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Scientific Research

Investigating the structural properties of nanoliposomes containing *Padina distromatic* algae extract fabricated by heating method

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ABSTRACT

Microencapsulation of bioactive compounds in lipid carriers, such as liposomes, in addition to improving stability during storage by increasing bioavailability and controlled release, increases the efficiency of these compounds in vivo. The studies conducted on Padina algae show the existence of a high level of phenolic and antimicrobial compounds. Also, this alga has a significant amount of polyphenols with antioxidant and anti-AChE (acetylcholinesterase) properties, which can be used as a supplement to improve neurological disorders. Therefore, the purpose of this research was to produce and investigate the structural properties of nanoliposomes containing Padina algae extract using the heating method. The particle size of nanoliposomes produced at varying levels of lecithin and loaded extract was obtained in the range between 318 and 60 nm. The resulting values for the polydispersity index and zeta potential indicate the uniformity of the produced particles along with the high electrostatic repulsion between the particles. The ability to load liposome particles at the lowest level of wall substance and the highest concentration level of the extract reached 52.8±0.3% in this research. Evaluation of the morphological characteristics of the structure using a transmission electron microscope shows the formation of uniform particles with a spherical geometry. The results of this research show the ability to produce a liposome structure containing Padina algae extract with suitable structural properties. These results can improve the prospect of possible use of this extract with a therapeutic approach.

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1- Introduction

Padina, a brown seaweed belonging to the family*Dictyotaceae* It is widely found throughout tropical waters and is usually found along coral reefs. So far, about 80 species of the genus Padina have been identified in the world. The habitat of this seaweed is reported in the tidal areas of 0-10 meters depth of warm waters and due to the special shape, size and color of its leaves, it can be easily identified from other seaweeds. 6 species of the genus Padina have been identified on the northern shores of the Persian Gulf. This algae is one of the most abundant algae on the northern shores of the Oman Sea and the Persian Gulf [1 and 2]. Halila et al. (2017), Ansari et al. (2019) and Yosaltrova et al. (2018) reported that brown algae have significant amounts of polyphenols with antioxidant, antimicrobial and anti-acetylcholinesterase properties. (AChE) (effective in the treatment of neurogenic disorders). Also, these algae contain omega-3 essential fatty acids and vitaminsB_{12} It is a rich source of vitamins$A$ ، B_1 ،B_2 ،B_6 ،C, niacin and minerals such as iodine, potassium, magnesium and calcium [3-5]. Shirani Bidabadi et al. (1401) to study the chemical composition of algae extract*Padinadistromatic* they paid. The results of these researchers showed that the most compounds in the extract of this algaeButanoic acid, butyl ester Andn-Hexadecanoic acid that had antioxidant and antimicrobial properties [6]. Also, Shirani Bidabadi et al. (1401) stated in a study that Padina brown algae extract (extracted by ultrasound method) had a high phenol and flavonoid content. These researchers also reported that Padina algae extract extracted by ultrasound and maceration method has acetylcholinesterase inhibitory activity.AChE) were [7]. In another study, these researchers investigated the antimicrobial effects of brown algae extracts (Padina and Sargassum). The results of studies by Shirani Bidabadi et al. (1401) showed that Padina algae extract had more antimicrobial properties compared to Sargassum algae extract. The minimum inhibitory concentration of Padina algae extract was 12.5 mg/ml on Staphylococcus aureus bacteria. Padina alga extract at 50 mg/ml had a bactericidal effect on Staphylococcus aureus bacteria [8]. Despite all the benefits of herbal extracts, it must be said Their bioactive compounds are sensitive to environmental conditions and undergo changes and decrease health effects during the storage period [9]. On the other hand, the effective compounds of plants in food products increase the performance characteristics (by controlling the release time) and improve the nutritional characteristics, which doubles their importance [10]. Therefore, the use of a method that protects the bioactive compounds of plant extracts against environmental conditions and achieves its release at the required time in a controlled manner is of great importance and a turning point in the production, trade and use of this It is considered compounds in the industry.

Microcoating is one of the new processes in the food industry that can protect beneficial compounds and release these compounds in a controlled manner at the required time [11]. In recent years, the application of nanoliposome creation method for encapsulating bioactive compounds and improving its efficiency and bioavailability has attracted a lot of attention in the field of food and medicine.[12 and 13]. A liposome is a microscopic vesicle containing two phospholipid layers that surrounds a fluid space. The thickness of this bilayer lipid is usually between 3-6 nm andLiposomeThe ones formed by them can have a drop between 50 nm and 50 micrometers

[14].LiposomeDue to the amphipathic properties of their components, they provide the possibility of delivering hydrophilic and lipophilic bioactive compounds. Features such as low inherent toxicity, biodegradability and lack of immunogenicity have caused thatLiposomeas a very suitable carrier in new delivery systems of bioactive compounds should be considered [15]. These small bag-like structures are similar to packages or capsules that can be used to carry the target compounds to different parts of the body by trapping drugs inside them (encapsulation). As a result, drug delivery is one of the important applicationsLiposomeis Therefore, the purpose of this research was to produce nanoliposomes of Padina algae extract (0.7% and 2% levels) with a wall of phosphatidylcholine (2.5% and 4.5% levels) in order to evaluate the ability to produce nanoliposome structure. containing Padina algae extract with appropriate structural characteristics, distribution and size of liposome particles, zeta potential of particles, encapsulation efficiency of the extract and transmission electron microscope imaging of the structure of liposomes were investigated.

2- Materials and methods

2-1- Materials

Phosphatidylcholine (lecithin) from the companyACROS ORGANICS Belgium and glycerol were obtained from Merck, Germany. Padina brown algae (*Padinadistromatic*) was collected from Sistan and Baluchistan province (Chabahar port).

2-2- extracting the extract from Padina algae

Extraction from Padina brown algae (*Padinadistromatic*) was done according to the method of Shirani et al. (1401) [7]. In this method, indirect ultrasonic waves with a frequency of 70 kHz at a temperature of 25 degrees Celsius were used for one hour. For this purpose, 10 grams of algae were poured into the glass along with methanol, n-hexane and n-butanol solvents. The mixture was placed in an ultrasonic bath for one hour. Then the solution was filtered and methanol and other solvents were removed by a rotary evaporator [16].

2-3- Preparation method of nanoliposomes containing Padina extract

Liposomes containing Padina algae extract were produced using the thermal method of Mozafari et al. (2005). First, the solution of Padina algae extract was prepared in 2 concentrations of 0.7 and 2% with deionized water. Then phosphatidylcholine was added to the solution containing the extracts at two levels of 2.5% and 4.5%. Finally, glycerol at a concentration of 3% was added to the prepared solutions. Then the samples were placed on a hot plate for one hour at 70°C and stirring speed of 1000 rpm. After the mentioned time, the liposomal samples were placed at room temperature (25°C) for one hour to increase their stability, and at the end, they were kept at 4°C until the tests were performed [17].

2-4-Measurement of particle size and distribution

Distribution of particle size and average diameter using particle size analyzer (Cordouan TechnologiesMade in France) and was measured based on light diffraction and according to the method of Pan et al. (2018) [18].

5-2-Measuring the zeta potential of particles

Zeta potential of liposome particles prepared using the device Colloids & Interface Instruments Zeta Compact The construction of France was

measured according to the method of Borab et al. (2014) [19].

6-2- Determination of total phenol content

Total phenol content was determined using the Folin-Cicocalto method. The amount of 100 microliters of the prepared extract was mixed with 2 milliliters of 2% sodium carbonate and after 2 minutes of storage at room temperature, 100 microliters of Folin-Cicocalto reagent (1-1 dilution with deionized water) was added to it. The produced sample was placed on a shaker to fully mix the reagent with the solution, and then it was placed at room temperature for 30 minutes to complete the reaction. The samples were absorbed at a wavelength of 720 nm using a spectrophotometer. Total phenol content is based on milligrams of gallic acid equivalent to grams of dry matter of the extract.GAE/gdw) was reported [20].

2-7-Determining the efficiency of the endocapsulation of the extract

The encapsulation efficiency of nanoliposomes was determined using Folin-Cicocalto method. Nanoliposomes using the company's centrifugeHelmer Germany model236HK (g 36000 for 30 minutes) was separated. Then the content of total phenol in the supernatant (the amount of free extract) was measured and the encapsulation efficiency of the extract was determined based on the following relationship [21].

Relationship 1-

$$\%EE=(P_i-P_S)/P_i)\times100$$

In this regardP_i Total phenol content andP_s It indicates the content of phenol placed in the supernatant.

8-2- Selection of transmission electron microscope

20 microliters of liposome suspension was placed on a copper-coated grid for 2 minutes, then stained with 20 microliters of uryl acetate for 1 to 2 minutes. After drying at room temperature, the morphology of the samples was analyzed using a transmission electron microscope (Omega,Leo912AB, Germany) was investigated at a voltage of 100 kW [22].

9-2-Statistical plan

The data obtained in the form of a completely random design using softwareMini-Tab17 was analyzed. In this way, the average of three repetitions was considered for all tests. Comparing means using Tukey's test ($P<0.05$) Making and drawing diagrams from the softwareExcel used.

3. Results and Discussion

3-1- Distribution and size of particles

Figure 1 shows the effect of changing the ratio of wall material (phosphotidylcholine) to core (Padina algae extract) on the size and distribution of liposome particles. As the results show, the size and distribution of the particles were influenced by the ratio between the core material and the phospholipid of the walls. Based on the results,

The average particle size was in the range of 59-318 nm. The largest particle size (318 nm) related to the sample with the highest level of phosphotidylcholine (lecithin) (4.5%) and extract (2%) was recorded for liposome production. By increasing the surface of the wall material to 4.5% w/w along with the highest level of the extract investigated in this research (2% w/w) with a ratio of nearly 2 times the wall material to the core, the highest particle size among the produced liposomes. was observed. In this regard, similar results were reported by Ramli et al. (2021). These researchers encapsulated propolis extract in a nanoliposome structure. Based on the results reported by these researchers, the size of the liposome particles increased with the increase in the extract level [23].

On the other hand, based on the results of the present study, the lowest particle size (59 nm) was observed in the sample containing the lowest level of wall material (2.5% w/w) and the highest level of extract (2%). In this sample, the ratio of the wall material to the core was at a relative level of almost equality. The reduction of the particle size to the lowest level obtained in this research shows the effect of the wall-to-core ratio on the change of the particle size. Similar results are available by Swaqbi et al. (2019). These researchers of nanoliposome system for encapsulation of sargassum algae extract they used Based on the reported results, changing the ratio of wall to core material caused a change in the particle size, and the smallest particle size was found in the optimal ratio between the extract and the liposome wall [9]. These results indicate that in nanoliposome systems, there is an optimal level of wall-to-core substance concentration. This is probably related to the decrease in size with increasing number of particles in the nanoliposomal system [24].

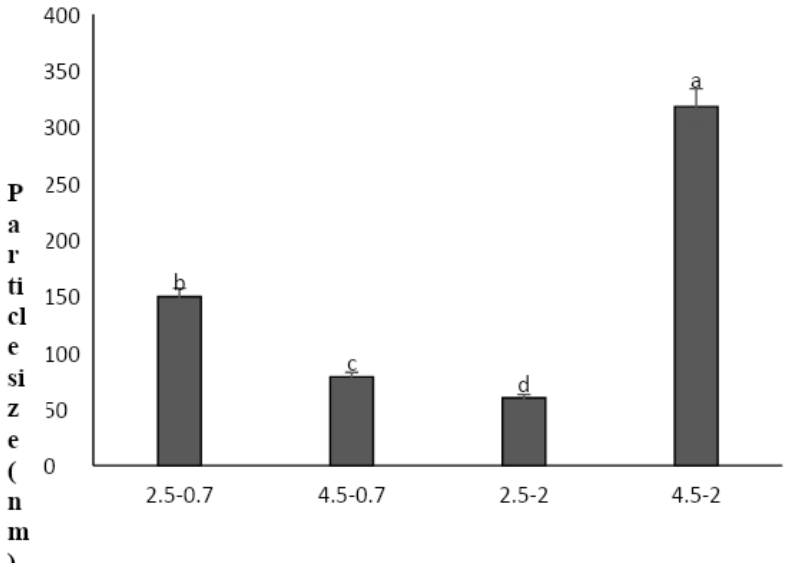

Fig 1- particle size of liposomes as different concentration of lecithin and Padina extract. Different letters represent significant difference from one another ($p< 0.05$).

The polydispersity parameter is one of the important parameters in evaluating the structural properties of nanoliposomes [25]. As can be seen in Table No. 1, the polydispersity index as a measure of the uniformity of particle size distribution in the rangePDI It was 0.15-0.35, which is dependent on the ratio between core and wall components. The obtained values of the polydispersity index indicate the uniform distribution of the formed particles.

Table 1- polydispersity index of liposomes as different concentration of lecithin and padina extract

Lecithin (%)	Padina extract (%)	Polydispersity index (PDI)
2.5	0.7	0.34±0.017[a]
4.5	0.7	0.35±0.021[a]
2.5	2.0	0.31±0.032[a]
4.5	2.0	0.15±0.016[b]

Different letters show significant differences, respectively ($p<0.05$)

3-2-Zeta potential

The zeta potential parameter is an indicator of structure evaluation in in vitro and in vivo conditions [9]. The size of zeta potential is also used as a parameter to predict the stability and durability of nanoliposome particles. High surface charge indicates high repulsion between particles and as a result the unwillingness to form mass and structural destruction. This parameter is a function of the surface charge of lipid vesicles, surface adsorbed layers and the nature and structure of the environment in which nanoliposomes are dispersed. In addition to the importance of this parameter in structural stability, in in vivo conditions and delivery of bioactive compounds to the target point, the surface charge of nanoliposomes also affects the circulation time of the particles in the blood [26].

Figure 2 shows the size of the zeta potential of nanoliposome particles containing Padina algae extract under the influence of changing the wall-to-core ratio. As the results show, this parameter varies between -33.8 to -40.35 mV for the produced nanoliposome particles depending on the change of core-to-wall ratio. Zeta potential less than -10 millivolts and as an ideaAlter less than -30 mV, by creating a high repulsion between colloidal particles present in the system, it creates favorable stability in suspensions containing nanoparticles [27]. Therefore, the results obtained in this research are proof of the stability of nanoliposomal systems. The highest value of zeta potential (-40.35 mV) for nanoliposome containing 4.5% of wall material and 0.7% of Padina algae extract and the lowest value (-33.8 mV) for liposome containing 4.5% of material walls and 2% extract was obtained. The change in the size of the zeta potential under the influence of the level of the extract and the diva substance can indicate the interaction between the composition of the walls and the phenolic components present in the extract of Padina algae [28]. The interaction between phenolic compounds and phospholipid walls determines the size of the zeta potential of nanoliposomes. The phenolic compounds present in the encapsulation medium are also absorbed in the internal structure of liposomes formed on the surface of the structure. Therefore, part of the phenolic compounds of algae extract has the ability to bind with the negative phospholipid groups placed on the surface of the nanoliposome membrane [29]. Also, compounds with relative positive charge present in the extract surround the surface of nanoliposome particles and increase the charge of formed vesicles [25]. Penila et al. (2017) observed a similar trend of changes in zeta potential size from -24.3 to -16.2 mV in nanoliposomes containing garlic extract [30]. Also, the results of the current research in the field of zeta potential changes of particles were similar to the results reported by Machado et al. (2019). In the study of surface charge changes of produced nanoliposomes, these researchers witnessed the change of zeta potential of particles towards positive values with the placement of the extract in the nanoliposome structure [29]. Similar findings have been published in connection with nanoliposomes containing Sargassum algae extract by Swaqbi et al. (2019) [9].

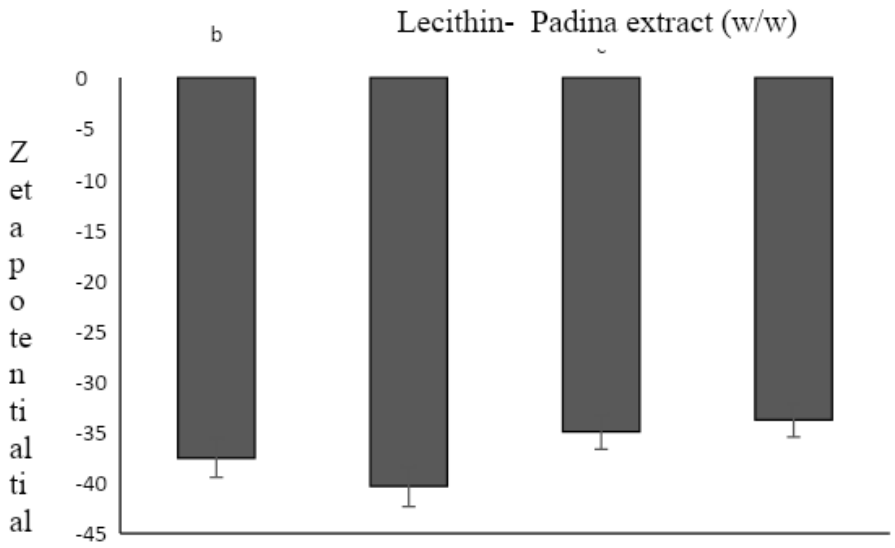

Fig 2- Zeta potential of liposomes as different concentration of lecithin and Padina extract. Different letters represent significant difference from one another ($p< 0.05$).

3-3-The efficiency of the cover

Figure 3 shows the changes in the encapsulation efficiency of Padina algae extract in the nanoliposome structure. As the results show, the encapsulation efficiency increased by increasing the concentration of the extract at a constant level of lecithin. On the other hand, increasing the level of lecithin decreased the encapsulation efficiency at both constant levels of the extract. Based on the results, the highest level of encapsulation (52.8 ± 0.3%) of Padina algae extract was obtained at a concentration of 2% of the extract and 2.5% of lecithin. Increasing the level of lestin by affecting the internal environment and reducing the fluidity reduces the free movement of phenolic compounds in the system. Hence, the encapsulation efficiency decreases and a lower level of phenolic compounds is trapped inside the structure of lipid vesicles [31]. A similar trend of changes in encapsulation efficiency with changes in the level of extract and wall material in the research conducted by Swaqbi et al. It has been reported [9]. Pagnasat et al. (2016) reported similar results of encapsulation of spirulina algae extract in the liposomal structure at a level of 55% [32]. Also, the encapsulation efficiency of 47.5% of garlic extract in liposomal structure was reported by Penila et al. (2017), which is in accordance with the results obtained in this research [30].

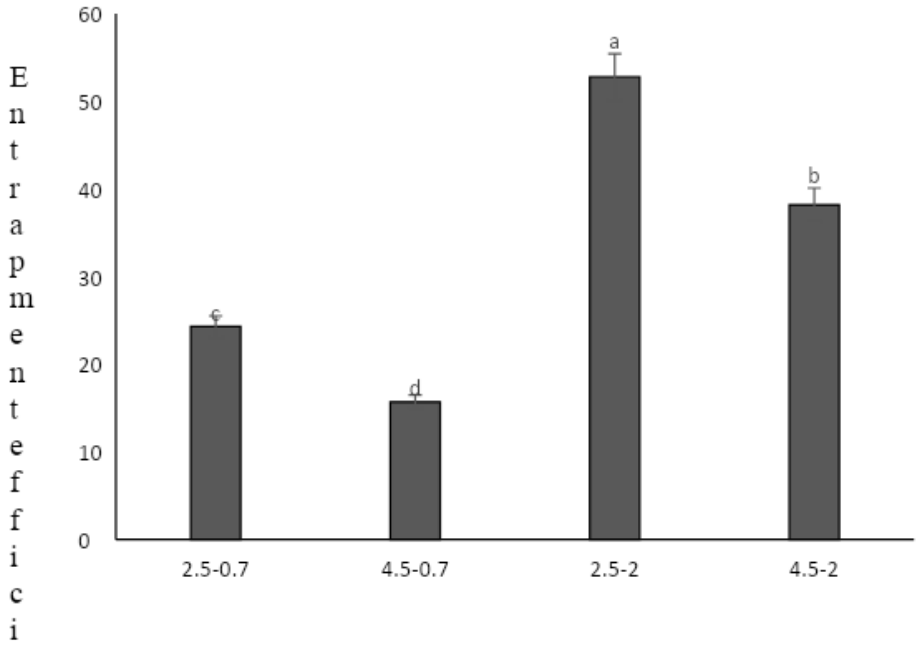

Fig 3- Entrapment efficiency of liposomes as different concentration of lecithin and Padina extract. Different letters represent significant difference from one another ($p< 0.05$).

3-4- transmission electron microscope

The structural characteristics of nanoliposomes were investigated using transmission electron microscopy. As can be seen in Figure 4, the formed nanoliposomes have a spherical structural morphology. The size of the particles as well as their dispersion distribution is consistent with the results of the laser light scattering method. Spherical morphology, the same particle size, as well as the reduction of particle size on the nanometer scale, in addition to improving the delivery of the loaded substance, make the nanoliposomal suspension stable during storage. In general, several factors such as the ratio of phospholipid walls to the loaded active substance, the method of nanoliposome production, temperature and production time are effective on the size and structural morphology of the formed particles [33]. The transmission electron microscope images show the lowest particle size of the sample with 2.5% of the wall material and 2% of Padina algae extract. The results obtained from this research are similar to the images published by Swaqbi et al. (2019). The results of these researchers indicated the formation of the optimal nanoliposome sample at the lowest level of wall composition with the highest level of phenolic composition of Sargassum algae extract [9].

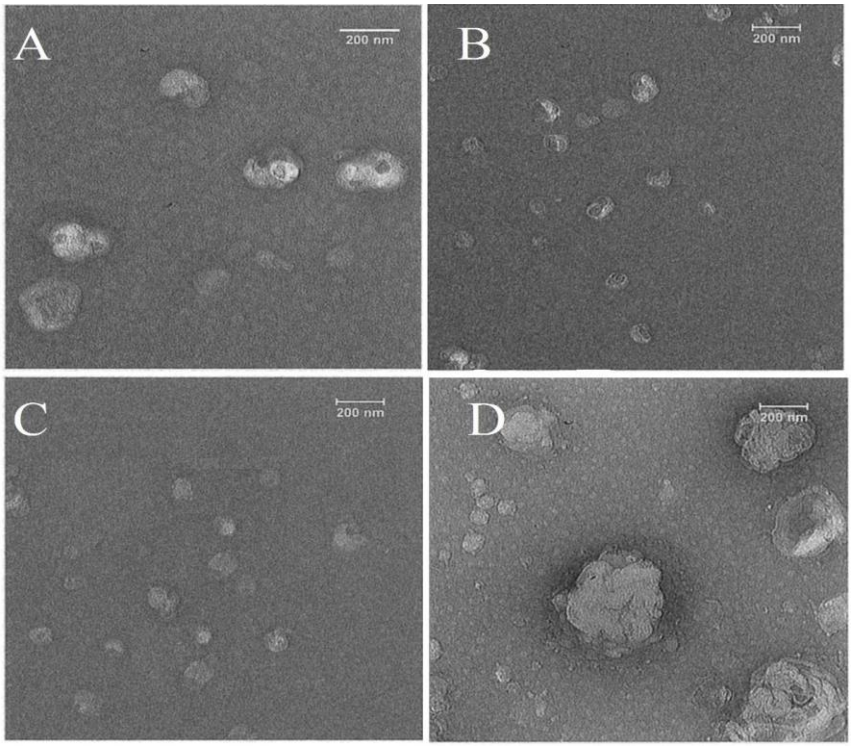

Fig 4- TEM images of liposomes as different concentration of lecithin and padina extract in 10000x magnitude. A) 2.5-0.7% lecithin-Padina extract (w/w), B) 4.5-0.7% lecithin-Padina extract (w/w),C) 25-2.0% lecithin- Padina extract (w/w), D) 4.5-2% lecithin-Padina extract (w/w).

4-Conclusion

Encapsulation was done using the thermal method in the nanoliposome structure to improve the shelf life and also increase the bioavailability of Padina algae extract. Based on the results, the change in the surface of the material of the walls and the loaded composition has an effect on the size and distribution of the particles, the surface charge and also the loading efficiency. Based on this, the best nanoliposome structure was observed in the lowest level of wall material (2.5%) and the highest concentration of Padina extract (2%). The results obtained from this research can be used as an approach in the preparation and production of food-pharmaceutical supplements from Padina algae extract.

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بررسی ویژگی‌های ساختاری نانولیپوزوم‌های حاوی عصاره جلبک پادینا (*Padina distromatic*) تولید شده به روش حرارتی

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چکیده

ریزپوشانی ترکیبات زیست فعال در حامل‌های لیپیدی از جمله لیپوزوم‌ها علاوه بر بهبود پایداری در زمان نگهداری با افزایش زیست‌دسترس‌پذیری و رهایش کنترل شده موجب ارتقاء کارایی این ترکیبات در شرایط درون‌تنی می‌گردد. جلبک پادینا دارای مقادیر قابل توجهی از پلی‌فنل‌ها با خواص آنتی‌اکسیدانی، ضدمیکروبی و مهار آنزیم استیل‌کولین‌استراز (AChE) است که قابلیت کاربرد به عنوان مکمل ضدسرطان و بهبوددهنده اختلالات نورولوژیک را دارد. از این رو هدف از انجام این پژوهش تولید و بررسی خصوصیات ساختاری نانولیپوزوم‌های حاوی عصاره جلبک پادینا با استفاده از روش حرارتی بود. بدین منظور آزمون‌های توزیع و اندازه ذرات نانولیپوزوم‌ها، پتانسیل زتا، راندمان درون‌پوشانی عصاره و تصاویر میکروسکوپ الکترونی عبوری انجام شد. نتایج نشان داد اندازه ذرات نانولیپوزوم‌های تولیدی در سطوح متفاوت فسفاتیدیل کولین (لسیتین) (۲/۵ و ۴/۵ درصد) و عصاره جلبک پادینا (۰/۷ و ۲ درصد) در محدوده ۳۱۸-۶۰ نانومتر بودند. نتایج آزمون شاخص چند پراکندگی و پتانسیل زتا نیز حاکی از یکنواختی ذرات تولیدی همراه با دافعه الکترواستاتیکی بالای میان ذرات بود. همچنین یافته‌های بدست آمده نشان داد قابلیت بارگذاری ذرات نانولیپوزوم (راندمان درون‌پوشانی عصاره) در کمترین سطح ماده دیواره‌ای (۲/۵ درصد) و بیشترین سطح عصاره جلبک پادینا (۲ درصد) ۵۲/۸ درصد بود. ارزیابی خصوصیات مورفولوژیک ساختار نانولیپوزوم با استفاده از میکروسکوپ الکترونی عبوری نیز نشان‌دهنده شکل‌گیری ذراتی یکنواخت با هندسه کروی شکل بود. با توجه به نتایج حاصله از این پژوهش می‌توان گفت که قابلیت تولید ساختار نانولیپوزومی حاوی عصاره جلبک پادینا با خصوصیات ساختاری مناسب مهیاست که این امر می‌تواند دورنمای کاربرد احتمالی این عصاره با رویکردی درمانی را بهبود ببخشد.