Boon-Beng Lee*, Rohaida Ibrahim, Sue-Yin Chu, Nurul Ainina Zulkifli and Pogaku Ravindra

Alginate liquid core capsule formation using the simple extrusion dripping method

Abstract: Liquid core capsules have been widely used in various biotechnological applications. The capsules could be formed by the simple extrusion dripping method. However, the method requires strict control of several process variables in order to form spherical capsules. The aim of this study was to systematically investigate the influence of process variables of the method on the capsule size and shape. The results showed that the capsules diameter was decreased when the concentration of alginate solution was increased. The capsule diameter was increased when the gelation time and dripping tip diameter were increased. The membrane thickness of the capsules was significantly increased by the concentration of calcium chloride, gelation time and dripping tip diameter. However, the concentration of alginate gave the opposite trend on the membrane thickness of the capsules. As a recommendation, uniform and spherical alginate liquid core capsules could be formed when concentration of calcium chloride was >10 g/l, the concentration of alginate solution was >5 g/l and <20 g/l, gelation solution height in between 1.7 cm and 3.2 cm, and stirring rate of the gelation bath was in the range of 400–500 rpm.

Keywords: diameter; dripping method; extrusion; liquid core capsule; membrane thickness; sphericity.

DOI 10.1515/polyeng-2014-0174
Received June 25, 2014; accepted October 2, 2014; previously published online November 21, 2014

1 Introduction

Encapsulation technology has been widely used in cells cultivation system, medicinal drug and nutritional supplements delivery, as well as liquid-liquid extraction [1–9]. In general, active ingredients such as living cells, drugs, extracts, nutrients and solvents are encapsulated (or confined) in a gel particle, either in the form of capsule or bead [1–9]. Liquid core capsule is a gel particle made up of an internal liquid core that contains the active ingredient and an external shell "membrane" layer surrounding the active ingredient [4–8]. Compared to beads, the liquid core allows homogeneous mass transfer of the ingredient through the shell membrane [4, 5, 7–10]. The liquid core also provides ample space for large active ingredient loading and cell growth without enlarging the membrane, thus prevents leakage of the ingredient load, especially the cells into the bulk [4, 6].

Liquid core capsules are generally made of alginate, and they are formed using the extrusion dripping method [4–7]. In the formation of liquid core capsules, the active ingredient is mixed with calcium chloride solution and then extruded as droplets into alginate solution. The calcium cations interact with alginate molecules to form gel following the well-known egg-box model [1]. The formation process of Ca-alginate liquid core capsules is basically inversed of that of the typical Ca-alginate beads. In the case of Ca-alginate bead gelation, the calcium cations diffuse inwardly through the alginate gel for cross-linking gelation. Conversely, the calcium cations diffuse outwardly from the liquid core of the droplet to form alginate gel surrounding the inner liquid core.

Furthermore, the "inversed" formation process of liquid core capsules involved the extrusion of low viscous liquid droplets into viscous alginate solution. Therefore, the process is more prone to produce deformed capsules, which are less spherical than the formation process of Ca-alginate beads. In order to ensure spherical capsules are formed, manipulation of several process variables of the formation process is needed. These variables included the physical properties of alginate solution and liquid core solution, types of alginate, cavity depth, collecting distance, dripping velocity and gelation time [4–7, 11–13]. However, the suitable process variables are obtained after many trials and errors, which are tedious and time consuming. In addition, the process variables are obtained based on one's past experience, as the information is generally not reported.

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The aim of this work was to systematically investigate the influence of the process variables on the size and shape of alginate liquid core capsules produced by simple extrusion dripping method. In this study, the process variables under investigation were alginate and liquid core solutions formulation, dripping tip diameter, gelation bath height, stirring rate (in gelation bath) and gelation time, as illustrated in Figure 1. Focus of this study was given to the size and shape of the capsules, because uniform size spherical capsules are desirable, as they give a predictable reaction, a homogenous mass transfer, a good appearance product and a controllable release [14]. These qualities are important for the application of the capsules in the pharmaceutical, nutraceutical, medicinal drug, fermentation and chemical industries [14].

2 Materials and methods

2.1 Materials

Liquid core (LC) solution consists of carboxyl methyl cellulose (CMC) (Calbiochem, Merk Millipore, Darmstadt, Germany), calcium chloride (R&M Chemical, UK) and calcium carbonate (HmbG Chemicals, Germany) was prepared. CMC was used as non-gelling agent to increase the viscosity and density of the liquid core solution to enhance the shape of the capsules [9, 11, 12, 15]. Calcium carbonate was added to contrast the liquid core from the formed membrane. In this work, seven different formulations of liquid core solution were prepared, as shown in Table 1. Gelation bath was prepared by dissolving sodium alginate I-3 (Kimitsu-Kimica, Chiyoda, Japan), and Tween 80 (Merck Group, Darmstadt, Germany) in distilled water.

2.2 Experimental set-up

Hypodermic needle (Terumo, Tokyo, Japan) of outer diameter 0.80 mm, 1.10 mm and 1.20 mm were used as dripping nozzle. Besides, the tube of inner diameter of 1.5 mm and 2.0 mm were also used as dripping nozzle. The length of the needles was shortened to 3 mm and the tip was blunted. The experimental set-up is shown in Figure 1. All studies were conducted at controlled temperature of 25°C. The collecting distance between the dripping tip and gelation bath was fixed at 3 cm. A motor agitator (IKA, Staufen, Germany) was used to stir the gelation bath. Once 50 capsules had been produced in the gelation bath (plastic container of 14.5 cm W×14.5 cm l×7.5 cm H), the capsules were filtered with wire mesh and rinsed with distilled water. Subsequently, the capsules were transferred into 5 g/l alginate solution for further gelation. The capsules were formed under the following conditions (unless otherwise stated): dripping tip diameter of 1.2 mm, alginate concentration of 15 g/l, liquid core solution formulation (LC5), stirring speed of 400 rpm, gelation solution height of 2.7 cm (equivalent to 500 ml) and gelation time of 30 min.

2.3 Capsule diameter, membrane thickness and sphericity analysis

The images of the capsules were captured using a digital camera (Samsung S760, Seoul, Korea). The images were

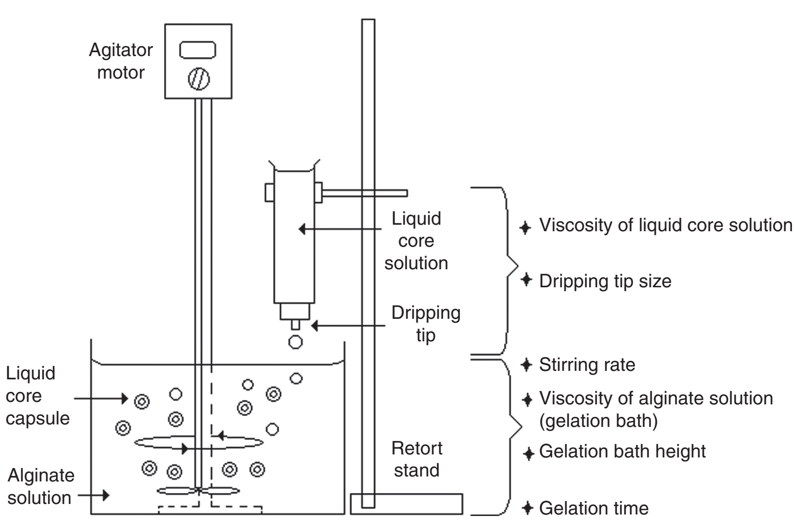


Figure 1 Schematic of the process variables studied in alginate liquid core capsules formation.

Table 1 Liquid core solution (LC) formulation.

Solution formulation identification	Calcium chloride concentration (g/l)	CMC concentration (g/l)	Calcium carbonate concentration (g/l)
LC1	2.5	15.0	100.0
LC2	5.0	15.0	100.0
LC3	7.5	15.0	100.0
LC4	10.0	15.0	100.0
LC5	15.0	15.0	100.0
LC6	15.0	10.0	100.0
LC7	15.0	20.0	100.0

analyzed using an image analyser (SigmaScan Pro 5, San Jose, CA, USA) to determine the diameter, membrane thickness and sphericity of the capsules in which the capsules diameter was determined from the area of particles on the captured image. While the membrane thickness of the capsules was determine based on the length of two points on the captured image, the sphericity of the capsules was quantified using sphericity factor (SF) (i.e., a dimensionless shape indicator):

$$SF=(d_{max}-d_{min})/(d_{max}+d_{min}) \quad (1)$$

where d_{max}=the largest diameter, and d_{min}=the smallest diameter perpendicular to d_{max}.

SF varies from 0 for a perfect sphere to approaching unity for an elongated object. Table 2 shows shape of the capsule with SF ranging from 0.02 to 0.20. The capsule with SF >0.07 was in elongated shape. Therefore, SF <0.07 was used as the criterion to justify the capsules as spherical in this study.

2.4 Statistical analysis

The diameter, membrane thickness and SF of the capsules were measured with sample size of 30 capsules. The standard deviation of the data was shown using an error bar in the graph.

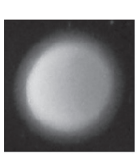
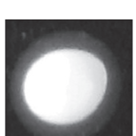
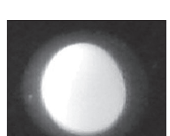
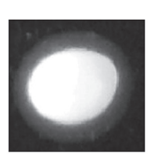
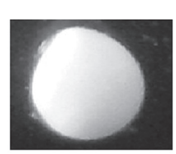
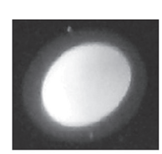
3 Results and discussion

3.1 Influence of liquid core solution formulation

Figure 2 shows the diameter, membrane thickness and SF of the capsules formed using different liquid core solution formulations. The effect of calcium chloride concentration (LC1–LC5) and CMC concentration (LC5–LC7) in the formulations on the size and shape of the capsules was studied. As shown in Figure 2A, the diameter of the capsule was neither influenced by calcium chloride concentration nor by CMC concentration. The reason could be that the calcium chloride concentration tested in this study was relatively low (<15 g/l) compared to previous studies [12, 16]. The previous studies reported the capsule diameter produced from calcium chloride concentration in the range of 13 g/l to 130 g/l [12, 16]. Because low concentration of $CaCl_2$ was used in this study, it is possible that the calcium cations are not in excess to react with alginate molecules at a given fixed gelation time. Therefore, the capsule diameter is not apparently influenced by the calcium chloride concentration. It has been reported that CMC concentration has no apparent effect on the capsule diameter [12], as it is used as a viscosifier and not a gelling agent.

As shown in Figure 2B, the membrane thickness of the capsules was in increasing trend when the calcium chloride concentration (LC1–LC5) was increased, and no apparent trend was observed when the CMC concentration was increased (LC5–LC7). The results were in agreement with previous studies [12, 15–17]. It has been reported that the membrane thickness of the capsules was increased as

Table 2 Sphericity of the capsules.

Image						
SF	0.02	0.04	0.05	0.07	0.10	0.20
Remarks	← Spherical →				← Elongated →	

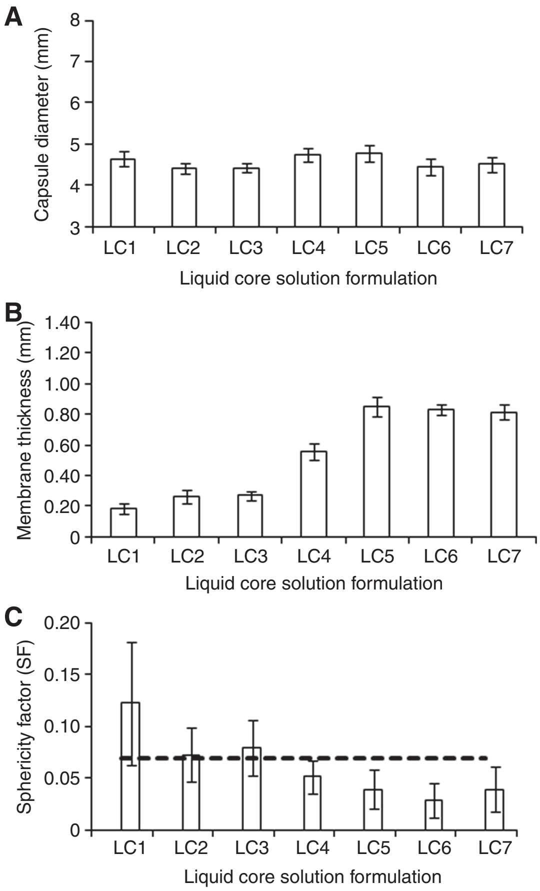


Figure 2 Diameter (A), membrane thickness (B) and SF (C) of the capsules produced from different liquid core solution formulations.

the calcium chloride concentration increased [12, 16, 17]. The membrane thickness of the capsules was found not to be apparently influenced by the CMC concentration [15]. When the calcium chloride concentration increased, there were more calcium cations available for the Ca-alginate gel formation and, hence, thicker membrane was formed. However, the growth of the internal membrane wall thickness has no significant effect on the capsule diameter as the calcium chloride concentration increased (see Figure 2A). This could be due to the formed membrane wall, which was relatively small compared to the capsule diameter.

Figure 2C shows that the SF of the capsules formed by different liquid core formulations. Formulations of LC1, LC2 and LC3 produced non-spherical capsules with SF >0.07, and a large variation of shape was observed for these formulations. This is because the concentration of calcium chloride below 10 g/l was insufficient to form strong gel membrane. When the concentration of calcium chloride below the critical value [18], the capsule membrane was weakly gelled and caused the formation of less spherical capsules [1]. Conversely, the CMC concentration has no apparent effect on SF of the capsules, as indicated by the results of the solution formulations of LC5–LC7. The results suggested that viscous liquid core solution forms spherical capsules, as long as the calcium chloride concentration is >10 g/l.

3.2 Influence of alginate solution formulation

The diameter, membrane thickness and SF of the capsules produced by different concentrations of alginate are shown in Figure 3. The diameter of the capsules decreases as the concentration of alginate solution increases, regardless of the gelation time (see Figure 3A). The membrane thickness of the capsules also exhibited a decrease as alginate concentration increased (see Figure 3B) for a given gelation time. The results were in good agreement with previous studies [12, 16, 17]. At high alginate concentrations,

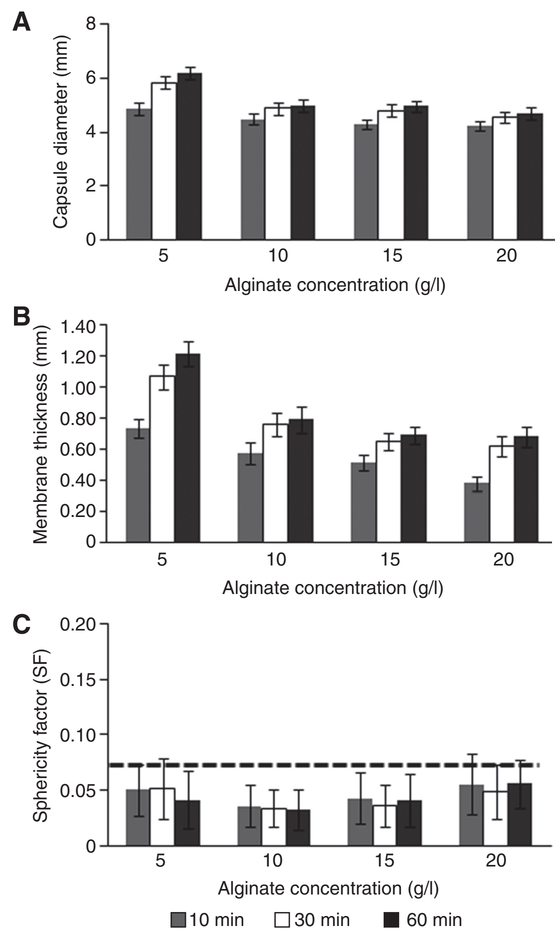


Figure 3 Diameter (A), membrane thickness (B) and SF (C) of capsules formed from solutions of different alginate concentrations. The gelation time of each experiment is indicated in the legend.

number of alginate molecules per unit volume and the number of binding sites for Ca^{2+} cation also increased. As a result, formation of high, densely cross-linked gel structure is expected [17]. This would explain why the diameter and membrane thickness of the capsules formed with high alginate concentration are lower than those formed with low alginate concentrations. Moreover, Figure 3A also shows that the capsules formed from 5 g/l alginate solution are relatively larger than those formed by high alginate concentrations. This could be due to the fact that gel structure of the capsules formed with 5 g/l alginate solution (low concentration) was highly loose [1].

Conversely, Figure 3C illustrated that the capsules produced by different concentrations of alginate were generally spherical with SF <0.07. The concentration of alginate solution has no significant effect on the SF of the capsules, and the results also are not dependent on the gelation time. This is because the liquid core solution (LC 5) is more viscous compared to that of alginate solutions (except for 20 g/l; data not shown). Therefore, the deformation of the liquid core solution droplets during impact on the alginate solution surface could be minimized and consequently spherical capsules could be formed, as reported in previous studies [10–12]. However, the sphericity variation of the capsules formed from 5 g/l and 20 g/l alginate solution were higher than the rest. This is because weak gel structure was formed with 5 g/l alginate solution; therefore, more deformed capsules were obtained [1]. For the case of 20 g/l alginate solution, the viscosity of the alginate solution was slightly higher than that of the liquid core solution (data not shown). Minor deformation may happen to the liquid core solution droplet prior to gelation due to impact on the alginate solution surface.

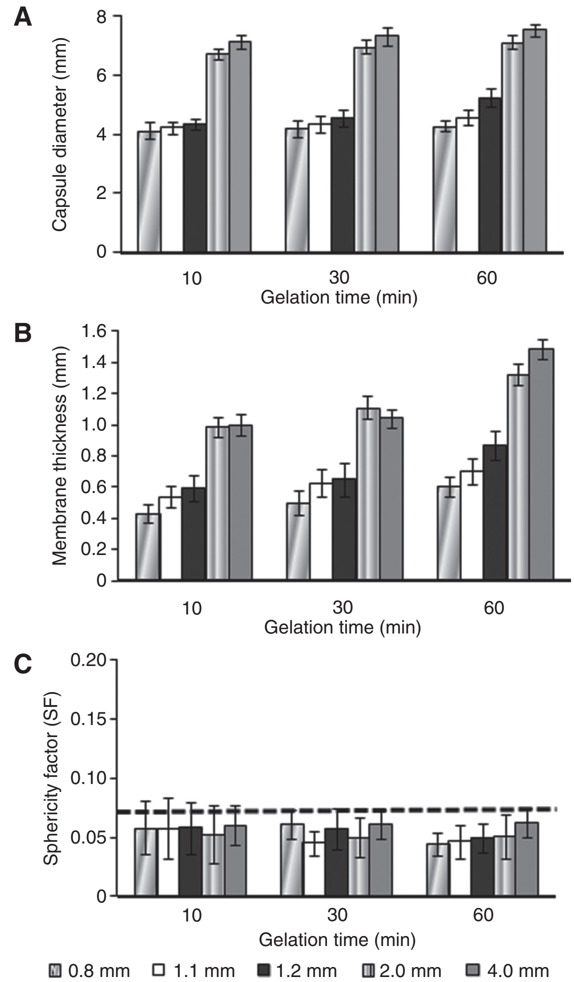


Figure 4 Diameter (A), membrane thickness (B) and SF (C) of the capsules produced from different gelation times. The diameter of dripping tip used is as indicated in the legend.

3.3 Influence of gelation time

The capsule diameter and membrane thickness formed with different gelation times are shown in Figure 4A and B, respectively. For a given dripping tip diameter, the capsule diameter and membrane thickness increases when the gelation time increases. The results were in good agreement with previous studies [6, 11, 12, 17]. After the core solution droplet detached from the dripping tip, it is speculated that instantaneous gelation occurred upon the droplet colliding with the alginate solution [11, 12]. The Ca^{2+} ions in the core solution penetrated through the alginate gel membrane that had been formed. As a result, the Ca^{2+} ions faced more resistance when the membrane grew thicker. Therefore, sufficient gelation time is needed for the capsule to reach equilibrium size [12]. It has been reported that 15 min of gelation is needed for the capsule to reach equilibrium size [11]. In the case of this study, the capsules reached their equilibrium size after a gelation time of 10 min.

As shown in Figure 4A and B, the capsule diameter is increased slightly compared to the increase rate of membrane thickness against gelation time. This could be explained by the “syneresis” phenomenon observed in Ca-alginate gel formation [1, 19, 20]. The Ca-alginate gel formation during cross linking reduces the space occupied by alginate that causes water loss and thus reduces the size of the capsules. In the case of liquid core capsule formation, the water may be “squeezed” into the capsule core, which can result in increased core solution volume along with Ca-alginate gel membrane formation. As a result, the diameter of capsules was not apparently increased with gelation time, although the membrane thickness increased with gelation time.

The sphericity of the capsules formed with different gelation time is shown in Figure 4C. In general, all the capsules formed were spherical in shape, as the SF value was <0.07, regardless of the gelation time. The studied gelation times have no significant influence on the sphericity of the capsules, because gelation time of about 10 min was found to form spherical capsules in previous studies [5, 6, 12].

3.4 Influence of dripping tip diameter

Figure 5A and B show that both the diameter and membrane thickness of the capsules increased as the dripping tip diameter increased. This is because a bulging droplet is formed at the small dripping tip [21]. Bulging droplet is defined as a pendant droplet where its diameter is greater than the diameter of the tip that holds it [21]. The droplet grows on the tip to its maximum weight and detaches from the dripping tip due to gravitational force [21]. It has been reported that the size of the detached (bulging) droplet is dependent on the dripping tip diameter [1, 21].

Figure 5C shows the SF of the capsule formed from different dripping tip diameters. The SF of the capsules was generally <0.07, and therefore the sphericity of the capsules was not influenced by the dripping tip diameter. This is because the capsules were formed with sufficient amounts of Ca^{2+} ions and gelation time, although the detached droplet size was varied using different dripping tips.

3.5 Influence of gelation solution height

In this study, the gelation solution height in the gelation bath was varied by adjusting the volume of alginate solution filled in the bath (i.e., 200–600 ml). The influence of the gelation solution height on the capsule size and shape is shown in Figure 6. The results show that the solution

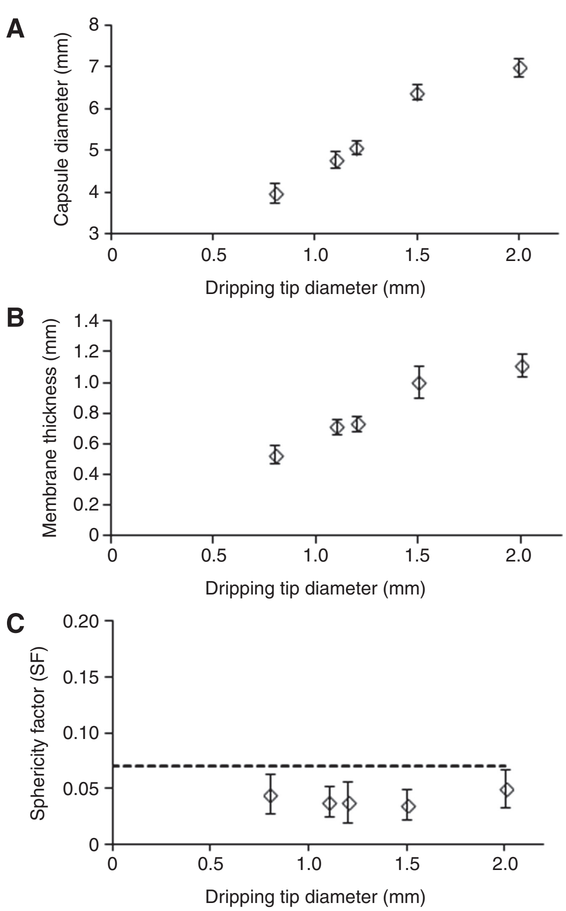


Figure 5 Diameter (A), membrane thickness (B) and SF (C) of the capsules produced from different dripping tip diameters.

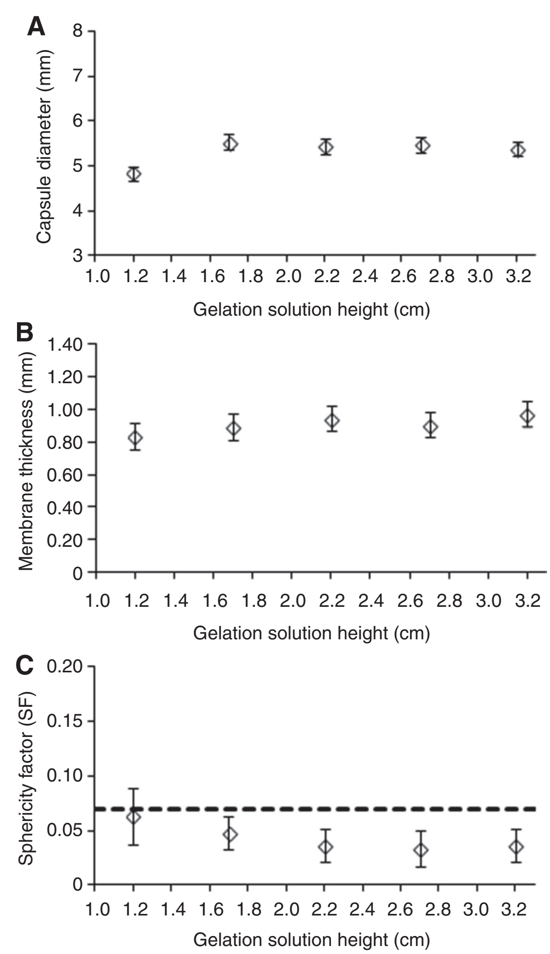


Figure 6 Diameter (A), membrane thickness (B) and SF (C) of the capsules produced from different gelation solution height.

height has no significant effect on the capsule diameter and membrane thickness, except for the solution height of 1.2 cm. The capsules formed at solution height of 1.2 cm are generally less spherical (elongated shape).

Figure 6C shows that the SF of the capsules is generally <0.07, and spherical capsules were formed. However, the SF of the capsules was consistently <0.07 when the solution height was between 1.7 cm and 3.2 cm (see Figure 6C). The solution height is important to generate the centrifugal force that is required to drag the droplet into the solution and gel rapidly [22]. The required centrifugal force could be indirectly determined by measuring the cavity height as mentioned by Cheong et al. [22]. In another study, a solution height of 1.0 cm was suggested to produce spherical capsules when using shallow gelation bath (i.e., 3 cm in height) [22]. In this study, a deep gelation bath was used and, hence, the results of both studies were not comparable.

3.6 Influence of stirring rate in gelation bath

During the capsule formation, the gelation bath was continuously stirred by an agitator motor to create fluid flow in the bath. Figure 7 shows the images of the liquid core solution droplet upon impact on the gelation bath surface without stirring. Figure 7 clearly illustrates that the elongated tear-shaped capsule was formed if the gelation bath was not stirred.

Figure 8 shows the diameter, membrane thickness and SF of the capsules formed at different stirring rates. The diameter and membrane thickness of the capsules formed at the lowest stirring rate was smaller than the others (see Figure 8A and B). This is because the capsules formed by 300 rpm were elongated and generally in tear shape (Figure 8C). Therefore, capsules with smaller diameter and membrane were produced. Moreover, these capsules gave a large variation in SF (see Figure 8C). As shown in Figure 8C, spherical capsules were generally produced at a stirring rate of 400 rpm and 500 rpm. Elongated capsules were formed at a low stirring rate (i.e., 300 rpm), because slow penetration of the droplet into the gelation bath tends to produce capsules with a tail (tear shape) (as illustrated in Figure 7). In the past, low stirring rates were also reported to cause formation of less spherical capsules [22].

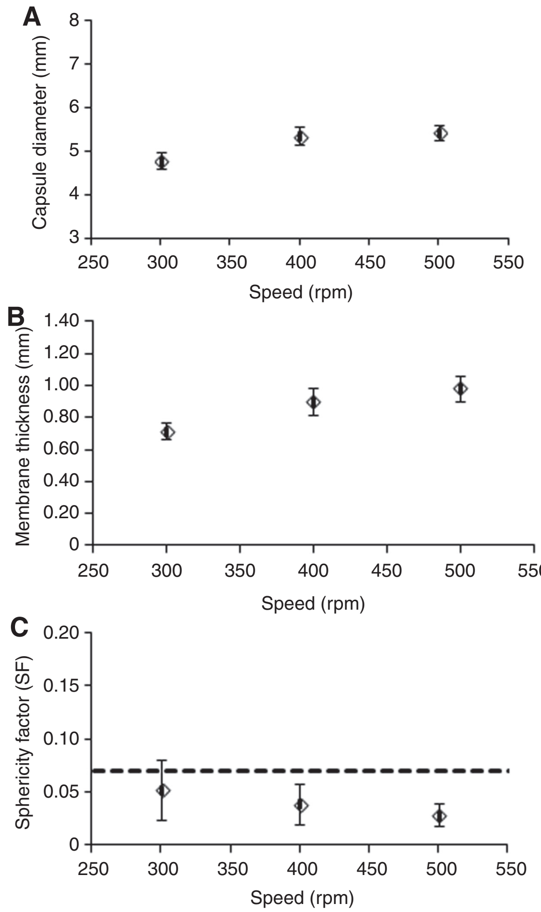


Figure 8 Diameter (A), membrane thickness (B) and SF (C) of the capsules produced from different stirring rates.

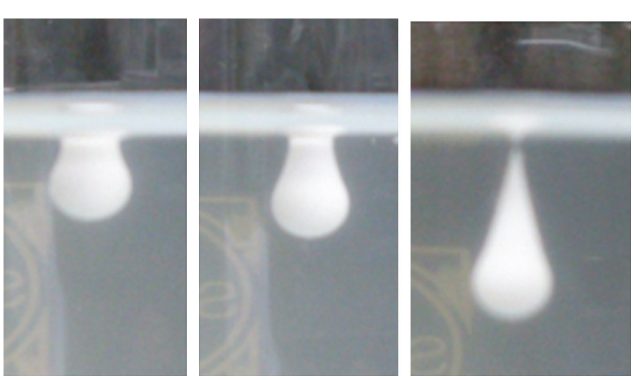


Figure 7 Penetration of liquid core solution droplet into the alginate solution upon impact on the gelation bath surface without stirring.

4 Conclusion

The influence of process variables on the size and shape of alginate liquid core capsules produced by extrusion dripping method was studied. The results showed that the concentration of CMC in the liquid core solution has no effect on the diameter, membrane thickness and SF of the capsule. As the concentration of $CaCl_2$ solution increased, membrane thickness of the capsule increased and SF of the capsule decreased. It was found that spherical capsules were produced when the concentration of $CaCl_2$ was >10 g/l. The results also showed that the diameter and membrane thickness of the capsule were large when the concentration of alginate solution was low. The diameter and membrane thickness results of 5 g/l alginate solution were noticeably larger than that of the rest. The variation of SF value of the capsules was large when the capsules were produced in alginate solution of low concentration (5 g/l) and high concentration (20 g/l). Deformed capsules were formed at low alginate concentration due to weak gel formation and at high alginate concentration due to impact force that distorted the shape of a droplet when it hit the surface of the viscous alginate solution. It was observed that gelation time of 10 min was sufficient to obtain equilibrium diameter of the capsules. The diameter and membrane thickness of the capsules was found to be proportional to the diameter of the dripping tip. The gelation solution height and stirring speed has great impact on the sphericity of the capsule, because elongated capsules were produced at low gelation solution height (<1.7 cm) and low stirring speed (300 rpm).

As a recommendation, uniform and spherical alginate liquid core capsules could be formed using the experimental set-up of this study and under the following conditions:

– Concentration of $CaCl_2$ solution: >10 g/l
– Concentration of alginate solution: >5 g/l and <20 g/l
– Gelation time: 10 min
– Gelation solution height: 1.7–3.2 cm
– Stirring speed: 400–500 rpm

Acknowledgments: Financial support from the Malaysia Toray Science Foundation (MTSF) [Project no: 9002-00015] and the Malaysian Ministry of Higher Education (KPM) [FRGS 9003-00358] is gratefully acknowledged.

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