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ABSTRACT

Background: Microencapsulation has received increasing attention across various industries, including pharmaceutics, cosmetics, foods, and biotechnology because of its many benefits. One method to prepare microparticles is an ionic gelation technique because of its simplicity and mild processing conditions. In this study, alginate beads encapsulating rice bran oil (RBO) for cosmetic applications were prepared using ionic gelation through vibration-nozzle coextrusion technology. **Objective**: The research aimed to investigate the effect of process parameters on the physical properties of alginate beads. **Methods**: Alginate beads encapsulating RBO were produced using Encapsulator B-390 with a concentric nozzle system. The shell material comprised 2% w/w sodium alginate, while 1.5% w/v calcium chloride served as the cross-linking agent. The study focused on the effects of various process parameters, including frequency, amplitude, electrode tension, and inner-outer flow rates on physical appearance, encapsulation of RBO in alginate beads, particle size, and size distribution. An experimental design was implemented to achieve alginate beads with optimal morphological characteristics. **Results**: The findings demonstrated that all investigated process parameters significantly influenced the physical properties of the alginate beads. The optimum process parameters were a frequency of 40 Hz, an amplitude of 6, an electrode tension of 0 V, an inner flow rate of 4 mL/min, and an outer flow rate of 14 mL/min. The resulting alginate beads exhibited an average size of 2.07 ± 0.27 mm, a spherical shape, and RBO encapsulation in the core, surrounded by a uniformly thick shell. **Conclusion**: These process parameters can be applied to develop alginate beads encapsulating cosmetic oils within a core-shell structure for application in cosmetic products.

Key words: Alginate, Beads, Encapsulator B-390, Process parameters, Rice bran oil

INTRODUCTION

Microencapsulation is a process of entrapment of an active substance (core), in liquid, solid particle, or gas form, inside the other material (shell)[1,2] to produce small particles. The objectives of microencapsulation are to protect reactivity between core substance and environment, convenient handling and transportation, easy to add to other components, able to modulate the release rate of core substance, mask unpleasant properties of active substance, and add value to a commercial product.[3,4] Microencapsulation has received increasing attention in industries, such as pharmaceutics, cosmetics, foods, and biotechnology. One method to prepare microparticles is an ionic gelation technique, which is a physicochemical method on ionic interaction between a polymer and a polycationic or polyanionic compound,[5] such as sodium alginate and calcium chloride, that is recommended for hydrophobic active substances. Ionic gelation can be operated by various processes such as atomization, extrusion and co-extrusion, or electrostatic pulverization. This method uses a simple and mild condition because it is conducted under room temperature, gently stirring, without using an organic solvent in a process, and can scale up industrially.[6,7] Co-extrusion process is capable of producing core-shell microcapsules through a vibration nozzle technology, that breaks a laminar fluid flow into droplets through a vibration. When these droplets fall into a gelling bath, the polymer membrane (shell) will cover the active ingredient (core).[8,9] To achieve

the desired characteristics of beads, such as a spherical shape, uniform and appropriate size to enhance visual appearance, and clear visibility of the active ingredients in cosmetic formulations, it was crucial to optimize the process parameters during their preparation using this technique. Furthermore, for effective core-shell encapsulation, the thickness of the shell surrounding the oil core should be consistently uniform.

Alginate has been widely used as a shell material because of its capability to form stronger gel structures in the presence of divalent cations (e.g., calcium ion), chemical stability, hydrophilicity, biocompatibility, biodegradability, non-toxicity and convenience of use. Alginate is a linear polysaccharide biopolymer obtained from brown seaweed and bacteria.[10,11] It consists of two monosaccharide units, α-L-guluronic acid (G) and β-D-mannuronic acid (M) joined by 1,4-glycosidic linkage to form monopolymeric G- or M-blocks and heteropolymeric MG-blocks. Gelation occurs when polyvalent cations participate in the interchain bonding between G-block in polymeric chain, creating a three-dimensional network. This binding zone between G-block forms cavity, which serves as binding sites for divalent cations, especially calcium ions, fitted into structures according to the egg box model.[8,12]

Rice bran oil (RBO) is a source of bioactive phytochemicals with rich antioxidant activities, such as gamma-oryzanol, tocopherols, and tocotrienols, and useful for topical applications. RBO has UV protection properties, can increase skin elasticity, and acts as a skin moisturizer,[13-15] therefore, it is often used in cosmetic industries.

This study aimed to prepare alginate beads encapsulating RBO by an ionic gelation method using Encapsulator B-390 with co-extrusion technology. Effects of the process parameters including vibration frequency, amplitude, electrode tension, and flow rates of inner and outer nozzles were investigated and optimized on physical characteristics, bead size and size distribution, and encapsulation of RBO in core-shell beads.

MATERIALS AND METHODS

Materials

Sodium alginate from brown algae (Product No. 71238, Lot No. BCBS1047V) was purchased from Sigma Aldrich (Norway). Refined RBO was purchased from Tropicalife Company Limited (Thailand). Calcium chloride dihydrate was purchased from Merck (Germany).

Preparation of Alginate Beads

Sodium alginate solution at a concentration of 2%w/w was prepared and kept at room temperature for 12 h to obtain a fully hydrated solution. The 1.5%w/v calcium chloride dihydrate solution was prepared as a cross-linking agent for sodium alginate. RBO-encapsulated alginate beads were obtained by ionic gelation method at room temperature using Encapsulator B-390 (Buchi, Germany) with a concentric nozzle system containing an inner nozzle diameter of 450 μm and an outer nozzle diameter of 900 μm. Effects of equipment variables including vibration frequency, amplitude, electrode tension, and flow rates of inner and outer nozzles were investigated on the physical properties of beads. In the first step, the process parameters of two levels were studied with a factorial design at the frequency of 40 and 200 Hz, the amplitude of 6 and 9, and the electrode tension of 0 and 1000 V [Formulations no. 1-8, Table 1]. The air pressure of 500 mbar, inner nozzle flow rate of 4 mL/min, and outer nozzle flow rate of 12 mL/min were fixed because they produced stable droplets of laminar fluid. The optimum process condition providing RBO-alginate beads with a spherical shape, narrow size distribution, and RBO encapsulation at the center of each bead with a uniform alginate shell thickness was selected to further study the effect of flow rates on the bead properties. The inner and outer nozzle flow rates were studied with a factorial design at three levels in the range of 4–8 mL/min and 12–16 mL/min, respectively, to obtain the perfectly formed core-shell beads. Upon formation, the beads were left in the gelling bath with 1.5% calcium chloride solution for 30 min with continuous stirring to complete the gelling. After that, the beads were collected using a sieve, thoroughly washed three times with Ultrapure® water to remove any residual calcium chloride solution from the bead surface, and then characterized for the subsequent analyses.

Characterization of RBO-alginate Beads

Physical appearance

The physical appearance of the wet beads was evaluated by visual examination and recorded by a digital camera. The

Table 1: Effect of the frequency, amplitude, and electrode tension on the size and size distribution of RBO-alginate beads (mean±SD, n=30)

Formulation No.	Formulation Code*	Process parameters			Particle size (mm)
		Frequency	Amplitude	Electrode tension	
1	F40A6E0	40	6	0	1.98±0.31
2	F40A6E1000			1000	1.77±0.43
3	F40A9E0		9	0	2.42±0.73
4	F40A9E1000			1000	2.02±0.35
5	F200A6E0	200	6	0	1.83±0.41
6	F200A6E1000			1000	1.86±0.44
7	F200A9E0		9	0	1.91±0.53
8	F200A9E1000			1000	1.83±0.46

*F: Frequency, A: Amplitude, E: Electrode tension

appearance of alginate beads should be a spherical shape and smooth surface.

RBO encapsulation in alginate beads

The core-shell structure of RBO-alginate beads was observed by visual examination and recorded by a digital camera. RBO should be encapsulated at the core of the bead and enclosed with uniform alginate shells to confirm complete encapsulation within the core-shell structure.

Particle size and size distribution

Particle sizes of alginate beads were measured with a digital Vernier caliper (Fowler Sylvac, Switzerland) by analyzing 30 beads for each formulation. The beads should have a uniform size with a narrow size distribution (no more than ±20% of the average size) and be sufficiently large (approximately 2–3 mm) to enhance visual appearance.

Data Analysis

All data were reported as mean ± standard deviation (SD). The statistical data of the frequency, amplitude, and electrode tension effects were performed using a three-way analysis of variance (ANOVA). Data of inner and outer nozzle flow rates effect were analyzed by two-way ANOVA followed by Tukey's and Bonferroni's multiple comparison tests. A significant difference was determined at $P < 0.05$.

RESULTS AND DISCUSSION

In the present study, the concentrations of sodium alginate and calcium chloride were determined based on our preliminary findings, using an inner nozzle diameter of 450 μm and an outer nozzle diameter of 900 μm. A 2% w/w sodium alginate solution produced spherical beads encapsulating RBO and less residue of fragmented alginate beads. Lower concentrations resulted in tear-shaped beads, while higher concentrations led to increased viscosity, producing pear-like beads. Furthermore, a 1.5% w/v calcium chloride solution was sufficient to generate beads with suitable mechanical properties for skin application.

RBO-encapsulated alginate beads in the core-shell structure could be prepared using Encapsulator B-390 with vibration-nozzle coextrusion technology. In the first step, the effects of process parameters including frequency, amplitude, and electrode tension were simultaneously investigated on the physical properties of beads. The result showed that alginate beads were primarily spherical, with some displaying a partially oval shape. Furthermore, the alginate shell could encapsulate RBO into the beads which could be observed by visual examination. Because the RBO has a dark yellow color, the alginate beads would be seen as a pale yellow core surrounded by a thin shell of alginate. The border and thickness of the shell were clearly observable in the beads remaining in the $CaCl_2$ solution. Only the formulations no. 1–2 (low frequency (40 Hz), low amplitude (6) with or without electrode tension) produced the completely formed beads, whereas the formulations no. 3–8 had some residue of calcium alginate fragments without RBO entrapment.

The size and size distribution of alginate beads prepared with different frequency, amplitude, and electrode tension parameters are present in Table 1. The average bead sizes of all formulations ranged from 1.77 to 2.42 mm. A three-way ANOVA indicated a significant difference in the bead size among each parameter ($P < 0.05$). The interactions between frequency and amplitude and between frequency and electrode tension had also a significant effect on the bead size ($P < 0.05$). The effect of frequency on their particle sizes was significantly obvious at the high amplitude of 9 and the electrode extension of 0 V ($P < 0.05$). The high amplitude had a strong intensity of vibration resulting in a high ability to split the droplet from the nozzle and the electrode tension of 0 V meant no effect of electrical force involved causing the bead size afforded by gravity force; therefore, the frequency affected significantly the droplet size. The high frequency (200 Hz) gave the alginate beads with significantly smaller size than the low frequency (40 Hz) ($P < 0.05$). The frequency is defined as the vibration that breaks the flow rate applied in the unit producing beads and affects the size of the droplet. A high frequency generated a smaller droplet size, in other words; as the frequency decreased, the droplet size increased.[6,16]

Moreover, the amplitude also had a significant effect on the size of alginate beads ($P < 0.05$). The bead size was bigger at the high amplitude and smaller at the low amplitude. This result became significantly apparent at the low frequency (40 Hz) providing slow vibration of nozzle and larger droplet size regardless of the electrode tension ($P < 0.05$). When the high amplitude was applied, the intensity of vibration became stronger making the bead size uncontrollable and resulting in larger size. Normally, amplitude refers to the intensity of vibration having minimal influence on the beads. However, too high value of the amplitude can cause an unstable laminar fluid[16] resulting in unstable particle size and wide size distribution.

In addition, the electrode tension resulted in the significantly smaller size of beads, especially at the low frequency and the high amplitude because these conditions provided the bigger droplet size ($P < 0.05$). The electrode tension created the electrical force which had a stronger ability to split the droplet than the gravity force and resulted in a smaller bead size. The previous study[17] provided similar results that when the electrode tension was increased, the sizes of alginate beads were smaller. Alginate solutions would be under gravity and electrical force when they were injected from a nozzle. Normally, a stronger electrical force from high electrode tension can split the solution droplets formed under gravity and results in smaller beads. However, some research reported that the electrode tension did not affect bead size, but only prevented agglomeration.[6,16]

From the results, the formulation no. 1 with the frequency at 40 Hz, amplitude at 6, and electrode tension at 0 V was selected for the further study of flow rate optimization because it provided spherical beads with RBO entrapment at the center of beads, larger size, and narrower size distribution as compared to the formulation no. 2 [Figure 1 and Table 1].

The flow rates of inner and outer nozzles were studied to optimize them for the preparation of alginate beads with core-shell structure and were increased from 4 to 8 mL/min and 12 to 16 mL/min, respectively. The results are presented in Figure 2. A two-way ANOVA showed that the inner and outer

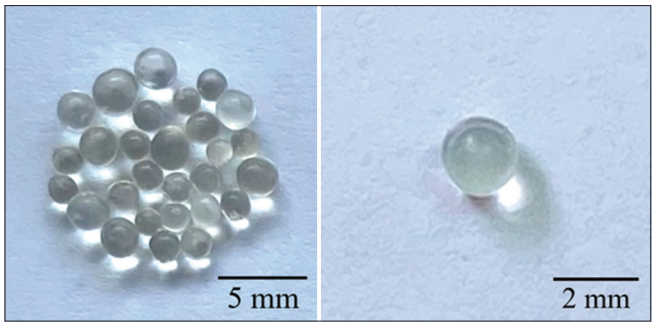

Figure 1: Physical appearance of the bead formulation no. 1 (F40A6E0) prepared with frequency of 40 Hz, amplitude of 6, and electrode tension of 0 V, inner flow rate of 4 mL/min, and outer flow rate of 12 mL/min

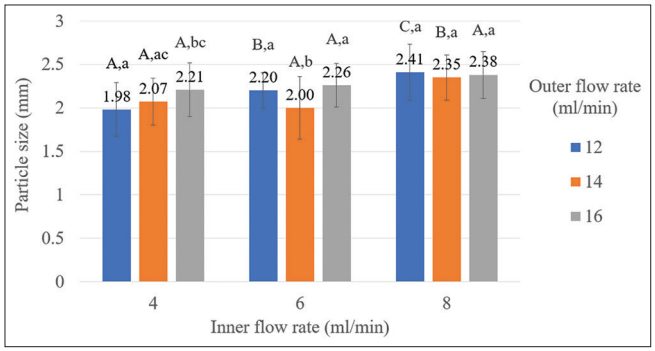

Figure 2: Effect of inner and outer flow rates on the size of alginate beads prepared with frequency of 40 Hz, amplitude of 6, and electrode tension of 0 V (mean±SD, n=30). Different capital letters on the columns (A-C) indicate significant differences among inner flow rates on each outer flow rate (P<0.05). Different lowercase letters on the columns (a-c) indicate significant differences among outer flow rates on each inner flow rate (P<0.05)

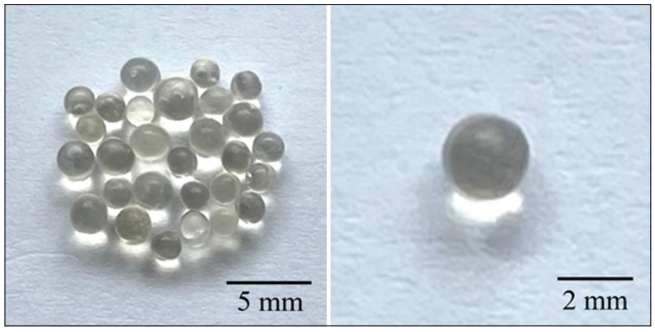

Figure 3: Physical appearance of the RBO-alginate beads obtained from the optimized conditions (frequency of 40 Hz, amplitude of 6, electrode tension of 0 V, inner flow rate of 4 mL/min, and outer flow rate of 14 mL/min)

flow rates significantly influenced the bead size. The interaction between the inner and outer flow rates also significantly affected the bead sizes (P < 0.05). The higher flow rate of the inner nozzle (8 mL/min) gave a significantly larger size of beads than the lower inner flow rates for the outer flow rates of 12 and 14 mL/min (P < 0.05), but no effect of the inner flow rate at the higher outer flow rate (16 mL/min) because it gave oval-shaped beads with a thicker shell. Moreover, the inner flow rates of 6 and 8 mL/min produced beads that were predominantly spherical but also included some irregularly shaped ones. In addition, these flow rates led to incomplete RBO encapsulation and the presence of fragmented alginate beads, regardless of the outer flow rates. This could be a result of an inappropriate correlation between the inner and outer flow rates. Therefore, the inner flow rate of 4 mL/min tended to provide the most suitable beads. For comparison of the outer flow rates at the lower inner flow rate (4 mL/min), the higher outer flow rate (16 mL/min) yielding oval-shaped beads with thicker shells gave significantly larger bead size than the lower outer flow rate (12 mL/min) (P < 0.05). The average sizes of beads obtained from the outer flow rates of 12 and 14 mL/min were insignificantly different (1.98 ± 0.31 mm and 2.07 ± 0.27 mm, respectively). However, the lower outer flow rate (12 mL/min) tended to produce beads smaller than the expected size (2–3 mm), whereas the outer flow rate of 14 mL/min yielded complete beads within the desired size range and resulted in less residue of the fragmented alginate beads. This finding is consistent with the previous research,[18] which observed that the alginate beads produced at the higher outer flow rate were significantly larger than those produced at the lower outer flow rate, irrespective of the inner flow rate. In addition, the study found that the higher inner flow rate resulted in insignificantly larger bead sizes compared to the lower inner flow rate. Therefore, the inner and outer flow rates of 4 and 14 mL/min, respectively, were considered promising for further investigation.

Therefore, the optimum process parameters for the preparation of RBO-alginate beads using Encapsulator B-390, as determined in this study, included a frequency of 40 Hz, amplitude of 6, an electrode tension of 0 V, an inner flow rate of 4 mL/min, and an outer flow rate of 14 mL/min. These conditions yielded beads with spherical morphology, an average diameter of 2.07 ± 0.27 mm, and effective encapsulation of RBO at the core, surrounded with a uniformly thick alginate shell [Figure 3].

CONCLUSION

In this study, microencapsulation of RBO was successfully prepared with an ionic gelation method using 2% w/w sodium alginate as a shell material and 1.5% w/v calcium chloride as a cross-linking agent. Alginate beads encapsulating RBO in core-shell structure were produced by Encapsulator B-390 with concentric nozzle system (inner diameter 450 µm and outer diameter 900 µm) and air pressure at 500 mbar. The process parameters including frequency, amplitude, electrode tension, and inner-outer flow rates had an impact on the formation of RBO-encapsulated alginate beads. The optimum conditions were the frequency of 40 Hz, amplitude of 6, electrode tension of 0 V, inner flow rate of 4 mL/min, and outer flow rate of 14 mL/min. These process parameters could be applied to develop alginate beads encapsulating cosmetic oils within a core-sell structure, suitable for incorporation into various cosmetic products such as gels, creams, and ointments. The encapsulated oils would be released upon application through direct pressure or rubbing. Further investigation is recommended to assess the physical and chemical stability of alginate beads encapsulating RBO.

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REFERENCES

1. Ferrandiz M, López A, Franco E, Garcia-Garcia D, Fenollar D, Balart R. Development and characterization of bioactive alginate microcapsules with cedarwood essential oil. Flavour Fragr J 2017;32:184-90.
2. Paulo F, Santos L. Design of experiments for microencapsulation applications: A review. Mater Sci Eng C Mater Biol Appl 2017;77:1327-40.
3. Chan ES. Preparation of Ca-alginate beads containing high oil content: Influence of process variables on encapsulation efficiency and bead properties. Carbohydr Polym 2011;84:1267-75.
4. Casanova F, Santos LC. Encapsulation of cosmetic active ingredients for topical application--a review. J Microencapsul 2015;33:1-17.
5. Benavides S, Cortés P, Parada J, Franco W. Development of alginate microspheres containing thyme essential oil using ionic gelation. Food Chem 2016;204:77-83.
6. Aizpurua-Olaizola O, Navarro P, Vallejo A, Olivares M, Etxebarria N, Usobiaga A. Microencapsulation and storage stability of polyphenols from *Vitis vinifera* grape wastes. Food Chem 2016;190:614-21.
7. De Moura SC, Berlingb CL, Garciac AO, Queirozd MB, Alvimd ID, Hubingera MD. Release of anthocyanins from the *Hibiscus* extract encapsulated by ionic gelation and application of microparticles in jelly candy. Food Res Int 2019;121:542-52.
8. Dolçà C, Ferrándiz M, Capablanca L, Franco E, Mira E, López F, *et al*. Microencapsulation of rosemary essential oil by co-extrusion/gelling using alginate as a wall material. J Encapsulation Adsorpt Sci 2015;5:121-30.
9. Lucía C, Marcela F, Ainhoa L. Encapsulation of almond essential oil by co-extrusion/gelling using chitosan as wall material. J Encapsulation Adsorpt Sci 2017;7:67-74.
10. Arriola ND, de Medeiros PM, Prudencio ES, Müller CM, Amboni RD. Encapsulation of aqueous leaf extract of *Stevia rebaudiana* Bertoni with sodium alginate and its impact on phenolic content. Food Biosci 2016;13:32-40.
11. Goh CH, Heng PW, Chan LW. Alginates as a useful natural polymer for microencapsulation and therapeutic applications. Carbohydr Polym 2012;88:1-12.
12. Cho AR, Chun YG, Kim BK, Park DJ. Preparation of alginate-$CaCl_2$ microspheres as resveratrol carriers. J Mater Sci 2014;49:4612-29.
13. Bernardi DS, Pereira TA, Maciel NR, Bortoloto J, Viera GS, Oliveira GC, *et al*. Formation and stability of oil-in-water nanoemulsions containing rice bran oil: *In vitro* and *in vivo* assessment. J Nanobiotechnol 2011;9:44.
14. Manosroi A, Chutoprapat R, Abe M, Manosroi W, Manosroi J. Anti-aging efficacy of topical formulations containing niosomes entrapped with rice bran bioactive compounds. Pharm Biol 2012;50:208-24.
15. Vorarat S, Managit C, Iamthanakul L, Soparat W, Kamkae N. Examination of antioxidant activity and development of rice bran oil and gamma-oryzanol microemulsion. J Health Res 2010;24:67-72.
16. Aguilar NN. Study of the Influence of Buchi Encapsulator Input Variables on Properties of Formed Alginate Beads. [Thesis]. Barcelona: Faculty of Chemical Engineering, Universitat de Barcelona; 2018.
17. Shi P, He P, Teh TK, Morsi YS, Goh JC. Parametric analysis of shape changes of alginate beads. Powder Technol 2011;210:60-5.
18. Chew SC, Nyam KL. Microencapsulation of kenaf seed oil by co-extrusion technology. J Food Eng 2016;175:43-50.