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Scientific Research

Production and Evaluation of Chitosan-Coated Nanoliposomes for Caffeine Encapsulation

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ABSTRACT

In this study, chitosan-coated nanoliposomes in ratios of 9-1, 8-2, 7-3 and 6-4 lecithin-cholesterol, were prepared using thin-film hydration method, as a practical delivery system for encapsulation of caffeine. Then, the particle size and zeta potential were measured to determine the characteristics of the produced particles. Average particle size (average hydrodynamic diameter) and particle size distribution for different lecithin-cholesterol ratios were in the range of 135.5-533.5 nm and 0.31-0.41, respectively. Zeta potential values were also obtained in the range of +40.96 to +50.5. After determining the encapsulation efficiency, FTIR method was used to investigate possible reactions between caffeine and nanoliposome wall materials. The morphology of chitosome with 9-1 ratio of lecithin-cholesterol loaded with caffeine, was shown by scanning electron microscopy (SEM). The stability of the chitosomal sample with a ratio of 1-9 lecithin-cholesterol was evaluated through visual observation of precipitation formation and calculation of the amount of release of encapsulated caffeine during 60 days of storage at ambient temperature. The results obtained in this research showed that nanochitosomes are an efficient system in maintaining and releasing caffeine.

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1. Introduction

caffeine1)،3 ،5- Trimethylxanthine, ($C_8H_{10}N_4O_2$ It is an alkaloid from the xanthine group and is found in the seeds, leaves or fruits of more than 60 plant species in the world. In its pure and solid form, this substance is a white crystalline powder with a bitter taste, which is soluble in water and some organic solvents such as chloroform and dichloromethane [1]. Caffeine is one of the most common bioactive substances in the world that can increase mental and physical performance, eliminate fatigue and reduce depression [2]. This compound occurs naturally in a number of common beverages such as tea and coffee, and is also commonly added to a wide variety of alcoholic and non-alcoholic beverages, pharmaceuticals, and cosmetics[3]. Consuming caffeine, as the main ingredient of many drinks, has become a social habit and with its uplifting and energizing effects, it has increasingly strengthened its place in our lives [1].

Numerous pharmacological activities, biological and physiological effects have been attributed to caffeine, making this compound the most widely studied stimulant in history [4]. The stimulant effects of caffeine are mainly related to the stimulation of the nervous, muscular and cardiovascular systems [5]. Caffeine metabolites lead to an increase in blood glucose levels, dilation of blood vessels, increase in urine volume and relaxation of muscles in the body. Consuming moderate amounts of caffeine (200 to 300 mg) is associated with increased physical and mental performance, resulting in people becoming more active and energetic. Also, caffeine with moderate doses leads to a decrease in the concentration of adenosine in the brain, which increases the user's attention and alertness [6]. In general, increasing blood circulation and reducing the risk of cardiovascular diseases, anti-inflammatory and anti-cancer effects, inhibiting the increase in body fat and modulating glucose metabolism are some of the pharmacological and biological effects of caffeine consumption [4]. This substance increases nerve stimulation by reducing the threshold of stimulation of neurons, therefore caffeine-containing drugs are used in the treatment of nasal congestion, asthma, headache and weight loss [1]. Also, caffeine is one of the few drugs allowed in sports, and its beneficial effects have been reported in disciplines that require endurance, such as cycling and football [3].

However, higher levels of caffeine consumption significantly cause negative effects such as anxiety, restlessness, and insomnia, which are mainly observed in caffeine-sensitive individuals [7]. Therefore, it is very important to monitor the caffeine content of different beverages. There are also concerns about the consumption of high amounts of caffeine by vulnerable groups such as children or pregnant women [3].

Therefore, considering the bitter taste and side effects of consuming high levels of caffeine, it is necessary to design a system for covering the taste and the possibility of programmable release of this bioactive compound in a long period of time. Microencapsulation technology is one of the best ways to overcome this challenge, this technique is widely used to improve the efficiency of bioactive materials [8]. To date, various studies on microcapsulation of caffeine such as; Caffeine encapsulation in alginate microhydrogels by electrospray method [9], caffeine encapsulation in lactoferrin-glycomacropeptide nanohydrogels [10] and caffeine microencapsulation in polysaccharide-based delivery systems [11] have been done.

In order to achieve a successful microencapsulation system, it is important to choose the appropriate carrier, depending on

the physical and chemical characteristics of the active substance. The carriers used for this purpose should be non-toxic, environmentally friendly and available [8]. Nanocarriers that can be used in food processing can be based on carbohydrates, proteins or lipids. Although many advantages have been attributed to carbohydrate and protein-based nanocarriers, their industrialization is not possible due to the need to use complex chemical or thermal treatments that are not fully controllable. On the other hand, lipid-based nanocarriers have the possibility of industrial production and also have high encapsulation efficiency and low toxicity [12]. Lipid-based nanocarriers in the food industry include nanocapsules, nanoliposomes, nanoemulsions, niosomes, phytosomes, and lipid nanoparticles with solid structures [13]. Nanoliposomes are considered as one of the most common and cost-effective nanocarriers in the food and pharmaceutical sectors, due to advantages such as high surface area, easy preparation methods, cheap raw materials, and better penetration potential [14].

However, low physical stability, degradation of encapsulated materials due to environmental variables (temperature,pH, light, oxygen and ionic strength of the environment) and rapid release of loaded compounds during storage are among the limitations of nanoliposomes in microencapsulation of bioactive compounds [14] and [15]. In order to solve these defects, researchers have focused on modifying the surface of nanoliposomes by polymers. Among all polymers, biopolymers are the most important because of their high biocompatibility. Among the biopolymers used to cover the surface of nanoliposomes, chitosan is a suitable choice since it is widely available and can be used as a layer on the surface of nanoliposomes due to its positive charge. Chitosan reduces the fluidity of the membrane and reduces the accumulation rate of the formed nanostructures [2]. The purpose of this research is to cover the flavor and control the release of caffeine using nanochitosomes.

2- Materials and methods

2-1- Materials

Pure caffeine powder was purchased from Nikoshimi Company and stored in a moisture-free environment at room temperature. Ethanol 96% and acetic acid were obtained from Majalli Company, Tween 80 from Merck, Germany, Lestin and cholesterol, as well as chitosan from Sigma Aldrich, Germany.

2-2- Test methods

1-2-2- Measuring the amount of caffeine obtained

First, to plot the caffeine standard calibration graph, the caffeine stock solution in concentrationppm250 was prepared. For this purpose, the amount of 25 mg of this substance was dissolved in distilled water and brought to a volume of 100 ml. Then, dilutions of 25-200 mg/liter (25-50-100-150 and 200 mg/liter) were prepared from the stock solution. In the following, the amount of absorption of the prepared solutions using a visible spectrophotometerUV- (BEL PHOTONICS) determined at a wavelength of 276 nm and the standard curve of caffeine was drawn ($y = 0.0035x + 0.0112$, $R^2 = 0.9988$) [2].

2-2-2-Preparation of liposomes containing caffeine

From the thin layer hydration method similar to the sealing method et al. (2019), with some modifications, was used to prepare nanochitosomes [16]. For this

purpose, caffeine-containing nanochitosomes were added in 1-9, 2-8, 7-3, and 6-4 lestin-cholesterol weight ratios. Next, different amounts of lestin in 10 ml of ethanol for 2 hours on a magnetic stirrer with a heater (Alpha model)D500 ،Iran) was mixed and kept overnight in the refrigerator to hydrate. Then, the mentioned amounts of cholesterol along with 0.02 grams of tween 80 were added to the hydrated lecithin samples. The resulting mixtures were stirred for complete mixing by a magnetic stirrer with a heater for 30 minutes. Then the final solutions were transferred to a round bottom flask and evaporated using a rotary evaporator (ThModelRV05basic, Germany) at a temperature of 40 °C, a duration of 20 minutes and in a rotating cyclerpm 60 Their solvent was evaporated. After the evaporation of the solvent, a thin layer was formed on the wall of the round-bottomed flask. In order to ensure complete removal of the solvent, the flask was kept overnight in a desiccator (at room temperature). Next, in order to hydrate the dried thin layer at the bottom of the flask, 250 mM sodium acetate buffer(Table 1) Was prepared. The amount of 25 mg of caffeine in 10 ml of prepared buffer was dissolved and added to the dried thin layer. After the complete hydration of the lipid layer under stirring, the prepared liposome solution is used to reduce the particle size to the nano scale by homogenizer.ThModelT25 digital, Germany) was homogenized for 5 minutes at 65 degrees (above Lestein's phase transition temperature) at 20,000 rpm. In the next step, the prepared liposomal solutions were placed in an ultrasonic bath with a frequency of 50 kHz for 15 minutes (45 seconds on and 15 seconds off). Then, the prepared chitosan solution (w/v 1% in acetic acid) was added to the produced nanostructures under constant stirring conditions to obtain chitosan-coated liposomes.

Table 1 Concentration of sodium acetate and acetic acid to prepare sodium acetate buffer (1L,1M)

pH	Acetic acid concentration (gr/l)	Sodium acetate concentration (gr/l)
3.5	5.7	0.72

The prepared nanochitosomes were evaluated in terms of particle size, zeta potential, loading capacity, and stability of chitosomal solutions.

2-2-3- Evaluation of nanochitosomes containing caffeine

2-2-3-1- Particle size, particle size distribution and zeta potential

Average hydrodynamic diameter and particle size distribution index[1](PI) One of the most important characteristics of nanoparticles for evaluating colloidal behavior is their stability and microcoating properties [17]. Dynamic light scattering method(DLS)[2] ، It is usually the preferred method for measuring particle size and size distribution; Because it is relatively easy and fast. With this method, after appropriate dilutions, the diffusion coefficient and hydrodynamic radius for vesicles can be obtained [18].

[1]- Polydispersity Index

[2]- Dynamic Light Scattering

In order to increase the distance between the particles and prevent different scattering effects, the chitosomal sample was diluted 10 times with distilled water and then injected into the capillary tube and in the special part of the device.DLS it placed. Particle size and chitosome zeta potential were determined for all samples at a temperature of 25 degrees Celsius and repeated in three stages.

2-2-3-2- Caffeine loading capacity in nanochitosomes [3] (**LC**)

To determine valuesLC, initially 10 ml of nanochitosomal solution, in order to separate free caffeine (not microencapsulated) for 15 minutes per roundrpm 3000 centrifuged(Hettich universal centrifuge model, Germany).Then, the sedimented cytosomes were separated by medium and their volume was measured. Next, 2 ml of the broth was mixed with 1 ml of chloroform (ratio 2:1) and shaken well for 10 minutes with a vortex machine so that the chitosomal membrane is well destroyed and caffeine is released. Next, the amount of loaded and unloaded (free) caffeine was measured using a spectrophotometer at a wavelength of 276 nm.[19].

Using equation (1), the percentage of caffeine loading was calculated in relation to lipid:

$$active\ material\ loading\ percentage = \frac{The\ liposome\ in\ the\ capsule\ active\ substance\ quantity}{Total\ lipid\ mass} \times 100 \quad (1)$$

[3] -Loading Capacity

2-2-3-3- Stability of caffeine in chitosan-coated nanoliposomes during 60 days of storage

The stability of the microcoating system is one of the most important factors that determine the ability of nanocarriers to maintain the active composition[2]. The amount of free and microencapsulated caffeine (after the destruction of the liposomal wall) during 2 months of storage at ambient temperature and on days 1-15-30-45 and 60 was measured by a spectrophotometer at a wavelength of 276 nm.

2-2-3-4- infrared spectroscopy[4]**(FTIR)**

In order to investigate the possible reactions of caffeine-phospholipid from the infrared spectrometer (FTIR) was used. Scan range in the wavelength rangecm^{-1} 400-4000 for each of the components of the wall, including phosphatidylcholine (lecithin), cholesterol and chitosan, as well as for the samples of caffeine powder, chitosomal solution containing caffeine and chitosomal solution without caffeine with a lecithin-cholesterol ratio of 1-9.

2-2-3-5-[5]**FESEM**

Investigating the surface properties and morphology of nanoparticles with a ratio of lestin-cholesterol of 1-9 by scanning electron microscopy.FEG-SEMModelMIRA3,Check) was made. For this purpose, a drop of chitosomal solution was placed on a laboratory slide and dried at ambient temperature. Next, the dried layer was covered with a gold sheet in order to be observed under an electron microscope by a sputtering device. The structure of nanochitosomes was observed and photographed with a magnification of 50,000[20].

[4]- Fourier-Transform Infrared Spectroscopy

[5]- Field Emission Scanning Electron Microscope

2-3- Data analysis

Statistical analysis of data in the form of a completely random design and comparing their average with Duncan's test(05/0P<)by statistical softwareSAS done. In order to check the stability of caffeine in nanochitosomes during the storage period, the data are repeated in time and their averages are compared through the test.LSD Done. The relevant charts were drawn with Excel 2016 software.

3- The results and findings of the research

3-1- Characterization of caffeine-containing nanochitosomes

3-1-1- Particle size, particle size distribution and zeta potential

Average particle size and size distribution (particle dispersion indexPI) are the most important characteristics of nanoparticles that affect their physical stability, solubility, biological function, diffusion speed and chemical stability [21]. Process parameters such as speed (probe speed of the homogenizer device, speed of mixing and stirring of materials), duration of homogenizer, duration of sonication, type of ultrasound used (bath or ultrasound probe) as well as the amount of active substance added to the formulation and the type of polymer used are among the factors. Effective in determining the size of the particles, the loading amount of the active substance and the regular crystal structure in the nanoparticles are [22]. The results ofDLS For chitosomal samples prepared with different lestin-cholesterol ratios in Table 2(Table2) Is expressed.

Table 2 Particle size, polydispersity Index and zeta potential of chitosome samples containing caffeine prepared with different lecithin-cholesterol ratios

Sample	Zeta potential (mv)	PI	Z-Average (nm)
Chitosomal nanocapsules with a 9-1 lecithin -cholesterol ratio	40.96 ± 0.46[d]	0.419 ± 0.04[a]	133.3 ± 7.27[d]
Chitosomal nanocapsules with a 8-2 lecithin -cholesterol ratio	45.16 ± 1.02[c]	0.360 ± 0.03[ab]	298.3 ± 10.33[c]
Chitosomal nanocapsules with a 7-3 lecithin -cholesterol ratio	48.36 ± 0.55[b]	0.318 ± 0.02[b]	443.6 ± 6.76[b]
Chitosomal nanocapsules with a 6-4 lecithin -cholesterol ratio	50.5 ± 0.3[a]	0.313 ± 0.05[b]	523.6 ± 3.96[a]

.Different letters in same column represent significant differences (P<0.05)

Particle size asZ-Average (average hydrodynamic diameter of particles) which was in the range of 133.5-533.5 nm for different lestin-cholesterol ratios. As can be seen in Table 2, the increase in the amount of cholesterol in the chitosomal structure led to an increase in the size of the particles, and with the increase in its concentration, the increase in the size of the particles intensified. In the explanation of this phenomenon, it can be stated that probably during the process of transforming phospholipid layered structures into mass vesicles, cholesterol settles in the created holes and molecular spaces and therefore to some extent causes a significant increase in the size of the vesicles [13]. On the other hand, in this research, the high average size

of the particles can be due to the surface coating of liposomes with chitosan solution. In this regard, it was reported in a research that by increasing the ratio of cholesterol to lestin, in the production of nanoliposomes containing vitaminC, the size of vesicles increases [23]. In another study, it was stated that the average diameter of liposomes containing cefoxitin increases by adding cholesterol to the liposome structure [24]. Also, in the research conducted by Malhiros et al. (2012), it was reported that the addition of cholesterol to liposomal structures containing nisin is associated with an increase in particle size [25]. However, the results of past studies indicate the absence of a uniform trend regarding the effect of cholesterol on the final size of nanoliposomes. In a research, different ratios of lestin-cholesterol were used to produce nanoliposomes containing beta-carotene; The obtained results showed that increasing the ratio of cholesterol to lestin does not have a significant effect on the size of vesicles [13]. Also, in another study, different ratios of lestin-cholesterol were used to make vitamin nanoliposomes.D_3 was used, it was observed that increasing the concentration of cholesterol had no effect on the particle size [26]. On the other hand, in a research, adding cholesterol to the structure of liposomes reduced the size of the particles [27]. Therefore, it seems that the increase in the size of liposomes depends on the type of phospholipid used, the nature of the microcoated material and their production method [13].

According to the results of Table 2, the valuesPI It is in the range of 0.31-0.41 for different ratios of lestin-cholesterol. Although the addition of cholesterol to the chitosomal structure was associated with an increase in the size of nanoparticles, however, it had a positive effect onPI created and caused the size difference to decrease and the size of the particles to become more uniform. the amount ofPI It usually ranges from 0 to 1. AmountsPI $\leq$ 0.1 indicates the highest quality of particle dispersion distribution, values of 0.3PI$\leq$ indicates optimal dispersion values and values of 0.5PI$\leq$ is also an acceptable value [28].

Another important parameter in the properties of liposomes is zeta potential. Zeta potential is the overall charge that a lipid vesicle acquires in a specific environment. Evaluation of zeta potential of nanoliposomes can help to predict their stability. Knowledge of zeta potential is also useful in controlling the aggregation, fusion and sedimentation of nanoliposomes, which are important factors affecting the stability of nanoliposome formulations [29]. High absolute values of zeta potential indicate more and higher stability of liposomal systems [8]. In general, nanoparticles with a zeta potential higher than 30± Millivolts have sufficient electrostatic repulsion between particles and show good physical stability [16]. As can be seen in Table 2, all the samples have high zeta potential, and with the increase in the ratio of cholesterol to lestin, the zeta potential has increased significantly, which indicates an increase in electrostatic repulsion and greater stability. It has been mentioned in various researches that increasing the amount of cholesterol causes a gradual increase in the zeta potential and improves the stability of nanoliposomes [13], [30] and [26]. In fact, the increase in nanoliposome stability with increasing cholesterol levels can be attributed to the fact that the hydroxyl group in cholesterol forms a hydrogen bond with the choline group in the polar lestin, and the choline group is pulled into the lipid membrane; As a result, the negative charge increases and then the electrostatic repulsion between the particles also increases [31]. However, coating the liposome samples with a fixed amount of chitosan solution changed the surface charge of nanoliposomes to

positive values due to the presence of free amine groups (positively charged)[32].

Due to the fact that the zeta potential is higher than the optimal range in all lecithin-cholesterol ratios, therefore, the optimal lecithin-cholesterol ratio is selected based on size and size distribution. For this purpose, sample 9-1 lestin-cholesterol, which has the smallest particle size and its particle size distribution is also optimal; It was chosen as the optimal ratio for the next tests.

3-1-2- Loading capacity of caffeine in nanochitosomes(LC)

Although phospholipids are known as non-toxic compounds, it should be noted that high amounts of lipids used in the structure of liposomes reduce the body's ability to digest them. Therefore, it is recommended that the loading percentage of the active substance to lipid is as high as possible or that the amount of lipid used is as low as possible [33]. The loading percentage of caffeine compared to lipid was measured for 1-9, 2-8, 3-7 and 4-6 lecithin-cholesterol ratios, the results of which are shown in Figure 1.(Fig 1) It has been reported.

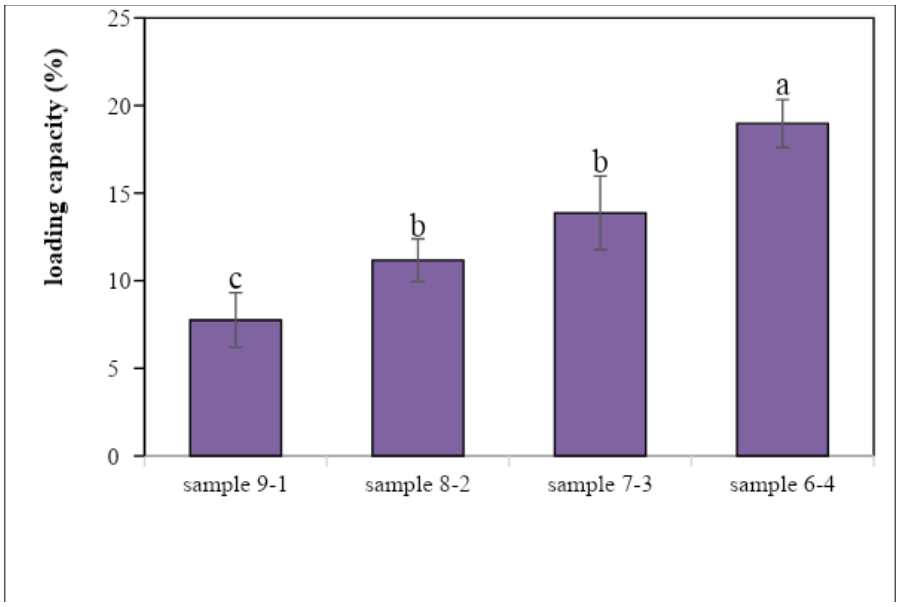

Figure 1 Loading capacity of caffeine to lipids at different amounts of lecithin -cholesterol ratios. Different letters in same column represent significant differences (P<0.05)

According to the results of Figure 1, it was observed that with the increase in the ratio of caffeine to lipid (lecithin), the loading efficiency of caffeine compared to lipid has increased, in other words, by decreasing the concentration of lecithin in the prepared chitosomal samples, the loading efficiency of caffeine has increased. In this regard, it was reported in a research that an increase in the ratio of active substance to lipid (up to the level of 5:1) is associated with an increase in loading efficiency [34]. In different studies, the loading rate of betaine [19], anthocyanin [35] and vitaminsC AndAND[36] 27, 24, 1.4 and 5.9% were reported in relation to lipid, respectively.

3-1-3- Stability of caffeine in nanoliposomes coated with chitosan during 60 days of storage

The stability of liposomes and the rate of

release of loaded compounds are significantly influenced by the composition of the formulation.pH, ionic strength and storage conditions (such as temperature, light) [15]. The stability of caffeine in nanochitosomes, according to the reduction of caffeine enclosed in nanochitosomes, the ratio of lecithin-cholesterol 1-9 and free caffeine in the control sample during storage at 25 degrees Celsius (ambient temperature) and for a period of 15, 30, 45 and 60 days. was investigated.

A decrease in the amount of microencapsulated caffeine during the storage period will indicate a decrease in stability. Corresponding results in Figure 2(Fig 2) is brought

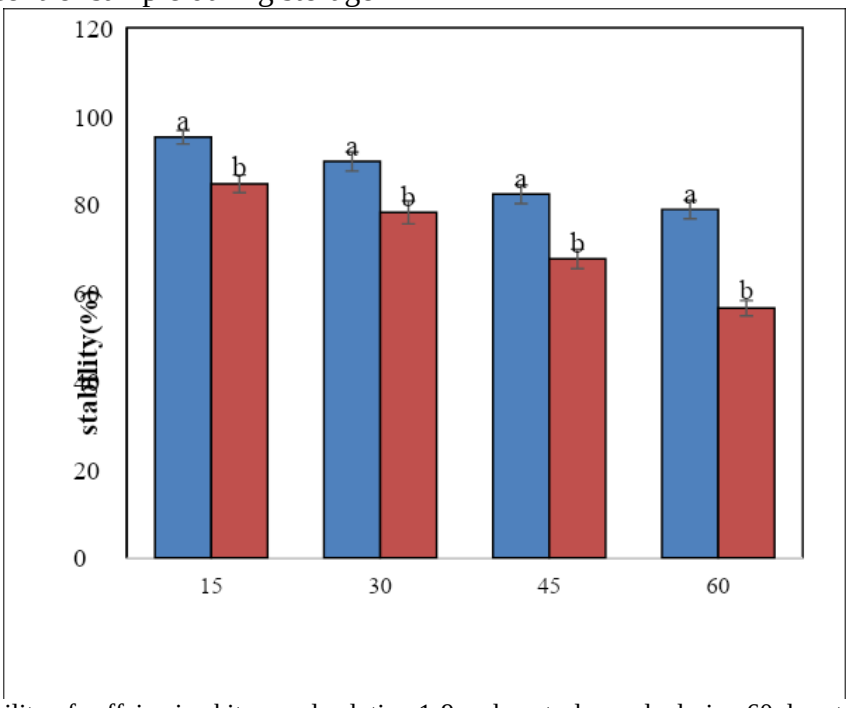

Figure 2 Stability of caffeine in chitosomal solution 1-9 and control sample during 60 day storage at room temperature

According to Figure 2, the obtained results showed that the stability of caffeine in nanochitosomes (1-9 lecithin-cholesterol ratio) during 60 days of storage at room temperature was always significantly higher than the control sample (uncoated caffeine).(05/0P<). On the other hand, during this period of time, the stability of caffeine in nanochitosomes was high and the release rate of this compound from lipid carriers was relatively low (around 21). The cause of this phenomenon can be attributed to two factors: 1) the presence of cholesterol in the structure of nanoparticles: cholesterol plays an important role in increasing the stability of the liposomal bilayer by significantly increasing the temperature of the phospholipid phase transition [27]. Also, certain and fixed concentrations of cholesterol cause the lipid bilayer to condense and harden, which reduces the permeability and fluidity of the lipid membrane and increases the stability of the liposome [13]. 2) Liposome coating with chitosan: In this study, in order to improve the stability of nanoliposomes, chitosan was used as an additional biopolymer on the outer surface of liposomes. In various researches, it has been shown that liposomes coated with chitosan show a greater ability

to maintain the core material, due to the formation of the chitosan electrostatic protective coating [2] and [37].

Of course, as seen in Figure (2), the stability of caffeine in nanochitosomes decreases during the storage period. The release of the active substance during storage due to the destruction of the wall of nanochitosomes can be one of the reasons for this. Because the permeability of the liposomal membrane and its destruction due to the oxidation of phospholipids, the release of the active substance (microcoated) is maintained over time [36]. Also, environmental conditions such as storage temperature are also effective factors in reducing the stability of liposomes. Probably, keeping chitosomal solutions at 25°C has caused more caffeine to leak out of the vesicles. Keeping liposomes at high temperature leads to changes in lipid crystal structure, softening of the membrane, increasing its fluidity and accelerating the oxidation of fatty acids, which leads to an increase in the rate of release of enclosed substances [37]. In this regard, in a research, the stability of fluconazole-containing liposomes during one month of storage at refrigerator temperature (2-9 °C), room temperature (2±25°C) and high temperatures (45°C) were evaluated. The results showed that keeping liposomes at refrigerator temperature has the highest level of stability and the lowest level of material release (less than 5%). It was also stated that high temperatures lead to an increase in the fluidity of the lipid bilayer and the release of more microencapsulated compounds [38]. Also, in the study conducted on vitamin-carrying nanoliposomesD_3 , storage of liposomes at low temperature (4°C) as the best condition to keep the vitamin stableD_3 In nanoliposomes, it was reported [26]. In another study, the rate of vitamin releaseANDOf the nanoliposomes carrying this substance, 62% was reported during one month of storage at room temperature (25°C), while this value was reported to be about 39% at 4°C [36].

3-1-4- Infrared spectroscopyFTIR

TestFTIRis a desirable method for identifying and separating organic compounds and functional groups in their structure. In this test, measuring the intensity of absorption in curved peaks makes it possible to identify different compounds and interactions between components. Since each of the functional groups has a certain intensity of absorption in a certain frequency, therefore, making changes in the structure of the compounds will cause a change in the intensity of absorption in them. In this research, the spectrumFTIR in rangecm^{-1} 4000-4000, related to the wall materials of nanochitosomes (lecithin-cholesterol and chitosan), caffeine, empty nanochitosome and loaded nanochitosome with a ratio of 9-1 lecithin-cholesterol are shown in Figure 3.

In the structure of cholesterol, a wide range in the range ofcm^{-1} 3400 to tensile bondOH is attributed Couriers 2899, 1674, 1464 and 1378cm^{-1} respectively to symmetric tensile vibrationsCH_2 , linkC═C In the second ring of cholesterol, the asymmetric stretching vibrations of groupsCH_2 AndCH_3 and bending vibrationsCH_2 AndCH_3 are related In addition, 1055 and 840 peakscm^{-1} are also related to the shape change of the cholesterol ring and C─C─C stretch bond in the cholesterol molecule [39]. In relation to lestin (phosphatidylcholine), absorption peaks 3432, 2924, 1735, 1225 and 1053cm^{-1} respectively related to vibrationsOH, asymmetric tensile vibrationsCH_2, groupC═O, groups$AFTER_2^-$asymmetric andC─O At$C─O─PO_2^-$ are[40]. Also in chitosan, peaks 3400, 2923, 1650 and 1095cm^{-1} respectively related to tensile bondOH , tensile bondCH, tensile bondC═O

in the amide I region and the stretch bondC–O are. Also, Couriercm^{-1} 894.91 also related to glycosidic bondβ1→4 is [41] and [42].

Regarding pure caffeine, peak 3441cm^{-1} Related to tensile bondOH and couriers 3111 and 2953 cm^{-1} respectively to the tensile vibrations of the bondsC–H AndC–H The methyl group is related. Also, couriers 1703 and 1656cm^{-1} They are related to C=O groups in the amide I region. Couriers 1547, 1356, 1071 and 1023cm^{-1} Also in order with regionsCH_3 , C–C andC–OH are related in amide II [2].

Comparing empty chitosomes with chitosomes loaded by caffeine, according to Figure 3, it can be seen that when caffeine enters the nanochitosomes with a 1-9 ratio of lecithin-cholesterol, most absorption peaks have an increase in absorption. Also, new peaks of 1284.96, 1238.82, 974.1 and 610.11cm^{-1} It was observed in caffeine-loaded nanochitosomes that it could possibly be the result of the interaction between the nanochitosome wall material and caffeine, which is a confirmation of the successful loading of caffeine into chitosomes.

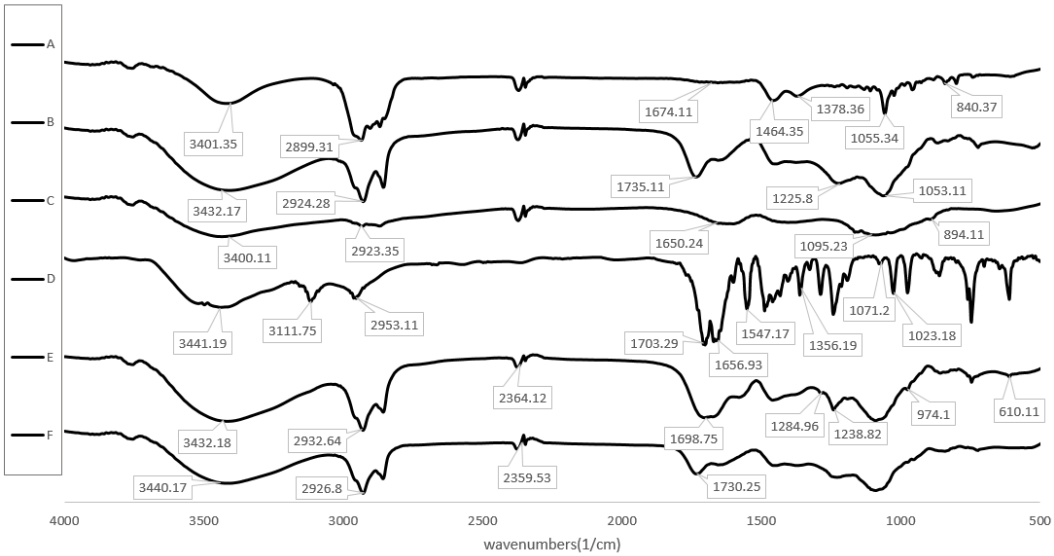

Figure 3 FTIR analysis results. (A=cholesterol, B= lecithin, C=chitosan, D=caffeine, E=lecithin-cholesterol 9-1 ratio nanochitosome containing caffeine, F=lecithin-cholesterol 9-1 ratio nanochitosome without caffeine)

3-1-5- Checking imagesFESEM

Morphology of caffeine-loaded chitosomes using scanning electron microscopy (WHICH) is shown in Figure 4 with a magnification of 50 thousand. This image results fromDLS It confirms the size of nanochitosomes; Because the size of most particles is less thannmIt is 150. According to Figure (4), the produced nanostructures show almost spherical shapes. Also, in this study, the surface coating of nanoliposomes with chitosan did not change the spherical morphology of nanoliposomes, which is consistent with the results of other studies [2] and [43].

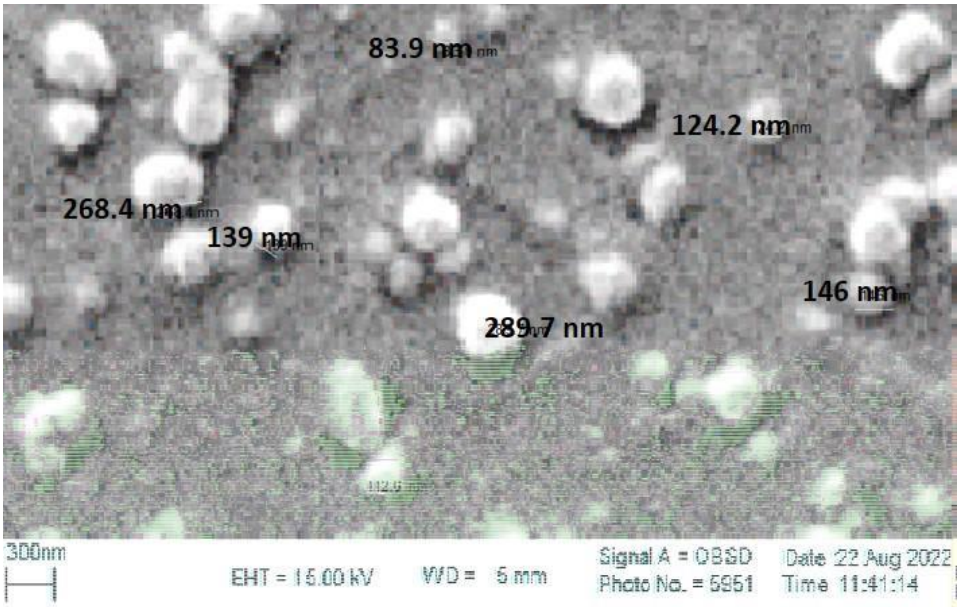

Figure 4 FESEM analysis for nanochitosome with 1-9 lecithin /cholesterol ratio containing caffeine

4 - Conclusion

In the present study, the effect of different lestin-cholesterol ratios (1-9, 2-8, 3-7 and 4-6) on changes in particle size, zeta potential and size distribution was significant. The results showed that with increasing cholesterol levels, the size and zeta potential of the particles increases. Due to the fact that the zeta potential of all ratios was in the desired range, therefore, the optimal ratio of lestin-cholesterol was selected based on the size and size distribution of the particles. The results of measuring the caffeine loading capacity showed that, with the increase in the ratio of caffeine to lipid (lecithin), the loading efficiency of caffeine increases compared to lipid. The results of the stability test showed the high stability of microcoated caffeine during two months of storage at ambient temperature. Test resultsFTIR, confirmed the successful loading of caffeine into nanochitosomes. Also, analysis of particle morphology images(FESEM),The results ofDLS Confirmed in relation to the size of the particles. Overall, the results of the present study showed the potential of nanochitosomes as an efficient system for the storage and sustained release of caffeine that can be used in the food industry.

5- References

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تولید و ارزیابی نانولیپوزوم‌های پوشش‌داده شده با کیتوزان برای درون‌پوشانی کافئین

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چکیده

در این پژوهش، نانولیپوزوم‌های پوشش‌داده شده با کیتوزان در نسبت‌های ۹-۱، ۸-۲، ۷-۳ و ۶-۴ لستین-کلسترول، با استفاده از روش هیدراتاسیون لایه نازک، به عنوان یک سیستم تحویل برای ریزپوشانی کافئین تهیه شدند. سپس، آزمون‌های تعیین اندازه و پتانسیل زتا برای تعیین ویژگی‌های نانوذرات تولید شده صورت گرفت. میانگین اندازه ذرات (میانگین قطر هیدرودینامیکی) و توزیع اندازه ذرات برای نسبت‌های مختلف لستین-کلسترول، به ترتیب در محدوده ۱۳۳/۵ تا ۵۳۳/۵ نانومتر و ۰/۴۱ – ۰/۳۱ قرار گرفت. مقادیر پتانسیل زتا نیز در محدوده ۴۰/۹۶+ تا ۵۰/۵+ بدست آمد. پس از تعیین ظرفیت بارگذاری، آزمون FTIR برای بررسی واکنش‌های احتمالی بین کافئین و مواد دیواره نانوکیتوزوم‌ها صورت گرفت. مورفولوژی کیتوزوم‌های نسبت ۹-۱ لستین-کلسترول بارگیری شده با کافئین، با استفاده از میکروسکوپ الکترونی روبشی (SEM) نشان داده شد. پایداری نمونه کیتوزومی با نسبت ۹-۱ لستین-کلسترول، از طریق مشاهده بصری ایجاد رسوب و محاسبه‌ی مقدار آزادسازی کافئین محصور شده در طول ۶۰ روز نگهداری در دمای محیط مورد بررسی قرار گرفت. نتایج بدست آمده در این پژوهش، نشان داد که نانوکیتوزم‌ها سیستم کارآمدی در حفظ و رهایش پایدار کافئین هستند.