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# Effects of process variables on the encapsulation of oil in ca-alginate capsules using an inverse gelation technique

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**Abstract**
The objective of this study was to investigate the effects of process variables on the encapsulation of oil in a calcium alginate membrane using an inverse gelation technique. A dispersion of calcium chloride solution in sunflower oil (water-in-oil emulsion) was added dropwise to the alginate solution. The migration of calcium ions to the alginate solution initiates the formation of a ca-alginate membrane around the emulsion droplets. The membrane thickness of wet capsules and the elastic modulus of dry capsules increased following first-order kinetics with an increasing curing time. An increase in the calcium chloride concentration increased the membrane thickness of wet capsules and the elastic modulus of dry capsules. An increase in the alginate concentration decreased the mean diameter of wet capsules but increased the elastic modulus of dry capsules.

**Keywords:** alginate, emulsion, encapsulation, inverse gelation, oil-core capsules

## Introduction

The encapsulation of oils in the form of oil-core capsules is used in agriculture, pharmaceuticals, foods, cosmetics and fragrances, and many other industries. Commonly, oils are encapsulated for controlled release, masking off-flavours or colours, increasing shelf life stability by protecting against oxidation, evaporation and incompatibilities with reactive substances, easy handling by converting sticky oils to free-flowing particles and for easy storage of dry particles compared to frozen oils. For instance, shark oil, as a supplementary food, is encapsulated because it has an offensive odour and taste. In the agricultural industry, volatile essential oils (e.g., *Zanthoxylum limonella*) are used as pest repellents and these are encapsulated to control the release of the oils into the environment.

Oils can be encapsulated in two structures, beads or capsules. A capsule consists of a well-defined core and an envelope, whilst a bead is made of a continuous phase of one or more miscible polymers in which encapsulants are dispersed (Mathiowitz et al., 1999). Different encapsulation methods are used to produce these two structures. Oils have been entrapped in beads using spray-drying and freeze-drying (Dzondo-Gaget et al., 2005; Jafari et al., 2008), emulsification/internal gelation (Ribeiro et al., 1999), co-crystallisation (Beristain et al., 1996), extrusion (Yilmaz et al., 2001), solvent evaporation (Aliabadi et al., 2007), emulsification (Miyazawa et al., 2000), ionic gelation (Chan, 2011), and molecular inclusions (Bhandari et al., 1999). On the other hand, oils can be encapsulated in capsules using a coacervation method (Bachtsi and Kiparissides, 1996; Katona et al., 2010), extrusion through concentric nozzles (Wyss et al., 2004), interfacial polycondensation (Bouchemal et al., 2006), internal phase separation (Dowding et al., 2004), and a microfluidic device (Ren et al., 2010). Spray-drying is the most economical and commonly used method to encapsulate oil; nonetheless, it has the disadvantage of using high temperature (up to 160°C) that accelerates the oxidation of oils (Kolanowski et al., 2006). The coacervation method is efficient but expensive, and has the limitation of using food-approved coating material in the food industry. Interfacial polymerisation allows high active loading (up to 90%) but involves high pH and toxic chemicals such as sebacoyl chloride (Yeo

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et al., 2001). On other hand, extrusion or cyclodextrin inclusion methods are mild but have a very low loading (Byun et al., 2010). An alternative method is needed to overcome some of these problems and to expand the availability of existing methods for encapsulating oils for the food, cosmetic, and agriculture industries.

Alginates are natural polysaccharides from brown algae consisting of long sequences of $\alpha$-L-guluronic acid and $\beta$-D-mannuronic acid (Fraser and Bickerstaff, 1997). They can easily be converted into gel in the presence of divalent cations ($Cd^{+}$, $Ba^{+}$, $Cu^{+}$, $Ca^{+}$, $Ni^{+}$, $Co^{2+}$, $Mn^{2+}$) through an ionic cross-linking (Ouwerx et al., 1998). Alginates have been widely used as carriers in many applications; for example, in enzyme immobilisation (Betigeri and Neau, 2002; Mondal et al., 2006; Bhushan et al., 2008) and the controlled release of drugs (You et al., 2001) because of their non-toxic, biodegradable, and biocompatible properties (Rinaudo, 2008).

The conventional method to produce calcium alginate beads through ionic gelation is by dropping an alginate solution into a calcium chloride solution. If the procedure is inversed, that is, calcium chloride solution is dropped into an alginate solution, aqueous-core calcium alginate capsules are produced (Soo et al., 1993; Blandino et al., 1999; Koyama and Seki, 2004; Sasaki et al., 2008). Recently, Anderson et al. (2005) patented an inverse gelation technique to produce oil-core capsules with a polysaccharide gel membrane. In this method, an emulsion comprising oil and calcium chloride solution is added dropwise to an aqueous gelling solution of ionic polysaccharide. However, little is known about the effects of the process variables on the physical properties of the oil-core capsules produced by this method. This knowledge is critical for developing formulations and processes to control the capsule properties.

The aim of this work was thus to investigate the effects of process variables on the physical properties of oil-core capsules produced by the inverse gelation method. In this study, alginate was used to form the capsule membrane and three different methods of incorporating the calcium source in oil were tested. The process variables examined were sodium alginate concentration, calcium chloride concentration, and curing time while the physical properties of the capsules investigated were membrane thickness and elastic modulus.

## Materials and methods

### *Materials*

Sodium alginate powder Algogel 3001 (mannuronic to guluronic acid ratio: 0.64) was kindly donated by Cargill (France). Calcium chloride powder ($CaCl_2 \cdot 2H_2O$) was purchased from Panreac Quimica Sau, Spain (purity 99%). Ultrafine calcium carbonate powder (2 μm) was provided by Omya (France) and sunflower cooking oil of commercial grade was purchased from Associated Oil Packers (France, brand name of Amphora de Risso). Miglyol 829 oil was kindly provided by Sasol (Germany). All other chemicals of analytical grade were obtained from Sigma Aldrich (France).

### *Experimental set-up*

Oil containing the calcium source (Figure 1(A)) was pumped at a flow rate of 6 mL/min using a peristaltic pump (Cole-Palmer Instrument Co., USA) (Figure 1(B)) to an eight-nozzle dispensing disc (Figure 1(C)) and dropped into 400 mL of alginate solution (Figure 1(D)). The multinozzle dispensing disc (80 mm diameter) consisted of two polyetheretherketone discs that were screwed together to form a chamber (Figure 1(g)). The top disc was connected to a pumping tube and the bottom disc was installed with eight luer lock connectors (Figure 1(h)). The disc was fitted with eight polyethylene helical-thread tapered tips (0.38 mm internal diameter) (Eleco Produits E.F.D., France) (Figure 1(i)). The alginate solution was stirred at 350–400 rpm with an 80-mm long wedge-shaped magnetic bar (Figure 1(E)). After the desired curing time (7.5, 15, 30 and 60 min) at ambient temperature ($20 \pm 2^{\circ}C$), the wet capsules were washed with demineralised water to remove excess calcium and to prevent the capsules from sticking to each other. The capsules were stored at 4°C until further use.

### *Preparation of alginate solution*

Alginate solution was prepared by dissolving alginate powder Algogel 3001 in demineralised water to make required concentrations of 5, 10 and 15 g/L. A surfactant (Tween 85) of 0.5% v/v was then added to the alginate solution.

### *Preparation of oil containing the calcium source*

Preliminary experiments were conducted to determine the best method of incorporating the calcium source in the oil. The three different sources of calcium are shown in Table 1.

#### *Method 1: Oil containing calcium carbonate powder*

Ultrafine calcium carbonate powder (5 g) was suspended in 100 mL of Miglyol 829 oil. Glacial acetic acid (4 mL) was added to sodium alginate solution (400 mL) to promote the dissolution of the calcium carbonate powder suspended in oil.

#### *Method 2: Oil containing calcium chloride powder*

Calcium chloride granules were ground using a pulveriser (Fritsch Pulverisette, France) and sieved through an 80 -μm mesh sieve (Prolabo, France). Three grams of calcium chloride powder was then dispersed in 100 mL of Miglyol 829 oil.

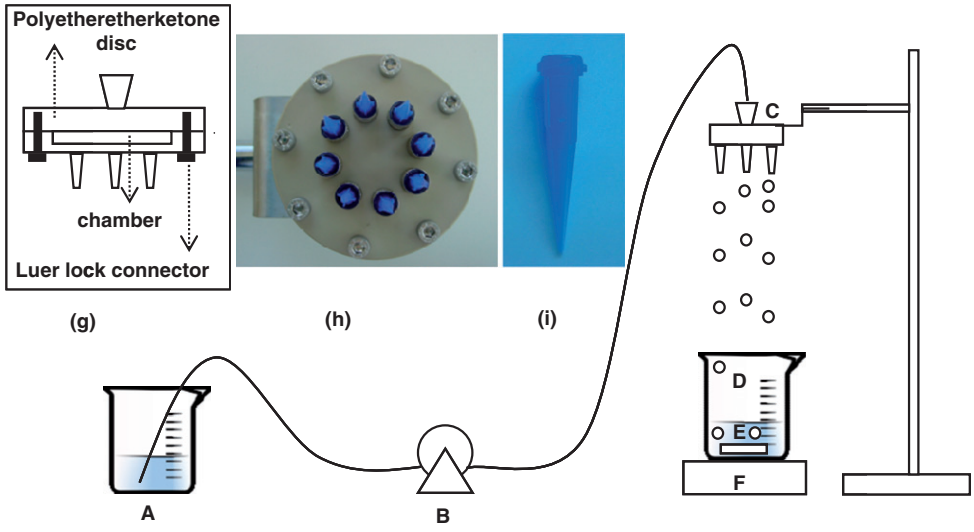


Figure 1. Diagram of the experimental set-up (A, vessel containing oil with calcium source; B, peristaltic pump; C, multinozzle dispensing disc; D, vessel containing sodium alginate solution; E, magnetic bar; F, magnetic stirrer). Inset pictures: (g) diagram of dispensing disc (side view), (h) top view of dispensing disc, (i) tapered tip.

Table 1. Composition of capsules using three different calcium sources.

| Method | Oil type (100 mL) | Calcium source | Type of droplet |
|---|---|---|---|
| 1 | Miglyol 829 | Calcium carbonate powder (5 g) | Oil containing calcium carbonate powder |
| 2 | Miglyol 829 | Calcium chloride powder (3 g) | Oil containing calcium chloride powder |
| 3 | Sunflower | Calcium chloride solution (30 mL) | Oil containing calcium chloride solution (water/oil emulsion) |

*Method 3: Oil containing calcium chloride solution (i.e., w/o emulsion)*

Calcium chloride solution (30 mL) was dispersed in 100 mL of sunflower oil (containing 0.50 mL of Tween 85 and 0.50 mL of Span 85) using a high shear mixer (Ultra-Turrax T25, IKA, Germany) at 13 500 rpm for 3 min.

*Viscosities of sodium alginate solutions and calcium chloride/oil emulsions*

The dynamic viscosities of the alginate solutions and the calcium chloride/oil emulsions were measured between a shear rate of 0.02 and 850 $s^{-1}$ with cone and plate (4°/60 mm) geometry using a Rheolyst AR1000-N rheometer (TA Instruments, France) at a constant temperature of 25°C. The viscosity was determined from the slope of a shear stress versus shear rate graph at a shear rate of 10 $s^{-1}$, which is a typical shear rate for extrusion under gravity (Richardson et al., 1999). Measurements were made in triplicate to obtain the averages and the standard deviations.

*Measurement of membrane thickness and diameter of wet capsules*

A confocal laser-scanning microscope (Nikon, France) with a 20X objective was used to measure the membrane thickness of the capsules. Image analysis was performed using ImageJ 1.42q software (USA). The wet capsules were labelled with fluorescent Rhodamine B (1 g/L) for 1 h. The average values and the standard deviations for 40 measurements of the membrane thickness from the same batch of capsules were reported. The external diameters of 40 wet capsules were measured manually using a vernier calliper to obtain the mean diameters and standard deviations of capsules.

*Measurement of elastic modulus of dry capsules*

The wet capsules were dried overnight at an ambient temperature (20°C ± 2°C) until a constant weight was reached. A uniaxial compression between two parallel plates was performed to measure the resulting stress when a constant strain of 5%/min was applied on the dry capsules using a Dynamic Mechanical Analyser Q800 (TA Instruments, France) at an ambient temperature (20°C ± 2°C). The individual elastic modulus for three capsules of the same size from the same batch was obtained by using best-fit linear regression in the linear region of 5% to 15% strain. The averages and standard deviations of the elastic moduli of three capsules were reported.

## Statistical analysis

The effects of calcium chloride and alginate concentrations on the mean diameter of wet capsules and the mechanical

strength of dry capsules were modelled using multiple linear regression analysis (SigmaPlot 11, Systat Software Inc.). *P*-values equal to or less than 0.05 were considered statistically significant. The standardised regression coefficient ($\beta_i$) was used to compare the relative influence of the individual independent variable on the dependent variables and was calculated as follows:

$$\left(\beta_i = b_i(s_i/s_y)\right),$$

where $b_i$ is the unstandardised regression coefficient, $s_i$ is the standard deviation of the independent variable *i,* and $s_y$ is the standard deviation of the dependent variable *y* (Bring, 1994).

## Results and discussion

*Preliminary studies on the production of oil-core capsules*

Oil-core capsules were produced by dropping oil containing a calcium source into an alginate solution. Preliminary experiments were conducted to determine the best method of incorporating the calcium source in the oil. The selection of the best method was based on the sphericity of the capsules, the simplicity of the method and the ability of droplets to form capsules once dropped into an alginate solution.

*Method 1: Oil containing calcium carbonate powder*

The concept of initiating the formation of a ca-alginate membrane by dissolving calcium carbonate powder when hydrated at low pH was adopted from the emulsification/internal gelation method described by Poncelet et al. (1992). In this experiment, fine calcium carbonate powder (i.e., about 2 μm) was used to allow a homogeneous dispersion of calcium carbonate in Miglyol 829 oil. This oil was used to facilitate the penetration of oil/calcium carbonate droplets into the alginate solution because of its high viscosity and density; 230–270 mPa.s and 1000–1020 kg/m$^3$, respectively.

The addition of 1% v/v of acetic acid to the alginate solution resulted in a pH drop, from 7.3 to 3.7. The low pH triggered the release of calcium ions and carbonate ions from the insoluble carbonate complex. Calcium ions diffused out of the oil phase to cross-link with alginate and form a calcium alginate membrane around the droplets. The presence of carbon dioxide bubbles due to the decomposition of carbonic acid could not be observed. The capsules were spherical and their size ranged from 2.5 to 3.5 mm. Under a microscope, the residual calcium carbonate powder was seen inside the core of the capsule (Figure 2(a)) due to its incomplete dissolution. Stoichiometrically, one mole of calcium carbonate reacts with two moles of acetic acid (Silva et al., 2006a). In this experiment, the acetic acid/calcium carbonate molar ratio (0.33) was below the stoichiometric proportion thus the amount of acetic acid was insufficient to dissolve the calcium carbonate completely. Increasing the concentration of acetic acid in the sodium alginate solution was not possible because sodium alginate precipitates to insoluble alginic acid at a pH below the pKa value of around 3.6 to 3.7 (Voo et al., 2011). On the other hand, decreasing the amount of calcium carbonate leads to weak capsules as the hydrogel network is less cross-linked (Chan et al., 2006). The residual calcium carbonate inside the capsules was dissolved after being stored in demineralised water for 5 to 7 days, indicating that the effect of the acetic acid/calcium molar ratio was imperceptible (Silva et al., 2006b). This method requires careful monitoring and control of pH to trigger the capsule membrane formation as well as an appropriate ratio between the amount of acetic acid and calcium carbonate to produce capsules. In view of the difficulties encountered with this approach, the second method was tested.

*Method 2: Oil containing calcium chloride powder*

The drawbacks of producing the oil core capsules from the suspension of calcium carbonate in oil led to the replacement of calcium carbonate powder by calcium chloride powder. The capsules produced were very delicate and easily broken during stirring, indicating that the membrane formed was soft and fragile. The capsules were also non-spherical and non-uniform in size (Figure 2(b)). This might be attributed to the undissolved calcium chloride in the oil that led to a low degree of cross-linking between the calcium ions and the alginate. Another problem encountered was the rapid sedimentation of calcium chloride grains at the tapered tip so that there was less calcium chloride in the oil droplets available for cross-linking in the alginate solution. In addition, the dripping process had to be completed in less than 1 min due to the blockage of the tip. In conclusion, this method is unsuitable for the production of oil-core capsules because the dripping process is difficult to handle and the capsules produced are not spherical or uniform.

*Method 3: Oil containing calcium chloride solution*

In this method, the calcium chloride was first dissolved in water then the solution was dispersed in oil to form a water-in-oil emulsion. In this case, sunflower oil was used instead of Miglyol 829 oil because it forms a stable emulsion with high viscosity. Nevertheless, several difficulties were encountered when the calcium chloride/oil emulsion droplets were dropped into the alginate solution. Firstly, it was difficult to obtain spherical capsules owing to the differences in viscosity and surface tension between the droplets and the alginate solution. The droplets must possess a higher viscosity than the alginate solution to overcome the deformation effect due to the impact between the droplets and the alginate solution surface. Secondly, the thickness of the capsules might be uneven as the membrane formation depends on the curing time or residence time in the alginate solution. Thirdly, the capsules agglomerated easily because of the continuous outward diffusion of calcium ions towards the outer surface of the capsules. The process conditions were thus optimised

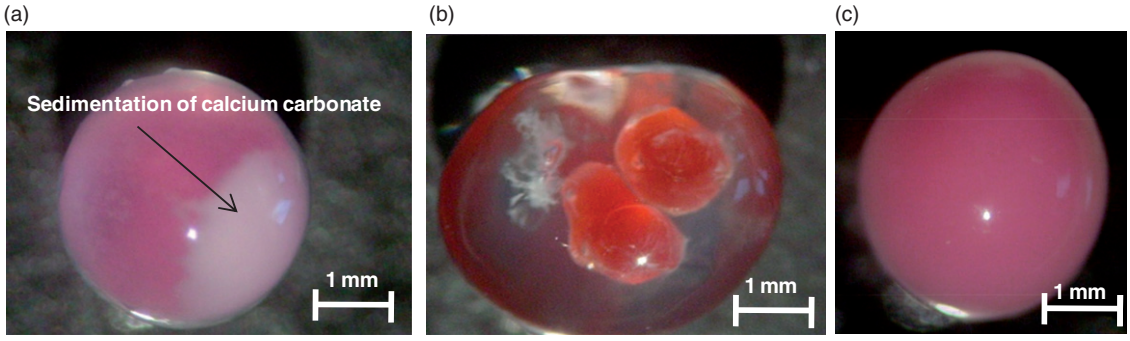


Figure 2. Wet capsules produced from droplets of (a) oil containing calcium carbonate powder, (b) oil containing calcium chloride powder, (c) oil containing calcium chloride solution (water/oil emulsion).



to solve these problems and capsules of spherical shape and uniform size were successfully formed (Figure 2(c)). Further details are described in the next section. In conclusion, this method was found suitable for the production of oil-core capsules. Further studies were carried out to investigate the effects of the process variables on the encapsulation of oil by dropping calcium chloride/oil emulsion droplets into an alginate solution.

### *Set-up of process variables for the production of oil-core capsules*

Following the preliminary studies, several operating parameters (dropping height, nozzle, dripping mode and stirring rate) that influenced the formation of the oil-core capsules were studied and then fixed at constant values.

Spherical capsules were formed at a dropping height, that is, distance between the dropping tips and the alginate solution surface, of 80–100 mm. Below 80 mm, the droplets could not penetrate the alginate solution surface, whereas at a dropping height above 100 mm, the droplets were broken on impact. This phenomenon can be explained by the kinetic energy of droplets during impact and their viscosity (Pregent et al., 2009). A dropping height was therefore fixed at 80 mm to ensure the formation of spherical oil-core capsules.

In this study, the calcium chloride/oil emulsion droplets were produced at a low liquid flow rate under dripping mode (drop per drop) in order to obtain uniform-sized droplets. The low production rate was overcome by using a multinozzle disc equipped with eight nozzles. Tapered tips were selected instead of hypodermic needles to reduce (by up to a factor of 4) the pressure required to extrude the highly viscous calcium chloride/oil emulsion. The droplets were dropped into a whirlpool cavity of the alginate solution that was developed by stirring the solution at 350–400 rpm. Stirring is required to facilitate the penetration of the droplets into the gelling bath solution and prevent agglomeration of the capsules. At the selected mixing speed, the capsules were mainly spherical with a uniform membrane thickness and none were broken.

Table 2. Physical properties of calcium chloride/oil emulsions.

| Calcium chloride concentration g/L | Density of emulsion* kg/m$^3$ | Viscosity of emulsion at 10 s$^{-1}$* mPa.s |
|---|---|---|
| 40 | 889 | 422 |
| 80 | 943 | 683 |
| 120 | 959 | 724 |

Note: *Measurements were made in triplicate with a coefficient of variance of less than 5%.

Table 3. Physical properties of alginate Algogel 3001 solutions.

| Alginate concentration g/L | Density of alginate solution* kg/m$^3$ | Viscosity of alginate solution at 10 s$^{-1}$* mPa.s | Surface tension without Tween 85 mN/m | Surface tension with Tween 85 mN/m |
|---|---|---|---|---|
| 5 | 997 | 6 | 59.0 | 34.6 |
| 10 | 1000 | 38 | 57.2 | 33.2 |
| 15 | 1006 | 127 | 55.8 | 32.5 |

Note: *Measurements were made in triplicate with a coefficient of variance of less than 5%.

### *Physical properties of droplets and gelling bath*

The viscosity and surface tension force of the droplets must be greater than the forces required to counteract the effects of impact and drag, so as to prevent the deformation of droplets during their impact with the gelling bath surface (Chan et al., 2009). The viscosities of the calcium chloride/oil emulsions and alginate solutions increased with increasing calcium chloride concentration and alginate concentration, respectively (Tables 2 and 3). The increase in the calcium chloride/oil emulsion viscosity with increasing calcium chloride concentration was attributed to the interfacial interaction between the surfactants and the electrolyte (calcium ions) that resulted in a stable and rigid interface, and thus shearing was more difficult (Kovalchuk et al., 2010). Similar observations have been previously reported (Aronson and Petko, 1993; Martinez et al., 2007). The viscosities of the emulsions were higher

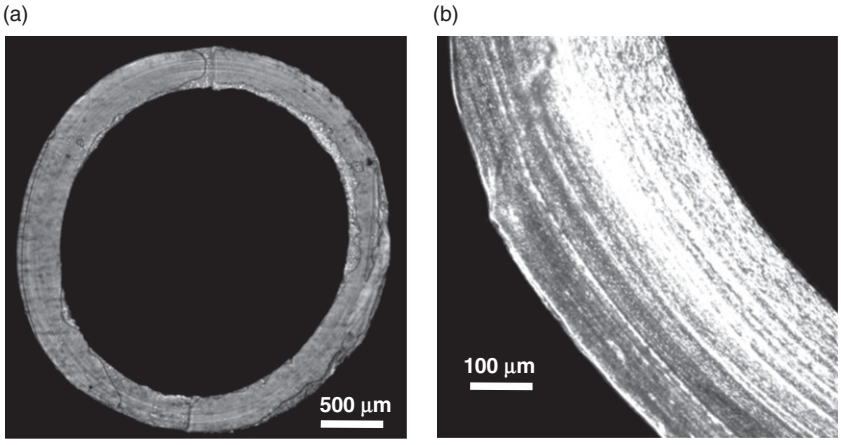


Figure 3. Confocal laser scanning microscope fluorescent images of (a) wet calcium alginate capsule and (b) wet membrane layer of calcium alginate.



than the viscosities of the alginate solutions (Tables 2 and 3). The deformation of droplets during impact with an alginate solution has been found to decrease with the increasing viscosity of the emulsion (Clanet et al., 2004). In addition, high viscosities of emulsions enhance the damping of the initial oscillations of the interface, remaining hanging from the nozzle immediately following the detachment of the previous drop as well as maintaining the about-to-fall droplet nearly spherical in shape as it detaches from the nozzle (Zhang and Basaran, 1995). However, when the viscosity of the emulsion was increased above 1000 mPa.s, the liquid gradually grew into a long thread from the dripping tip and this thread stretched under its own weight until it finally ruptured to form elongated droplets.

A previous study has shown that a stable water-in-oil emulsion can be described by an increase in the viscosity of the emulsion at least 3 orders of magnitude higher than the starting oil (Fingas and Fieldhouse, 2003). A stable calcium chloride/oil emulsion was achieved by applying a hydrophile-lipophile balance (HLB) system (Griffin, 1949). The HLB value of a mixed surfactant system $(HLB)_m$ was calculated by using HLB values ($HLB_A$ and $HLB_B$) and mass fractions of individual surfactants ($X_A^W$ and $X_B^W$) as follows: $(HLB)_m = X_A^W(HLB_A) + X_A^W(HLB_B)$ (Sahin and Sumnu, 2006).

The calcium chloride/oil emulsions that were produced from different calcium chloride concentrations were stable throughout the dripping. The viscosities of the emulsions were between 8 and 13 orders of magnitude higher than that of the starting oil with increasing calcium chloride concentration. The stability of the emulsions could also be attributed to the stabilising effect of calcium salt, which Marquez et al. (2010) reported as being due to the reduction in the water droplet size, the decrease in the attractive force between the water droplets, and the increase in the adsorption density of the emulsifier.

The degree of deformation during the production of capsules has been correlated to the differences in the viscosity of the droplet solution and the gelling bath solution as well as the kinetics of membrane formation (Dautzenberg et al., 1996). An alginate with the lowest viscosity (Algogel 3001, 38 mPa.s at 1%) was thus selected to give minimum resistance to the penetration of calcium chloride/oil emulsion droplets. The droplets penetrated the alginate solution to form spherical capsules, indicating that their viscosity was resistant enough to overcome the forces during impact and the shear forces from the stirring of the alginate solution. In addition, a hydrophilic non-ionic surfactant, 0.5% v/v Tween 85, was added to the alginate solution to modify its interfacial properties, and thus facilitate the penetration of droplets. The addition of surfactants is an efficient way to adjust the surface tension of a solution and several studies have been carried out to investigate the interactions between alginate and surfactants (Neumann et al., 2003; Yang et al., 2008; Yang et al., 2009). The addition of Tween 85 was found to reduce the surface tension of alginate solutions by 40% (Table 3).

### *Effect of curing time on the membrane thickness of wet capsules*

Figure 3(a) and (b) show photographs of a wet calcium alginate capsule and the calcium alginate membrane observed using a confocal laser scanning microscope. The capsule is spherical in shape and has a uniform membrane thickness encircling the oil core. The sphericity of the wet capsules generated using the dripping mode was dependent on the appropriate selection of flow rate, emulsion viscosity, dropping height, and stirring rate of the alginate solution.

The changes in membrane thickness of the wet capsules with curing time were modelled using non-linear regression analysis (Figure 4). The data were curve-fitted with a binomial diffusion equation described by Chrastil (1988, 1991) as shown below:

$$L = L_{max}[1 - \exp(-kt)]^n \quad (1)$$

where $L$ is the membrane thickness at time $t$, $L_{max}$ is the maximum membrane thickness (completion of membrane formation), $k$ is the membrane formation rate constant, and $n$ is the heterogeneous structural resistance constant. When the calcium chloride/oil emulsion droplets are

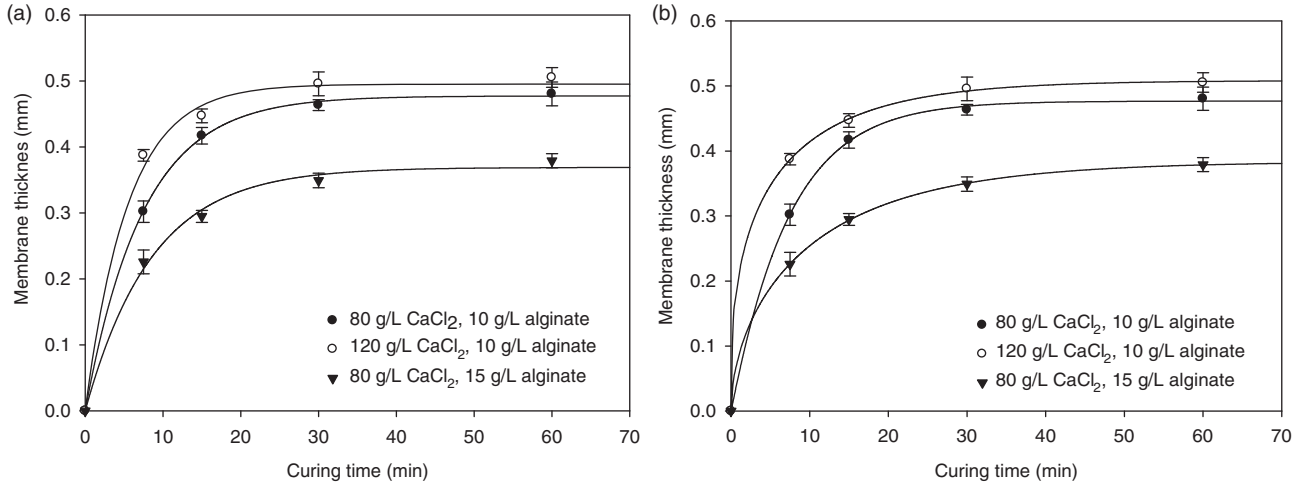


Figure 4. Effect of curing time on the membrane thickness of wet capsules (a) $n=1$, (b) $n\neq 1$. Capsule size: 2.9–3.5 mm with a coefficient of variance of less than 5%.

dropped into the alginate solution, calcium ions diffuse to the droplet interface to cross-link with the alginate solution. This phenomenon gives rise to a sharp increase in the membrane thickness. As the membrane thickness increases, the diffusion of calcium ions through the gel network becomes limiting because of the mass transfer resistance of the thicker membrane. Once the amount of calcium ions inside the core of the capsule is depleted, the gelation process ends. The rate of membrane formation decreases continuously until the calcium ions are depleted.

Two conditions were considered with respect to the $n$ value in fitting the data with the non-linear regression model: $n=1$ for a first-order reaction rate and $n\neq 1$ for a diffusion-limited reaction (Chrastil, 1988). A comparison of the fitted curves with a non-linear regression model for $n=1$ and $n\neq 1$ shows that the data fitted well in both conditions as the values of $r^2$ are approximately equal to unity (Table 4). A comparison of the kinetic parameters for both conditions shows that an increase in the calcium chloride and alginate concentrations altered the values of $k$ for $n\neq 1$ more than they did for $n=1$. Furthermore, analytically the values of $k$ for $n\neq 1$ seemed incoherent with the increase in calcium chloride and alginate concentrations. The comparison suggests that the value of $n=1$ is more suitable because it fitted coherently with the variation in the experimental parameters.

The membrane formation rate constant, $k$, increased by 35% and decreased by 13% with a 50% increase in the calcium chloride and alginate concentrations, respectively. Clearly, the membrane thickness was influenced by both concentrations. Chrastil (1991) reported that the membrane rate formation constant, $k$, was linearly dependent on alginate concentration, calcium chloride concentration and alginate composition. Leick et al. (2010) showed that the membrane thickness of aqueous-core capsule formation was dependent on curing time. The authors presumed that the gelation kinetics was not purely diffusion-controlled and that calcium alginate gel formation might be affected by other processes, such as the reorientation of the alginate chains, ionic interactions or cooperative processes of the extended ionic junction zones. Consequently, further experimental investigations are required to study other factors that may influence the gelation kinetics of membrane thickness and to develop a new modelling equation. The membrane thickness of wet capsules increased linearly with the mean diameter of wet capsules over the range of the curing time investigated (Figure 5).

One unanticipated finding was that, at a curing time of more than 60 min, the capsules disintegrated and dissolved in the alginate solution. This phenomenon was also observed by Koyama et al. (2004) in the production of aqueous-core calcium alginate capsules that were formed by dropping calcium chloride polyethylene glycol solution into an alginate solution. The authors assumed that a large amount of water entered the capsules from the alginate solution due to osmotic action, thus causing their deformation and rupture. However, the oil core of the capsule was not in a hypertonic state relative to the bulk solution. A possible explanation for this phenomenon may be that the complexation of calcium ions with alginate is reversible, which leads to the migration of calcium into the alginate solution. To confirm this assumption, calcium alginate beads were suspended in an alginate solution. The results showed a complete dissolution of these beads, which suggests that the suitable curing time for complete membrane formation is in the range of 30 to 40 min.

### *Effects of curing time on the mechanical strength of dry capsules*

Prior to the mechanical strength tests, the wet capsules were air-dried at an ambient temperature ($20^{\circ}C\pm 2^{\circ}C$) until constant weight was achieved. Air-drying was selected to ensure mild drying conditions. The wet capsules were essentially elastic hydrogel membranes. After drying, these membranes shrank significantly to form thin (less than

Table 4. Influences of calcium chloride and alginate concentrations on the kinetic parameters of membrane formation. Capsule size: 2.9–3.5 mm with a coefficient of variance of less than 5%.

| Heterogeneous structural resistance constant, $n$ | [$CaCl_2$] g/L | [Alginate] g/L | Maximum membrane thickness, $L_{max}$ mm | Membrane formation rate constant, $k$ $min^{-1}$ | $r^2$ |
|---|---|---|---|---|---|
| 1 | 80 | 10 | $0.477 \pm 0.003$ | $0.135 \pm 0.003$ | 1.000 |
| 1 | 120 | 10 | $0.495 \pm 0.003$ | $0.182 \pm 0.004$ | 0.996 |
| 1 | 80 | 15 | $0.369 \pm 0.002$ | $0.117 \pm 0.005$ | 0.996 |
| $1.039 \pm 0.016$ | 80 | 10 | $0.477 \pm 0.004$ | $0.138 \pm 0.006$ | 1.000 |
| $0.347 \pm 0.013$ | 120 | 10 | $0.508 \pm 0.004$ | $0.081 \pm 0.004$ | 1.000 |
| $0.532 \pm 0.013$ | 80 | 15 | $0.384 \pm 0.002$ | $0.062 \pm 0.003$ | 1.000 |

Note: *Results are given with ± standard error from the non-linear regression.



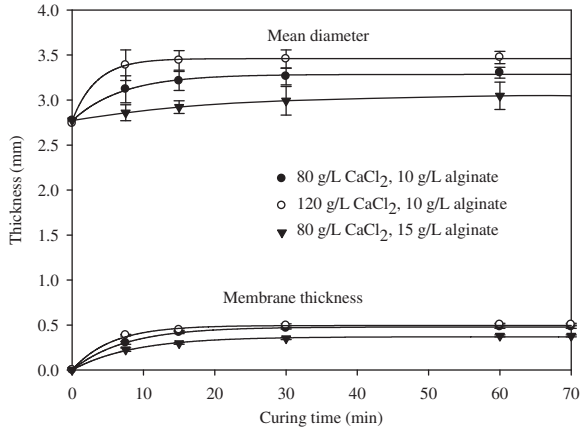


Figure 5. Effect of curing time on the membrane thickness and mean diameter of wet capsules. Capsule size: 2.9–3.5 mm with a coefficient of variance of less than 5%.

65 μm) rigid membranes. The dry capsules were spherical with a smooth surface (Figure 6). Smrdel et al. (2008) suggested that the slow water removal during air-drying leads to a slow and uniform shrinkage of beads. It has also been proposed that an incomplete dehydration in air-drying might significantly reduce the pore size of the alginate beads and prevent the surface from cracking (Das and Senapati, 2008).

The elastic modulus characterises the stiffness of the calcium alginate membrane, that is, the material resistance against the deflection of an applied force (Junter and Vinet, 2009). The elastic modulus of the dry capsules increased with increasing curing time (Figure 7). This was attributed to more interactions between calcium ions and the guluronic acid monomers in alginate chains to form a gel matrix as curing time increases. This was expected because the elastic modulus is a measure of the resistance offered by the gel to the stretching of ionic bonds that bind the polymer chains (Junter and Vinet, 2009). The elastic modulus of dry capsules also increased with an increase in membrane thickness. This shows that it is greatly governed by the membrane thickness of the capsules. Rehor et al. (2001) studied the influence of the membrane thickness of aqueous-core microcapsules that were formed by dropping sodium alginate/sodium cellulose sulphate into the calcium chloride solution. The authors reported that the resistance to smaller deformations was positively correlated to membrane thickness. A similar result was described by Rachik et al. (2006) who found that the Young modulus of aqueous-core capsules increased with increasing membrane thickness. Additionally, increasing both the calcium chloride and the alginate concentrations increased the elastic modulus of dry capsules; however, the former had a more marked effect. This can be explained by the diffusion of calcium ions, which mainly controls the mechanical properties and the membrane thickness of the capsules (Leick et al., 2010).

### *Effects of alginate and calcium chloride concentrations at constant curing time on the diameter of wet capsules and the mechanical strength of dry capsules*

The previous findings showed that the changes in membrane thickness and mechanical strength of capsules with curing time relied strongly on the amounts of the two cross-linkers: alginate and calcium chloride. Therefore, further investigations were carried out by preparing capsules with different reactant concentrations at a constant curing time of 40 min. The capsules were produced by the dispersion of three different concentrations of calcium chloride (40, 80 and 120 g/L) in sunflower oil to make calcium chloride/oil droplets that were then added dropwise to three different concentrations of alginate solution (5, 10 and 15 g/L). The influences of calcium chloride and alginate concentrations on the mean diameter of wet capsules and the elastic modulus of dry capsules were studied separately. As the change in the calcium alginate membrane over time was also exhibited by the mean diameter of capsules as mentioned previously, the following section will discuss the membrane thickness in terms of the mean diameter of capsules.

### *Mean diameter of wet capsules*

At constant alginate concentrations, the mean diameter of wet capsules increased by a factor of approximately 1.2 with an increase in the calcium chloride concentration (Figure 8(a)). When the amount of calcium ions increases, the higher gradient of calcium ion concentration between

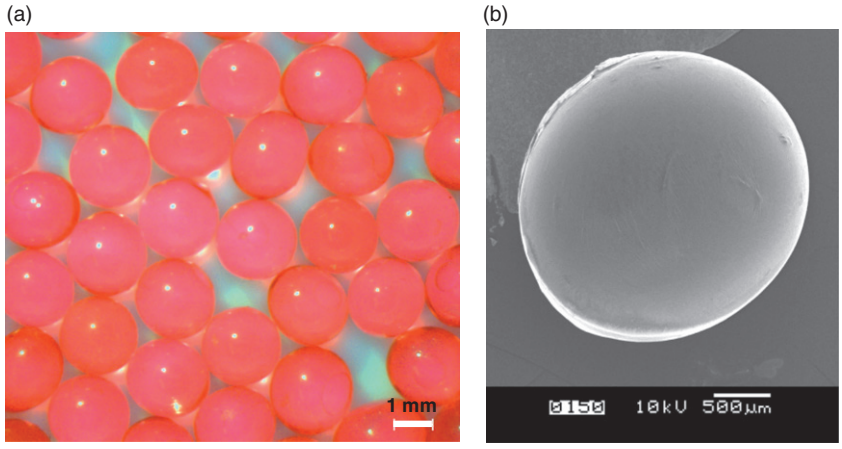


Figure 6. Photographs of dry capsules under a light microscope and a scanning electron microscope. Capsule size: 2.0 mm with a coefficient of variance of less than 5%.



the emulsion droplets and the alginate bath initiates more free calcium ions to diffuse and bind with the guluronic fraction in the alginate chains. As a result, the binding sites increase, which leads to an increase in the membrane thickness of the capsules. At constant calcium chloride concentrations, an increase in the alginate concentration led to a decrease in the mean diameter of wet capsules by a factor of 0.9 (Figure 8(b)). This can be explained by the increase in the number of alginate molecules per unit volume in close proximity to the droplet/alginate solution interface. As a result, calcium ions diffuse through a shorter distance to cross-link with the alginate, which leads to a compact cross-linked polymer network. These close and firm gel structures create a thinner membrane, resulting in the reduction of the mean diameter of wet capsules.

To determine the correlation between calcium chloride concentration ([Ca]) and alginate concentration ([NaAlg]) on the mean diameter of wet capsules ($\phi$), the data obtained were modelled using a multiple linear regression, which is represented by the following equation:

$$\phi(\text{mm}) = 0.00854[\text{Ca}] - 0.0262[\text{NaAlg}] + 2.788 \qquad (2)$$

The second coefficient is negative, indicating an inverse relationship between the membrane thickness of wet capsules and alginate concentration. On the contrary, the calcium chloride concentration shows a direct relationship with the membrane thickness of wet capsules. The standardised regression coefficients are 0.906 and 0.347 for calcium chloride and alginate concentrations, respectively. This suggests that the calcium chloride concentration has a greater influence on the membrane thickness of wet capsules compared to the alginate concentration.

### *Mechanical strength of dry capsules*

The elastic modulus of dry capsules increased by a factor of 2 to 3 with an increase in the calcium chloride concentration at constant alginate concentration (Figure 9(a)). This was attributed to the increase in the membrane thickness of capsules, which led to a stiff membrane shell. Previous studies (Leick et al., 2010) showed that the deformation of wet aqueous-core calcium alginate capsules under centrifugal forces decreased with an increase in the calcium ion concentration. Similarly, Chai et al. (2004) showed that the compression intensity of aqueous-core calcium alginate capsules increased with increasing calcium chloride concentration. Increasing the alginate concentration at constant calcium chloride concentrations increased the strength of the dry capsules by a factor of 1.1 to 1.7 (Figure 9(b)). This was attributed to an increase in the number of calcium ions binding to the alginate network to form stiff gel structures, indicating that the elastic modulus of the capsules was governed not only by their membrane thickness but also by the structure of the gel. Similar results were reported by Chai et al. (2004) and Leick et al. (2010), where the mechanical stability of calcium alginate capsules was enhanced by increasing the alginate concentration.

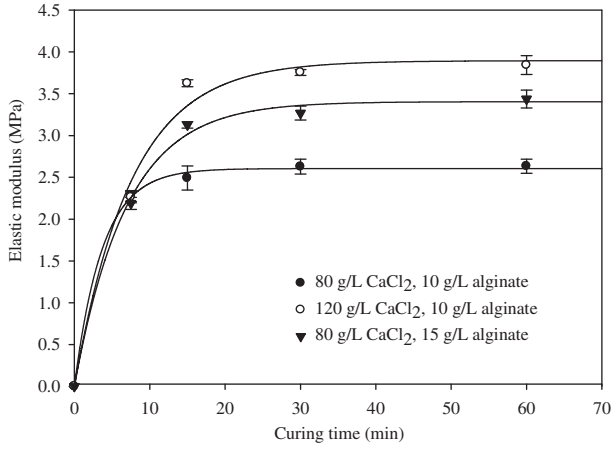


Figure 7. Effect of curing time on the elastic modulus of dry capsules. Capsule size: 2.0–2.5 mm with a coefficient of variance of less than 5%.

To estimate the effects of both calcium chloride concentration ([Ca]) and alginate concentration ([NaAlg]) on the elastic modulus of dry capsules ($E$), the data obtained were

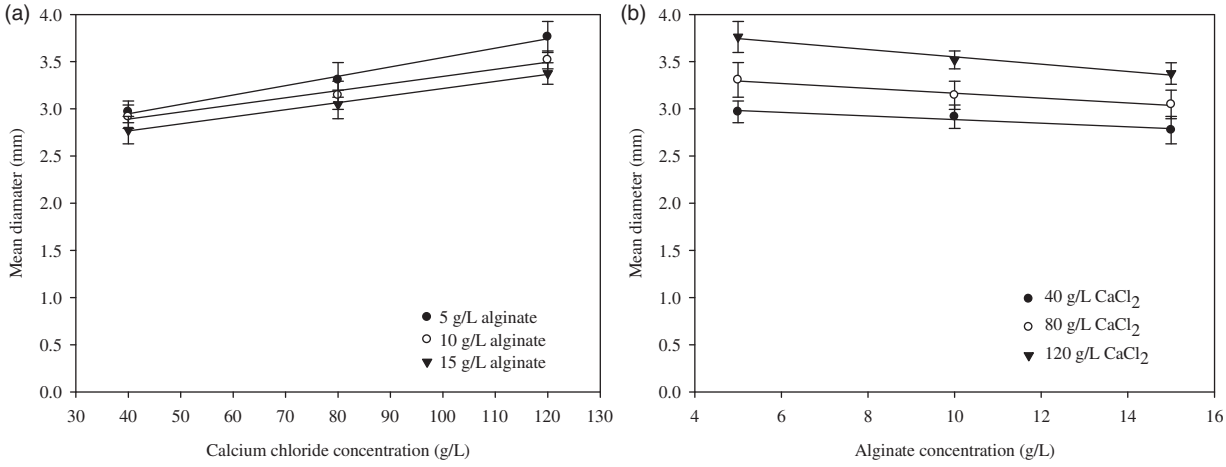


Figure 8. Effects of calcium chloride and alginate concentrations on the mean diameter of wet capsules.

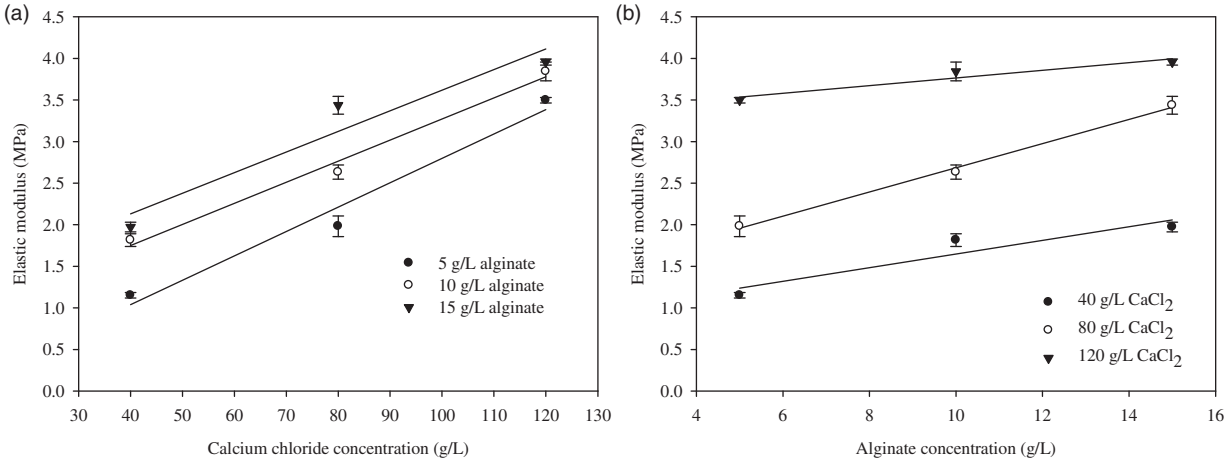


Figure 9. Effects of calcium chloride and alginate concentrations on the elastic modulus of dry capsules. Capsule size: 2.0–2.5 mm with coefficient of variance of less than 5%.

modelled using a multiple linear regression, which is represented by the following equation:

$$E(\text{MPa}) = 0.0269[\text{Ca}] + 0.0912[\text{NaAlg}] - 0.370 \quad (3)$$

The coefficients of calcium chloride and alginate concentrations are both positive, indicating a direct relationship with the elastic modulus of dry capsules. The calculated standardised regression coefficients are 0.925 and 0.391 for calcium chloride and alginate concentrations, respectively. These results suggest that the calcium chloride concentration has a greater influence on the elastic modulus of dry capsules, which is similar to the case of the membrane thickness of wet capsules.

## Conclusions

The effects of process variables on the encapsulation of oil in calcium alginate capsules using an inverse gelation technique were investigated. The membrane thickness and the mechanical resistance of the capsules can be customised by modifying the process conditions: gelation time, calcium chloride concentration and sodium alginate concentration. The oil phase may represent more than 95% of the dry capsules, indicating that the method allows for a high loading of encapsulants. At a flow rate of 360 mL/h, the present set-up of a simple dripping process can produce between 16 000 and 28 000 capsules/h, which makes the process attractive for the production of speciality products on a small scale. The biodegradable and renewable nature of alginate may open up the possibility of its application in the controlled release of compounds such as insect repellents and aromatherapy oils. Our future studies will involve the encapsulation of different active compounds (e.g., enzymes and plant extracts) using the inverse gelation method and the study of the loading, yield, release profile, integrity and functionality of the encapsulants.

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## Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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