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Preparation and investigation of sodium alginate nanocapsules by different microemulsification devices

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ABSTRACT: Numerous techniques of preparation have been reported for the encapsulation of the core material, but the microemulsion technique is the most effective and commonly used for encapsulation. In this article, three microemulsification devices including ultrasonic stirrer, labo ratory reactor, and mechanical stirrer were used to produce the alginate nanocapsules containing peppermint oil. The effect of different parameters on surface morphology, mean particle size, and size distributions were investigated. The results indicated that the ultrasonic stirrer is the best device to make the nanocapsules containing essential oils prepared by microemulsion method, so that the size of nanocapsules prepared by ultrasonic stirrer was about 56 nm. Also, the results of GC-MS, FTIR, and TGA show that quantities of the remaining compounds in nanocapsules prepared by ultrasonic stirrer are higher than other devices.
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INTRODUCTION

Encapsulation is a technique for preparing the tiny packaged materials that have many attractive features.[1] The encapsulated materials are utilized in pharmaceutical, food, agricultural, cosmetic textile, paper, paint, and printing industries, among others. However, the encapsulation technology is effectively used for such purposes as protection, compatibility, and controlled release of core materials.[1–6] Different methods are available for encapsulation such as extrusion, electrospraying, emulsion, and microemulsion methods. Extrusion is the oldest method for manufacturing capsules with hydrocolloids.[7] Electrospraying is a process of synchronous production of droplets and their charging using electrical forces that were used for produce the micro and nanocapsule in our previous works.[8,9] In emulsion technique, the mixture of core compound and polymer solution is emulsified in an immiscible liquid, and the solvent is then removed by such processes as evaporation, extraction, etc.[10] The microemulsion method that firstly reported by Stoffer and Bone in 1980[11] involves mixing the appropriate ratios of the aqueous and oil components in the presence of a surfactant. The adsorption of surfactant in the interface reduces the surface tension and the microemulsion forms with low energy consumption.[12] This method compared with conventional emulsion method has many advantages such as rapid polymerization rate, high polymer molecular weight, less particle size, narrower molecular distribution, and thermodynamically stable.[12,13] Since the type of stirrer used for microemulsificaton process, could perform a significant role in making the microemulsion and form and size of the produced nanocapsules, can be used different stirrers to assign the effects of the various parameters and select the best device.[14]

The core contents may be released due to friction, pressure, temperature changes, diffusion through the polymer wall, dissolution of the wall coating, biodegradation, etc.[15,16] Among the various biopolymers that can affect the formation of semipermeable membranes, alginate is one of the most frequently used owing to the fact that the encapsulation process is carried out under mild conditions.[17,18] Considering their excellent physical properties and good compatibility, biodegradability, and capability of drug delivery, alginates are used in several industrial applications such as medical, food, and textile industries.[19–21] Figure 1 illustrates the structure of alginate, which is a natural polysaccharide consisting of linear copolymers of 1–4 glycosidically linked β-D-mannuronic acid (M) and α-L-guluronic acid (G) residues.[18] Alginate solution can form a three-dimensional gel network in the presence of bivalence or multivalence cations, a property which is similar to that of an "egg-box" model as observed in Figure 2.[22,23]

Generally, the physical properties of alginate gels depend on the affinity of divalent cations of the alginate, quantity of ions must be bound to the alginate, the molecular size and concentration

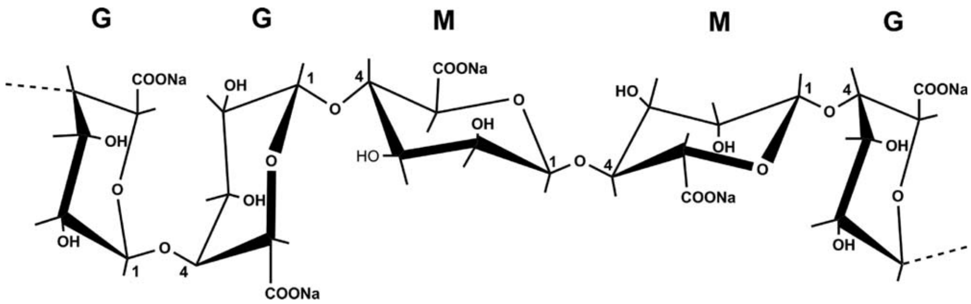

Figure 1. Chemical structure of alginate (G: guluronic acid, M: mannuronic acid).

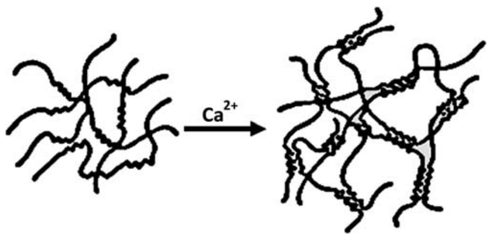

Figure 2. Formation of alginate gel in the presence of cations and construction of the egg-box model.[22]

of alginate polymer, the ratio of mannuronic and guluronic acids and sequential arrangements within the polysaccharide chain, and the type and concentration of cations.[24,25]

In this work, in order to investigate the effect of stirrer type in size and morphology of capsules, three device; ultrasonic stirrer, laboratory reactor, and mechanical stirrer were used for microemulsion formation and preparation alginate nanocapsules. Considering to importance of application of aromatic compound and herbal plants in different industries such as food, pharmaceutical, and cosmetic manufacturing, the peppermint essential oil was used as the core material. Finally, the effects of the various parameters on the morphology of obtained nanocapsules using different device were studied by scanning electron microscope (SEM), gas chromatography-flame ionization detector (GC-FID), gas chromatography-mass spectrometry (GC-MS), fourier transform infrared (FTIT) spectroscopy, and thermogravimetric analysis (TGA).

EXPERIMENTAL

Materials

Sodium alginate [Manutex FAV, ISP Alginates, (UK)] as wall material, calcium chloride (Merck, Germany) as a gelling agent, and Tween 20, (Merck KGaA, Darmstadt, Germany), Span 20 (Sigma-Aldrich) and Span 80 (Sigma-Aldrich) as emulsifiers were purchased. Fragrant peppermint oil as the core material was obtained from Barij Essence Pharmaceutical, Iran. Sodium citrate, ethyl acetate, and methanol were purchased from Merck, Germany. Water used in all experiments was purified by deionization.

Methods

The aim of this work is to prepare alginate nanocapsules containing peppermint oil and to investigate its properties. Figure 3 shows a schematic of the preparation of the alginate nanocapsules containing essential oils by microemulsion method.

Preparation of Nanocapsules

In this study, emulsified nanocapsules were prepared by use the sodium alginate solution as an aqueous phase, peppermint oil as the oil phase, and emulsifier. The microemulsion process was accomplished using the three devices (ultrasonic stirrer, laboratory reactor, and mechanical stirrer) to evaluate the influence of the type of stirrer applied on shape, size, and stability of the nanocapsules produced.

Production of Nanocapsules Using Ultrasonic Stirrer

A mixture of sodium alginate solution, peppermint oil, and emulsifier was produced, using a 200 W and 24 kHz ultrasonic wave generating device (UP200H Hielscher, Germany). Waves were transferred from the device to the mixture using a sonotrode 3 mm in diameter and made of titanium located at a depth of 1 cm below the surface of the liquid. The sonification process was done in certain times. Finally, the calcium chloride solution was added to the microemulsion thus obtained in order to produce nanocapsules. The process temperature was fixed at 25°C during the mixing operation.

Production of Nanocapsules Using Laboratory Reactor

Capsules were made using a laboratory reactor (IKA-Werke GmbH, Germany) equipped with a mechanical stirrer and homogenizer. To create the microemulsion, a certain ratio of oil, emulsifier and the alginate solution were mixed at 24,000 rpm. Then the calcium chloride solution added in order to make the ion crosslink.

Production of Nanocapsules Using Mechanical Stirrer

The alginate solution was procured by dissolving sodium alginate in deionized water. Then, 30 g of peppermint oil containing emulsifier was added. This mixture, enclosed in a chamber, was stirred vigorously by a mechanical stirrer (Greave Mixer, England) at 2000 rpm until it was emulsified and appeared creamy. Then, calcium chloride solution was added quickly to the mixture and stirred. Finally, the phase separation of oil/water microemulsion occurred.

Collecting Nanocapsules

To recover and collect nanocapsules, different methods such as deposing, flotation, and filtration by repeated washing were examined. The nanocapsules produced using a centrifuge for 5 min at a speed of 2000 rpm were isolated from the reaction environment and washed with deionized water twice.

Drying Nanocapsules

Two different methods were used for drying the nanocapsules. In the first method, the nanocapsules were rinsed in acetone

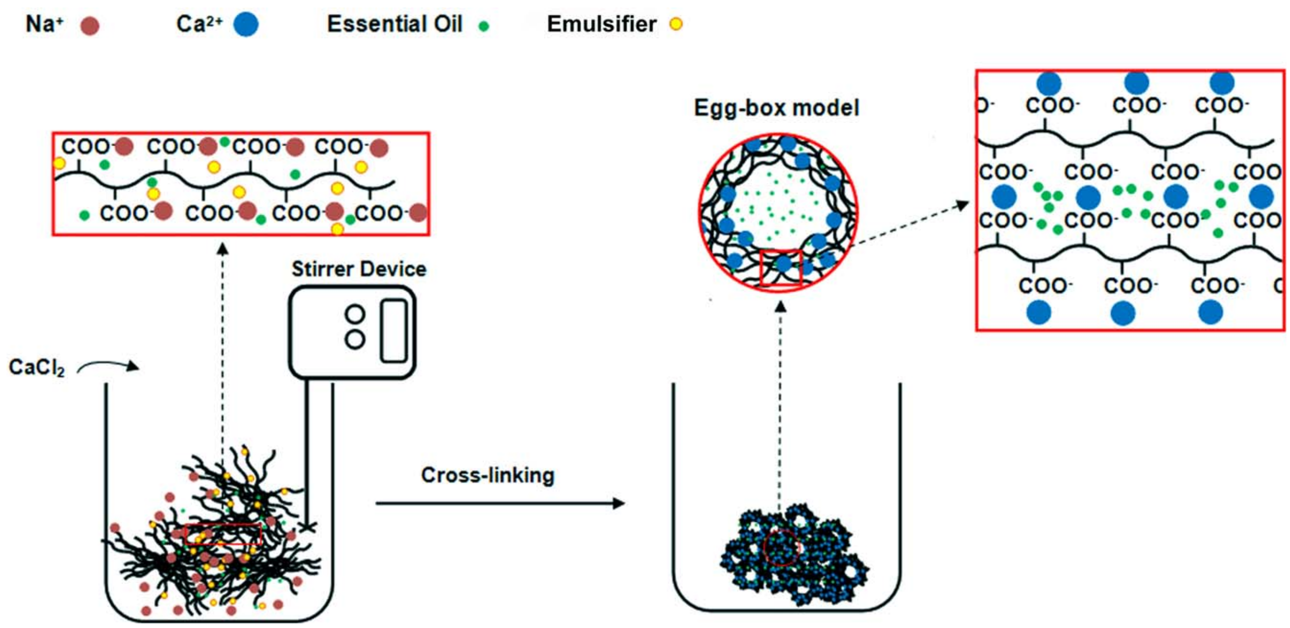

Figure 3. Preparation of the alginate nanocapsules containing essential oils by microemulsion method. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

several times to extract the residual water. Dry granules were subsequently obtained by allowing the residual acetone to evaporate from the nanocapsules (if necessary, they were exposed to a gentle flow of air for 0.5–1 h).

In the second method a freezing dryer (Heto-Holten, Denmark) was used. In this method, the capsules were frozen at 20°C for 12 h and then transferred to freeze dryer at 20°C under vacuum with pressure less than 0.61 kPa. They were left for 5 h during, which their water was vaporized completely.[8] The first method was preferable as it not only dries the nanocapsules rapidly but removes the residual oil as well. Moreover, it is appropriate in that it maintains and stabilizes materials and useful combinations, especially those sensitive to heat. Despite the intensive time and energy required in this method. The product is of higher quality.[26] The nanocapsules thus dried were easily kept at the ambient temperatures.

Investigation of Nanocapsules

The size and surface morphology of the nanocapsules was specified by SEM. For this purpose, nanocapsules were poured on a bilateral strip to be coated by a layer of gold particles before they were studied under a microscope.

Chemical compounds of the peppermint oil before and after encapsulation using different devices (ultrasonic stirrer, laboratory reactor, and mechanical stirrer) were recognized using GC-FID and GC-MS. The GC-FID apparatus was a HP-6980 Agilent gas chromatograph equipped with a flame ionization detector (FID) with a fused silica capillary column of HP-5MS (I.D. = 0.25 mm, 30 m long, and 0.25 μm film thickness and 5% phenyl methyl siloxane). GC-MS analysis was done using a HP 6890 GC system coupled with a 5973 network mass selective detector. The compounds of the essential oil were identified by comparison of their retention indices (RIs), mass spectra fragmentation with those on the stored Wiley 7n.1 mass computer library, NIST (National Institute of Standards and Technology) and data published in the literature of Adams.[27]

FTIR spectroscopy was used to examine the presence of essential oil in capsules. This study was done using a BOMEN MB-series 100 spectrophotometer (Hartman & Braun, Canada). Spectra were collected at a resolution of 4 cm^{-1} and given as the ratio of 21 single beam scans to the same number of background scans in pure KBr.

The stability of peppermint oil in the produced capsules was evaluated using a Thin Layer Chromatography (TLC) and Thermogravimetric Analysis (TGA). TLC was performed using silica gel plates in the moving phase of ethyl acetate/methanol. Alginate nanocapsules were solved in 0.1*M* sodium citrate, and the methanol served as an oil solvent. To study the thermal characteristics of the nanocapsules, 40 mg of the sample was heated to 600°C at an increasing rate of 10°C min^{-1} under N_2 steady flow.

RESULTS AND DISCUSSION

Alginate biopolymer is an anionic poly-electrolyte with too many ionized groups. Once ionized, the active carboxyl and hydroxyl group creates a poly ion with negative charges in the presence of water that can be easily linked with many oppositely charged ions. In preparing the microemulsion, constant stirring leads formation of spherical particles of different sizes. By adding calcium chloride, alginate chain crosslinks are quickly formed to produce nanocapsules.[28]

Effect of Some Process Variables on the Size and Shape of Nanocapsules

The size and shape of nanocapsules can be controlled by effective and appropriate changes in conditions of process. In this study, alterations were incurred in certain parameters to investigate their effects on the reduced size of nanocapsules prepared by ultrasonic stirrer, laboratory reactor, and mechanical stirrer.

It should be noted that the results of each parameter are obtained from the three times repetition of measurements.

Sodium Alginate Concentration

To study of the effect of sodium alginate concentration on the size and shape of nanocapsules, 0.2% solution of Tween 20 was used as emulsifier and the concentration of calcium chloride and the time of the process were 2% and 5 min, respectively. Concentration of sodium alginate was changed between 1, 1.5, 2, 2.5, and 3%. Sodium alginate as the capsules wall is affecting on the size and shape of prepared capsules, so that increasing sodium alginate concentration improved the viscosity of the solution and the liquid resistance against dispersion caused by stirring. The results of this study that are shown in Figure 4 confirm also the above statements. As it can be observed, a larger size capsules were formed with higher concentration. However, as has been predicted, the capsules prepared using the ultrasonic stirrer (63 nm by 1.5% sodium alginate to 125 nm by 3% sodium alginate) are small against to capsules prepared by the laboratory reactor (80 nm by 1.5% sodium alginate to 135 nm by 3% sodium alginate) and mechanical stirrer (98 nm by 1.5% sodium alginate to 150 nm by 3% sodium alginate). According to the obtained results, the optimum sodium alginate concentration is 1.5% that was used in other stages of work.

Type and Amount of Emulsifier

The distribution of particles during the emulsifying process increases with reducing surface tension. Therefore, it will be possible to produce small capsules in the presence of an emulsifier that can minimize surface tension. The emulsifier will also create a protective layer around the particles and prevents their binding together.[29] To investigate the type of emulsifier, the concentrations of calcium chloride and sodium alginate were considered 2% and 1.5%, respectively and the time of the process was set at 5 min. Also, 0.2% solutions of Tween 20, Span 20, and Span 80 were used in which the best results were obtained by Tween 20 (Figure 5). The capsules prepared using all three devices by Tween 20 (65, 88, and 90 nm) as the emulsifier are smaller than those prepared by Span 20 (78, 99, and 100 nm) and Span 80 (88, 110, and 112 nm). Tween 20 with wide applications in the encapsulation process is capable to reduce the evaporation rate of peppermint oil.

To study of emulsifier concentration, the concentrations of calcium chloride and sodium alginate and also time were constant and 0.1, 0.2, 0.3, 0.4, and 0.5% solutions of Tween 20 were used. The results indicate that using low amounts of emulsifier will increase the emulsifying capacity of the polymer. At high concentrations, however, over-changing the polymer structure will result in inadequate coverage of the water/oil interface and develops the nanocapsule diameter. As can be seen in Figure 6, the capsules prepared by the ultrasonic stirrer (60 nm by 0.1% Tween 20–127 nm by 0.5% Tween 20) are small than capsules prepared by the laboratory reactor (74 nm by 0.1% Tween 20–134 nm by 0.5% Tween 20) and mechanical stirrer (88 nm by 0.1% Tween 20–155 nm by 0.5% Tween 20). According to the above results, 0.1% solution of Tween 20 was selected as the optimum concentration of emulsifier.

Amount of Calcium Chloride

To investigation of the influence of calcium chloride concentration on the obtained capsules, 1.5% solution of sodium alginate, and 0.1% solution of Tween 20 were used and the encapsulation process was done during 5 min. Also, the calcium chloride concentration was changed between 0.05, 1, 2, 3, 4, and 5%. Figure 7 indicates the effect of calcium chloride amount in size and shape of capsules prepared by different devices. As it can be seen, increasing the amount of the used calcium chloride not only gives rise to smaller formed particles but also improves the final process yield. The cause of increasing absorption efficiency of emulsifier molecules on the surface of particles shows that they did not bind together and lead to formation of smaller capsules with a greater distribution. Also the capsules prepared by the ultrasonic stirrer (110 nm by 1% $CaCl_2$ to 56 nm by 5% $CaCl_2$) are small than capsules prepared

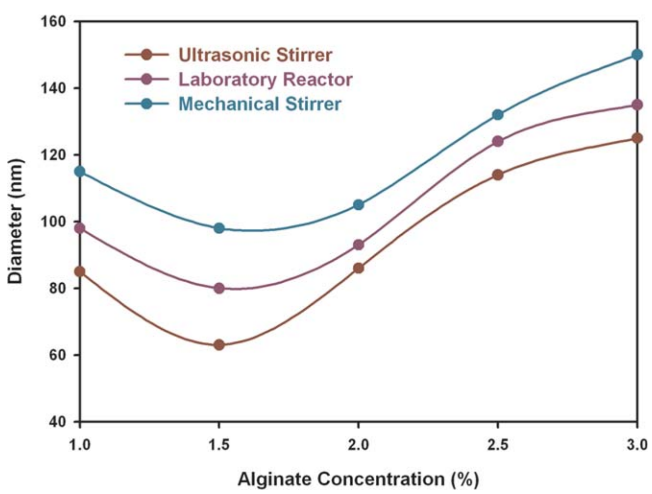

Figure 4. Effect of alginate concentration on the average size of nanocapsules using ultrasonic stirrer, laboratory reactor, and mechanical stirrer. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

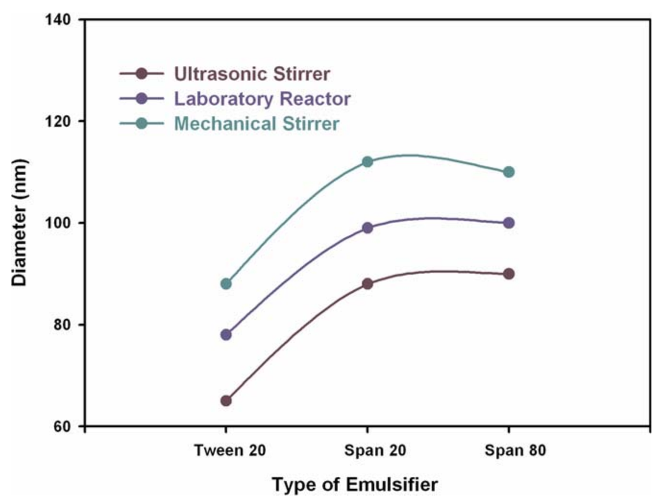

Figure 5. Effect of the type of emulsifier on the average size of nanocapsulesusing ultrasonic stirrer, laboratory reactor, and mechanical stirrer. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

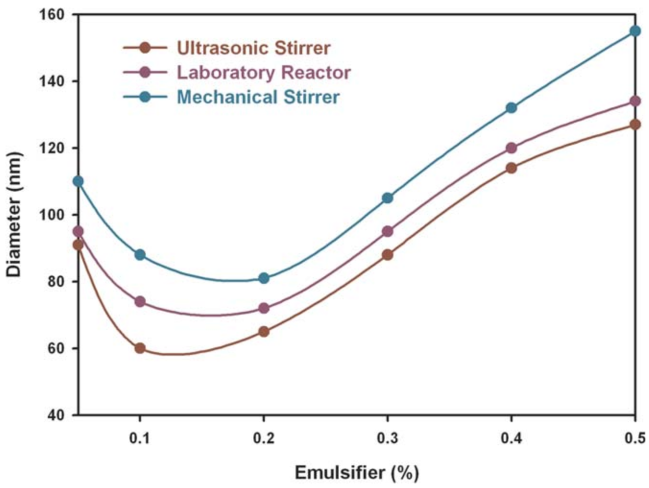

Figure 6. Effect of different amounts of emulsifier on the average size of nanocapsulesusing ultrasonic stirrer, laboratory reactor, and mechanical stirrer. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

by the laboratory reactor (121 nm by 1% $CaCl_2$ to 72 nm by 5% $CaCl_2$) and mechanical stirrer (128 nm by 1% $CaCl_2$ to 94 nm by 5% $CaCl_2$). Finally, the concentration of 5% was considered as the optimum calcium chloride concentration.

Time of Process

This stage was done at different times of 5, 10, 15, and 20 min and the concentrations of sodium alginate, calcium chloride, and Tween 20 were considered 1.5%, 5%, and 0.1%, respectively. The results of our experiments showed that the time of the process had a little effect on increasing the number of nanocapsules produced (Figure 8). In other words, the number of nanocapsules produced during the first minutes of the reaction did not increase as the process proceeded. Generally, the capsules prepared by the ultrasonic stirrer ($\approx$56 nm) are small than capsules prepared by the laboratory reactor ($\approx$74 nm) and mechanical stirrer ($\approx$95 nm) at the different times. Because of the obtained results, 5 min was considered as the optimum time of process.

SEM Images

The size and morphology of the capsules obtained by optimum conditions (1.5% solution of sodium alginate, 5% solution of calcium chloride, 0.1% solution of Tween 20, and the time of 5 min) was studied by SEM images. As can be observed in Figure 9, the nanocapsules produced by the ultrasonic stirrer (55–60 nm) are smaller in size than those produced by the laboratory reactor (65–75 nm) and mechanical stirrer (85–90 nm). It can, therefore, be claimed that the three stirring employed methods are capable of producing nanocapsules. The images also indicate the formation of spherical and small alginate capsules on the nanoscale. It should be noted that we prepared encapsulated peppermint oil by a coaxial jet electrospray technique in our previous works and the size of produced capsules was at micro-nanometer range.[8,9] In this work, we used a microemulsion technique and prepared alginate nanocapsules by changing affective parameters on their size.

GC-MS Analysis

To studying the efficiency of three devices in storage of peppermint essential oils, GC-FID, and GC-MS analysis were done. The results are indicated in Table I. Quantities of the most important compounds exiting in pure peppermint essential oil and nanocapsules obtained by ultrasonic stirrer, laboratory reactor, and mechanical stirrer are; menthol (49.30%, 49.12%, 49.11%, and 48.85%), menthone (18.38%, 18.21%, 18.22%, and 18.20%), piperitone (7.55%, 7.42%, 7.44%, and 7.38%), Menthofuran (6.52%, 6.45%, 6.42%, and 6.39%), Menthyl acetate (6.21%, 6.02%, 6.00%, and 5.95%), and 1,8-Cineole (3.30%, 3.30%, 3.28%, and 3.20%), respectively. These results showed that no significant changes are observed in the peppermint oil compounds during encapsulation by all devices, but generally, quantities of the remaining compounds in encapsulated

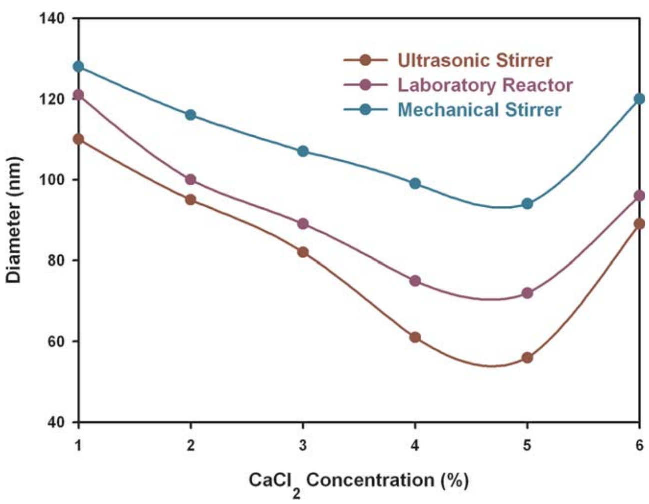

Figure 7. Effect of $CaCl^{-2}$ concentrations on the average size of nanocapsulesusing ultrasonic stirrer, laboratory reactor, and mechanical stirrer. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

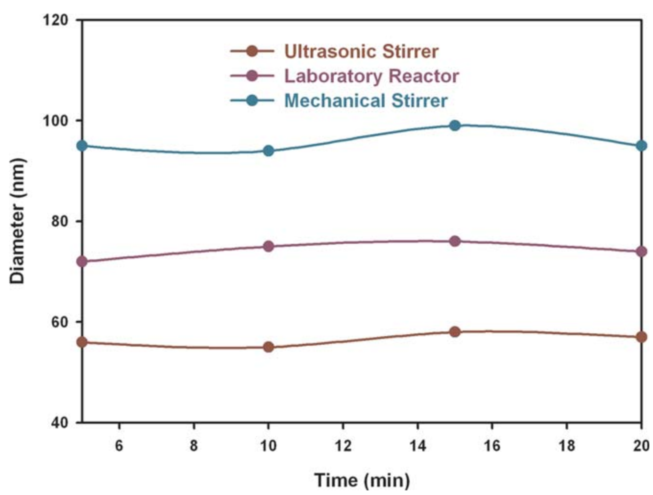

Figure 8. Effect of time on the average size of nanocapsulesusing ultrasonic stirrer, laboratory reactor, and mechanical stirrer. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

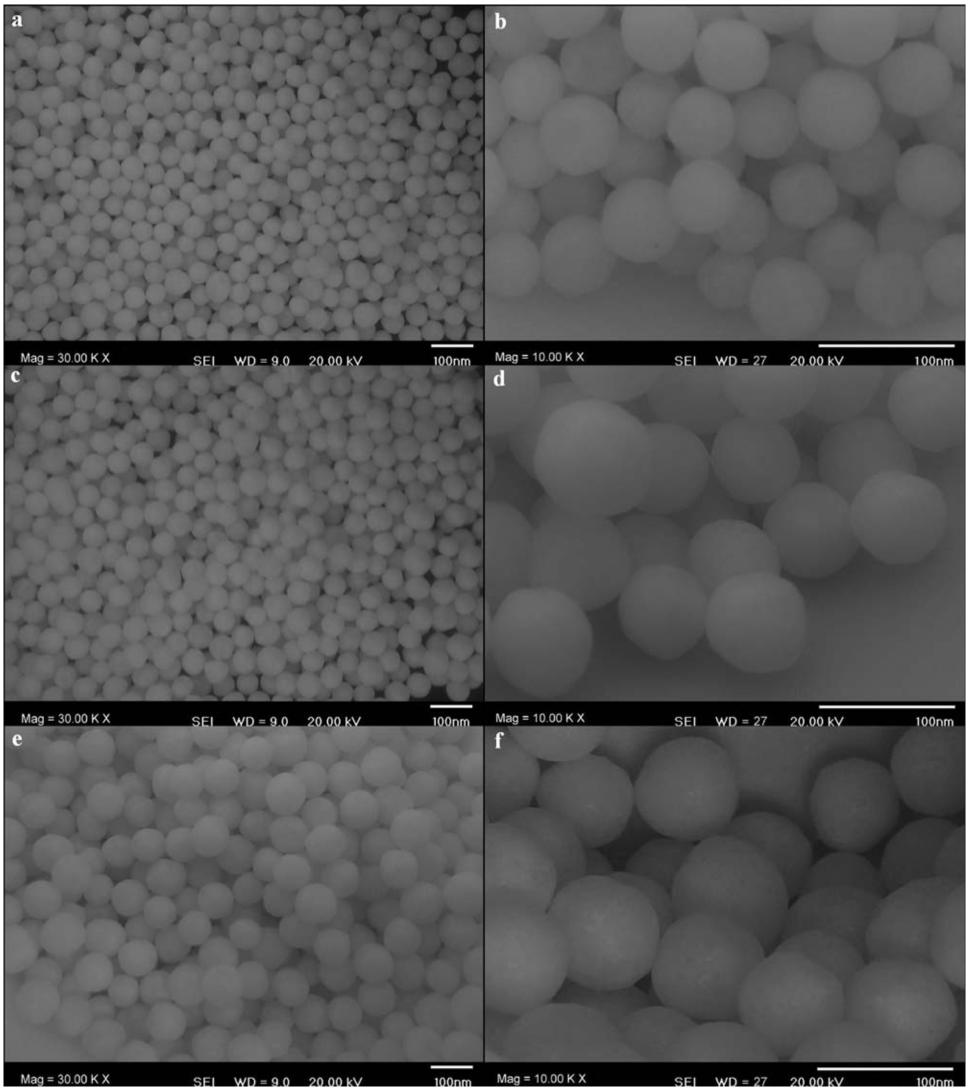

Figure 9. SEM photographs of nanocapsules obtained using (a, b) ultrasonic stirrer, (c, d) laboratory reactor, and (e, f) mechanical stirrer at different magnification.

peppermint essential oil by ultrasonic stirrer are higher than other devices.

Stability of Nanocapsules

The size of nanocapsules affects their quality and stability, so that the smaller the particles, the more stable they are. The results of our tests indicate that the nanocapsules produced by the ultrasonic stirrer were more stable than were those formed by the laboratory reactor and the mechanical stirrer. This may be due to differences in the type of stirrer blades.

Thin Layer Chromatography

Thin layer chromatography is a suitable way to check the amount of oil in the nanocapsules produced. On the basis of the requirements of this test and after the proper choice of a solvent system useful for almost any material, the value for R_f obtained for unknown samples (nanocapsules dissolved in sodium citrate) and that obtained for standard samples (peppermint oil) were found to be exactly the same, which explains the reason for identifying essential oil in nanocapsules.

FTIR

FTIR spectra of alginate and nanocapsules prepared by mechanical stirrer, laboratory reactor, and ultrasonic stirrer are indicates in Figure 10. The absorbance peaks at 3444, 1628, and 1428 cm^{-1} in alginate spectrum were attributed to stretching vibrations of O—H bonds, stretching vibrations of carbonyl groups (C=O) and bending vibrations of CH_2 groups, respectively. The appearance of two peaks at 1164 and 1293 cm^{-1} in spectra of encapsulated essential oils [Figure 10(b–d)] can be attributed to the stretching vibration of C—O bands in menthol that is the main compound of peppermint oil. Increasing the height of the peak in 1628 cm^{-1} may be due to the stretching vibration of carbonyl groups in various compounds of peppermint oil such as menthon. Also, the height of peak at 1428 cm^{-1} is increased in the spectrum of encapsulated essential oils. Above points are indicates the presence of oil in the produced capsules. On the other hand, increasing the height of the peaks (1164, 1293, and 1428 cm^{-1}) in spectra of nanocapsules prepared by ultrasonic stirrer shows the encapsulation efficiency is further by this method.

Table I. Some of the Composition of Pure Peppermint Oil and Encapsulated Peppermint Oil by Mechanical Stirrer, Ultrasonic Stirrer, and Laboratory Reactor

Compound	RIs[a]	RRIs[b]	Pure peppermint oil (%)	Ultrasonic stirrer	Laboratory reactor	Mechanical stirrer
α-pinene	938	939	1.34	1.22	1.20	1.16
β-Pinene	980	979	0.34	0.30	0.30	0.25
Limonene	1028	1029	0.62	0.50	0.45	0.45
1,8-Cineole	1032	1031	3.30	3.30	3.28	3.20
Menthone	1153	1153	18.38	18.21	18.22	18.20
Iso-menthone	1162	1163	3.58	3.52	3.45	3.46
Menthofuran	1166	1164	6.52	6.45	6.42	6.39
Menthol	1173	1172	49.30	49.12	49.11	48.85
α-terpineol	1190	1189	2.11	2.05	2.05	2.06
Carvone	1242	1243	0.16	0.15	0.15	0.13
Piperitone	1252	1253	7.55	7.42	7.44	7.38
Menthyl acetate	1293	1295	6.21	6.02	6.00	5.95
β-caryophyllene	1421	1419	1.46	1.40	1.34	1.32

[a] RIs: Retention Indices that obtained in our experiment.
[b] RRIs: Reference Retention Indices that published in the literature of Adams.[27]

Thermal Properties of Nanocapsules

To specify the effect of temperature on nanocapsules, TGA curves for nanocapsules without essential oil and with essential oil encapsulated by ultrasonic stirrer, laboratory reactor, and mechanical stirrer were obtained and compared. TG analysis determines the weight of the remaining materials according to their temperature changes. In TGA curves, horizontal areas related to temperatures that the compounds presents were stable. As shown in Figure 11, significant differences were observed between the weight loss percentage in samples containing oils and those without oils. This change in the weight of nanocapsules containing oil may be due to evaporation because the weight loss occurred at temperatures higher than 100°C where aromatic materials are volatile. Also, weight loss in nanocapsules prepared by ultrasonic stirrer was further than other nanocapsules. These results show that a greater amount of essential oil is stored in nanocapsules prepared by ultrasonic stirrer.

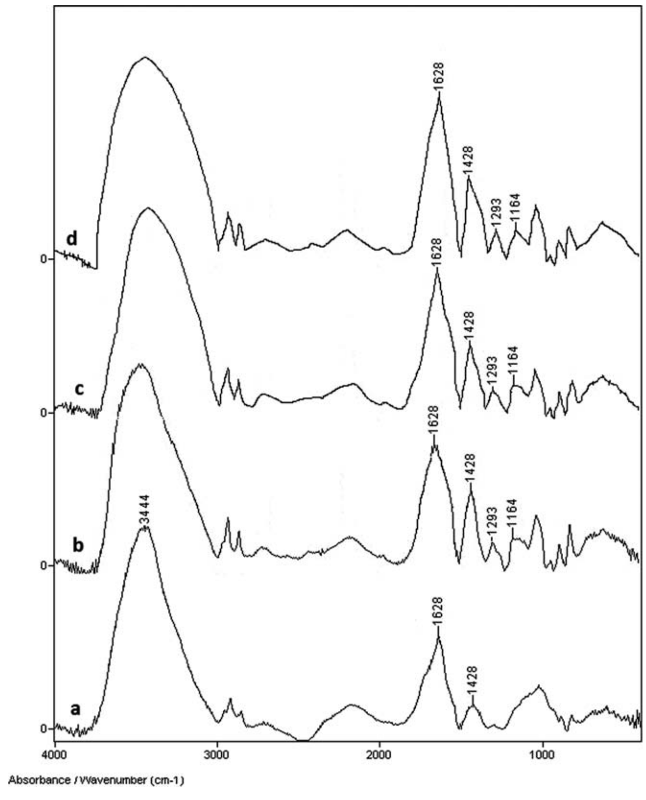

Figure 10. Infrared spectra of (a) alginate and encapsulated essential oils by (b) mechanical stirrer, (c) laboratory reactor, and (d) ultrasonic stirrer.

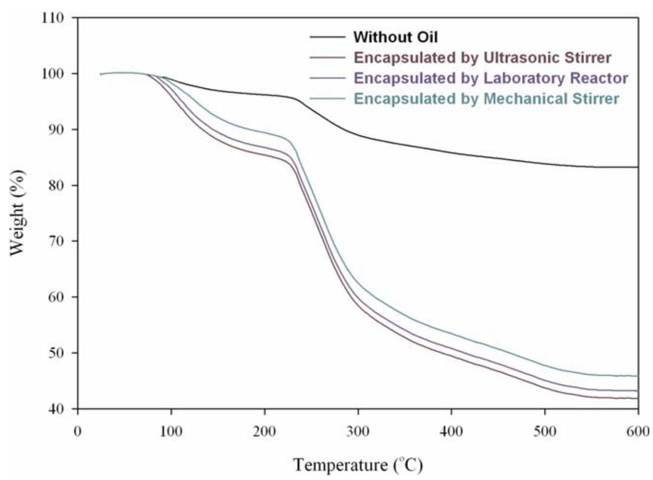

Figure 11. TGA curves of nanocapsules without essential oil and nanocapsules containing essential oil prepared by ultrasonic stirrer, laboratory reactor, and mechanical stirrer. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

CONCLUSIONS

The results indicate that it is possible to manipulate the retention and release of fragrant compounds by creating a specific microenvironment for these volatile materials. One of the major advantages of oil encapsulation in alginate capsules is that the encapsulation does not adversely affect the flavor release during the lifetime and use of the product.[30] In this research, the effects of ultrasonic stirrer, laboratory reactor, and mechanical stirrer on manufacturing alginate nanocapsules using the microemulsion method was investigated. Also, size distribution, morphologies, thermal properties, and GC/MS analysis and FTIR spectroscopy were examined. Producing microcapsules on the nanoscale demonstrated the ability of the three methods employed. The method of preparing a microemulsion is a significant parameter affecting the size and shape of nanocapsules. During the stirring operation, the liquid assumes different speeds while moving from the center (near the stirrer) toward the container walls; hence, there is no uniform distribution of shear stresses in the container, which explains why the nanocapsules have different sizes. It was found that type of stirrer, polymer concentration, as well as type and quantity of emulsifier are some of the effective factors on producing more homogeneous and smaller nanocapsules. The resulting nanocapsules were found to have a narrow size distribution and surface morphology with great smoothness while also capable to preserve the peppermint oil over long periods**.**

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