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Monodisperse core-shell alginate (micro)-capsules with oil core generated from droplets millifluidic

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ABSTRACT

Droplets millifluidic devices are efficient tools to produce monodisperse emulsions that can be used as template for the production of core-shell capsules. This work aims to develop an original alginate inverse gelation method to produce (micro)-capsules with a narrow size distribution using droplets millifluidic. Water-in-oil (W/O) emulsion dispersed phase containing Ca^{2+} ions was directly injected into a continuous alginate phase to generate a secondary W/O/W emulsion. Due to the cross-linking of alginate molecules by Ca^{2+} ions release, core-shell (micro)-capsules were formed with a very high oil loading. This study demonstrated for the first time the production of (micro)-capsules based on the inverse gelation mechanism using a simple millifluidic device. Monodisperse core-shell capsules with sizes ranging from 140 μm to 1.4 mm were produced by tuning flow rates of the continuous and dispersed phases and by varying internal diameter of the capillary tubing. The use of millifluidic devices paves the way to an integrative formulation of core-shell materials with very large characteristic sizes and new complex architectures. This should lead to the rapid emergence of new products in cosmetics or food where the texture and visual aspect play a key role for sale.

1. Introduction

Emulsions with controlled size are produced and used as templates to design microcapsules for applications in food formulation or controlled release for instance (Schmit, Courbin, Marquis, Renard, & Panizza, 2014; Theberge et al., 2010). Contrary to the dispersion techniques that produce microcapsules with large size distribution, droplets millifluidic devices allow the easy production of highly controlled and uniform microcapsules (Duncanson et al., 2012; Theberge et al., 2010).

In droplets millifluidic, a dispersed phase is introduced through a capillary or needle into the co-flowing continuous phase (Sun, Liu, & Xu, 2014) to generate droplets. The structure of the particles can be determined by the flow properties in the devices while the chemical composition is dictated by selected fluids. For the use of basic junction such as co-axial or T-junction, millifluidic offers more advantages than soft lithography and microfluidic techniques. The millifluidic method consists of using an assembly of capillaries or flexible tubes (plastic or silica tubing with diameters ranging from 50 μm to a few mm) put together with elementary home-made or commercial modules. The elementary modules are able to achieve the basic functions used in microfluidic devices such as for instance the formation of periodic trains of monodisperse droplets with very good control over their size or the dilution-concentration of these trains while keeping the volume of droplets unchanged (Engl, Backov, & Panizza, 2008). Modular millifluidic set-ups can then be designed to produce newly controlled integrated architectures limited only by the number of combinations possible and one's creativity (Engl et al., 2008). The connecting capillary tubes and the various modules can assemble and disassemble easily so that modular set-ups can be designed on demand in a short time. The great versatility of this method gives to millifluidic strategy several advantages over microfluidic synthesis while keeping its specificities to deeply study critical parameters involved in microcapsules production for instance.

Among the polymers used for oil encapsulation, alginate has attracted much attention for its low cost, easy to use, biodegradability and non-toxicity. Alginates are algal polysaccharides consisting of a linear chain of (1–4) linked residues of β-D-mannuronic acid (M) and α-L-guluronic acid (G) in different proportions and sequential arrangements (Ouwerx, Velings, Mestdagh, & Axelos,

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1998). This polymer has a remarkable property of quick gelation in presence of divalent ions such as calcium ions (Ca^{2+}). Indeed, alginate gelation can be obtained by two different mechanisms: external and internal gelation.

Based on the external gelation mechanism, Ren et al. (Ren, Ju, Xie, & Chu, 2010) proposed a technique of oil encapsulation in Ca-alginate membranes using a micro-capillary microfluidic device. Briefly, oil was injected into alginate solution forming an oil-in-water (O/W) emulsion which is dispersed in a second oil phase forming a secondary oil-in-water-in-oil (O/W/O) emulsion. The secondary emulsion is injected in a capillary system and put in contact with a $CaCl_2$ solution. O/W emulsion droplet migrates to calcium solution leading to the formation of multicore oil-in-alginate capsules.

Based on the internal gelation mechanism, Schmit et al. (Schmit et al., 2014) proposed to inject an oil using a co-flow junction into the alginate/$CaCO_3$ solution forming an O/W emulsion. O/W emulsion was added drop wise to the oil-acetic acid bath where acidification triggered the release of Ca^{2+} ions and gelation. This pendant drop millifludic method, where the core was first formed at the tip of the inner nozzle, allowed a good control over the number of core droplets in the millimetre-sized capsules.

In both methods described above, a large quantity of oil is used either to generate emulsion drops or when collecting (micro)-capsules. This volume of oil is generally wasted unless in few cases where the oil is recycled during or after the process. In addition, a thorough rinsing is necessary to remove the oil covering the (micro)-capsules surface.

In the inverse gelation mechanism, oil and $CaCl_2$ solution are emulsified and added dropwise into alginate solution bath (Abang, Chan, & Poncelet, 2012). Ca^{2+} ions diffuse from the emulsion drop to the alginate bath cross-linking the surrounding alginate molecules. As a result, core-shell capsules can be produced with an oil loading of 90% (v/v) for dry capsules (Andersen, Gaaseroed, & Larsen, 2005). The oil encapsulation using this gelation mechanism is a quite recent approach and remains few studied (Abang et al., 2012; Martins, Renard, Davy, Marquis, & Poncelet, 2015). In addition, the (micro)-capsules production using droplets millifluidic has never been performed and can be a promising strategy for oil encapsulation before to extend the process at large scale.

This work aims therefore to propose a new droplets millifluidic/inverse gelation based process to produce core-shell alginate (micro)-capsules with a narrow size distribution. Therefore, water-in-oil (W/O) emulsion drops containing Ca^{2+} were directly injected into alginate solution within glass capillary to form downstream of the circuit the (micro)-capsules.

2. Experimental

2.1. Materials

Sodium alginate powder (Saltialgine S 60 NS), with a ratio of mannuronic (M) over guluronic (G) acid units (M/G) and molar mass equal to 1.37 and 1.57 $\times$ 10^5 g/mol, respectively, was kindly donated by Cargill (France). Calcium chloride powder ($CaCl_2$. $2H_2O$) (Panreac Quimica Sau, Spain), sunflower cooking oil (Associated Oil Packers, France), PGPR 90 (Danisco, 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

Ten grams of alginate powder were dissolved in 1 L of demineralized water using a paddle stirrer. Tween 20 (5–20 mL/L) and pure ethanol (5–20% v/v) were added or not to the alginate solution. During the ethanol addition, the alginate solution was mixed constantly at 400 rpm to avoid the precipitation of the polymer.

Calcium chloride solutions were prepared by dissolution of 120–480 g of $CaCl_2 \cdot 2H_2O$ in 1 L of demineralized water (i.e. molar concentrations ranging from 0.8 to ~3.3 M).

2.3. Preparation of W/O emulsions

One hundred millilitres of sunflower oil containing 0.048–0.96 g of the surfactant (PGPR 90) was mixed using a high shear mixer (Ultra-Turrax T25, IKA, Germany) at 18 000 rpm during 1 min. Sixty millilitres of calcium chloride solution was then added slowly and an additional shear mixing at 18 000 rpm for 3 min was performed.

2.4. Conductivity of the emulsions

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

2.5. Stability of the emulsions

One hundred millilitres of emulsions were placed in graduated tubes at ambient temperature. The time of stability was defined when a phase separation (1% v/v) was observed (Martins et al., 2015).

2.6. Capsules production by droplets millifluidic

A millifluidic device with a co-axial flow focusing geometry was used (Fig. 1). The dispersed phase, a W/O emulsion freshly prepared, was pumped (Harvard Apparatus PHD 2000, France) through a fused silica capillary tube (interior diameter (ID) 530 μm and outside diameter (OD) 660 μm) at a rate (Q_{emul}) varying between 0.5 and 8 mL/h. The continuous phase, an alginate solution added or not with Tween 20 or Tween 20/ethanol, was pumped through a Teflon tube (ID = 1.57 mm and OD = 0.5 mm) at a rate (Q_{alg}) varying between 2 and 8 mL/h. The W/O emulsion and the alginate solutions co-flowed in the glass tube (ID = 0.8 or 2, OD = 4 mm and length of 10 cm) (inset Fig. 1). As the generation of capsules using W/O emulsion requires hydrophilic surfaces, the glass tube was previously immersed in a saturated-NaOH solution for 5 min at room temperature and rinsed using tap water (Schmit et al., 2014).

W/O emulsion drops were formed and dispersed in the continuous alginate phase. Calcium ions (Ca^{2+}) diffused from the W/O emulsion drop cross-linking the alginate molecules. The capsules were collected in a distilled water, alginate/Tween 20 or alginate/Tween20/ethanol bath stirred at 50 rpm. After a curing time (time of contact between the capsules and the alginate solution) varying between 8 s and 20 min, the capsules were sieved, suspended in calcium chloride solution (15 g/L) and stored at 4 °C until use.

2.7. Capsules imaging and characterization

The Olympus IX51 microscope (Olympus, France) with a 4X objective was used to image microcapsules. The diameter (d), membrane thickness (M_t) and core size of capsules were deduced from image analyses on 20 capsules per sample using the ImageJ 1.47 v freeware (National Institutes of Health, USA). The experiments and image analyses were performed in triplicate.

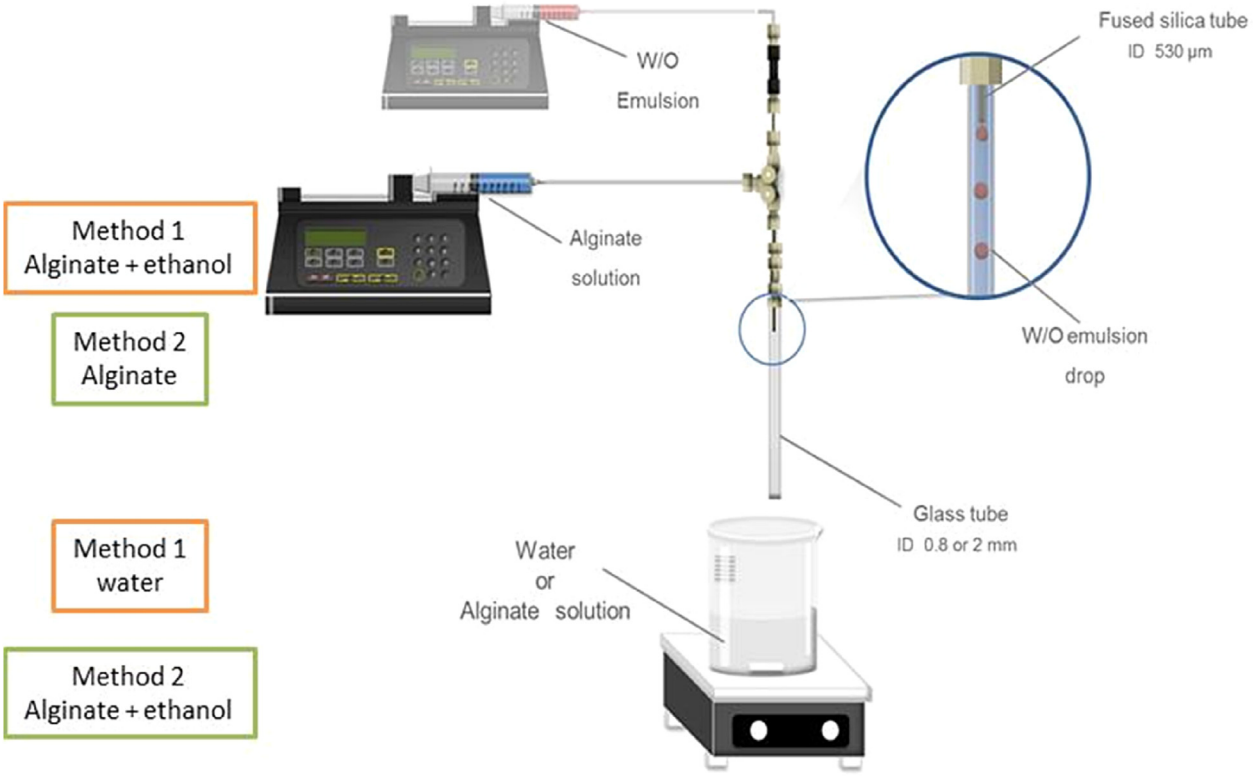

Fig. 1. Droplets millifluidic device used to produce alginate core-shell capsules by inverse gelation. Methods 1 and 2 refer to the effect of ethanol addition on the membrane thickness and morphology of the capsules (see Fig. 8).

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 PGPR 90 concentration on the emulsion stability

By inverse gelation, the dispersed W/O emulsion phase contains the Ca^{2+} source necessary to cross-link the continuous alginate phase in order to form the membrane of the capsule. Emulsion properties, such as stability time, can have a direct impact on the Ca^{2+} release during the membrane formation. In order to vary the stability time, emulsions were formulated using increasing concentrations of surfactant. PGPR 90 was chosen as emulsifier due to its low hydrophilic lipophilic balance value (HLB = 1.5), which made it prone to stabilize W/O emulsions (Al-Sabagh, 2002).

Emulsions containing sunflower oil, $CaCl_2$ solution (240 g/L) and PGPR 90 were prepared using a high shear mixer. W/O emulsion structure was confirmed by the absence of electrical conductivity. By increasing emulsifier concentration, W/O emulsions with stabilities increasing from 0.9 to 132 h were found (Fig. 2). According to Márques et al. (Marquez, Medrano, Panizzolo, & Wagner, 2010) and Su et al. (Su, Flanagan, Hemar, & Singh, 2006), the increase of surfactant concentration decreased the size of water droplets in the W/O emulsion, increasing both its viscosity and stability. According to Park et al. (Park, Cho, & Lee, 2003), the addition of electrolytes in the aqueous phase decreased the attractive force between the water droplets resulting in more stable W/O emulsions. Calcium salt would increase the adsorption of PGPR 90 at the interface resulting in more stable emulsions (Paunovic & Schlesinger, 2006). Marquez et al. (Marquez et al., 2010) also detected a significant interaction between PGPR 90 and calcium, confirming a synergistic effect between the emulsifier and the salt. The stabilizing effect of calcium salt could be partially explained by the decrease of water droplets in the W/O emulsions due to a decrease of the attractive force between the water droplets (Marquez et al., 2010). An

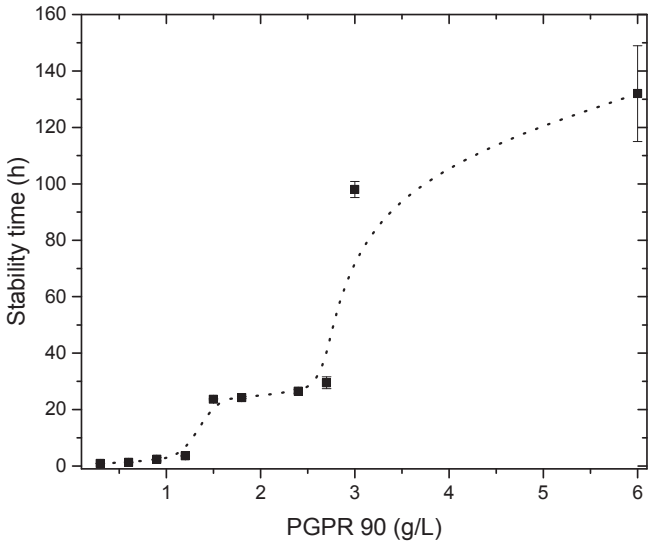

Fig. 2. Effect of surfactant concentration on the stability time of W/O emulsions. Emulsion production conditions: 100 mL of sunflower oil, 60 mL of $CaCl_2$ solution (240 g/L) and PGPR 90.

alternative way by which calcium salt could increase stability to coalescence would be through its effect on the adsorption density of PGPR 90 at the interfacial film by reducing the oil-water interfacial tension in the presence of the emulsifier. Calcium would therefore bind ionic surface active components such as free fatty acids from the emulsifier and/or from the oil phase, reducing their competitive adsorption and favoring the adsorption of PGPR at the interface (Marquez et al., 2010). The synergistic effect between $CaCl_2$ salt and PGPR 90 could explain the high values of emulsion stability observed even at low PGPR 90 concentrations (Fig. 2). In addition, correlating the emulsion stability with the calcium ions release, the higher stability of the emulsions at high PGPR 90 concentrations probably decreased the calcium ions release from the emulsion drop. This hypothesis helps to explain the formation of capsules with thinner membranes.

3.2. Effect of emulsion stability on the capsules production

To investigate the influence of emulsion stability on capsules production, all formulations prepared in the previous section were tested. The dispersed W/O emulsion phase was co-flowed with the continuous alginate phase in order to generate monodisperse W/O emulsion drops. For PGPR 90 concentrations lower than 1.2 g/L, an emulsion-jetting or a non-uniform dripping regime was observed (Fig. 3) leading to fibres or irregular shaped capsules. In this case, during pumping through the feeding capillary tubes, a pronounced emulsion phase separation (oil and $CaCl_2$ solution) occurred leading to a quick and uncontrollable gelation. The emulsion phase separation was probably favoured by mechanical stress during the extrusion. Bremond et al. (Bremond, Thiam, & Bibette, 2008) demonstrated that the extrusion of emulsion through capillary tubes induced a cascade of coalescence events leading to phase separation. Moreover, Davies et al. (Davies, Dickinson, & Bee, 2000) reported that the shear sensitivity of emulsions was dependent on the concentration of emulsifier in the droplets.

For PGPR 90 concentrations between 1.5 and 2.7 g/L, an ideal dripping regime was obtained (Fig. 3). Capsules ($d = 1080 \pm 30$ μm) with core-shell structure were recovered; however, they showed a long-tear-shaped membrane. For PGPR 90 concentrations between 3.0 and < 6.0 g/L, a transition between a jetting and dripping regime was obtained. Su (Su, 2008) demonstrated that the increase of PGPR 90 concentration resulted in more viscous emulsions ($\eta = 0.67$ Pa s for 5 g/L PGPR 90 *vs* $\eta = 1.01$ Pa s for 80 g/L PGPR 90) and, according to Cramer et al. (Cramer, Fischer, & Windhab, 2004), an increase of the viscosity of the dispersed phase stabilized the drop interface by damping interfacial oscillations. Therefore, longer threads were generated between capillary outlet and droplet at higher viscosities of the dispersed phase (Cramer et al., 2004; Zhang, 1999), leading to a jetting regime. The transition between a jetting and dripping regime at high PGPR 90 concentration could therefore be explained by a decrease of interfacial tension at the emulsion-alginate interface leading to the detachment of the drop during co-flowing of the dispersed phase in the continuous alginate phase. The interfacial tension force is the main force which holds the drop at the needle, so that equilibrium of forces is reached earlier for systems with lower interfacial tension (Cramer et al., 2004). This phenomenon did not occur at low PGPR 90 concentration where a constant jetting regime was observed due to a slight decrease of interfacial tension.

In the case of very stable emulsions (132 h; PGPR 90 at 6 g/L), capsules were not produced. It was supposed that the high emulsion stability limited the $CaCl_2$ release preventing the membrane formation. This finding was in accordance with those of Su (Su, 2008) who also observed weaker ions release from stable W/O emulsions. In addition, using optical microscopy, it was found that more stable emulsions displayed smaller inner water droplets (Martins, 2015). The release of calcium ions thus depended on coalescence of water droplets and their expulsion from the emulsion drop. Moreover, it was also observed the reduction of core volume over time indicating a gradual loss of water from W/O emulsions. This observation would evidence that the mechanism of calcium release involved the expulsion of inner water droplets especially those closed to the oil-alginate interface.

As a general rule, unstable emulsions (PGPR 90 < 0.6 g/L) led to high calcium release and to fibers formation downstream the glass capillary tube. By the contrary, calcium ions were not released from emulsions with high stability (PGPR 90 > 2.7 g/L) and membrane was not formed. Emulsions with moderate stability were necessary to generate an ideal dripping regime and form the membrane. A homogeneous drop generation was only observed for emulsions containing between 1.5 and 2.7 g/L of PGPR 90. This range of emulsifier concentration was thus kept in further experiments to produce capsules with narrow size distribution. However, further experimental improvements were necessary to solve the problem of long-tear-shaped capsule (Fig. 3).

3.3. Effect of emulsion destabilization on the spherical capsules production

To solve the problem of long-tear-shaped capsules, the emulsion

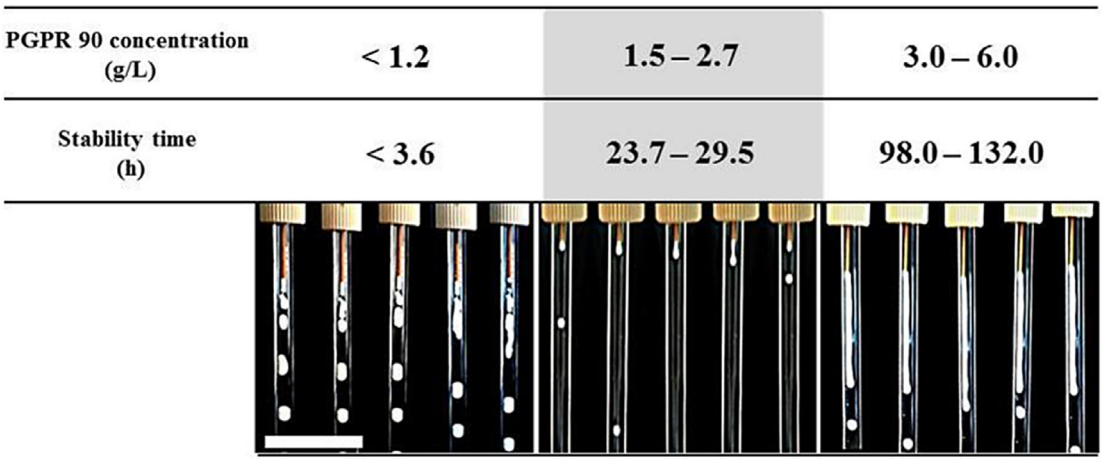

Fig. 3. Influence of PGPR 90 concentration on the emulsion drop generation. Scale bar: 10 mm. *Capsule production conditions*: $CaCl_2$ concentration: 90 g/L; alginate solution: 10 g/L; curing time: 8 s; *ID* glass tube: 2 mm; Q_{alg}: 4 mL/h and Q_{emul}: 2 mL/h.

drop thus needed to be detached more quickly. Yeom and Lee (Yeom & Lee, 2011) reported that the drop detachment time was influenced by the physical properties of the emulsion such as its viscosity and interfacial tension.

The emulsion viscosity (i.e. stability) was governed both by PGPR 90 concentration, aqueous fraction (Φ = water volume/emulsion volume) or shearing rate used to disperse oil and water phases, as it was previously demonstrated for W/O and O/W emulsions (Christiansen & Norn, 2015; Martins, 2015; Martins et al., 2015). The change in one of these parameters would have a consequence on the emulsion stability that may compromise the capsule production. For this reason, it seemed obvious and easier to decrease the interfacial tension of the emulsion drop by the addition of Tween 20 in the alginate solution. In addition, it was previously shown that emulsion with a lower stability released more Ca^{2+} ions (see Fig. 3). The emulsion was therefore co-flowed with alginate solutions added with Tween 20 at different concentrations.

The addition of Tween 20 (0.5 and 1.0% v/v) resulted in capsules perfectly spherical (Fig. 4). Tween 20 efficiently reduced the surface tension of alginate solution from 55 to 33 mN/m and therefore the interfacial tension between the emulsion drop and the alginate solution. For this reason, the drop "neck" break-up was speeded up allowing the quick detachment of the drops and the production of spherical capsules (Cramer et al., 2004). In accordance with our findings, Lepercq-Bost et al. (Lepercq-Bost, Giorgi, Isambert, & Arnaud, 2008) reported that lower interfacial tension contributed to a lower force retaining the drop at the capillary outlet. By addition of 0.5% v/v of Tween 20, it was therefore demonstrated that the core diameter sharply decreased from 1100 to 900 μm (Fig. 4B). For Tween 20 concentrations comprised between 0.5 and 2.0% v/v, no significant reduction of the core size was however observed. By the contrary, other works reported that, by using microfluidic, the increase of surfactant concentration resulted in smaller drops diameters (Schroder, Behrend, & Schubert, 1998; van der Graaf, Steegmans, van der Sman, Schroen, & Boom, 2005; Wu et al., 2008). According to Pawlik (Pawlik, 2012), the critical micelle concentration (CMC) of Tween 20 was approximately 6.0 10^{-3}% v/v. The range of surfactant concentrations used in our study was therefore much higher than the CMC and did not result in a further interfacial tension reduction and explained why additional core size reduction was not observed with the increase of Tween 20 concentration.

Moreover, the membrane thickness linearly increased with the increase of surfactant concentration (Fig. 4C). According to Pays et al. (Pays, Giermanska-Kahn, Pouligny, Bibette, & Leal-Calderon, 2001), Tween 20 is probably adsorbed by the emulsion destabilizing the PGPR 90 films that recovers the aqueous phase droplets ($CaCl_2$ solution) inside the emulsion. In addition, authors reported that the release of encapsulated compound was fast in presence of hydrophilic surfactant at concentrations higher than the CMC. The $CaCl_2$ droplets near the oil-alginate interface would therefore coalesce in presence of Tween 20 resulting in both emulsion destabilization and higher Ca^{2+} release with increasing surfactant concentration.

For Tween 20 concentration at 2% v/v, the membrane was not spherical and inhomogeneous in thickness resulting in the formation of tear-shaped capsules (Fig. 4A). Due to the faster release of Ca^{2+} ions in presence of Tween 20, the membrane would be formed

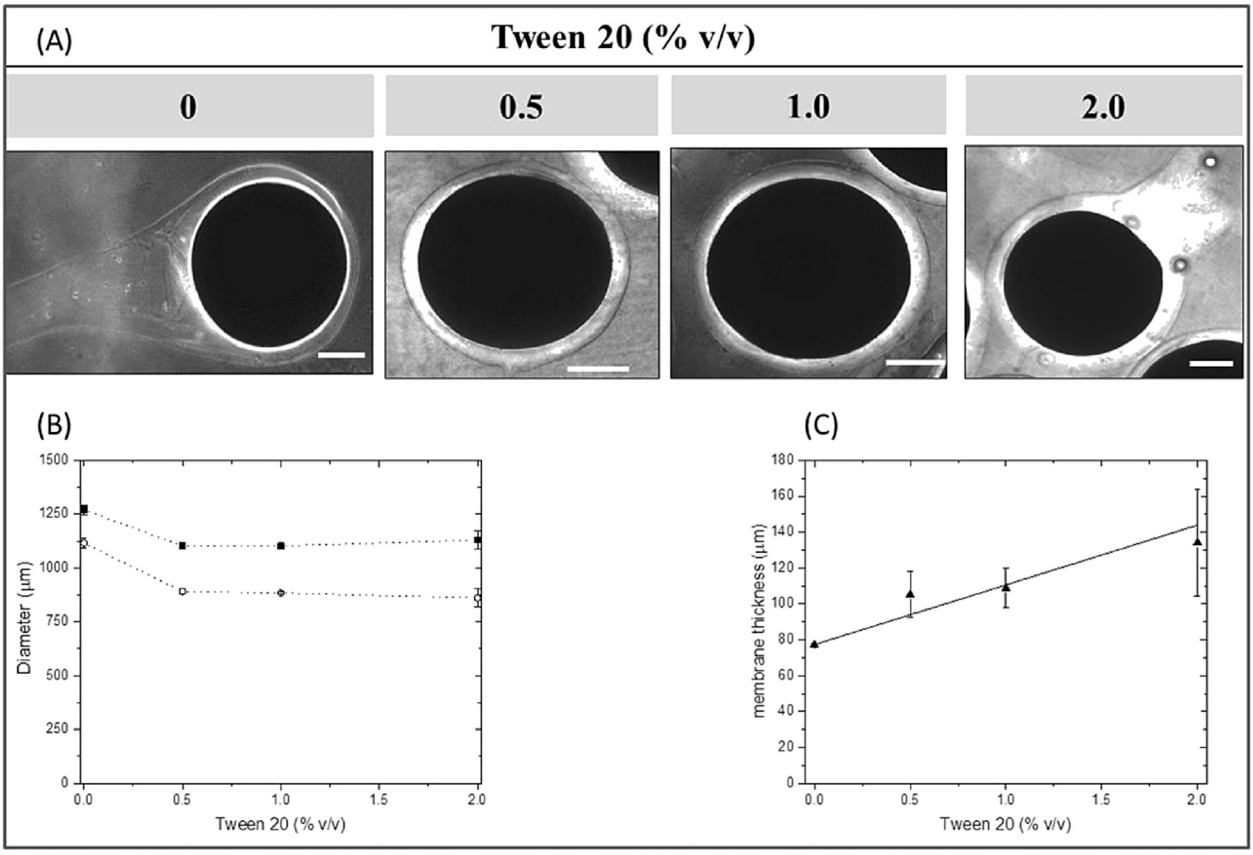

Fig. 4. Effect of Tween 20 concentration on (A) the capsules shape (scale bar: 250 μm), (B) the core diameter and (C) the membrane thickness. (●) Capsule diameter; (○) Core diameter. *Capsule production conditions*: Emulsion: PGPR 90 and $CaCl_2$ concentrations of 1.5 and 90 g/L, respectively. Alginate solution at 10 g/L added or not with Tween 20. Curing time: 8 s; *ID* glass tube: 2 mm; Q_{alg}: 4 mL/h and Q_{emul}: 2 mL/h.

before the break-up of the drop "neck". As no driving force was present to restore the spherical shape, the capsule kept a tear-shaped morphology.

As Tween 20 destabilized emulsion, coalescence and larger $CaCl_2$ droplets near the emulsion-alginate interface would be expelled from the emulsion drop leading to an increase of the membrane thickness (see Fig. 4C).

Tween 20 is known to be quite hydrophilic and could not then penetrate deeply in the emulsion. Only a limited corona around the emulsion would be destabilized. Ethanol by dissolving in the oil would make this oil more hydrophilic. In this case, Tween 20 could therefore be transfer in a deeper corona and destabilize a large part of the emulsion.

3.4. Parameters influencing the capsules size

3.4.1. Influence of alginate concentration

For fixed values of flow rates of alginate solution and W/O emulsion, the core and capsule sizes linearly decreased with the increase of alginate concentration (Fig. 5). The capsules and cores diameters exhibited a two-fold decrease with a 4-fold increase of alginate concentration. In addition, it was also found that the size of capsules was proportional to the size of cores according to the equation: $D_{capsule} = 1.18\ D_{core} + 24.6$, with a R-square value of 0.99. The size of capsules would be therefore governed by the size of the cores.

The increase of viscosity at high alginate concentrations (>20 g/L) made it very difficult to extrude alginate solutions through the capillary tube. Scheele & Meister (Scheele & Meister, 1968) demonstrated that the drag force was proportional to the viscosity of the continuous phase. In our experiments, the increase in alginate concentration led to an increase of the viscosity and therefore, a higher drag force was exerted on the emulsion. Consequently, the emulsion drop detached more quickly from the capillary outlet leading to the formation of smaller drops (cores). In agreement with our results, Erni et al. (Erni, Cramer, Marti, Windhab, & Fischer, 2009) also reported that the size of drops formed in microfluidic devices was reduced by the increase of the continuous phase viscosity.

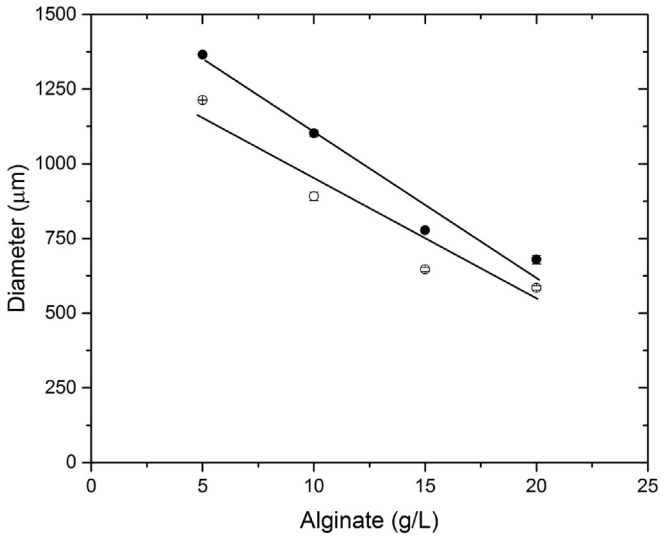

Fig. 5. Effect of alginate concentration (g/L) on the core and capsule diameter (µm). (●) Capsule diameter; (○) Core diameter. *Capsule production conditions*: Emulsion: PGPR 90 and $CaCl_2$ concentrations of 1.5 and 90 g/L, respectively. Alginate solution added with Tween 20 at 0.5 % v/v. Curing time: 8 s; *ID* glass tube: 2 mm; Q_{alg}: 4 mL/h and Q_{emul}: 2 mL/h.

3.4.2. Influence of alginate and W/O emulsion flow rates

The control of the capsules size was performed by tuning the continuous alginate phase (Q_{alg}) and the dispersed W/O emulsion phase (Q_{emul}) flow rates and internal diameter (*ID*) of the glass tube. Using a constant flow rate for alginate solution ($Q_{alg} = 2$, 4 or 8 mL/h), the cores and capsules size increased with the increase of emulsion flow rate, whatever the internal diameter of the glass tube (Fig. 6A and B). On the contrary, by keeping constant Q_{emul}, the cores and capsules diameter decreased with the increase of the alginate flow rate, whatever the internal diameter of the glass tube.

Similar findings were also observed by Zhang et al. (Zhang et al., 2006). In the microcapsule production using a microfluidic device, alginate solution (dispersed phase) was injected into undecanol added of CaI_2 (continuous phase). The authors observed that the microcapsules size decreased by increasing undecanol flow rates. By studying the formation of droplets in microfluidics flow-focusing devices, Zhou (Zhou, Yue, & Feng, 2006) also demonstrated that the drop size increased with the increase of flow rate of the dispersed inner fluid and decreased with the increase of the continuous outer fluid.

For a glass tube of $ID = 2$ mm, (micro)-capsules with diameters varying from 1470 to 670 µm were recovered (Fig. 6A, Table 1). Although a wide range of capsules size was obtained by tuning flow rates and using a capillary tube with an *ID* of 2 mm, (micro)-capsules smaller than 670 µm were not produced. With increasing alginate flow rate (Q_{alg}), capsules sizes were two-fold reduced. In order to further decrease the capsules size, a glass tube with a narrower internal diameter ($ID = 0.8$ mm) was chosen. The capsules sizes were therefore drastically reduced where microcapsules with diameters ranging from 490 to 140 µm were obtained (Fig. 6B, Table 1). In addition, membranes thicknesses were also reduced with values between 65 and 35 µm with increasing Q_{alg}. Whatever the conditions used, the capsules diameters exhibited standard deviation lower than 5% indicating the production of monodisperse (micro)-capsules (Table 1). By reducing the internal diameter of glass tube from 2 to 0.8 mm, the capsule diameters were approximately 3.5 fold reduced (Table 1). Christopher and Anna (Christopher & Anna, 2007) indicated that by reducing the internal diameter of the glass tube, the droplet size (or core size in our case) can also be reduced.

Figure 6C showed an asymptotical decrease of the capsule/core diameter ratio from ~1.18 to 1.10 with the increase of the Q_{emul}/Q_{alg} flow rates ratio, $D_{capsule}/D_{core}$ becoming stable when Q_{emul}/Q_{alg} ratio was higher than 1.5.

This slight dependence of capsule morphologies by tuning emulsion and alginate flow rates confirmed again the robust and highly reproducible character of the droplets millifluidic technique to generate monodisperse (micro)-capsules.

3.5. Parameters influencing the membrane thickness

3.5.1. Impact of curing time

Emulsions drop was formed in the glass tube and kept in contact with alginate solution by increasing curing time (Fig. 7). In few seconds (8s), and until 5 min of curing time, an average membrane thickness of 110 ± 15 µm was formed. This surprising result was reproducible indicating a fast membrane formation. A calculation of the average linear distance *d* covered by calcium ions in a time *t*, using the relationship $d = \sqrt{2Dt}$, with D the diffusion coefficient of $CaCl_2$ in water ($1.312\ 10^{-9}\ m^2\ s^{-1}$) (Ribeiro et al., 2008), and time t = 8s, gave 145 µm. This maximum linear distance was therefore in agreement with the average membrane thickness experimentally found (from 40 to 147 µm) whatever the $CaCl_2$ concentration and alginate concentration used. Calcium ions diffusion would therefore be the main limiting factor in the progress of the shell thickness.

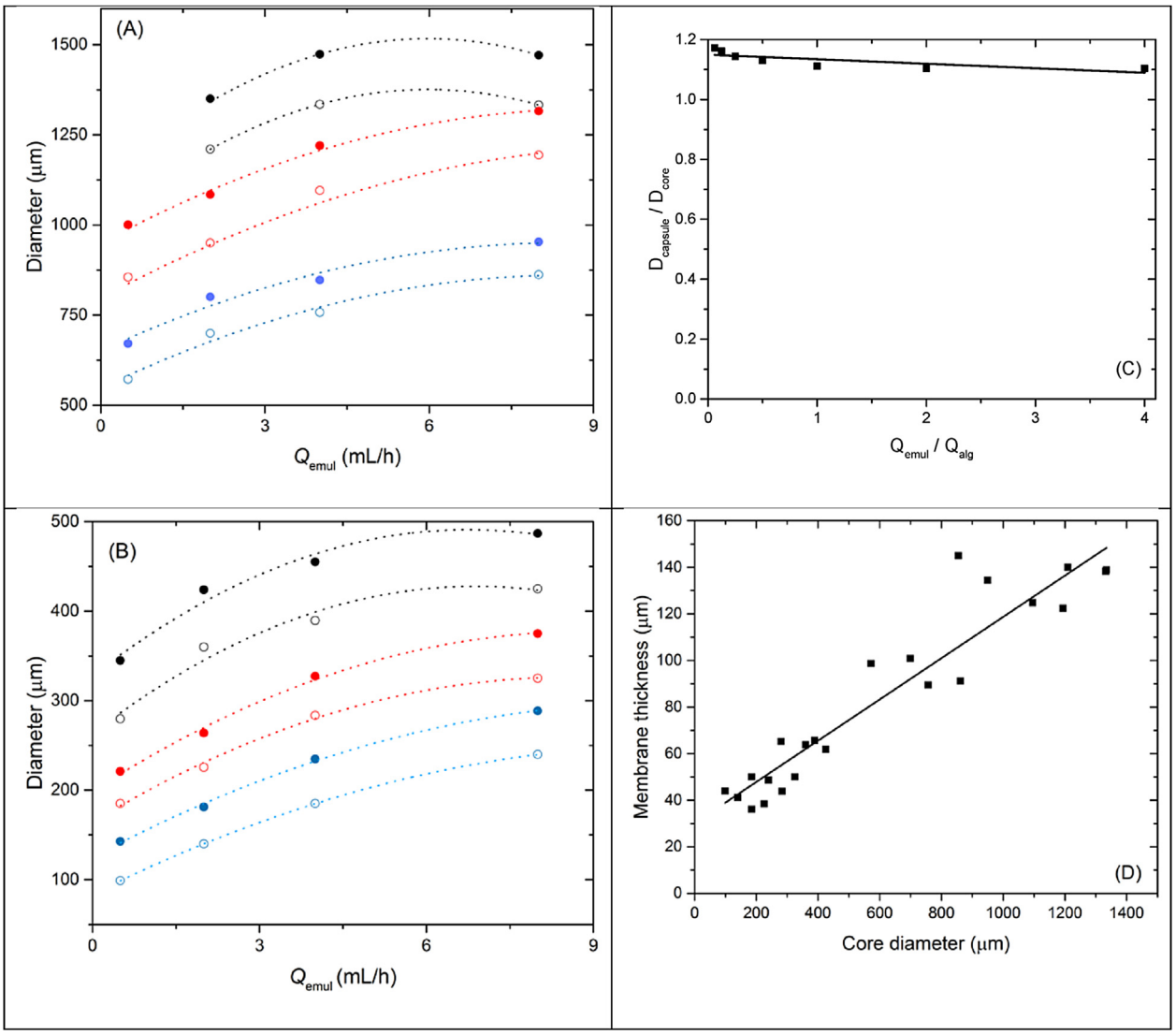

Fig. 6. Effect of emulsion (Q_{emul}) and alginate (Q_{alg}) flow rates and glass tube internal diameter (*ID*) on the core and capsule diameters. (A) *ID* = 2 mm, (B) *ID* = 0.8 mm. (●) Capsule diameter; (○) Core diameter. Q_{alg}: 2 mL/h (●,○); 4 mL/h (●, ○); 8 mL/h (●, ○). (C) Dependence of the capsule/core size ratio with the Q_{emul}/Q_{alg} flow rates ratio; the solid line corresponded to the fitting of the data by a linear function (see text for details). (D) Dependence of the membrane thickness (μm) with the core diameter (μm); the solid line corresponded to the fitting of the data by a linear function (see text for details). *Capsule production conditions*: Emulsion: PGPR 90 and $CaCl_2$ concentrations of 1.5 and 90 g/L, respectively. Alginate solution at 10 g/L added with Tween 20 at 0.5 % v/v. Curing time: 8 s.

Table 1
Variation of capsule diameter (μm ± standard deviation) as a function of the alginate flow rate (Q_{alg}), the emulsion flow rate (Q_{emul}) and the glass tube internal diameter (*ID*).

	Q_{emul} (mL/h)	Q_{alg} (mL/h)		
		2	4	8
ID = 2.0 mm	0.5	–	994 ± 6 ^{g}	671 ± 3 ^{m}
	2.0	1342 ± 9 ^{a}	1085 ± 29 ^{h}	806 ± 3^{n}
	4.0	1474 ± 4 ^{b}	1214 ± 9 ^{i}	847 ± 5^{o}
	8.0	1471 ± 4 ^{b}	1316 ± 11 ^{a}	953 ± 12^{p}
ID = 0.8 mm	0.5	315 ± 5^{c}	221 ± 12 ^{j}	143 ± 5^{q}
	2.0	424 ± 13 ^{d}	264 ± 7 ^{k}	181 ± 8^{r}
	4.0	449 ± 3 ^{e}	327 ± 9 ^{c}	237 ± 8^{j}
	8.0	487 ± 20 ^{f}	378 ± 7 ^{l}	289 ± 3^{s}

Different letters in the same column or line indicates significant difference ($p < 0.05$).

A linear decrease of the membrane thickness was however observed after 5 min of curing time. After 30 min, no capsules were recovered which probably indicated the complete dissolution of the membrane. In the capsule production using an extrusion-dripping/inverse gelation technique, Abang et al. (Abang et al., 2012) found that for curing time higher than 60 min, the capsules disappeared and the membrane was dissolved in the alginate solution. Similar phenomenon was also described by Koyama et al. (Koyama & Seki, 2004) in the production of aqueous-core calcium alginate capsules by dropping calcium chloride polyethylene glycol solution into an alginate solution.

The results obtained in this study together with those from literature confirmed that the progressive dissolution of the membrane thickness with increasing curing time would be related to the fact that, as complexation of calcium ions with alginate is known to be reversible, the migration of calcium into the alginate bath would be favoured. Abang et al. (Abang et al., 2012) confirmed that by suspending calcium alginate beads in an alginate solution, a complete dissolution of the beads was observed indicating the dissolution of the Ca-alginate gel with time.

In conclusion, a curing time between 8 s and 5 min was ideal to

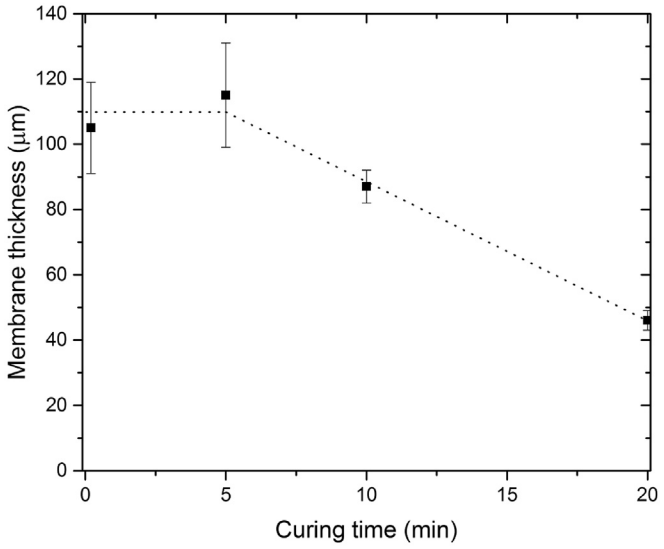

Fig. 7. Effect of curing time on the membrane thickness. *Capsule production conditions*: (A) Emulsion: PGPR 90 and $CaCl_2$ concentrations of 1.5 and 90 g/L, respectively. Alginate solution at 10 g/L added with Tween 20 at 0.5 % v/v. *ID* glass tube: 2 mm; Q_{alg}: 4 mL/h and Q_{emul}: 2 mL/h.

reach the maximal membrane thickness in the capsules produced by droplets millifluidic.

3.5.2. Impact of $CaCl_2$ concentration

For $CaCl_2$ concentrations lower than 45 g/L, capsules were not recovered. It was assumed that these $CaCl_2$ concentrations generated very thin membranes or the membranes were not enough resistant to stabilize the capsules. For $CaCl_2$ concentrations higher than 45 g/L, capsules were obtained. However, the membrane thickness was then independent on $CaCl_2$ concentration. A minimal $CaCl_2$ concentration (45 g/L) was thus necessary to create a cohesive membrane.

3.5.3. Impact of Q_{alg}, Q_{emul} and ID glass tube

The control of the membrane thickness, in line with the control of capsules or cores size, was performed by tuning the continuous (Q_{alg}) and dispersed (Q_{emul}) flow rates together with the internal diameter (*ID*) of the glass tube. Fig. 6D clearly demonstrated that the membrane thickness (M_t) was linearly dependent on the core diameter (D_{core}). A 7-fold increase in core diameter led to a 3.5-fold increase in membrane thickness. Considering that only calcium ions near the core surface participated to the alginate gelation, it was assumed that bigger cores had more available calcium ions to produce thicker membrane.

3.5.4. Impact of ethanol

As well as Tween 20, ethanol can also be applied as a destabilizer of W/O emulsions and improves Ca^{2+} ions release (Andersen et al., 2005). To evaluate the effect of ethanol on the membrane thickness, two methods of capsule production were tested (Fig. 1):

1) Emulsion was injected into the alginate solution supplemented with ethanol and the capsules were collected in a water bath
2) Emulsion was injected into the alginate solution and the capsules were collected in an alginate bath supplemented with ethanol

In method 1, ethanol was in contact with the surface of the droplets during the transfer through the capillary (8 s) and then was diluted in the water bath. In such conditions, the membrane thickness was quite similar to the membrane formed in absence of ethanol (Fig. 8A). When the concentration of ethanol was equal or higher than 20%, membrane thickness was homogeneous, cores were therefore deformed and capsules had a tear-shaped morphology (Fig. 8B). Ethanol would therefore have no effect on the W/O emulsion destabilization at the time scale of the experiment leading to the same content of calcium ions released from W/O droplets and the absence of alginate membrane growth.

On the other hand, by collecting the capsules into alginate bath added with ethanol (method 2), the membrane thickness increased with ethanol concentration then a plateau was reached for ethanol concentration higher than 10% (Fig. 8C). Spherical capsules were obtained with membrane thicknesses varying between 105 and 140 µm. Ethanol in the collecting bath would have therefore an effect on the alginate membrane growth.

To understand the effect of ethanol on the differences observed on the membrane thickness, it was first checked that the osmotic pressure difference between the internal and external aqueous phases was not at the origin of the diffusion of water/$CaCl_2$ outside W/O emulsion droplets leading to the increase of membrane thickness. Using the van't Hoff law, it was found that the osmotic pressure of the internal aqueous phase ($\Pi_{int} = 1.2\ 10^7$ Pa) was very similar to those of the external aqueous phase ($\Pi_{ext} = 6.9\ 10^6$ Pa). The increase of membrane thickness with the ethanol content, whatever the method used to add ethanol, could not therefore be ascribed to an osmotically driven process.

One hypothesis to explain the specific effect of ethanol present in the collecting bath would be that ethanol could dehydrate part of alginate macromolecules present in the collecting bath. Ethanol is considered as a non-solvent for alginate: its presence in the solution facilitates inter-chain interaction even in the absence of demixing (Borgogna, Bellich, Zorzin, Lapasin, & Cesàro, 2010). The desolvation process of alginate chains would therefore improve their deposition on the neo-forming alginate membrane. As a consequence, for the same quantity of calcium, an increase in the membrane thickness of the core-shell capsules was observed for increasing ethanol content in the collecting alginate bath.

If a $CaCl_2$ droplet is in contact or very near of the emulsion surface, the Laplace pressure, which tends to shrink and facilitate spherical shape of the emulsion drop, would tend to expel $CaCl_2$ droplets.

4. Conclusion

This study demonstrated the success of the production of (micro)-capsules based on the inverse gelation mechanism using droplets millifluidic. Monodisperse (micro)-capsules with sizes ranging from 1.4 mm down to 140 µm were produced by direct injection of the W/O emulsion phase containing ionic cross-linker in the alginate solution.

This simple droplets millifluidic method based on the use of pendant drops does not require sophisticated equipment and only parameters such as flow rates and internal diameter of the capillary tube are needed to be controlled to ensure (micro)-capsules production. Moreover, it was found that the addition of Tween 20 surfactant affected capsule shape and increased the membrane thickness. In addition, the membrane thickness was also tuned by the addition of ethanol in the alginate solution.

The inverse gelation method is simpler and easier to perform approach compare to those based on external or internal gelation because a simple extrusion process is needed. Moreover, the method allows the production of core-shell capsules while polynuclear capsules are obtained by direct gelation (external or internal gelation). Finally, (micro)-capsules are neither formed nor

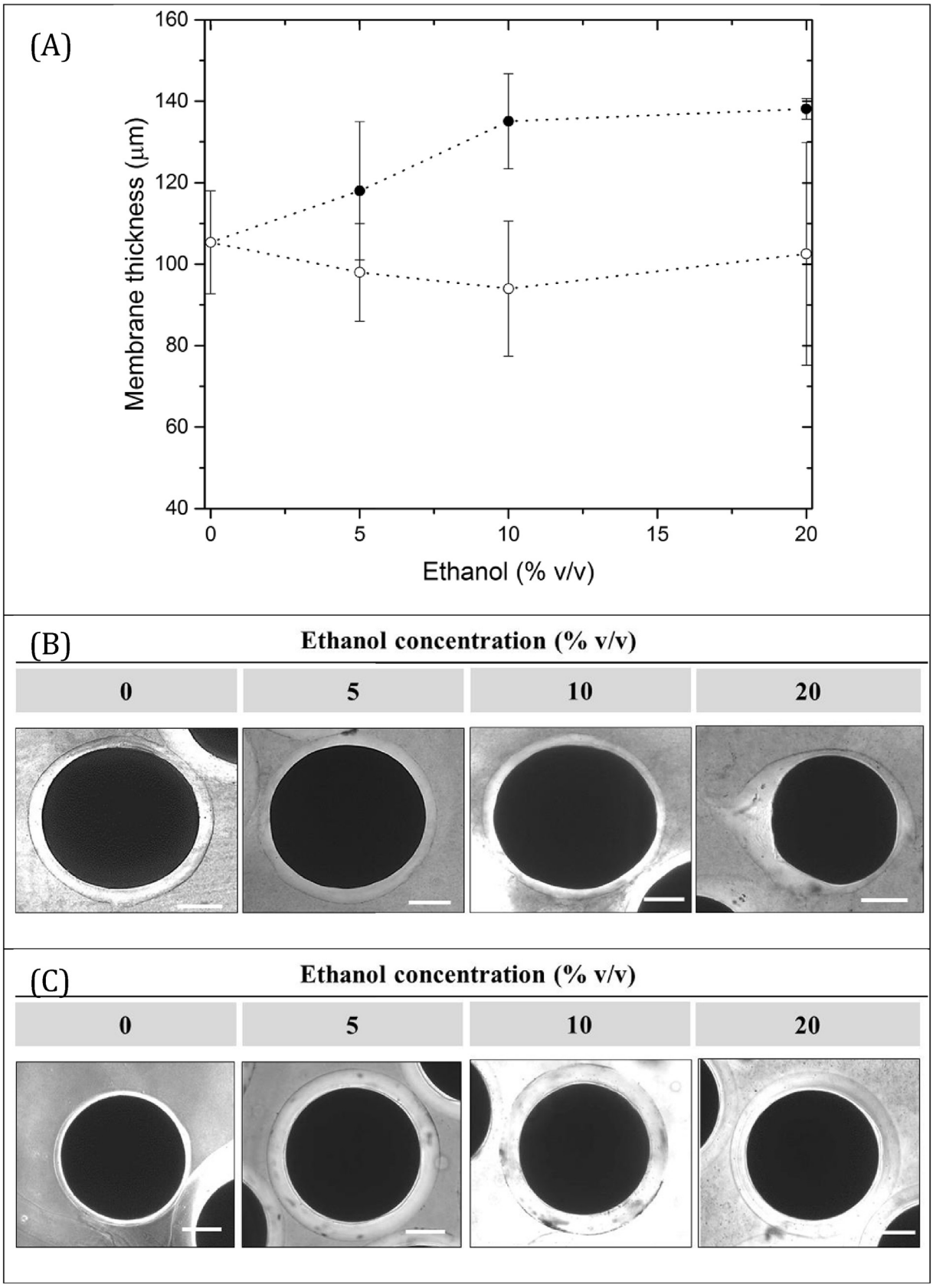

Fig. 8. Effect of ethanol addition on the membrane thickness (A) and morphology of the capsules depending on method 1 (B) and method 2 (C). (○) Method 1: capsules collected in water bath; ethanol was still present in the alginate solution (●) Method 2: capsules collected in alginate bath added with ethanol. Scale bars: 250 μm. *Capsule production conditions*: Emulsion: PGPR 90 and $CaCl_2$ concentrations of 1.5 and 90 g/L, respectively. Alginate solution at 10 g/L added with Tween 20 at 0.5 % v/v with ethanol (method 1) and without ethanol (method 2), ethanol being present in the collecting bath . *ID* glass tube: 2 mm; Q_{alg}: 4 mL/h and Q_{emul}: 2 mL/h. Curing time: 5 min.

collected into an oil phase, consequently reducing the volume of oil necessary during the process and simplifying the (micro)-capsules rinsing procedure.

The scaling up for the production of monodisperse capsules could be performed using membrane technology, the emulsion being extruded through a porous membrane forming a high number of drops per time unit. Another alternative would be the parallelization of the devices in order to multiply the production yield by the number of devices used. For instance, Engl, Tachibana, Panizza, and Backov (2007) (Engl et al., 2007) used « tubular millifluidic » consisting in a simultaneous operation of 50-reactors devices in parallel, and were able to produce up to 1.8 10^4 particles per hour.

The use of millifluidic devices pave the way to an integrative formulation of dispersed materials with very large characteristic sizes ranging from typically 100 μm to several mm and new complex architectures. This should lead to the rapid emergence of new products in cosmetics or food where the texture and visual aspect play a key role for sale. In particular, these capsules could find applications as colored capsules in food drinks or vitamin or oil flavors loaded capsules in nutrition. They could also find applications as fragrance or pigment or essential oil carriers in shampoos or creams in cosmetics.

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