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PREPARATION OF LYSOZYME ENZYME COATED WITH CHITOSAN NANOPARTICLES

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ABSTRACT

In this study, the lysozyme enzyme was encapsulated with chitosan nanoparticles by adopting the ionic gelation technique, where samples were prepared from different concentrations of lysozyme and chitosan nanoparticles. With the presence of triphosphate polyphosphate (TPP). Lys-CS-NPs particle sizes ranged between 30-105 nm for the treatment A3 and a rate of 68.7 nm and for the A4 treatment between 30-90 nanometers and a rate of 67.79 nm, meaning that they were within the nanometer, and their Zeta potential reached +50.41 and +47.55 mV for the two treatments respectively. That is, it was of good stability. SEM microscope images showed that the liquid particles were of uniform swollen spherical shapes for the two parameters and their sizes were larger than the freeze particles, which had a solid structure and a spherical, orderly and symmetrical shape, but the particles in treatment A3 were more organized and coordinated than the treatment A4. The AFM images showed the surface shape of the samples for the two parameters A3 and A4, which were divided into three regions according to color, a region with white spherical shapes representing chitosan particles, and a small white point area representing chitosan nanoparticles, while the third region appeared in the dark golden brown color and in small, inconsistent shapes. Size They represent Lys-CS-NPs .The enzymatic activity of Lys-CS-NPs was assessed to see if the chitosan-coated lysozyme still retained its efficacy after release by comparing its enzymatic activity with the activity of the non-coated enzyme, after keeping the enzymes by cooling for 48 hours to allow it to be released from particles showed high efficacy. Compared with uncoated lysozymes, treatment A3 reached 1.657 units / ml, and for treatment A4 1.480 units / ml, while the activity of the uncoated enzyme was 1.159 units / ml.

Keywords: lysozyme enzyme, chitosan, nanoparticles.

Introduction

Lysozyme is a small monomers protein. It's a glycoside hydrolase composed of 129 amino acid residues with a molecular weight of 14.3 kDa, that carries a net positive charge to it within a wide range of pH (Jing *et al*., 2016; Jing *et al*., 2016; Jiménez *et al*., 2013) and The enzyme lysozyme belongs to the hydrolases that carry the classification number (EC3.2.17) which is B-1-4 N-acetyl muramidase (Mine & Lauriau, 2004) It's an enzyme that is stable over a wide range of pH, and temperature (Losso *et al*., 2000). Lysozyme has anti-bacterial properties , and it is of a widespread nature found or excreted in both plants and animals and participates in the defense against bacteria as well as anti-inflammatory, anti-cancer and analgesic properties (Ercan and Demirci, 2016) many of studies have indicated that the enzyme lysozyme is a Peptidoglycan N -acetylmuramoyl hydrolases which is also called Muramidases. analyses the cellular walls of bacteria (Araki *et al*., 2003; Coma, 2008; Primo *et al*., 2018).

Chitosan is a multi-sugar and is a vital polymer derived from chitin after removing the acetyl group, it carries a positive charge because it contains the amine group and is composed of -4-1-4 N-acetyl glucose amin, glucosamine units, which is a white inelastic solid non-flexible sugar (Badawy & Rabea, 2011). Chitosan is one of the suitable polymers to form edible and biodegradable films and wrappers and possesses good mechanical properties as well as acting as antimicrobial of various types of microorganisms and prolongs the life of foodstuffs (Pascall and Lin, 2013) it's a β-1-4 bond that connects β-1-4 Chitin monomer, 2- amino2-deoxy -B-D-glucopyranase (Park *et al*., 2011; Puvvada, 2012). Nanotechnology systems have the ability to improve food quality and one of the most important aspects that nanotechnology can cover are nano additives (Jeon, 2016), smart nanostructured packaging, control and transport of nutrient compounds (Bhushani *et al*., 2014; Ghaani *et al*., 2016; Ghaani *et al*. & Farris, 2016).

Ionotropic gelation : This technique was first reported by Calvo *et al*. (1997) and has been widely examined and developed. The method utilizes the electrostatic interaction between the amine group of chitosan and a negatively charged group of polyanion such as tripolyphosphate. Chitosan can be dissolved in acetic acid in the absence or presence of the stabilizing. agents, such as polymer. Poly anion was then added, and nanoparticles were formed spontaneously under mechanical stirring at room temperature. The size and surface charge of particles can be modified by changing the ratio of chitosan to the stabilizer. A general increase in particle compactness and size was observed on increasing the chitosan concentration and on increasing the polymer to poly anion ratio (Jonassen *et al*., 2012).

Materials and Methods

Materials

Lysozyme enzyme (from Hen egg white) it's activity 100.00 unit/mg and Molecular weight of lysozyme enzyme of 14.600 Dalton and attended prepared by HIMEDIA CO., India. Chitosan nanoparticle with a molecular weight of 161.2 KDa, with a percentage of the degree of deacetylation was about 95.7% and sodium tri poly phosphate TPP were purchased from supplied by Sagherb CO, China.

Preparation of lysozyme enzyme coated with chitosan nanoparticles

Prepare lysozyme solution : The molecular weight lysozyme solution attended 14,600 Dalton and 100,000 unit / mg activity in three different concentrations, dissolving (0.01, 0.04 mg) of the enzyme/1 ml distilled water Each separately at room temperature.

Prepare chitosan solution : The use of nanoparticle chitosan with a molecular weight of 161.2 KDa and with a percentage of removed acetic groups 95.7%, Prepare chitosan solution in three different concentrations by dissolving 0.025 and 0.15 mg of chitosan nanoparticles in 1 ml of aqueous solution of dilute acetic acid with a concentration of (1%), and adjust the pH to 6.7 using sodium hydroxide 1 standard.

Preparation of the aqueous solution of sodium triphosphate : Prepare by dissolving 0.15 mg of sodium triphosphate in 1 ml of distilled water.

Method : The method described by Deng *et al*. (2006) for the preparation of lysozyme coated with chitosan nanoparticles using different concentrations of lysozyme and chitosan nanoparticles. Add lysozyme enzyme prepared in paragraph (1-2-1) in the form of drops through a burette (volume of 25 ml) to the chitosan solution prepared in paragraph (1-2-2) and placed in a glass container quickly and stirred continuously using a magnetic mixing device, then add 2 ml of trisodium phosphate in drops to 5 ml of a mixture of chitosan with lysozyme, with continuous stirring at room temperature, then the samples were exposed to 400 ultrasound apparatus resulting in an ice bath for 6 minutes to obtain small particles.

Preparation of dried lysozyme enzyme coated chitosan nanoparticles

To obtain dehydrated nanoparticles, use the Lyophilizer dehydration apparatus, in which the solution of the separate nanoparticles prepared in paragraph (2-1) is exposed to -60 °C, Then the lyophilized particles were preserved with petroleum dishes after being coated with a polyethylene layer and an aluminum foil layer at a temperature of 4 °C.

Estimating the size of lysozyme enzyme particles coated with chitosan nanoparticles using an atomic force microscopy (AFM)

The size of the lysozyme enzyme coated with chitosan nanoparticles was estimated for all parameters by means of the atomic microscopy technique, which clarified the size and dimension of the nanoparticles using the Grobelny *et al*. (2010) method, as a sample of the sample prepared in paragraph (2-1) was set into a device tube and measured. The size of the molecules formed.

Determination of the shape of lysozyme particles coated with chitosan, lyophilized nanoparticles using a SAM scanning electron microscope

The sample was prepared by drying 7 ml of the solution prepared in paragraph (2-1) By spreading the sample on a slide and placing this slide in the dryer, then the form of lysozyme particles coated with chitosan nanoparticles prepared and dried using the SEM scanning electron microscope was detected, as the tests were carried out in the Islamic Republic of Iran (CAC Center for Chemical Tests) and the models were developed. Lyophilized on a slice of pure aluminum, and the slice was then examined in a SEM microscope by placing the samples inside the microscope chamber and a flow of electrons of LEO (1450vP / 20kv) was projected into different areas of the sample.

Determination of the Formation of Lysozyme Enzyme Coated Liquid Nanoparticles Using Atomic Force Microscopy

The shape of the lysozyme enzyme coated with chitosan nanoparticles was detected using its liquid solutions using the AFM atomic force. Liquid samples were prepared by taking a drop of the lysozyme-chitosan nanoparticle Ly-CS-NP solution prepared in paragraph (2-1) on the slide assigned to examine the shape The surface of the sample particles and the different places of the sample with an image area estimated at nm2482, 2484 nm, and with a magnification strength of px469,49.

Zeta potential measurement

The Zeta Potential was estimated for the transactions using the Zeta Plus device as a sample was taken from the sample prepared in paragraph (2-1) and placed in a tube for the device and the Zeta potential was measured for each sample.

Measurement of enzyme activity of lysozyme enzyme coated by chitosan nanoparticles

Phosphate buffer solution : Bring a potassium phosphate buffer solution at a concentration of 0.06M with a pH of 6.24.

Stuck of bacteria cells : To Prepareation of solution dissolve 1.5 mg of *Micrococcus lysodeikticus*, supplied by Sigma Chemical CO. In 10 mL of the potassium phosphate buffer solution prepared in paragraph (2-7-1) at room temperature and stir the suspension well before use.

Method for measuring the enzymatic potency of lysozyme enzyme coated with chitosan nanoparticles : The Jolles Jollès, & Jollès (1961). method was adopted to measure the enzymatic efficacy of the lysozyme enzyme particles coated with the prepared chitosan nanoparticles, The activity was estimated by the Turbidmetric method determined by Sigma Chemical Co. the reaction mixture was prepared by adding 2.5 mL of the suspended bacteria solution *M. lysodeikitcus* and prepared simultaneously to 0.1 mL of the lysozyme solution, and mixed well by shaking, then the optical absorption values were recorded at a wavelength of 450 nanometers per 30 seconds and for 4 minutes using the optical spectrometer Spectrophotometer, equipped by Bio Engineering Mangment. The enzymatic activity was measured at different times immediately after preparing the nanoparticle solution and after the passage of 24 and 48

hours, then the enzymatic activity was calculated using the following equation:

$$\text{Unit/ml} = \frac{\Delta A450\,\text{nm/min}}{0.001 \times \text{Reaction mixture} \times \text{time(min)}}$$

Unit/ml: The unit of enzyme activity as the amount of the enzyme that causes a decrease in photosynthesis by 0.001 per minute along a wavelength of 450 nanometers and at a temperature of 37 °C by using *Micrococcus lysodeiticus* cells as a base material under reaction conditions.

Results and Discussion

Preparation of lysozyme enzyme coated chitosan nanoparticles

Lysozyme enzyme nanoparticles were prepared according to the Ionic gelation method as described by (Deng *et al*., 2006), using different concentrations of lysozyme enzyme and chitosan nanoparticles. On lyophilized samples, the diagnostic process for Ly-CS-NP coated lysosomal enzyme Ly-CS-NP is performed.

Many studies followed the same method of preparation with the aim of preparing different packages. In a study conducted by Duan *et al*. (2007), this method was used to prepare wrappers for packaging mozzarella cheese to prolong its preservation period, and the method was used in packaging boiled eggs to enhance microbial safety and prolong the period of egg preservation. (Kim *et al*., 2008), also in a study conducted by Zhang *et al*. (2020), the lysozyme solution loaded with nano chitosan was enhanced with cellulose to improve the antibacterial properties of this solution.

Diagnosis of lysozyme coated chitosan nanoparticles the size of nanoparticles

The size of Ly-CS-NP coated lysozyme particles was measured with ion gel using an AFM device. Particle size ratios in the samples ranged, which is represented by A3 (0.01 mg lysozyme / 1 ml 1% acetic acid + 0.15 mg nano-chitosan / 1 ml 1% acetic acid) and A4 (0.04 lysozyme / 1 ml distilled water + 0.025 mg nano-chitosan / 1 ml 1% acetic acid) 24 hours after preparation. . The results are shown in the table (1) The particles of treatment A4 whose sizes were (30 - 90 nm), then the particles of treatment A3 where their sizes were (30-105 nm). The reason for obtaining these small sizes is to expose the nanoscale solutions prepared for the mixing and mechanical homogenization process and to expose them to high sound waves (Sonication) at specific times of time in 6 separate minutes, as these sound waves break down the nanoparticles into smaller sizes and enhance the enzyme encapsulation process further. Therefore, the results indicated that nanoparticles with sizes less than 100 nm were formed, and an ice bath was used in the mechanical homogenization process to maintain the enzyme activity.

Table 1 : The size of lysosome enzyme particles coated with chitosan nanoparticles prepared by Ionic gelation method measured in nanometers using different concentrations of lysosome and chitosan nanoparticles.

Samples	Sample concentrations		The size of the nanoparticles nm	average size of the nanoparticles nm
	Mg lysozyme / ml distill water	Nano-chitosan mg / ml acetic acid		
A3	0.01	0.15	30-105	68.7
A4	0.04	0.025	30-90	67.79

A study of the properties of lysozyme enzyme coated chitosan nanoparticles

Study the relationship between size and diameter of nanoparticles : To find out the relationship between the percentage of the nanoparticle size to its diameter, use the AFM (Atomic Force Microscopy) device. It ranged between 30 to 105 nanometers and the average size of the nanoparticles was about 68.70 nanometers. In the treatment A4, the size of the particles ranged between 30 to 95 nanometers and the average diameter of the nanoparticles formed was 67.79 nanometers in the treatment A3. These sizes are close to the particle sizes obtained by Lee *et al*. (2018), which were with an average size between (64.34 ± 2.1) and (147.93 ± 2.9) nm for chitosan particles loaded with N-CS nanoparticles, while the sizes we obtained were less compared to the sizes. That Wu *et al*. (2016) found an increase in the size of chitosan nanoparticles when loaded with lysozyme enzyme, rising from 488 nm to 613 nm, and the sizes that we found in this study are also less compared to the sizes obtained by *et al*. Wang (2020) when preparing nanoparticles by incorporating lysozymes into chitosan (CS-NPs) by ionic coagulation technique to obtain lysozyme fixed carbon nanoparticles (Lys-CS-NPs), as the size of the nanoparticles reached (243.1 ± 2.1 nm), Also, the sizes we obtained were less than the sizes reached by (Deng *et al*., 2006), who stated that the average sizes of lysozyme coated with chitosan nanoparticles ranged between 50 - 280 nm, which is also less than the sizes obtained by Piras *et al*. (2014) When preparing LZ-loaded nanoparticles (LZ-NPs) by ionotropic gelation, they were 150 nm in diameter.

Diameter (nm)<	Volume (%)	Cumulation (%)	Diameter (nm)<	Volume (%)	Cumulation (%)	Diameter (nm)<	Volume (%)	Cumulation (%)
30.00	3.52	3.52	65.00	5.42	46.88	100.00	4.61	81.57
35.00	5.96	9.49	70.00	6.23	53.12	105.00	4.07	85.64
40.00	5.15	14.63	75.00	6.78	59.89			
45.00	7.59	22.22	80.00	5.42	65.31			
50.00	5.69	27.91	85.00	3.52	68.83			
55.00	7.05	34.96	90.00	4.61	73.44			
60.00	6.50	41.46	95.00	3.52	76.96			

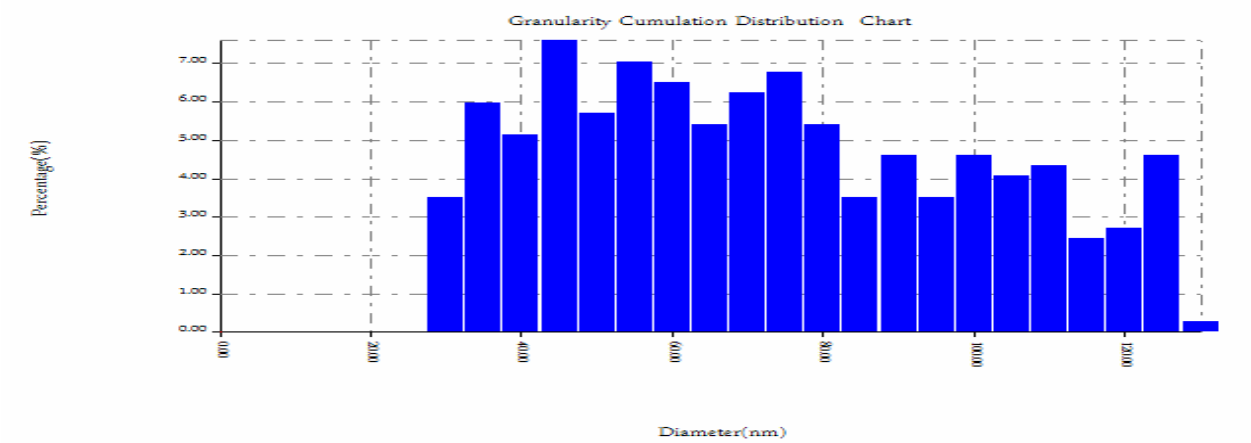


Fig. (1): A graph showing the relationship between the percentage of the size of the nanoparticles to their diameter in nanometers for the treatment A3 (consisting of 0.01 mg lysozyme + 0.15 mg nano chitosan) using the AFM device (the samples used for the measurement were dried on the surface of the aluminum foil that is part From the AFM

Diameter (nm)<	Volume (%)	Cumulation (%)	Diameter (nm)<	Volume (%)	Cumulation (%)	Diameter (nm)<	Volume (%)	Cumulation (%)
30.00	1.61	1.61	55.00	5.65	18.55	80.00	12.90	76.61
35.00	0.81	2.42	60.00	11.29	29.84	85.00	9.68	86.29
40.00	1.61	4.03	65.00	13.71	43.55	90.00	6.45	92.74
45.00	4.84	8.87	70.00	7.26	50.81	95.00	7.26	100.00
50.00	4.03	12.90	75.00	12.90	63.71			

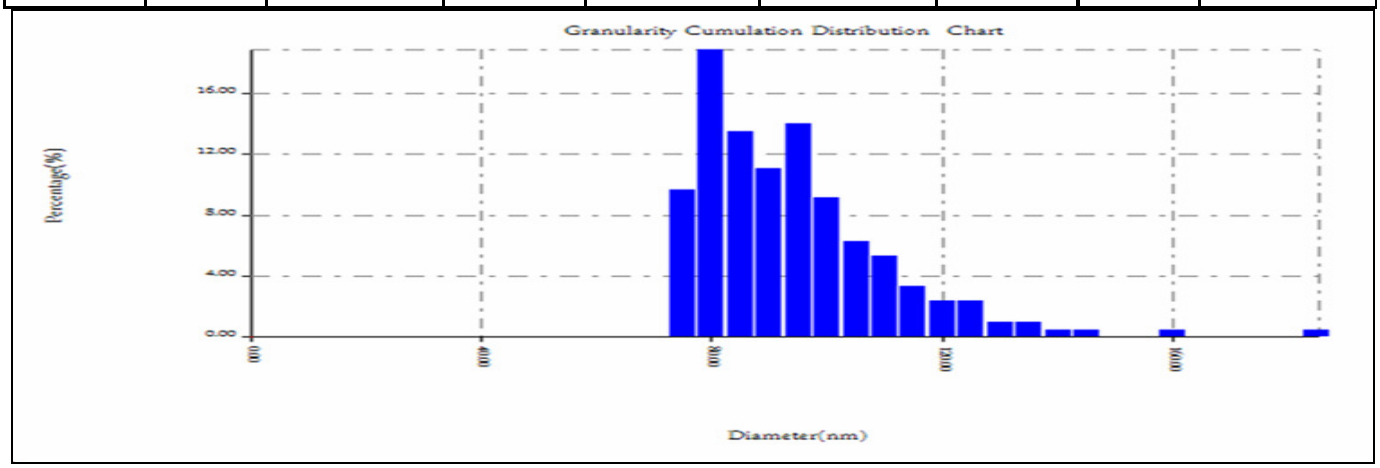


Fig. (2): A graph showing the relationship between the percentage of nanoparticle size to its nanometer diameter in nanometers for treatment A4 (consisting of 0.04 mg lysozyme + 0.025 mg nano chitosan) using an AFM device (the samples used for the measurement were dried on the surface of the aluminum foil which is Part of the AFM).

Determination of the shape of the nanoparticles:

Determining the shape of the nanoparticles using a scanning electron microscope:

The shape of the lysozyme enzyme particles coated with liquid and lyophilized nanoparticles was investigated using a Scanning Electron microscope (SEM), as Figure (3) shows that the shapes of LNP nanoparticles differ with each other with the different state in which they are freeze-dried or liquid. In the case of the lyophilized particles, the particles were of a solid and consistent structure in the two parameters A3 and A4 (Fig. 3 a and b) and appeared spherical, orderly and symmetrically, but we note that the particles in the sample A3 were more organized and coordinated than in the sample A4. The reason for this may be due to The difference in the concentrations used for each of the nano chitosan and the lysosome enzyme, these results are in line with the findings of Janes & Alonso (2003) when using nano chitosan in the encapsulation of peptides and transporting them inside the vivo. The nanoparticles increased in size and were of a uniform and symmetrical shape.

Also, the particles in the lyophilized samples were of smaller dimensions, as the sodium triphosphate (TPP) material used in the preparation of lysozyme-loaded nanoparticles has a role in the formation and formation of spherical nanoparticles as a result of its association with chitosan and glutamic acid, forming a network of regular nanoparticles with dimensions ranging from 50 - 100 nm (Liu *et al*., 2013), and these results are in line with the results obtained by Lee *et al*. (2018) for chitosan loaded with N-CS nanoparticles, as the composite nanoparticles were spherical and homogeneous.

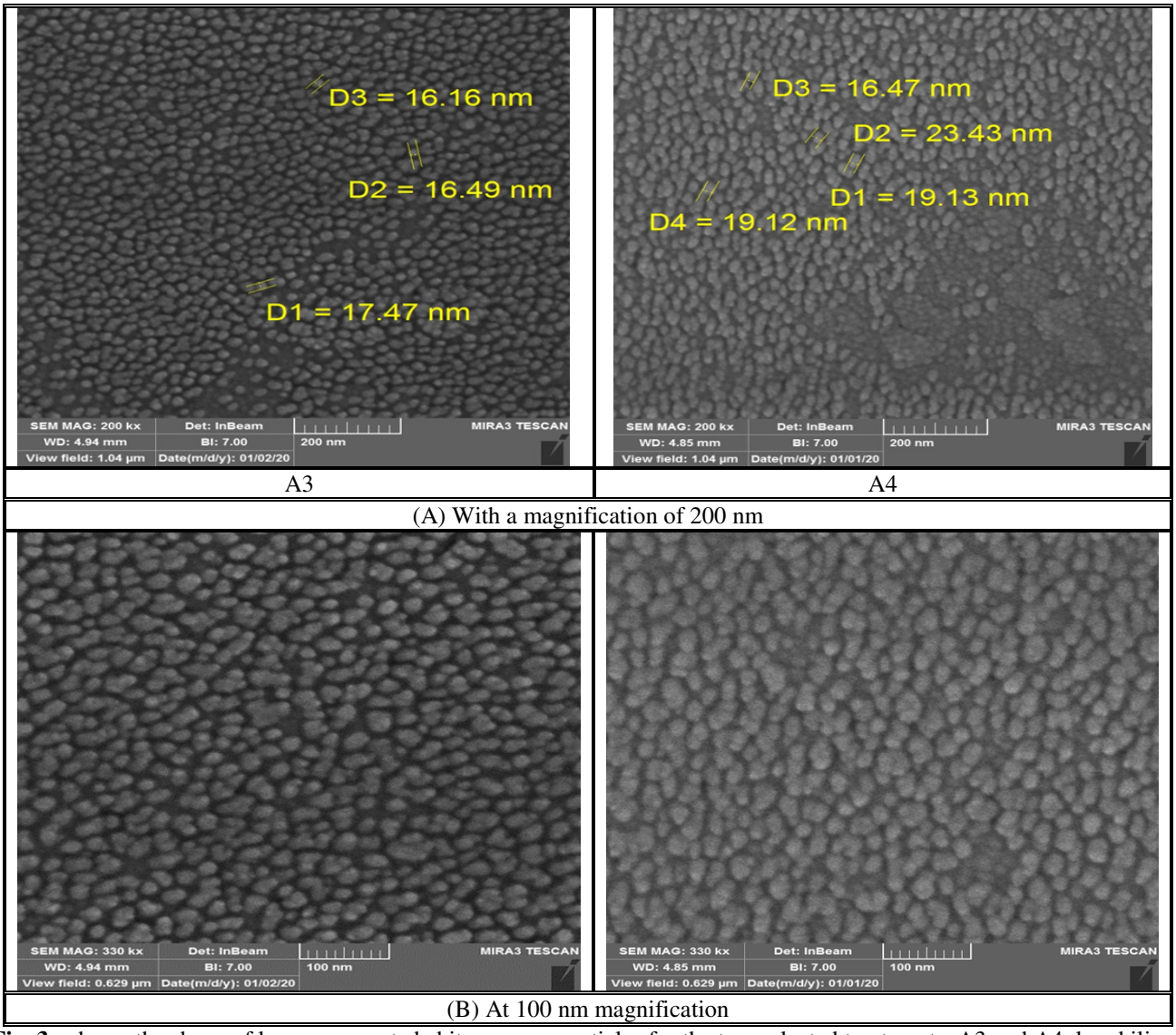


Fig. 3 : shows the shape of lysozyme coated chitosan nanoparticles for the two selected treatments A3 and A4, lyophilized using a SEM scanning electron microscope with a magnification of 100 and 200 nm.

The SEM images showed that the samples of lysozyme enzyme particles coated with liquid chitosan nanoparticles were of different shapes and sizes from their lyophilized counterparts and in both the selected treatments A3 and A4, as shown in Figure (4) when using a magnification force of 1 micrometer, as the shapes of the lysosome enzyme particles coated with chitosan were Bulky spherical liquid nanoparticles are regular and larger sizes for samples A3 and A4.

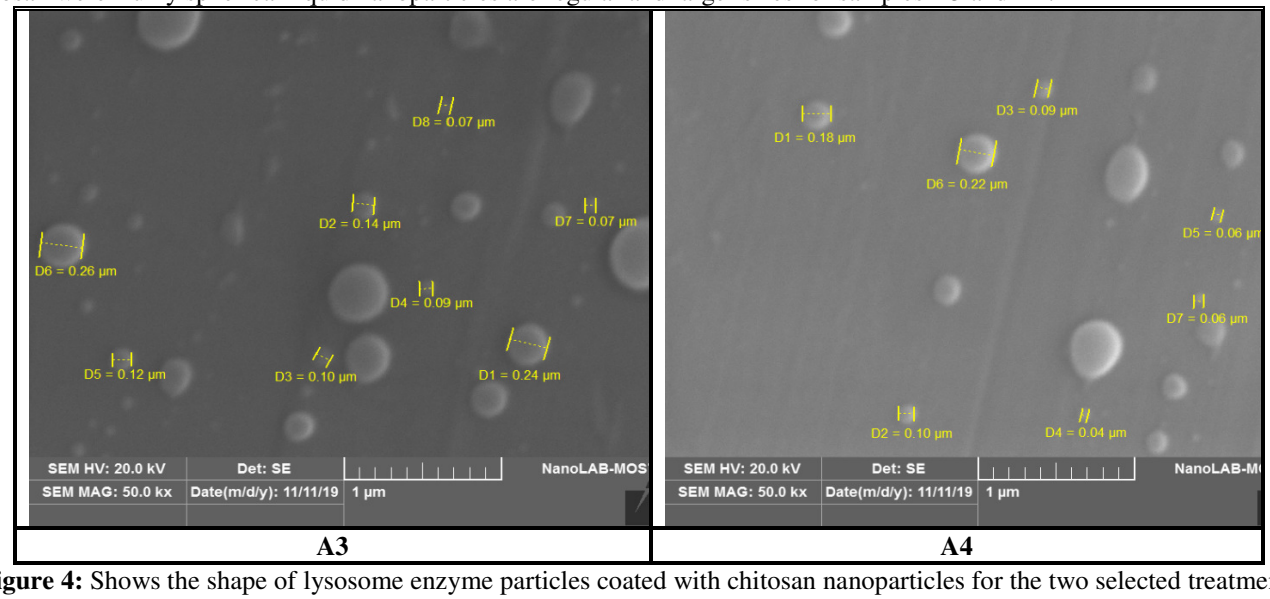
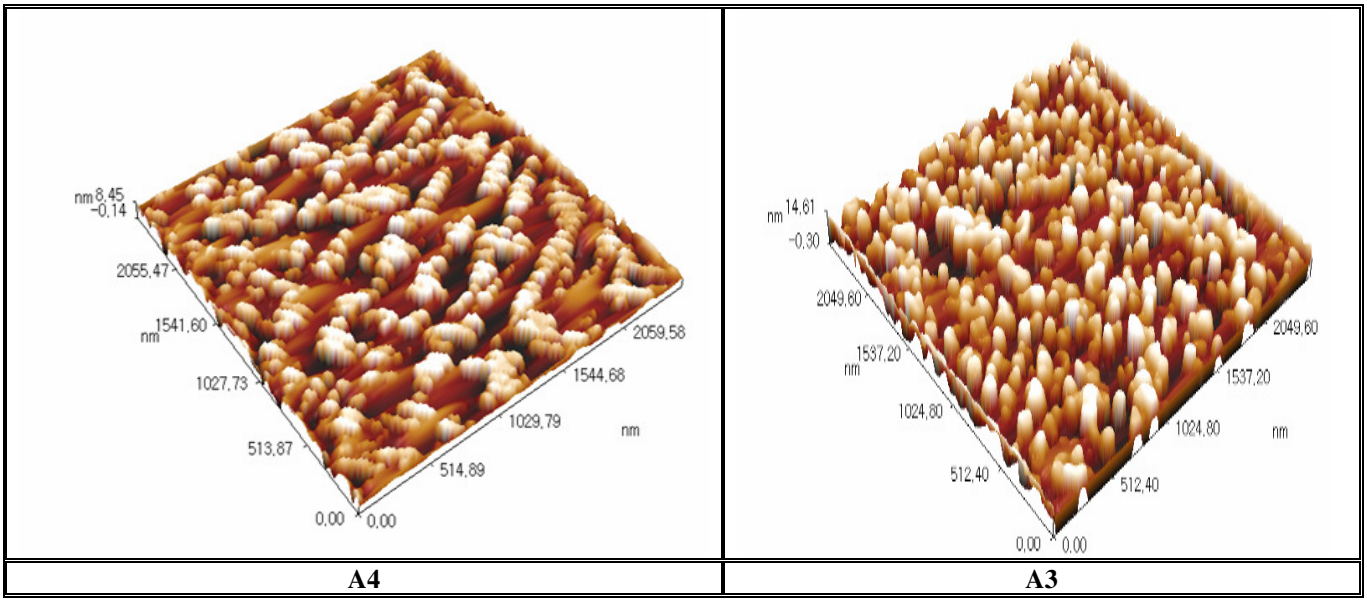


Figure 4: Shows the shape of lysosome enzyme particles coated with chitosan nanoparticles for the two selected treatments A3 and liquid A4 using a SEM scanning electron microscope with a magnification power of 1 mμ

Determine the shape of nanoparticles using atomic force microscopy : Atomic Force Microscopy (AFM) was used to scan the surface of the samples of lysosomal enzyme particles coated with chitosan nanoparticles. White spherical shapes appear to represent the SC chitosan particles, while the chitosan nanoparticles CS-NPs appear in the form of small white dots, while the Ly-CS-NPs of chitosan appear in dark brown and gold with small, inconsistent shapes.

Also in the figure, the size of the CS-NPs was smaller than the size of Ly-CS-NPs, which resulted from the incorporation of lysozyme Ly into the chitosan nanoparticles. Nanoparticles are effective in determining the particle size (Wu *et al*., 2017). Moreover, the difference in the distribution of nanoparticles per unit volume can be because the pH can affect the protein of the CS amino groups.

The decrease in the size of the nanoparticles is attributed to the strength of the electrostatic bonding that results between the amines group in the nano-encapsulated lysosome chitosan and the TPP as a result of their different charges, so the TPP material binds to chitosan by means of Ionic gelation technology and when a high-speed centrifugation process and exposing the particles Sonication to create nanoparticles with dimensions smaller than 100 nanometers.



A4 **A3**

Fig. 5 : Illustrates the shape of lysosome coated chitosan nanoparticles of the two selected parameters A3 and A4 using atomic force microscopy AFM.

Zeta potential measurement : The zeta potential gives an indication of the degree of aggregation of colloidal particles due to the electrical gravity resulting from the charges on the surface of these particles, and the amount of the zeta potential indicates the degree of electrical repulsion between the adjacent and similarly charged particles in the dispersion medium (Dounighi *et al*., 2012), when the zeta potential is high The formed particles are more stable, that is, the solution or the dispersion medium will resist the aggregation of these particles, but when the value of the zeta potential is low, the attractive forces may exceed this repulsion and this dispersion may be refracted, so colloids with a high oil voltage (negative or positive) are fixed. Electrically while colloids with low oil voltage tend to aggregate (Hanaor *et al*., 2012).

Figures (6) and (7) show that the zeta potential value of the parameters A3 and A4 was 50.41 + and + 47.55 mV, respectively. The positive charges appearing on the surface of the nanoparticles are due to the amine groups in chitosan, according to Singh and Lillard Jr, (2009) that nanoparticles with an zeta potential above ± 30 mV are stable in the suspension, while the surface charge reduces their aggregation (Singh and Lillard Jr, 2009).

The similar charges cause the particles to repel each other, while the different charges cause the particles to be attracted to each other, and the higher the zeta potential value, the more repulsion with it, and thus the particle pool in the suspension is less.

The zeta potential must be higher than 30 mV or less than -30 mV to maintain the stability of the nanoparticles in the suspension and to have sufficient repulsion force to prevent them from aggregating (Couvreur *et al*., 2002).

These results are similar to those of (Lee *et al*. 2018) who mentioned that the value of zeta potential of N-CS-loaded chitosan and nano chitosan nanoparticles were 49.3 + and 33.4 + mV, respectively, and similar results were obtained before. Khan *et al*. (2017), where the values of zeta potential 48.34 + and 39.4 mV for chitosan, nisin , and chitosan nanoparticles loaded with N-CS, respectively, also agree with the results with the results of (Agarwal *et al*., 2015). Zeta of chitosan nanoparticles with limits of 50+ mV. The appearance of positive charges explained the presence of amino groups on the surface of chitosan (Alishahi, 2014; Dorkoosh *et al*., 2002).

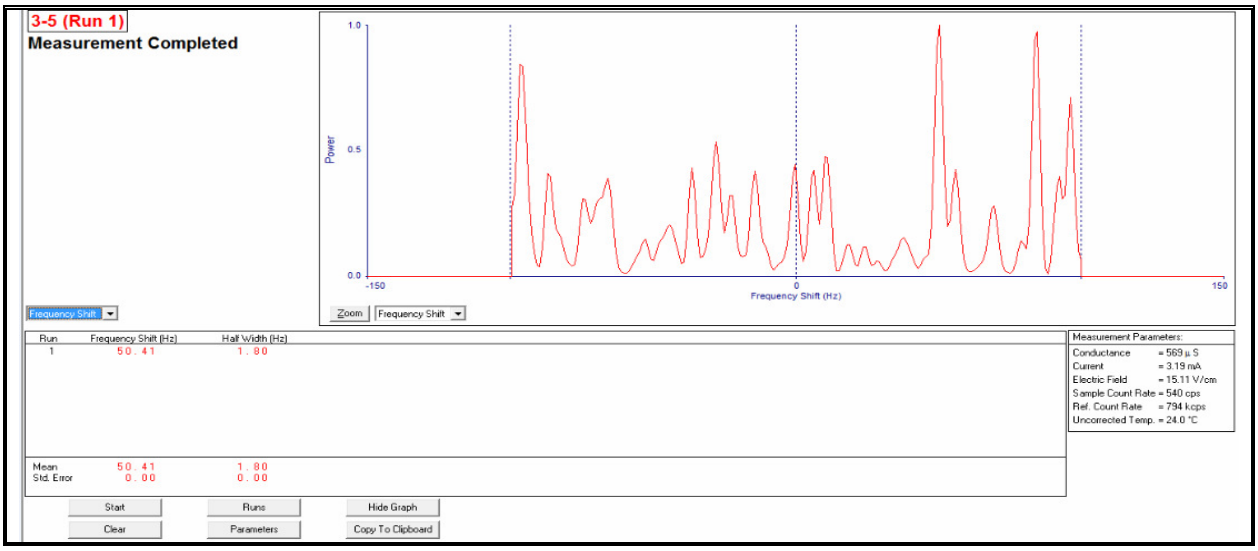


Fig. 6 : Indicates the Zeta Potential (mV) for transaction A3, measured with the Zeta Potential Analyzer

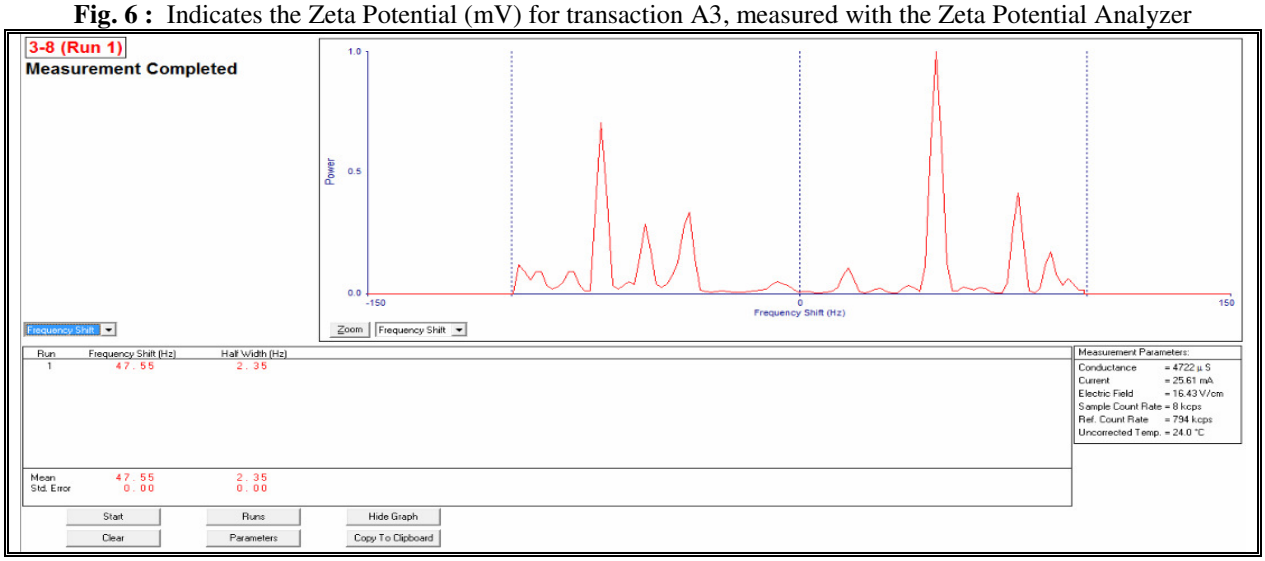


Fig. 7 : Shows the value of the zeta potential (mV) for transaction A4 measured by Zeta Potential Analyzer

Estimation of enzymatic activity

It is important for lysozyme to maintain its enzymatic activity after release from CS-NP, and to ensure that the activity of the lysozyme enzyme after release from chitosan nanoparticles (CS-NP) encapsulated for this enzyme has a similar activity to that of pure or free lysosome enzyme. The solution containing lysozyme enzyme particles coated with chitosan nanoparticles for the two treatments A3 and A4 at a temperature of 7 °C (in the refrigerator) for 48 hours, and compared with the activity of the free, uncoated enzyme, the readings showed that the activity of the free, uncoated enzyme reached 1.157 units / ml after 48 hours after If it was 1.075 units/ml when estimated before preservation, and when encapsulating the enzyme with Chitosan, it was noticed that this process enhanced the enzyme's activity, so the activity of the lysozyme enzyme increased from 0.480 units/ml when it was inside chitosan nanoparticles to 1.657 units/ml after it was released from the particles in the treatmentA3 ,And In treatment A4, the enzymatic activity increased from 0.442 to 1.480 units/ml after 48 hours (Table 8), indicating that the activity of the enzyme released from nanoparticles was good. At the same time, it was higher than the free enzyme activity when compared. This increase in the activity of the lysozyme enzyme released from nanoparticles is due to the presence of chitosan, as the lysozyme enzyme analyzes chitosan and makes use of it. As the results of the statistical analysis indicated that there were significant statistical differences at the level of probability ($P \leq 0.05$) between the enzymatic activity of the two treatments A3 and A4, respectively, compared with the results of the statistical analysis of the free enzyme A, where the results indicated that there were no statistical differences during a period from after preparation to 48 hours, while There were significant statistical differences in the enzymatic activity of the two treatments A3 and A4 at the time of preparation compared to the free enzyme not encapsulated, and there were no significant statistical differences on the probability level ($P \leq 0.05$) between the two treatments A3 and A4 after 48 hours of preparation, as well as compared to the free enzyme.

The results regarding the activity of the lysozyme enzyme that we obtained in this study were similar to the results obtained by Wu *et al*. (2018). When encapsulating the lysozyme enzyme with chitosan and genes, the lysozyme enzyme, which was loaded with chitosan and genes, showed a higher efficacy rate than the effectiveness of free lysozyme, which was 87.72 ± 3.96%, but it differs from the results of the study conducted by Pérez De & Griebenow (2002), which stated that the lysozyme enzyme lost a lot. Of its activity when it is encapsulated in micro-lactic-glycolic granules**.**

Table 8 : Estimation of the enzymatic activity of free lysosome and chitosan coated lysosomes for two treatments A and B

Treatment	Concentrations		Enzymatic activity (units / ml) at a time		LSD
	Mg lysosome enzyme / 1 ml distilled water mg	chitosan nano size / 1 ml 1% acid	0	48 ساعة	
A	-	-	1.075	1.159	0.288 NS
A3	0.01	0.025	0.480	1.657	0.367*
A4	0.04	0.075	0.442	1.480	0.319*
LSD			0.427*	0.353*	-

- The readings represent an average of three replications.
- The lowest significant difference in probability level * (P≤0.05).

The results were also similar to the study of Yanan Wang, 2020, which showed that the process of fixation or restriction of lysosomes on chitosan nanoparticles greatly enhanced the thermal stability of the enzyme and the possibility of re-activity and efficacy of the lysosome enzyme after its release from its enveloping nanoparticles and restricting its action.

Conclusion

One of the most important conclusions reached by this study is the possibility of encapsulating the lysozyme enzyme with nano chitosan using a simple chemical method, which is the ion gelation method.

In this method, the lysozyme enzyme maintained good activity after being covered with chitosan nanoparticles, and this activity increased after the enzyme was released from the particles. It could be used as a biological preservative for dairy products.

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