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Formation Of Chitosan-Alginate Capsules Using Extrusion-Dripping Method: Effect Of Stirring Speed And Biopolymers Types

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Abstract: The aim of this study was to investigate the effect of stirring speed and biopolymer types on size and shape of chitosan-alginate capsules produced through extrusion-dripping method. Chitosan-alginate capsules were produced by extruding chitosan-calcium chloride solution into sodium alginate solution. As a result, capsules with defined inner core and membrane were formed. Under the tested conditions, chitosan-alginate capsules with diameter in a range of 3.6 mm to 4.1 mm were produced. The result shows that the shape of chitosan-alginate capsules was significantly affected by the stirring speed. At the stirring speed of 600 rpm, mainly small and spherical capsules were produced. It was found that chitosan-alginate capsules produced from guluronic acid-rich alginate (AHG) were larger in term of diameter and membrane thickness as compared to those produced from mannuronic acid-rich alginate (AHM). The molecular weight of chitosan has no significant effect on diameter, shape and membrane thickness of the capsules.

Key words: Extrusion dripping, Chitosan-alginate capsules, Size, Shape, stirring speed

INTRODUCTION

Encapsulation is defined as a process of entrapping biological active compounds within a matrix in particulate form (Chan *et al*., 2012). Encapsulation has been widely applied in biotechnology related industries such as pharmaceutical, environmental, food industry and dairy product industries due to its unique characteristics (Lee *et al*., 2005). In these applications, predictable and controllable release characteristics as well as good aesthetic appearance of product are important (Chan *et al*., 2009). Therefore, the capsule formed for the application is desirable to have narrow size distribution and spherical shape.

Alginate is composed of natural polysaccharide derived from brown seaweed and its basic structure consists of linear unbranched polymers containing β-(1-4)-linked D-mannuronic acid (M) and α-(1-4)-linked L-guluronic acid (G) residues. It is one of the most widely applied biopolymers for encapsulation because it is abundantly available and non toxic. Moreover, it can easily form biocompatible gel matrix in the presence of calcium ions under mild conditions. Despite all the attractive features of calcium alginate matrix, it has limitation upon interaction with chelating agents such as phosphate, citrate and lactate (Smidsrod and Skjak-Braek, 1990). Therefore, one of the solutions to resolve the chemical instability issue of calcium alginate matrix is by introducing the chitosan to the calcium alginate matrix via external coating or internal gelation.

Chitosan is obtained by partial alkaline N-deacetlylation of chitin which found in the shell of crustaceans. It is polycationic biopolymer consist of glucosamine and N- acetylglucosamine. Similar to alginate, it has been applied in wide applications because it is abundantly available, nontoxic and biocompatible. The polycationic nature of chitosan leads to a strong interaction with polyanionic biopolymer such as alginate (due to having an opposite charge). The formation of polyelectrolyte complex could be used to stabilize the capsule from chelating agents.

Chitosan-alginate capsules can be produced through ionic gelation. It is commonly conducted by dropping an alginate solution into gelling medium consist of an aqueous solution of calcium chloride and chitosan (Lee *et al*., 1997 and Albarghouthi *et al*., 2000). In the inverted procedure, the mixture of chitosan and calcium chloride solution is dropped into an alginate solution. As a result, a liquid chitosan core surrounded by interphasic chitosan-alginate membrane is produced (Daly *et al*., 1988).

The size and shape of the chitosan-alginate capsules are influenced by formulation of the biopolymer solution, gelling agent solution and process variables. The process variable influences the size and shape based on previous works are concentration of chitosan and alginate, types of biopolymer as well as process variable like dropping height and stirring speed (Takka *et al*., 1999;Blandino *et al*., 2000; Chai *et al*., 2004; Abang *et al*., 2012).To date, there are no studies reported to systematically study the effect of stirring speed and biopolymer types on size and shape of chitosan-alginate capsules.

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The aim of this study was to investigate the effect of process variable on physical properties of chitosan-alginate capsules produced through extrusion-dripping method. The studied process variable includes stirring speed and types of chitosan and alginate. The physical properties of chitosan-alginate capsules determined were size, shape and membrane thickness.

MATERIALS AND METHODS

Materials:

Calcium Chloride (Bendosen, Norway), Tween 80 (Merck, Germany), acetic acid glacial 99.8% (Bendosen, Norway). Sodium alginate with different M/G ratio was purchased from Kimitsu Chemical Industries Japan. Chitosan of different molecular weight were provided by Acros Organic, Belgium. The details of chitosan and sodium alginate listed in Table 1 and Table 2. Alginate solution 1 % (w/v) was prepared by dissolving sodium alginate in distilled water. Chitosan solution 1 % (w/v) was prepared by dissolving chitosan in distilled water containing concentrated acetic acid glacial 98.8%. Calcium chloride was also added into chitosan solution. The chitosan-calcium chloride solutions 1 % (w/v) were maintained at pH 2.5.

Table 1: Physical properties of types of chitosan

Type	Identification	Molecular weight[a][12]	Degree of deacetylation(%)
Chitosan	CMW	10000-30000g/mol	82±2[a]
Chitosan	CHW	60000-80000g/mol	80-90[b]

[a] The value was obtained from (Munro and McGrath , 2012).
[b] Information was provided from manufacturer.

Table 2: Physical properties of types of sodium alginate [a]

Type	Identification	Viscosity 1 % solution (mPa.s)[a]	M/G ratio[a]
Sodium alginate (Kimitsu Algin 1-3)	AHM	50-80	55:45
Sodium alginate(Kimica Alginex)	AHG	200-400	45:55

[a] Information was provided from manufacturer.

Preparation Of Chitosan-Alginate Capsules:

The experimental set-up for chitosan-alginate capsules formation is shown in Figure 1.The chitosan-calcium chloride solution was extruded into the sodium alginate solution through the hypodermic needles (Terumo Corporation, Australia) with outer diameter 0.8 mm needle. The distance between the edge of the needle and surface of the sodium alginate solution was fixed to 2 cm. The depth of the gelation bath was fixed to 4 cm. The gelation time of the chitosan-alginate capsules was fixed at 30 min under constant stirring speed. After gelation, the chitosan-alginate capsules formed were filtered and washed thoroughly with distilled water to remove unreacted reagents. The capsules were stored in 0.5 % (w/v) calcium chloride prior to size and shape analysis.

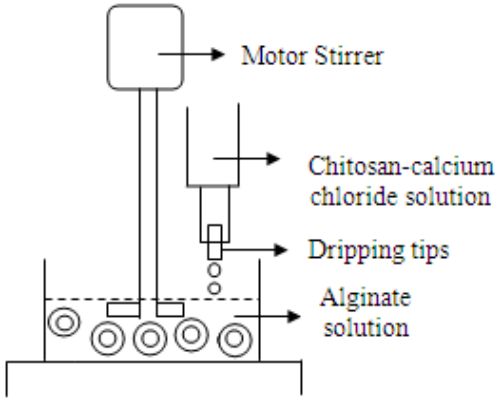


Fig. 1: The schematic diagram for extrusion dripping method

Characterization of Chitosan-alginate capsules:

The size, shape and membrane thickness of the chitosan-alginate capsules were characterized. An average of 30 capsules was taken for each analysis. The images of the capsules were captured using a digital camera (Canon Power Shot A2200, Japan). The images were analyzed using Sigma ScanPro5 software (USA) to determine diameter and minimum and maximum diagonal length of the capsules. The data were then used for

calculation using Microsoft Excel Office 2007 (USA). In which, the sphericity factor (SF) was calculated according to Eq.1 (Chan *et al*., 2009)

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

Where d*max* is maximum diagonal length (mm); d*min* is minimum diagonal length (mm)

The sphericity factor was used to determine the roundness of the capsules. In this study, the shape of capsules was further classified into three types: spherical, egg and deformed as shown in Table 3.

Table 3: Shape of capsules are classified by sphericity factor (SF)

0<SF≤0.07	0.07<SF≤0.10	SF>0.10
Spherical	Egg	Deformed
		

The membrane thickness of the capsules was measured by Microscope (Motic, USA) which was equipped with Motic Image Software (USA). The membrane thickness of each capsule was taken by averaging four measurements using Microsoft Excel Office 2007 (USA).

Statistical Analysis:

The diameter and SF of the capsules was measured from 30 samples. The membrane thickness of capsules was calculated based on measurement of five capsules. All the standard deviation of each measurement were calculated and showed in the graph.

RESULTS AND DISCUSSION

Effect of Stirring Speed:

Figure 2 shows the diameter of the capsules formed at different stirring speeds. As presented in Figure 2, the diameter of the capsules decreases from 4.10 mm to 3.60 mm when the stirring speed increases from 400 rpm to 600 rpm. However, the diameter of the capsules was approximately 3.60 mm even though the stirring speed was further increased to 800 rpm. The capsules produced at stirring speed of 400 rpm and 500 rpm was larger than the rest. This is because those capsules were deformed (not spherical) at low stirring speed. Therefore, the diameter of the deformed capsules was deviated (became larger) from the ideal value if they were spherical. In other words, the stirring speed has no significant effect on the diameter of the capsules.

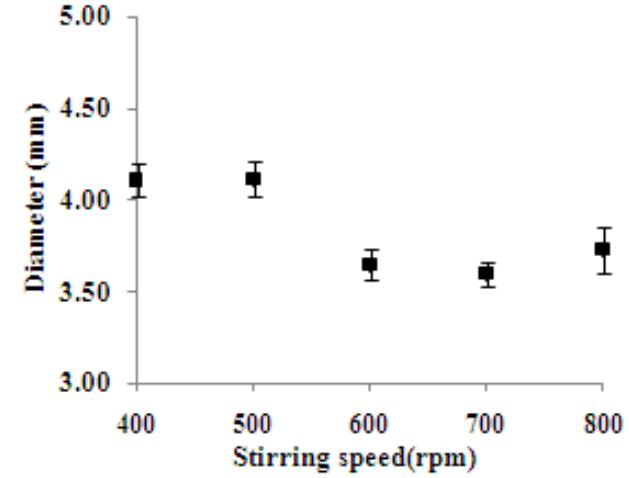
Figure 3 shows the shape of the capsules formed at different stirring speeds (ranging from 400 to 800 rpm). At stirring speed of 400 rpm to 500 rpm, some of the capsules were in egg shape and some of them were deformed (see Figure 3). When the stirring speed was increased from 600 rpm to 800 rpm, the capsules formed was mainly in spherical shape. The effect of stirring speed on shape of the capsules can be explained based on the formation of whirlpool cavity. When the alginate solution was stirred with low speeds (i.e. 400 rpm and 500 rpm), the whirlpool cavity formed in the solution did not generate enough centrifugal force to facilitate the droplets to penetrate into the gelation bath. Similar observation was reported (Abang *et al*., 2012). On the other hand, when the alginate solution was stirred with high speeds (in the range of 600 rpm to 800 rpm), the centrifugal force generated by the whirlpool cavity was sufficient to 'pull' the droplet into the gelation bath. Based on the authors' observation, the droplets floated at the surface of the bath and their shape began to distort while gelling in the bath to reach higher density than the bath density so that they can penetrate into the gelation bath.

Effect Of Types Chitosan:

Figure 4 shows the diameter of chitosan-alginate capsules formed using different types of chitosan. As shown in Figure 4, the diameter of the capsules from AHG alginate was averagely 4.5 cm, regardless of types chitosan (CHW and CHW). Similarly, the diameter of the capsules from AHM alginate was 3.86 mm, regardless of chitosan types. The result indicated that chitosan (CMW and CHW) has no significant effect on diameter of the capsules. These results are good agreement with previous work (Takka and Acarturk , 1999 and Cekik *et al*., 2007).

Figure 5 shows the membrane thickness of chitosan-alginate capsules formed using different types (molecular weight) of chitosan. As shown in Figure 5, the membrane thickness of the capsules from AHG alginate was averagely 0.175 mm, regardless of types chitosan (CMW and CHW). In the same manner, the

membrane thickness of the capsules from AHM alginate was 3.90 mm, regardless of chitosan types. The results showed that chitosan (CMW and CHW) molecular weight has no significant effect on the membrane thickness of capsules. It is speculated that the molecular weight cut off of the alginate gel could be much lower than the molecular weight of both chitosan types tested. As a result, the gelation process between alginate and chitosan was not significantly affected by the chitosan types.



Working condition:

Chitosan(CHW) concentration	: 1 % w/v
Calcium Chloride concentration	: 1 % w/v
Alginate (AHM) concentration	: 1 % w/v
Tween 80	: 0.1 %w/v
Gelation Time	: 30 min
Needle Size	: 0.8mm

Fig. 2: Diameter of chitosan-alginate capsules formed under various stirring speeds.

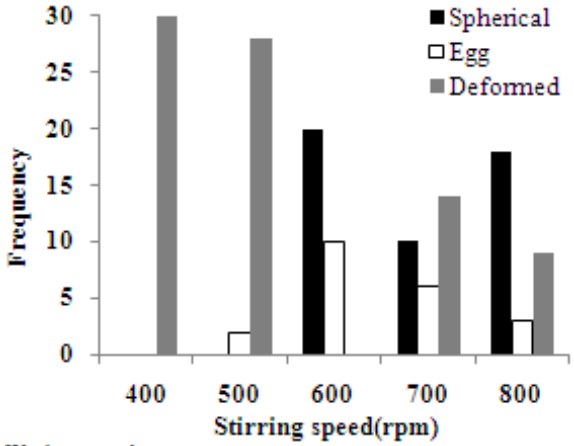


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Fig. 3: Shape distribution of chitosan-alginate capsules formed under various stirring speeds.

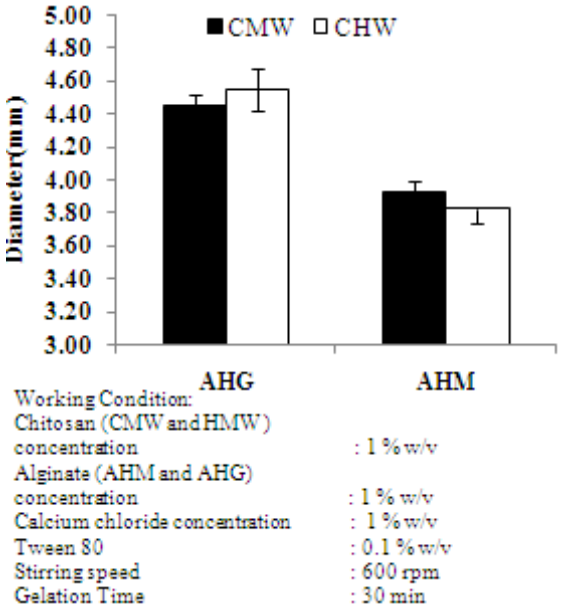

Fig. 4: Diameter of chitosan-alginate capsules formed using different types of chitosan and sodium alginate.

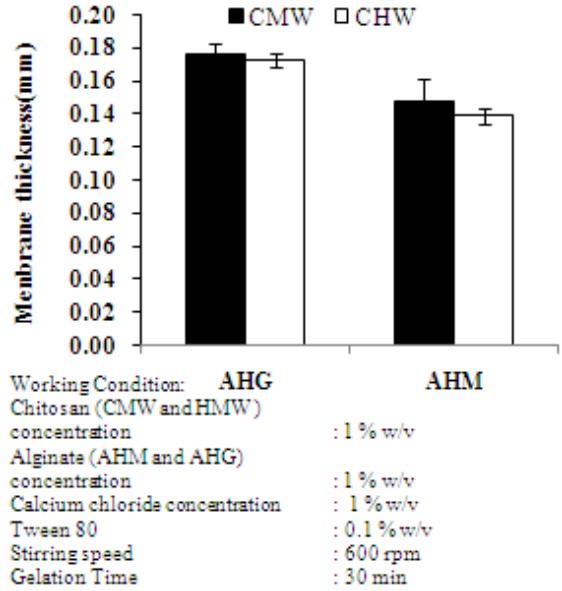

Fig. 5: Membrane thickness of chitosan-alginate capsules formed using different types of chitosan and sodium alginate.

Figure 6(a) and (b) show the shape distribution of chitosan-alginate capsules formed by different types of chitosan (CMW and CHW) and sodium alginate. As shown in both Figures, more than 50% of the capsules formed were in spherical shape, regardless of the types of chitosan and sodium alginate used. However, there were some deformed capsules when CHW chitosan was used in the formation. Since the high molecular weight chitosan (CHW) produces viscous liquid core solution, the solution droplet may need longer travelling distance than 2 cm (fixed in this study) before penetrating into the gelation bath. Long traveling distance is needed by the viscous solution droplet to regain spherical shape before gelation (Chan *et al*., 2009 and Chan *et al*., 2012).

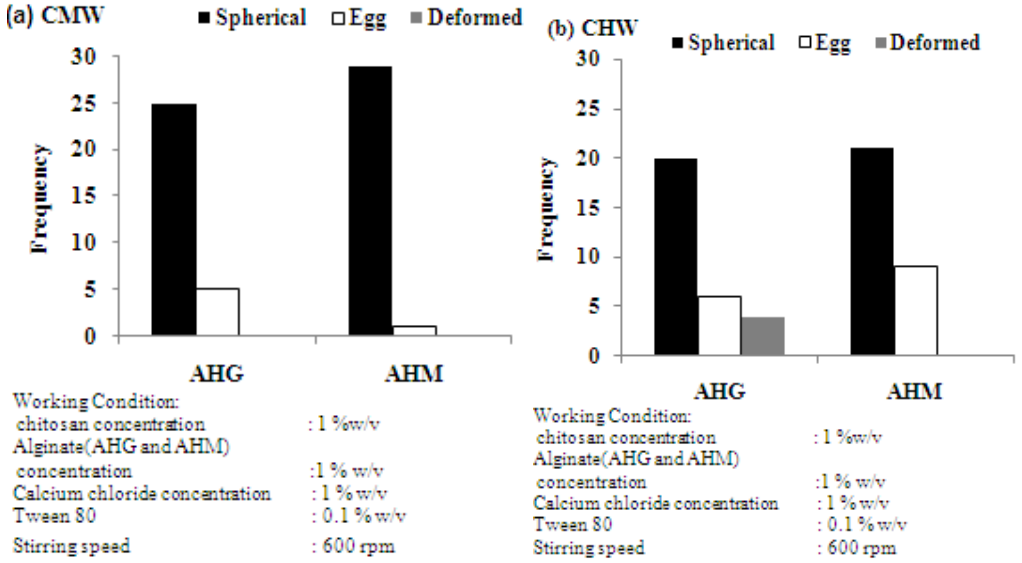

Fig. 6: Shape distribution of chitosan-alginate capsules formed by different types of chitosan (a) CMW (b) CHW and sodium alginate.

Effect Of Alginate Types:

Figure 4 illustrates the diameter of the capsules formed by different types of chitosan and sodium alginate. As presented in Figure 4, the diameter of chitosan-alginate capsules formed by AHG alginate and AHG alginate was averagely 4.5 mm and 3.9 mm, respectively. The diameter of the capsules was not influenced by the chitosan type. The results indicated that alginate type has significant effect on the diameter of the capsules. This could be explained by the M/G ratio of the alginate used. It has been reported that the guluronic acid-rich alginate produced larger gel particles than that of mannuronic acid-rich alginate (Chan *et al*., 2009). This could be attributed to the gels formed by the guluronic acid-rich alginate which are rigid, open and static (Draget *et al*., 1997).

The membrane thickness of chitosan-alginate capsules formed by different types of chitosan and sodium alginate was illustrated in Figure 5. The membrane thickness of the capsules formed by AHG alginate and AHM alginate was significantly different, regardless of the chitosan type. The membrane thickness of the AHG alginate capsules was approximately 0.04 mm thicker than that of AHM alginate capsules. As mentioned earlier, the guluronic-acid rich alginate forms open, rigid and static gel which eases the diffusion of gelating agents (i.e. calcium cation and chitosan) to cross-link or complex with the free binding site across the gel (Draget *et al*., 1997). As a result, thick membrane is formed by guluronic acid-rich alginate.

Shape distribution of the capsules formed by different types of chitosan and sodium alginate is shown in Figure 6. In regard to the alginate type, AHM alginate produces more spherical capsules than AHG alginate. In reference to Table 2, the viscosity of 1 % (w/v) AHG alginate solution was higher than that of AHM alginate solution and hence, the AHG alginate solution induced higher shear force to the capsule during formation. As a consequence, the capsules formed by AHG alginate solution are expected to have higher tendency to deform than that of AHM alginate solution (see Figure 6).

Conclusion:

The diameter, membrane thickness and shape distribution of the chitosan-alginate capsules formed under different stirring speeds was studied. In general, the diameter of the capsules was not affected by the stirring speed. At low stirring speeds (400 and 500 rpm), the capsules were deformed/elongated and hence, the capsule diameter was deviated and became larger than that of spherical capsules. At high stirring speeds, the centrifugal force generated by whirlpool cavity in the gelation bath was large enough to facilitate the penetration of the droplets into the gelation bath. As a result, spherical capsules were formed. In addition, the chitosan type was found to have no significant effect on the diameter, membrane thickness, and shape distribution of the capsules. This could be due to the molecular weight of the chitosan tested in this study was far higher than the molecular weight cut off of the alginate gel. Lastly, the guluronic acid-rich alginate (AHG) was found to form capsules with larger diameter and membrane thickness. This is because the AHG alginate tends to form open, rigid, and static gel than AHM alginate. However, the number of spherical capsule formed by AHG alginate was less than that of mannuronic acid-rich alginate (AHM). This is because the viscosity of the AHG alginate solution used in this study is higher than that of AHM alginate solution. In conclusion, the diameter and membrane thickness of

chitosan-alginate capsules were affected by the alginate type. The shape of the capsules was influenced by the stirring speeds and alginate type.

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REFERENCES

Abang, S., E.S. Chan and D. Poncelet, 2012. Effects of process variables on the encapsulation of oil in ca-alginate capsuleusing an inverse gelation technique. Journal of Microencapsulation, 29(5): 417-28.

Albarghouthi, M., D.A. Fara, M. Saleem, T. El-Thaher, K. Matalka and A. Badwan, 2000. Immobilization of antibodies on alginate-chitosan beads. International Journal of Pharmaceutics, 206(1-2): 23-34.

Blandino, A., M. Macias and D. Cantero, 2000.Glucose oxidase release from calcium alginate gel capsules. Enzyme Microbial Technology, 27: 319-324.

Chai, Y., L.H. Mei, G.L. Wu, D.Q. Lin and S.J. Yao, 2004. Gelation conditions and transport properties of hollow calcium alginate capsules. Biotechnology and Bioengineering, 87(2): 228-233.

Chan, E.S., Lee, B.B., Ravindra, P and D. Poncelet, 2009. Prediction models for shape and size of ca-alginate macrobeads produced through extrusion–dripping method. Journal of Colloid and Interface Science, (338): 63-72.

Chan, E.S., Lim, T.K., Ravindra, P., Rachel, F.M and A. Islam, 2012. The effect of low air-to-liquid mass flow rate ratios on the size, size distribution and shape of calcium alginate particles produced using the atomization method. Journal of Food Engineering, (108): 297-303.

Cekik, N.D., Savic, S., Savic, M.M and Z. Jovic, 2007. Preparation and characterization of phenytoin – loaded alginate and alginate-chitosan microparticles. Drug Delivery, (14): 483-490.

Daly, M.M and D. Knorr, 1988. Chitosan-alginate complex coacervate capsules: Effects of calcium chloride, plasticizers, and polyelectrolytes on mechanical stability. Biotechnology Progress, (4): 76-81.

Draget, K.I., G. Skjak-Braek and O. Smidsrod, 1997. Alginate based new materials. International Journal Biology Macromolecule, (21): 47-55.

Lee, B.B., Hong, W.O., Ravindra,P and Chan, E.S., 2005. Production of alginate-membrane capsules by using co-extrusion dripping method. In the Proceedings of the 2005 International conference on Chemical and Bioprocess Engineering.

Lee, O.S., Ha, B.J., Park, S.N and Y.S. Lee, 1997. Studies on the pH –dependent swelling properties and morphologies of chitosan/ calcium alginate complexed beads. Macromolecule Chemical Physical, 198: 2971-2976.

Munro, N.H and K.M. McGrath, 2012. Biomimetic approach to forming chitin/aragonite composites. Chemical Communications, **(**48): 4716-4718.

Smidsrod, O and G. Skjak-Braek, 1990. Alginate as immobilization matrix for cells. Trends Biotechnology, (8): 71-78.

Takka, S and F. Acarturk, 1999. Calcium alginate microparticles for oral administration: I: effect of sodium alginate type on drug release and drug entrapment efficiency. Journal of Microencapsulation, 16(3): 275-290.