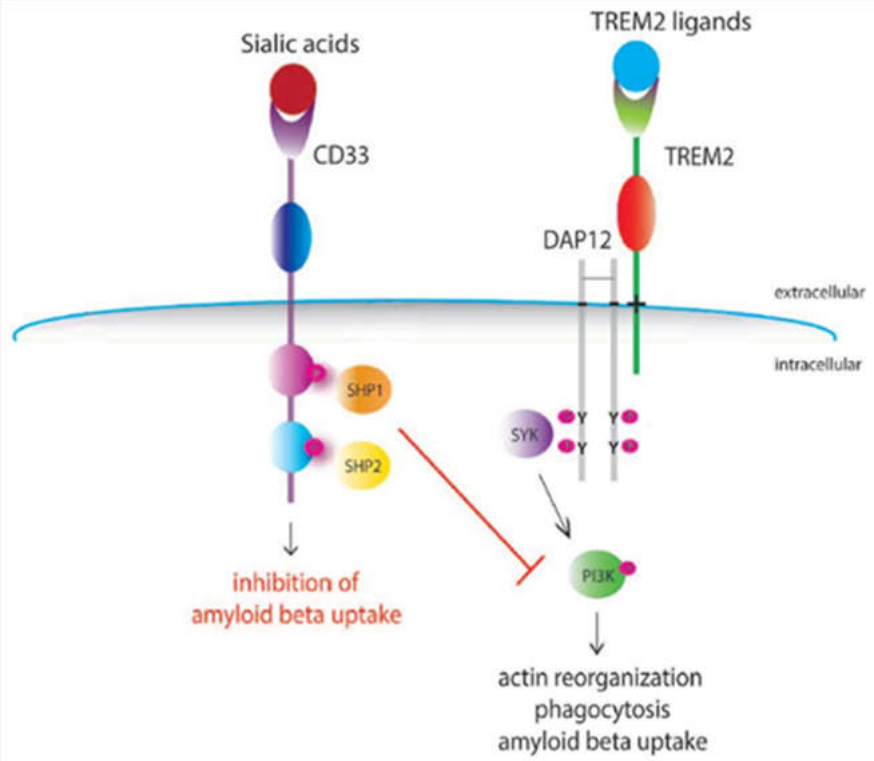

Figure 3. Working model of interference between two microglial receptors, CD33 and TREM2 (4).

5. Conclusion

AD is one of the main causes of disability and decreased quality of life among the elderly. Despite active research in the field, many fundamental questions remain about the molecular context of the disease. Old theories linking AD to genes directly related to Aβ formation and Tau hyperphosphorylation cannot explain the complexity of AD and do not provide a clear target for treatment. In fact, conventional treatments based on the Aβ hypothesis have failed to treat or prevent AD. About 99.6 percent of drugs targeting amyloid pathways, including beta-secretase inhibitors, gamma-secretase inhibitors and Aβ itself, do not have a significant therapeutic effect on people with AD. Recent studies and new hypotheses for AD have revealed many potential new targets, even though we are still far from a definitive solution. The evidence from the study of the disease and its treatment, together with the current concept of AD as "multifactorial pathology," clearly underlines the need to consider broader approaches that involve complex interactions between different pathways. Some of these biological processes were introduced and described in this study (11).

Interestingly, Griciuc et al. (2013) (12) showed that expression of CD33 receptor (Siglec-3), a vital immune suppressor receptor, was greatly increased in the microglia of patients with AD. They also showed that CD33 signaling inhibits TREM2-mediated phagocytosis in mouse microglia. Accordingly, the removal of CD33 receptors in AD genetically modified mice significantly reduced AD pathogenesis (8).

CD33 and TREM2 microglial receptors modulate microglial pathology and neuroinflammation and have emerged as targets for drug development in Alzheimer's disease. CD33 counteracts the signaling effects of TREM2 and makes an attractive target because it can potentially be inhibited, for example with gene therapy, small molecules, or immunotherapy. TREM2 appears to be a promising therapeutic target, with several agonist antibodies activating the receptor signal. Clinical trials underway with monoclonal antibodies that target CD33 and TREM2 are major steps toward targeted immunotherapy

for Alzheimer's disease. In summary, CD33 inhibition or TREM2 activation represents valuable therapeutic strategies for strengthening neuroprotective microglia and reducing neuroinflammation, which is crucial for the prevention and treatment of Alzheimer's disease. Targeting neuroinflammation through CD33 inhibition or activation of TREM2 with gene therapy, small molecules, or immunotherapy may have important implications for neurodegeneration in Alzheimer's disease and may be complementary to anti-Aβ monoclonal antibody therapies that target plaques which removes plaques without reducing neuroinflammation. Advances have been made in the inhibition of CD33 by gene therapy, small molecules, or immunotherapy, and increased activity of TREM2 by immunotherapy, which may reduce neuroinflammation in the brains of Alzheimer's patients (4).

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Fabrication of low-cost superhydrophobic coating on low-carbon steel using liquid flame spray

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Abstract

A nano-engineered superhydrophobic coating was fabricated using a two-step and low-cost method employing a liquid oxy-acetylene flame spray mechanism on mild A516 steel. Oxygen and acetylene were used as flammable gases. Nanostructured coating was obtained by flame spraying aluminum nitrate solved in ethanol on the substrate. To lower the surface energy Perfluorodecyltriethoxysilane (PFDTES) and silicone elastomer were used. Results revealed that the wettability of the surface strongly depends on the precursor concentration so that at 3.5% concentration of aluminum nitrate in ethanol, maximum contact angle (157°) occurs. The distance between the substrate and nozzle is another key parameter to control and it directly affects the contact angle. Sandpaper abrasion test showed outstanding mechanical durability so that the coating maintained in hydrophobicity range under the load of 100 grams of weight and moving 10 cm on sandpaper number 1000 for 8 cycles. The parameters affecting the process were thoroughly analyzed according to the applied liquid flame spray mechanism and the appropriate performance range of each was obtained that According to the experiments, the precursor flowrate and the distance between the substrate and nozzle should be repectively 0.9-1.9 ml/min and 14-22 cm.

Key words: Liquid flame spray, Nanostructured coating, Mechanical stability, Aluminum nitrate, Oxy-acetylene flame spray

1. Introduction

Fabrication of superhydrophobic surfaces by thermal spray methods due to their durability has drawn much attention in the past decades (1). The most widely used thermal methods include flame spray (2, 3, 4, 5, 6), plasma spray (7, 8), arc spray (9), HVOF (high velocity oxygen fuel) spray (10), and VPS (vacuum plasma spray) (11). Liquid flame spray pyrolysis (LFS) is a thermal spray method that can produce metal oxide powders from highly volatile gaseous metal chlorides that are decomposed in flame to form nano-oxide powder. The production of nano-engineered superhydrophobic coatings by the LFS method can bedone in two different ways; the direct use of nano-powder homogenized in the solvent which is recomended to use with micro-powder, due to its low mechanical durability (6), or simultaneous production of nano-powder during the process .

Regarding the direct use of nano-powder during thermal coating methods, In previous studies, rutile and anatase titanium nanoparticles have been used and the mechanical and photocatalytic

properties of the coatings have been investigated (7, 8). Among the various thermal methods, mostly plasma spray and flame spray have been used for this purpose (6, 7, 8). Titanium nanoparticles change at about 900k from anatase structure to rutile. Coatings produced by the direct use of nano-powder, less emphasis is placed on the superhydrophobicity and mostly titanium-coated properties based on hydrophilicity have been studied. Dimension and structure of the produced nanopowder are a function of temperature, so that at different temperatures, the rutile or anatase structure may be produced and as a result the dimensions of the sedimentary grains on the coating will vary (8).

Regarding the production of nano-particles during coating process, the liquid flame spray method has been used to produce different nanoparticles, e.g. titanium (5, 12, 13, 14), aluminum (4, 15, 16), zirconium (17), and silica (2, 13, 18), from ceramics. To produce nanopowder simultaneously during the process, liquid feedstock containing metal chlorides sprayed into high-temperature flame and after solvent evaporation, particles of the desired element settled on the surface under a complex process that occurs. In this process liquid precursor evaporates in the flame and form nanoparticles in the gas phase. The solvent material burns into the fire and then the evaporated precursor decomposes into the vapour of the product material. Next, nucleation occurs and after that, coagulation and sintering take place on the nucleation molecules. As the temperature of the flame decreases along the fire stream, sintering turns into aggregation and finally agglomeration (2). In many studies, titanium and aluminum nanoparticles have been produced by the LFS process and validated using X-ray diffraction analysis. Many parameters in the LFS process affect the diameter of the produced nanopowder e.g. injection flow rate If the injection rate increases, the observations indicate an increase in the average diameter of the formed nanopowders. Previous work results revealed that if 14% of the total steel surface is covered by nanopowder, it still has superhydrophilic properties (5).

Generally, studies have used oxygen and hydrogen gases to create a flame, and only a limited number of them have used other gases such as acetylene or propane (6). Using acetylene instead of hydrogen has some advantages. First, acetylene produces a higher flame temperature than hydrogen. The acetylene flame will have a maximum temperature of about 3100°C, while hydrogen will eventually create a temperature of 2800°C. Hydrogen is a more dangerous gas and more complicated and expensive equipment is needed to work with. These reasons make acetylene a more suitable and applicable option for liquid flame spray instead of hydrogen .

A good way to increase the adhesion of a nano-coating to the surface is using a hybrid micro and nanopowder at the same time (6). The increase in adhesion appears to be due to an increase in the momentum of the colloidal particles which contains micro-particles surrounded by nano-particles moving toward the steel surface, which results in higher intensity and consequently an increase in mechanical strength so that the produced nanopowders surround the microparticles and move rapidly. The results were compared with those which used only nanopowder, in which case the adhesion was up to 9 times higher (6). Affecting parameters generally include (14): Precursor injection flow rate, Distance between the substrate and the torch, Movement speed of the sample and Concentration of solute in the solvent.

As the flow rate of injecting liquid increases, the production rate of the nano powder increases. If the injection flow increases, the concentration of the solution should be reduced to prevent the small particles from joining and becoming bullet-shaped.

As the distance between the substrate and the flame increases, the solution with a higher concentrate should be used, respectively. On the other hand as the distance decreases, there is not enough time for liquid flame spray to take place so nano powder production process can't occure perfectly. Based on the working mechanism and all of the affecting parameters, the optimum distance between the substrate and the torch can be detected.

As the substrate moves faster in front of the flame, the nanopowder settles less on the surface and eventually exhibits less superhydrophobicity (14, 19).

The solvent is one of the most effective parameters in the LFS process. Two of the most widely used solvents are ethanol and isopropanol that ethanol is hygroscopic and isopropanol is not. Due to the higher percentage of water in ethanol in comparison with isopropanol at the same concentration, it delays the opportunity for evaporation and reassembly, so using isopropanol as a solvent seems to lead to a much more nanopowder production (19). The interaction between the parameters should be observed more closely. Imagine if the injection flow rate increases, in order to keep the production rate of nanopowder constant, the concentration of the solution must be decreased. In this case of increasing the injection flow rate, It is practically similar to the situation where the solute percentage has increased, which causes the flame to be longer and wider, and as a result, the maximum temperature of the flame is moved forward and the solution has more opportunity to evaporate. This means that despite the decrease in the concentration of the solution, there might be no difference in the amount of nanopowder produced (19). Reducing the energy level of superhydrophilic surfaces is done by low-energy substances such as silicon elastomers (20) and epoxy resins (21, 22, 23, 24, 25), polypropylene (26), silane derivatives (27, 28, 29) and some low-energy acids. Typical cured epoxies have surface energy around 45 dyne/cm (30) while silicon rubber which is used as lowering surface energy material has surface energy about 19-22 dyne/cm (31).

2.Methodology

Low-Carbon Steel (A516 Gr 60) is used as a working substarate. First,the substrate polished by 400-2000 sandpapers from rough to soft ones, then the samples are washed and cleaned in acetone using ultrasonic to clean all the impurities off the surface. Steel samples with 7 mm thickness and 20 mm× 30 mm size is used as shown in Figure 1.

Figure 1: Low-carbon steel samples

The mechanism used for the liquid flame spray process is a simple set up cosisting: oxygen and acetylene capsule, two Volcano manometers, Volcano cutting head Eniso 5172, syringe pump, 1 ml insulin syringe and connector hoses. All the components and the operating system showed in Figure 2.

Figure 2: 1:Oxygen cylinder, 2: Oxygen manometer, 3: Acetylene cylinder, 4: Acetylene manometer, 5: Cutting Head, 6: Nozzle, 7: Hoses, 8: Operating mechanism

In this work both mentioned methods to fabricate superhydrophobic coatings are used but mainly emphasized on the method which the nano powder produced simultaneously due to more stability and low-cost of the fabricated coating.

As mentioned before, some have used water-soluble salt of various metals with LFS and by the means of XRD test, they assured that during the thermal process, nano-powder has been produced, e.g. (2, 4, 9). Moreover, another reason that can guarantee the formation of nano-powder is that the final surface after the LFS process shows excellent superhydrophilicity. In this work the $Al(No_3)_3$ is used as basic metal salt with melting temperature of 73°C and melting temperature of nano-powder 660°C. As you know the maximum temperature of acetylene can be 3100°C and varying along the fire stream. So if the injection takes place at the optimal position, aluminum nano-powder will extract and settle on the surface.

In this process there are parameters to be optimized, e.g. solvent density, solution injection flowrate, oxygen and acetylene flowrate, nozzle geometry, distance between the injection position and nozzle tip, distance between the substrate and nozzle and the speed of moving substrate in front of the flame.

2-1- Parameters Optimization

Based on the experiments the gas pressure on oxygen and acetylene considered 4 bar and 1 bar, respectively. Figure 3 shows the flame morphology at different Flow rates of oxygen while acetylene flow rates is kept constant.

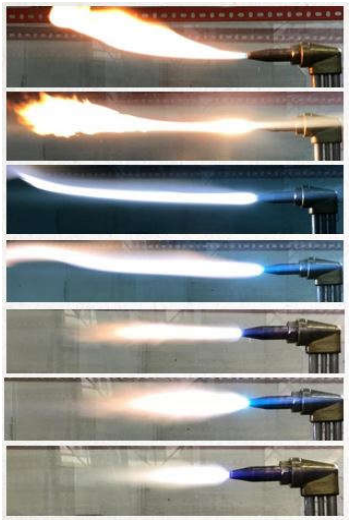

Figure 3: Flame morphology

In general, to optimize the distance between the substrate and the nozzle, thermal process was implemented at different distances and analyzed the appropriate distance according to the properties of the produced coating. The evidence showed that for distances greater than 22 cm between the substrate and the nozzle, the adhesion of nano powders on the surface decreases, so that the sandpaper wear tests also showed a decrease in mechanical resistance with increasing distance.

At distances less than 14 cm, the direct impact of the flame on the steel quickly caused the formation of iron oxide on the sample, which prevents proper adhesion between the produced nanoparticles and the steel. Therefore, to carry out the tests of this method and avoid the mentioned problems, the distance between the substrate and the nozzle is set in the range of 14 to 22 cm.

To optimize the injection flow rate of precursor material into the flame by keeping the previous parameters constant, the experiments were performed again with different flow rates and according to the experimental observations and the thermodynamic properties of the produced coatings, we suggest the optimal range for the injection flow rate. To inject the precursor into the flame, we used a system including a syringe pump with ability to adjust the flow rate. The most ideal situation occurs when the precursor solution penetrates the center of the flame without scattering. Thus, according to observations, when the injection flow rate is less than 0.9 ml/min, the solution does not penetrate the flame, and due to the very high speed of the flame, it is completely scattered before entering it.

On the other hand, for flow rates higher than 1.9 ml/min, the injected precursor solution started moving away from the center of the flame, while the amount of material consumption was higher in this case, and the amount of nanopowder production did not increase, because the solution injected into the flame is not given enough time to produce nanopowder. According to the optimization that was done for different parameters, the tests of this method were designed and implemented in the range of the table below.

Table 1: Range of test parameters of liquid flame spray method

Oxygen and acetylene pressure (*Bar*)	Precursor solution	Distance between the substrate and nozzle (Cm)	Precursor flowrate $(ml / \min)$
4, 1	Aluminum nitrate dissolved in ethanol	$14 < L < 22$	$0.9 < Q < 1.9$

Based on the suitable range of parameters, several methods were designed to fabricate superhudrophobic coating. The LFS parameters and the contact angle of the manufactured samples are listed in the table below.

Table 2: Coating specifications

	Precursor flowrate $(ml/\min)$	Distance between the substrate and nozzle (Cm)	Method of lowering surface energy	Contact angle
Aluminum nitrate 3.5% in isopropanol	1.15	22	RTV/Powersil552	133±3°
Aluminum nitrate 3.5% in ethanol	1.5	20	RTV/Powersil552	138±3°
	1.15	22	FAS-17	150±3°
	1.15	17	FAS-17	157±3°

3. Results and discussion

In order to reduce the energy of the surfaces produced by liquid flame spray, various low energy materials were used. It should be noted that the silicone elastomer used in this section is Powersil RTV1-552. Both static and dynamic contact angles were taken by Jikan CAG-20 contact angle goniometer device and analyzed in ImageJ software. Static Contact angle of the produced coating showed in Figure 4.

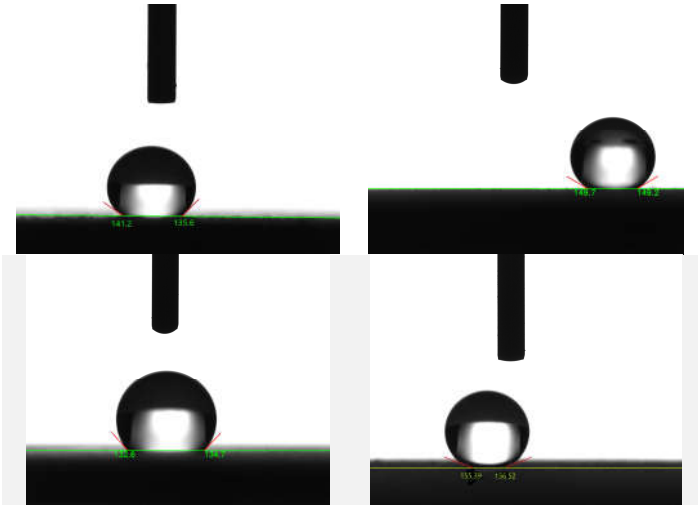

Figure 4: Static contact angle

The best coating made by this method had a contact angle of 157°, advancing and receding angles of 159° and 154° respectively and hysteresis less than 5°. To produce this coating, 3.5% aluminum nitrate in ethanol precursor injected with a flow rate of 1.15 (ml/min) at a distance of 0.5 cm from the nozzle head into the fire stream. The distance between the sample and the nozzle kept 17 cm and the whole process of LFS completed in 180 seconds. The lowering surface energy completed by immersing in FAS-17 solution and drying at 250° C in oven. Figure 5 indicates dynamic contact angles measured by Jikan CAG-20 goniometer.

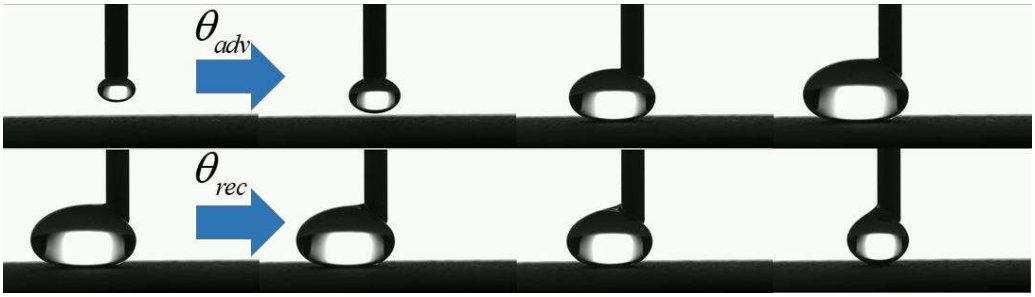

Figure 5: Dynamic contact angles $\theta_{advancing}$ & $\theta_{receding}$

The coatings produced by this method before applying the low-energy material are superhydrophilic with a contact angle of less than 5°, with a very high mechanical resistance to wear, so that even after washing with very rough items, They have kept their superhydrophilic state.

The abrasion test mechanism is shown in Figure 6. Prepared sample after the LFS method pushed on 1000 grit SiC sandpaper with a weight of 100 gr which provides 1.56 kPa of applied pressure.The sample moved 80 Cm in one direction. This test showed high mechanical durability of the superhydrophilic coating so that the coating still maintained its superhydrophilic properties and contact angle maintained less than 5°.

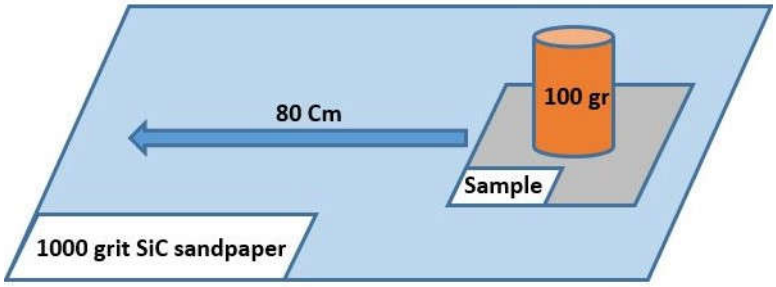

Figure 6: Abrasion test on superhydrophilic coating

In this research, the effective parameters were optimized for this mechanism and the performance range was presented.

4. Acknowledgments

Low cost of producting method was the main idea of considered mechanism for LFS process and as a result of which the produced coatings will be prone to use in large industries. In this work oxy-acetylene flame used instead of oxy-hydrogen which is more dangerous gas than acetylene so cheaper and simpler equipment for the implementation of the liquid flame spray process is needed.

Considering the number of effective parameters and the complexity of the LFS process, it seems more research and development on parameters optimization should be and also considering the gap in the literature of working gases in this method, other gases that are economically more affordable and less dangerous, such as Butane and propane can also be considered. As mentioned before, one of the most influential parameters in the liquid flame spray process is the flame morphology, which directly affects the amount of nanopowder production and momentum of the produced particles towards the surface. Therefore the issue of designing and manufacturing special nozzles aiming to control the morphology of the flame and the integrated injection system on it can be investigated.

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Biotechnological Applications of Plant Proteases Inhibitors

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Abstract

Protease inhibitors are regulatory proteins found in numerous animal tissues and fluids, plants, and microorganisms that reduce and inhibit the exacerbated and uncontrolled activity of the target proteases. Recently, novel biologic activities have been reported for plant protease inhibitors, including antimicrobial, and anticoagulants, thus pointing to possible applications in and biotechnology. In this review, we provide a comparative overview of plant protease inhibitors classifying them into four groups according to their thermal and pH stability, then emphasizing the relevance of the physicochemical characteristics of these proteins for potential biotechnological and industrial applications. Finally, we analyze the biological activities of the stable protease inhibitors previously characterized that are the most relevant to potential applications in the food industry, and agriculture

Key words: Protease Inhibitors, Biotechnological Applications, Physicochemical Characteristics, Industrial Applications.

1. Introduction

1.1. Protease inhibitors

Among the natural compounds, protease inhibitors are regulatory molecules found in numerous animal tissues and fluids, plants, and microorganisms that control the activity of their target proteases; in some instances, blocking their exacerbated and uncontrolled activity (1).
Among the natural compounds, protease inhibitors are regulatory molecules found in numerous animal tissues and fluids, plants, and microorganisms that control the activity of their target proteases; in some instances, blocking their exacerbated and uncontrolled activity (1). The major physiologic application of endogenous protease inhibitors is the prohibition of unwanted proteolysis and, therefore, in most normal physiologic processes, as well as in pathologic circumstances. That involvement in the regulation of proteolytic activity includes the activation of coenzymes and the release of biologically active polypeptides (2). Among protease inhibitors, those of a protein nature are small molecules ranging from 15 to 60 amino acids with a high content of cysteine residues in the form of disulfide bridges that confer resistance to heat treatment, extreme pHs or ionic strengths, and proteolysis In whole organisms, these molecules represent an efficient way to control the activity of endogenous proteases, which enzymes need to be balanced in a normal state to effect controlled proteolysis (3).

1.2. Inhibitors in plant protease

plant protease inhibitors have also been extensively studied owing to their physiologic role in the regulation of endogenous proteases, storage, and potential function including protecting plants against herbivorous insects by inhibiting digestive proteases (4). High levels of plant protease inhibitors are often found in plants belonging to the Solanaceae, the Leguminosae (Fabaceae), and the Gramineae (Poaceae) families (5). plant protease inhibitors of Kunitz and Bowman-Birk are found in members of the leguminous family. These inhibitors are classified on the basis of their cysteine-residue content and the number of protein-binding sites. Of the

two, the Kunitz-type inhibitors are proteins that usually exhibit a molecular mass of 18–24 kDa, with one or two polypeptide chains and 4 cysteine residues forming 2 disulfide bridges, and with a single protein binding site (6). The second class of more extensively studied inhibitors in plants is the cysteine proteases. Plant cystatins or phytocystatins are classified into 3 subfamilies: Group I, comprising members of molecular mass 12–16 kDa with a single cystatin domain; Group II, of molecular mass 23 kDa and with domains in their N- and C-termini that confer the ability to bind to and thus inhibit cysteine proteases of type C13, and finally, group III, composed of multi cysteines that contain two or more cystatin-type domains (7). plant protease inhibitors are widely found in crop plants, and though displaying a particular abundance in legumes, are also present in cereals and tubers, where they form part of the plant's defense against pest attack. That defensive role of plant protease inhibitors is based on their inhibition of the digestive enzymes of insects and of other pathogenic proteases involved in certain vital processes, either causing a critical shortage of essential amino acids (8). In particular, high levels of plant protease inhibitors are associated with plant resistance against insects and microbes (9). Although this latter function has been well documented, the mechanism for plant protection per se still remains unclear. That many plant protease inhibitors act as defensive compounds against pests has been demonstrated in direct trials or indirectly by expression in transgenic crop plants (10).

2. Applications

2.1 Biomedical applications

The use of natural products for the prevention or treatment of human diseases continues to be an area of intense research. Approximately 3 billion people are at a risk of contracting infectious diseases (11). That danger is greater under poor living conditions, where treatments are inadequate or inaccessible, and in those geographical regions of warm climate and high humidity; with the latter constituting suitable conditions for the development of diseases caused by different types of pathogenic microorganisms. This situation had previously prevailed principally in underdeveloped countries, but now under the influence of the global climate change, has also obtained in regions not previously under risk, but currently exhibiting an increased incidence of several diseases transmitted by warm-climate vectors (i. e., malaria, dengue). An estimation has been made that, between 2030 and 2050, the present climate change could lead to another 250,000 annual deaths caused by malaria, diarrhea, extreme heat, and malnutrition (12).

2.2 Agricultural applications

In plant biotechnology, interest has arisen in new molecules that counteract diseases of mammals and also in new biotechnological weapons that serve to prevent microbial attacks on plants and to eradicate those pathogenic microorganisms along with infectious insects, especially in crops of biotechnology, agricultural, and/or cultural interest. Plants are subjected to a great number of pests and pathogenic infections, whose actions contribute substantially to an overall reduction in crop yield. Chemical pesticides have been used for several decades in order to diminish the damage caused by the different invasive species. The agricultural industries employ numerous pesticides to combat this problem, but those compounds are fraught with serious drawbacks because of a lack of specificity, the development of resistance after prolonged use, and a danger to human health resulting from residual toxicity. For this reason, the inadequate use of many of those agrochemicals in combination with the possibility of resistance acquired by various invading organisms has promoted an acute interest in the search for new alternatives to the treatment of those diseases. Thus, biodegradable biologic

control agents and/or natural products constitute the most promising alternatives since those recourses are free of pollutant residues and have a reduced incidence in the development of microbial resistance (13).

Several plant protease inhibitors —from both the wild-type and nonhost relatives— are more effective than the plant protease inhibitors of the host plants in the management of insect pests, since the digestive enzymes present in the insect organs have not been adapted to those nonhost plant protease inhibitors (14). In recent years, several plant protease inhibitors from plant seeds with insecticide activity have been reported whose mode of action involves a reduction in the hydrolytic processes of the dietary proteins in the intestine of the insect in order to decrease the availability of endogenous amino acids, particularly those essential for larval development (15).

2.3 Applications to the food industry

In recent years, the food industry has been forced to search for new preservatives that are both natural and stable in order to extend the half-life of food and to preserve the safety of food quality, whether in a natural way or by altering the microflora of the food, because the deterioration and microbial contamination of food has become a worldwide concern (16). Microbes can not only cause foodborne illnesses but also result in significant economic losses during a postharvest period, with that spoilage through microbial activity being estimated at up to 25% of the food produced (17). Microorganisms can contaminate food in several ways: at the farm through irrigation water, field workers, insects, or exposure to the feces of wild animals; by inadequate postharvest preservation in the transport vehicles, processing equipment, and washing water; or by contamination from other foodstuffs in the processing plant or marketplace (18).

Plant protease inhibitors constitute a potential alternative to chemical antimicrobials as stable and natural additives for food-preservation processes, not only because of their suppression of the growth of pathogenic microorganisms, but also because of their inhibition of proteases (19).

3. Materials and methods

the data on the physicochemical properties of plant protease inhibitors and biologic properties with biotechnological application including the food industry and agriculture were collected and analyzed. In an effort to compile all the information keywords such as plant protease inhibitor, pH and thermal stability was used in the following databases: NCBI-PubMed (http://www.ncbi.nlm.nih.gov/pubmed); Science direct (https://www.sciencedirect.com/); Wiley online library (https://onlinelibrary.wiley.com/); Scholar Google (https://scholar.google.com.ar/).

4. Results and discussion

4.1 Thermostable plant protease inhibitors

Group IA: Inhibitors with high thermal stability can be classified into the following 3 groups based on the activity that they retain at high temperature. Within this group are plant protease inhibitors that retain 25–50% of the original inhibitory activity at temperatures over 90 ºC after 5–10 min incubations. Within Group IA are inhibitors that were found to be stable at temperatures below 60 °C with a 50% loss of inhibitory activity at 80 °C namely, the soybean serine-protease inhibitor (20).

Inhibitors that retain 50–75% of the inhibitory activity at 90 ºC classified as Group IB, where we grouped together the inhibitors that retained 50–75% of the original inhibitory activity

after incubation at 90 ºC for 5–30 min. Within these plant protease inhibitors are BvvTI, a Kunitz trypsin inhibitor isolated from Bauhinia variegata seeds (21) and RflP1, a Kunitz trypsin inhibitor isolated from Rhamnus frangula leaves (22).

Inhibitors that retain greater than 75% of the initial inhibitory activity at 90 ºC This last group of plant protease inhibitors with especially high thermal stability corresponds to the Group IC, where we grouped together the inhibitors that retained over 75% of the starting inhibitory activity after 5–30 min at 90 °C. The main characteristics of these inhibitors, with the following being typical examples: CSTI is a Kunitz trypsin inhibitor isolated from the pink powderpuff Calliandra selloi Macbride seeds that remained quite stable at increasing temperatures, retaining more than 75% of the original inhibitory activity after incubation at 80 °C for 30 min. At 100 °C, CSTI lost 50% of the initial activity owing to protein denaturation (23). Group IIA: Inhibitors that retain the initial inhibitory activity after a 30-min incubation at temperatures over 90 ºC Within Group IIA are plant protease inhibitors that maintain the inhibitory activity at temperatures over 90 ºC after 30 min of incubation. Notable among these inhibitors were the following: A barley-cysteine-protease inhibitor isolated from Hordeum vulgare seeds was found to be stable at temperatures below 80 °C, but lost only 15% of the initial activity at 100 °C (24). Group IIB: Inhibitors that retain the initial inhibitory activity after a 60-min incubation at temperatures over 90 ºC, where the inhibitors are grouped that - quite remarkably-maintain a total inhibitory activity after 60 min of incubation at temperatures above 90 ºC. Notable among these plant protease inhibitors were WSTI-I and WSTI-IV, both BowmanBirk trypsin inhibitors isolated from G. max seeds, whose inhibitory activities were retained even after heating at 90 °C for 60 min (25) .Group IIC: Inhibitors that that retain the initial inhibitory activity after a 120-min incubation at temperatures over 90 ºC. In this last category of plant protease inhibitors exhibiting an extreme hyper-thermostability are grouped the inhibitors that maintain the initial inhibitory activity after a 120-min incubation at temperatures over 90 °C. These molecules accordingly are extraordinarily heat-resistant, representing the most thermostable peptides characterized in the present research. The following examples have been cited: RcTI, a trypsin inhibitor isolated from Ricinus communis seed cake, maintained 80% of the initial inhibitory activity even after heating at 100 °C for 2 h, (15).

4.2 Plant protease inhibitors with antimicrobial activity

RcTI, the trypsin inhibitor isolated from R. communis seed cake and a member of Group IIC, possesses antifungal activity as evidenced in an inhibition of the spore germination of Colletotrichum gloeosporioides (15); while IETI, the Kunitz trypsin inhibitor isolated from I. edulis seeds and a member of Group IB, exhibited an antimicrobial activity against Candida ssp., including Candida buinensis and Candida tropicalis triggering a membrane permeability in that yeast, thus decreasing cell viability (26). JcTI-I, the trypsin inhibitor isolated from J. curcas seed cake and a member of Group IIC, inhibits the growth of the bacteria Salmonella enterica subsp. Enterica serovar choleraesuis and Staphylococcus aureus (27).

5. Conclusions

During the most recent decades, an increasing interest has developed in the use of natural products for different applications. Among the naturally occurring compounds, proteins, and peptides are widely explored for various applications. Plant protease inhibitors offer certain advantages, being small molecules with satisfactory solubility in aqueous solutions and resistant to proteolysis. These plant protease inhibitors display several proven invitro and/or

in-vivo bioactivities in which more studies on them will lead to economic and biotechnology improvement.

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Application of alumina nanoparticles in thermoluminescence dosimetry

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Abstract

In this research, alumina nanoparticles were prepared by a sol-gel method, and characterized via X-ray diffraction (XRD) and scanning electron microscope (SEM) to understand the structure, size, and shape of particles. In addition, the thermoluminescence radiation curve of these nanoparticles, irradiated with gamma rays from the ^{60}Co source, was fitted with a computer program and their trapping parameters were calculated numerically. Two overlapping peaks at temperatures of 443 and 461 K were observed in the thermoluminescence curve of the nanoparticles. The obtained results show that the alumina nanoparticles are suitable for use in dosimetry.

Key words: Nanoparticles, sol-gel, TL, kinetic parameters, Al_2O_3

1. Introduction

Today, it has become important to measure the dose that a person is exposed to in the work environment [1-2]. Considering the importance of this issue and as a result of dosimetry and radiation protection, thermoluminescence is one of the most effective practical methods for dosimetry applications . Thermoluminescence is a luminescence phenomenon, and luminescence excitations in materials can be created by various types of sources such as ultraviolet rays, electric fields (electroluminescence), cathode rays (cathodeluminescence), etc. [3]. When the materials are irradiated for the thermoluminescence phenomenon, the energy of the beam is excited by the electrons of the valence layer of the charge carriers, and they are separated from their alignment, thus beginning to move freely throughout the crystal lattice to finally reach the trap points caused by lattice imperfections or impurities. In fact, they are created in the host substance and get trapped. Accordantly, the energy of the radiated rays is stored in the material, so that the trapped charge carriers are excited and released from the dopant centers by heat [4]. The transition of electrons and holes from the trapping levels to their ground state leads to photon emission. These photons have the extra energies released by charge carriers. On the other hand, materials in nanodimensions show behavior and characteristics that will induce different properties compared with the bulk state [5]. In other words, nanotechnology has created a clear perspective for us in this field, and today the study of the properties of nanomaterials has become one of the research topics [6]. Alumina structure with heat resistance is used to make flame retardant coatings in fire extinguishers. At the nanoscale, alumina nanoparticles are used as an electrical insulator and as a very high thermal conductor due to their hardness. Alumina nanoparticles are also employed as one of the biomaterials in the medical and health industries to replace hip joints [7]. In this research, alumina nanoparticles are synthesized by a sol-gel method, and their dosimetric properties are studied.

2. Experimental details

Alumina nanoparticles were synthesized using a sol-gel method. The precursor materials used were aluminum nitrate [Al $(NO_3)_3$] and oleic acid ($C_{18}H_{34}O_2$), and obtained from Merck, Germany with high purity. Initially, 0.5 g of aluminum nitrate was mixed with 10 ml of water for half an hour under magnetic stirring, followed by adding oleic acid. The remaining solution was then kept at 180°C for 3 h until the water completely evaporated, thereby forming a black gel. We calcined the gel at different temperatures, and heated the sample to temperatures of 650°C and 1200°C for 20 min and 3 h, respectively. Finally, a transparent white precipitate was obtained, containing alumina nanoparticles. An X-ray diffraction (XRD) device (model: Rigaku D/Max diffractometer) was used to investigate the structure and to confirm the formation of Al_2O_3 nanoparticles. A scanning electron microscope (SEM; Philips XL30) was employed to observe the shape and size of the nanoparticles. In this research, a TLD reader (model: 4500 Harshaw) was used to read the irradiated samples. All the samples were irradiated under the same conditions using a ^{60}Co source in the range from 50 °C to 250 °C with a temperature rate of 2 °C/s.

3. Results and discussion

The XRD spectrum of alumina nanoparticles is shown in Fig. (1).

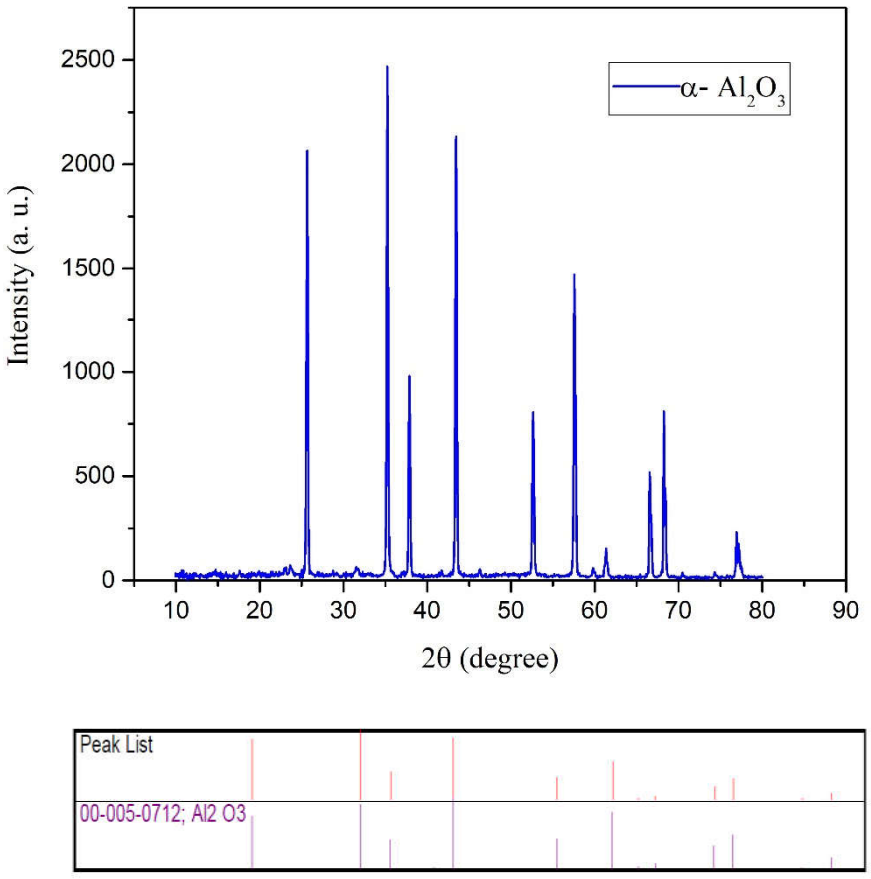

Figure 1 :XRD spectrum of alumina nanoparticles synthesized at 1200°C.

According to this figure, peaks at 2θ=25.58°, 35.13°, 37.78°, 43.61°, 52.78°, 57.59°, 66.43°, 68.51° and 77.25° are attributed to (012), (104), (110), (113), (024), (116), (214), (300), and (1010) planes, respectively. These planes are in complete agreement with the alumina reference sample with the standard card number [0712-005-00], indicating the formation of Al_2O_3 crystals with a rhombohedral structure.

Using the Scherrer formula (equation (1), the average size of the crystal can be estimated as follows:

$$D = \frac{0.9\lambda}{\beta Cos(\theta)} \quad (1)$$

where β is the full width at half maximum (FWHM; in terms of radians), θ is the Bragg angle related to the diffraction peak, and λ is the X-ray wavelength [8]. The size of the crystal was obtained to be 55 nm. The result of SEM analysis of the alumina nanoparticles is shown in Fig. 2. The nanoparticles are observed to have good aggregation and dispersion. Also, the size of the nanoparticles is in agreement with the crystal size obtained from the Scherrer formula.

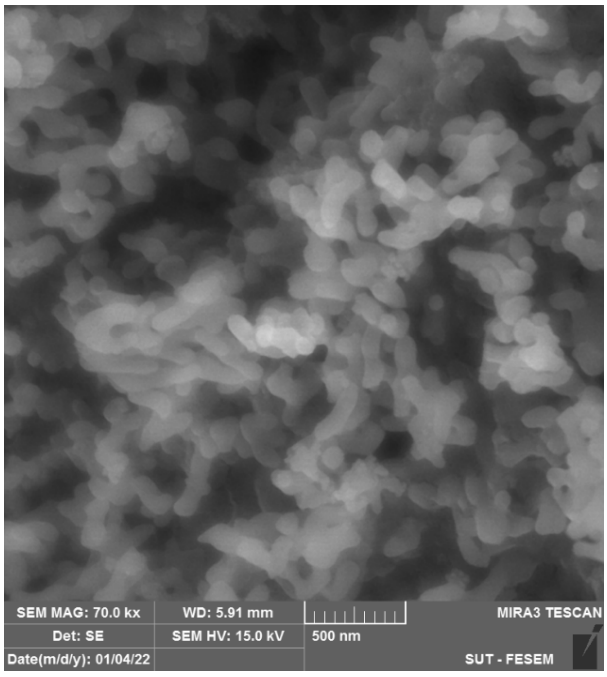

Figure (2): SEM image of Al_2O_3 nanoparticles at a magnification of 500 nm.

According to the literature, the general order kinetic model was used and curve fitting was performed by software based on the Levenberg-Marquardt algorithm. The following relationship was used to calculate the kinetic parameters E and b [9]:

$$I(T) = I_m b^{\frac{b}{b-1}} exp\left(\frac{E(T-T_m)}{KTT_m}\right) \times \left\{\frac{T^2}{T_m^2}(b-1)\left(1-\frac{2KT}{E}\right)exp\left(\frac{E(T-T_m)}{KTT_m}\right)+1+(b-1)\frac{2KT_m}{E}\right\}^{\frac{-b}{b-1}} \quad (2)$$

whereb is the kinetic parameter (ranging between 1–2), E is the activation energy, T is the temperature (in Kelvin), T_m is the maximum temperature, and k is the Boltzmann constant.

According to Fig. 3, the glow curve of the nanoparticles is formed from two peaks at temperatures of 443 and 461 K. Equation (3) was used to determine the degree of conformity between theoretical and experimental thermoluminescence curves:

$$FOM = \frac{\sum |y_i - f_i|}{\sum y_i} \times 100 \qquad (3)$$

where y_i denotes to the original values or experimental data, and f_i is the best value that can be obtained through this fit [10]. The FOM amount is found to be 2.17, indicating a good fit between the theoretical and experimental curves. Table (1) shows the results of kinetic parameters obtained from the fitted curve.

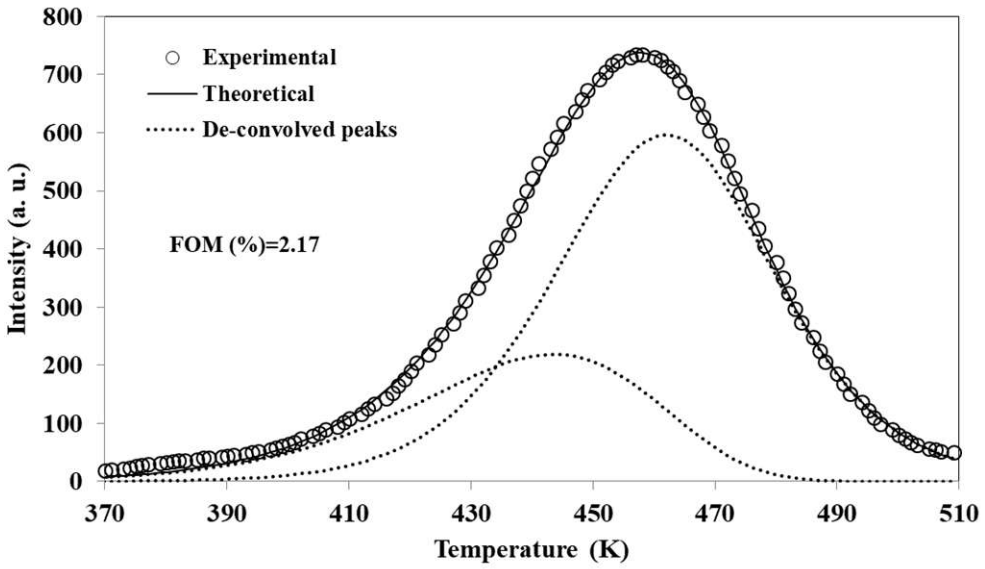

Figure (3): Fitted thermoluminescence curve of Al_2O_3 nanoparticles.

Table 1: Kinetics parameters of Al_2O_3 nanoparticles.

Peak	b	E (eV)	T_m (K)	I_m (a.u)
1	1.00	0.82	443	218
2	1.70	1.33	461	596

4. Conclusion

In this research, alumina nanoparticles were synthesized by a sol-gel method in order to study their dosimetric properties. Among the notable points regarding the sol-gel method, one can mention about its cost-effectiveness. Also, the method used was advantageous for the synthesis of the nanoparticles, because the formed particles were uniform in size and the resulting structure was in complete agreement with the Al_2O_3 crystal. The formation of the nanoparticles was confirmed by XRD analysis. SEM analysis showed the formation of rhombohedral particles. The irradiated nanoparticles consisted of a broad curve, indicating the overlapping of two peaks at temperatures of 443 and 461 K. This provided a suitable temperature for dosimetry purposes. Therefore, the synthesized thermoluminescence sample can be considered a good candidate for dosimetry.

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Advances in CRISPER/Cas9 technology

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Abstract

Genome editing is an important biotechnological tool that enables genetic engineering of many living organismsby inserting, deleting, or replacing DNA at a precise location in the genome. The most commonly used technologies for this purpose include transcription activator-like effector nucleases (TALENs), endonucleases or meganucleases homing, zinc finger nucleases (ZFNs), and clustered regularly interspaced short palindromic repeats (CRISPR) associated protein 9. Among these tools, CRISPR/Cas9 is preferred more. The CRISPR/Cas9 system can specifically identify any target and activate mutation correction or expression regulation through the NHEJ or HDR pathway, providing a powerful platform for revolutionary treatments of various diseases. CRISPR applications have not only grown rapidly but also transformed biological sciences. Currently, this technology is being used in various fields such as agriculture, food biotechnology, medicine, cancer, and targeted genome therapies. In particular, these tools have provided an extraordinary new avenue for gene therapy and cell therapy in particular.

Key words: Genome Editing, Molecular Markers, *CRISPR/Cas9*

1- Introduction

Genome editing is an important biotechnological tool that enables genetic engineering of many living organisms through insertion, deletion, or replacement of DNA at a precise location in the genome (1). Genome editing technology allows scientists to make changes in the DNA of model organisms at the genomic level to obtain important biotechnological products from them. The most commonly used technologies for this purpose include transcription activator-like effector nucleases (TALENs), endonucleases or homing meganucleases, zinc finger nucleases (ZFNs), and clustered regularly interspaced short palindromic repeats associated protein 9 (CRISPR/Cas9). Among these tools, CRISPR/Cas9 is preferred more because it is easy to use, has a low mutation rate, has excellent efficiency, and has low development costs (2). The CRISPR-associated protein 9 (Cas9) system associated with CRISPR, which is a bacterial defense mechanism against phage infection and plasmid transfer in nature, has been used regularly as a powerful DNA targeting platform with RNA guidance for genome editing, transcriptional disruption, epigenetic modulation, and genome imaging (3).

Recent advances in genome engineering technologies based on the Cas9 endonuclease guided by CRISPR-associated RNA provide the ability to systematically interrogate the function of mammalian genomes. Similar to searching for keywords in modern word processors, Cas9 can be directed to specific locations in complex genomes by a short RNA search string. Using

this system, DNA sequences within the genome can now be easily edited or modified as well as their functional outputs in any selected organism. The CRISPR/Cas system has revolutionized biological research and offers a plethora of options for manipulating, detecting, imaging, and annotating specific DNA or RNA sequences of different organisms. CRISPR arrays are genes that encode Cas proteins. Cas proteins provide the necessary enzymatic machinery to obtain new spacers that target invading elements. Many bacteria and most archaea have developed complex RNA-guided immune systems that are encoded by CRISPR loci and associated cas genes to confer acquired immunity against bacteriophage infection and plasmid transfer. During immunization after exposure to invading genetic elements from phages or plasmids, short pieces of foreign DNA are integrated into the host chromosome as new spacers within the CRISPR array as a result of which a genetic memory of previous infections is provided, allowing the host to prevent future invasion by the same invader.

2- CRISPER/Cas9 technology

Genome editing techniques require programmable endonucleases with specific sequence recognition to create single-strand breaks (SSBs) or double-strand breaks (DSBs) at the desired location, allowing for repair mechanisms to fill in the breaks. These breaks are repaired by one of two main mechanisms, homology-directed repair (HDR) and non-homologous end joining (NHEJ).(7). Structurally, Cas9 is a helicase that can bind to RNA transcribed using palindromic DNA repeats and RNA-spacer cleavage sites that match external DNA. Thus, CRISPR-RNA activating trans (tracrRNA) is named for copying palindromic DNA repeats while spacer copies are known as crRNA (CRISPR-RNA). CRISPR-RNA and crRNA activating trans form a unique guide RNA (sgRNA) that can direct Cas9 towards the target DNA and cleave it, creating a double-stranded DNA break (DSB) at PAMs (proximity-adjacent motifs). PAMs are small protein motifs with 3-6 base pairs that are stationary in external DNA and identified by Cas proteins(8). S. pyogenes Cas9 (hereafter referred to as SpyCas9) is a large multi-domain DNA endonuclease (1368 amino acids) with multiple functions. It separates the 3 base pairs upstream of PAM through its distinct nuclease domains: an HNH nuclease domain that breaks the complementary DNA strand of the guide RNA sequence (target strand), and a RuvC-like nuclease domain responsible for cleaving DNA. In addition to its vital role in CRISPR interference, Cas9 also participates in crRNA maturation and spacer acquisition(9).
The versatility of the Cas9 nuclease and its derivatives has led to its applications in various fields, from basic sciences to clinical settings. The Cas9 system's adaptability now allows for almost all types of genome editing, including the formation of unintended indels, base substitutions, designed deletions, or insertions. Such editing strategies can be used to disrupt or restore gene function, regulate gene expression, and insert therapeutic DNA fragments for the treatment of a variety of disorders, including genetic diseases, metabolic disorders, cancer, infectious diseases, and more (10).The adaptability of the Cas9 nucleases and its derivatives has led to their applications in various fields, from basic sciences to clinical settings. The Cas9 system's adaptability now allows for almost all types of genome editing, including the formation of unintended indels, base substitutions, designed fragment deletions or insertions. Such editing strategies can be used to disrupt or restore gene function, regulate gene expression, and insert therapeutic DNA fragments for treating a variety of disorders, including genetic diseases, metabolic disorders, cancer, infectious diseases, and more(10).

3- Applications of CRISPR technology

CRISPR applications have not only grown rapidly, but have also transformed the field of biological sciences. Currently, this technology is being used in various fields such as agriculture, food biotechnology, medicine, cancer research and targeted genome therapies(11). Cancer occurrence and progression involve a series of genetic mutations or expression disorders. The CRISPR/Cas9 system can specifically identify any target and activate mutation correction or expression regulation through NHEJ or HDR pathways, providing a powerful platform for revolutionary treatments for various diseases. In particular, the convergence and development of CRISPR/Cas9 technology with nanotechnology in recent years has led to a wide range of nanomedicine-based technologies(12). Eye diseases are a very heterogeneous group of phenotypes that are created by a spectrum of genetic types and environmental factors that exhibit various clinical symptoms. Genome editing technology is rapidly evolving; CRISPR is a precise molecular gene editing tool that has tremendous potential in treating human eye diseases by changing defective gene sequences and rescuing phenotypes. As discovered in the late 1980s, it was reported that CRISPR/Cas9 successfully edited mammalian genomes for the first time in 2013. Its application later expanded to various diseases including retinal dystrophy (a group of eye phenotypes) and other neurodegenerative conditions. In addition, the use of in vivo CRISPR/Cas9 to correct mutations in mouse models has yielded promising results(13).

The dense and complex parenchyma of the brain and the state of neurons after mitosis pose a challenge to efficient genome editing. Delivery systems for CRISPR-Cas proteins and RNA guide molecules (sgRNA) include viral vectors and non-viral strategies, each offering different advantages and disadvantages for clinical applications. In a study, non-viral, biodegradable PEGylated nanocapsules (NCs) were created to deliver pre-assembled ribonucleoprotein complexes (RNPs) of Cas9-sgRNA. RNP NCs were found to result in strong genome editing in neurons following intracerebral injection into the body of healthy mice. Genome editing was mainly observed in medium spiny neurons (over 80%), with occasional editing in cholinergic, calretinin, and parvalbumin neurons. Glial activation was minimal and localized along the needle tract. The results of this study demonstrate that RNP NCs are capable of safe and efficient neural genome editing within the body(14).

Soil pollution by toxic metals is a major health problem that can be partially solved through the use of genetically modified plants. Many plants, including agricultural crops, have the potential to tolerate, stabilize, and transform both organic and metallic pollutants, and have previously been modified using CRISPR technology. In addition, many genes necessary for increasing metal absorption and tolerance have been identified. Therefore, using CRISPR, target genes can be activated or suppressed for optimizing phytoremediation in active plants(15).

Another example of genetic modification using CRISPR is rice with low cadmium absorption. Researchers were able to create a mutation in the gene responsible for cadmium absorption in rice that has the ability to grow and survive in soils with high levels of cadmium(16).

CRISPR technology has great potential for treating diseases such as cancer, genetic diseases, and even diseases caused by microbes such as HIV.(17)

Overall, CRISPR technology has the potential to revolutionize various fields, including medicine and agriculture

4- Conclusion

In the past decade, we have witnessed an explosion in genome editing technology, to the point where genome editing tools derived from Cas9 have been discovered and evolved more and more. The ability of these tools to effectively and accurately install a wide range of changes in the genome has transformed basic life science and clinical medical research. In particular, these tools have offered a new and extraordinary direction for gene therapy and cell therapy.

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Misconception of Students in Teaching Thermochemistry

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Abstract

The purpose of the present study is to investigate students' understanding of the concept of heat and temperature. Concepts that are very important in school curriculum. This concept is one of the concepts related to daily life that forms the basis of physics, chemistry and biology, and in most curricula, it is suggested to learn it from the very first basics of the elementary school. The present article showed that the students of different levels of elementary, middle school (guidance) and middle school (high school) all have a misconception in the topics of heat and thermochemistry. Conceptual understanding of heat and temperature and the difference between these two is a problem that is seen by some students, but applying it and relating it to different quantities such as mass and temperature is a problem that is more comprehensive than the previous problem. The educational planners of the country should benefit from the researches of researchers in the field of educational content and identification of misconception. Teachers, by conducting continuous formative evaluations, inquiring about initial ideas and paying attention and correcting them during teaching, are among the factors that can help neutralize misconception in the early stages and fix them.

Key words: Misconception, Thermochemistry, Chemistry Education, Physical Chemistry

1- Introduction

Misconceptions appear in all fields of science and among all age groups. Experimental evidence shows that children have qualitative differences in their understanding of science concepts, which often contradicts what the teacher has told them through education. A Misconception is different from a Misconception about a concept. In order to overcome Misconceptions and solve them, strategies have been presented in research articles. The teacher is an essential component in the success of these strategies, who often facilitates students' thinking through questions and student dialogue. What the limited research on teachers' misconceptions shows is that novice teachers are often unaware that their students may have misconceptions. In addition, even when teachers are aware of students' Misconceptions, they may use wrong knowledge in their teaching. Also, studies show that experienced teachers have very complex perceptions of prior knowledge and make significant use of their students' prior knowledge, such as Misconceptions, in education (Trend, 2001). Research shows that teachers sometimes have the same Misconceptions as their students. Many of these Misconceptions appear in the planning and teaching of their courses, and instead of correcting them with scientific facts, it leads to an increase in students' Misconceptions (Hashweh, 1987).

There are many researches that have investigated students' Misconceptions about scientific phenomena. Although less research has been done to identify teachers' Misconceptions, the results of the few available studies show that teachers and students have similar Misconceptions. The study of the understanding of physical science concepts by elementary science teachers shows that after three decades of research on Misconceptions, teachers still have the same ideas as students (Burgoon et al., 2010). The comparison of response patterns in two groups of learners (high school students and student teachers) to a diagnostic tool about some scientific concepts in the same educational field in Singapore showed that although student teachers provide some types of wrong answers less than high school students, they have a high level They keep the alternative concepts that usually exist among

high school students, and also according to some special notes, certain alternative concepts were found that were more common among student teachers (Taber and Tan, 2011). Therefore, it is very important to study and investigate teachers' views on scientific concepts and identify their Misconceptions (Saadati, 2019).

One of the effective factors in dealing with Misconceptions is accepting its existence among teachers and student teachers. In a study, Gomes has tried to determine how much teachers with different experiences are aware of how Misconceptions develop in their students. Are these teachers aware of the methods of correcting students' Misconceptions and do they use these methods to correct their students' Misconceptions? In a research involving 30 teachers from California with at least 1 year of teaching experience in third to fifth grades, semi-structured interviews were used to collect data. The researcher coded the interview text in the categories of definition of Misconception, roots of Misconceptions, development of Misconceptions and teaching strategies to address Misconceptions and analyzed them. The results indicate that although most teachers are aware of Misconceptions, they do not understand how Misconceptions develop or have a full understanding of their impact in their teaching (Gomez-Zwiep, 2008). Also, it is very important to identify children's mental preconceptions in elementary school science education. Identifying these concepts by teachers while teaching science in the first years of elementary school is a subject that researches on them are still limited. Teachers agree that daily activities enable children to learn some science even before entering preschool, and children's ideas are part of the classroom. Some of these ideas are not completely correct and Misconceptions are related to children's incorrect or incomplete ideas. The results of some researches show that most teachers do not confirm the existence of these Misconceptions and this is probably an obstacle for children's learning (Kambouri-Danos, 2014).

Another study, based on the theoretical framework of constructivism, defines preconceptions as children's misconceptions before formal education, has addressed this issue. The results indicate that even when confirming the possibility of preconceptions, teachers do not allocate the necessary time to identify children's preconceptions when planning and teaching science. This shows the lack of understanding of the importance of children's preconceptions due to their neglect. The results also indicate the need for more training and professional development in relation to the teaching of science in the first elementary grades, especially that only a very small percentage of the teachers in the first elementary grades are inclined to study science during the compulsory academic years (Kambouri, 2016).

Mir and his colleagues, understanding the importance of combining science education with engineering, have studied about the Misconceptions that may arise for high school and elementary school science teachers in their reflections on science and engineering. By using studies and films related to real science and engineering works, teachers' thoughts were used to discover the infrastructures of their understanding. Six science teachers (two elementary and four high school teachers) participated in this study as part of an online professional development experience. The analyzes indicate the existence of Misconception in eight science and engineering activities related to the next generation science standards of the United States in four areas. The purpose of this study is to discuss the nature of this understanding among participants and the implications for the professional development of engineering education for science teachers (Meyer and Meyer, 2016). The amount of knowledge of student teachers about the greenhouse effect with the aim of determining their Misconceptions and classifying their knowledge level by studying 71 third-year student teachers in the science education department of the Faculty of Education of the State University of Turkey by a research group. In this research, student teachers were asked to present their knowledge about the greenhouse effect by drawing and writing. The resulting drawn designs and written expressions have been evaluated by descriptive analysis of the answers given to the questions. According to the results of the research, it has been observed that student teachers have both insufficient knowledge and misconceptions about the greenhouse effect (Çelikler and Aksan, 2014).

The investigation of the Misconceptions of student teachers in Iran has also attracted the attention of researchers. Badrian investigated the Misconceptions of experimental science student teachers about the nature of evaporation, surface evaporation rate and vapor pressure and came to the conclusion that experimental science student teachers have many Misconceptions in the field of

evaporation, surface evaporation rate and vapor pressure and they cannot simulate in many cases. to put their learning about basic concepts to good use. Based on these findings, it is necessary to be more careful in organizing the concepts related to evaporation, evaporation rate, vapor pressure and their modeling in the textbooks in the revision of the curricula and educational materials of the master's and associate's field of experimental sciences (Badrian, 2016). Also, similar researches have been conducted on the Misconceptions of the student teachers of chemistry and elementary science of Farhangian University, and the results indicate the existence of Misconceptions about different concepts of science among them (Saadati, 2017 and 2018).

Many of the students' mental ideas are the result of daily experiences, observing scientific phenomena and the application of science and technology in human life, and when they are discussed in the classroom, they can be shown as preconceptions or previous learning and affect the teaching-learning process. put Alternative and non-scientific ideas of students are important factors that prevent meaningful and effective learning and have a negative effect on the continuation of learning in higher grades (Gönen and Kocakaya, 2010).

Studies have shown that elementary school students have various misconceptions about scientific concepts (Allen, 2010). Among the multitude of scientific concepts, heat and temperature are among the concepts related to daily life that form the basis of physics, chemistry and biology, and in most curricula, their learning is suggested from the very first basics of the elementary school (Badrian, 2011). Among the multitude of scientific concepts, the concept of "energy" is one of the concepts related to daily life that forms the basis of physical sciences and is used to explain many phenomena and concepts such as work, force, movement, photosynthesis, chemical reactions, chemical bonds, etc. is used (Else, 1988). Also, everyone deals with this word in their daily life in social, political and economic discussions related to energy sources, energy consumption, energy shortage, energy waste, etc. (Taber, 1989).

Heat and temperature are two quantities related to each other, but this does not mean that both are exactly the same quantity in the same sense. Temperature should not be confused with heat, which is a form of energy. Temperature shows the speed of atoms and molecules of a body, while heat not only shows the speed of movement of atoms and molecules, but also determines the number of atoms and molecules that are affected by it. Also, thermal energy can be transferred without changing the temperature of the object. For example, in the conversion of zero degree Celsius ice to zero degree Celsius water, the energy or heat of the system increases, while the temperature of the object remains constant (Sozbilir, 2003).

Since in the curriculum of experimental sciences of different educational courses, the concept of heat and temperature is taught from the first elementary level to the higher level of secondary school in the form of advanced topics such as thermodynamics and thermochemistry, discovering the students' misconceptions and then trying to correct them is of great importance. It has a lot. Investigations conducted in the elementary course (Naseri Azar, 2012). It shows that elementary school students do not have a correct conceptual understanding of the concept of heat and temperature and the difference between these two scientific words. Ahmadi's studies (2012) show that the students of guidance course could not use the concept of heat and temperature correctly in new learning situations. Even middle school students have Misconceptions in the conceptual application of heat, temperature, and thermal balance in the thermochemistry section (Badrian, 2009) and various sections of physics course 1, 2 and 3 (Sadr al-Ashrafi, 2011).

Since the concept of heat is considered one of the main concepts in the teaching of experimental sciences, especially physics and chemistry, and the understanding of many physical and chemical phenomena depends on the correct understanding of such a concept. Using this concept, students should study the physical and chemical properties of materials, the behavior of simple and complex materials, the change of state of materials, the effect of temperature change on materials, the behavior of materials in individual and aggregated states, kinetic energy and types of material movement. (Niaz, 2006).

Despite the conceptual understanding of heat and temperature on the part of some students, there is still a need to pay a lot of attention and precision in teaching the concepts related to heat and temperature. Also, many students have problems in understanding the relationship between mass and

heat, the difference between heat and temperature, and the exchange of thermal energy in the presence of a temperature difference. They cannot apply what they have learned about heat and temperature well in many simulated cases. Various factors can be introduced as the origin of such Misconceptions. The experiences and prior learning of the students in the previous years, the abstractness of the concepts and the inadequacy of the presented scientific content with the level of the student's cognitive development, the inappropriate organization of the educational content without taking into account the prerequisites and appropriate longitudinal and transverse connections, as well as the use of inappropriate analogs and simulations by the teachers, all of them They are considered to be the causes of Misconception in students (Badrian, 2013).

When planning and writing textbooks, all challenging concepts that are likely to create a Misconception in students should be investigated. The use of diagnostic and formative evaluations and teachers' awareness of students' views and opinions regarding challenging concepts helps to adopt appropriate teaching methods. Research findings can help lesson planners, authors of experimental science textbooks for general education, and also relevant teachers to take steps towards improving the quality of the teaching-learning process of concepts related to heat and temperature. Based on the findings of this research, it is suggested to the planners and writers of the experimental science textbooks of the primary course that in the new experimental science textbooks of the fourth and fifth grades, the concepts of heat, temperature and heat exchange should be given more attention in order to avoid such Misconceptions. be prevented (Badrian, 2013).

2- Acknowledgments

Students of different levels of primary, first secondary (guidance) and second secondary (high school) all have a Misconception in the topics of heat and thermochemistry. Conceptual understanding of heat and temperature and the difference between these two is a problem that is seen by some students, but applying it and relating it to different quantities such as mass and temperature is a problem that is more comprehensive than the previous problem. The students' preconceived notions, the abstractness of the material, the teacher's lack of proper presentation of the lesson to the students, the lack of compliance with the prerequisites, the lack of scientific and educational literacy of the teacher, the teacher's lack of familiarity with the common Misconceptions of the students in the topics, etc. And the growth of Misconceptions in students of different levels is considered.

One of the reasons that causes Misconception among secondary school students is the use of vague words and definitions when explaining basic chemical concepts. Concepts are the root of chemistry. Therefore, if the correct concepts are not taught, the students will suffer in the continuation of the education process. The widespread use of scientific expressions and terms that have been removed in the new educational system has created doubts in the minds of students and disturbed the organization of their minds. Some of these concepts are essential for students to understand. The problem here is that some of the omitted concepts are prerequisites for some other topics. But in some cases, teachers should refrain from mentioning such terms. (Salehi Avval, 2023)

The educational planners of the country should benefit from the researches of researchers in the field of educational content and identification of Misconceptions. Teachers, by conducting continuous formative evaluations, inquiring about initial ideas and paying attention and correcting them during teaching, are among the factors that can help neutralize Misconceptions in the early stages and fix them.

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Certain and peripheral neuropeptides as signaling neurotransmitters

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Abstract

Nervous human neurotransmitters are necessary for sending nerve signals in the whole body in humans. Neuropeptides are small proteinaceous substances produced and released by neurons through the regulated secretory route and acting on neural substrates. They are including monoamines, amino acids, peptides, purines, and catecholamines. In the present study, the various types of neuropeptides and their applications in the nervous and peripheral systems were investigated. For this purpose, all data collection was performed in the general and specific databases. Finally, we proposed that critical neuropeptides such as neuropeptide y have a crucial role in certain and peripheral nervous systems.

Keywords: Nervous neurotransmitters, Neuropeptides, Peripheral systems, Whole body.

1. Introduction

Towards this objective, the first question is “Just what are neuropeptides?” One definition is: “Neuropeptides are small proteinaceous substances produced and released by neurons through the regulated secretory route and acting on neural substrates” (2). The key word in this definition is “neurons” because the only distinction between neuropeptides and other peptides, such as peptide hormones, is that a neuropeptide is synthesized and used by a neuron. Both neuropeptide and peptide hormones are synthesized, modified, and degraded by the same sets of enzymes. Furthermore, both can act nearby as autocrine and paracrine agents and at a distance as endocrine agents. Indeed, nearly all neuropeptides are also found as peptide hormones and vice/versa. So, while we use the term neuropeptide, it is important to keep in mind that neuropeptides are not just in the nervous system – they act both in and out of the CNS. The pleiotropic potential of neuropeptides was best stated by an early pioneer in the field, Candace Pert, who eloquently stated: “As our feelings change, this mixture of peptides travels throughout your body and your brain. And they’re literally changing the chemistry of every cell in your body”. With this perspective, it is not surprising that neuropeptides are emerging as key players in migraine.

The objective of this overview is to provide an appreciation of how the diversity of neuropeptides in the body may contribute to the pathophysiology of migraine by modulating synaptic transmission in the CNS and acting in peripheral tissues to alter cellular activities.

2. Material and methods

We described the data on the neuropeptides as well as some of their properties with potential application in biomedicine, and neurosciences. In an effort to compile all the information on neuropeptide applications were available in the public domain, a key word research -i.e., neurotransmitters, neuropeptides applications, peripheral and certain neuropeptides- was performed in the following databases: NCBI-PubMed (https://www.sciencedirect.com/); Wiley online library (https://onlinelibrary.wiley.com/); Scholar Google (https://scholar.google.com.ar/). Only recent keywords reported within the last 20 years were considered.

3. Results and discussion

3.1. Diversity of neuropeptides

A little appreciated fact is that over the past several decades, the number of known neuropeptides in the human brain has grown to over 100 distinct molecules. An internet resource of data on all known neuropeptides expressed in the human brain has been compiled by the Human Genome Organization Gene Nomenclature Committee (http://www.neuropeptides.nl/tabel%20neuropeptides%20linked.htm), which has been further annotated in another database (http://isyslab.info/NeuroPep). Based on structural homologies, many of these peptides can be grouped into families (Table 1). This table of neuropeptide gene families includes only a partial, representative list of biologically active products within each family. For a complete listing of all the known neuropeptide genes and mature peptides, the reader is referred to the neuropeptides website listed above.

Table 1. Neuropeptide families and migraine.

Families possibly involved in migraine[+]	**Other families**
CGRP: CGRP (α, β), calcitonin, amylin, adrenomedullin (AM1,2)	Opioids:enkephalins, dynorphin, endorphins, nociceptin
	Somatostatin/cortistatin
Glucagon/secretin: PACAP, VIP, glucagon, secretin, GHRH, GIP	Natriuretic factors: ANF, BNF, CNP
	GRP, neuromedins
Vasopressin/oxytocin	Endothelins
	CCK/gastrin
F- and Y-amides: NPY, PPY, NPFF	Insulins: insulin, IGFs, relaxins
	Motilin/ghrelin
Tachykinins: Sub P, neurokinin A, neuropeptide K, neuropeptide gamma	Galanins
	Gonadotropin releasing hormones
Tensins: angiotensin, neurotensin, bradykinin	Neuropeptide B/W/S
	Neurexophilins
CRH-related: CRH, urocortins, urotensins	Cerebellins
	Granins: chromogranins, secretogranins
Adipose neuropeptides: leptin, adiponectin, resistins	
Family-less: orexins, MCH, TRH, PTHrP, CART, AGRP, prolactin, diazepam-binding inhibitor peptide, kisspeptins, etc	

[+] neuropeptides that may potentially be involved in migraine pathogenesis are indicated in bold.

A number of neuropeptides could potentially play roles in migraine. While in most cases, a role in migraine is very speculative, about a dozen candidates are highlighted in Table 1. Discussion of the speculation behind these candidates is beyond the scope of this introductory article and are indicated mainly to emphasize that there is a fairly wide frontier remaining to be explored. The two top peptides, CGRP and PACAP, and several candidates, oxytocin, orexin, and amylin, are discussed in the accompanying articles of this issue. It is intriguing that CGRP and PACAP share many features, which may underlie their roles in migraine (7). In addition, special attention should be paid to NPY based on its autonomic and anti-nociceptive actions.

Likewise, hypothalamic CRH is an interesting candidate based on its sexually dimorphic expression and activity (levels are higher and activation of stress/anxiety pathways is not inhibited by oxytocin in females), including recently described actions in the trigeminal nucleus, as well as its release from the paraventricular nucleus being controlled by CGRP. The expression of many of these neuropeptides in the hypothalamus raises the possibility that

they may contribute to descending pain modulation and autonomic and premonitory symptoms of migraine (8).

While the number of known neuropeptides is somewhat daunting, even more impressive is when all peptides, not just neuropeptides, are considered. There are > 1000 total peptides in Homo sapiens based on genome homologies of neuropeptides, peptide hormones, cytokines, growth factors, antimicrobial peptides, toxins & venom peptides, and antifreeze proteins (www.peptides.be/?p=home). While the functions of many of these peptides in human health and disease remains to be uncovered, the strong evolutionary conservation of peptides and their receptors, argues that this cast of a thousand may play important undiscovered roles, including possibly in migraine.

3.2. Common features of neuropeptides

The common features of neuropeptides can be grouped into three categories: (1) posttranslational processing from precursor proteins and release from dense core vesicles, (2) activation of cell-surface receptors over a relatively large distance, and (3) modulation of target cells in the periphery and the brain, which we suggest could alter sensory perception. These features are described below.

3.3. Neuropeptide processing and release

All neuropeptides are processed from precursor proteins and released from vesicles (Figure 1). The precursor proteins (called pro-peptides) are proteolytically cleaved and many, but not all, are also modified by C-terminal amidation, which is required for biological activity. The biochemistry of peptide proteolysis and amidation was largely worked out in the labs of Eipper and Mains and has been recently reviewed.

An important feature of the processing pathway is that it is a mechanism that can generate a diverse portfolio of peptides from a single gene. In this way, a single precursor protein can encode multiple neuropeptides based on processing, which can dictate generation of the final mature neuropeptides. For example, cell-specific cleavage of the proopiomelanocortin precursor protein can generate either adrenocorticotropic hormone or β-endorphin, which have very different biological activities.

Peptide processing occurs as a progressive mechanism from the endoplasmic reticulum and Golgi apparatus into a subset of secretory vesicles called dense core vesicles (Figure 1). Synthesis and removal of the signal peptide sequence from the pre-propeptide occurs in the endoplasmic reticulum to generate the propeptide. As the peptides traverse the secretory route through the Golgi apparatus, proteolytic cleavage and other modifications occur, such as glycosylation on some peptides. Proteolytic cleavage by endopeptidases, often next to basic residues (lysine or arginine) continues in the secretory vesicles. In addition, cleavage of C-terminal residues and C-terminal amidation also occurs for some peptides in the vesicles. The secretory vesicles, which are called dense core vesicles because of accumulated peptides causing dense staining in electron micrographs, are transported from the cell body down the axon. Depending on the length of the axon, transport can take hours or even a day. The dense core vesicles are present throughout the neuron, but are especially abundant in dendrites, the cell body, and varicosities in the axon. At synapses, they are colocalized with clear synaptic vesicles that contain classical neurotransmitters, such as glutamate. Dense core and clear synaptic vesicles are often co-released, although by somewhat different machineries. Like neurotransmitters, neuropeptides are released by calcium-dependent exocytosis in response to depolarization or other signals. However, in contrast to clear synaptic vesicles, dense core vesicles do not require specialized presynaptic machinery for release. Vesicular release of

neuropeptides from dendrites and cell bodies has been observed in a variety of neurons, including neuronal cell bodies in dorsal root ganglia (6). Combined with features discussed below, these properties are consistent with the view that neuropeptides yield relatively slow and prolonged actions over a larger area than classical neurotransmitters.

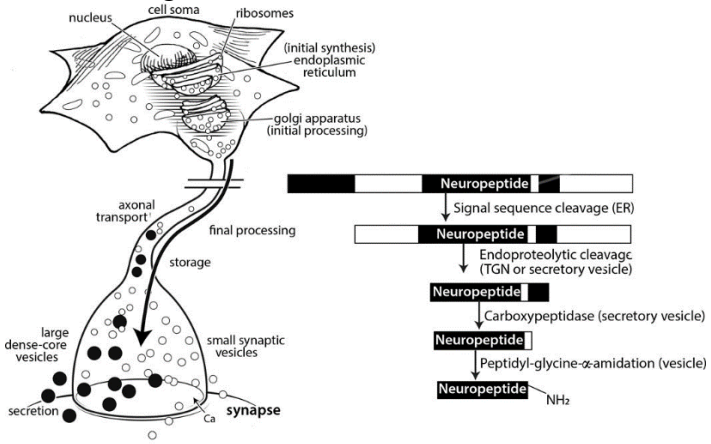

Figure 1. Neuropeptide activation requires multiple processing steps in the cell A representative neuropeptide is shown with sequential processing steps within the neuron beginning in the endoplasmic reticulum, followed by endoproteolytic cleavages in the trans- Golgi network (TGN) or secretory vesicles. Further processing in the vesicles at the C-terminus removes amino acids and adds a C-terminal amide group. The vesicles are transported down the axon and are stored at varicosities (not shown) and near the synapse. Neuropeptides are stored in dense core vesicles, which are larger and functionally distinct from the small, clear synaptic vesicles.

3.4. Activation of receptors at a distance

A perhaps under-appreciated feature of neuropeptides is that, in contrast to classical neurotransmitters, neuropeptides diffuse from their point of release and hence can act at a relatively large distance. This diffusion-driven distribution is referred to as volume transmission or dispersion (Figure 2). Volume transmission is a nonsynaptic dispersion in the extracellular fluid and cerebrospinal fluid (5). It should be noted that this mechanism can also apply to neurotransmitters in certain situations, for example, dopamine and norepinephrine, which can be released from axonal varicosities.

Once released, the peptides are only slowly removed from the extracellular space. This slow removal is due to the lack of reuptake machinery for peptides. In contrast, classical neurotransmitters are rapidly removed from the synaptic cleft by reuptake pumps. The combination of volume transmission and lack of reuptake contributes to the relatively long lasting effects of neuropeptides.

All neuropeptides act as signal transducers via cell-surface receptors. Nearly all neuropeptides act at G-protein coupled receptors (Figure 2). This is an important distinction from ion channel-coupled receptors, since G-protein coupled signaling is consistent with neuropeptides inducing a slower and modulatory response compared to neurotransmitters. In addition, neuropeptide receptors have relatively high ligand affinities (nanomolar Kds), compared to neurotransmitter receptors. This allows a small amount of diffused peptide to still activate receptors. In summary, the combination of these features allows neuropeptides to be active at relatively large distances at relatively low concentrations.

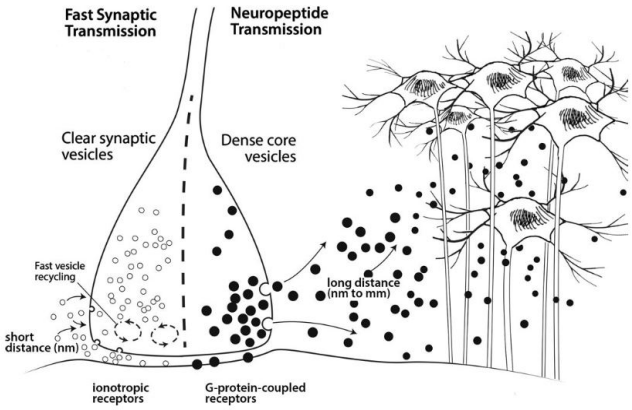

Figure 2. Neuropeptides broadly diffuse and act beyond the synapse Distinguishing features of fast synaptic transmission versus neuropeptide transmission are shown. Classical small molecule transmitters, such as glutamate, are stored in clear synaptic vesicles, while peptides are stored in dense core vesicles. The synaptic vesicles are rapidly recycled and refilled with neurotransmitter close to the synaptic cleft. Once released, neuropeptides are not taken back up into the neuron, so dense core vesicles are not regenerated at the synapse. Instead, dense core vesicles are replenished by axonal transport of new vesicles from the cell body. In addition, dense core vesicles are released from non-synaptic sites as indicated. Once released, classical neurotransmitters bind to ion channel receptors, while nearly all neuropeptides bind to G-protein coupled receptors.

3.5. Modulation in the brain and periphery

Neuropeptide modulation of target cells can occur by two distinct, but overlapping, mechanisms. They can act within the brain as neuromodulators and within the periphery as signaling molecules. Given these features, neuropeptides are well poised to alter sensory perception in migraine. An example is CGRP actions in the periphery and the central nervous system (Figure 3). CGRP can alter the microenvironment of the trigeminovasculature to potentially sensitize trigeminal nociceptors, as well as act in the brain to enhance glutamatergic signaling of CNS neurons. The two mechanisms are not necessarily independent since altered peripheral signals could lead to altered neuromodulatory activity in the brain (10). For example, it has recently been reported that changes in vascular flow in parenchymal arterioles can alter pyramidal neuron activity in cortical slices. And conversely, centrally released CGRP can also alter in vivo blood flow deep in the brain (3).

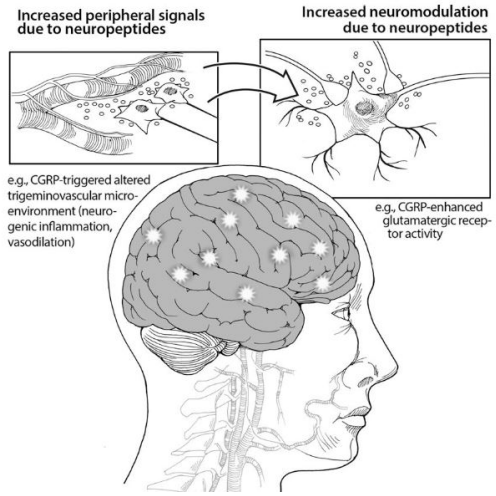

Figure 3. Enhancement of neural activity by neuropeptides acting via peripheral and central Mechanisms. An example of peripheral actions of the neuropeptide CGRP to alter the microenvironment of the trigeminovasculature is shown. A conceptual link between peripheral and central CGRP actions is indicated by the arrow.

The consequence is that nearly all bodily functions can be modulated by neuropeptides. An example of neuropeptide peripheral actions that is relevant to migraine might be at the

cerebrovascular. The vasculature is heavily innervated by sensory, parasympathetic, and sympathetic nerves (4).

4. Conclusion

Members of the diverse families of neuropeptides share common features that could potentially contribute to migraine pathogenesis. Despite these shared properties, each neuropeptide has its own specific retinue of targets and activities that could add to the complexity of migraine (9). Clearly CGRP will not be the only neuropeptide involved in migraine and given the emerging evidence for other peptides, it seems likely that altered neuropeptide actions may be a general theme underlying the heightened sensory state of migraine (1). Such a mechanism could increase the perception of sensory input from the visual, auditory, gustatory, olfactory, and somatosensory systems (Figure 4).

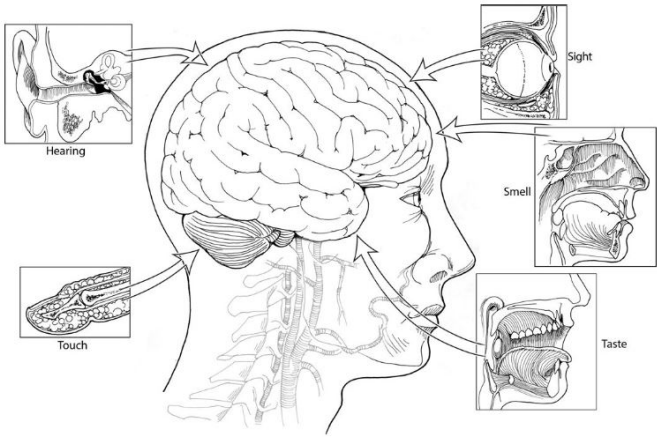

Figure 4. Model of neuropeptide induction of a heightened sensory state in migraine. The senses of sight, smell, taste, hearing, and touch are indicated, with transmission of their sensory information to the CNS represented as arrows. Neuropeptides are predicted to enhance the perception of these sensory signals and in the case of migraine, the signals are suggested to reach a threshold that triggers a pain response.

It is intriguing to speculate that if our senses are heightened by neuropeptides sensitizing the brain, then perhaps migraine may represent a state of too much of a good thing. It will be interesting to see how neuropeptides may help awaken our senses and why for some people that heightened sensitivity triggers the pain and discomfort associated with migraine. recent observations, although preliminary, indicate a role for endogenous neuropeptides in the regulation of autophagy which deserves to be further investigated. This may provide a better knowledge of the molecular mechanisms and functional dynamics of autophagic process as well as its pathophysiology.

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Medicinal application of phycocianin extracted from *Spirulina* algae

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Abstract

Phycocyanin is a natural blue pigment found in *Spirulina* algae, and it has been shown to have numerous medicinal applications. Phycocyanin has potent anti-inflammatory and antioxidant effects, making it useful in the management of conditions such as arthritis, asthma, and colitis, and in reducing the risk of chronic diseases such as cancer, heart disease, and diabetes. Phycocyanin can also stimulate the immune system and protect nerve cells, the liver, and other organs from oxidative stress and inflammation. Furthermore, phycocyanin has been shown to have anti-cancer, anti-viral, and anti-obesity effects. While more research is needed to fully understand its mechanisms of action and optimal dosages, phycocyanin has the potential to be a valuable therapeutic agent in the prevention and treatment of various diseases.

Key words: Medicinal application, *phycocianin, Spirulina,* algae.

1- Introduction

Phycocyanin is a blue pigment that is commonly found in various species of cyanobacteria and algae, including *Spirulina* (1). It has been extensively studied for its potential medicinal applications due to its antioxidant, anti-inflammatory, and immune-boosting properties (2). In this study, we will discuss the medicinal applications of phycocyanin extract from *Spirulina* algae. *Spirulina* is blue - green algae that have been used for centuries as a dietary supplement due to its high protein content and other beneficial nutrients (3). Phycocyanin is one of the major pigments found in *Spirulina*, and it has been shown to have numerous health benefits (4, 5).

2- Chemical Properties of phycocyanin

Phycocyanin is a water-soluble pigment that gives *Spirulina* its blue-green color. It is composed of two subunits, alpha, and beta, and has a molecular weight of approximately 104 kDa (6). Phycocyanin has been shown to have antioxidant, anti-inflammatory, and immunomodulatory properties. Phycocyanin is belongs to the phycobiliprotein family. It is a complex molecule composed of a protein subunit, called alpha or beta phycocyanin, and a chromophore, called phycocyanobilin (PCB). The PCB molecule is responsible for the blue color of phycocyanin and has a similar structure to heme, which is the molecule that gives blood its red color (7). The chemical properties of phycocyanin are primarily determined by its chromophore, PCB. PCB is a tetrapyrrole molecule that can exist in different oxidation states depending on the pH and the presence of oxygen or other oxidizing agents (8). In its oxidized form, PCB absorbs light in the blue region of the spectrum and emits fluorescence in the red region (9). This property makes phycocyanin useful in various applications, such as fluorescent labeling and detection in biological research (10).

Phycocyanin is also a stable molecule with a high resistance to heat, pH changes, and proteolytic enzymes (11). This stability is attributed to the protein subunit that protects the chromophore from degradation and denaturation. However, phycocyanin can be denatured by harsh conditions such as extreme pH, high temperature, and high pressure (11, 12). Phycocyanin has been found to have some unique chemical properties that make it useful in various fields. For example, it has been used as a natural food colorant in the food industry because of its blue color and stability (13). It has also been used as a fluorescence probe in biomedical research due to its strong fluorescence emission (14).

3- Medicinal application of phycocianin

Phycocyanin has many medicinal uses, some of which we will discuss below. Phycocyanin is a safe and natural compound with no reported side effects and is a promising alternative to synthetic drugs with potential toxicity (15). While more research is needed to fully understand its mechanisms of action and optimal doses, phycocyanin has the potential to be a valuable therapeutic agent in the prevention and treatment of various diseases (16).

3-1- Anti-inflammatory

Phycocyanin has been shown to have potent anti-inflammatory effects by inhibiting the production of pro-inflammatory cytokines and some enzymes (17). This makes it useful in the management of conditions such as arthritis, asthma, and colitis. Phycocyanin has been found to be as effective as some non-steroidal anti-inflammatory drugs (NSAIDs) in reducing inflammation, but without the potential side effects.

3-2- Antioxidant

Phycocyanin is a powerful antioxidant that can help to protect the body against oxidative stress. It can scavenge free radicals and reduce the damage caused by reactive oxygen species (ROS) in cells and tissues (18). This can reduce the risk of chronic diseases such as cancer, heart disease, and diabetes (19). Studies have also shown that phycocyanin can protect the kidneys and other organs from oxidative damage caused by various toxins (20, 21).

3-3- Immune-boosting

Phycocyanin can stimulate the production of white blood cells, which are responsible for fighting off infections and diseases (22). It can enhance the activity of natural killer (NK) cells, which are important in the body's defense against cancer and viruses (23). Studies have shown that phycocyanin can increase the production of cytokines such as interleukin-1β (IL-1β) and tumor necrosis factor-alpha (TNF-α), which are important in the immune response (24, 25).

3-4- Neuroprotective

Phycocyanin has been studied for its potential to protect nerve cells from damage caused by oxidative stress and inflammation (26). It can reduce the production of ROS and prevent the activation of inflammatory pathways in the brain (27). Studies have shown that phycocyanin can improve cognitive function and memory in animal models of Alzheimer's disease (28, 29). It may also have potential in the treatment of other neurodegenerative diseases such as Parkinson's and Huntington's disease (30, 31).

3-5- Liver-protective

Phycocyanin can protect the liver from damage caused by various toxins such as alcohol and drugs. It can reduce oxidative stress and inflammation in the liver, and improve liver function (32). Studies have shown that phycocyanin can reduce liver damage in animal models of liver injury (33, 34).

3-6- Anti-cancer

Phycocyanin has been shown to have anti-cancer properties. It can inhibit the growth and proliferation of cancer cells by inducing cell cycle arrest and apoptosis. Studies have shown that phycocyanin can reduce the growth of tumors in animal models of cancer. It may also have potential in the prevention and treatment of various types of cancer in humans (35, 36).

3-7- Anti-viral

Phycocyanin has been studied for its potential to inhibit the replication of viruses such as HIV and hepatitis C. It can prevent the entry of viruses into cells and inhibit viral replication by interfering with viral proteins. Studies have shown that phycocyanin can reduce the viral load and improve immune function in animal models of viral infections (37, 38).

3-8- Anti-obesity

Phycocyanin has been shown to have anti-obesity effects. It can reduce body weight and improve lipid metabolism by regulating adipocyte differentiation and lipid metabolism-related gene expression (39).

3-9- Other Medicinal Effects

Phycocyanin has also been shown to have other medicinal effects, such as antidiabetic, antihypertensive, and hepatoprotective properties (40). It can reduce blood glucose levels, lower blood pressure, and protect the liver from damage. These properties may have potential as therapeutic agents for diabetes, hypertension, and liver diseases (41).

4- Conclusion

Phycocyanin extract from *Spirulina* has numerous medicinal properties, including antioxidant, anti-inflammatory, immunomodulatory, antidiabetic, antihypertensive, and hepatoprotective properties (42). These properties may have potential as therapeutic agents for various diseases, such as cancer, cardiovascular disease, neurodegenerative diseases, arthritis, inflammatory bowel disease, asthma, diabetes, hypertension, and liver diseases (43). However, further research is needed to fully understand the therapeutic potential of phycocyanin extract from *Spirulina* and to develop it into safe and effective treatments for these diseases (23).

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Algae as a Promising Source for Biodiesel Production

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Abstract

Biodiesel production has become a global concern due to the increasing demand for alternative and sustainable energy sources. Algae have been recognized as a promising source for biodiesel production due to their fast growth rate, high lipid content, and ability to grow in various environments. This review paper aims to provide a comprehensive overview of the current research on algae as a source for biodiesel production. The paper discusses the different algae species that have been investigated for biodiesel production, the cultivation techniques, lipid extraction methods, and the advantages and disadvantages of using algae as a source for biodiesel production. The paper also highlights the current challenges faced in the production of biodiesel from algae and suggests future directions for research in this field.

Key words: Algae, Biodiesel, Lipid Extraction, Cultivation Techniques, Challenges.

1- Introduction

Biodiesel is a renewable and sustainable energy source that has gained global attention due to its potential to reduce greenhouse gas emissions and dependence on fossil fuels (1). Biodiesel is typically produced from vegetable oils, animal fats, and used cooking oil including triglycerides (2). However, the use of these feedstocks has limitations, such as competition with food production, limited availability, and high production costs. Algae have emerged as a promising alternative source for biodiesel production due to their high lipid content, fast growth rate, and ability to grow in various environments (3, 4). Biodiesel production from algae is a promising and sustainable source of renewable energy that has gained significant attention in recent years (5). The use of algae as a source for biodiesel production offers several benefits over traditional feedstock (6). We discuss some of the benefits of producing biodiesel by algae and how it can contribute to a cleaner and greener energy:

Firstly, algae have a fast growth rate and can be cultivated in a variety of environments, such as freshwater, saltwater, and wastewater (7). Algae can also be grown on non-arable land, reducing the impact on food production (8). This makes algae an ideal feedstock for biodiesel production as it does not compete with food production, which is a significant issue for traditional biodiesel feedstocks (3). Additionally, algae can be grown in large quantities and can produce a high yield of lipids, which can be converted into biodiesel (9).

Secondly, biodiesel produced from algae has several environmental benefits. Biodiesel is a renewable and sustainable source of energy that can reduce greenhouse gas emissions, which are a major contributor to climate change (3). The production and use of biodiesel can reduce emissions of carbon dioxide, particulate matter, and other pollutants compared to fossil fuels. The use of biodiesel can also reduce our dependence on imported oil (10).

Thirdly, biodiesel produced from algae has excellent energy content, which makes it a suitable substitute for diesel fuel (11). Biodiesel can be used in existing diesel engines without any modifications, and it has similar energy content to petroleum-based diesel (12). Biodiesel also has a higher cetane number, which indicates better ignition quality, resulting in better fuel efficiency and reduced emissions (13).
Fourthly, biodiesel production from algae can provide economic benefits, particularly in rural areas (14). Algae cultivation can create jobs and provide income for farmers, particularly those who have non-arable land (15). The production of biodiesel from algae can also create new industries and businesses, which can contribute to local economies and provide economic growth (16).
Finally, the production of biodiesel from algae can help improve energy security and reduce dependence on oil (17). Biodiesel can be a stable and reliable source of energy and reduce the impact of price fluctuations and supply disruptions (18).

2- Algae Species for Biodiesel Production

Various algae species have been investigated for biodiesel production, including microalgae and macroalgae (19). Microalgae are unicellular organisms that can be grown in open ponds, photobioreactors, and closed systems. They have high lipid content and can produce lipids under different environmental conditions (20). Some of the commonly investigated microalgae species for biodiesel production include *Chlorella*, *Nannochloropsis*, and *Dunaliella* (21). Macroalgae, on the other hand, are multicellular organisms that can be harvested from natural seawater or cultivated in marine farms (22). They have a lower lipid content compared to microalgae but can still produce biodiesel. Some of the commonly investigated macroalgae species for biodiesel production include *Ulva*, *Gracilaria*, and *Saccharina* (23).

3- Cultivation Techniques

The cultivation of algae for biodiesel production involves the optimization of environmental conditions, such as light, temperature, nutrients, and pH (24). Algae can be grown in open ponds, which are the most common and cost-effective cultivation method, or in closed systems, such as photobioreactors, which allow better control over environmental conditions (25). Closed systems can be expensive, but they offer higher yields and reduce the risk of contamination (26).

4- Lipid Extraction Methods

The extraction of lipids from algae is a crucial step in biodiesel production. Various methods have been developed, including mechanical methods, such as pressing and sonication, and chemical methods, such as solvent extraction and supercritical fluid extraction (27). Solvent extraction is the most common method used for lipid extraction, but it requires large amounts of solvents and can be expensive (28). Supercritical fluid extraction is an emerging technology that uses supercritical carbon dioxide to extract lipids, which is more environmentally friendly and efficient (29).

5- Advantages and Disadvantages of Algae for Biodiesel Production

The use of algae as a source for biodiesel production has several advantages, such as their high lipid content, fast growth rate, and ability to grow in various environments (4, 30). Algae also have a lower impact on food production compared to other feedstocks, such as vegetable

oils (3). However, the use of algae for biodiesel production also has some limitations, such as the high capital and operational costs of cultivation and lipid extraction, the need for optimized environmental conditions, and the potential for contamination (31).

6- Challenges and Future Directions

The production of biodiesel from algae is still facing several challenges, such as the high costs of cultivation and lipid extraction, the low lipid productivity of some algae species, and the need for sustainable and scalable cultivation methods (32). Future research should focus on optimizing cultivation conditions, developing more efficient lipid extraction methods, and exploring the use of genetic engineering and biotechnology to enhance lipid productivity and reduce costs (33). Furthermore, the potential environmental impacts of large-scale algae cultivation should also be considered (34).

7- Conclusion

Algae have emerged as a promising source for biodiesel production due to their high lipid content, fast growth rate, and ability to grow in various environments (3). The use of algae for biodiesel production has several advantages over other feedstocks, such as their lower impact on food production (3, 4). However, the production of biodiesel from algae still faces several challenges, such as high costs and low lipid productivity (32). Future research should focus on optimizing cultivation and lipid extraction methods and developing more efficient and cost-effective technologies for large-scale algae cultivation. Additionally, the use of genetic engineering and biotechnology to enhance lipid productivity and reduce costs should also be explored (35). In conclusion, the production of biodiesel from algae is a promising alternative to conventional feedstocks due to their high lipid content and fast growth rate (36, 37). In summary, research and development on algae-based biodiesel production has the potential to become a sustainable and economical source of renewable energy in the future.

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Role of *spirulina* algae in reducing the effects of environmental stress on plants

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Abstract

Environmental stress, such as drought, salinity, high temperature, and heavy metal toxicity, is a major concern for crop productivity worldwide. *Spirulina*, a blue-green microalga, has been found to have beneficial effects on plant growth and stress tolerance. In this paper, we review the current literature on the role of *spirulina* in reducing the effects of environmental stress on plants. We discuss the potential mechanisms by which *spirulina* improve stress tolerance, including the regulation of antioxidant enzymes, modulation of gene expression, enhancement of nutrient uptake, and hormonal regulation. We also highlight the potential applications of *spirulina* in sustainable agriculture, including its use as a biofertilizer, biostimulant, and biopesticide.

Key words: *Spirulina,* Algae, Environmental Stress, Plants, Antioxidant.

1- Introduction

Environmental stress is a major factor limiting crop productivity and quality worldwide (1). These stresses can have a significant impact on crops, resulting in reduced yields, lower quality produce, and even crop failure. Drought, extreme temperatures, salinity, and nutrient deficiencies are just a few examples of stressors that can negatively affect plant growth and development (2). These stressors can lead to changes in plant physiology, including reduced photosynthesis, altered metabolic pathways, and increased susceptibility to pests and diseases (3). As a result, farmers and researchers are increasingly seeking solutions to mitigate the effects of environmental stress on crops to maintain food security and ensure sustainable agriculture (4, 5).

Nowadays, due to the global increase in population and the need for more food and agricultural products, it is necessary to develop strategies to increase plant tolerance to environmental stresses for sustainable agriculture (6). *Spirulina* is a blue-green microalga that has been widely used as a nutritional supplement due to its high protein content and health-promoting properties (7). In recent years, *spirulina* has attracted increasing attention for its potential role in improving plant growth and stress tolerance (8). The aim of this paper is to review the current literature on the role of *spirulina* in reducing the effects of environmental stress on plants.

2- Mechanisms of *spirulina* in reducing the effects of environmental stress on plants

Spirulina has been shown to improve plant growth and stress tolerance through several mechanisms (9, 10). One of the main mechanisms is the regulation of antioxidant enzymes. Under stress conditions, plants produce reactive oxygen species (ROS), which can cause cellular damage and oxidative stress (11). *Spirulina* has been shown to increase the activity of antioxidant enzymes, such as superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), which scavenge ROS and protect the plant from oxidative damage (12, 13).
In addition, *spirulina* has been shown to modulate gene expression in response to stress (14). Furthermore, *spirulina* has been shown to enhance nutrient uptake and utilization in plants. *Spirulina* contains high levels of micronutrients, such as iron, zinc, and magnesium, which are essential for plant growth and stress tolerance (15). *Spirulina* has been shown to enhance the uptake of these micronutrients by plants, which can improve plant growth and stress tolerance (16). Moreover, *spirulina* has been found to regulate plant hormones, such as abscisic acid (ABA) and auxins, which play a critical role in plant growth and stress tolerance (17). *Spirulina* has also been found to enhance auxin levels, which can improve root growth and nutrient uptake (16, 17).

3- Potential applications of *spirulina* in sustainable agriculture

Spirulina has potential applications in sustainable agriculture as a natural and environmentally friendly strategy to enhance plant growth and stress tolerance (8). *Spirulina* can be used as a biofertilizer, as it contains high levels of nitrogen, phosphorus, and potassium, which are essential for plant growth (18, 19). Furthermore, *spirulina* can be used as a biostimulant, as it enhances plant growth and stress tolerance (8). *Spirulina* can also be used as a biopesticide, as it contains bioactive compounds that have been shown to have antifungal and antibacterial properties (20).

4- Conclusion

In conclusion, *spirulina* has been found to have beneficial effects on plant growth and stress tolerance under various environmental stress conditions, such as drought, salinity, high temperature, and heavy metal toxicity (21). The mechanisms by which *spirulina* improves stress tolerance include the regulation of antioxidant enzymes, modulation of gene expression, enhancement of nutrient uptake, and hormonal regulation (22). *Spirulina* also has potential applications in sustainable agriculture as a natural and environmentally friendly strategy to enhance plant growth and stress tolerance, as a biofertilizer, biostimulant, and biopesticide (23, 24). Further research is needed to fully understand the mechanisms of *spirulina* in improving plant stress tolerance and to optimize its use in sustainable agriculture. Nevertheless, *spirulina* holds great promise for addressing the challenges of environmental stress on plant growth and sustainable agriculture (25).
Overall, *spirulina* algae have shown great potential in reducing the effects of environmental stress on plants and improving sustainable agriculture (26). Its ability to enhance plant growth and stress tolerance through various mechanisms makes it a promising natural and environmentally friendly strategy to overcome the challenges of environmental stress on crop productivity (27). With further research and development, *spirulina*-based products could become an important tool for sustainable agriculture and food security (28).

5- Suggested future research

Future research could focus on investigating the effects of *spirulina* on different crops and under different environmental stress conditions. Also, the potential use of *spirulina* in combination with other plant growth-stimulating microorganisms, such as mycorrhizal fungi

and rhizobacteria, to increase plant growth and tolerate stress synergistically can be interesting. Additionally, research could focus on the production and application of *spirulina*-based biofertilizers, biostimulants, and biopesticides at a larger scale, as well as their efficacy in field trials.

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Medicinal application of phycocianin extracted from *Spirulina* algae

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Abstract

Phycocyanin is a natural blue pigment found in *Spirulina* algae, and it has been shown to have numerous medicinal applications. Phycocyanin has potent anti-inflammatory and antioxidant effects, making it useful in the management of conditions such as arthritis, asthma, and colitis, and in reducing the risk of chronic diseases such as cancer, heart disease, and diabetes. Phycocyanin can also stimulate the immune system and protect nerve cells, the liver, and other organs from oxidative stress and inflammation. Furthermore, phycocyanin has been shown to have anti-cancer, anti-viral, and anti-obesity effects. While more research is needed to fully understand its mechanisms of action and optimal dosages, phycocyanin has the potential to be a valuable therapeutic agent in the prevention and treatment of various diseases.

Key words: Medicinal application, *phycocianin*, *Spirulina*, algae.

1- Introduction

Phycocyanin is a blue pigment that is commonly found in various species of cyanobacteria and algae, including *Spirulina* (1). It has been extensively studied for its potential medicinal applications due to its antioxidant, anti-inflammatory, and immune-boosting properties (2). In this study, we will discuss the medicinal applications of phycocyanin extract from *Spirulina* algae. *Spirulina* is blue - green algae that have been used for centuries as a dietary supplement due to its high protein content and other beneficial nutrients (3). Phycocyanin is one of the major pigments found in *Spirulina*, and it has been shown to have numerous health benefits (4, 5).

2- Chemical Properties of phycocyanin

Phycocyanin is a water-soluble pigment that gives *Spirulina* its blue-green color. It is composed of two subunits, alpha, and beta, and has a molecular weight of approximately 104 kDa (6). Phycocyanin has been shown to have antioxidant, anti-inflammatory, and immunomodulatory properties. Phycocyanin is belongs to the phycobiliprotein family. It is a complex molecule composed of a protein subunit, called alpha or beta phycocyanin, and a chromophore, called phycocyanobilin (PCB). The PCB molecule is responsible for the blue color of phycocyanin and has a similar structure to heme, which is the molecule that gives blood its red color (7). The chemical properties of phycocyanin are primarily determined by its chromophore, PCB. PCB is a tetrapyrrole molecule that can exist in different oxidation states depending on the pH and the presence of oxygen or other oxidizing agents (8). In its oxidized form, PCB absorbs light in the blue region of the spectrum and emits fluorescence in the red region (9). This property makes phycocyanin useful in various applications, such as fluorescent labeling and detection in biological research (10).

Phycocyanin is also a stable molecule with a high resistance to heat, pH changes, and proteolytic enzymes (11). This stability is attributed to the protein subunit that protects the chromophore from degradation and denaturation. However, phycocyanin can be denatured by harsh conditions such as extreme pH, high temperature, and high pressure (11, 12). Phycocyanin has been found to have some unique chemical properties that make it useful in various fields. For example, it has been used as a natural food colorant in the food industry because of its blue color and stability (13). It has also been used as a fluorescence probe in biomedical research due to its strong fluorescence emission (14).

3- Medicinal application of phycocianin

Phycocyanin has many medicinal uses, some of which we will discuss below. Phycocyanin is a safe and natural compound with no reported side effects and is a promising alternative to synthetic drugs with potential toxicity (15). While more research is needed to fully understand its mechanisms of action and optimal doses, phycocyanin has the potential to be a valuable therapeutic agent in the prevention and treatment of various diseases (16).

3-1- Anti-inflammatory

Phycocyanin has been shown to have potent anti-inflammatory effects by inhibiting the production of pro-inflammatory cytokines and some enzymes (17). This makes it useful in the management of conditions such as arthritis, asthma, and colitis. Phycocyanin has been found to be as effective as some non-steroidal anti-inflammatory drugs (NSAIDs) in reducing inflammation, but without the potential side effects.

3-2- Antioxidant

Phycocyanin is a powerful antioxidant that can help to protect the body against oxidative stress. It can scavenge free radicals and reduce the damage caused by reactive oxygen species (ROS) in cells and tissues (18). This can reduce the risk of chronic diseases such as cancer, heart disease, and diabetes (19). Studies have also shown that phycocyanin can protect the kidneys and other organs from oxidative damage caused by various toxins (20, 21).

3-3- Immune-boosting

Phycocyanin can stimulate the production of white blood cells, which are responsible for fighting off infections and diseases (22). It can enhance the activity of natural killer (NK) cells, which are important in the body's defense against cancer and viruses (23). Studies have shown that phycocyanin can increase the production of cytokines such as interleukin-1β (IL-1β) and tumor necrosis factor-alpha (TNF-α), which are important in the immune response (24, 25).

3-4- Neuroprotective

Phycocyanin has been studied for its potential to protect nerve cells from damage caused by oxidative stress and inflammation (26). It can reduce the production of ROS and prevent the activation of inflammatory pathways in the brain (27). Studies have shown that phycocyanin can improve cognitive function and memory in animal models of Alzheimer's disease (28, 29). It may also have potential in the treatment of other neurodegenerative diseases such as Parkinson's and Huntington's disease (30, 31).

3-5- Liver-protective

Phycocyanin can protect the liver from damage caused by various toxins such as alcohol and drugs. It can reduce oxidative stress and inflammation in the liver, and improve liver function (32). Studies have shown that phycocyanin can reduce liver damage in animal models of liver injury (33, 34).

3-6- Anti-cancer

Phycocyanin has been shown to have anti-cancer properties. It can inhibit the growth and proliferation of cancer cells by inducing cell cycle arrest and apoptosis. Studies have shown that phycocyanin can reduce the growth of tumors in animal models of cancer. It may also have potential in the prevention and treatment of various types of cancer in humans (35, 36).

3-7- Anti-viral

Phycocyanin has been studied for its potential to inhibit the replication of viruses such as HIV and hepatitis C. It can prevent the entry of viruses into cells and inhibit viral replication by interfering with viral proteins. Studies have shown that phycocyanin can reduce the viral load and improve immune function in animal models of viral infections (37, 38).

3-8- Anti-obesity

Phycocyanin has been shown to have anti-obesity effects. It can reduce body weight and improve lipid metabolism by regulating adipocyte differentiation and lipid metabolism-related gene expression (39).

3-9- Other Medicinal Effects

Phycocyanin has also been shown to have other medicinal effects, such as antidiabetic, antihypertensive, and hepatoprotective properties (40). It can reduce blood glucose levels, lower blood pressure, and protect the liver from damage. These properties may have potential as therapeutic agents for diabetes, hypertension, and liver diseases (41).

4- Conclusion

Phycocyanin extract from *Spirulina* has numerous medicinal properties, including antioxidant, anti-inflammatory, immunomodulatory, antidiabetic, antihypertensive, and hepatoprotective properties (42). These properties may have potential as therapeutic agents for various diseases, such as cancer, cardiovascular disease, neurodegenerative diseases, arthritis, inflammatory bowel disease, asthma, diabetes, hypertension, and liver diseases (43). However, further research is needed to fully understand the therapeutic potential of phycocyanin extract from *Spirulina* and to develop it into safe and effective treatments for these diseases (23).

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Novel drug delivery systems

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Abstract

One of the key areas in pharmaceuticals is the precise targeting of drugs to selected cells or tissues. Drug targeting systems must control the fate of the drug entering the body. Today's delivery technologies are numerous, with the so-called "magic bullet" scheme proposed by Paul Ehrlich at the beginning of the 20th century, in which the drug is delivered precisely to the target. Nano drug delivery systems (NDDSs) are categories of nanomaterials that increase the stability and solubility of drugs in water, prolong the cycle time, increase the absorption rate of target cells or tissues, and reduce enzymes, thus improving safety. And drug efficacy NDDS can be administered through existing routes including inhalation, oral administration or intravenous injection and remains better bioavailability. Nanoparticles are classified as nanoparticles with a diameter between 10 and 100 nm. During the design of nanoparticles, some controls such as release patterns and surface properties should be taken into account, which, with an appropriate dosing scheme, determine the activation of specific sites at optimal rates. Many new carriers have been registered for implants in the last ten years, including nanosomes, nanophytosomes, nanoemulsions, transferosomes, and autosomes. The aim of this study is to synthesize various newly developed technologies for drug delivery of herbal medicines to improve therapeutic response.

Key words: Phytochemical, Nanomedicine, Phytosome, Novel Drug Delivery System

1- Introduction

One of the most important aspects of drug delivery is the precise targeting of drugs to specific cells or tissues. To achieve this, drug targeting systems must be able to control the entry of drugs into the body. However, current delivery technologies are still far from Paul Ehrlich's "magic bullet" design, which aimed to precisely target drugs to their site of action(1). Nanotechnology presents a new challenge in this field, as it aims to deliver drugs to the right place at the right time. Nanotechnology involves creating and utilizing materials, devices, and systems on a nanometer length scale, which allows for atomic, molecular, and supramolecular level control(2). Nanoparticulate drug delivery systems (NDDSs) are a category of nanomaterials that can increase stability and solubility in water for drugs, prolong cycle time, increase cellular or tissue uptake rate, reduce enzyme degradation and ultimately improve safety and efficacy of drugs. NDDSs can be administered through various routes such as

inhalation, oral administration or intravenous injection and provide better biological availability(3). The advancements in biotechnology and related fields have greatly aided the discovery and rational design of new drug classes. However, specific drug delivery methods must be improved to ensure clinical efficacy. Many drugs are limited by poor solubility, high toxicity, high dosage requirements, accumulation due to poor solubility, non-specific delivery, in vivo degradation, and short half-life in circulation(4). Fortunately, the field of drug delivery is rapidly developing and providing opportunities for therapeutic methods that were previously eliminated by conventional dosing forms. Nanoparticles with diameters between 10-100 nanometers are being used to overcome targeting difficult tissues such as the blood-brain barrier(5). These new systems can prevent solubility problems, protect drugs from external environments such as light degradation and pH changes while reducing dosage by controlling release profiles. Additionally, controlled targeting at the site of action and reduced exposure time to non-target tissues increase treatment efficacy while reducing toxicity and side effects The first documented nanoparticles were based on a non-biodegradable polymer framework (polyacrylamide, polymethyl methacrylate, polystyrene) (6). Many new carriers have been registered in the past decade for implants, including nanoliposomes , nanoniosomes , nanophytosomes , nanoemulsions , transferosomes and autosome. Various newly developed technologies for delivering herbal drugs are examined in this article to improve therapeutic responses(7).

2- Liposome

Liposomes, which are bilayered vesicles composed mainly of natural or artificial phospholipids, have dense, water-filled interiors. They come in different sizes as single-layer or multilayer structures, and their name comes from the phospholipids they are made of, rather than their size. Liposomes have no lipophobic substance, such as water, despite it being an exception rather than the rule. Artificial vesicles made up of bilayered lipids are known as liposomes(8). The size of liposomes typically ranges from 0.08 to 10.00 micrometers and they can be categorized based on their size and the presence of two layers of phospholipids, including small unilamellar vesicles (100-100 nanometers), large unilamellar vesicles (100-300 nanometers), and multilamellar vesicles. Liposomes are considered excellent delivery systems for ophthalmic applications due to their exceptional biocompatibility, similarity to cell membranes, and capacity to encapsulate drugs that are both hydrophilic and hydrophobic(9). Three major achievements of liposome application: steric stabilization, remote loading of drugs by pH and ion gradients, and lipoplexes based on complexes of cationic liposomes with anionic nucleic acids or proteins extended research toward liposome application and opened the way for development of a large spectrum of products.(10)

3- Nanophytosome

Phytozomes, also known as herbosomes, are a plant-based transport system that enhances the absorption and availability of poorly soluble drugs. They are composed of a combination of active natural phospholipids and phytochemicals that are structurally linked through a reaction between plant extracts and phosphatidylcholine in a solvent. The resulting structure is comprised of amphiphilic molecules that completely cover the phosphatidyl moiety. Phytozomes offer numerous benefits, including high drug encapsulation, improved stability due to chemical bonds between the amphiphilic molecule polar head and plant material, and increased biological availability. They are ideal for both active compounds that require a biological effect and polar plant compounds(9). Nanotechnology opened a pioneer field in cancer therapy by modifying significant properties of drugs and their carriers. Nanotechnology utilizes various nanostructures to transport anti-cancer agents to the site of action. The greater stability of nanophytosomes is due to formation of chemical links between

phospholipid molecules and phytoactive agents. Among several new drug delivery systems, phytosomes depict an advanced technology to deliver phytoactive compounds to the target site of action, and at present, several phytosome formulations are in clinical use.(12)

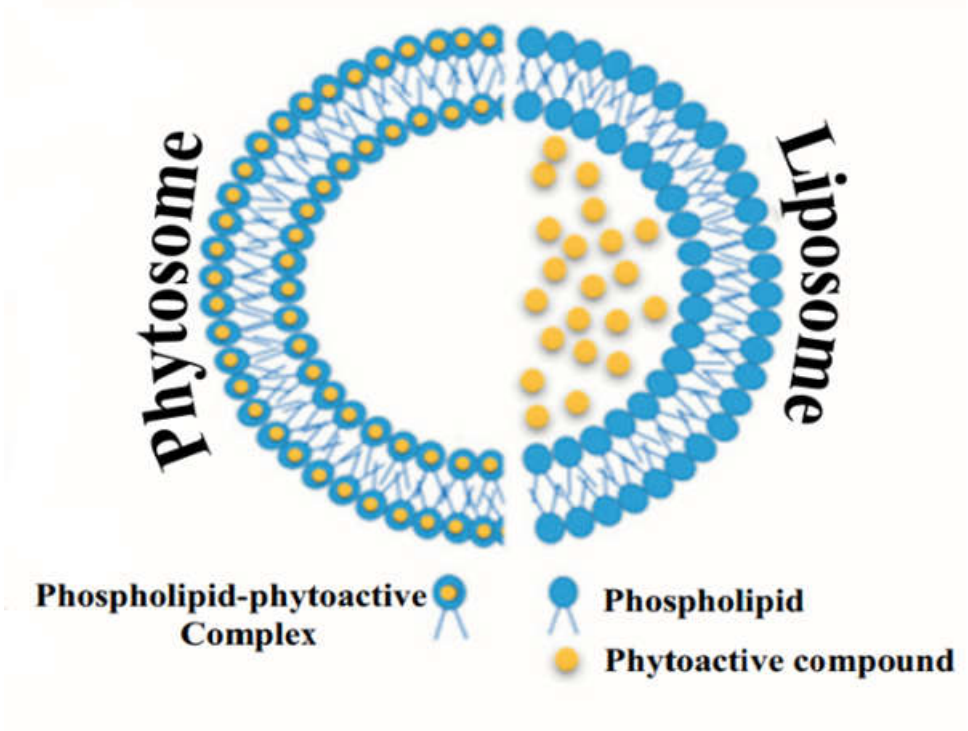

Figure 1:Structural differences between phytosome and liposome.(12)

4- Nanoniosome

The stiffness of bilayer structures can be enhanced by combining non-ionic surfactant and cholesterol. Niosomes, which are nanocarriers based on non-ionic surfactants, have become increasingly popular due to their exceptional characteristics. Niosomes are an alternative to other carriers, particularly liposomes, as they offer several advantages such as easy preparation, affordability, excellent stability, and the ability to encapsulate both water-soluble and water-insoluble drugs simultaneously(11).Newsomes are more desirable than liposomes due to their enhanced stability and cost-effectiveness. They improve the therapeutic effectiveness of drugs by prolonging their presence in the bloodstream and safeguarding the surroundings(13).Newsome vehicles are tiny particles made up of amphiphilic molecules that enclose a water-filled chamber. These particles consist of both hydrophilic (water-loving) and hydrophobic (water-repelling) components, which allow them to form different shapes like micelles or flat layers. In the layered structure, the hydrophobic parts are pushed away from the water while the hydrophilic heads remain in contact with it. By adjusting factors such as composition, size, layering, volume, surface charge, and concentration, the properties of these particles can be modified(14).As application of nanonisome, we can name drug delivery, vaccination and gene delivery.(15)

5- Transferrosomes

Transferosomes are a new type of lipid vesicle that surpasses the limitations of ordinary liposomes by deeply penetrating the skin and reaching deeper layers of the stratum corneum. These highly flexible lipid-based elastic vehicles have membranes that are easily deformable,

allowing for non-obstructive delivery of substances to deeper skin tissues. Through osmotic force or skin hydration, the substance is able to penetrate into the intercellular regions between lamellar layers. Composed of phospholipids and a single-chain surfactant, transferosomes possess elastic and deformable properties that make them ideal for providing local nutrients to maintain healthy skin(7). Delivering a drug to the brain is a formidable phenomenon that can be achieved by three main approaches. s. One of them is intranasal administration of therapeutic compounds, which bypasses the BBB and delivers the drug directly to the brain through the olfactory region, or delivers the drug to the blood and then to the brain, therefore improving the drug bioavailability and efficiency.Olanzapine-loaded deformable vesicles, transferrosomes and nanocubic vesicles have been observed to be more effective in delivering drug to the brain than less deformable and larger vesicles following intranasal administration in rats.(16)

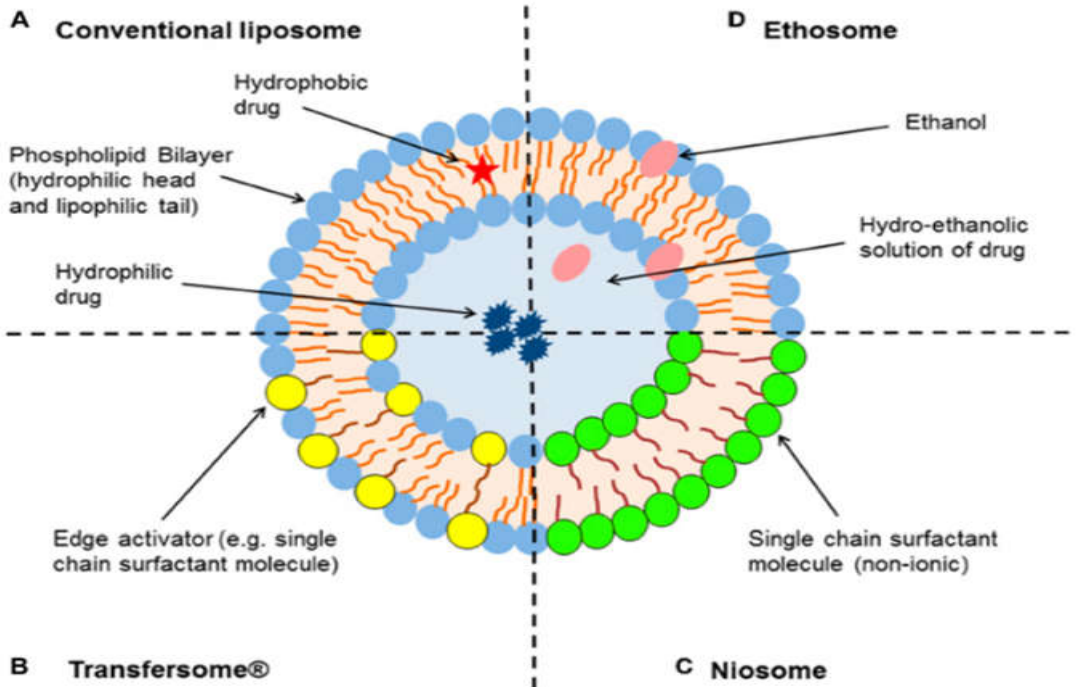

Figure 2: A schematic illustration of liposome (A), transferosome (B), niosome (C) and ethosome (D).(7)

6- autosome

A novel autosome has been created as a gentle and non-invasive lipid-based vesicle for local, transdermal, and systemic use with exceptional effectiveness for both hydrophilic and lipophilic medications, as well as the transportation of active ingredients to deeper skin layers. The autosome is made up of water, specific phospholipids (phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, and phosphatidylglycerol), and a relatively high concentration of alcohol (30 to 45 percent) (ethanol and isopropyl alcohol)(17). This innovative autosome heightens the localized delivery of concentrated active substances while improving the efficiency of skin penetration. Additionally, the flexibility of bilayer lecithin in comparison to longer liposomes enhances the physical stability of the autosome(18).

7- Nanoemulsion

Nanoeemulsions are tiny particles that serve as vehicles for drug molecules, with sizes ranging from 10 to 1000 nanometers. These particles are solid spheres with a negatively charged surface that is compatible with lipids. Magnetic nanoparticles can be incorporated to improve their targeted properties. By acting as a drug delivery system, nanoeemulsions enhance the effectiveness of drugs while reducing the risk of side effects and toxicity.

Emulsion, on the other hand, is a two-phase system where one phase is dispersed in the other as small droplets ranging from 0.1 to 100 micrometers in diameter (19) . The aim of nanotechnology-based drug delivery systems and lipid-based formulation systems is to enhance the solubility and bioavailability of drugs and active ingredients in food that are not soluble in water. Various bioactive food components, such as flavonoids (flavanols, flavones, flavonones, and isoflavones), non-flavonoids (hydroxybenzoic acids, acylbenzenes, and curcuminoids), and carotenoids (carotenes and xanthophylls), have been effectively encapsulated in nanoformulations (20) .Nanoeemulsions refer to two fluids that cannot mix, such as oil in water (O/W) or water in oil (W/O), that are stabilized by a suitable surfactant. These emulsions typically have droplets that are smaller than 500 nanometers in size, resulting in a transparent or cloudy appearance. This is different from coarse emulsions, which have larger droplets and appear milky white due to multiple light scattering. While the terms submicron emulsion or mini-emulsion may also be used, nanoeemulsion should not be confused with microemulsions (21) .

Advantages of nanoemulsion:

1. Nanosuspensions have a higher surface area and free energy, making them an effective transport system.
2. They do not exhibit inherent problems such as creaming, clotting, aggregation, and settling.
3. They can be formulated in various forms such as foam, cream, liquid, and spray.
4. They are non-toxic and non-irritating, making them easily applicable to skin and mucous membranes.
5. If the formulation contains biocompatible surfactants, it can be prescribed orally.
6. They do not harm healthy human and animal cells, making them suitable for human and veterinary therapeutic purposes.
7. They provide better absorption of oil-soluble supplements in cell culture technology to improve the growth of cultured cells and enable studies on the toxicity of oil-soluble drugs.
8. They may be used as a substitute for liposomes and vesicles, and there is a possibility of creating liquid-layered crystal phases around nanodroplets of nanosuspensions.
9. Due to their small size, nanosuspensions can penetrate through the "rough" surface of the skin, increasing the penetration of active substances (22).

8- Conclusion

There is great promise in using nanoparticles as a controlled and selective mechanism for drug delivery. The development of nanotechnology will have significant impact on the pharmaceutical industry, impacting various routes of administration. Predictions include less toxic drugs, lower treatment costs, increased biological accessibility, and extended economic lifespan for registered drugs. This will result in more efficient drug therapies and reduced side effects. The potential of herbal medicines for therapeutic use should be explored through value-added drug delivery systems. Lipid solubility and molecular size are the main obstacles for drug molecules to pass through the biological membrane for systemic absorption after oral or topical administration. Despite demonstrating excellent biological activity in laboratory conditions, herbal extracts and plant molecules exhibit weak in vivo activity due to poor solubility in fat or inappropriate molecular size, leading to poor absorption and low biological availability. Standardized plant extracts or polar compounds like flavonoids, terpenoids, tannins, and xanthones demonstrate much better absorption profiles when administered using new drug delivery systems, allowing them to pass through the biological membrane and improving their bioavailability. As a result, there is a lot of potential in developing new drug delivery systems for active ingredients and herbal extracts.

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Mathematical Misconception in Physical Chemistry

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Abstract

Misconception refers to any kind of false notions that cause non-scientific beliefs, confused concepts, simple concepts and theories without scientific roots. Misconceptions cover a wide range of scientific concepts. Misconception exist not only in different disciplines, but also in interdisciplinary areas. Understanding the basic concepts in thermodynamics greatly helps students' ability to establish a connection between mathematical equations and macroscopic phenomena. The present article is a review article in which the issue of students' skill in applying mathematical equations in thermodynamic laws is discussed. Many students do not have serious problems in mathematics. They can interpret the first and second order partial derivatives well and they got good marks in math in the last semesters, but the major problem arises when these people cannot apply math in chemical physics. Perhaps because of this abstract nature, it is challenging for students to interpret mathematical expressions and relate them to information related to macroscopic systems.

Key words: Misconception, Mathematics, Physical Chemistry, Mathematics in Chemistry.

1.Introduction

Learning science, including topics from chemistry, biology, physics, etc., has always been a concern of researchers, and efforts to find the reasons for science learning problems have been reported in scientific sources for relatively long years. Many researchers have tried to answer the question "why learning science is difficult?" In their study, they came to the conclusion that part of the difficulties in learning science are related to the nature of science and another part to science teaching methods (Johnstone, 1991). Since experimental science has conceptual issues and most of its discussions are related to invisible issues, there is a possibility of creating some misconception about the concepts of experimental science among students and student teachers and even teachers (Saadati, 2018).

Basically, misconception do not exist independently, but appear in a specific conceptual format, and therefore it is possible that they change or disappear when the format is changed. If the improvement of learning is done in the conceptual fields and also different levels of learning, the recognition of misconception and the roots of their creation takes place. Because misconception form part of the conceptual structure of the learner's mind that interacts with the new concept. Because errors are caused by misconception, these effects are often negative (Strike, 1983, cited in Olivier, 1989).

Misconception are one of the reasons for not learning effectively and meaningfully. In the past years, many researchers have studied the understanding of students and their misconception of chemical phenomena. Chemistry is one of the sciences that includes concepts, calculations and their combination. Due to the abstract nature of chemistry, some

students face problems in understanding chemical concepts. Since there is a large overlap in content between related domains such as chemistry, physics, and mathematics, there is a critical need for more work to study student's misconceptions in mathematics. So that chemistry teachers can help students to reinterpret mathematics in the fields of chemistry (Becker and Towns, 2012).

Misconception are one of the important factors that cause major problems in learning mathematics. Misconception arise in the form of a specific concept and they can be eliminated by making positive changes (Shahverani and others, 2013, quoted by Haqkhah and Davoudi, 2019). Understanding mathematical concepts has always been accompanied by mistakes that may be due to lack of focus and precision, haste or misinterpretations of the problem; But some errors are due to incomplete and wrong understanding of the concepts and are not the result of carelessness and lack of concentration, but have a specific structure and system and do not arise by chance, which are called misconception (Reihani et al., 2015).

Physical chemistry is the study of the underlying physical principles that govern the properties and behavior of chemical systems. A chemical system can be studied from either a microscopic or a macroscopic viewpoint. The microscopic viewpoint is based on the concept of molecules. The macroscopic viewpoint studies large-scale properties of matter without explicit use of the molecule concept. We can divide physical chemistry into four areas: thermodynamics, quantum chemistry, statistical mechanics, and kinetics. Thermodynamics is a macro-scopic science that studies the interrelationships of the various equilibrium properties of a system and the changes in equilibrium properties in processes (Levine, 2009).

2.Results and discussion

Thermodynamics (from the Greek words for "heat" and "power") is the study of heat, work, energy, and the changes they produce in the states of systems. In a broader sense, thermodynamics studies the relationships between the macroscopic properties of a system. A key property in thermodynamics is temperature, and thermodynamics is sometimes defined as the study of the relation of temperature to the macroscopic properties of matter. Equilibrium thermodynamics deals with systems in equilibrium. (Irreversible thermodynamics deals with nonequilibrium systems and rate processes.) Equilibrium thermodynamics is a macroscopic science and is independent of any theories of molecular structure. Strictly speaking, the word "molecule" is not part of the vocabulary of thermodynamics. However, we won't adopt a purist attitude but will often use molecular concepts to help us understand thermodynamics. Thermodynamics does not apply to systems that contain only a few molecules; a system must contain a great many molecules for it to be treated thermodynamically (Levine, 2009).

Undergraduate physical chemistry courses require students to be proficient in calculus in order to develop an understanding of thermodynamics concepts (Becker and Towns, 2012). Physical chemistry uses calculus extensively (Levine, 2009). Understanding the basic concepts in thermodynamics greatly helps students' ability to make connections between mathematical equations and macroscopic phenomena. Therefore, students should be guided by professors to understand mathematical expressions from a physical point of view and use them to predict and justify the chemical behavior of studied systems (Panq and Saeedi, 2019).

The use of mathematical equations allows physical chemists to have a common language for inquiry (Kozma and Russell, 1997). Becoming fluent with mathematical representations is thus essential for the acquisition of expertise in chemistry (Kozma et al., 2000; Kozma and Russell, 1997). Mathematical inscriptions related to derivatives and integrals are a fundamental part of the language of physical chemistry. Consider, for instance, a type of mathematical relationship commonly encountered in a junior-level chemical thermodynamics course, the Maxwell Relations. Also referred to as the equality of mixed second partial

derivatives of exact differentials, the Maxwell Relations provide a way to relate macroscopic observable quantities such as temperature and pressure to more abstract chemical properties such as entropy or Gibb's Energy (Becker and Towns, 2012).

Many of the abstract equations students encounter in physical chemistry are quite distant from the natural phenomenon they represent. Perhaps because of this abstract nature, interpreting mathematical inscriptions and connecting them to information about macroscopic or microscopic systems has been shown to be quite challenging for students (Greenbowe and Meltzer, 2003; Hadfield and Wieman, 2010; Jasien and Oberem, 2002) due to students' lack of skills in the application of mathematical equations in thermodynamic laws and the abstractness of some thermodynamic concepts such as entropy, Gibbs free energy, and Helmholtz free energy are necessary for students to gain a correct understanding of chemical processes, especially in thermodynamics, as well as the analysis of its results in chemistry (Panq and Saeedi, 2019). Even students who are successful according to course metrics, such final exam scores and course grades, may fail to correctly interpret and use mathematical inscriptions in new contexts, such as that of thermodynamics (Hadfield and Wieman 2010; Thompson et al., 2005). An understanding of science content does not always enable students to successfully relate prior mathematics knowledge to problem solving tasks (Becker and Towns, 2012).

It may be challenging for students to transfer mathematical understanding successfully to physics and chemistry contexts (Rebello et al., 2005). Though students' may possess understandings related to mathematics use they may fail to apply those resources if they focus on surface features such as notational differences (Hammer et al. 2005; Rebello Zollman and Allbaugh, 2005). Students who rely primarily on rote memory learning may be particularly vulnerable to this type of difficulty (Yeatts and Hundhausen, 1992).

When students are asked to use mathematics in science contexts such as physics, they seldom are able to recall and apply mathematics ideas in a straightforward fashion (Meredith and Marrongelle, 2008). One way to help instructors to better scaffold students' use of mathematics in chemistry contexts may be to engage students in collaborative problem solving. For instructors of chemistry, observing students working together may offer instructors an opportunity to observe facets of students' reasoning that may not be made explicit from an examination of student homework and exams. Observing students' difficulties as they occur may provide unique opportunities for instructors to give formative feedback that may help students develop a deeper understanding of content. Furthermore, such collaborative activity may provide students a space to negotiate understandings of concepts with their peers permitting them to make connections between symbolic representations and physical understandings (Towns and Grant, 1997).

Interdisciplinary approaches to thermodynamics and other highly mathematical science content may be one way to facilitate productive use of mathematical inscriptions in science contexts. One current curriculum development relevant to upper-division chemistry instructors is entitled "Creating a Common Thermodynamics." This project aims to develop an interdisciplinary core curriculum for thermodynamics courses in biology, chemistry, and physics (Klymkowsky, 2011).

Students' ability to interpret and understand mathematical equations and symbols to describe the macroscopic system has been less studied in scientific researches. For example, the study of Thomas and Schwezs (1998) focused on the misconception of chemistry students in the course of chemistry and physics on the first and second laws of thermodynamics. These studies mainly focus on the conceptual understanding of thermodynamic content rather than students' physical interpretation of mathematical content in chemistry and physics fields.

Therefore, if chemistry professors want to facilitate students' reasoning with mathematical expressions in chemistry, more research is needed on how students interpret and apply mathematical equations to reason about chemical concepts (Panq and Saeedi, 2019).

Few studies have been done on the problems related to the methods of interpretation and students' use of mathematical expressions in thermodynamics. One of these studies is the research of Hadfield and Weiman (2010), who investigated the problems of students in interpreting mathematical expressions related to the first law of thermodynamics (Panq and Saeedi, 2019).

In another study, Thompson et al. (2006) studied the way of reasoning related to mathematical expressions in the subject of physics in eight final year physics students to discover their understanding of the combination of partial derivatives and Maxwell's relations. Students were asked to explain the difference between partial derivatives and total derivatives and express their understanding of mathematics and physics related to Maxwell's relations as part of a pre-examination survey. Their findings showed that even the students who had good marks at the end of the semester in math courses failed to interpret and apply mathematical sentences in thermodynamics. Students' correct understanding of mathematics is not always successful in solving the problems of other subjects (Panq and Saeedi, 2019).

Despite the fact that many research studies have been reported regarding mathematics education and physics education in the field of students' approaches in interpreting and using mathematical expressions to solve problems, very few of these researches are about the reasoning method of chemistry students about mathematical expressions in the fields of They have studied thermodynamics. In order to improve students' learning performance in the subject of thermodynamics, it is necessary to study the methods of understanding the concept of mathematical expressions in chemistry courses, especially in chemical physics (Panq and Saeedi, 2019).

3.Conclusions

Chemical physics has strong empirical foundations for accurate prediction and calculation of objective observable quantities. The mathematical content related to derivatives and integrals is a basic and important part of the subject of chemistry. For example, consider a mathematical relationship commonly used in a chemical thermodynamics course known as Maxwell's relations. These relationships, which actually represent the relationships of partial derivatives of thermodynamic quantities, provide a method to relate macroscopic observable quantities such as temperature and pressure to more abstract thermodynamic quantities such as entropy or Gibbs free energy. The use of mathematical equations allows chemical physicists to have a common language for research (Panq and Saeedi, 2019).

Many students are not aware of their misconception. This issue makes them not take a step to resolve their misconception and their problem deepens. Especially when it is an interdisciplinary topic. Teaching mathematics in chemistry by professors who have a doctorate in chemical physics is an important issue that has been well paid attention to and is observed in the planning approved by the Ministry of Science. Misconception related to the two fields of mathematics and chemistry should be solved by expert teachers. In this matter, an expert teacher is someone who, in addition to chemistry and mathematics, has educational sciences and psychology and knows how to apply these sciences in teaching.

Some of the solutions that make students' misconception to be discovered and then fixed are:

1) Increasing students' activity with frequent teacher questions and answers
2) Correct, principled and timely conduct of all kinds of evaluations

3) not limiting chemical physics to a theoretical and abstract course
4) Coordination of mathematics professors in chemistry and chemical physics in teaching, evaluation and...

Many students do not have serious problems in math. They can interpret the first and second order partial derivatives well and they got good marks in math in the last semesters, but the major problem arises when these people cannot apply math in chemical physics. Some other problems are carelessness in quantity units and its misinterpretation, abstractness of some quantities and its misinterpretation. One of the reasons for this work can be examined in this case that the chemical physics course is not presented in a problem-oriented manner.

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An Overview of Different Types of Adjuvants and Their Application

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Abstract

Vaccination is one of the most efficient strategies for the prevention of infectious diseases. Vaccines, including subunit, recombinant, and conjugate vaccines, require the use of an adjuvant for maximum efficacy (They are safe, but poorly immunogenic). Adjuvants not only enhance the strength and longevity of immune responses but may also influence the type of immune system responses. Nevertheless, few adjuvants are licensed for human vaccines and various formulations are now being accepted in clinical trials. There is no universal adjuvant to cover all vaccine needs. The appropriate selection of adjuvants to match the antigens is key to the formulation of novel and efficacious vaccines. In this review, we briefly describe the most well-known adjuvants used in experimental and clinical settings based on their main mechanisms of action.

Keywords: Vaccines, Adjuvants, Drug delivery, Emulsions, Liposomes, Alum

1. Introduction

Vaccinations are certainly one of the most striking health achievements of human history (1). According to the World Health Organization (WHO), vaccination saves 5 lives every minute and will save over 25 million lives from 2011 to 2020 (2). Vaccination was started by Edward Jenner based on the usual notice that milkmaids who had suffered from cowpox were protected against smallpox (3). Long-lasting and effective of immune system response can be achieved by conventional vaccines composed of live attenuated or inactive pathogens. Meanwhile, subunit vaccines are safer but are less immunogenic (4). Thus, adjuvants are used to improve the immune response and to increase the immunogenicity of these vaccines (5).

2. What is adjuvant?

Vaccine adjuvants are defined as components that enhance the adaptive immune responses to an antigen (6). The efficacy of a vaccine depends not only on the antigen elements, but also on adjuvants that are often used in order to accelerate the immune system in a more effective way (1). These additional substances provide the ‘‘help’’ (from adjuvare, to help) needed to improve the immunogenicity of vaccine antigens (7). Adjuvants may be compounds, molecules, or macromolecular complexes that boost the potency, longevity, or quality of specific immune responses to antigens that should cause minimal toxicity (8).

3. History

The French veterinarian Gaston Ramon, who worked at the Pasteur Institute in 1920 discovered that horses vaccinated against diphtheria had stronger antibody titres if they developed inflammatory abscesses at the site of injection. Ramon represented that adding different substances, such as starch or breadcrumbs, to the inactivated diphtheria toxin caused boosting antibody production in response to the vaccine and inflammation at the injection site (9).

4. Adjuvant classification

Adjuvants may act with combination of various mechanisms including enhancement of antigen uptake and presentation, depot effect, induction of chemokines and cytokines, promoting antigen transport to draining lymph nodes and recruitment of immune cells (10). Adjuvants present many different natural and synthetic substances, and can be divided in two main classes based on their detected mechanism of action: delivery systems and immunestimulators (11).

1-4- Delivery systems

The process of developing a vaccine consists of two key steps: identifying an antigen and developing a delivery system for the antigen to enhance cellular and humoral immunity (12). The size and properties of the delivery system influence the entry of an antigen into APCs (13).

1-1-4- Aluminum salt-based adjuvants (Alum)

Aluminum salt-based adjuvants (alum) were the first adjuvants used in licensed human vaccines (14). They enhance the immune response and thus ensure the potency and efficacy of typically less available antigen (15). They have been widely used in tetanus, diphtheria, and hepatitis B vaccines, and etc (16). Alum has been the most widely used adjuvant for over 90 years (17). In spite of their longstanding use, their mechanism of action is poorly realized (18).

The types of aluminum salt-based adjuvants in FDA approved several vaccines include aluminum phosphate, aluminum hydroxide, and amorphous aluminum hydroxyphosphate sulfate (AAHS) (16). Alum's mechanism of action is widely believed to involve acting like a matrix into which antigen can be adsorbed and stabilized. The slow release of antigen from alum at the inoculation site was involved to support the induction of immune response for a long time (depot effect) . The function of alum is dependent on activation of innate immunesensing pathways, induction of inflammatory cell death at the injection site and production of chemokines and cytokines at the injection site and/or draining lymph nodes (LNs) (19). Aluminium salts are poor inducer of T-cell responses when evaluated in humans. This may be due to the lack of potent stimulation of the innate immune system, in comparison to TLR stimulation (20). It has been reported that alum suppresses secretion of IL-12, a critical third signal for TH1 cell differentiation. Perhaps this is one of the ways that alum biases responses of Tcell differentiation into TH2 type cells (21). One of the main discoveries of alum adjuvants is their ability to activate the NLRP3 inflammasome (22). In addition to being widely available and cheap, alum also allows for single-vial refrigerated liquid vaccines that are safe (23).

For a long time, expert international congresses claimed that aluminum given by vaccination route removed quickly from the body via the kidneys, and official information sites repeated this claim to the general community. But, the latest data on the pharmacokinetics of aluminum has demonstrated that this is not correct (24). It is still the only adjuvant licensed for human use by the U.S. Food and Drug Administration (FDA), but the European Medicinal Evaluation Agency has licensed three additional adjuvants in the past decade: the TLR4-agonist monophophoryl lipid A formulated in alum (AS04), and the oil-in-water emulsions AS03 and MF59 (25).

1-2-4- Emulsions

Exception for alum, emulsion-based vaccine adjuvants are prescribed to far more people than other adjuvants, particularly since the 2009 H1N1 influenza pandemic. In 1990s, it was discovered that oil-in-water emulsion is an effective adjuvant for the influenza vaccine (26). The number of clinical and safety evaluations of vaccines containing emulsion adjuvants has

grown accordingly (27). An emulsion is a mixture of two or more liquids that are not normally miscible and stabilized by the presence of a surfactant. In an oil-in-water (w/o) emulsion, oil is dispersed in water (28). The best example of this is MF59, a commercial adjuvant used in human vaccines. A water-in-oil (W/O) emulsion includes aqueous droplets suspended in the oil phase (29). MF59 is the only safe and effective oil-in-water (o/w) emulsion adjuvant included in a licensed vaccine for use in seasonal influenza (30). Recent studies show that MF59 induces more cytokines and chemokines than alum and recruits CD11b+ cells more rapidly at the injection site (31), but Intramuscular injection of MF59 adjuvant was found to induce cell deaths and tissue stress (32). Emulsion adjuvants are stable, cheap and relatively easy to prepare into vaccine formulations (33). The most widely used oil-water emulsion adjuvant in animal experimentations is Freund's adjuvant. There are two types: Incomplete Freund's Adjuvant (IFA) and the (CFA) adjuvant. For improving the immune response, the CFA contain killed Mycobacterium tuberculosis which are responsible for attracting macrophages and other cells to the injection site, and due to that, it is usually applied in the initial immunizations. Because of its secondary reactions and toxicity, the use of CFA in humans is ineligible as a proper adjuvant; however, IFA is less toxic and suitable for its clinical usage (34). Emulsion adjuvants can be manufactured at a large scale, but they need a complex manufacturing facility (35).

1-3-4- Liposomes

A liposome is a spherical-shaped structure including one or more phospholipid bilayers surrounding an equal numbers of aqueous compartments (36). It is effective for encapsulation of hydrophobic antigens (into the bilayer of the liposome membrane) and hydrophilic proteins (into the inner water core of liposomes). Antigen localization in liposomes can enhance the immunogenicity of vaccines (37). They are biocompatible, biodegradable and safe (38). Cationic liposomes are preferred to be more utilized as vaccine carriers, because the positive charge provides prolonged exposure time of antigen at the mucosal surface (depot effect), reduced clearance rate, and enhanced endocytosis of liposomes by APC (39). In contrast to their activation of innate immunity, further induction of APCs for the secretion of cytokines that trigger differentiation of naive T lymphocytes into different subpopulations of CD4+ and/or CD8+ T cells depends on the physicochemical characteristics of liposomes: lipid composition; size, determining the phase state of the bilayer and liposome charge; and the presence of costimulatory molecules (immunomodulators) (40). The size of liposomes has an important impact on the immunostimulatory activity of the vaccine adjuvant-delivery system and may dictate the immune reactions to proceed toward the Th1- or Th2-biased pathway. For example, it is reported that when administered to mice by either subcutaneous injection or oral uptake, smaller lipid vesicles (< 150 nm) improved the development of Th2 response while larger lipid vesicles (> 200 nm) promoted typical Th1 response and IFN-γ (41).

1-4-4- Iscoms

ISCOMs (Immune-stimulating complexes) are cage-like nanoparticles of about 40 nm (42), obtained by combining lipids, Q. saponaria saponins, and protein antigens (43). They were first described by Morein et al. (1984) as a vaccine delivery vehicle (44). The physical characteristics of ISCOM contribute to reducing the hemolytic effects associated with saponins, antigen stability, and improve interaction with DCs. ISCOMs improve the cross-presentation of the incorporated antigen, generating both antibody and CD4+ and CD8+ T cell responses. ISCOM upregulates Th1 and Th2 responses as well as stimulates a strong humoral response (IgG1, IgG2a and IgG2b) by inducing cytotoxic T cell (45). The only concern while employing ISCOMs is the severe toxicity leading to hemolysis or granulomas (46).

2-4- Immunostimulatory adjuvants

A new approach is to use 'pattern recognition receptors' (PRR) including cytokines and toll like receptors (TLRs) as adjuvants, which can result in the development of both innate and adaptive immune responses with fewer side effects (47).

2-1-4- TLR agonists

TLRs are transmembrane receptors, which are predominantly expressed by innate immune cells. They can be classified into cell surface (TLR1, TLR2, TLR4, TLR5, TLR6) and intracellular TLRs (TLR3, TLR7, TLR8, TLR9), expressed on endosomal membranes. Besides a transmembrane domain, each TLR possesses a leucine-rich repeat (LRR) segment that mediates PAMP/DAMP recognition and a TIR domain that delivers the downstream signal transduction and initiates an inflammatory response (48). Thus, TLRs are excellent targets for adjuvants to provide a "danger" signal to induce an effective immune response that leads to long-lasting protection (49). TLR agonists can be combined with alternative adjuvants or other TLR to create combination adjuvants with modulatory or synergistic effects (50). TLR agonists such asCpG-1018 and monophosphoryl lipid A (MPL) have been formulated in licensed vaccines for their adjuvanticity, and other TLR agonists developing for this purpose (51). However, they may also act as a double-edged sword, and dysregulated TLR responses may improve immune-mediated pathology, instead of providing protection (52).

2-2-4- NOD agonists

NOD (nucleotide-binding and oligomerization domain)-like receptors (NLRs) are kinds of PRRs that recognize danger-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs) and activate the downstream signaling pathways (53). Targeting specific PRRs from different classes gives a wide range of immune responses because each receptor activates a distinct signaling pathway and this can influence innate and subsequent adaptive immune responses to produce defined cellular and antibody responses (54). In the last decade, different Nod-like Receptor (NLR) agonists were examined as vaccine adjuvants and showed promising results in clinical studies (55), however, these small molecules diffuse from the injection site into the blood freely and rapidly, reducing their ability to prime immune cells and often causing systemic side effects, such as a cytokine storm (56).

2-3-4- Bacterial toxins

Immunostimulatory molecules like bacterial toxins enhance immune responses by interacting with specific receptors. Heat-labile toxins (HLTs) are produced by some enterotoxigenic E. coli strains and are able to fuse with other antigenic proteins to act as adjuvants (57). Cholera toxin is the most powerful known mucosal adjuvant but is much too toxic for human use (58). Another approach is the use of the nontoxic B subunit of the toxins, but this subunit shows less immunogenicity (10).

2-4-4- Cytokines

Cytokines are molecular messengers of the adaptive and innate immunity that enable cells of the immune system to communicate over short distances in autocrine and paracrine manner (59). They are a class of highly active glycoproteins and low-molecular-weight that have important cell-to-cell communications roles . The selection of a cytokine for use as an adjuvant is based on its known influence on immune cells and the desired immune response to vaccination (60). IL-1 is the main representative of anti-inflammatory cytokines produced by neutrophils, Mo/Mp, dendritic cells (DCs), NK-cells and activated B- and T-Lp. IL-1 plays an important role in development of an inflammatory response (61). As a member of the IL-1 cytokine family, IL-33 can act as a pro-inflammatory cytokine and has been reported to drive protective antiviral CD8+ T cell responses (62). Cytokines are known to be strong and safe as adjuvants for vaccines, including mucosal vaccines. Different types of cytokines, such as interferon, interleukins, and TNF-α have been examined for mucosal vaccines against

respiratory tract disease. Interleukin-17B (IL-17B), a member of the IL-17 family, plays a key role in regulating the expression of pro-inflammatory cytokines (63).

5. Conclusion

The goal of vaccination is to make protection against disease-causing pathogens. Protective immunity against different pathogens requires different immune responses that can be produced by using suitable vaccine adjuvants. So, detailed knowledge of the mechanisms of action of adjuvants is very important in vaccine design. In recent years, significant advances have been made in understanding the mechanisms of action of different adjuvants, especially the activation of innate immunity. Safety is an important factor for licensing new adjuvants. We hope to learn more details of their mechanisms of action, which will lead to novel adjuvants for use in human vaccines being approved.

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A Comprehensive Study on Vehicular Ad-hoc Network Routing Protocols

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Abstract

Vehicular Ad-hoc Network (VANET) create all types of vehicle-to-vehicle and vehicle-to-infrastructure communications using radio waves. All the services available in the Vehicular Ad-hoc Network are classified into three different groups, which are safety, traffic control and applications. Safety messages must be exchanged between vehicles to prevent road accidents. Traffic control is used to improve the quality of travel by preventing excessive traffic. Applications are also included in order to create entertainment and services for passengers in the car. In this article, Vehicular Ad-hoc Network (VANET), their applications, challenges and problems are described, and routing protocols in Vehicle Ad-hoc Networks are comprehensively investigated.

Key words: Vehicular Ad-hoc Network (VANET), Routing, Routing Protocols, Smart Systems.

1. Introduction

The use of intelligent systems and Vehicular Ad-hoc Network to manage and control the traffic is one of the most important issues today. Routing the packets sent in this type of networks effectively time is of particular importance with maximum delivery rate and minimum delivery delay. In this article, Vehicular Ad-hoc Network (VANET), their applications, challenges and problems are described, and routing protocols in vehicle ad hoc networks are comprehensively investigated.

2. Vehicular Ad-hoc Network

Vehicular Ad-hoc Networks create all kinds of vehicle-to-vehicle and vehicle-to-infrastructure connections using radio waves. The main difference between Vehicular Ad-hoc Networks and cellular and dedicated networks is that no central station or node is responsible for managing and controlling the network, and the network consists of a series of vehicles (here, let's assume a network node) that are mobile and do not have a fixed place and do not play the role of a router or a base station. In fact, Vehicular Ad-hoc Networks are a special type of mobile Ad-hoc networks whose nodes are on board unit vehicles. Every vehicle can identify the cars around it at any moment and can form a network and establish the necessary communication by connecting to them. This car will create another network a little later with new cars around it and roadside units, which are fixed infrastructures and usually deployed on the roadside. The main basis of automotive case networks is their unstructured nature and the use of 802.11p and DSRC standards. Therefore, these types of networks can quickly change their topology and create a lot of flexibility due to the fact that they do not have any problems in terms of energy consumption and computing resources. For example, a car can be

connected to several Vehicular Ad-hoc Networks at the same time and receive the necessary information [1].

2-1- The components of Vehicular Ad-hoc Networks

This type of network consists of three general components: users, which are on-road vehicles, and roadside units, which are called RSUs. Wireless communication in the Vehicular Ad-hoc Network is divided into two categories: vehicle-to-vehicle communication and communication between the vehicle and roadside units. To be added to a car Ad-hoc Network, a car needs a receiver/transmitter of signals and information (like a wireless network card) and a control device including a central chip. The main protocol of the Vehicular Ad-hoc Network is DSRC. Also, the coverage range of this equipment should be from three hundred meters to several kilometers (in the latest designs, it is even two to three kilometers). Roadside stations and antennas exchange information with the car's wireless device by connecting to the Internet and urban traffic network. In fact, the most important part of the Vehicular Ad-hoc Network is the sensors that should be used in different parts of the car and report the status of the car and the external environment to the driver and the controller, or vice versa, apply the driver's commands or received information from other cars [1].

2-2- Applications of Vehicular Ad-hoc Network

All the services available in the Vehicular Ad-hoc Network are classified into three different groups, which are safety, traffic control and applications. Safety messages must be exchanged between vehicles to prevent road accidents. Traffic control is used to improve the quality of travel by preventing excessive traffic. Applications are also included in order to create entertainment and services for passengers in the car (such as sharing music and video, the possibility of conversation, games and access to the Internet) [1].

2-3 –Challenges in Vehicular Ad-hoc Network

There are many challenges in Vehicular Ad-hoc Network. Here, the case-by-case description of these challenges is discussed [1].

1) One of the important issues for VANET applications is data release.
2) Network design resistant to various destructions and security attacks.
3) Adaptive and reliable transmission.
4) The ability to handle dynamic communications, such as broken links, congestion, proper bandwidth, transmission interference, etc.
5) Efficient cross layer design.
6) Presenting an effective plan for car traffic estimation.
7) A network is scalable that can work well in scenarios with low and high density of nodes.
8) Compatibility with network saturation problem.
9) Resistant to the hidden terminal problem.
10) Prevent channel saturation.
11) The transmission of messages between nodes must be completely free of any errors.
12) Bandwidth sharing.

3- Routing in automotive Ad-hoc Networks

Dissemination of information in the automotive Ad-hoc Network environment is very important and routing plays a vital role in disseminating information. The main requirement of a routing protocol is that it can guarantee minimum resource consumption by considering bandwidth and network transmission delay with minimum communication time. Due to the dynamic nature of mobile nodes in Vehicular Ad-hoc Networks, finding and maintaining routes in these networks is very challenging. Routing in Vehicular Ad-hoc Networks (with

special architectures of this category of networks) has been studied [2]. These protocols are categorized as follows:

1) Ad-Hoc routing 2) Broadcast routing 3) Geo-cast routing 4) Uni-cast routing

3-1- Ad-hoc Routing Protocols

Vehicular Ad-hoc Network is an example of mobile Ad-hoc Network that have a common principle: they have no fixed infrastructure for communication and have many similarities; for example, low bandwidth self-organization and short radio range. Therefore, many mobile ad-hoc protocols such as DSVN [3], AODV [4] and DSR [5] can be used in vehicular ad hoc networks. However, considering the unique features of vehicular ad-hoc networks, using protocols designed for mobile Ad-hoc Networks may not be a suitable solution. Direct use of AODV is not able to quickly find and maintain long routes. AODV has been modified to increase route stability and reduce network overhead, and PAODV and DAODV protocols were presented, which are suitable for mobile Ad-hoc networks and can reduce link breakage and routing overhead by choosing less routing.

3-2- Broadcast Routing Protocols

Broadcast routing is widely used in mobile Ad-hoc networks. These protocols distribute packets to all nodes in the broadcast domain, which is a suitable solution for information exchange such as information sharing services, emergency accident, weather, advertising and notification. In general, the purpose of broadcast routing is that vehicles can send packets to nodes that are outside their transmission range through multi-hop communication.

Appropriate broadcast protocols have been designed to respond to dynamic and complex conditions in mobile Ad-hoc networks, which include the Urban Multi-hop Broadcast Protocol (UMB) and the Ad- hoc Multi-hop Broadcast Protocol (AMB), which are used for solving hidden node, broadcast storm and reliability problems. Among the broadcast protocols, there is the BROADCOMM protocol, which deals with the problem of transporting emergency broadcast communications in highways [5].

3-3- Geo-cast Routing Protocols

Different from broadcast routing that broadcasts packets to all nodes in the domain, geo-cast routing sends packets from one vehicle to another in the zone of relevance (ZOR) [6]. which is a transportation domain throughout the destination area. Robust vehicular routing (ROVER) and distributed robust geo-cast (DRG) are two examples of geographic routing protocols. ROVER protocol, due to the increase in the number of sent packets, the data packets are transmitted with a delay, which leads to an increase in the overhead of the control packet [7]. DRG is also developed for fast and reliable transmission.

3-4- Uni-cast routing protocol

Unicast routing sends the message from a single source node to a single destination node using wireless multi-hop method. In most cases, topology-based routing, location-based routing based on the first place can be divided into proactive protocol and reactive protocol. In location-based routing, each node knows geographic locations and uses a neighboring hop to reach the destination. All nodes sense their locations using the on-board GPS device. Location-based routing is classified into three distinct groups: 1) non-delay-tolerant (None-DTN), 2) delay-tolerant, and 3) hybrid.

The first case is used for emergency and life-related applications, where it is forbidden to cut the path of two nodes. The second case allows to keep the message in the sending node until the node finds the nearest node to deliver the packet. Since None-DTN is related to safety, it is mainly preferable to check this class, which includes the following:

Geographic Perimeter Source Routing (GPSR)

Geographic SOURCE Routing (GSR)

Anchor-based Street and Traffic Aware Routing (A-STAR)
Greedy Traffic Aware Routing (GySTAR)
Direct use of AODV is not able to quickly find and maintain long routes [8].

1. Comprising Routing Protocols in Vehicular Ad-hoc Networks

Table 1. comprising routing protocols in vehicular Ad-hoc networks

Scenario	Transportation method	Digital map	Routing type	Routing protocols
-	Multi-hop	x	Uni-cast	AODV
-	Multi-hop	x	Uni-cast	DSR
Urban	Multi-hop	x	Uni-cast	PFQ-AODV
Urban	Multi-hop	x	Uni-cast	PRAODV
Urban	Multi-hop	x	Uni-cast	PRAODVM
Urban	Multi-hop	x	Uni-cast	PAODV
Urban	Multi-hop	x	Uni-cast	DAODV
Urban / Highway	Multi-hop	√	All-cast	UMB
Urban / Highway	Multi-hop	√	All-cast	AMB
Highway	Multi-hop	x	All-cast	BROADCOMM
Highway	Multi-hop	√	All-cast	Weighted p-persistence
Urban	Greedy	x	Topology	ROVER
Highway	Greedy	x	Topology	DRG
Highway	Greedy	x	Topology	IVG
Urban	Greedy	√	Uni- cast	GPSR
Urban	Greedy	√	Uni- cast	GSR
Urban	Greedy	√	Uni- cast	A-STAR
Urban	Transport and send	√	Uni- cast	GYSTAR

5. Conclusion

According to the comprehensive studies conducted on routing algorithms, many routing algorithms have been presented for the vehicular ad-hoc network. The geographic resource routing algorithm is not suitable due to the need for a map. In the traffic-aware routing protocol, it is difficult to design a mobile mode for cars because this protocol transmits information without considering the direction and speed of the cars. The performance of the greedy traffic-aware routing protocol is better than the geographic source routing protocol, but if the nodes are unsuccessful in maintaining the packets, this protocol will fail. Also, it fails to find the route by predicting the movement of cars. The geographic resource routing protocol performs better in urban environments and fails in dealing with the problem of network dispersion and is not suitable due to the need for a range map. In the methods and works done in the past, it has been tried to react to the problem of local minima by providing methods called improvement strategies, so that whenever an in-vehicle unit does not have any next step towards the destination from improvement strategies such as stateless greedy routing algorithm can be used. However, the stateless greedy routing algorithm is designed for highly mobile scenarios, which often fails in vehicular ad-hoc networks with a significant increase in the number of transmissions and without a higher delivery rate. The greedy routing algorithm has poor performance in urban mode because it faces more local optimality problems in complex environments. In addition, the bandwidth overhead increases rapidly in the mode of

environmental transport, and in the urban environment, the loss of routes increases with the density of cars [9]. The three main issues that limit the performance of stateless greedy routing algorithm are: network disconnection, large number of steps, infinite routing loops. According to the mentioned requirements, the simple and at the same time effective solution is to improve the greedy algorithm. However, due to the problems mentioned in this method, such as the local minimum, and since the destinations are fixed in traffic-sensitive vehicle networks and the delay is tolerable, instead of using an improvement method, the traffic flow can be arranged those that do not lead to local minimization.

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On the Number of Spanning Trees of Rooted Product of Graphs

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ABSTRACT
In this paper, we obtain some exact formulas for the number of spanning trees of rooted product of graphs. Also we obtain the number of spanning trees of the cluster and the thorn graphs.
Keywords: Spanning tree, Rooted product, Cluster, Thorn graph.

1. Introduction

The evaluation of the number of spanning trees in graphs or networks not only is interesting from a mathematical (computational) perspective but also is an important measure of reliability of a net work or designing electrical circuits. In reliable network synthesis, given the class of all connected graphs with n vertices and m edges, it is very important to seek graphs (known as the t-optimal graphs) with the most number of spanning trees, so the number of spanning trees is closely connected to reliable network design [1]. When using a probabilistic graph to model a communication network, the reliability of a network can be expressed as a function of the number of connected spanning subgraphs (spanning trees) of diffrent orders. Thus, the number of spanning trees of a graph describing a network is one of the most natural characteristics for its reliability, and deriving closed formulae of the number of spanning trees for various graphs has attracted the attention of a lot of researchers [7, 9].

Throughout this paper, we will consider simple connected graphs. Given a graph G with the vertex set $V(G)$ and the edge set $E(G)$, there are two natural ways of deriving smaller graphs from G. If e is an edge of G, we may obtain a graph on $|E(G)| - 1$ edges by deleting e from G but leaving the vertices and the remaining edges intact. The resulting graph is denoted by $G\backslash e$. Similarly, if v is a vertex of G, we may obtain a graph on $|V(G)| - 1$ vertices by deleting from G the vertex v together with all the edges incident with v. The resulting graph is denoted by $G - v$. To *identify* vertices x and y of a graph G is to replace these vertices by a single vertex incident to all the edges which were incident in G to either x or y. We denote the resulting graph by $G/\{x, y\}$. To *contract* an edge e of a graph G is to delete the edge and then identify its ends. The resulting graph is denoted by G/e. A subgraph obtained by vertex deletions only is called an *induced subgraph*. If X is a subset of $V(G)$, the subgraph of G that induced by X is denoted by $G[X]$ and it is the graph whose vertex set is X and whose edge set consists of all edges of G which have both vertices in X.

An *acyclic* graph is one that contains no cycles. A connected acyclic graph is called a *tree*. According to these definitions, each component of an acyclic graph is a tree. For this reason, acyclic graphs are usually called *forests*. Each vertex of degree one in a tree is called a *leaf* of the tree. A *subtree* of a graph is a subgraph which is a tree. If this tree is a spanning subgraph, it is called a *spanning tree* of the graph. There is a remarkably simple formula for the number of labeled trees on n vertices or, equivalently, for the number of spanning trees in the complete graph K_n. This formula was discovered by Cayley [4], who was interested in representing certain hydrocarbons by graphs and, in particular, by trees. We denote the number of spanning trees in an arbitrary graph G by $t(G)$. Cayleys Formula says that $t(K_n) = n^{n-2}$. There is a simple recursive formula relating the number of spanning trees of a graph G to

the numbers of spanning trees in the two graphs $G\backslash e$ and G/e obtained from G by deleting and contracting an edge e.

Theorem 1.1. *[2, Proposition 4.9.] Let G be a graph and e an edge of G. Then* $t(G) = t(G/e) + t(G\backslash e)$.

Let H be a labeled graph on n vertices and let G be a sequence of n rooted graphs $G_1, \ldots, G_n$. According to [5], the *rooted product* of H by G, denoted by $H(G) = H(G_1, \ldots, G_n)$ is the graph obtained by identifying the root vertex of G_i with the i-th vertex of H for all $i = 1, \ldots, n$. In the special case when the components G_i, $i = 1, \ldots, n$, are mutually isomorphic to a graph K, the rooted product of H by G is denoted by $H\{K\}$ and called the *cluster* of H and K. Any other notations are standard and taken mainly from [2].

2. Number of spanning trees of rooted product of graphs

Let H be a labeled graph on n vertices with the vertex set $V(H) = \{1, \ldots, n\}$ and let G be a sequence of n rooted graphs $G_1, \ldots, G_n$. In this section, we compute the number of spanning trees of the rooted product of H by G. Then the number of spanning trees of cluster of graphs and thorn graphs are determined.

Theorem 2.1. *The number of spanning trees of rooted product* $H(G)$ *is given by:*

$t(H(G)) = t(H)\, t(G1) \ldots t(Gn)$

Corollary 2.2. *The number of spanning trees of the cluster* $H\{K\}$ *is as:*

$t(H\{K\}) = t(H)\, (t(K))^{|V(H)|}$

Corollary 2.3. *Let* $T_1, \ldots, T_n$ *be some trees and H be a graph with n vertices. Then*

$t(H(T_1, \ldots, T_n)) = t(H)$:

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Fuel Cell Power System Developments for Electric City Bus

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Abstract

Considering the pollution issues in recent years, the electric city bus has played an important role in intra-city transport. On the other hand, the merit of hydrogen energy as one of the main energy carriers which has higher priorities, mainly due to its high energy density and low pollution, has led to its developments in various industries. Great developments especially can be seen in the electric vehicle (EV) industry. Therefore, fuel cells (FCs), as the main hydrogen energy converters to electric energy, have been developed as well. In this paper, the developments of fuel cells in the electric city bus industry are investigated. The design details of the fuel cell hybrid power subsystems for city buses are described and the main specifications of the fuel cell hybrid power subsystems for city buses are presented. Also, the target technical specifications of the fuel cell buses are discussed.

Key words: Hydrogen, Fuel cell, Power system, City bus

1. Introduction

Due to the recent issues mainly caused by air pollution in cities, electric transportation industries have been considerably developed. Reaching a higher range in the limited space of electric vehicles has led to the use of energy carriers with higher energy density. Hydrogen energy is one of the energy carriers which meets the requirements of EVs. Therefore, fuel cell electric vehicles have been considerably developed in recent years. As a result, the fuel cell city buses for intra-city transportation have been introduced and their design and construction have become applicable research. Several projects about fuel cell electric buses (FCEBs) have been accomplished. These projects have been launched in the last two decades in North America, Europe, and Asia.

In Europe during the recent twenty years, more than five mega-projects were launched for fuel cell bus technology demonstration. During these projects, more than 200 fuel cell-powered buses have been developed. The buses were demonstrated in several cities such as Hamburg, London, Barcelona, Stockholm, Porto, Stuttgart, Amsterdam, Luxembourg, and Madrid. The projects aimed to demonstrate the feasibility of an innovative, highly energy-efficient, clean intra-city public transport system. Different hydrogen production and refueling infrastructures were established in each of the cities. These projects greatly improved public acceptance of the hydrogen fuel cell transport system and contributed to the development of a more secure energy supply in Europe. The last project is 3Emotion. This project stands for environmentally friendly Efficient Electric Motion. The project will fulfill the gap between current fuel cell bus demonstration projects and the larger-scale deployment and procurement foreseen by the FCH-JU Bus commercialization study [1].

In the USA according to the NREL report about the fuel cell bus transit fleet, the technology readiness level (TRL) for fuel cell electric buses is currently in the latter half of the technology/commissioning phase including TRL 7 through 8 [2].
A fuel cell hybrid electric bus is an electric bus in which the fuel cell and battery supply the propulsion and service loads. By using a fuel cell in conjunction with a battery bank, the size of each can be optimized considering the load profile.
The fuel cell system generates electric energy through an electrochemical reaction and the other by-products are only water and heat and there are no emissions. Because the fuel cell generates only water as an emission, the fuel cell bus can be considered a zero-emission bus. The produced water is drinkable. The fuel cell's electric energy is used to charge the batteries in cases in which the demand is lower than the fuel cell's power. The produced heat can be in the bus air conditioning system which leads to a considerable energy efficiency increment. The batteries also provide storage for regenerated braking energy. All the energy required for the bus to operate is provided by hydrogen stored on board.
Hydrogen provides higher energy density compared to batteries, this enables a longer range compared to electric buses solely based on batteries. Refueling the bus for charging the high-pressure hydrogen tanks takes less than 10 minutes.
In this paper, the details of the fuel cell power system for electric buses are investigated. The fuel cell power subsystem ranges and the target specifications of fuel cell buses are discussed and presented.

2. Fuel Cell Power System Design

The overall arrangement of the fuel cell power system in an electric bus is shown in figure 1.

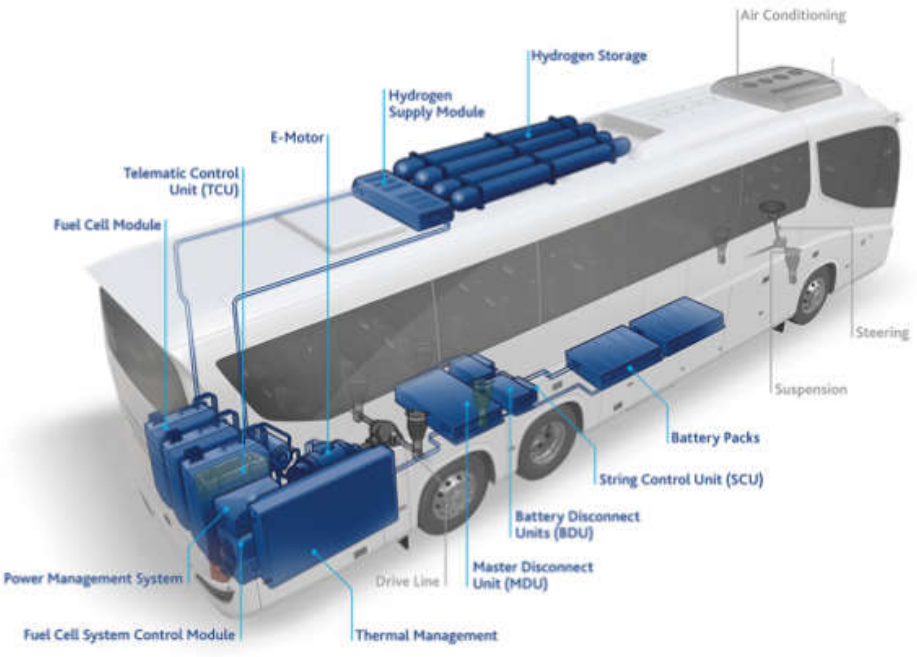

Figure۶ : Arrangement of the fuel cell power system in an electric bus [3]

The arrangement presented in figure 1, is not a unique configuration, but it is a popular configuration. Currently, 350 bar composite hydrogen tanks are used for hydrogen storage in buses. The hydrogen tanks are usually stored on the roof of the bus, while the fuel cell stacks are located at the back of the

bus. The fuel cell buses are mainly categorized into 3 classes, single deck, articulated and double deck. The bus lengths, ranges, and costs can be seen in figure 3.

Figure 2: The fuel cell bus classes [4]

A block diagram of the fuel cell power system of a city bus is presented in figure 3. The power system is developed in the ThunderPower fuel cell bus. Also, the suppliers of the different subsystems can be seen in figure 4.

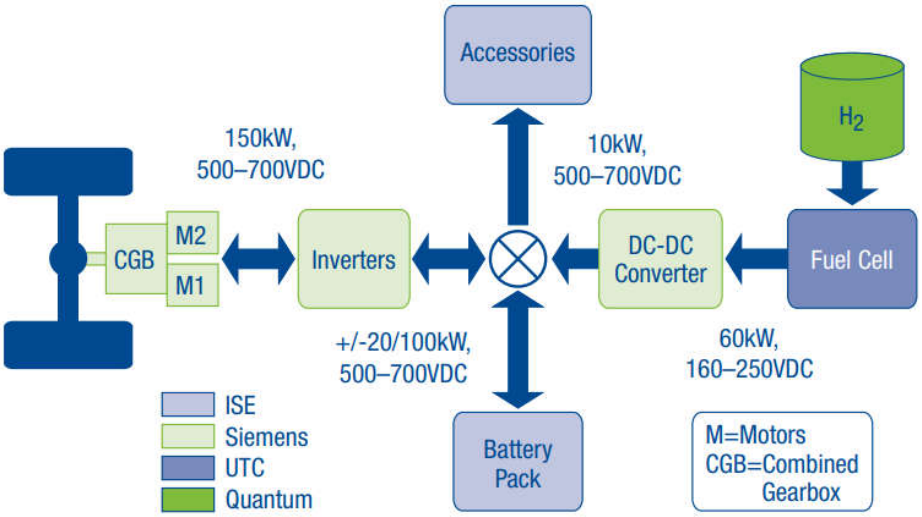

Figure 3: The fuel cell power system block diagram developed for a fuel cell bus [5]

The fuel cell power system includes the following components:

1- Fuel cell stacks and their auxiliary devices which are usually called balance of plants (BOP)

2- Hydrogen cylinders

3- The power electronics interface which mainly consists of a DC-DC converter to match the fuel cell and battery bank's voltages to hybridize the system's power supplies to feed the bus's propulsive and service loads and the fuel cell's BOP consumption.

4- Battery bank with its battery management system (BMS).
5- DC/AC inverter which acts as the propulsion motor's driver.
6- Electric motors which are usually induction or permanent magnet motors.
7- Central control unit.
8- Switchboxes and the required cables and wires.
In the next section, the general ranges of the fuel cell bus power system are presented.

3. Fuel cell bus subsystems' specifications

Considering the investigation results of various intra-city fuel cell buses, the prevalent specifications of the fuel cell bus subsystems are presented in table 1. It should be noted that the fuel cell implementation in electric buses follow two concepts. One of them is the fuel cell implementation as the bus's main power and the other one is its implementation as the bus's range extender. It is obvious that the fuel cell power range is much higher when it is installed as the main power supply of the bus.

Table۲ : Fuel cell bus subsystem specifications

Subsytem's Specification		Range/Value
Fuel cell power	Main	150-200 kW
	Range Extender	30-60 kW
Fuel Type		Gasous Hydrogen
Hydrogen Pressure		350 bar
Tank Type		Composite
Hybrid Sytem Configuration		Fuel Cell/Battery
		Fuel Cell/Super Capacitor
Power Plant Lifetime[6]		25,000 hours

The target technical specifications for fuel cell buses are presented in table 2 [6].

Table 2: The target technical specifications for fuel cell buses

Characteristics	Units	Targets
Bus Lifetime	years/miles	12/500,000
Bus Availability	%	90
Fuel Fills	per day	1 (<10 min)
Bus Cost	$	600,000
Power Plant Cost	$	200,000
Hydrogen Storage Cost	$	50,000
Road Call Frequency (Bus/Fuel Cell System)	miles between road calls	4,000/20,000
Operation Time	hours per day/days per week	20/7
Scheduled and Unscheduled Maintenance Cost	$/mile	0.40
Range	miles	300
Fuel Economy	miles per gallon diesel equivalent	8

4. Conclusions

Considering the issues concerning the air pollution and the merits of the hydrogen energy, the electric transportation based on fuel cell has been greatly developed in recent years. In this paper, the developments of the fuel cell city buses were investigated. The configuration of the fuel cell power system in the city buses was presented and the subsystems were described. Also, the prevalent specifications of the fuel cell city bus subsystems and its technical targets were presented.

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Some traveling wave solutions of (3+1)-dimensional nonlinear Schrödinger equation

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Abstract

In this work, our main motivation was to find the traveling wave solutions of the (3+1)-dimensional nonlinear Schrödinger equation (NLSE), which describes wave propagation dynamics in a nonlinear medium using the extended rational sinh−cosh method. The partial differential equation (PDE) under consideration was transformed into an ordinary differential equation (ODE) using a wave transformation. The resulting solutions of the NLSE are expected to be expressed in the rational forms of hyperbolic functions. After substituting the solutions to the ODE and performing some fundamental calculations, a set of algebraic equations were obtained. Therefore, obtaining solutions for the PDE became equivalent to solving a set of algebraic equations. The unknown coefficients in the solutions in the rational form were found by solving the obtained system. These methods were highly effective and could be employed for discovering exact solutions to a wide range of PDEs in mathematical physics.

Keywords: *(3+1)-dimensional nonlinear Schrödinger equation; Traveling wave solutions; Extended rational sinh−cosh method*

1. Introduction

Nonlinear partial differential equations (NLPDEs) are frequently encountered in some models, such as the reaction-diffusion model in chemistry, disease spread model in biology, machine learning, and neural networks in computer systems. The modified nonlocal Boussinesq equation [1], KdV-type equations [2], Biswas–Milovic equation [3], and Whitham-Broer-Kaup Equations [4] are some of the important examples of NLPDEs. Besides, its variant, the nonlinear Schrödinger equation (NLSE), is extensively used in the sub-branches of physics such as mechanics, magnetics, optics, thermodynamics, solids, and energy. The NLSE has been solved by many methods, including the extended Jacobi elliptic function [5], the first integral [6], the exp-function [7], the projective Riccati equation [8], simple equation [9], as well as rational sine−cosine and sinh−cosh methods [10,11]. The present article applied the extended rational sinh−cosh method to derive exact traveling wave solutions for the (3+1)-dimensional NLSE. It is a mathematical model that describes light propagation in nonlinear optical fibers. The relevant equation is written as follows

$$i\mathrm{P}_t + \mathrm{P}_{xx} + \mathrm{P}_{yy} + \mathrm{P}_{zz} + \gamma\left|\mathrm{P}\right|^2 \mathrm{P} = 0, \qquad (1)$$

where i is the imaginary unit, t denotes time, x, y, z imply spatial coordinates, γ denotes a constant that depends on the properties of the fiber, and P is a complex-valued function of x, y, z and t.

In recent years, some exact solutions for (3 + 1)-dimensional NLSE have been obtained using different methods. For example the Fan's F-expansion method was used and Jacobi elliptic function techniques were extended to analyze this equation by Bhrawy et al. [12]. Moreover, Gagnon and Winternitz researched the symmetry reductions in the (3 + 1)-dimensional nonlinear model [13]. In addition, the NLSE was investigated using the $(\frac{G'}{G})$-expansion method in a study conducted by Somayeh Arbabi and Mohammad Najafi [14]. Dianchen Lu et al. [15] used the extended form of the simple equation method (SEM) to acquire solutions for NLSEs. Furthermore, in an investigation by Hashemi, new integrable nonlinear Schrödinger type equation obtained by Nucci's reduction to solutions for NLSEs [16].

This paper proposes an extended rational sinh-cosh method and demonstrates its effective application in solving nonlinear equations in section 2. Section 3 explains the proposed method in detail, and demonstrates its application to explain how, the proposed method is applied to solve a nonlinear equation, and several solutions are obtained. The results are presented through figures at the end of the paper. Finally, Section 4 presents the conclusions from this study.

2. Extended Rational Sinh-Cosh

Now we discuss the process of identifying traveling wave solutions for nonlinear evolution equation [10]. Let us assume that we are given a nonlinear equation in two independent variables, x and t as the following:

$$F(\mathrm{P}, \mathrm{P}_t, \mathrm{P}_x, \mathrm{P}_{tx}, \mathrm{P}_{xx}, \ldots) = 0, \tag{2}$$

where $\mathrm{P} = \mathrm{P}(x,t)$ and F is a polynomial of P and its derivatives, in which the highest order derivatives and nonlinear terms are involved. In the following, we outline the initial steps.

Firstly, whereas

$$\mathrm{P}(x,t) = \mathrm{P}(\xi), \qquad \mathrm{P}(\xi) = x + wt, \tag{3}$$

then, by using (3), Eq. (2) can be turned into following ODE w.r.t. ξ

$$F(\mathrm{P}, \mathrm{P}', \mathrm{P}'', \mathrm{P}''', \ldots) = 0. \tag{4}$$

Secondly, suppose that the solution of equation (4), can be represented by a polynomial in sinh-cosh as follows:

$$\mathrm{P}(\xi) = \frac{a_0 \sinh(\eta\xi)}{a_2 + a_1 \cosh(\eta\xi)}, \qquad \cosh(\eta\xi) \neq -\frac{a_2}{a_1}, \tag{5}$$

$$\mathrm{P}(\xi) = \frac{a_0 \cosh(\eta\xi)}{a_2 + a_1 \sinh(\eta\xi)}, \qquad \sinh(\eta\xi) \neq -\frac{a_2}{a_1}, \tag{6}$$

introduce that a_0, a_1 and a_2 are parameters to be found in terms of the other parameters, and the non-zero constant η is the wave number. The derivatives of the predicted solutions are as the following:

$$\mathrm{P}'(\xi) = \frac{a_0 \eta \left[\cosh(\eta\xi) a_2 + a_1\right]}{\left[a_2 + a_1 \sinh(\eta\xi)\right]^2}, \tag{7}$$

$$\mathrm{P}''(\xi) = -\frac{a_0 \eta^2 \sinh(\eta\xi)\left[2a_1^2 + a_1 \cosh(\eta\xi) a_2 - a_2^2\right]}{\left[a_2 + a_1 \cosh(\eta\xi)\right]^3}, \tag{8}$$

In the first form,

$$P'(\xi)=\frac{a_0\eta\left[\sinh(\eta\xi)a_2+a_1\right]}{\left[a_2+a_1\sinh(\eta\xi)\right]^2}, \tag{9}$$

$$P''(\xi)=-\frac{a_0\eta^2\cosh(\eta\xi)\left[2a_1^2-a_1\cosh(\eta\xi)a_2+a_2^2\right]}{\left[a_2+a_1\sinh(\eta\xi)\right]^3}. \tag{10}$$

Moreover, in the second form, we replaced the equations (7) or (9),in to the reduced form of the governing equation attained above in equation (4). collecting all the terms with the same power of m together, and equating each coefficient to zero, yields a set of simultaneous algebraic equations as follows and placed coefficients of each term with the same power in either $\sinh(\eta\xi)^m$ or $\cosh(\eta\xi)^m$ to zero, which leads us to a system of algebraic equations. After solving these algebraic equations by Maple software, we get the following collection of solutions for the sinh-cosh method.

3. Applications

In this section, the sinh-cosh method is applied to solve (3+1)-dimensional NLSE.

First, we use the wave transformation as follows

$$p(x,y,z,t)=e^{i\theta}P(\xi),\ \ \xi=x+y+z+vt\ ,\theta=\alpha x+\beta y+rz+lt. \tag{11}$$

Then introduce $V=-2(\beta+\alpha+l)$ into Eq. (12) to obtain an ODE ξ:

$$2P''-(\beta^2+\alpha^2+l^2+r)P+\gamma P^3=0. \tag{12}$$

3.1. Solutions via Extended Rational Sinh Method

Considering that Eq. (2) possesses a solution in the form of Eq. (5), by changing the equation and its derivative into Eq. (1) and the coefficients with the same power of equating to zero, we get the following sets of algebraic equations using the Maple software.

$$\cosh(\eta\tau)^0=-4\eta^2a_1^2+2\eta^2a_2^2-a_0^2\Delta_1+\Delta_2a_2^2,$$

$$\cosh(\eta\tau)^1=-2\eta^2a_1a_2+2\Delta_2a_1a_2,$$

$$\cosh(\eta\tau)^2=a_0^2\Delta_1+\Delta_2a_1^2,$$

by solving these algebraic equations, we get the following solutions.

Family 1: When

$$\eta=\frac{\sqrt{\Delta_2}}{2},\qquad a_0=\pm\sqrt{-\frac{\Delta_2}{\Delta_1}}a_1,\qquad a_2=0,$$

after replacing them into Eq. (5) and its derivative into Eq. (8) ,and put them in Eq. (12) we get the solution for Eq. (1) as below:

$$P_1^{\pm}(t,x,y,z)=\pm\sqrt{\frac{\Delta_2}{\Delta_1}}\tanh\left(\pm\frac{\sqrt{\Delta_2}}{2}(x+y+z-2(\beta+\alpha+l)t)\right)\exp[i(\alpha x+\beta y+rz+lt)]. \tag{13}$$

Family 2: When

$$\eta=\sqrt{\Delta_2},\qquad a_0=\pm\sqrt{-\frac{\Delta_2}{\Delta_1}}a_1\ ,\qquad a_2=a_1,$$

after replacing them into Eq. (5) and its derivative into Eq. (8), and put them in Eq. (12) we get the solution for Eq. (1) as below:

$$P_2^{\pm}(t,x,y,z)=\frac{\pm\sqrt{\frac{\Delta_2}{\Delta_1}}\sinh\left(\sqrt{\Delta_2}(x+y+z-2(\beta+\alpha+l)t\right)\exp^{I(\alpha.x+\beta.y+r.z+l.t)}}{1+\cosh\left(\sqrt{\Delta_2}(x+y+z-2(\beta+\alpha+l)t)\right)}. \quad (14)$$

Family 3: When

$$\eta=\sqrt{\Delta_2}, \qquad a_0=\pm\sqrt{-\frac{\Delta_2}{\Delta_1}}a_2, \qquad a_1=-a_2,$$

after replacing them into Eq. (5) and its derivative into Eq. (8) and put them in Eq. (12) we get the solution for Eq. (1) as below:

$$P^{\pm}{}_3(t,x,y,z)=\frac{\sqrt{-\frac{\Delta_2}{\Delta_1}}\sinh\left(\sqrt{\Delta_2}(x+y+z-2(\beta+\alpha+l)t\right)\exp^{I(\alpha.x+\beta.y+r.z+l.t)}}{1-\cosh\left(\sqrt{\Delta_2}(x+y+z-2(\beta+\alpha+l)t)\right)}. \quad (15)$$

3.2. Solutions via Extended Rational Cosh Method

Considering that Eq. (2) possesses a solution in the form of Eq. (6), we can simplify the process of solving for the coefficients in this equation by substituting Eq. (12) and its derivative into it and equating the coefficients with the same power to zero. Therefore, we get the following sets of algebraic equations using the Maple software.

$$\sinh(\eta\tau)^0=(-2\eta^2\Delta_3+\Delta_2)a_1^2-a_2^2(\eta^2\Delta_3+\Delta_2),$$

$$\sinh(\eta\tau)^1=a_1a_2(\eta^2\Delta_3-2\Delta_2),$$

$$\sinh(\eta\tau)^2=-a_0^2\Delta_1-\Delta_2a_1^2,$$

by solving these algebraic equations, we get the following solutions.

Family 4: When

$$\eta=\pm\frac{\sqrt{\Delta_2}}{2}, \qquad a_0=\pm\sqrt{-\frac{\Delta_2}{\Delta_1}}a_1, \qquad a_2=0,$$

after replacing them into Eq. (5) and its derivative into Eq. (10) and put them in Eq. (12) we get the solution for Eq. (1) as below:

$$P^{\pm}{}_4(t,x,y,z)=\sqrt{-\frac{\Delta_2}{\Delta_1}}\coth\left(\sqrt{\frac{\Delta_2}{\Delta_3}}(x+y+z-2(\beta+\alpha+l)t)\right)\exp^{I(\alpha.x+\beta.y+r.z+l.t)}. \quad (16)$$

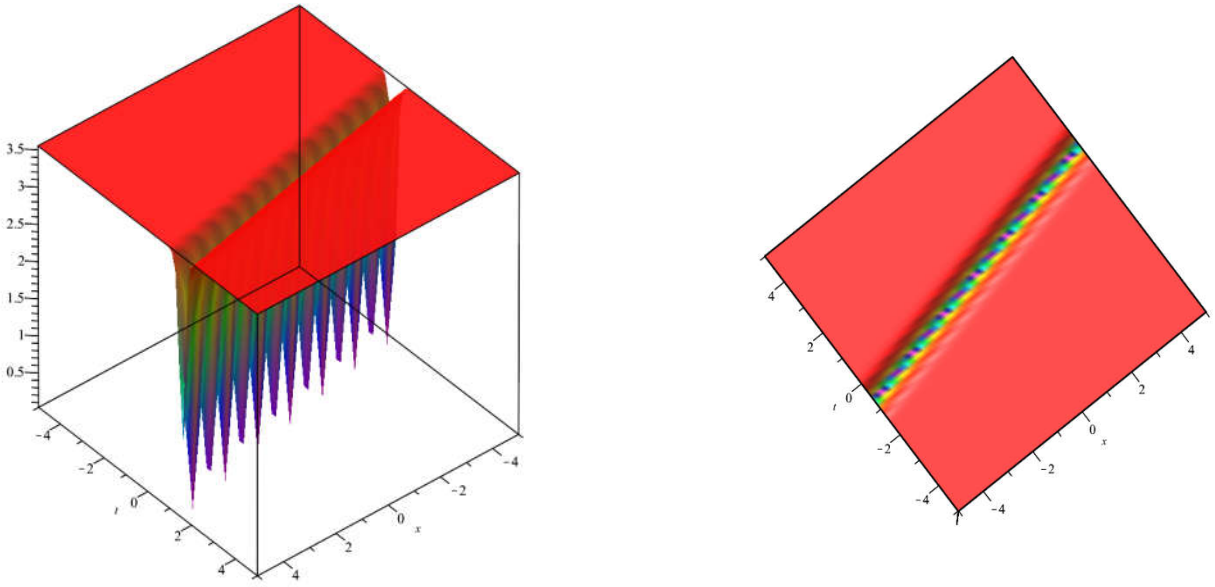
Family 5: When

$$\eta=\pm\sqrt{\Delta_2}, \qquad a_0=\pm\sqrt{\frac{\Delta_2}{\Delta_1}}a_2, \qquad a_1=Ia_2,$$

after replacing them into Eq. (5) and its derivative into Eq. (10) and put them in Eq. (12), we get the solution for Eq. (1) as below:

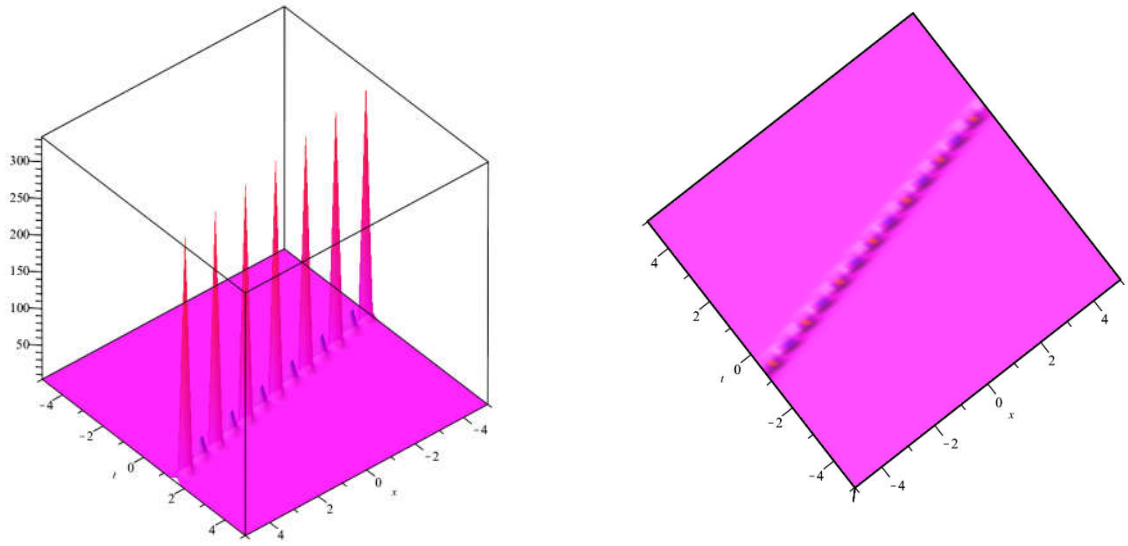
$$P^{\pm}{}_5(t,x,y,z)=\frac{\pm\sqrt{\frac{\Delta_2}{\Delta_1}}\cosh(\sqrt{\Delta_2}(x+y+z-2(\beta+\alpha+l)t)\exp^{I(\alpha.x+\beta.y+r.z+l.t)}}{1+I\sinh(\sqrt{\Delta_2}(x+y+z-2(\beta+\alpha+l)t)}.$$

(17)



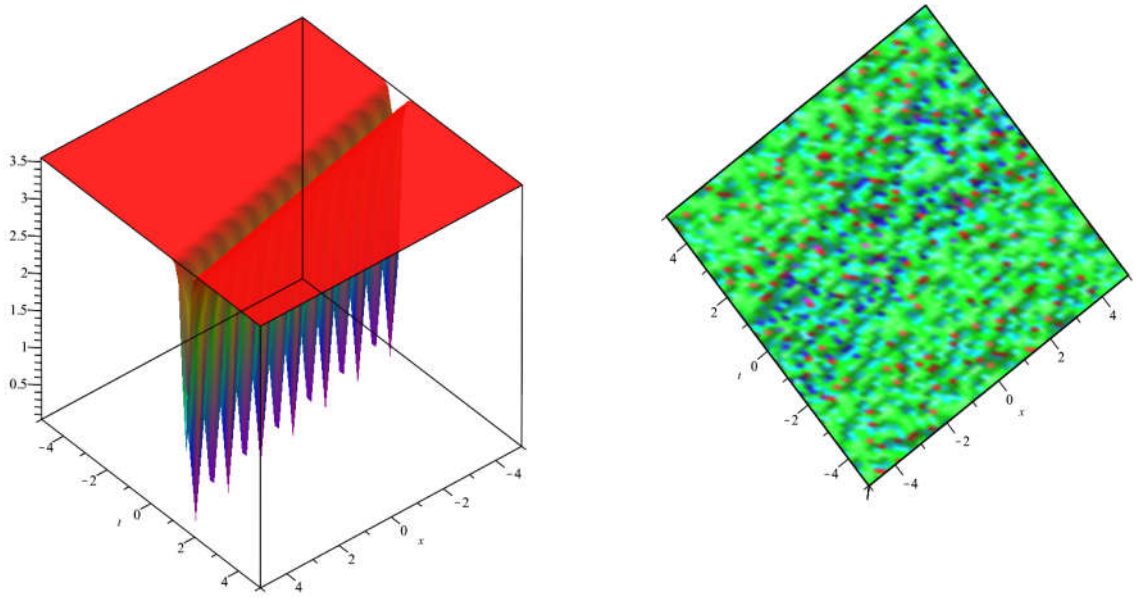
(a) **(b)**

Figure 1. (a) 3D-plot of the modulus and (b) the contour plot of the modulus for P_1 at r = -0.75, $\beta=0.5$, $\alpha=2.5$, $\mu=1.5$, $\gamma=-0.5$, $l=-0.75$, $y=1$, $z=1$.



(a) **(b)**

Figure 2. (a) 3D-plot of the modulus, (b) the contour plot of the modulus, for P_3 at r = -0.75, $\beta=0.5$, $\alpha=2.5$, $\mu=1.5$, $\gamma=-0.5$, $l=-0.75$, $y=1$, $z=1$.



(a) **(b)**

Figure 3. (a) 3D-plot of the modulus, (b) the contour plot of the modulus, for P_5 at r = -0.75, $\beta = 0.5$, $\alpha = 2.5$, μ=1.5, $\gamma = -0.5$, $l = -0.75$, $y = 1$, $z = 1$

4 .Conclusion

The present paper employs the extended rational sinh-cosh method with wave transformation to find solutions for (3 + 1)-dimensional NLSE. This equation is considered a PDE that describes the evolution of a complex-valued wave function. It is a nonlinear extension of the standard Schrödinger equation and is often used to model an extensive range of physical phenomena, including fluid dynamics and nonlinear optics. We have confirmed the accuracy of these solutions using Maple software. Our findings demonstrate that the proposed method is highly effective and can be applied to solve other NLPDEs in various fields, such as engineering, mathematical biology, physics, and chemistry.

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An Overview of Algal and fungi pigment sources in foods

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Abstract

Color of the food is associated with the safety, flavor, and its nutritional value. Synthetic food colorants have been used because of their low cost and high stability. Natural food colorants have received particular attention, not only because they are potent substitutes for synthetic additives but also because they provide health and security benefits to consumers such as their antimicrobial, anti-inflammatory, anti-amyloid, and antitumor proprieties. Additionally, these compounds have been associated with reductions in several diseases, such as diabetes and obesity. Natural pigment applications can be limited by weaker tinctorial strength, lower stability, and interactions with food ingredients. Nowadays, microorganisms, including algae and fungi have been shown to be an excellent source of natural pigments. For the large-scale production of pigments, microorganisms are more suitable, due to processing and ease of handling, a clear understanding of their cultural techniques. Natural pigments from microbes, especially from algea and fungi, have been reported worldwide by many researchers. This review summarizes characteristics of prevalent pigments, including anthocyanins, betalains, carotenoids, and chlorophylls and examples of their fungal and algal sources.

Keywords: *natural pigments, colorants, anthocyanins, betalains, carotenoids, chlorophylls*

1. Introduction

Color is the first and most impacting attribute affecting directly the consumer's selection, preference, and desire. Synthetic colorant ingredients are used by the food and pharmaceutical industries. However, there are concerns about the use of these pigments because of their adverse health effects. These issues have increased the replacement of synthetic pigments by natural ones (1). Historically, natural colorants or pigments used in food applications were obtained from renewable resources such as from plants or from microbes (e.g. yeasts, algae, bacteria, and fungi) and insects. Extracts of: paprika, saffron, turmeric, and various flowers are some examples from which natural pigments were traditionally derived with wide applications, synthetic pigments gained popularity as one of the main food coloring compounds. These synthetic pigments have been related with high stability, low production costs, and high tinctorial strength as well as ease of application in the food system (2). natural pigments have strong advantages like simple extraction methods, low cost, sustainability, and

high relative abundance (3). However, the most common pigments are typically yellow, red or orange. The disadvantages generally reported for microbial pigments include potential contamination with secondary toxic metabolites, and unsatisfactory production levels. Optimization of the culture medium combiation and other experimental conditions is usually carried out to improve the production yields. On the other hand, to avoid the undesirable production of toxins, a careful search and selection of safe microorganism strains should be performed (4). Fungi have immense advantages over plants such as easy and fast growth in a cheap culture medium, season-independent pigment production, production of pigments with different color shades and of more stable, easy processing, and soluble pigments. Fungi belonging to the *Trichocomaceae*, *Nectriaceae*, *Pleosporaceae*, *Hypocreaceae*, *Cordycipitaceae*, *Chlorociboriaceae* *Xylariaceae*, *Herpotrichiellaceae*, *Sordariaceae*, *Chaetomiaceae*, *Hyaloscyphaceae*, *Ophiostomataceae*, *Hymenochaetaceae*, *Polyporaceae*, *Tremellaceae*, and *Tuberaceae* families have been described as potent pigment producers. These fungi are known to synthesize different kinds of pigments as their secondary metabolites. They are prolific producers of pigments belonging to several chemical classes, such as melanins, carotenoids, flavins, azaphilones, phenazines, monascin, quinones, indigo, violacein, etc. (5). Algae have been known as natural producer of bioactive commercial pigments. To perform photosynthesis, algae use pigments to harvest sunlight energy. The pigments found in algae are categorized in carotenoids, chlorophylls, and phycobilins. Popular carotenoids include astaxanthin, fucoxanthin, lutein, β-cryptoxanthin, canthaxanthin, zeaxanthin, and finds application as anti-inflammatory, antioxidant, immunoprophylactic, antitumor activities among others (6).

2.Anthocyanins

Anthocyanins are water-soluble pigments that belong to the group of secondary metabolites within the class of phenolic compounds. They are found as glycosides of their respective aglycones, called anthocyanidins. Cyanidin, delphinidin, malvidin, pelargonidin, peonidin, and petunidin are major anthocyanidins present in foods. Anthocyanins offer a wide range of health benefits to human health (7) These compounds are widely distributed in fruit and vegetables such as blueberry (*Vaccinium myrtillus*), blackberry (*Rubus fruticosus*), cranberry (*Vaccinium macrocarpon*), grapes (*Vitis sp.*), purple corn (*Zea mays*), grumixama (*Eugenia brasiliensis*), juçara (*Euterpe edulis Mart.*), fig (*Ficus carica L.*), and petals of saffron (8). Anthocyanins are widely spread in nature and exhibit multiple health-promoting effects and brilliant colors; so, they are extensively incorporated into foods, cosmetic industries, and pharmaceuticals (9). Production of natural products using biotechnological methods, especially plant cell and tissue cultures and microbial cell factories is considered as a promising option since 1950s and has been widely studied. Depending on the culture conditions and cell type, cell suspension cultures enable more direct pigment production, preferably in amounts superior to those of the intact plants. Similarly, the genetic engineering of microorganisms can also provide an efficient way to produce and modulate anthocyanin biosynthesis. unicellular prokaryotic cells such as *Streptomyces venezuelae* and *E. coli* are considered to be the most suitable hosts for heterologous production of anthocyanins (10).

3. Betalains

Betalains are tyrosine-derived, pigments that can be biochemically discribed by their inclusion of betalamic acid as the central chromophore (11). According to their spectral properties they have been classified into two main sub-groups: the red-violet betacyanin and the yellow betaxanthin (12). Betalains are compounds of great interest because they have bioactive properties. Their health-promoting effect that has been demonstrated in the in

vivo model *Caenorhabditis elegans*, increase lifespan and reduce oxidative stress (13). Some fungi of the *Hygrocybe* and *genera Amanita* produce betalain-related pigments (14). These are restricted to Caryophyllales in higher plants while some are also found in fungi like *Hygrosporus* (15). the discovery of *Gluconacetobacter diazotrophicus* as the first betalain-forming bacterium opened a new field in the search for novel biological systems able to produce betalains (16).

4. Carotenoids

The most studied natural pigments are carotenoids. They are fat-soluble that can be found in almost all vegetables and fruits, providing the colors yellow, orange, and red, due to their chromophores, which consists mainly of a chain of conjugated double bonds. Carotenoids have also been reported in fungi, algae, birds, insects, fishes, and crustaceans (17). Carotenoids can be categorized into two groups: oxygen-containing xanthophyllls (e.g., astaxanthin and zeaxanthin) and carotenes which are pure hydrocarbons with no oxygen (β-carotene and lycopene). Carotenoids absorb light within a wavelength of 400–550 nm. They range from colourless to deep-red, with their colour dependent upon the number of conjugated double bonds. Carotenoids cannot be synthesized by humans; we must obtain a sufficient amount from our diet (18). The group of yeast that can synthesize carotenoids includes *Phaffia rhodozyma* (and its teleomorph *Xanthophyllomyces dendrorhous*) and species of the genera *Sporobolomyces*, *Rhodosporidium*, *Rhodotorula*, and *Sporidiobolus*. The *Blakeslea* trispora species is of the greatest importance (19). The main sources of astaxanthin are the microalgae *Chlorella zofingiensis*, *Haematococcus pluvialis*, and *Chlorococcum sp*. Lutein and zeaxanthin are mainly found in algal species such as *Rhodophyta spp.*, *Scenedesmus spp.*, *Chlorella spp.*, or *Spirulina spp*. However, the extraction and purification processes of xanthophylls from algae need to be standardized to facilitate their commercialization (20).

5. Chlorophylls

Chlorophylls are green pigments found in all higher plants which participate in the photosynthesis. Chlorophyll is a macrocyclic tetrapyrrole with coordinated magnesium in the center. There are two types of chlorophyll (chlorophyll a and b) (21). Chlorophylls are porphyrins, which are macrocyclic tetrapyrrole pigments in which the pyrrole rings are joined by methyne 884 D. B. Rodriguez-Amaya bridges and the double bond system forms a closed, conjugated loop (22).

5. Conclusion

In recent decades, there has been an increasing trend to replace artificial colors with natural pigments due to the intense consumer preference for natural products. Natural pigments are used in various fields of life such as food production, textile production, agricultural research, water science and technology. Not only increase the marketability of products, but also show beneficial biological activities as antioxidants and anticancer agents. On the other hand, synthetic pigments cause considerably environmental pollution and adverse toxicological side effects. It is essential to explore different natural sources of food grade colorants and their use potentials. Use of microbial pigments in processed food is an area of promise with large economic potential. However, microbial pigments offer challenges due to lower stability, high cost, and variation in shades due to changes in pH. For the large-scale production of pigments, microorganisms are more suitable, due to a clear understanding of their cultural techniques, processing, and ease of handling. Future research on natural pigments should also be done to expand the colors that can be obtained, and to promote pigments with beneficial properties for health treatments. Also, more research is used on natural fixations, which until today are addressed using different methods of different dyes on molecular complexation or

microcoating. However, currently only scarce data, currently contradicting laboratory results, are available on the stability of natural pigments in food systems.

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Green synthesis of the Ag/Fe_3O_4 nanocomposites and their catalytic application for the degradation of organic pollutants

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Abstract

The present study is the first report representing the biosynthesis and characterization of Ag/Fe3O4 nanocomposites using *Eryngium caucasicum* extract and evaluation the nanocomposites for decolorization of nitro-amines and azo dyes, known as important biological pollutants. The Ag/Fe3O4 nanocomposites also demonstrated a high catalytic activity and 100%, 93.6%, 92.7% and 82.5% of the CR, RhB, MB and MO dyes were degraded in 200s, 80s, 520s, and 80s, respectively. Nanocomposite catalyst can be recovered from the reaction mixture and reused for three-cycle without remarkable loss of catalytic activity. Kinetics parameter of the reaction showed that the catalytic degradation of CR, RhB, MB and MO dyes followed the pseudo- first-order reaction. Results obtained through catalytic evaluation suggested that the Ag/Fe3O4 nanocomposite could be helpful for eliminate the nitro-amines and azo dyes in a very effective manner.

Key words: *Ag/Fe3O4 nanocomposites, Eryngium caucasicum, Azo dyes, Pseudo-* first-order reaction

1. Introduction

Organic dyes are chiefly used in pharmaceuticals fields. Moreover, these dyes have various applications in other industries such as textile, leather, cosmetic, food, and publication [1]. These dyes are released from several industries, which can be considered as the most critical environmental pollutant agents. Also, normal water purification processes cannot degrade industrial dyes due to their intricate aromatic structures, hydrophilic property, and numerous stabilities against water, temperature, light, and light chemicals [2]. Degradation of the dyes to Non-toxic compounds is a very critical reaction [3]. For degradation the nitro-amines and azo dyes, none of the strategies are completely successful, as most of them are very expensive and produce hazardous by-products [4]. Due to diverse bioactive compounds along with exceeding decolorization activities, many researchers are looking for bioactive to design catalytic compounds with promising decolorization effect. Looking for bioactive compounds with catalytic efficacy, here metal nanoparticles were extensively examined as a suitable

approach for degradation of the dyes to Non-toxic compounds [5]. Among the known metal nanoparticles, Ag and Fe are broadly used in the field of catalysis, sensors, electronics, medicine, and pharmaceutical science because of their excellent physicals and chemical properties [6]. In this innovative study, we have utilized a new plant named *E. caucasicum* as a capping and reducing agent for the synthesis of Ag/Fe_3O_4 nanocomposites. Catalytic dye degradation potential of these Ag/Fe_3O_4 nanocomposites was studied for Congo red, methylene blue, methyl orange and rhodamine B.

2. Material and methods

The catalytic activity of Ag/Fe_3O_4 nanocomposites was evaluated for reduction of CR, MB, MO, and RhB dyes. 200 µg of Ag/Fe_3O_4 nanocomposites was added into a series of 15mL falcon tubes containing 6 ml aqueous solution of the respected dyes and 0.5 ml NaBH4. The changes were recorded by an ultraviolet-visible (UV-Vis) spectrophotometer (8453, Agilent) at 493, 664, 554, and 468 nm wavelengths, respectively. The percentage degradation of CR, MB, RhB, and MO was calculated using the following formula:

$$C=\left[\frac{A0-At}{A0}\right]\times 100$$

C is the percentage degradation of CR, MB, RhB, and MO, A_0 and A_t are the absorbance of the dyes at time zero and t, respectively. A pseudo-first-order kinetics was carried out to evaluate the reaction kinetics of CR, MB, RhB, and MO, expressed in the following equation:

$$\text{Ln}\ (C_t/C_0) = -k_t$$

Where k is the rate constant at the given time and t is the reaction time. C_0 and C_t are the concentrations of the dyes at time zero and t, respectively.

3. Results and discussion

The catalytic behavior of Ag/Fe3O4 nanocomposites in degrading four dyes (CR, MB, MO, and RhB) was investigated. It is clear that the color of solutions quickly faded and ultimately became colorless indicating dye degradation through the Ag/Fe3O4 nanocomposites. The reduction was found to happen only in the presence of our catalyst, and no remarkable reduction happened in the absence of the catalyst due to the large kinetic barrier and high activation energy. NaBH4 operates as an electron donator, and CR, MB, MO, and RhB operate as electron captors in the redox reaction. Dyes accept electrons from the surface of Ag/Fe3O4 nanocomposites which in turn receive electrons from NaBH4. Ag/Fe3O4 nanocomposites serve as electron relay, accelerating the redox response between CR, MB, MO, RhB, and NaBH4. The Ag/Fe3O4 nanocomposites showed high catalytic activity as

compared to some of the conventional methods. Fig.1 (a, b, c, and d) showed the UV–visible spectra of CR, MB, MO and RhB degradation by NaBH4 in the presence of Ag/Fe3O4 nanocomposites. This reduction in the peaks intensity indicates the gradual disintegration of the dye as time goes on. Our results showed that, within 80-520 second, based on the dye, the reduction process completed entirely. As shown in Fig. 1, reduction of CR, MB, MO, and RhB occurred within 200s, 80s, 520s, and 80s, respectively. Ag/Fe3O4 nanocomposites catalyzed 100%, 93.6%, 92.7% and 82.5% degradation of CR, RhB, MB and MO, respectively. The results revealed that the green synthesized Ag/Fe3O4 nanocomposites using *E. caucasicum* extract had a higher destruction percentage compared to AgNPs synthesized by *Crataegus pentagyna* fruit extract. The apparent pace constants (Kapp) for the decomposition of organic dyes were calculated from the slope of the linear plots of ln (Ct/C0) vs. time. The first-order rate constants (k) for CR, MB, MO and RhB degradation were KCR= 9×10^{-3} s^{-1}, KMB=5.4×10^{-3} s^{-1}, kMO=1.38×10^{-2} s^{-1} and kRhB=3.82×10^{-2} s^{-1} which is more acceptable than the previous reports. In the case of RhB, the Ag/Fe3O4 nanocomposites showed an approximately 2.4, 4.2-, and 7.1-times higher reaction rate than MO, CR, and MB, respectively. The reasons for the difference in the reaction rate are perhaps due to the different hydrophobicity, chemical structures, reduction potential, charge and presence of donor atom, which modify the activation power for catalytic reduction reaction. Our results showed that the catalyst was retrieved and reused for at least three consecutive runs with no obvious decrease of catalytic activity.

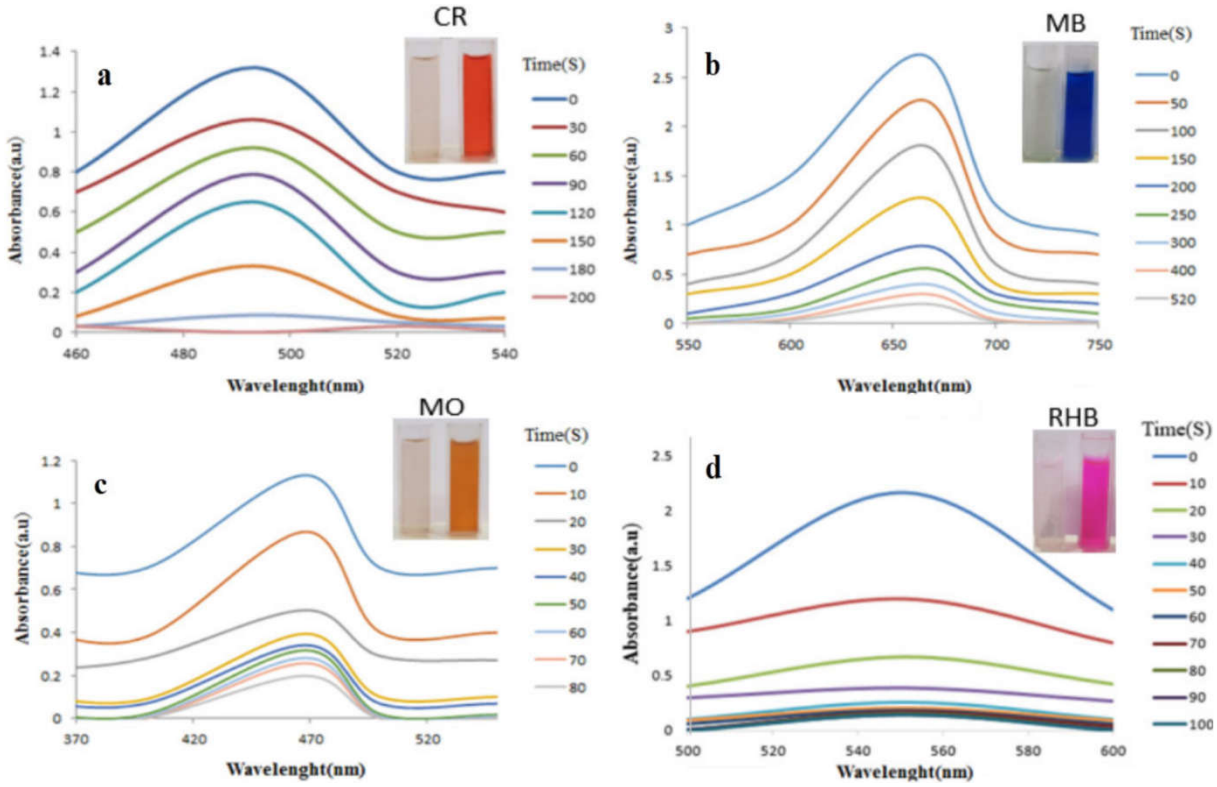

Fig. 1. UV-Vis spectra of CR (a), MB(b), MO(c) and RhB(d) with NaBH4 and Ag/Fe_3O_4 nanocomposites

Conclusions

Also, the results showed that Ag/Fe_3O_4 nanocomposites was an effective catalyst that exhibited high catalytic activity for the reduction of dyestuffs. According to the rate constants, Ag/Fe_3O_4 nanocomposites exhibited better catalytic activity for RhB ($kRhB=3.82\times10^{-2}$ s^{-1}) than MO ($kMO=1.38\times10^{-2}$ s^{-1}), CR ($KCR= 9\times10^{-3}$ s^{-1}) and MB ($KMB=5.4\times10^{-3}$ s^{-1}). We offered that the green synthesized Ag/Fe_3O_4 nanocomposites is good alternatives in medicinal and therapeutic usages and the elimination of hazardous materials from industrial wastewaters. Compared with previous methods for the decomposition of azo dyes, this method provided several benefits such as lesser reaction time and higher performance, simple work-up procedure, and *recycled* at least three *times without loss of its catalytic*

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A Comprehensive Review of *Ligula intestinalis*

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ABSTRACT

This review article provides a comprehensive overview of the infection of fish by Plerocercoeid of *Ligula intestinalis* and the role of eating birds as final hosts in the epidemiology of this infection. The paper begins with an introduction, presenting various data on this parasite, emphasizing different research on *L. intestinalis* in Iran. The article then explores the collection and processing of results from different regions of the world, highlighting the significance of management and prevention strategies in parasite control. Furthermore, the zoonotic potential of Plerocercoeid and the occurrence of zoonotic cases are discussed, shedding light on the importance of understanding the zoonotic aspects of *Ligula* plerocercoid. The efficacy of available treatments and prevention methods is evaluated, and the evolutionary dynamics of it and related genera and species within the same family and order are investigated. Specifically, the paper analyzes the relationship between *L. intestinalis* and *Digramma interrupta* in terms of morphology, molecular structure, and epidemiology. Finally, the taxonomy and nomenclature of these two genera, as well as their family and order, are examined to determine if any rearrangements or updates have occurred. due to the importance of infection in wild and domestic cycle as threating of public health and aquaculture industry Losses, is seem to be necessary design a comprehensive prevention program based on more study on high-risk areas on appropriated intermediated fish host.

Key words: *Ligula intestinalis, Digramma interrupta,* morphology, *Fish.*

1. Introduction

This review article provides a comprehensive overview of the infection of fish by Plerocercoeid of Ligula intestinalis and the role of eating birds as final hosts in the epidemiology of this infection. The paper begins with an introduction, presenting various data on this parasite, emphasizing different research on *L. intestinalis* in Iran. this article by collection and processing of data from whole the world, highlighting the significance of management and prevention strategies in parasite control. Furthermore, the zoonotic potential of Plerocercoeid as an unclear argument and the occurrence of zoonotic cases are discussed, shedding light on the importance of understanding the zoonotic aspects of *Ligula* plerocercoid. The efficacy of available treatments and prevention methods is evaluated, and the evolutionary dynamics of that and related genera and species within the same family and order are investigated. Specifically, the paper analyzes the relationship between them and *Digramma interrupta* in terms of morphology, molecular structure, and epidemiology. Finally, the taxonomy and nomenclature of these two genera, as well as their family and order, are examined to determine if any rearrangements or updates have occurred (1,2).

1. *Ligula intestinalis*:

L. intestinalis is a parasitic tapeworm that infects fish, particularly in freshwater environments. It has a complex life cycle involving intermediate hosts and final hosts, including various species of birds. Epidemiological Data and researchers like as (Ahmadiara et.al) in Iran This section presents an overview of epidemiological data on this pleroercoeid in fish body cavities as seconf final host (2,3). emphasizing the research conducted by a few of researchers in Iran, some work provides valuable insights into the prevalence, distribution, and impact of *L. intestinalis* infection in fish populations in Iran (3,4,5).
Collection and processing of results by global surveillance of this plerocercoeid infection Providing comprehensive survey of this infection in various regions of the world is discussed in this section. for example Ligulosis due this plerocercoid is one of the most common infection of freshwater intermediated host in lakes and water reservoirs (Lott et al 2002). We confront by this fact that there are many reports on internal infections of various kinds of cyprinidae fishes with plerocercoid of *L. intestinalis* from many regions like Iran. However, the reports on infestation of some species Compared with other ones like *Alburnoides bipunctatus* are limited (6,7,8).
The collection and processing of data from different studies contribute to our understanding of the global epidemiology of this parasite. Role of Management and Prevention Strategies Effective management and prevention strategies are essential for controlling *L. intestinalis* infection. This section examines various approaches, such as habitat management, host control, and prevention measures, and their impact on reducing the prevalence and transmission of the parasite (7,8).

2. Zoonotic Potential

Zoonotic Potential of Plerocercoeid. Zoonotic Cases: Fact or Myth? The zoonotic potential of Plerocercoeid, including *L. intestinalis*, is a topic of concern. This section evaluates the occurrence of zoonotic cases and explores the evidence supporting or refuting the zoonotic nature of *Ligula* plerocercoid infections in humans. Exploring the Zoonotic Aspect of *Ligula* plerocercoid Building upon previous research, this section delves into the zoonotic aspect of *Ligula* plerocercoid infections, examining potential routes of transmission, risk factors, and the importance of surveillance and awareness in addressing zoonotic implications.
Treatment and Prevention: Efficacy of Medications for Treatment and Prevention The efficacy of available medications for treating this intestinalis infection in fish and preventing its transmission is assessed. This section reviews the current treatment options and their effectiveness in reducing the burden of the parasite. it is very important from standpoint of nutritional health. When a huge number of fishes are infested with the parasite, a main source of high quality protein is jeopardized. Overall regarding economic losses and zoonotic importance of the food borne infection, prevention programs, conducting tough measures and performing comprehensive studies seem necessary. Strategies for Preventing plerocercoeid of *L.intestinalis* Infection (8).
As mentioned in many lakes, water reservoirs, dame, local rivers and another fresh water resource like local rivers in country, remarkable infection in many genouse of cyprinid fish were seen. By attention to that points that is be very necessary to finding an appropriate solution to control this widespread fresh water resource and fish infection with some pseudophyllidean cestoda (3).

3. piscivorous birds role

In this way 3 main objectives must be considered with whole attention. First of all, we must know piscivorous birds have the most important role in the epidemiology of ligulosis infetions. Because they

can flied and transmitted easily the adult intestine parasite. They spread infection with flying from one location to another regions without any limitation. After that, secondly and thirdly important issue is the pollution of water with eggs and coracidium of *L. intestinalis* and contamination of copepods and fish with procercoid and plerocercoid respectively. So the prevention programs should breaking the lifecycle of parasite is so nesesary for preserve of public health (3,4).

4. treatment

However treatment the birds as the final host is possible with some chemotherapeutic agents like Praziquantel (Droncit) by a single dose of 5-10 mg/kg body weight or a single dose of 2g Niclosamide but since that birds are wild and inaccessible is not available and Logical (10). On the other hand ligulosis infection duration in a bird intestine is very short and usually lasting under 4–5 days, then treatment process of birds for preventing actually is impossible. In fact in wild cycle the most effective try is decrease the infectious birds by monitoring them in high infection location and elimination and high reduction of infected fish by monitoring and collecting of them and destroyed the copepods by preventive medicine as mentioned below (7,8).

5. prevention program

Base on Ahmadiara et al. (2013a) study, copepods and cyclops as the first intermediated hosts often habitat in the slow-flowing water. So we except pollution be more in reservoirs and lakes into fast flow rivers (Ahmadiara et al., 2013a). The result of different surveys is coordinate with this theory. So this resource need to more attention, however is very difficult control of natural resources and wild populations. But in Fish farming pool control preventing are rather more practical. So more efforts should do on ponds and limited water treatment to eliminate infectious fish and copepods. For this purpose for free or semi-free water, such as lakes, there is no certain treatment, but for breeding pools and indoor ones, immersion of fish in a 3/100000 solution of picric acid for one hour or use of di-n-butyl tin oxide by 250 mg/kg dose for elimination of infected copepods is practical (6).
Any way it's so better prevention of ligulosis than waiting for outbreak of them. Since there are several reports of human ligulosis infection (Eslami, 2006), and due to the importance of infection in wild and domestic cycle as threating of public health and aquaculture industry Losses, is seem to be necessary design a comprehensive prevention program based on more study on high-risk areas on appropriated intermediated fish host (8).

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A comparative study of *Shewanella oneidensis MR-1* and *Escherichia coli* pure cultures and co-cultured inocula in microbial desalination cell

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Abstract

Energy, food and water supply are the most challenging demands in modern and developing societies. Microbial desalination cell technology is a promising technology which can perform saltwater desalination, electricity generation and wastewater treatment simultaneously. Various factors influence overall performance of MDC technology which is significantly dominated by inoculum composition in mutual interaction to other factors. Bacterial metabolism is inhibited due to salt stress occurs during system operation and desalination process, which in a challenging limitation in scaling up such technology. In this paper inoclum of *Shewanella oneidensis* MR-1 and *Escherichia coli* pure culture have been compared to co-cultured system, and the OCV and desalination efficiency have been evaluated on a basic three-chambered MDC equipped with an air cathode fed sodium lactate and sodium acetate as anolyte.

Key words: *Microbial fuel cell, Desalting, Wastewater treatment, Bacteria inocula*

1. Introduction

The rapid growth of modern societies and the population has increased the demand for energy, food, and water, and fulfilling these needs has left significant environmental pollution. Electricity providing, as well as greenhouse gas reduction, has led global communities to make use of renewable energies [1]. Also, by increasing worldwide population growth and climate change, the freshwater supplement will be one of the main problems in different regions in the future. Although numerous technologies have been developed for wastewater treatments and desalination of brackish and saltwater, these technologies are both energy- and cost-intensive, raising a paradox between electricity generation and freshwater supplements, as long as reducing greenhouse gas emissions. Energy consumption of desalination technologies are dissimilar, and some technologies such as mechanical vapour compression (MVC), reverse osmosis (RO) and electrodialysis (ED) are optimized in term of energy consumption and currently are being used in different part of the

world [2]. Also municipal and industrial wastewater are treated through various technologies such as activated sludge, membrane technologies, anaerobic decomposition and electrochemical oxidation, which 3% of global electricity are used for wastewater treatment [3].

Biofuel cells (BFCs) as a renewable and sustainable energy source have attracted much attention recently, directly converting chemical energy stored in organic matter to electrical energy using renewable bio-catalysts. Microbial fuel cell technology (MFCs) is the most important branch of BFC technology, which utilizes microorganisms as catalysts to capture bioelectricity[4], which municipal, industrial and agricultural wastewater as long as plant residues and sewage sludge can be used as organic fuel [5]. Oxidation of dissolved organic matter in wastewater by current generating microbial catalyst of MFC causes reduction of chemical oxygen demand (COD), describing MFC as a merged technology that simultaneously provides bioelectricity and wastewater treatment. The combination of Electrodialysis (E.D) as a commercialized desalination technology [2] and MFC technology has led to the development of Microbial desalination cell (MDC) technology in 2009, showing that desalination can be accomplished without electricity energy input [6] as long as electricity generation and wastewater treatment can be achieved simultaneously. Mehana et al. investigate the effect of substrate and salt concentration and membrane ion exchange capacity on three-chambered MDC's desalination, power production, and COD removal performance [7]. *Qu et al.* reported that higher buffer concentration could enhance power production, desalination efficiency, and COD removal and introduced a novel MDC system that recirculates the system's solution to decrease buffer consumption as one of the commercialization limitations [8]. Lou et al. investigate the long-term performance of MDC, defining how membrane biofouling can affect the overall performance of MDC[9].

Beside various development of MDC, this promising technology faces various challenges. Ion transport which is basic of desalting process cause pH unbalance which overshadows microbial community metabolism [10]. *Shewanella oneidensis* is a gram-positive facultative anaerobic species, which prefers lactate as the sole electron donor in anaerobic conditions, and can transfer released electrons during substrate oxidation to an electron acceptor outside the cell through it's EET pathway, making this species as a potential exoelectrogen for BFCs applications. The culture of *S. oneidensis*, in combination with other microorganisms, e.g., *Escherichia coli*, can enhance the electrochemical behavior of the MFCs. In the co-cultured microbial community, the non-exoelectrogenic bacteria can scavenge leaked oxygen to the anolyte solution and maintain the anaerobic condition of the cell as long as enhancement of COD removal[11].

In this study, we have investigated the pure culture of *S. oneidensis* MR-1 and *E. cloi* and the co-cultured inocula, and compare OCV and desalination efficiency of three systems.

2. Materials and methods

2.1 Bacterial strains and culture

Pure culture of *S. oneidensis* MR-1 and *E. coli* was purchased from the Iranian Biological Resource Center (IBRC). The strains were cultured at 37ºC in LB broth medium for 24 hours, and then the stocks were prepared by 50 v/v of 30% glycerol and stored at -70ºC refrigerator for further use. Before incubation of microorganisms into the cell, one stock of each bacteria was centrifuged at 6000 rpm for 10 minutes at 4ºC to separate glycerol. Then the sediment was cultured in autoclaved LB broth medium for 12 hours.

2.2 Equipment and methods

A PC-connecting GDM-461 multimeter (GW Instek co.) was used for voltage recording, and the cell's open circuit voltage (OCV) was recorded every 2 minutes. NaCl concentration, pH, and dissolved oxygen (D.O) were measured by an az-561 multiparameter calibrated through the company procedure. Medium, buffer, and electron donors were sterilized by autoclavation. Other components, such as cells, membranes, and electrodes that were unable to autoclavation, were sterilized by the circulation of ethanol 70% and 30 minutes of U.V. radiation. OCV decline and desalination rate stop were presumed as the end of the cycle, and closed circuit voltage was measured from 25 to 25000 ohms and normalized base on anode surface area to evaluate power output and final internal resistance of the system, which maximum power density is achieved when internal resistance and external resistance of the system are equal [12].

2.3 MDC design and startup

A cubic structure of Plexiglas of 5 cm × 10 cm × 7 cm was prepared and was split into three equal chambers (70 mL of working volume) by inputting AEM and CEM (2.5×2.5 Cm^2) to prepare three equal chambers with a desalination chamber in the middle of the cell. The cell was initially employed under MFC mode to reach a stable OCV voltage and then switched to MDC mode, which the procedure is described as follows;

MFC mode: a 100mM buffer solution of sodium bicarbonate was prepared and was N_2 aerated for 20 minutes to remove dissolved oxygen (O.D. less than 1 gr/L) to induce biofilm formation of exoelectrogens; as reported before, anaerobic conditions can induce EAB cells to adhere to electrode surface to form biofilms [13]. The anode chamber was filled with 50mL buffer solution and 20 mL LB medium under a sterilized microbial hood. The desalination and cathode chamber were filled with 70 mL of buffer solution as the same. After adjusting the pH of the system to 6.8, the whole system was U.V. radiated for 30 minutes to sustain the sterilized condition. The microorganisms were incubated in the system, and electron donors were added after incubation. The system was packed under the hood to maintain the inner sterilized condition of the system and then went under OCV measurement. At the same time, the catholyte was aerated at 0.5 $gr.L^{-1}.min^{-1}$, controlled by Dohar technology flowmeter.

MDC mode: after stabilization of OCV, the buffer solution at the middle chamber was replaced by 70 mL of 26 gr/L NaCl solution, the anolyte was recharged, and the catholyte solution was replaced by 100mM autoclaved buffer solution under a sterilized microbial hood. The OCV measurement of MDC and desalination rate was performed during the cell running.

3. Results and discussion

3.1 The effect of Co-culture system in MFC mode

S. oneidensis enhances transferring electrons to electron acceptors outside the cell by secretion of electron shuttles such as FMN and Flavin. Researches on removing electron shuttles showed a decline in electrode-assisted electron transfer [14], [15]. Studying the monoculture of *S. oneidensis* and *E. coli* in contribution to the mutualistic interaction of *S. oneidensis* and *E. coli* elucidate that the highest flavin secretion occurs in co-culture condition, which enhances the current generation [16]. On the other hand, *E. coli* can scavenge leaked oxygen into the anolyte, which maintains the anaerobic condition of the anode chamber and enhances the electrochemical behavior of the cell. [17], Runs Sh, Ec and Sh-Ec regarding *S. oneidensis* and *E. coli* pure culture and co-culture of *S. oneidensis-E. coli*, respectively. Sodium lactate (4gr/L) and sodium acetate (4gr/L) were used as the sole electron donor for *S. oneidensis* and *E. coli* pure cultures respectively. A combination of sodium lactate and sodium acetate (each 4 gr/L) was utilized for the co-culture inocula of *S.*

oneidensis-E. coli. Figure 1 represent the voltage-time curve of MFC phase of these runs. Despite the pure culture of *E. coli* and *S. oneidensis* showing a decrease in OCV after 2400 and 1400 minutes, respectively, the co-culture of *S. oneidensis- E. coli* represented the higher and more stabilized voltage. Also, the higher OCV of 500±9 for co-culture inocula was obtained compared to 327±6 and 420±21 mV for the pure culture of *S. oneidensis* and *E. coli*.

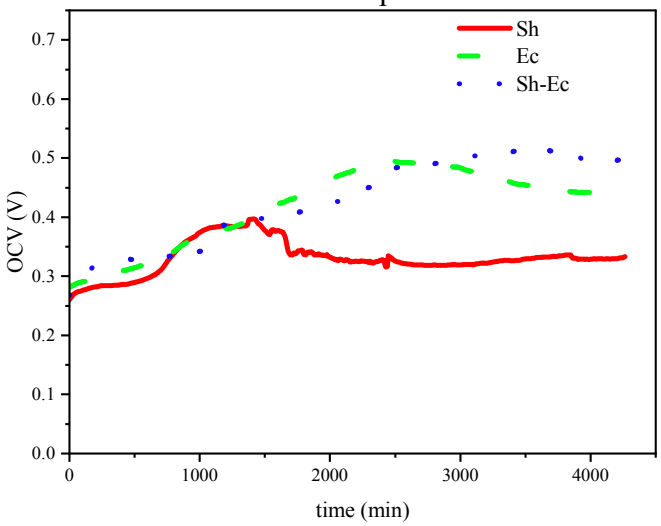

Figure 1: voltage-time curve of Pure culture of *S. oneidensis* and *E. coli*, and co-culture of *S.oneidensis-E. coli* in MFC phase

3.2 Effect of co-cultured inoculum in MDC mode

The mutual interaction of mono- and co-culture of *S. oneidensis* and *E. coli* on desalination efficiency have been evaluated. The OCV-time curves of these systems are presented in Figure 2. Co-culture of *S. oneidensis-E. coli* (Sh-Ec) shows the highest stabilized OCV of 490±19 mV compared to the pure culture of *E. coli*, which presented an OCV of 447±9 mV. The pure culture of *S. oneidensis* presented a significant increase in OCV after switching to MDC mode, reaching OCV of 553±9 mV. Although the OCV of the *S. oneidensis* pure culture was higher than that of the co-cultured and pure culture of *E. coli*, respectively, the desalination percentage of 52.38% was observed, and the concentration of dissolved salt ions in the middle chamber during the desalination process is presented in Figure 3. The desalination percentage of 58.78 and 56.77 was observed for the pure culture of *E. coli* and co-cultured system, respectively. As *Wang et al.* reported before [16], co-culture inoculum produces more current than pure culture inoculum, and hydrogen and formate are the primary metabolites of the co-cultured system; we assume this phenomenon is the reason for higher desalination efficiency in the co-culture of *S. oneidensis-E. coli* inoculum.

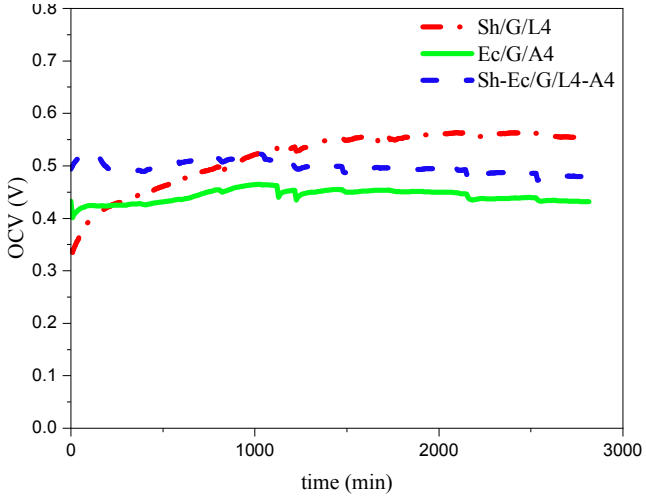

Figure 2: voltage-time curve of Pure culture of *S. oneidensis* and *E. coli*, and co-culture of *S.oneidensis-E. coli* in MDC phase

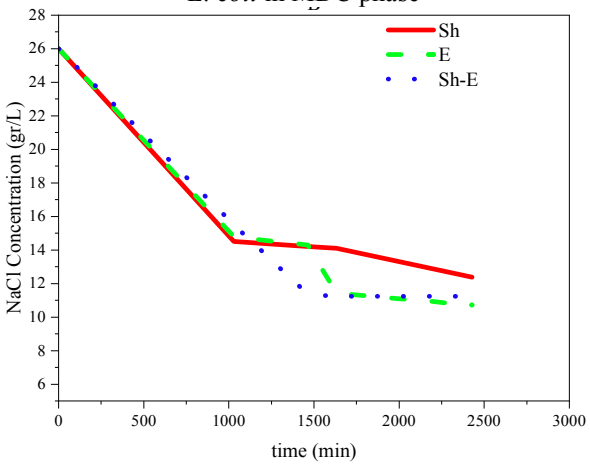

Figure 3: Desalination rate of pure culture of *S. oneidensis* and *E. coli* and co-culture of *S. oneidensis-E. coli*

4. Conclusion

Utilization of mono- and co-cultured inocula of *S. oneidensis* in MDC system, enhanced OCV of the system compared to mixed cultured systems investigated in other studies. Considering the equal volume of the system chambers, desalting capacity of the mono- and co-cultured systems investigated in this study showed a significant improvement compared to other studies utilizing equal chambered system inoculated with mixed cultures. These results demonstrate the importance of microbial biocatalyst composition to achieve higher yields of MDC technology.

5. References

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