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RESEARCH ARTICLE

Oil core microcapsules by inverse gelation technique

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Abstract

A promising technique for oil encapsulation in Ca-alginate capsules by inverse gelation was proposed by Abang et al. This method consists of emulsifying calcium chloride solution in oil and then adding it dropwise in an alginate solution to produce Ca-alginate capsules. Spherical capsules with diameters around 3 mm were produced by this technique, however the production of smaller capsules was not demonstrated. The objective of this study is to propose a new method of oil encapsulation in a Ca-alginate membrane by inverse gelation. The optimisation of the method leads to microcapsules with diameters around 500 μm. In a search of microcapsules with improved diffusion characteristics, the size reduction is an essential factor to broaden the applications in food, cosmetics and pharmaceuticals areas. This work contributes to a better understanding of the inverse gelation technique and allows the production of microcapsules with a well-defined shell–core structure.

Keywords

Alginate, emulsion, encapsulation, mixing, optimisation

History

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Introduction

Hydrophobic components in food, cosmetics and pharmaceutics are essential for the quality and the activity of the related applications. However they are often volatile, labile and sensitive to environmental factors such as light, water and oxygen. Therefore their encapsulation is necessary to protect and release them at the right place and time (Sauvant et al., 2012). Microencapsulation also facilitates incorporation of lipophilic materials into hydrophilic matrices and masking undesirable tastes (Chan, 2011).

The industrial technologies for oil phase encapsulation are mainly spray drying and coacervation (Cárdenas-Bailón et al., 2014; Oliveira et al., 2014). However, spray drying does not provide a strong protection and often degrades the product due to high process temperatures while microcapsules produced by coacervation require cross-linking with gluteraldehyde to be stable upon drying (Jerobin et al., 2012; Beirão-da-Costa et al., 2013; Carneiro et al., 2013).

One alternative method proposed in the literature consists of dispersing the oil in a polysaccharide solution (such as alginate) and produce beads by extruding the emulsion dropwise (dripping) into a gelation bath (calcium solution) (Chan et al., 2009). This technique leads to hydrogel beads containing a dispersion of oil droplets with entrapped oil content greater than 87% (w/w) (Peniche et al., 2004). However, since microcapsules are generally porous in dry form, the oil load should be kept lower to avoid losses via expulsion.

Better oil encapsulation loading was obtained by coextrusion-dripping of oil in a polysaccharide solution, and gelation into a divalent ion solution bath. Core–shell capsules were produced with high oil loading (>90%) after drying (Whelehan and Marison, 2011). While this technique is very promising, the scale-up of this method remains a bottleneck.

Inverse gelation consists of dripping oil, containing calcium dispersion into an alginate solution. The migration of calcium ions from oil phase to the alginate forms a hydrogel shell around the oil droplets (Anderson et al., 2005). In a previous work performed in our laboratory (Abang et al., 2012), sunflower oil and calcium chloride solution were emulsified and extruded through a nozzle dropwise into an alginate solution (Figure 1). After completion of the gelation process, wet capsules with diameter around 3 mm with an oil core surrounded by a Ca-alginate membrane were formed. After drying, capsules of 2 mm were obtained with oil loading up to 95 vol% (Abang et al., 2012).

Except for a few applications where capsules have to be visible (i.e. cosmetics), lower capsule sizes are required to allow incorporation into target matrix without changing the texture and augment the mechanical resistance of the end product. In addition, desired release scenarios from prolonged release by diffusion through the capsule wall to quick burst release may be required depending on applications. These scenarios will greatly depend on the ability to modulate capsules size. The main objective of this work is therefore to develop an inverse gelation method to produce small size capsules. The mechanism of microcapsule formation and some process variables that can influence the production are also discussed.

Materials and methods

Materials

Sodium alginate powder Saltialgine S60 NS was kindly donated by Cargill (France). The ratio of mannuronic (M) to guluronic (G) units (M/G = 1.37) and the molar mass of alginate ($M_w = 1.57 \times 10^5$ g/mol) were determined respectively by ^{1}H NMR spectroscopy and HPSEC-MALLS. Calcium chloride

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powder ($CaCl_2 \cdot 2H_2O$) (Panreac Quimica, Spain) and sunflower cooking oil (Associated Oil Packers, France) were used to prepare the oil-in-water (O/W) emulsions. All other chemicals of analytical grade were obtained from Sigma Aldrich (France).

Preparation of alginate and $CaCl_2$ solutions

Alginate powder was dissolved in demineralised water to get final concentrations of 5, 10, 15 and 20 g/L. A surfactant (Tween 85) at 0.5% v/v was then added to the alginate solutions. Tween 85 reduces the surface tension between the alginate solution and the oil phase. It supports both the dispersion of oil into the alginate solution and the removal of surface oil adhering to the outer surface of the capsules at the rinsing step.

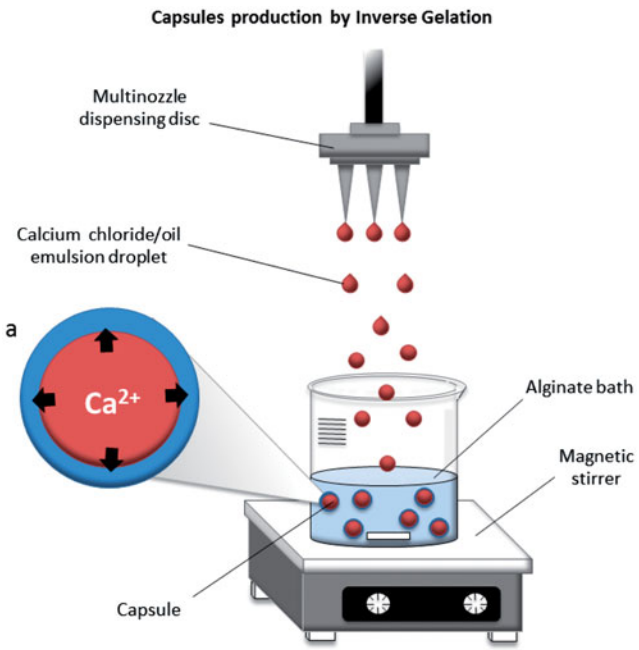

Figure 1. Capsules production by the inversion gelation technique. (a) Schematic representation of the calcium ions (Ca^{2+}) migration during the gelling process.

The calcium chloride solutions used in the primary O/W emulsions were prepared by dissolving $CaCl_2$ powder in demineralised water to get final concentrations of 10, 20, 30, 60, 120 and 240 g/L.

Preparation of primary O/W emulsions

The primary O/W emulsions containing the source of calcium were produced as described by Abang et al. (2012) with some slight modifications. Briefly, 100 mL of sunflower oil containing 0.5 mL of Tween 85 (0.5% v/v) and 0.5 mL of Span 85 (0.5% v/v) and 30 mL of calcium chloride solution (at 10, 20, 30, 60, 120 or 240 g/L) were stirred using a high shear mixer (Ultra-Turrax T25, IKA, Germany) at 13 500 rpm during 3 min (Figure 2A).

To obtain a stable O/W emulsion formulation, firstly the optimal hydrophilic–lipophilic balance value ($HBL_{optimal}$) of the emulsion was calculated using the equation: $HBL_{optimal} = \Phi_{oil} \cdot HLB_{oil}$, with Φ_{oil} the fraction of oil in the emulsion (0.77) and HLB_{oil} the hydrophilic–lipophilic balance value (7.0) of the sunflower oil. As a result of the previous equation, the optimal HLB value of the emulsion was 5.4. However, to prepare stable emulsions in practice, the HLB value of the surfactant alone or in mixture (HLB_m) can vary in the range $HLB_{optimal} \pm 1.0$ in relation with the $HLB_{optimal}$ of the emulsion (Griffin, 1949; ICI Americas Inc, 1980). To stabilise the O/W emulsion, a 1:1 mixture of a hydrophilic surfactant Tween 85 (HLB = 11) and alipophilic surfactant Span 85 (HLB = 1.8) were chosen. The HLB_m was calculated according to the equation: $HLB_m = X_A^W (HLB_A) + X_B^W (HLB_B)$, where HLB_A and HLB_B are the HLB values of surfactants A and B respectively, while X_A^W and X_B^W are the corresponding mass fractions of these components (Sahin and Sumnu, 2006). A HLB_m value of 6.4 was then calculated.

A primary O/W emulsion containing 27.7 g/L of $CaCl_2$ and 0.08 g/L of fluorescein was also produced using the same preparation procedure for confocal microscopy observations.

Preparation of the secondary O/W/O emulsions

For the secondary O/W/O emulsions, 100 mL of sunflower oil was put in a reactor (Ø 6.0 cm and *H* 6.5 cm) containing four deflectors (3 × 5 cm) symmetrically positioned. Ten millilitres of primary O/W emulsion was dispersed in oil using a paddle stirrer

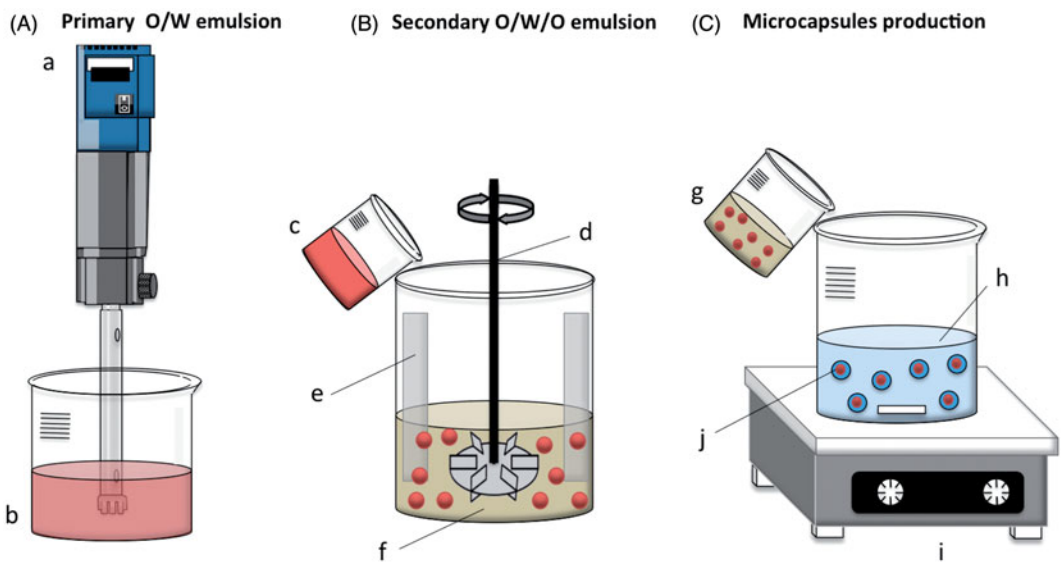

Figure 2. Diagram of the experimental set-up: Production of the primary O/W emulsion using sunflower oil and calcium chloride ($CaCl_2$) solution (A), secondary O/W/O emulsion by dispersing the primary O/W emulsion in sunflower oil (B) and microcapsules by mixing secondary O/W/O emulsion in alginate solution (C). (a) High shear mixer; (b) and (c) primary O/W emulsion; (d) turbine paddle; (e) deflector; (f) and (g) secondary O/W/O emulsion; (h) alginate solution bath; (i) magnetic stirrer and (j) microcapsules.

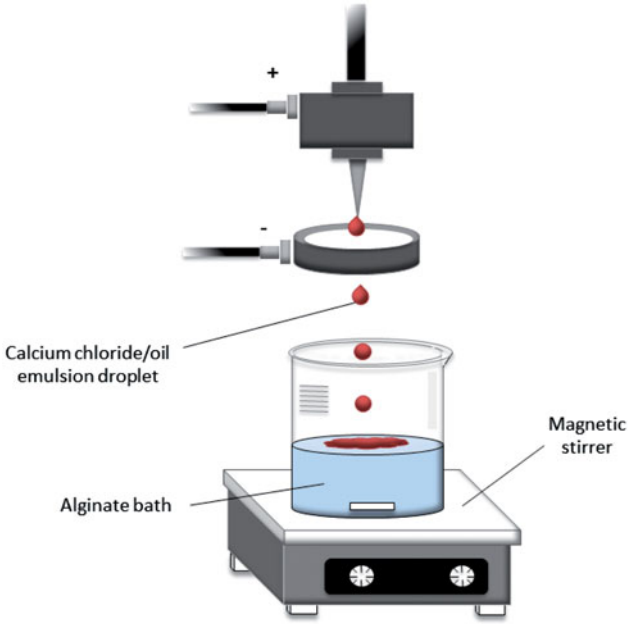

Figure 3. Schematic representation of the electrospray device.

(Eurostar digital, IKA, Germany) with a turbine paddle (Ø 3.7 cm) (Figure 2B). The secondary O/W/O emulsions were mixed at 150, 200, 350, 500 or 650 rpm for 2 min in order to evaluate the effect of stirring rate on the mean diameter of the microcapsules.

Microcapsules production

Production by electrostatic technology

An electrospray device consisting of a syringe pump, a polyethylene tip (Ø 0.38 mm), a ring electrode (Ø 50 mm) and one high voltage generator was used for the microcapsule production. Primary O/W emulsion containing 27.7 g/L of calcium chloride was extruded through the needle and positive voltages (0–4000 V) were applied between it and the ring electrode (Figure 3). The primary O/W emulsion droplets were dripped into a bath of 1% alginate solution under stirring (350–400 rpm).

Production by mixing technique

For microcapsule production, 110 mL of secondary O/W/O emulsion was added to 300 mL of alginate solution containing 1.5 mL Tween 85 (0.5% v/v) and stirred at 350–400 rpm with an 80 mm long wedge-shaped magnetic barrel (Figure 2C). To evaluate the effect of curing time on the production, the secondary O/W/O emulsion was mixed in the alginate solution during 5, 10, 15, 20, 30 and 60 min at ambient temperature (20 ± 2 °C). The wet microcapsules were sieved with a mesh of 0.16 mm (Prolabo, France) and washed with demineralised water to remove excess alginate. The microcapsules were then suspended in calcium chloride solution (15 g/L) to prevent sticking to each other.

Characterisation of the primary O/W and secondary O/W/O emulsions

Stability of the emulsions

The stabilities of the primary O/W and secondary O/W/O emulsions were determined according to Züge et al. (2013) with some modifications. Hundred millilitres of primary O/W or secondary O/W/O emulsions were placed in graduated tubes at 25 °C and the emulsions were visually inspected as a function of time in order to evaluate the critical time where a phase separation was clearly visible.

Observation of the emulsions by microscopy

Primary O/W emulsion labelled with fluorescein in the aqueous phase was observed using a Nikon Eclipse Ti inverse confocal scanning laser microscope (Nikon, France). Fluorescein emission fluorescence was recorded between 500 and 530 nm after excitation at 488 nm.

Secondary O/W/O emulsions were observed using a phase contrast microscope using an Olympus IX51 inverse microscope (Olympus, France) equipped with phase contrast illumination and a digital camera (Sony, SCD-SX90).

Primary O/W emulsion size distributions

The oil droplet size distribution in the primary O/W emulsion was measured by laser diffraction using a Mastersizer (Malvern Instruments, UK). The analysis was performed in wet mode where the primary O/W emulsion containing 27.7 g/L of calcium chloride was dispersed in distilled water.

Conductivity of primary O/W emulsion

Conductivity of the primary O/W emulsions was measured in triplicate at ambient temperature using a conductimeter (Mettler –Toledo, Analytical, Switzerland).

Measurements of microcapsules diameters, core diameters and membrane thicknesses

A Nikon Eclipse Ti inverse confocal scanning laser microscope (Nikon, France) with a 4X objective was used to capture the images of microcapsules after alginate labelling using fluorescein. The size of microcapsules, core diameters and membrane thicknesses were determined by image analysis using ImageJ 1.47v software (USA) on 100 capsules per sample. All samples were assayed in triplicate.

Results and discussion

Production of microcapsules by dripping

According to Abang et al. (2012), calcium chloride/oil emulsion (containing 27.7 g/L of $CaCl_2$) was pumped at a flow rate of 360 mL/h and extruded through a tip with a 0.38 mm internal diameter, leading to capsules with a mean diameter around 3 mm and an average production rate within 16 000–28 000 capsules/h.

In extrusion-dripping, the capsule size is limited by the syringe needle diameter and the viscosity of the solution. For this reason, capsules smaller than 1 mm are difficult to produce (Poncelet et al., 1992).

Additional external force in the dripping system needs to be applied to decrease the calcium chloride/oil emulsion droplet size, which is proportional to the capsule size (Brandenberger et al., 1999). In this study, we mainly use the electrospray technique consisting of the application of an electrostatic potential between the needle and a ring (Figure 3). Small calcium chloride/oil emulsion droplets with a diameter around 500 µm were produced using 0.1 mL/min flow rate of the emulsion passing through an electrostatic potential of 4000 V. Unfortunately, the small droplets were not able to penetrate into alginate solution bath (Figure 3). To penetrate into the bath, the emulsion droplets need to achieve a kinetic energy sufficient to break the surface tension of the alginate solution. The kinetic energy of the droplets, E, is given by:

$$E = \frac{\pi}{12}\rho d^2 v^2 \quad (1)$$

with v the fall velocity, ρ the density and d the diameter of the droplet.

The kinetic energy of the droplet increases proportional to the third power of the droplet size. The energy of small droplets is then insufficient to break the alginate solution surface. Ergo, instead of penetrating in the alginate solution, the droplets break at the solution surface and no capsules are formed.

Production of microcapsules by direct injection of calcium chloride/oil emulsion into alginate solution

To bypass droplet penetration problem, the calcium chloride/oil emulsion was injected directly into the alginate solution. A polyethylene tip (Ø 0.38 mm) connected at a pump was immersed in the alginate solution (10 g/L) under stirring (magnetic stirrer, 300 rpm/min). The calcium chloride/oil emulsion was pumped at different flow rates (0.1–6 mL/min). Instead of getting microcapsules, Ca-alginate fibres were formed in all tested conditions.

The minimum alginate gelation time (t_{gel}) can be estimated from the simplified Fick's second law (Cuadros et al., 2012):

$$t_{gel} \approx L^2/D \quad (2)$$

where L is the thickness of Ca-alginate shell in the fibre ($\approx 8\,\mu m$), and D the diffusion coefficient of calcium chloride in water at 25 °C ($1.3 \times 10^{-9}\,m^2/s$) (Ribeiro et al., 2008). The reaction between calcium ions and alginate occurred in ~0.05 s (from Equation (2)), which is not sufficient for the breakup of the injected emulsion into droplets before gelation takes place.

Production of microcapsules by injection of calcium chloride/oil emulsion into water or oil

As the direct injection in the alginate bath was not a viable strategy for microcapsule production, the injection of calcium chloride/oil emulsion in an aqueous phase was tested as alternative to form a dispersion of emulsion droplets, then alginate was added to induce gelation and produce microcapsules. However, resulting dispersions in aqueous phase consisted of very small oil droplets (4–16 µm) even in very gentle mixing conditions (Figure 4A). In presence of water, the emulsion was diluted compromising its structure and stability.

The calcium chloride/oil emulsion was injected in an oil phase in similar conditions. Surprisingly, large emulsion droplets (100–600 µm) dispersed in continuous oil phase were obtained (Figure 4B).

Better understanding of the calcium chloride/oil emulsion was therefore necessary to control the microcapsule formation.

Analysis of the calcium chloride/oil emulsion

The conductivity of the calcium chloride/oil emulsion was compared to the conductivity of the two phases present in the emulsion. The emulsion conductivity values (K_{em}) were also estimated based on the approximation (Allouche, 2003):

$$K_{em} = f_{cp}^{\frac{3}{2}} K_{cp} \quad (3)$$

where f_{cp} is the volumetric fraction and K_{cp} is the conductivity of the continuous phase.

The conductivity of the calcium chloride/oil emulsion was closer to the expected value if one assumed that the aqueous phase was the continuous phase (Table 1). Furthermore, according to Mayer et al. (2013), the high electrical conductivity of the emulsion indicated that the continuous phase was in fact aqueous. To confirm this observation, the calcium chloride/oil emulsion was viewed by confocal microscopy while adding some fluorescein dye in the aqueous phase. For the concentration used, the fluorescein is completely soluble in water and does not interfere on the size of oil droplets dispersed into aqueous phase.

Figure 5(A) shows that the aqueous phase (green) represented the continuous phase while oil phase was dispersed as small droplets. The size distribution of the calcium chloride/oil emulsion revealed that the volumetric mean diameter of the oil droplets was equal to 14 µm (Figure 5B).

Despite the fact that the emulsion formulation was designed to get a water-in-oil emulsion (choice of surfactants, aqueous phase volume of 23%, see Abang et al. (2012)), it appeared that the emulsion was in fact an oil-in-water type. Such structure may be advantageous while producing capsules by dripping, allowing a faster release of calcium.

In addition, no phase separation occurred over 15 days, showing a very high stability of the emulsion.

As initially conducted by Abang et al. (2012), the emulsion displayed the ideal characteristics to produce capsules by dripping, no investigation about the effect of the surfactants concentration on the properties of the emulsion was therefore performed.

Producing microcapsules by a secondary O/W/O emulsion

Previous experiments and observations led to test the production of microcapsules based on the following scheme:

(1) The calcium chloride/oil emulsion was prepared as above (called hereafter primary O/W emulsion).
(2) The primary O/W emulsion was dispersed in oil using a turbine impeller leading to an oil/water/oil emulsion (called hereafter secondary O/W/O emulsion).
(3) The secondary O/W/O emulsion was put in contact with an alginate solution leading to a transfer of the primary O/W emulsion into the alginate solution and formation of microcapsules.

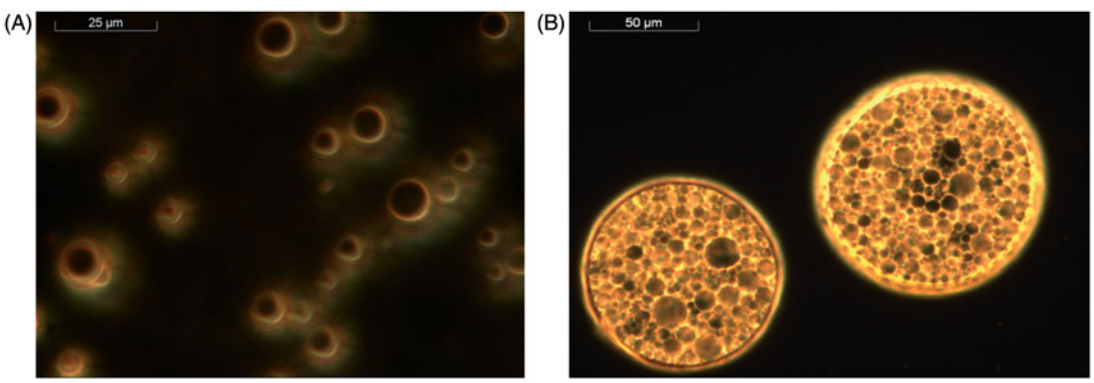

Figure 4. Optical microscopy images of the calcium chloride/oil emulsion diluted in water (A) or in oil (B). (A) Oil droplets (yellow circles) dispersed in water (black background); (B) calcium chloride/oil emulsion droplets dispersed in sunflower oil (black background). A secondary oil-in-water-in-oil (O/W/O) emulsion was obtained in this last condition.

Table 1. Conductivity of the two phases and the emulsion containing 100 mL of sunflower oil and 30 mL of calcium chloride solution at 120 g/L.

Experimental value of the oil phase	Experimental value of the aqueous phase	Experimental value of the primary O/W emulsion	Theoretical value of the primary emulsion assuming aqueous continuous phase	Theoretical value of the primary emulsion assuming oil continuous phase
0	28 000 ± 2500 µS/cm	6800 ± 300 µS/cm	3103 µS/cm	0

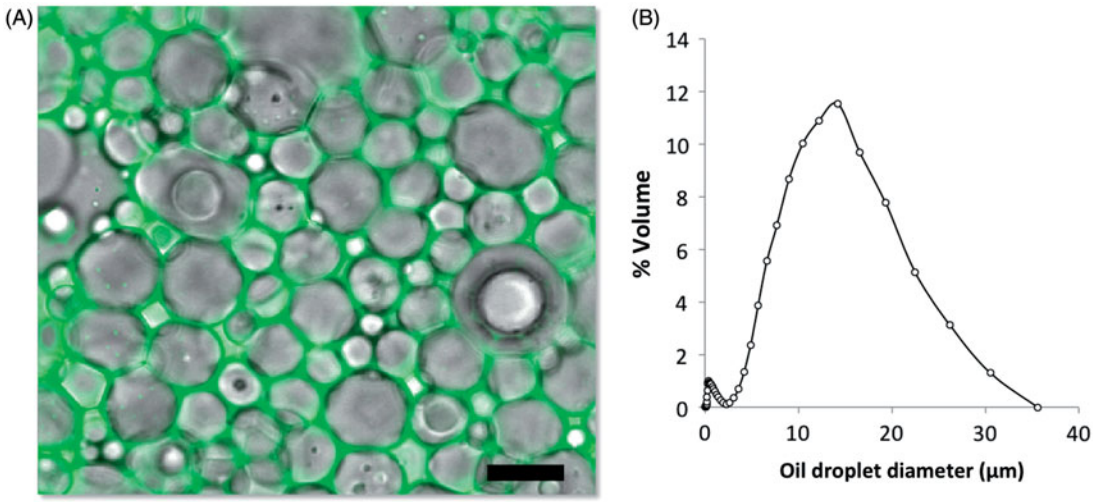

Figure 5. Optical microscopy image and volume size distribution of the calcium chloride/oil emulsion. (A) Confocal microscopy image of the calcium chloride/oil emulsion. Green: calcium chloride solution labelled with fluorescein; grey: sunflower oil. Scale bar = 10 µm. (B) Size distribution (% volume) of the calcium chloride/oil emulsion.

(4) The microcapsules were collected by filtration on 125 µm mesh and rinsed with distilled water.

Figure 4(B) shows an optical microscopy image of the secondary O/W/O emulsion in which primary O/W emulsion droplets (100–300 µm) were visible.

The stability of the secondary O/W/O emulsion was evaluated by the time of phase separation. In contrast to the primary O/W emulsion, the secondary O/W/O emulsion showed a high instability, leading to phase separation in less than 10 min. The instability of the secondary O/W/O emulsion favoured the migration of the primary O/W emulsion droplets from the oil phase to alginate solution. On the other hand, the fast phase separation necessitated the immediate use of the emulsion after its production.

The secondary O/W/O emulsion was then quickly poured into an alginate solution. The droplets of the primary O/W emulsion sedimented just above the oil–alginate interface however they did not cross over the alginate phase.

To promote the transfer, the secondary O/W/O emulsion and alginate solution were stirred at 350–400 rpm for 10 min using a magnetic stirrer. The stirring of the alginate solution increased the kinetic energy of the primary O/W emulsion droplets facilitating their transfer to the aqueous phase. However, the transfer was still incomplete and too slow (more than 60 min).

To overcome this inconvenience, a surfactant (Tween 85, 0.5 % v/v) was added to the alginate solution. The surfactant acts on the oil/water interface by decreasing its interfacial tension (Adamczak et al., 2013).

This procedure finally led to a fast and efficient transfer of the primary O/W emulsion droplets into the alginate solution. Microcapsules were then sieved and collected after 15 min of contact with alginate (Figure 6A).

Figure 6(B) presents the different steps of the mechanism of microcapsules formation process. Secondary O/W/O emulsion was poured into the alginate bath and was fragmented in drops containing droplets of primary O/W emulsion (Figure 6B, a). Due to thestirring of the alginate solution and the surfactant added into the aqueous phase, primary O/W emulsion droplets migrated outside external oil phase (Figure 6B, b). As a result, the release of calcium ions promoted the formation of an alginate membrane around released droplets of primary O/W emulsion (Figure 6B, c). The oil phase from secondary O/W/O emulsion was broken in droplets and dispersed in the alginate solution (Figure 6B, d).

When primary O/W emulsion droplets crossed secondary O/W/O emulsion–alginate interface, a deformation generally occurred leading to tear-shaped microcapsule cores (Figure 6B, d). Dang and Joo (2013) produced beads by dispersing the alginate solution in oil and contacting it with a calcium solution. Tear-shaped alginate beads were formed while alginate droplets crossed the oil–water interface.

The core deformation at the oil–water interface can compromise the microcapsule structure under certain conditions. If the core is sharply deformed due to excessive shear forces, microcapsules with elliptical form can be obtained. In conditions of extreme shearing, the core may also suffer from breakage. As a consequence, multi-core microcapsules can be formed. In addition, highly deformed cores will compromise the microcapsule shape and therefore the release profile of encapsulated actives in the core.

To circumvent these limitations, the ideal is to work with a moderate agitation of the alginate bath and using high concentrations of calcium chloride in the O/W primary emulsion. By using higher concentrations of calcium chloride, thicker membranes are quickly formed protecting the core from deformation. Microcapsules with thicker membranes then tend to contain more spherical cores.

The core and microcapsule sphericity contributes to a more homogeneous release of actives through the membrane.

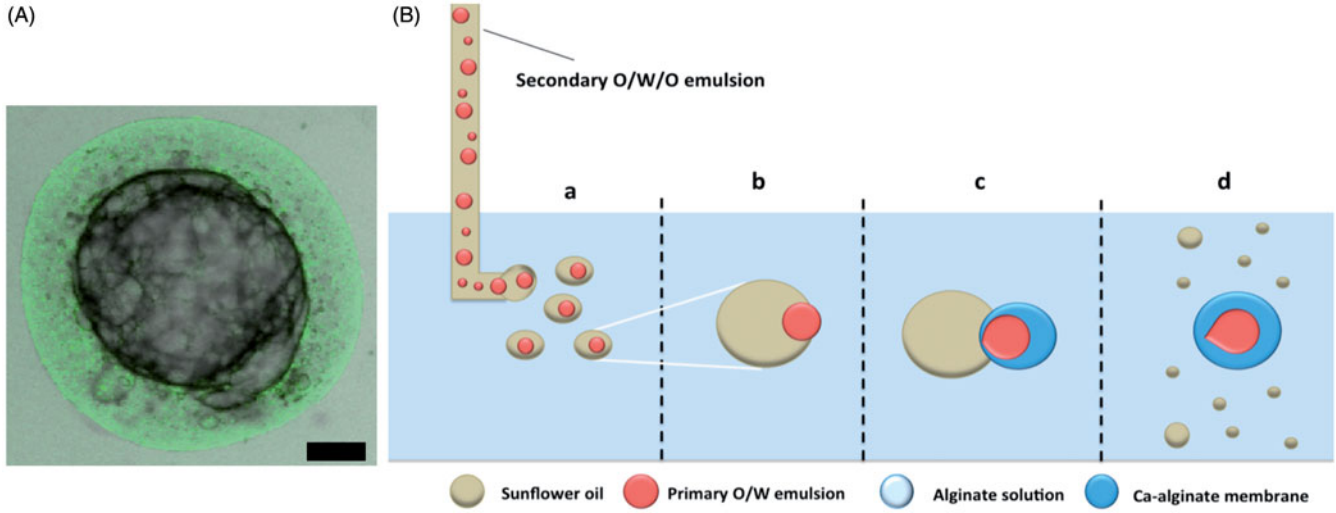

Figure 6. (A) Confocal microscopy image of a microcapsule produced by the inverse gelation method. Green area: Ca-alginate gel membrane; dark area: core formed by the primary O/W emulsion. Scale bar = 100 µm. (B) Mechanism of microcapsules formation by mixing technique. The secondary O/W/O emulsion was poured into the alginate solution and broken in droplets (a). The primary O/W emulsion droplets migrated outside oil phase and came into contact with the alginate solution (b). Calcium ions were released and Ca-alginate gel membrane began to be formed around primary O/W emulsion droplets (c). Microcapsules with an oil core and a gelled alginate membrane were produced (d).

Effect of the alginate and calcium chloride concentrations

Microcapsules were produced using different calcium chloride ($CaCl_2$) concentrations in the primary O/W emulsion and different alginate concentrations in the gelling bath. Stirring rate to form secondary O/W/O emulsion (350 rpm for 2 min) and curing time (10 min) were kept constant in the next series of experiments.

For $CaCl_2$ concentrations lower than 2.3 g/L, microcapsules broke during the stirring of the alginate bath or the filtration/rinsing step. The amount of Ca^{2+} ions was insufficient to form a thick and resistant alginate membrane. For $CaCl_2$ concentrations higher than 4.6 g/L, stable microcapsules were collected (Figure 7). Increasing $CaCl_2$ concentration led to an increase of the membrane thickness (Figure 7A).

For the highest $CaCl_2$ concentration (55.4 g/L), membranes of 188 µm thickness were obtained corresponding to 88% of the microcapsule volume. The high membrane percentage can be advantageous as thicker membranes are more mechanically resistant and impermeable (Ma et al., 2012).

Oil–core microcapsules with a high membrane percentage (~95 %) can also be achieved by microfluidics devices (Ren et al., 2010). In contrast to the mixing technique proposed here, the microfluidics devices do not allow a high production of microcapsules.

Figure 7(C) displays the structure of the microcapsules as a function of $CaCl_2$ and alginate concentrations. At low concentrations of $CaCl_2$ (4.6 g/L), the membrane was thin and oil droplets escaped from the microcapsules. For high concentrations of alginate (15 g/L), oil droplets were entrapped in the membrane. Well-structured core–shell microcapsules were obtained for alginate lower than 15 g/L and $CaCl_2$ concentration larger than 4.6 g/L (Figure 7C).

The mean diameter of the microcapsules increased with increasing $CaCl_2$ concentration (Figure 7B). This was explained by the high percentage of volume of membrane that contributed mainly to the final volume of microcapsules.

Alginate concentrations higher than 20 g/L prevented the penetration and dispersion of the secondary O/W/O emulsion. As a consequence, no microcapsules were obtained. For lower alginate concentrations, the membrane thickness was constant (Figure 7A). Therefore, the membrane thickness seems to be mainly governed by the migration of calcium ions.

However, the yield of produced microcapsules was significantly lower while using alginate concentration equal to 5 g/L. It was assumed that the microcapsules were more fragile and broke down during filtration and rinsing. The susceptibility of Ca-alginate membranes towards mechanical stress is due to the fact that alginate gels less dense and more porous at these low concentrations (Soliman et al., 2013).

In conclusion, alginate solution at 10 g/L and $CaCl_2$ concentration at 55.4 g/L were considered to be the best compromise to obtain well-structured core shell microcapsules with high percentage of membrane.

Effect of curing time

Microcapsules were prepared using different curing times (time in contact with alginate solution) using the following constant parameters:

(a) $CaCl_2$ concentration of 55.4 g/L in the primary O/W emulsion;
(b) Alginate concentration of 10 g/L;
(c) Stirring rate of 350 rpm during 2 min for the preparation of the secondary O/W/O emulsion.

In these experimental conditions, after 20 min of curing time, the maximum in membrane thickness and wet microcapsules weight were obtained (Figure 8A and B). In addition, by using this curing time, the maximal amount of calcium ions was released from the oil core forming thicker membranes. This is advantageous because thicker membranes provide the greatest protection of encapsulated material. Furthermore, the high value of weight of obtained microcapsules implies a higher amount of alginate incorporated into the membrane. In this case, the membrane tends to be more structured and resistant to mechanical stress.

For long curing time, the membrane thickness decreased weakly and this was also reflected on the weight of wet microcapsules. In the dripping method presented by Abang et al. (2012), a re-dissolution of the alginate calcium membrane was observed after long contact periods between the Ca-alginate membrane and the alginate solution. The cross-links between calcium ions (Ca^{2+}) and alginate chains are reversible, Ca^{2+} being able to migrate from the membrane to the solution compromising the integrity of membrane structure.

Based on these observations, it was concluded that a curing time of 20 min was ideal to form microcapsules.

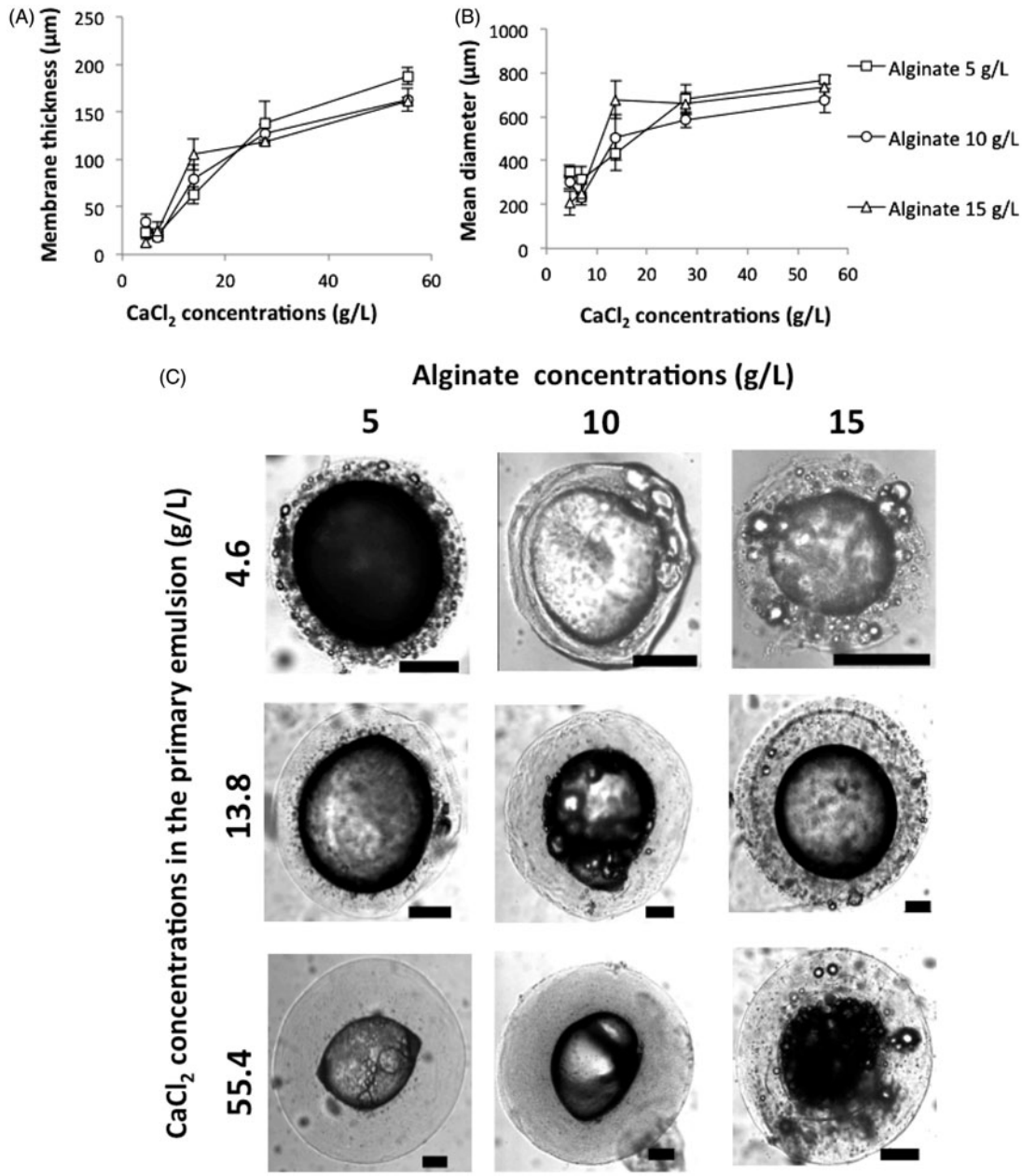

Figure 7. Membrane thickness (A) and mean diameter (B) of microcapsules produced using different $CaCl_2$ and alginate concentrations. Optical microscopy images of microcapsules produced using different concentrations of $CaCl_2$ and alginate (C). Scale bar = 100 µm.

Effect of core size on the membrane thickness and size diameter of microcapsules

The size of the microcapsules would depend on:

(a) the size of the core
(b) the size of the primary O/W emulsion droplets
(c) the thickness of the membrane, defined by the quantity of calcium ions released from the core.

In the previous sections, some parameters that control the membrane thickness ($CaCl_2$ concentration and curing time) were studied. However, the effect of the core size on the membrane thickness and microcapsule diameter has not yet been discussed.

The core constitutes the Ca^{2+} ions source used in the membrane formation. Figure 8(C) shows that the volume of the membrane increases with an increase of the core volume. Consequently, larger cores liberate more Ca^{2+} ions and thicker membranes are formed. For the range of evaluated core diameters (200–700 µm), the relation between the membrane (V_m) and core volume (V_c), both measured in μm^3, was described by the equation:

$$V_m = -810^{-3}V_c^2 + 1.8V_c + 0.8 \quad (4)$$

with a correlation coefficient of 0.99.

Assuming the microcapsules are perfectly spherical, the microcapsule volume (V) and the volumetric percentage of membrane in the microcapsules (% Memb.) can be described by the Equations (5) and (6), respectively:

$$V = V_c + V_m \quad (5)$$

$$\%\ \text{Memb} = V_m/V \quad (6)$$

Figure 8(D) shows that the percentage of membrane in the microcapsule decreases exponentially with the increase of the core volume.

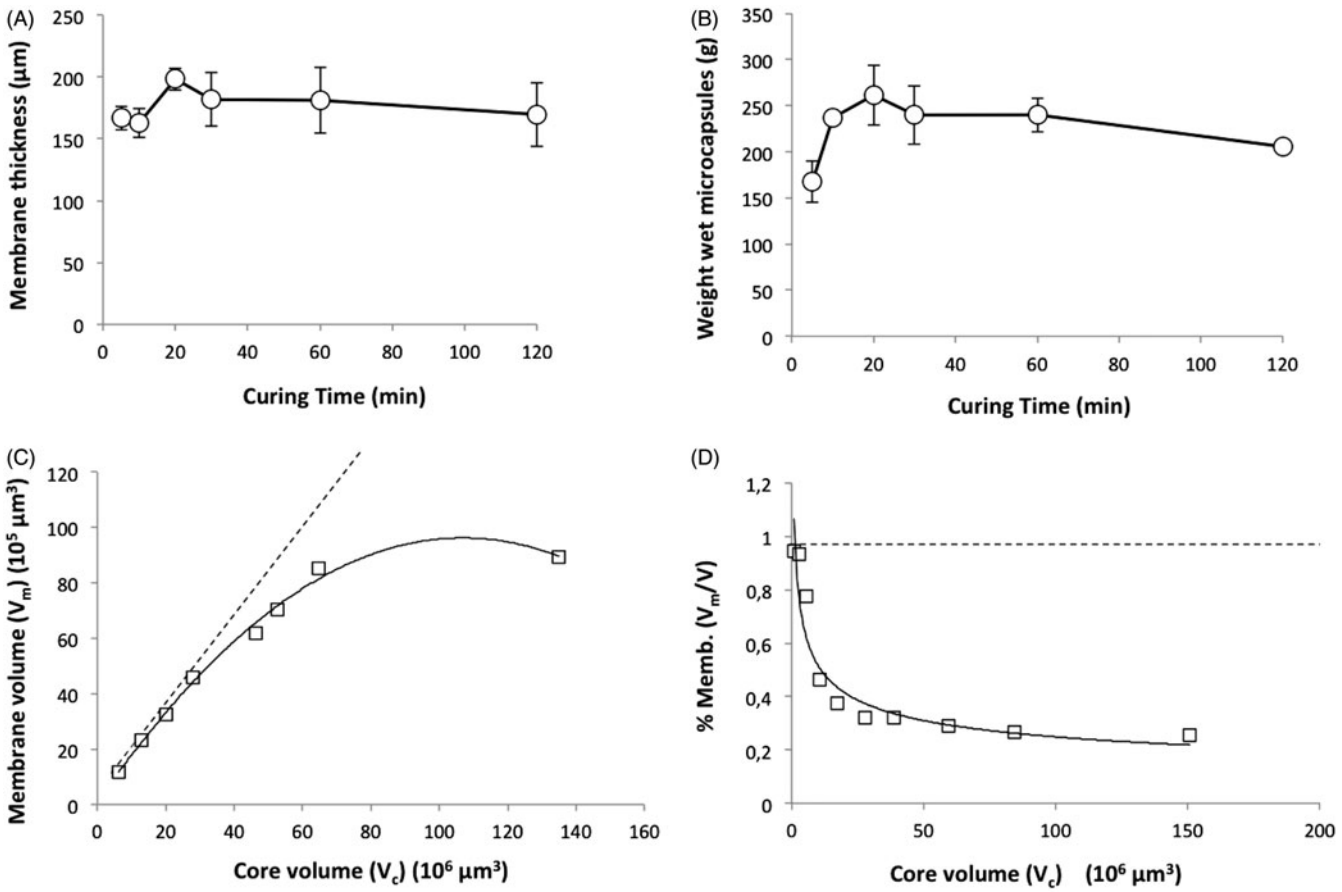

Figure 8. Effect of curing time (time in contact with alginate solution) on the membrane thickness (A) and weight wet microcapsules (B). Influence of core volume on the membrane volume (C) and membrane percentage (D). Dashed lines: theoretical curves considering a complete release of Ca^{2+} ions from the core.

Assuming that all Ca^{2+} ions inside the core is released and consumed in the membrane formation, two theoretical considerations can be done:

(1) V_m is directly proportional to V_c (Figure 8C).
(2) The % Memb. is constant and maximal with the increase of the values of V_c (Figure 8D).

However, the experimental results shown in Figure 8(C) and (D) do not respect the two theoretical considerations. For this reason, it was concluded that the Ca^{2+} ions inside the core are not completely released during the gelation process.

The most probable reason for the bigger cores is that Ca-alginate membranes are formed more quickly and are denser and less porous. Due to the cohesive structure of alginate gel in the membrane, the diffusion of Ca^{2+} ions in the gel tend to be limited or ceased.

The primary O/W emulsion droplets in the secondary O/W/O emulsion will be at the origin of the microcapsule core during mixing with alginate solution. Therefore, the size of the microcapsule cores and the primary O/W emulsion droplets can be considered equivalents.

The microcapsule diameter was directly proportional to the diameter of the primary O/W emulsion droplets (Figure 9A) and this relation was described by:

$$D_{cap} = 1.64D_{em} + 144 \quad (7)$$

where D_{cap} and D_{em} are the diameters (µm) of the microcapsule and primary O/W emulsion droplet, respectively. The correlation coefficient for the linear regression was 0.98.

Therefore, to control the mean diameter of microcapsules produced by the mixing technique, it is necessary to control the size of primary O/W emulsion droplets in the secondary O/W/O emulsion.

Effect of stirring rate to form the secondary O/W/O emulsion

For the production of the secondary O/W/O emulsions, a dispersion process was used. The greatest limitation of the dispersion methods is the large size polydispersity of the emulsion droplets (Poncelet et al., 1992). To reduce the polydispersity, three stirring systems were evaluated: turbine paddle, propeller paddle and magnetic barrel. The narrowest size distribution of the primary O/W emulsion droplets in the secondary O/W/O emulsion was obtained using a turbine paddle.

To evaluate the effect of stirring rate to form the secondary O/W/O emulsion on the size distribution of microcapsules, $CaCl_2$ concentration in the primary O/W emulsion (55.4 g/L), alginate concentration (10 g/L) and curing time (20 min) were kept constant.

During mixing of the secondary O/W/O emulsion in the alginate solution, a soft mixing system (magnetic stirrer) was used to avoid further breakage of the secondary O/W/O emulsion. Figure 9(B) shows that the mean size of microcapsules was linearly decreasing with stirring rate:

$$D_m = -0.67S + 806 \quad (8)$$

where D_m is the mean diameter of the microcapsules (µm) and S the stirring rate of the secondary O/W/O emulsion (rpm). The correlation coefficient for the linear regression was 0.96.

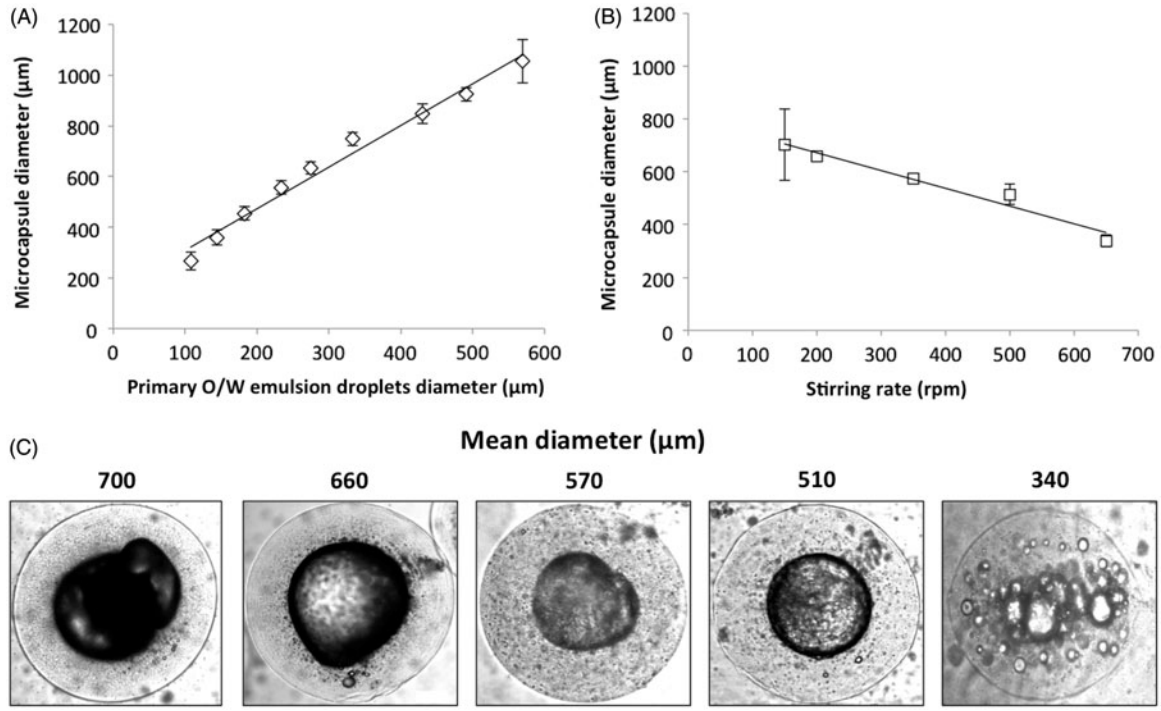

Figure 9. Influence of primary O/W emulsion droplets diameter on the microcapsules diameter (A) and effect of stirring rate of the secondary O/W/O emulsion on the diameter of microcapsules (B). Alginate shell–oil core structure of microcapsules as a function of mean diameter (C).

By increasing the stirring rate to produce secondary O/W/O emulsion, smaller primary O/W emulsion droplets and consequently smaller microcapsules were obtained. For low stirring rates, the mean microcapsule diameter showed a large variation from batch to batch. Low stirring rates (<150 rpm) were not sufficient to mix the system homogeneously and secondary O/W/O emulsions were not reproducible (Figure 9B).

Within the stirring rates of 150–650 rpm, microcapsules with mean diameter between 340 and 700 µm were produced (Figure 9B and C). Most microcapsules showed a core-shell structure. However, the smallest microcapsules tended to present a multi-core structure (Figure 9C), in accordance with the largest stirring rates (>500 rpm) used.

Conclusion

Core–shell microcapsules made of an alginate membrane and an oil core were produced by a three step emulsification/mixing process:

- Formation of a primary O/W emulsion with oil and calcium chloride solution.
- Formation of a secondary O/W/O emulsion by dispersing primary O/W emulsion in oil.
- Mixing secondary O/W/O emulsion in an alginate solution; the transfer of the primary O/W emulsion droplets in the alginate solution and the release of calcium ions leading to alginate membrane formation around oil core.

The process was optimised by a systematic study of the experimental conditions used: alginate and calcium chloride concentrations, curing time of the primary O/W emulsion and stirring rate of the secondary O/W/O emulsion. In the following conditions, alginate solution of 10 g/L, $CaCl_2$ concentration of 55.4 g/L in the primary O/W emulsion, curing time of 20 min and stirring rate of 350 rpm for 2 min, well-defined core–shell microcapsules were produced with a mean diameter of 500 µm.

The new method based on the inverse gelation technique can be used in the encapsulation of lipophilic actives like fragrances, pigments and vitamins. Therefore these microcapsules can be potentially applied in cosmetics, foods and pharmaceuticals areas.

Future research direction will be to dry the microcapsules and determine their properties in terms of mechanical resistance and controlled release after encapsulation of actives.

In addition, this work will be extended to the application of inverse gelation technique to capillary flow-based approach using microfluidics. The capillary flow-based approach offers a number of advantages over conventional emulsification process such as the control of size of microcapsules and membrane thickness due to geometries and flow rates used. In addition, monodisperse microcapsules will always be produced.

Declaration of interest

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