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A novel method of oil encapsulation in core-shell alginate microcapsules by dispersion-inverse gelation technique

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ABSTRACT

Oil-core microcapsules may be produced by dispersing a calcium solution-oil emulsion into an alginate solution. The release of calcium from the emulsion leads to the gelation of alginate around the oil droplet and therefore to the formation of microcapsules. This work aims to propose a new method of microcapsule production by dispersion-inverse gelation technique. Therefore, W/O emulsions were dispersed in alginate solution and led to the formation of capsules with varying diameters depending on the stirring rate of the alginate bath. The membrane thickness varied between 35 and 200 μm depending on the type of emulsion destabilization treatment used. Oil was encapsulated at a yield of 100% allowing the extrapolation of this method at pilot scale. In addition, microcapsules released hydrophilic dye in few hours while hydrophobic dye was retained in the core due to interaction with the oil phase. Core-shell alginate microcapsules produced by dispersion-inverse gelation technique displayed interesting property suitable for applications where actives need to be retained during long times or for volatile compounds.

1. Introduction

Oils are widely applied in the formulation of foods, pharmaceutical and cosmetics products; however, they are subjected to oxidation in presence of heat, light, metals ions or oxygen [1–4].

An efficient strategy to decrease the sensitivity of oils towards environmental conditions consists of its encapsulation in inert polymer matrix using gelation/emulsification technology [5–7]. Many methods have been developed to form capsules of alginate gel via ionotropic gelation. Though both liquid-air and liquid-liquid methods have their advantages and limitations, the selection of a suitable dispersion method typically depends on the target particle size and size distribution, economics of production, productivity, and technical constraints [8].

Oil encapsulation by inverse gelation is an innovative approach recently described in the literature [1,9–11]. An emulsion of oil and $CaCl_2$ solution is extruded dropwise into an alginate bath. Upon contact, Ca^{2+} ions diffuse to the outer periphery of the drop and ionotropically cross-link with the alginate polymer chains at the drop interface. The ionotropic gelation process continues until the free Ca^{2+} ions are depleted. At the end of the process, the initial liquid drop is engulfed by a continuous semi-permeable Ca-alginate membrane leading to core-shell capsules. This method is promising since it is environmentally safe and allows the capsule production by simple extrusion-dripping system [9]. Wet capsules with approximately 3–7 mm of diameter can contain both hydrophilic and hydrophobic actives like proteins, dyes or enzymes, which is an advantage compared to other methods of oil encapsulation [11]. Furthermore, after drying, capsules display high oil loading (>90% w/w).

While millimetric capsules may find applications in food, agricultural, cosmetics and home care products, as fragrance diffusers, decorative pearls or for crops protection (i.e. pesticides, herbicides) [12–14], microcapsules can be necessary in specific applications because of their reduced size [15]. For example, microcapsules can be found in products such as chewing gums, dairy powders, nutritional supplements, sportswears, cosmetics and home care products as detergent and bed linen. The advantages of the microcapsules in comparison with the millimetric capsules is due to the fact that microcapsules had low or no impact on the visual and sensorial aspects of the final products (food and cosmetics) and that they can be better dispersed in food formulations.

Several strategies have been developed to reduce the capsule size including introducing vibration or electrostatic force to induce detachment of the compound droplets from the nozzle tip [16,17]. However these approaches were not successful for oil encapsulation by inverse gelation [1]. On the other hand, in a previous work performed in our laboratory, capsules of 500 μm were obtained using a dispersion-inverse gelation technique [1]. In this study, an oil-in-calcium emulsion (O/W primary emulsion) was first prepared then mixed in an oil phase to

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form an oil-in-calcium-in-oil emulsion (O/W/O secondary emulsion). Finally, the secondary emulsion was dispersed in alginate solution to produce the microcapsules [1].

This technique was easy to perform at laboratory scale and did not need complex equipment; however, a disadvantage prevented its scale-up: >90% of the oil used during the process was not encapsulated and could not be recovered at the end of process.

In order to reduce the substantial oil loss occurring during the oil encapsulation by the dispersion-inverse gelation technique, the aim of the present work was to propose an alternative method based on the use of water-in-oil (W/O) emulsions. From our recent work, it was demonstrated that the release of Ca^{2+} ions from water-in-oil (W/O) emulsions was slow and could be finely tuned by emulsion destabilizers [18]. The objective of the present work was therefore to develop a scale-up of oil encapsulation in core-shell capsules taking advantages of the dispersion-inverse gelation technique and the W/O emulsion destabilization mechanism in order to control the alginate membrane formation.

2. Materials and methods

2.1. Materials

Sodium alginate powder (Saltialgine S 60 NS) with a mannuronic (M) to guluronic (G) acid unit ratio (M/G) and a molar mass equal to 1.37 and 1.57×10^5 g/mol, respectively, was kindly donated by Cargill (France). Calcium chloride powder ($CaCl_2 \cdot 2H_2O$) (Panreac Quimica Sau, Spain), sunflower cooking oil (Associated Oil Packers, France), PGPR 90 (Danisco, France) and SPAN 85 (Sigma Aldrich, France) were used to prepare the W/O emulsions. Other chemicals reagents were obtained from Sigma Aldrich (France).

2.2. Preparation of alginate and calcium chloride solutions

For alginate solution, 10 g of alginate powder was dissolved in 1 L of demineralized water using a paddle stirrer. The calcium chloride solution was prepared by dissolving 60 to 480 g of $CaCl_2 \cdot 2H_2O$ in 1 L of demineralized water.

2.3. Preparation of the W/O emulsions

One hundred millilitres of sunflower oil containing 0.01, 0.1 or 1 g of surfactant (SPAN 85 or PGPR 90) was mixed using a high shear mixer (Ultra-Turrax T25, IKA, Germany) at 13,500 rpm for 1 min. Thirty millilitres of calcium chloride solution was then added slowly and a new shear mixing at 13,500 rpm for 3 min was performed (Fig. 1A).

2.4. Characterization of the W/O emulsions

2.4.1. Stability

The W/O emulsion stabilities were performed as described by Martins et al. [1]. Hundred millilitres of W/O emulsions were placed in tubes of 100 mL with graduation of 1 mL. The emulsions were kept at ambient temperature (20 ± 2 °C) and visually inspected as a function of time in order to assess the critical time for which 1% of phase separation occurred (i.e. corresponding to 1 mL of phase separated liquid).

2.4.2. Electrical conductivity

Conductivity of the emulsions was measured in triplicate at ambient temperature using a conductimeter (Mettler-Toledo, Analytical, Switzerland).

2.4.3. Microscopic observations

The W/O emulsions were examined using an optical microscope (Leica Microsystems, France). The size of $CaCl_2$ solution droplets (dispersed phase) was determined by image analysis using the ImageJ 1.47v freeware (USA). Three different zones (1.1 × 1.5 mm) for each sample were imaged and approximately one hundred droplets per zone were measured. The measurements were carried out in triplicate with three repetitions. In other words, nine W/O emulsions of each formulation were analyzed by optical microscopy.

2.5. Microcapsules production by dispersion technique

Two hundreds millilitres of alginate solution was introduced into a beaker of 600 mL (Ø = 95 mm; h = 120 mm) and stirred at 350–400 rpm with a 80 mm long wedge-shaped magnetic barrel during 1 min. Two millilitres of emulsion were dispersed into alginate

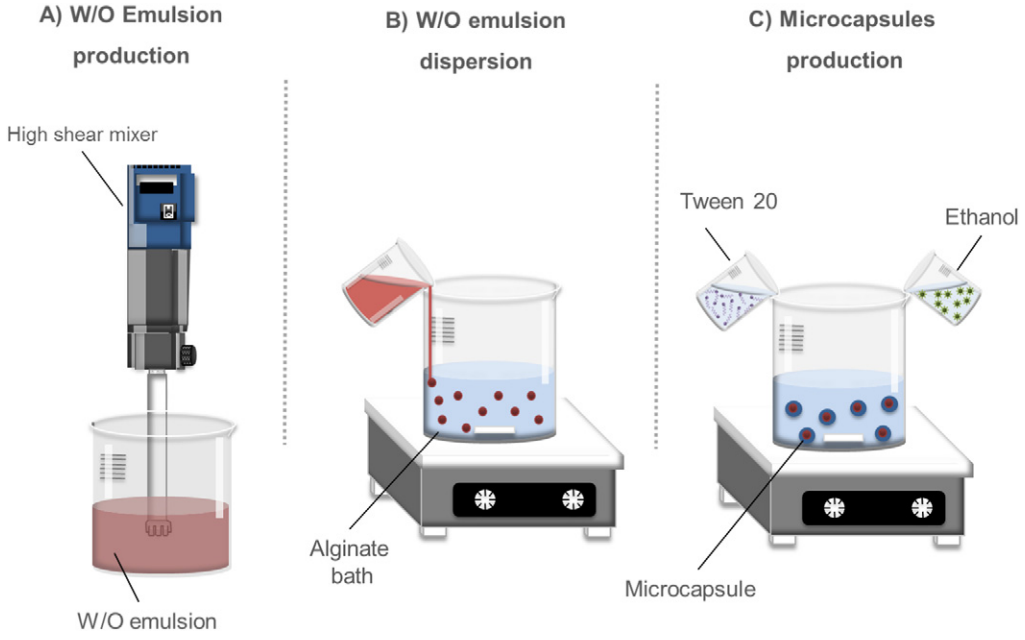


Fig. 1. Schematic representation of the experimental set-up used to produce microcapsules by inverse gelation using W/O emulsions.

solution during 2 min (Fig. 1B). Tween 20 (2 mL) and/or ethanol 99% (20 mL) were then added to destabilize the emulsion (Fig. 1C). Wet microcapsules were sieved with a mesh of 50 μm (Grosseron, France) and washed with demineralized water. The microcapsules were stored in calcium chloride solution at 15 g/L [19].

2.6. Visualization and measurements of microcapsules

Optical microscope (Leica Microsystems, France) was used to image microcapsules. The diameter (d_{cap}), membrane thickness (Mt) and core size (d_{core}) of the microcapsules were measured using ImageJ 1.47 v freeware (USA). One hundred microcapsules were analyzed per sample (3 mL) from three different batches.

2.7. Controlled release studies

Controlled release experiments were conducted using a hydrophilic (Methyl Orange) and a lipophilic (Sundan Red) dye.

For Methyl Orange release studies, 7 mg of dye were dissolved into 30 mL of $CaCl_2$ solution at 240 g/L. One hundred millilitres of oil and $CaCl_2$ solution was then used to prepare the emulsion as described in Section 2.3 "Preparation of the W/O emulsions". The final Methyl Orange concentration in the emulsion was of 0.05 mg dye/g emulsion. Seventy grams of emulsion was encapsulated and the wet microcapsules were kept in 175 mL of isotonic saline solution (NaCl 0.85% w/v, pH 7.0). During release experiments, pH of the isotonic saline solution was constantly maintained at 7.0 by addition of NaOH 0.1 N.

Aliquots of 1.5 mL of the supernatant were collected and centrifuged at 10,000 rpm for 3 min (Thermo Scientific, Germany). The amount of dye released in the supernatant was determined at 480 nm using a spectrophotometer (UNICAM UV1 Spectrometer, USA).

For Sudan Red release studies, 7 mg of dye was dissolved in 100 mL of sunflower oil. The oil and $CaCl_2$ solution (30 mL at 240 g/L) were used to prepare the emulsion. The initial concentration of Sudan Red was of 0.05 mg dye/g emulsion. Seventy grams of emulsion was encapsulated and the wet microcapsules were dispersed in 175 mL of sunflower oil. Aliquots of 1.5 mL external media (oil) were collected and the amount of dye was determined by spectrophotometry at 550 nm.

Release experiments were performed in triplicate at room temperature (20 ± 2 °C) and were stopped when a variation in the absorbance value ≤1% was observed.

Considering that loss of actives during the encapsulation process was negligible, the maximal concentration of active released ($R\infty$) was calculated by Eq. (1):

$$R\infty = \frac{M_{dye}}{V} \quad (1)$$

where M_{dye} is the mass of dye encapsulated (3.5 mg) and V is the volume of isotonic saline solution or oil (0.175 L). $R\infty$ was equal to 20 mg/L.

The cumulative release (Cr) of the actives was calculated using Eq. (2):

$$\mathrm{Cr} = \frac{Rt^{*}100}{R\infty} \quad (2)$$

where Rt is the concentration of active released at time t.

2.8. Statistical analysis

The results were compared using the Student's t-test statistical method, which compares the actual difference between two means in relation to the variation in the data. A significant difference at p-value < 0.05 was assumed.

3. Results and discussion

3.1. Effect of surfactant type on the W/O emulsion stability and the production of capsules

In order to determine the effect of surfactant on the emulsion stability, emulsions were produced using SPAN 85 or PGPR 90. The surfactants chosen have low hydrophobic lipophilic balance (HLB) values ($HLB_{SPAN\ 85} = 1.8$; $HLB_{PGPR\ 90} = 1.5$), a suitable criterion for W/O emulsions stabilization [20]. The surfactants were tested by varying their concentration in emulsions (0.01, 0.1 or 1 g/100 mL of oil). The characteristics of the emulsions were displayed in Table 1.

All emulsion formulations showed an absence of electrical conductivity (Table 1) indicating that oil was the continuous phase and that emulsions were therefore water-in-oil emulsions.

W/O emulsions produced with SPAN 85 displayed a stability lower than 20 min (Table 1). Furthermore, when in contact with alginate solution, unshaped gels containing several small cores were formed. High amount of Ca^{2+} could be released from SPAN 85 based emulsions, probably due to its low stability, leading to the encapsulation of several oil cores by a single alginate gel (Table 1).

On the other hand, emulsions produced with the surfactant PGPR 90 showed higher stability (from 50 min to 5 days) (Table 1). The higher stability of the PGPR90-emulsions allowed a better controlled release of Ca^{2+} ensuring the production of mononuclear capsules with a thin Ca-alginate membrane (Table 1).

By increasing PGPR 90 concentration, W/O emulsions were considerably more stable (23 h to 5 days) and no capsules were produced (Table 1). It was assumed that the high emulsion stability prevented the Ca^{2+} release from the oil core and therefore the alginate membrane formation.

3.2. Effect of the W/O emulsion destabilization on the membrane thickness

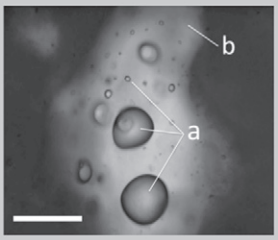
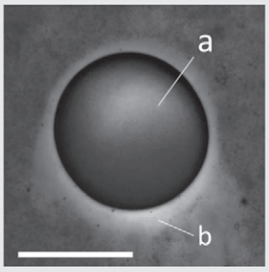
The dispersion of the W/O emulsion into the alginate bath led to microcapsules with a thin and fragile membrane (even by using optimum surfactant type and concentration, i.e. PGPR 90 at 0.08 g/L, Table 1). Emulsions with similar concentration of PGPR 90 were therefore dispersed into alginate bath during 2 min and emulsion destabilization treatments were then applied:

1) No destabilizer (control)
2) Addition of ethanol: 20 mL (10% v/v) of ethanol was added to alginate bath
3) Addition of Tween 20: 2 mL (1% v/v) of Tween 20 was added to alginate bath
4) Addition of Tween 20 and ethanol: 2 mL of Tween 20 was added to alginate bath, then after 5 s of stirring (350 rpm), 20 mL of ethanol was added.

To evaluate the effect of each destabilization treatment on the release of Ca^{2+} ions, the membrane thickness was measured and compared to the control (Fig. 2A). No variation in the membrane thickness ($p < 0.05$) was observed while adding only ethanol. The addition of Tween 20 or Tween 20 and ethanol led to a 3 fold and 5 fold increase of the membrane thickness compare to the control, respectively.

Fig. 3 showed a detailed micrograph of the microcapsule produced with Tween 20 and ethanol where a thick alginate membrane was obtained (Fig. 3A). An interspace was observed between the alginate membrane and the oil core (Fig. 3B) while the core still contained a large quantity of calcium solution droplets (Fig. 3C). It was supposed that the detachment of the oil core from the membrane was due to the diffusion of the $CaCl_2$ solution droplets from the oil core after contact

Table 1
Characteristics of the W/O emulsions produced with SPAN 85 or PGPR 90.

Surfactant		Surfactant concentration (g/L)	Conductivity (μS/cm)	Size of the $CaCl_2$ solution droplets in the emulsion (μm)	Stability time	Microcapsules produced
Surfactant	SPAN 85	0.08	0	18.0 ± 8.0[a]	15 ± 3 min[d]	Complete gelation of the alginate bath
		0.8	0	15.3 ± 9.1[a]	18 ± 2 min[d]	
		8	0	14.0 ± 8.1[a]	20 ± 4 min[d]	
	PGPR 90	0.08	0	15.0 ± 3.6[a]	51± 10 min[e]	
		0.8	0	7.0 ± 0.8[b]	23 ± 1 h[f]	No microcapsules production
		8	0	0.7 ± 0.1[c]	5 ± 1 day[g]	

a: oil core; b: alginate membrane; white bar: 400 μm.
Different letters in the same column indicated

with ethanol and Tween 20. Microcapsules were therefore made of an oil core, an interspace between the core and the membrane and a thick alginate membrane.

Oil cores with initial sizes between 100 and 500 μm reduced its diameter by ~30% after emulsion destabilization by Tween 20 + ethanol (Fig. 4A). By contrast, bigger oil cores (>700 μm) displayed a lower reduction in size (7%) after emulsion destabilization treatment (Fig. 4A). These results suggested that the W/O destabiliser effect was more pronounced on small emulsion drops. In other words, more aqueous phase ($CaCl_2$ solution droplets) was removed from the smaller cores.

During the destabilization, $CaCl_2$ droplets diffused from the oil core to the alginate bath. If all $CaCl_2$ solution droplets had left the emulsion drop, only oil would have been observed in the microcapsule core. However, further investigation revealed the presence of $CaCl_2$ solution droplets in the oil core even after W/O emulsion destabilization (Fig. 3B). This observation highlighted the fact that a certain amount of Ca^{2+} was not released and used to form the alginate membrane.

Some calculations were thus performed to estimate the percentage of Ca^{2+} released from the oil core. The loading of Ca^{2+} ions in the oil core can be determined by Eq. (3) (see also Supplementary material):

$$N_{Ca,core} = [Ca]_{core} \frac{\pi}{6} d_{core}^3 \quad (3)$$

where $N_{Ca,core}$ is the number of Ca^{2+} ions in the oil core (mol), $[Ca]_{core}$ is the Ca^{2+} ions concentration in the core (0.4 mol/L) and d_{core} is the oil core diameter (dm).

The number of moles of Ca^{2+} in the membrane ($N_{Ca,m}$) can be estimated by Eq. (4):

$$N_{Ca,m} = [Ca]_m \frac{\pi}{6} \left(d_{cap}^3 - d_{core}^3\right) \quad (4)$$

where $[Ca]_m$ is the Ca^{2+} concentration in the membrane (0.011 mol/L) and d_{cap} is the capsule diameter (7×10^{-2} dm).

The percentage of calcium released from the oil core φ can therefore be estimated by Eq. (5):

$$\varphi = \frac{N_{Ca,m}}{N_{Ca,core}}^{*} 100 \quad (5)$$

Using Eq. (5), the percentage of calcium released from the oil core was estimated for each W/O emulsion destabilization treatment (Fig. 4B). Fig. 4B showed that the release of Ca^{2+} from the control and the ethanol-treated microcapsules were very low (1–10%) and quite similar. On the other hand, for the microcapsules treated with Tween 20 and Tween 20 + ethanol, the percentage of calcium released was much higher and varied from 20 to 80% depending on the core volume. Regardless of the applied W/O emulsion destabilization treatment, the percentage of calcium released was always higher for smaller oil cores. By decreasing the oil core diameter, the contact area between the emulsion drop and the alginate bath became higher, improving the diffusion of Ca^{2+} ions and therefore the percentage of calcium ions released.

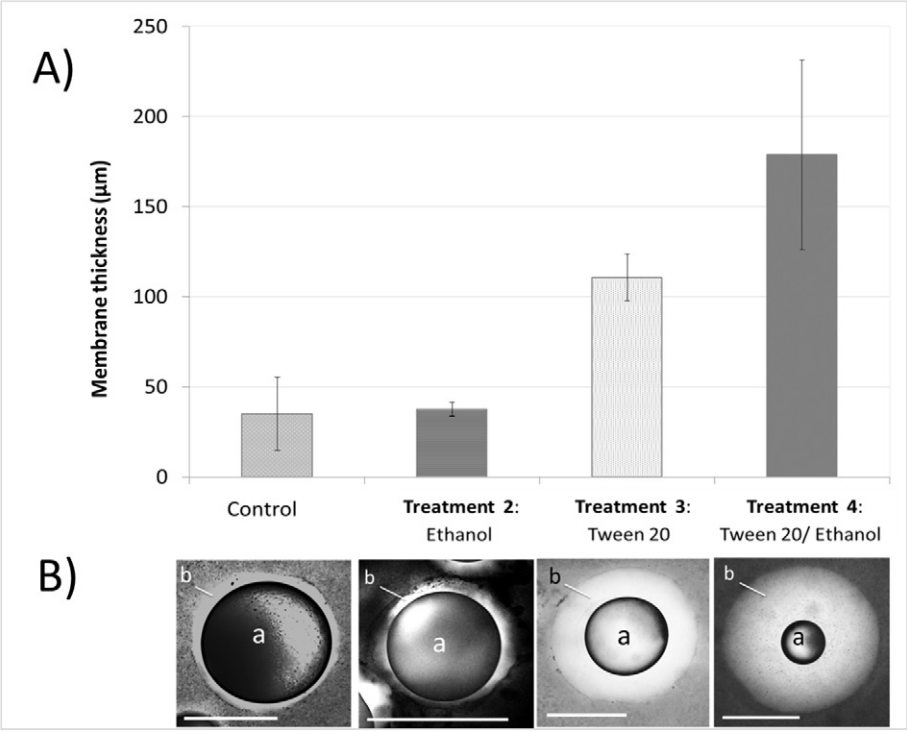


Fig. 2. A) Membrane thickness before and after emulsion destabilization by addition of ethanol and/or Tween 20. Measurements were carried out on microcapsules with cores varying between 200 and 400 µm. B) Micrographs of microcapsules obtained after different W/O emulsion destabilization treatments (from left to right): no destabilizer (control), ethanol, Tween 20 and Tween 20/ethanol. a: core; b: membrane. Scale bar: 400 µm.

3.3. Mechanism of microcapsules formation

Based on the previous results and some literature reports, a hypothetical mechanism of microcapsules formation by inverse gelation will be discussed in this section. To better understand this process, the formation of the microcapsules was described in six steps as follows (Fig. 5).

During step I, W/O emulsion was fragmented in small drops by the shear forces applied in the alginate bath.

During step II, solution droplets near the oil-alginate interface diffused from the emulsion to the alginate bath. The diffusion may be due to a simple emulsion destabilization due to the agitation or by a mechanism of diffusion/permeation through the oil layer [21]. In this latter case, $CaCl_2$ solution droplets either diffused through the thin

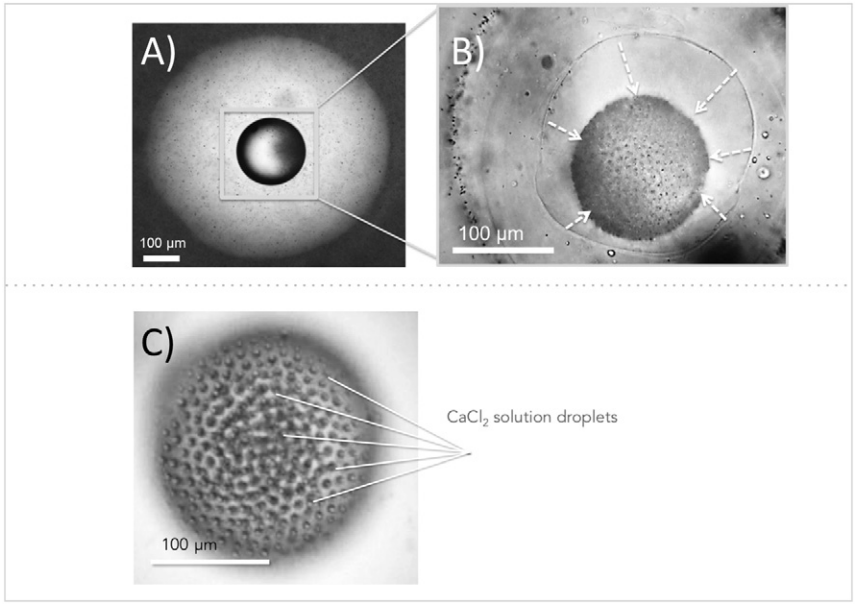


Fig. 3. Microscopy of a typical microcapsule produced from W/O emulsion destabilization using Tween 20 and ethanol. A) Microcapsule. B) Detail of core shrinking (arrows delimited the area of shrinking). C) Presence of $CaCl_2$ solution droplets in the core.

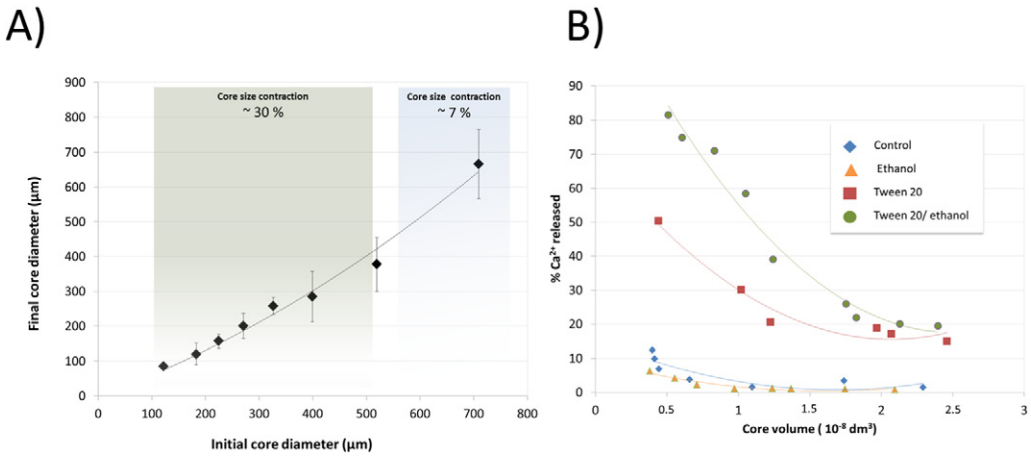


Fig. 4. A) Relation between initial and final oil core size during microcapsules production; W/O emulsions were destabilized with Tween 20 and ethanol. B) Percentage of calcium released from the oil core to form the membrane for each W/O emulsion destabilization treatment.

films of PGPR 90 present in the oil layer, or diffused through the incorporation of "reverse micelles" [21–24]. However, the $CaCl_2$ solution droplet diffusion remained low and not uniform resulting in microcapsules with thin and irregular membranes (step III).

During step IV, Tween 20, a non-ionic surfactant with high HLB value ($HLB_{Tween\ 20} = 16.7$) was added to alginate bath. Tween 20 would cross the alginate-oil core interface and destabilize the PGPR 90 film stabilizing the $CaCl_2$ solution droplets (step IV.a). As a consequence, $CaCl_2$ solution droplets fused themselves (step IV.b) and the transition from the diffusion to the coalescence regime led to a larger release of calcium (step IV.c) [24].

In step V, ethanol was added into the alginate bath. The mechanism of ethanol action under membrane formation is not yet clear. One hypothesis would suggest that the solvent increases the osmotic pressure of the polysaccharide solution favouring the $CaCl_2$ solution diffusion towards the alginate bath [21,25]. Another hypothesis would be that the ethanol could increase the solubility of Tween 20 into the oil phase favouring the destabilization of PGPR 90 film at the interface of the $CaCl_2$ solution droplets.

The emulsion dehydration in steps IV and V shrunk the oil core volume (Fig. 3A). As there were no hydrophobic interactions between the oil core and the membrane [14], the oil core detached from the membrane while the membrane remained unchanged. An interspace between the oil core and the alginate membrane was thus formed (step VI).

3.4. Effect of curing time on the membrane thickness

Curing time is defined as the time of contact between the emulsion drops and the alginate bath after the addition of destabilizer (Tween 20 and ethanol). To define the effect of curing time on the membrane thickness, the emulsion was dispersed into alginate bath during 2 min at 350 rpm. Emulsion destabilizers were then added and the alginate bath continued to be stirred during 3 to 30 min. After 3 min of curing time, microcapsules reached the maximum of membrane thickness and no variation was observed in time ($p < 0.05$). However, after 15 min, microcapsules with broken membranes were observed (Fig. 6A), maybe caused by the stirring of the alginate bath. To overcome this drawback, the alginate was mixed at 400 rpm during 3 min and the stirring was then reduced to 100 rpm during 15 to 30 min. Following this procedure, microcapsules with intact membranes were recovered.

Curing times between 3 and 5 min were therefore sufficient to achieve the maximal Ca^{2+} release and membrane thickness.

3.5. Effect of $CaCl_2$ concentration on the membrane thickness

To evaluate the effect of $CaCl_2$ concentration on the membrane thickness, emulsions with increasing $CaCl_2$ concentrations were mixed with alginate followed by the destabilization process. Similar membrane thicknesses ($Mt = 200 \pm 25$ µm) were obtained regardless the $CaCl_2$ concentration used (Fig. 6B). Previous study using droplets millifluidic demonstrated that the Ca^{2+} release occurred only during the first seconds of contact between the W/O emulsion and alginate bath [18]. It was thus suggested that membrane thickness was only limited by the distance travelled by Ca^{2+} ions during alginate gelation, this distance being independent of the $CaCl_2$ concentration in the W/O emulsion.

3.6. Factors influencing microcapsules size

3.6.1. Influence of the stirring rate on the microcapsules size

Microcapsules were produced using increasing stirring rates of the alginate bath (Fig. 6C).

The core and microcapsule diameters decreased with the increase of stirring rate. By tuning the stirring rate, microcapsules with diameters between 370 and 600 µm were produced. This behaviour was also observed by Aravand & Semsarzadeh [26]. According to these authors, in dispersion techniques, an increase in the rotational speed of the mixer reduced the size of the particles.

3.6.2. Influence of the core size on the microcapsule diameter

From Eq. (3), the Ca^{2+} ion loading in the core (number of moles) is proportional to the cube of the core diameter. Considering that the membrane thickness was associated to the amount of Ca^{2+} ions released from the emulsion destabilization, it was assumed that a proportional relationship existed between the membrane thickness and the core size.

However, experimental results demonstrated that the proportionality between membrane thickness and oil core size was only valid for core sizes between 140 and 200 µm (Fig. 6D). For cores bigger than 440 µm, the membrane thickness together with the microcapsule diameter decreased with the increase of core size.

The variation of membrane thickness (Mt) and microcapsules diameter (d_{cap}) with the oil core size (d_{core}) were given by the following equations obtained by fitting parabolic functions (second order polynomial functions) to the experimental results plotted in Fig. 6D:

$$Mt = -0.002{d_{core}}^2 + 1.1 d_{core} + 34 \qquad R^2 = 0.95 \qquad (6)$$

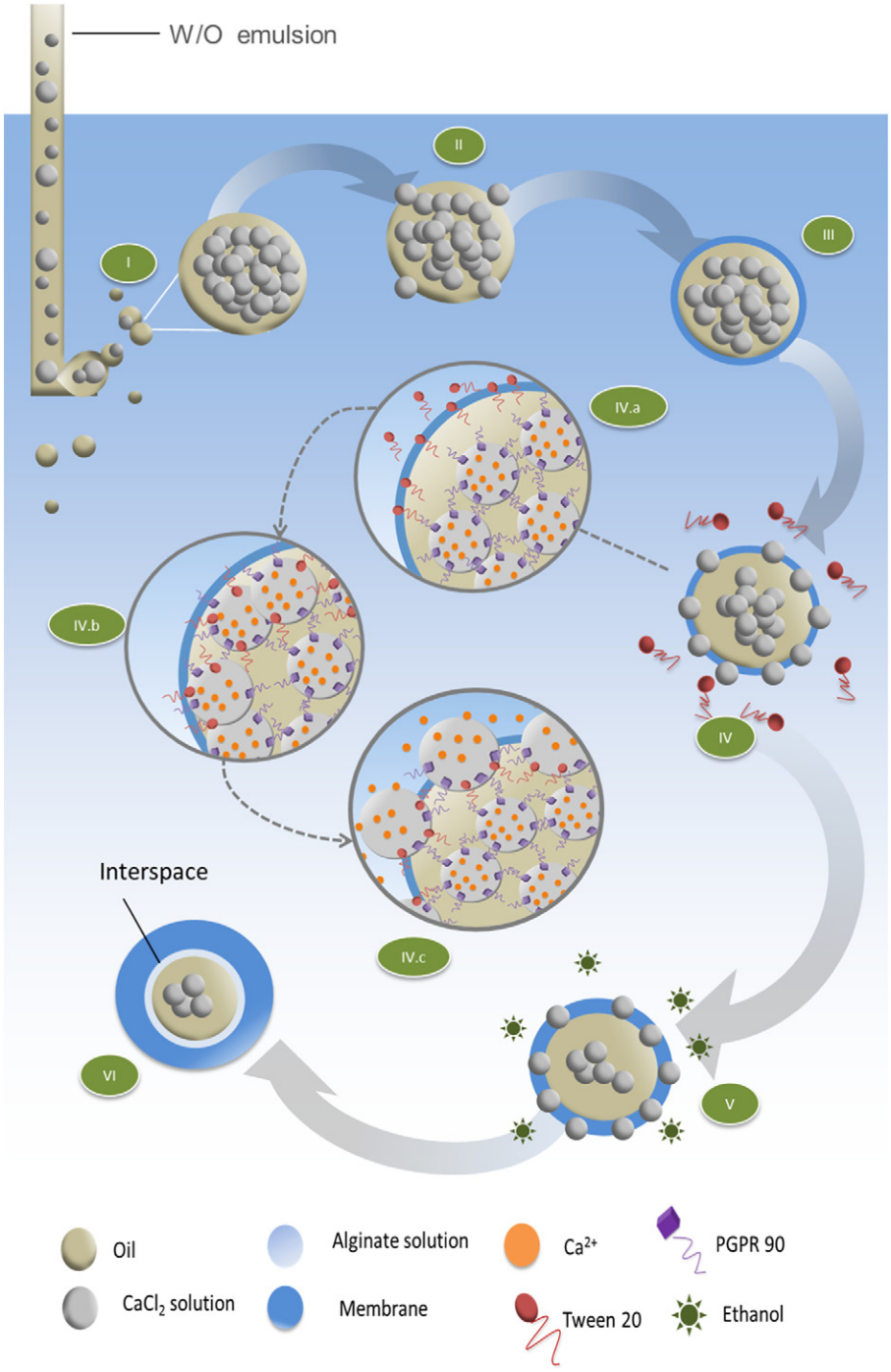


Fig. 5. Schematic representation of the formation of microcapsules by inverse gelation technique. The formation of the microcapsules was described in six steps as follows: step (I): fragmentation of W/O emulsion by the shear forces applied in the alginate bath; step (II): diffusion of $CaCl_2$ solution droplets from the W/O emulsion to the alginate bath; step (III): formation of a thin Ca^{2+}-alginate membrane; step (IV): larger release of Ca^{2+} ions after destabilization of PGPR 90 film stabilizing $CaCl_2$ solution droplets by addition of Tween 20; step (V): shrinking of the oil core due to W/O emulsion dehydration after addition of ethanol and formation of an interspace between oil core and alginate membrane; step (VI): final morphology of the capsules.

$$d_{cap} = -0.004{d_{core}}^2 + 3.2d_{core} + 69 \qquad R^2 = 0.99 \qquad (7)$$

These equations indicated that for smaller cores (<200 μm), the membrane thickness and the capsule diameter increased linearly with the increase of core size. In other words, larger the core was, larger the content of Ca^{2+} was released to form the membrane.

By contrast, it was demonstrated that the amount of Ca^{2+} released was inversely related to the core volume as shown in Fig. 4B. It was therefore assumed that the emulsion destabilization induced by Tween 20 and ethanol was less effective on capsules with bigger cores due to the lesser diffusion of Tween 20 and ethanol in the emulsion droplet (see Fig. 5.IVa). This hypothesis would therefore explain the pseudo-plateau observed in the size – core diameter relationships with increasing core volume of the capsules displayed on Fig. 6D.

3.7. Release profile

The release profile studies were carried out using hydrophilic (Methyl Orange) and hydrophobic (Sudan Red) dyes. Methyl Orange

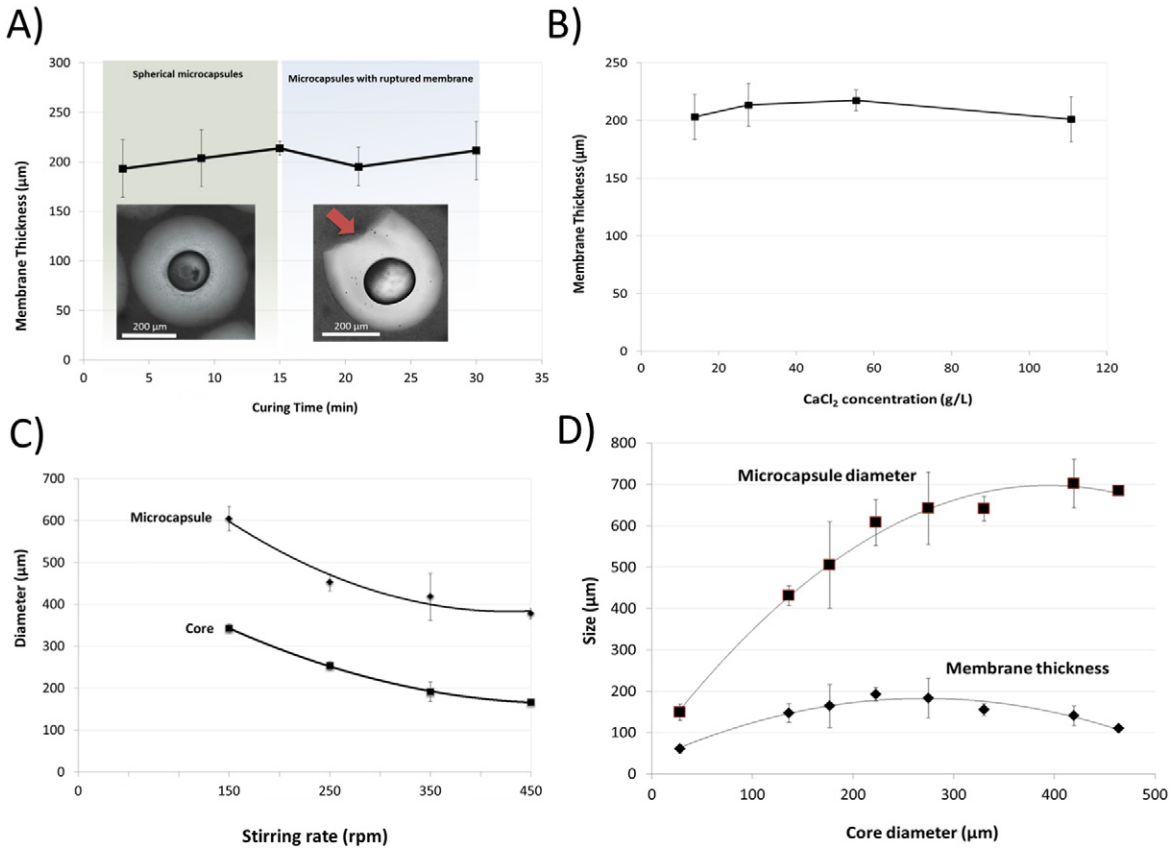


Fig. 6. A) Effect of curing time on the membrane thickness. Measurements were carried out on microcapsules with cores varying between 200 and 400 µm. Red arrow: breakdown of the membrane. $CaCl_2$ concentration in W/O emulsion: 55.4 g/L; B) effect of $CaCl_2$ concentration on the membrane thickness. Measurements were carried out on microcapsules with cores varying between 200 and 400 µm. Curing time: 3 min; C) effect of stirring rate of the alginate bath on the core and microcapsules size. $CaCl_2$ concentration in the emulsion: 55.4 g/L, curing time: 3 min; D) effect of the core size on the microcapsule diameter and membrane thickness and fitted data according to Eqs. (6) and (7) (see text for details). $CaCl_2$ concentration in the emulsion: 55.4 g/L, curing time: 3 min.

is a pH indicator whose colour varies from red below pH 3.1 to yellow above pH 4.4. The pH values of the microcapsules suspensions were therefore continuously monitored and adjusted to 7.0.

Around 40% of Methyl Orange was released after 6 h (Fig. 7A). In the first hours, there was a steep increase in the Methyl Orange release ascribed to the release of adsorbed dye at the surface (or close to) of alginate membrane [27]. Between 6 and 48 h, the release rate was reduced, with a pseudo-plateau in the curve, and the maximal cumulative release of 87% was reached after 144 h (Fig. 7A). The possible explanation of this release profile would come from the fact that Methyl Orange was dissolved in $CaCl_2$ solution and that, when the emulsion was put in contact with the alginate bath, both Ca^{2+} ions and dye diffused from the emulsion to the alginate bath. As a consequence, a fraction of dye was entrapped in the membrane of the microcapsule and was therefore quickly released during the first hours. However, once the dye in the membrane was depleted, its release was considerably slowed down. By microscopic observations (Fig. 7B), it was found that the core destabilized in time leading to a progressive release of dye in the internal part of the membrane (pseudo-plateau in Fig. 7A).

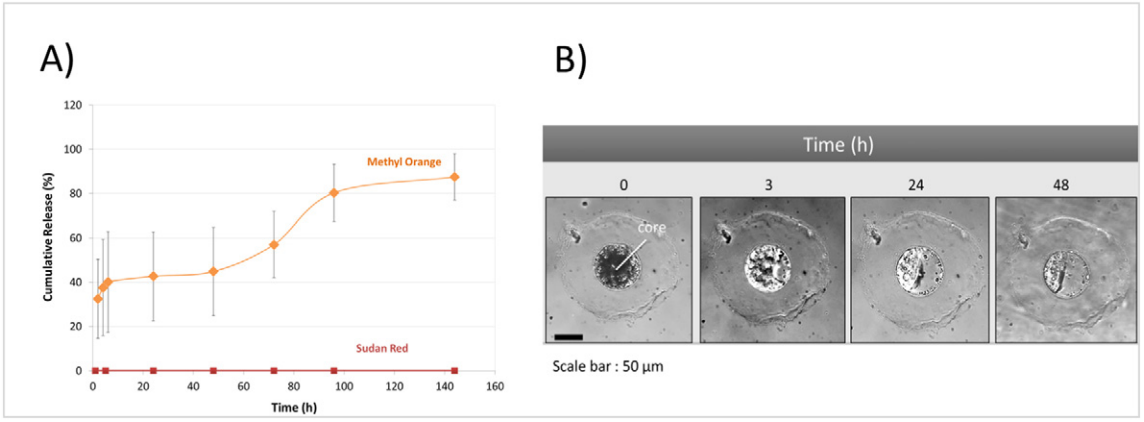


Fig. 7. A) Release profile of hydrophilic (Methyl Orange) and lipophilic (Sudan Red) dyes. B) Core destabilization of a microcapsule with time.

Contrary to what was observed for the hydrophilic Methyl Orange dye, Sudan Red was not released from the microcapsules (Fig. 7A). The hydrophobic dye interacted with the oil phase and remained in the oil core. In addition, the high degree of hydration of alginate membrane (94.9 ± 0.4%, w/w) would have certainly slowed down or even prevented Sudan Red diffusion from the oil core.

4. Conclusion

A novel method with a very simple experimental set-up of oil microencapsulation by inverse gelation of alginate was proposed using W/O emulsions as templates to generate microcapsules. It was demonstrated that microcapsules with (controllable) diameter between 370 and 600 μm were able to be produced by tuning stirring rate of the alginate bath. It was also possible to simply tune the membrane thickness by using emulsion destabilizers (Tween 20 and ethanol). Most important, the robustness of the method came from the fact that almost 100% of oil was encapsulated with a fast curing time. The microcapsules displayed no release of a hydrophobic dye, a property suitable for applications where actives need to be retained during long times or for volatile compounds. Moreover, this technique allows the microcapsules to be formed in a single step at room temperature, which is particularly important in the case of actives susceptible to thermal degradation.

In future works, it could be interesting to produce microcapsules using a static mixer, where the shear forces applied could generate smaller microcapsules with a narrow polydispersity, with the aim to have a continuous production and scale the process up to a pilot process.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.reactfunctpolym.2017.03.006.

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