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Preparation of alginate nanocapsules containing turmeric oil

Pranee Lertsutthiwong [a,*], Khanittha Noomun [a], Nutthapon Jongaroonngamsang [b], Pornchai Rojsitthisak [b], Ubonthip Nimmannit [c]

[a] Center for Chitin-Chitosan Biomaterials, Metallurgy and Materials Science Research Institute, Chulalongkorn University, Soi Chula 12, Phyathai Road, Pathumwan, Bangkok 10330, Thailand
[b] Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand
[c] Pharmaceutical Technology Program (International), Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand

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ABSTRACT

To encapsulate turmeric oil, a model oily compound, with an alginate biopolymer coating, alginate nanocapsules were prepared in a three-step procedure using emulsification, crosslinking with calcium chloride, and solvent removal. The type of solvent, concentration of turmeric oil, sonication, and oil/alginate mass ratio affected the characteristics of the nanocapsules in terms of average size, zeta potential, morphology, loading capacity, and stability at 4 °C and 25 °C. Dissolution of turmeric oil in ethanol and presence of Tween 80® in the formulation were found to be optimal in the preparation process. An increase in the oil concentration or oil/alginate mass ratio resulted in an increase in the average size of the nanocapsules. To obtain uniform-sized nanocapsules, sonication is required. In addition, alginate nanocapsules show good physical stability in long-term storage at 4 °C and data on loss of oil in key steps in the process may facilitate improvement in the procedure to produce an increased loading capacity.

1. Introduction

Oily compounds are used widely in pharmaceutical and cosmetic applications (Edris, 2007). Oily drugs are conventionally prepared as emulsions and are preferably administered orally, but may be unacceptable to patients due to their taste (You et al., 2005). In cosmetic applications, poor aqueous solubility and instability of oily compounds cause problems in formulation and product stability (Müller, Petersen, Hommoss, & Pardeike, 2007). One approach to overcome these difficulties is to encapsulate oily compounds in capsules, and for a drug delivery system such an approach is more attractive if the size of the capsules is reduced to a nanometer scale (Bouchemal, Briançon, Perrier, & Fessi, 2004; Letchford & Burt, 2007; Müller-Goymann, 2004; Müller, Mäder, & Gohla, 2000; Pandey, Ahmad, Sharma, & Khuller, 2005). Biopolymeric nanocapsules are used in a variety of applications, and particularly in drug delivery systems. These nanocapsules are colloidal-sized, vesicular systems in which the drug is confined to a reservoir or within a cavity surrounded by a polymer membrane or coating (Soppimath, Aminabhavi, Kulkarni, & Rudzinski, 2001). Frequently, the vesicle comprises a core of oily liquid surrounded by a single layer of polymer, and the most common method of producing nanocapsules is by interfacial deposition of preformed polymers with subsequent solvent displacement (Fessi, Puisieux, Devissaguet, Ammoury, & Benita, 1989). However, some polymers are toxic and immunogenic when injected into the body (De & Robinson, 2003) and some require solvents that cause unwanted effects (Soppimath et al., 2001). To avoid these problems, sodium alginate, which is a naturally occurring biopolymer that is non-toxic and water soluble, was selected for preparation of nanocapsules.

Alginate is an anionic biopolymer produced by marine brown algae, and is of interest as a biopolymer to prepare nanocapsules owing to its good biocompatibility, biodegradability, non-toxicity, mucoadhesion, gelation, and film formation properties (Lapasin & Pricl, 1995; Phillips, Williams, & Wedlock, 1990). Alginate consists of linear chains of α-L-guluronic acid (G) and β-D-mannuronic acid (M) residues joined by 1,4-glycosidic linkages (Fig. 1) (Johnson, Craig, & Mercer, 1997). Soluble sodium alginate can be crosslinked using calcium chloride, with formation of insoluble calcium alginate particles on a nanometer scale depending on the concentrations of sodium alginate and calcium chloride (Rajaonarivony, Vauthier, Couarraze, Puisieux, & Couvreur, 1993). Alginate has been widely studied for particle formation in the size range of 100 nm to 2 mm for drug delivery (Anal & Stevens, 2005; Babu, Sairam, Hosamani, & Aminabhavi, 2007; Liu et al., 2004; Martins, Sarmento, Souto, & Ferreira, 2007; Rajaonarivony et al., 1993;

* Corresponding author. Tel.: +66 2 218 4221; fax: +66 2 611 7586.
E-mail address: Pranee.L@chula.ac.th (P. Lertsutthiwong).

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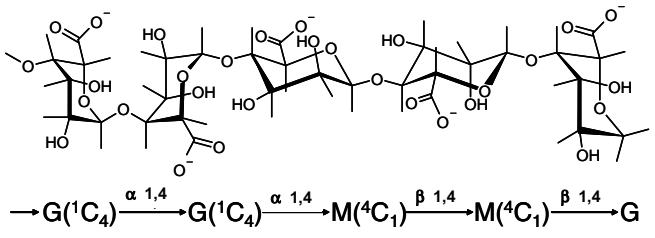

Fig. 1. Molecular structure of alginate.

Sarmento, Ferreira, Jorgensen, & van de Weert, 2007; Sarmento, Ferreira, Veiga, & Ribeiro, 2006; Zhao et al., 2007), but it has not been used for formulation of nanocapsules containing a volatile oily drug.

Turmeric (from the rhizome of *Curcuma longa*) belongs to the family of Zingiberaceae and is widely used as a food additive, condiment, and household medicine in Southern Asia. Turmeric is composed of curcuminoids, essential oil, sugars, oleoresin, and other compounds (e.g. minerals, vitamins, protein, etc.) (Leung, 1980). The essential oil, turmeric oil, in which the major constituent is ar-turmerone (2-methyl-6-(4-methylphenyl)-2-hepten-4-one) (Chang, Jong, Huang, Nien, & Chang, 2006; He, Lin, Lian, & Lindenmaier, 1998; Raina et al., 2002), is used in pharmaceutical applications for its antifungal (Apisariyakul, Vanittanakom, & Buddhasukh, 1995; Gowda, Malathi, & Suganthi, 2004), antibacterial (Negi, Jayaprakasha, Rao, & Sakariah, 1999), insect repellent (Roth, Chandra, & Nair, 1998), antioxidant, antimutagenic (Jayaprakasha, Jena, Negi, & Sakariah, 2002), and anticarcinogenic (Aratanechemuge et al., 2002) activities. However, turmeric oil itself is volatile and insoluble in water, and this has limited development of new formulations. Preparation of nanocapsules containing turmeric oil may provide a solution to these problems, and therefore the current study was undertaken to examine the influence of solvent, turmeric oil concentration, sonication, and turmeric oil/alginate mass ratio on the size, zeta potential, physical stability, and loading capacity in the preparation of alginate nanocapsules containing turmeric oil.

2. Materials and methods

2.1. Materials

Commercially available turmeric oil containing 21.5% (w/v) of ar-tumerone was provided by Thai-China Flavours and Fragrances Industry Co., Ltd., Ayuthaya, Thailand. Sodium alginate with a molecular weight of 80,000–120,000 Da and a low guluronic acid content ($F_G = 0.39$) was purchased from Sigma Chemicals, St. Louis, MO, USA. A 2% (w/v) aqueous solution of sodium alginate exhibited a viscosity of 2808 ± 43 cps at 25 °C. Standard ar-turmerone was purchased from ChromaDex™, Santa Ana, CA, USA. All other chemicals, including Tween 80®, acetic acid, ethanol and calcium chloride, were of analytical grade and acetonitrile was of HPLC grade.

2.2. Preparation of alginate nanocapsules

A sodium alginate solution was prepared by dissolution in ultrapure water at 50 °C for 45 min and filtration using glass microfiber filters (GF/C) for removal of any impurity before use. Turmeric oil was diluted with either ethanol or acetone. Alginate nanocapsules were prepared using o/w emulsification, followed by crosslinking using calcium chloride and solvent removal, using modified versions of the methods described by Bouchemal et al. (2004), De and Robinson (2003), and Malone and Appelqvist (2003). Briefly, the sodium alginate o/w emulsion was made by dispersion of diluted turmeric oil into appropriate volumes of 0.6 mg/ml alginate solution containing 1% Tween 80® (w/v) under continuous mechanical stirring at room temperature. An appropriate volume of 0.67 mg/ml $CaCl_2$ was then added into the resulting emulsion and stirred for an additional 30 min. The turmeric oil-loaded nanocapsule suspension was equilibrated overnight before removal of the solvent under reduced pressure at 40–45 °C for 20 min. Nanocapsules containing turmeric oil were obtained as a dispersion in aqueous solution.

2.3. Variation of parameters in the formulation

Factors affecting the size of nanocapsules were investigated with the turmeric oil to alginate ratio fixed at 1:1. Turmeric oil concentrations of 0.5–10% (w/v) in ethanol were used, the effects of using acetone and ethanol solvents were examined, and nanocapsule formation with or without surfactant (Tween 80®) was also investigated. Once the turmeric oil concentration was optimized, the effect of sonication on the size of the nanocapsules was investigated by varying the time of sonication from 0 to 60 min. Sonication was applied either before or after addition of diluted oil with a fixed turmeric oil to alginate ratio (1:1) and a fixed Tween 80® content (1% w/v). To study the influence of the turmeric oil to alginate mass ratio on the characteristics of the nanocapsules, formulations were prepared using mass ratios of 1:1, 1:2, 1:4, and 4:1, with a constant Tween 80® content of 1% (w/v). The effect of the preparation process on loading capacity was examined using the turmeric oil to alginate mass ratios of 0.1:1, 0.25:1, 0.5:1, and 1:1. The turmeric oil solutions were prepared from a stock ethanol solution of appropriate concentration.

2.4. Characterization of nanocapsules

The average size and zeta potential of alginate nanocapsules containing turmeric oil was measured using a Zetasizer model Nano-ZS, Malvern Instruments, England. Nanocapsules were visualized using atomic force microscopy (AFM, model multimode SPM, nanoscope 4, Veeco, USA). Freshly prepared nanocapsules were diluted with distilled water and deposited onto a glass substrate, followed by drying in a desiccator. The morphology of the nanocapsules was obtained using the tapping mode with a silicon canteliver (resonance frequency 280 KHz). Nanocapsules were also observed by transmission electron microscopy (TEM, model JSM-2100, Japan). The nanocapsule suspension was deposited onto a Formvar-coated copper grid and stained with a 1% uranyl acetate solution. The physical stability of the turmeric oil-loaded nanocapsules was determined by assessment of average size after storage at 4 °C and 25 °C over a period of 4 months.

The amount of turmeric oil in the formulated alginate nanocapsules was assayed using HPLC, according to the method of Chang et al. (2006) with modifications. Briefly, the sample was diluted with ethanol and injected onto an Altima C18 column (4.6 mm × 150 mm i.d., 5 μm particle size) at 33 °C. The mobile phase consisted of a mixture of acetonitrile and water (65:35, v/v). The injection volume was 20 μl, and elution was isocratic with a flow rate of 1.0 ml/min. Detection was carried out at 254 nm. The total chromatographic analysis time per sample was 25 min with ar-turmerone eluting at a retention time of 11.5 min. The ar-turmerone content in the commercially available turmeric oil was found to be 21.5% (w/v). Turmeric oil loading capacity was calculated from the total amount of turmeric oil in the nanocapsules after ultracentrifugation as a percentage of the total dry mass of nanocapsules.

2.5. Statistical analysis

All experiments were repeated a minimum of three times and measurements were performed in triplicate in each experiment. Results are presented as means ± SD. Statistical analysis was performed by one-way ANOVA using Microsoft Excel (Microsoft Corporation) with $p < .05$ considered to indicate statistical significance.

3. Results and discussion

3.1. Effects of solvent and turmeric oil concentration on formation of nanocapsules

Preparation of alginate nanocapsules containing turmeric oil was carried out using a multistep process of o/w emulsification, gelification, and solvent removal. Dispersion of diluted turmeric oil in an aqueous alginate solution containing Tween 80® caused immediate formation of micelles with an oil core. The alginate shell was then solidified by crosslinking with calcium chloride, and subsequently the solvent (ethanol or acetone) used to dissolve the turmeric oil was removed from the system by evaporation under pressure.

Previous work (Lertsutthiwong et al., unpublished data) suggested that formation of turmeric oil-loaded nanocapsules using the proposed protocol is unsuccessful if the turmeric oil is not diluted before use, with appearance of droplets of oil on the surface of the aqueous solution being a particular problem. Therefore, turmeric oil was diluted in ethanol or acetone at a concentration of 1% (w/v) before use. Dropwise addition of turmeric oil in acetone into sodium alginate solution containing Tween 80® resulted in immediate turbidity, whereas use of ethanolic turmeric oil gave a less turbid solution. Nanocapsules formed with turmeric oil in acetone were larger than those formed with ethanol as solvent ($p < .05$), but the zeta potentials of the capsules did not differ significantly ($p > .05$) between the solvents (Table 1). The effect of ethanol and acetone on the average size of the nanocapsules can be explained by the lipophilicity and dielectric constant of the solvents. Acetone is more lipophilic ($\log P = -0.24$) and has a lower dielectric constant ($k = 20.7$) compared to ethanol ($\log P = -0.30$ and $k = 24.3$). Therefore, acetone used to solubilize turmeric oil slowly diffused from the oil droplet into the aqueous phase during nanocapsule formation, causing a large particle size for the nanocapsules. In contrast, ethanol is more miscible with water and diffused from the oil droplet into the aqueous phase much faster than acetone, resulting in smaller nanocapsules.

Turmeric oil in ethanol was able to form oil droplets in alginate solution without surfactant, but the average size of the nanocapsules was larger and zeta potential was more negative than in the presence of surfactant ($p < .05$), as shown in Table 1. These results suggest that interactions occurred among the compounds in the system. That is, Tween 80® formed a micelle surrounding the oil droplets and the hydrophilic head of Tween 80® bound with the alginate shell following crosslinking using $CaCl_2$. This led to a reduction in charge density on the surface of the alginate nanocapsules, resulting in a less negative zeta potential. In addition, the average size of the nanocapsules in the presence of surfactant was about 60% smaller than those formed without Tween 80®. This may be accounted for by a decrease in the interfacial tension and migration of the insoluble polymer towards the o/w interface, where it is deposited to form the nanoparticle membrane (Fessi et al., 1989).

Table 1
Effects of solvent and surfactant on average size and zeta potential of alginate nanocapsules containing turmeric oil (oil/alginate ratio of 1:1)

Solvent	Surfactant (Tween 80®)	Average size (nm)	Zeta potential (mV)
Acetone	Yes	373 ± 100	−17.3 ± 2.2
Ethanol	Yes	263 ± 68	−17.4 ± 1.2
Ethanol	No	677 ± 336	−41.2 ± 0.5

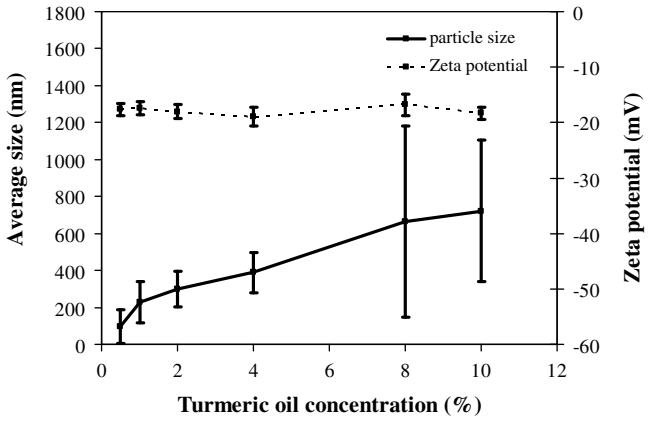

Fig. 2. Effect of turmeric oil concentration on average size and zeta potential of alginate nanocapsules produced using mechanical stirring.

As expected, an increase in the turmeric oil concentration resulted in a significant increase in the average size of the nanocapsules ($p < .05$) (Fig. 2), since the droplet contains a higher concentration of turmeric oil and relatively less ethanol. With less ethanol to diffuse out of the droplet during nanocapsule formation, a higher volume of oil is included in the inner phase of the capsules during preparation, and this leads to an increased capsule size (Peltonen, Koistinen, Karjalainen, Häkkinen, & Hirvonen, 2002). To get the average size <300 nm, a turmeric oil concentration of $\leqslant$2% was required (Fig. 2). However, the zeta potential of the nanocapsules showed no dependence on turmeric oil concentration ($p > .05$).

3.2. Effect of sonication on nanocapsule size homogeneity

With mechanical stirring only, the nanocapsules showed a wide range of sizes at each concentration of turmeric oil (Fig. 2). This suggests that stirring is insufficient to prepare nanocapsules of uniform size. Landfester, Bechtold, Tiarks, and Antonietti (1999) and Pérez-Maqueda, Duran, and Pérez-Rodríguez (2005) suggested that sonication was essential for production of uniform-sized nanocapsules, and that the size of the nanocapsules could be controlled by the time of sonication. Therefore, the current study was carried out by applying sonication for 0, 15, 30, and 60 min either before or after addition of turmeric oil to the sodium alginate solution. The results indicated that the average size of nanocapsules was more uniform than that achieved with mechanical stirring only, regardless of the timing and extent of sonication (Fig. 3). For example, the average size of nanocapsules without sonication was 199 ± 37 nm, whereas a size of 83.6 ± 4.4 nm was achieved with sonication for 15 min after addition of oil. The zeta potential showed little variation with the timing or period of sonication, remaining within a range of −13 to −20 mV for all periods of sonication (Fig. 3).

An increased sonication time either before or after addition of turmeric oil resulted in smaller capsules (Fig. 3), but sonication applied after addition of turmeric oil gave the smallest capsules; for example, sonication for 15 min after adding turmeric oil produced nanocapsules that were about 50% smaller than those formed with sonication before addition of oil. Sonication of the sodium alginate

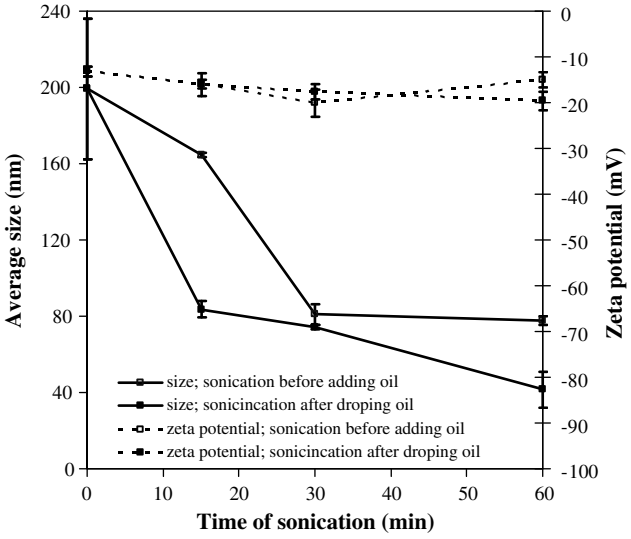

Fig. 3. Effect of sonication before and after addition of turmeric oil on average size and zeta potential of alginate nanocapsules.

solution before dropwise addition of oil may cause some degradation of the sodium alginate chains in aqueous solution via mechanical force (Wasikiewicz, Yoshii, Nagasawa, Wach, & Mitomo, 2005), and the presence of degraded lower molecular weight polymer could conceivably produce smaller nanocapsules relative to those formed with a higher molecular weight polymer. However, the more important process may be disturbance of oil droplets surrounding the alginate wall via sonication after addition of oil, which might split the capsules into larger numbers of smaller nanocapsules with a uniform size. The morphological surface of the uniform-sized nanocapsules formed using ethanol as a solvent appeared to be round (Fig. 4), as also observed by Peltonen et al. (2002).

The results also indicate that the optimal sonication time depends on the size requirement. For example, the dynamic light scattering data in Fig. 3 show that preparation of nanocapsules of <100 nm requires sonication for 15 min after dropwise addition of oil. To confirm the dynamic light scattering data, capsule sizes were determined by AFM and TEM following preparation of nanocapsules under these conditions. The morphology and horizontal width of nanocapsules determined by AFM are shown in Fig. 4a, and these data indicate a nanocapsule diameter of 83.5 nm. Similarly, the average size of nanocapsules estimated from TEM was about 95 nm (Fig. 4b). Therefore, both AFM and TEM give nanocapsule sizes in good agreement with the sizes predicted from light scattering data.

3.3. Effect of the core-to-wall ratio

The influence of the oil/alginate (core-to-wall) ratio on the characteristics of nanocapsules containing turmeric oil is shown in Table 2. An oil/alginate mass ratio of 1:1 gave nanocapsules almost 30% smaller than those formed at a ratio of 1:2, and a 1:4 ratio produced an aggregated gel. An increase in the amount of oil to give a 4:1 ratio resulted in a large increase in the size of the capsules ($p < .05$). A higher amount of alginate resulted in a more negative zeta potential, whereas the zeta potential did not show a strong dependence on the amount of oil in the preparation (1:1 vs. 4:1 ratios, Table 2). The observation is in agreement with that of Vanichtanunkul, Vayumhasuwan, and Nimmannit (1998), and it was concluded that a high core-to-wall ratio resulted in an increased particle size.

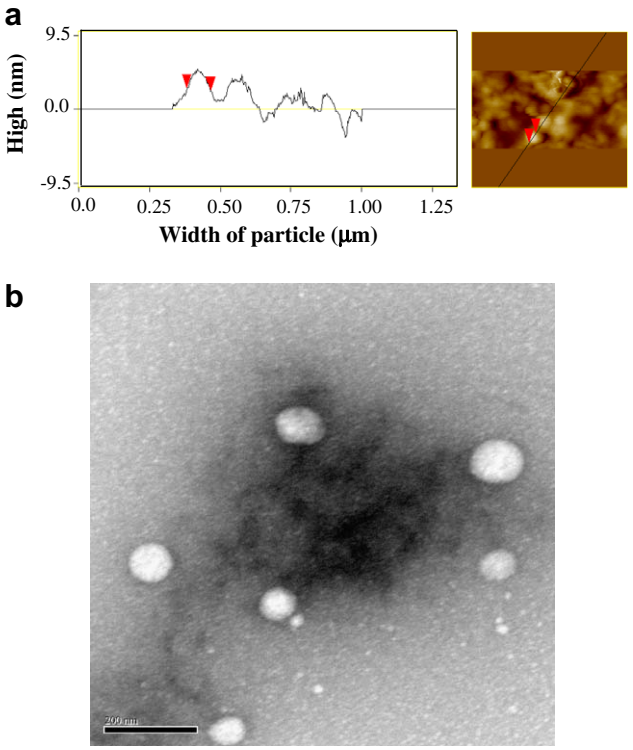

Fig. 4. Morphology and size of turmeric oil-loaded alginate nanocapsules prepared with 15 min of sonication after dropwise addition of turmeric oil in ethanol at a concentration of 2% (oil/alginate ratio of 1:1). (a) AFM characterization of nanocapsules indicating a diameter of 83.5 nm. (b) TEM characterization of nanocapsules, indicating an average size of about 95 nm.

Table 2
Effect of turmeric oil/alginate ratio on average size and zeta potential in formulations prepared with sonication for 15 min after addition of turmeric oil

Oil/alginate mass ratios	Average size (nm)	Zeta potential (mV)
1:1	78.0 ± 0.6	−14.3 ± 0.3
1:2	116 ± 14	−24.3 ± 2.7
1:4	Gel aggregate	−32.5 ± 3.3
4:1	234 ± 21	−16.4 ± 0.6

3.4. Physical stability

The effects of storage temperature (4 °C and 25 °C) and duration of storage on the physical stability of nanocapsules containing turmeric oil (oil/alginate ratio = 1:1) were evaluated. The size of the capsules was unchanged with storage at 4 °C for 45 days, but at 25 °C the size increased by about 14% (Table 3). The zeta potential increased by about 50% over 45 days of storage at 4 °C and 25 °C. Storage for 90 days at 25 °C resulted in an increase in size of about 25%, with no further change after storage for 120 days. In contrast,

Table 3
Physical stability of turmeric oil-loaded alginate nanocapsules with a turmeric oil/alginate ratio of 1:1

Time (days)	Average size (nm)		Zeta potential (mV)	
	4 °C	25 °C	4 °C	25 °C
0	78.0 ± 0.6	78.0 ± 0.6	−14.8 ± 1.2	−14.8 ± 1.2
45	75.0 ± 0.6	89.2 ± 1.1	−21.5 ± 3.1	−23.5 ± 2.6
90	66.3 ± 2.5	97.9 ± 0.7	−21.7 ± 3.2	−15.0 ± 2.8
120	66.2 ± 5.4	96.8 ± 0.6	−22.3 ± 1.2	−15.7 ± 1.8

Table 4
Effect of the preparation process on the amount of turmeric oil in the system

Oil/alginate ratio	Solvent evaporation	Amount of turmeric oil (mg)				Loading capacity (%)
		Total added	In suspension	In supernatant	In nanocapsules	
0.1:1	Yes	1.20	0.74	0.48	0.05	0.78 ± 0.01
0.25:1	Yes	2.91	1.80	1.23	0.12	1.62 ± 0.15
0.5:1	Yes	5.79	3.61	2.36	0.25	3.21 ± 0.20
1:1	Yes	11.66	6.55	3.45	0.39	5.47 ± 0.21
1:1	No	11.75	8.26	4.06	0.64	10.38 ± 0.50

the size of the nanocapsules decreased after storage at 4 °C for 90 days and remained at this size after 120 days. The preferred temperature for storage of the nanocapsules was 4 °C, since aggregation occurred more quickly at 25 °C.

3.5. Loading capacity

The loading capacity of turmeric oil in the alginate nanocapsules increased in proportion to the amount of turmeric oil added in the process (Table 4). A loading capacity of 1.62 ± 0.15% (based on the dry mass of nanocapsules) was obtained with an oil/alginate ratio of 0.25:1 and the loading capacity reached 3.21 ± 0.20% and 5.47 ± 0.21% at oil/alginate ratios of 0.5:1 and 1:1, respectively. However, the loading capacity of turmeric oil in alginate nanocapsules is relatively low under all conditions, because turmeric oil is volatile and easily lost during processing. Teixeira, Alonso, Pinto, and Barbosa (2005) have also suggested that a drug with low affinity for the polymer might diffuse from the organic phase to the external aqueous medium during formation of nanoparticles, thus leading to a low drug loading capacity.

The loading capacities of 5.47 ± 0.21% and 10.4 ± 0.50% were obtained for processes with and without solvent evaporation, respectively. Without solvent evaporation, about 30% of the turmeric oil was lost during formation of the turmeric oil-loaded nanocapsules (Table 4). Addition of solvent removal to the process caused a total loss of about 42% of the turmeric oil, suggesting that the evaporation process is responsible for loss of 14% of the total oil. About a further 40% of the oil was lost during separation using ultracentrifugation at 45,000 rpm for 1 h.

4. Conclusion

Alginate is an effective biopolymer for encapsulation of a volatile oily drug using a three-step process of o/w emulsification, gelification, and solvent removal. Sonication is required for size homogeneity and the choice of solvent for the oil and the presence of a surfactant influence the size of the nanocapsules. Dissolution of turmeric oil in ethanol and inclusion of Tween 80® in the sodium alginate were found to be optimal in the preparation process. Alginate nanocapsules also show good physical stability in long-term storage at 4 °C. However, the loading capacity of turmeric oil in the nanocapsules is relatively low. To address this issue, the loss of turmeric oil in key steps in the process was quantified and these data may facilitate improvements in the procedure to produce an increased loading capacity.

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