Formation of Alginate-Membrane Capsules by using Co-Extrusion Dripping Technique

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Introduction

Bioencapsulation could be defined as a process of engineering 'artificial skin' or membrane for confinement or localization of biological active materials. Bioencapsulation is a widely used tool in biotechnology and its related fields such as pharmaceutical and therapeutical, environmental, agriculture and food industries to achieve certain purposes. Previous works have reported successful use of various types of hydrogel materials to encapsulate biomaterials. Among all, alginate is the most widely used and investigated polymer for cell encapsulation. This is because it is nontoxic, biocompatible, abundant, cheap and its gelling process can be performed under mild conditions.

Single nozzle extrusion is a generally known technique to form or produce alginate-membrane liquid core capsules [1-4]. The cell suspension is mixed with calcium chloride solution and extruded towards nozzle and allowed to drop into sodium alginate solution. However, there are several problems when applying this method to produce liquid core capsules. First, it is difficult to obtain spherical capsules due to the difference in viscosity and surface tension between sodium alginate solution and the mixture solution of cell suspension and calcium chloride. Therefore, the viscosity and surface tension of both solutions have to be modified by adding thickener and surfactant. Second, the thickness of the capsules produced might not be uniform because it is dependent on the gelation time or residence time and concentration of the calcium ion in liquid core. In addition, the capsules produced could easily agglomerate, stick together and thus difficult to handle. This could be due to the continuous outward diffusion of calcium cation from the surface of capsule towards outside to the surface of another nearby capsule.

To solve these problems, co-extrusion technique was applied to produce alginate-membrane liquid core capsules in this research. Liquid core solution was co-extruded simultaneously with the alginate polymer solution, and they were dropped into a calcium chloride gelling bath. The effect of flow rate, concentration and nozzle size on capsules properties like capsules diameter, size distribution or coefficient variance, shape factor, membrane thickness and center factor were studied.

Materials and methods

Reagents: Sodium alginate manugel DMB (ISP Technologies Inc, UK) in medium range (mannuronic acid 37%, guluronic acid 63%), xanthan gum (TIG GUMS, USA), calcium chloride (MERCK, Germany), Tween 80 (SIGMA ALDRICH Chemical Co., USA), Tapioca starch (Thye Huat Chan Sdn. Bhd, Malaysia), cooking oil (Yee Lee Edible Oils Sdn. Bhd., Malaysia).

Preparation of alginate-membrane capsules: 1.0 – 3.0% w/v of alginate solution was prepared by dissolving 1.0 – 3.0 g alginate in 100 ml distilled water. Liquid core solution was prepared by dissolving 3.0% w/v starch as a model of biomaterial in xanthan gum solution (0.3 – 2.0% w/v). Cooking oil was also used as model liquid core. Both solutions were then extruded drop wise towards a concentric nozzle (Figure 1) into 0.01 M calcium chloride solution, which contained Tween 80 (0.5% v/v). Different flow rates of alginate solution and liquid core solution were attempted by manipulating the pumps speed. These capsules were hardened for 1 hour under appropriate agitation, followed by rinsing with distilled water. The experiment of co-extrusion technique is illustrated in Figure 2.3.

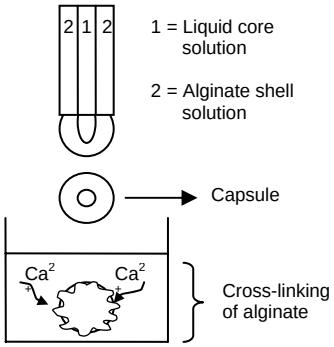


Figure 1: Schematic of capsule formation during extrusion from concentric nozzle and gelling bath

Characterization of capsules: The capsules were characterized by diameter and membrane thickness. An average of 30 capsules was taken for each analysis. The image of the capsules was captured by using a digital camera. An image analyzer was used to analyze and determine the capsules size and coefficient variance and shape factor. Theoretical diameter is determined by using Tate's Law. The membrane thickness of the capsules was measured by cutting the capsules into two halves. Nine capsules were measured for each analysis. The membrane thickness of the capsules was determined by electronic digital caliper. If the liquid core was not located in the center position of capsules, the maximum and minimum portion of the membrane thickness were measured. Center factor, CF was used to determine the location of liquid core solution within a capsule. The center factor of the capsule was determined based on the difference between the maximum portion and minimum portion of the capsules membrane thickness. Coefficient variance or CV was used to determine the size distribution of the capsules produced. Shape factor, SF (or sphericity coefficient) is used to indicate the sphericity of an object, with a value of 1.0 for a perfect.

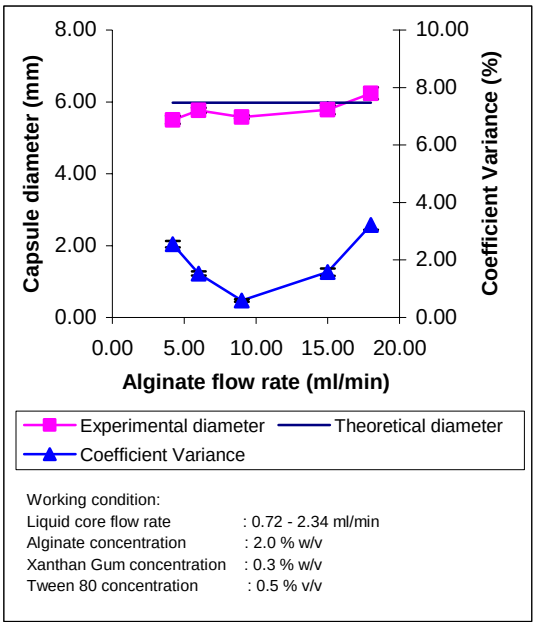
Statistical analysis: The diameter and shape factor of the capsules were determined based on the measurement of 30 capsules while membrane thickness and the center factor were determined based on 9 capsules. All standard errors in the graph were calculated based on 95.5% confidence level by using t-test.

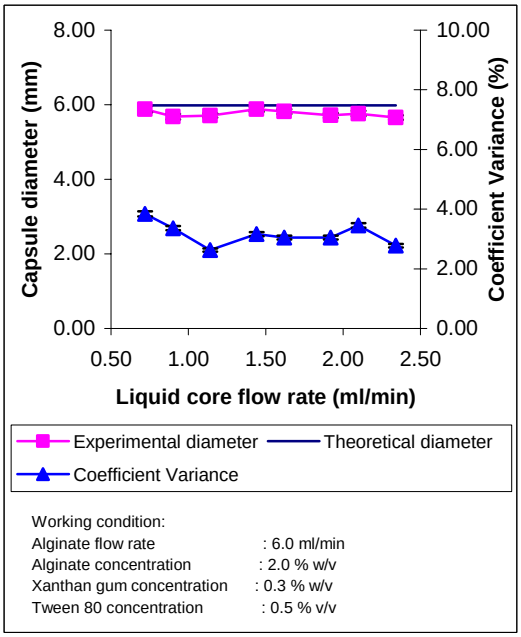
Results and Discussion

Effect of flow rate: Figure 2 shows the effect of alginate flow rate on capsule diameter. There was no significant change in capsule diameter when the flow rates of the alginate solution increased from 4.2 ml/min to 15 ml/min. The capsule diameter was around 5.6 mm. This was very close to the theoretical value of 5.9 mm, which was determined by using Tate's Law. However, capsule diameter increased from 5.8 to 6.2 mm when flow rate increased from 15 to 18 ml/min. This increment may be due the destabilization of the dripping process when liquid jet was started to form. Consequently, this might cause an additional kinetic force or momentum to the gravitational force during droplets formation, which results in bigger droplet. On the other hand, Figure 3 shows

the effect of liquid core solution flow rate on capsule diameter. There was no significant effect on capsule diameter when liquid core flow rate increased from 0.72 to 2.3 ml/min. The capsule diameter remained constant around 5.8 mm. The experimental value deviated only 2.0% with the theoretical value, 5.9 mm.

Figure 2 and 3 also clearly show that the solutions flow rate has no effect on CV of capsules produced. The CV of the capsules was very small, less than 5.0%. This means that it is possible to produce capsules with narrow size distribution by using this technique. In addition, it was found that the SF of the capsules was not significantly affected by solutions flow rate (data not shown). The SF of the capsules was in the average range of 0.70. Furthermore, it was found that the solutions flow rate has no effect on membrane thickness (data not shown). The maximum portions were approximately 2.9 mm whereas minimum portions were 0.07 mm.





The error bars indicate standard error at 95.5% confidence level

Figure 2: Effect of alginate flow rate on capsule diameter and coefficient variance

Figure 3: Effect of liquid core flow rate on capsule diameter and coefficient variance

Effect of alginate concentration: Figure 4 shows the effect of alginate concentration on capsule diameter. At 1.0% alginate concentration, the viscosity was too low to sustain the shape of a droplet when colliding with gelling bath. The impact caused leakage of liquid core solution from alginate membrane that resulted in smaller capsules of 4.7 mm. On the other hand, the capsules diameter remained unchanged at 5.8 mm in alginate concentration range of 1.5-2.5%. Beyond 2.5%, the alginate solution became very viscous and it was difficult to form proper capsules.

Figure 4 also shows clearly that an alginate concentration below 2.5% has CV below 5.0%. However, at higher concentration of 3.0%, the CV increased dramatically to 11%. This again was due to the difficulty in extruding a relatively higher viscosity solution which resulted in formation of capsules with tail (Figure 5). In addition, it was found that alginate concentrations have no effect on SF & CF with a value of 0.70 and approximately 3.0 mm respectively.

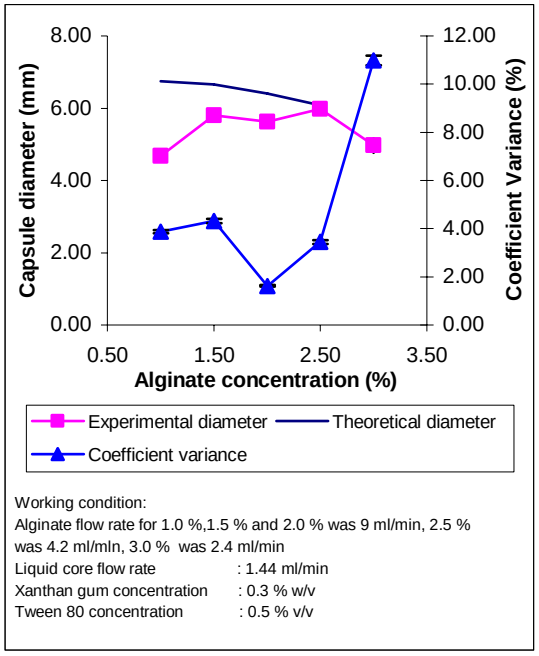


Figure 4: Effect of alginate concentration on capsule diameter and coefficient variance.

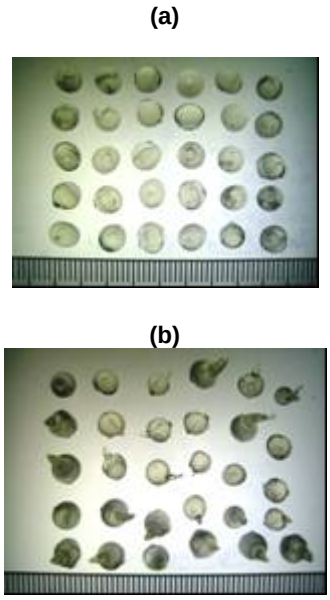


Figure 5: Capsules formed with different Alginate concentration (a) 2% w/v b) 3% w/v

Encapsulation of oil within alginate-membrane: Figure 6 provides the visual evidence of capsules encapsulating oil and starch-xanthan gum solution. It can be clearly seen that the type of core liquid has effect on the CF as it was found that the CF of capsule containing oil and starch-xanthan gum solution were 1.2 mm and 2.8 mm respectively. This might be due to the immiscible property of oil and the effect of its interfacial tension with alginate polymer. Figure 7 shows the effect of nozzle diameter on size and center factor of capsule encapsulating oil. As expected, it was found that nozzle diameter has significant effect on capsule diameter.

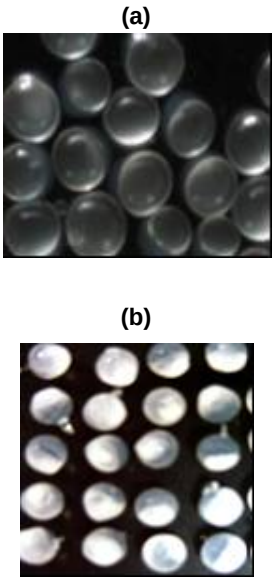


Figure 6 Encapsulation of a) oil b) starch-xanthan gum solution

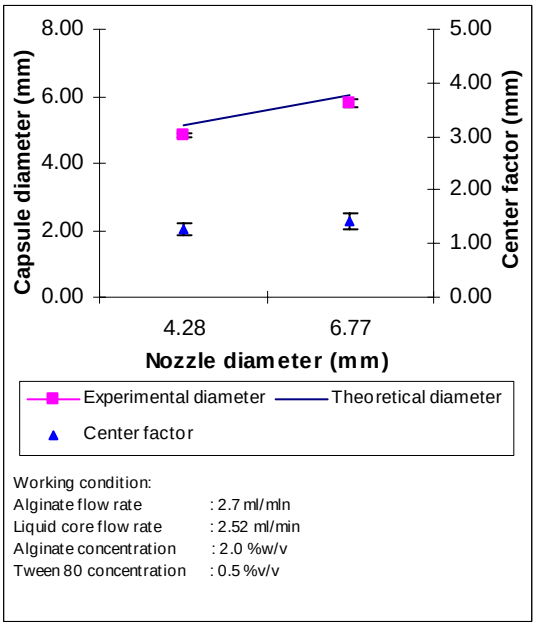


Figure 7 Effect of nozzle diameter on size and center factor of capsule encapsulating oil

Conclusion

This work clearly shows that it is possible to form liquid-core capsules by using co-extrusion dripping technique. The set-up of this method is simple and overcome some problems of liquid-core capsule formation using extrusion technique. However, the problem is that the core liquids were not positioned at the center of capsules which resulted in uneven shell thickness. Understanding of mechanism of droplet formation during extrusion is required to solve the problem and this forms the basis for future work.

References

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