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Oil encapsulation in core–shell alginate capsules by inverse gelation II: comparison between dripping techniques using W/O or O/W emulsions

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ABSTRACT

In the first part of this article, it was described an innovative method of oil encapsulation from dripping-inverse gelation using water-in-oil (W/O) emulsions. It was noticed that the method of oil encapsulation was quite different depending on the emulsion type (W/O or oil-in-water (O/W)) used and that the emulsion structure (W/O or O/W) had a high impact on the dripping technique and the capsules characteristics. The objective of this article was to elucidate the differences between the dripping techniques using both emulsions and compare the capsule properties (mechanical resistance and release of actives). The oil encapsulation using O/W emulsions was easier to perform and did not require the use of emulsion destabilisers. However, capsules produced from W/O emulsions were more resistant to compression and showed the slower release of actives over time. The findings detailed here widened the knowledge of the inverse gelation and gave opportunities to develop new techniques of oil encapsulation.

Introduction

Mainly industrial technologies for oil phase encapsulation need high temperatures or organic solvents, which is a drawback for some applications (Cárdenas-Bailón et al., 2014; Oliveira et al., 2014). Spray-drying, for example, does not provide a strong protection and can degrade the product due to high temperature application while the oil microencapsulation by coacervation requires the use of glutaraldehyde as a cross-linking agent (Jerobin et al., 2012; Beirão-da-Costa et al., 2013; Carneiro et al., 2013).

One alternative method consists of dispersing the oil in alginate solution and produce beads by extruding the emulsion drop wise into a calcium chloride bath (Chan et al., 2009). Although simple and practical, these capsules contain low oil loading since losses via diffusion can occur through the pores of hydrogel beads (Peniche et al., 2004). By the contrary, core–shell capsules produced by co-extrusion dripping display higher oil loading (Whelehan and Marison, 2011), but the scale-up of this method remains a bottleneck. In most cases, the co-extrusion dripping requires a vibration system to form the drops and the liquid phases (oil and alginate solution) are pumped separately through concentric nozzles. Therefore, it is necessary to control the frequency of vibration, to regulate the

pumping rates of each fluid and to avoid the clogging of the concentric nozzles (which may frequently occur depending on the fluid viscosities and the hole of the nozzles).

The oil encapsulation in Ca-alginate membranes by inverse gelation consists of adding drop wise oil/calcium chloride emulsions into alginate bath (Andersen et al., 2005).

Depending on the oil/$CaCl_2$ solution ratio and the emulsifier selected, the emulsion can be of the oil-in-water (O/W) or water-in-oil (W/O) type (Griffin, 1949; ICI Americas Inc., 1980; Züge et al., 2013). This alternative method in comparison with the oil encapsulation by the extrusion-dripping technique using alginate external gelation leads to core–shell capsules with a high oil loading. In a previous work, the production of capsules using O/W emulsions was therefore demonstrated by Abang et al. (2012). Briefly, the technique consists in added drop wise an O/W emulsion containing $CaCl_2$ into a stirred alginate bath containing the surfactant. At the end of the process, the initial liquid droplet is engulfed by a continuous semi-permeable Ca-alginate membrane leading to core-shell capsules; dried capsules contain more than 95% w/w of oil (Abang, 2011; Abang et al., 2012). Using a similar approach, oil encapsulation using W/O emulsions was

recently described where it was evidenced that the W/O emulsion stability and the addition of emulsion stabiliser (ethanol) were the main factors influencing the capsules production (Martins et al., 2017a). Both wet and dry capsules were obtained with a spherical shape where all the volume of oil was encapsulated.

The dripping-inverse gelation techniques using W/O or O/W emulsions display some peculiarities and a comparison between both methods is necessary in order to highlight the advantages and drawbacks of each process. Furthermore, this article aims to compare capsules produced from both emulsions in terms of mechanical resistance and release profile of hydrophilic and lipophilic actives.

Materials and methods

Materials

Sodium alginate powder Satialginate S 60 NS (Cargill, France) was used to prepare the alginate bath. Calcium chloride powder ($CaCl_2 \cdot 2H_2O$) (Pancreac Quimica, Spain) and sunflower cooking oil (Associated Oil Packers, France) were used to prepare the W/O and O/W emulsions. The surfactants Span 85 (Sigma Aldrich, France), Tween 85 (Sigma Aldrich, France) and PGPR 90 (Danisco, France) were used to stabilise the emulsions. Hydrophilic dye (Congo Red, Sigma Aldrich, France), Bovine Serum Albumin (BSA, ICN Biomedicals) and a lipophilic dye (Sudan Red, Sigma Aldrich, France) were used for the release profile studies. All other chemicals of analytical grade were obtained from Sigma Aldrich.

Preparation of alginate and CaCl$_2$ solutions

Ten grams of alginate powder was dissolved in 1 l of demineralised water.

The calcium chloride solutions were prepared by dissolving $CaCl_2 \cdot 2H_2O$ powder in demineralised water to get final concentrations of 240 and 300 g/l. These calcium chloride concentrations were chosen in order to obtain, respectively, capsules from W/O and O/W emulsion with similar membrane thickness.

Capsules production

Capsules based on W/O emulsions

W/O emulsion was produced by dispersion of 11.2 mg of PGPR90, 100 ml of sunflower oil and 43 ml of calcium chloride solution (300 g/l) using a high shear mixer (Ultra-Turrax T25, IKA, Germany) at 13 500 rpm during 4 min. The emulsion was pumped at a flow rate of 30 ml/h through a tip (2 mm of internal diameter) and added drop wise into alginate solution containing Tween 20 (1% v/v) and ethanol (20% v/v). The alginate solution put into a beaker (Ø 6.0 cm and H 6.5 cm) was stirred at 300 rpm during 5 min and wet capsules were recovered by filtration. The detailed protocol of capsules production was previously optimised in the first part of this article (Martins et al., 2017a).

The capsules were washed with distilled water and transferred into a calcium chloride solution (1.5 g/l) to stabilise the membrane (Degen et al., 2015).

Dry capsules were obtained by absorption of the excess of water using a paper towel followed by air-drying ($20 \pm 2\,°C$) during 2 days.

Capsules based on O/W emulsions

The production of capsules using O/W emulsions was performed as described by Abang et al. (2012). Briefly, O/W emulsion was prepared by dispersion of 470 mg of Span 85, 570 mg of Tween 85, 100 ml of sunflower oil and 30 ml of calcium chloride solution (240 g/l) using a high shear mixer (Ultra-Turrax T25, IKA) at 13 500 rpm during 4 min (Abang et al., 2012). The emulsion was pumped at a flow rate of 30 ml/h through a tip (2 mm of internal diameter) and added drop wise into alginate solution containing Tween 20 (1% v/v). The alginate solution was stirred at 300 rpm during 25 min and wet capsules were recovered by filtration.

Dry capsules were obtained by absorption of the excess of water using a paper towel followed by air-drying ($20 \pm 2\,°C$) during 2 days.

Measurement of capsule diameter and membrane thickness

For the determination of the capsule diameter (d), 20 capsules were photographed using a Dino-lite digital microscope pro (Taiwan) and the diameter was measured by image analyses using ImageJ 1.47v freeware. The capsules were then half-cut and the membrane thickness was measured, in eight different points symmetrically positioned, using the Dino-lite digital microscope pro. The membrane thickness (Mt) of each capsule was calculated from the mean of the eight measurements performed.

All measurements were assayed in triplicate corresponding to a total of 60 capsules.

Mechanical resistance

Capsules were compressed at 5 mm/min until rupture using MTS-Synergie 100 compressive strength machine

Table 1. Experimental details of the active release studies using wet and dry capsules.

		Wet capsules			
Emulsion type	Active	(M_{act}) Mass of active (mg)	(M_{emul}) Mass of emulsion (g)	(V) Volume of NaCl solution or oil (l)	($R\infty_{wet}$) Maximal concentration of active released (mg/l)
W/O	Congo Red	10	130	0.07	20
	BSA	1000	131	1.96	80
	Sudan Red	10	130	0.07	20
O/W	Congo Red	10	120	0.50	20
	BSA	1000	121	2.08	80
	Sudan Red	10	120	0.50	20
		Dry capsules			
	Active	(ρ) Mass of active/ Mass of capsule (mg/g)		(V) Volume of NaCl solution or oil (l)	($R\infty_{dry}$) Maximal concentration of active released (mg/l)
	Congo Red	0.11		0.05	20
	BSA	11.30		1.41	80
	Sudan Red	0.11		0.05	20

Note: Release kinetics was made in triplicate at room temperature ($20 \pm 2\,°C$) and was stopped when no more variation in the absorbance value ($\leq 1\%$) was observed.

(Minnesota). The capsules were placed on the plate of the compression cell after gentle drying on the filter paper. Deformation (ε) was calculated according to Equation (1):

$$\varepsilon = \frac{d - d_{final}}{d} \times 100 \qquad (1)$$

where $\varepsilon =$ deformation, $d =$ initial diameter (mm) before compression and $d_{final} =$ final diameter (mm) after compression.

The compression pressure (σ) exerted on the capsule was calculated by the ratio between the force (F) during compression and the cross-sectional area of the initial capsule (Moura et al., 2009). The elastic modulus (E) was calculated from the initial slope of the σ versus ε curve, where a linear response between compression pressure and deformation was observed. Measurements were made on five capsules with a diameter of 7.0 ± 0.1 mm from three different batches corresponding to a total of 15 capsules.

Release profile of actives

Calcium chloride solutions containing hydrophilic actives (Congo Red or BSA) or oil added with lipophilic dye (Sudan Red) were used to prepare the emulsions. Ten milligrams of Congo Red or 1000 mg of BSA were dissolved into 43 ml of calcium chloride solution at 300 g/l or 30 ml of calcium chloride solution at 240 g/l before the production of the emulsions. In the same way, 10 mg of Sudan Red was dissolved into 100 ml of oil before to produce the emulsions according to sections Capsules Based on W/O Emulsions and Capsules Based on O/W Emulsions. Calcium chloride solution or oil containing actives were centrifuged at 10 000 rpm

for 3 min (Thermo Scientific, Germany) and no precipitate was formed, indicating complete dissolution of actives into the continuous phase.

The amount of actives (M_{act}) used for each emulsion was presented in Table 1.

Twenty grams of each emulsion was encapsulated in alginate membrane according to the methods presented in section Capsules Production. Wet or dry capsules containing hydrophilic actives were suspended in isotonic NaCl solution (0.85% w/v, pH 7.0) while capsules containing lipophilic actives were suspended in sunflower oil.

During the encapsulation process, alginate bath samples were evaluated by spectrometry and no variation in the absorbance was noticed. This indicated that the loss of actives during the encapsulation process was negligible and the maximal concentration of active released from wet (R_{wet}^{∞}) and dry (R_{dry}^{∞}) capsules was therefore calculated, respectively, using Equations (2) and (3):

$$R_{wet}^{\infty} = \frac{M_{act}\ ME_{emul}}{M_{emul}\ V} \qquad (2)$$

$$R_{dry}^{\infty} = \frac{M_{cap}\ \rho}{V} \qquad (3)$$

where M_{act} is the mass of active used to prepare the emulsion (mg), ME_{emul} is the mass of emulsion encapsulated (20 g), M_{emul} is the mass of emulsion prepared (g) containing the mass (M_{act}) of active (mg), V is the volume of NaCl solution or oil used for the dispersion of capsules (L), M_{cap} is the mass of dry capsules (10 g) and ρ is the mass of active divided by the mass of dry capsule (mg/g). The values of R^{∞} for wet and dry capsules were listed in Table 1.

The cumulative release of the actives (Cr) was calculated by Equation (4):

$$Cr = \frac{R_t}{R^\infty} * 100 \qquad (4)$$

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

The determination of the amount of active released was determined by spectrophotometry (UNICAM UV1 Spectrometer). Absorbance was measured for Congo Red and Sudan Red at 500 and 550 nm, respectively. BSA release was quantified using the Micro BCA™ Protein Assay Kit (Thermo Scientific, Rockford, IL) by measuring absorbance at 562 nm.

Release kinetics were made in triplicate at room temperature ($20 \pm 2\,^\circ$C) and was stopped when no more variation in the absorbance value ($\leq 1\%$) was observed.

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 values < 0.05 was assumed.

Results and discussion

The production of capsules by dripping-inverse gelation technique can be divided into four main steps:

1. Preparation of an emulsion containing calcium chloride.
2. Extrusion and dripping of the emulsion.
3. Penetration of emulsion drop into alginate solution.
4. Membrane formation.

Even if all experimental steps are intimately connected, the conceptual division in four steps will be respected in order to compare the methods of capsules production from W/O and O/W emulsions.

Preparation of an emulsion containing calcium chloride

W/O emulsion

For oil encapsulation by inverse gelation, emulsions are produced from oil, calcium chloride solution and surfactants. The hydrophilic-lipophilic balance (HLB) value of the surfactant is the main factor that determines the type of emulsion (W/O or O/W). As a general rule, surfactants with low HLB value are used to produce W/O emulsions while surfactants with high HLB value are used to stabilise O/W emulsions (ICI Americas Inc., 1980).

In this study, the stabilisation of W/O emulsions was guaranteed by the addition of PGPR90 surfactant. This surfactant exhibits low HLB value ($HLB_{PGPR90} = 1.5$) and can stabilise W/O emulsions even with high water content. Furthermore, the phase inversion of the emulsion was never observed.

More information about the PGPR90 structure and the chemical interaction with the emulsion components was previously detailed (Martins et al., 2017a).

W/O emulsion stability, i.e. time necessary to observe a phase separation, was checked depending on PGPR90 concentration. At low surfactant concentration ($\sim$0.08 g/l), the emulsion was very unstable leading to a high release of Ca^{2+} ions. By contrast, using high surfactant concentration ($\sim$0.8 g/l), the emulsion became very stable and Ca^{2+} was entrapped in the emulsion. Therefore, it was determined that PGPR90 concentration of 0.8 g/l was the best compromise between stability and Ca^{2+} release. The emulsion kept stable during approximately 50 min and phase separated after this time. The emulsion was therefore not able to be stored for further use, a disadvantage for the production of capsules at large scale.

By optical microscopy analyses, it was found that the emulsion was recovered by an oil film that hindered the release of $CaCl_2$ solution droplets (see Martins et al., 2017a).

In other words, the release of Ca^{2+} was restricted and needed higher $CaCl_2$ concentration (90 g/l of emulsion) to circumvent this drawback. Furthermore, the emulsion destabilisation was provoked by addition of W/O emulsion destabilisers (Tween 20 and ethanol) to improve Ca^{2+} release.

O/W emulsion

The O/W emulsion was produced according to the method proposed by Abang et al. (2012) where a combination of two surfactants with different HLB values (Tween 85 ($HLB_{Tween\,85} = 11$) and Span 85 ($HLB_{Span\,85} = 1.8$)) was used. To determine the proportion of each surfactant, the optimal HLB value ($HLB_{optimal}$) of the emulsion was calculated by Equation (5):

$$HLB_{optimal} = \Phi_{oil} * HLB_{oil} \qquad (5)$$

where Φ_{oil} is the oil fraction in the emulsion ($\Phi_{oil} = 0.77$) and HLB_{oil} the HLB value of the sunflower oil ($HLB_{oil} = 7.0$). Equation (5) gave, therefore, an optimal

HLB value of 5.4. From this value, to produce stable emulsions, the HLB value resulting from the mixing of surfactants (HLB_m) should be equivalent to $HLB_m = HBL_{optimal} \pm 1$ (Griffin, 1949; ICI Americas Inc., 1980). Based on this principle, Abang et al. (2012) produced an O/W emulsion from a 1:1 mixture of Tween 85 and Span 85 where the HLB_m was calculated from Equation (6):

$$HLB_m = W_{Tween85} * HLB_{Tween85} + W_{Span85} * HLB_{Span85} \tag{6}$$

where HLB and W represent the HLB and mass fraction of each surfactant, respectively (Sahin and Sumnu, 2006). An HLB_m value of 6.4 was thus obtained. This formulation could, therefore, form both O/W and W/O by catastrophic phase inversion depending on the stirring conditions (Bouchama et al., 2003). Slight variations in the parameters of production (oil/water ratio, amount and type of surfactants, stirring rate and stirring time) could, therefore, compromise the emulsion structure. During phase inversion, O/W emulsion (Figure 1(A)) is converted into W/O emulsion (Figure 1(B)) changing the emulsion properties (viscosity, surface tension, stability time) and Ca^{2+} release during encapsulation. Furthermore, the addition of hydrophilic or hydrophobic actives also promoted the inversion phase of emulsion (data not shown). In some cases, the addition of dyes (Fluorescein, Congo Red, Sudan Red, Methyl Orange and Crystal violet) resulted in the emulsion inversion. As a general rule, the actives should be added at low concentrations or, in the case of hydrophilic dyes, mixed to the emulsion after its preparation. In this last case, a higher concentration of active can be added without disrupting the emulsion structure.

This drawback should be considered for productions at large scale where the phase inversion can occur by emulsion breaking/fragmentation within the pipes, by thermal destabilisation or by ingredients addition to the emulsion (Züge et al., 2013).

When the formulation is adequately performed, O/W emulsion is formed where oil droplets are surrounded by a thin layer of the $CaCl_2$ solution and display high stability time (>15 days), which allows the storage under refrigerated conditions (Figure 1(A)).

As $CaCl_2$ solution represents the dispersed phase of the O/W emulsion, Ca^{2+} is released more efficiently. It means that lesser amount of $CaCl_2$ (concentration of 6.9 g/l in the emulsion) is necessary to produce the capsules and emulsion destabilisers are not necessary.

Extrusion and dripping of the emulsion

After preparation, the emulsions were placed into a syringe and extruded through a tip to form drops. During the extrusion process, the stability of emulsions was a key factor to guarantee the reproducibility of the production of capsules. As a general rule, less stable emulsions are more easily prone to phase separation (oil and $CaCl_2$ phase separation) during the extrusion process.

As previously discussed, the W/O emulsions showed lower stability and should be quickly extruded through tips with a large internal diameter (2 mm) to avoid phase separation. Using tips with smaller internal diameter resulted in the phase separation of W/O emulsion due to high shear forces during the extrusion.

On the other hand, O/W emulsions were very stable and extruded more easily through tips with smaller
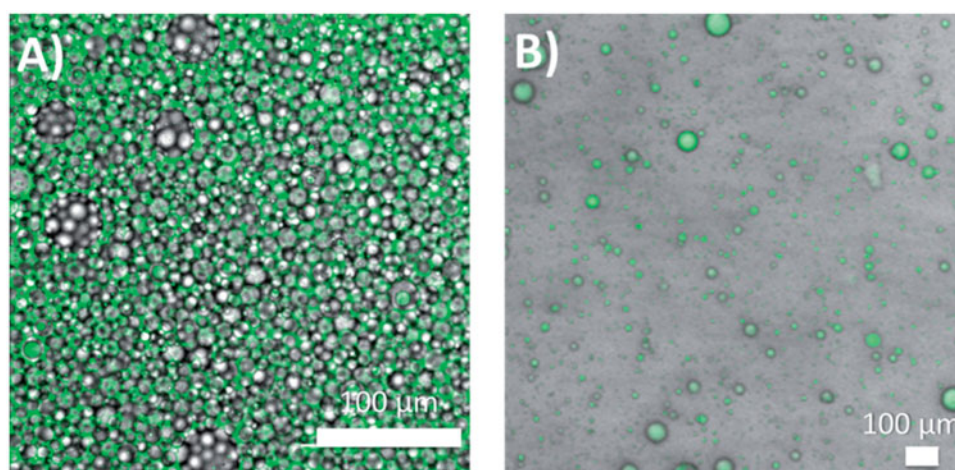

Figure 1. Confocal microscopy images of emulsions produced with sunflower oil (100 ml), $CaCl_2$ solution (30 ml), Span 85 (470 mg), Tween 85 (570 mg) and fluorescein (10 mg). (A) O/W emulsion prepared using 13 500 rpm of stirring rate. (B) W/O emulsion produced from phase inversion. Green: $CaCl_2$ solution; grey: oil.

diameters (0.38 mm) (Abang et al., 2012). In this case, the drop size could be slightly controlled by tuning tip internal diameter.

Penetration of emulsion drop into alginate solution

For W/O and O/W emulsions, similar problems of drop penetration were observed. Alginate solutions displayed high viscosity (85–260 mPa.s) and surface tension (55 mN/m) values hindering the penetration of the emulsion drops in the alginate bath. In the previous article, it was shown that a compromise of alginate concentration of 10 g/l was the better choice in order to limit the effect of viscosity on the emulsion drop penetration (Martins et al., 2017a).

The surface tension of the alginate solution is associated with the emulsion drop deformation during its penetration into the bath. To reduce the surface tension, Tween 20 was added, decreasing the surface tension of alginate bath from 55 to 33 mN/m. By tuning the physical conditions of alginate bath (viscosity, surface tension), the production of spherical capsules was possible.

Membrane formation

After penetration into the alginate bath, emulsions drops releases Ca^{2+} resulting in the cross-linking of the surrounded alginate molecules and further membrane formation. Considering that W/O and O/W emulsions showed different structures and mechanisms to release Ca^{2+}, the main factors associated to the membrane formation will be discussed in more details in the following sections.

Membrane formation using W/O emulsions

It was demonstrated that the membrane thickness was not dependent on the calcium chloride concentration ($CaCl_2$) in the emulsion. However, a minimal $CaCl_2$ concentration of 90 g/l was necessary to produce spherical capsules (Figure 2(A)). Furthermore, the membrane thickness did not depend on the curing time (time of contact between the emulsion drop and the alginate bath) (Figure 2(B)) and capsules achieved maximal membrane thickness in less than 5 min, allowing a fast production.

In a complementary experiment, W/O emulsion was added drop wise into alginate solution and, after 5 min of curing time, the capsules were transferred into the alginate bath during 5 min containing titanium dioxide at 1% w/v. The transfer of capsules (from alginate bath to alginate/TiO$_2$ bath and visa-versa) was repeated five times corresponding to 25 min of curing time. By cutting-half the capsules, it was possible to observe a colourless membrane formed only by alginate without titanium dioxide (Figure 3(A)). This result reinforced the idea that, using W/O emulsion, Ca^{2+} was quickly released in one step just after contact of the emulsion drop with the alginate bath. In other words, the membrane was fully formed in a very short curing time, as pointed out by a recent study that determined the membrane formation in few seconds after contact of W/O emulsion with alginate solution (Martins et al., 2017b).

Based on the results presented in Figure 2(A,B) and some reports found in the literature, a mechanism of membrane formation was suggested. The emulsion drop (just after leaving the tip) (Figure 4(A), step I) was made of $CaCl_2$ solution droplets dispersed in the oil phase acting as a membrane that hinders the contact of Ca^{2+} with the alginate solution. To break this barrier, emulsion destabilisers as Tween 20 and ethanol were added to the alginate bath.

The mechanism of action of destabilisers of W/O emulsion was not completely elucidated, but it is known that a synergistic effect of Tween 20 and ethanol exists (Martins et al., 2017c). Some authors suggested that the emulsion destabilisation by ethanol was caused by an increase in osmotic pressure between the emulsion and the alginate bath (Cárdenas and Castro, 2003). Another hypothesis suggested that the penetration of Tween 20 in the W/O emulsion was improved by ethanol facilitating the destabilisation of the PGPR90 film that covered the $CaCl_2$ solution droplets within the emulsion (Martins et al., 2017c).

Due to the action of destabilisers, $CaCl_2$ droplets close to the oil/alginate interface left the emulsion (Figure 4(A), step II) and alginate macromolecules were instantaneously cross-linked leading to the membrane formation (Figure 4(A), step III).

Membrane formation using O/W emulsions

By using O/W emulsions, the membrane thickness increased with both the increase of $CaCl_2$ concentration in the emulsion and the curing time (Figure 2(C,D)). Depending on the $CaCl_2$ concentration, maximal membrane thickness was achieved after 20–30 min of curing time. To demonstrate the evolution of membrane, O/W emulsion was added drop wise into alginate bath. After 5 min of curing time, the capsule was transferred into an alginate/TiO$_2$ bath. The process of transfer was repeated five times
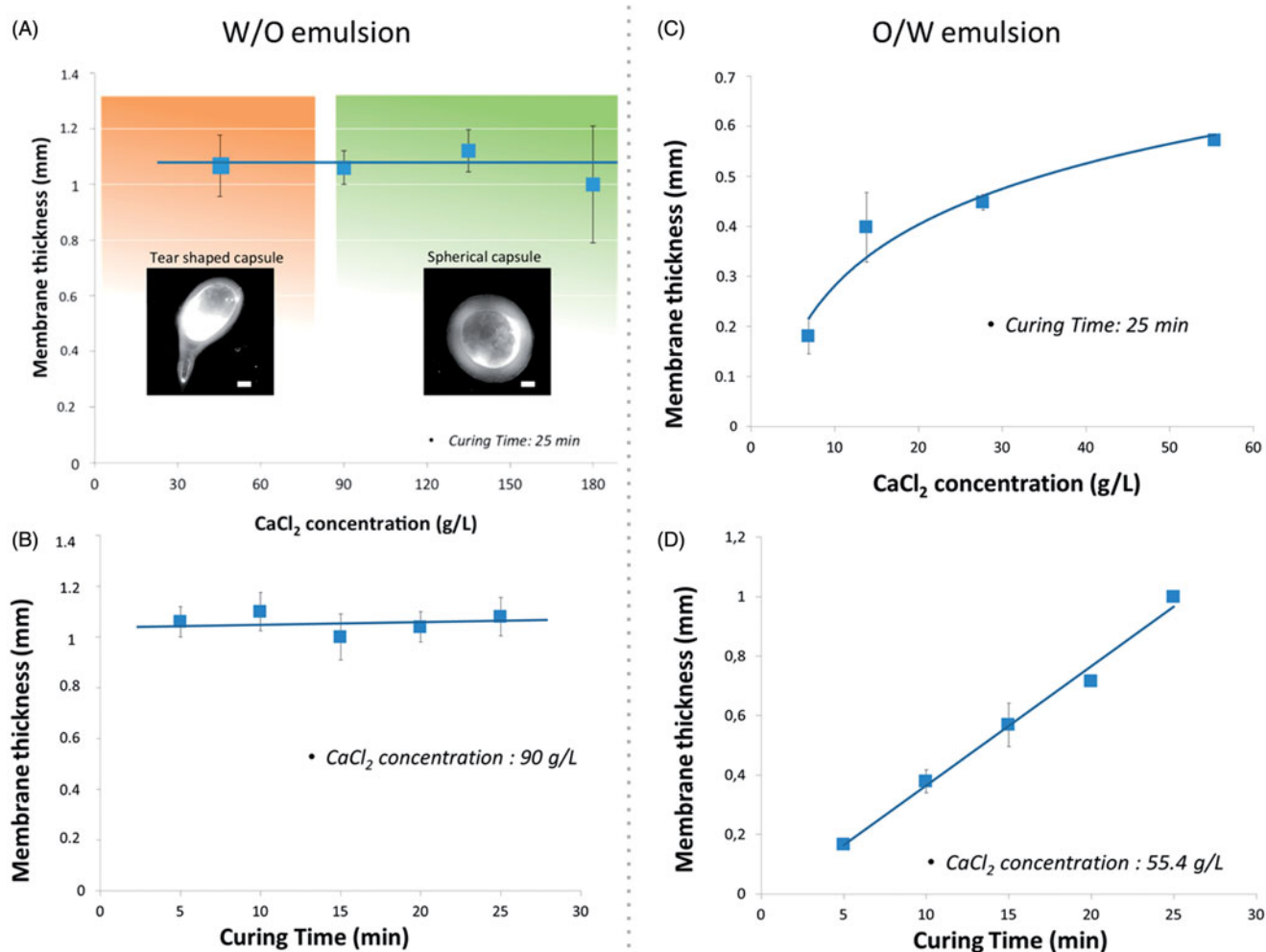
Figure 2. Effect of the CaCl$_2$ concentration and curing time on the membrane thickness of capsules produced using W/O emulsion (A and B) or O/W emulsion (C and D). (A) The membrane thickness was not dependent on the CaCl$_2$ concentration in the emulsion. A minimal CaCl$_2$ concentration of 90 g/l was necessary to produce spherical capsules. (B) The membrane thickness did not depend on the curing time. (C,D) The membrane thickness increased with both the increase of CaCl$_2$ concentration in the emulsion and the curing time. All measurements were assayed in triplicate corresponding to a total of 60 capsules measured by each CaCl$_2$ concentration or curing time evaluated. Scale bar: 1 mm.

corresponding to 25 min of curing time. By cutting-half the capsules, it was possible to observe successive colourless shells and TiO$_2$ pigmented shells in the membrane (Figure 3(B)). This simple experiment demonstrated that the Ca^{2+} release occurred in time in the O/W emulsions and that the membrane was consequently gradually built.

The use of O/W emulsion allowed the production of spherical capsules using smaller concentrations of CaCl$_2$ in the emulsion (Figure 2(C)). In addition, the progressive release of calcium ions allowed the determination of the membrane thickness by controlling the curing time. Similar observations were also found in previous works carried out in our laboratory by Abang et al. (2012) and Martins et al. (2015).

Based on our previous results (Abang et al., 2012; Martins et al., 2015), some empirical observations and literature reports, a hypothetical mechanism of capsules formation by inverse gelation was discussed. In O/W emulsions, the emulsion drop had a hydrophilic surface charged in calcium ions (Figure 4(B), step I). Immediately after its contact with the alginate bath, calcium ions diffused outside the emulsion (Figure 4(B), step II) and cross-linked the alginate macromolecules (Figure 4(B), step III). A thin membrane ($Mt \sim 0.2$ mm) was formed preventing the emulsion drop fragmentation. By increasing the curing time, more calcium ions diffused outside the emulsion cross-linking additional alginate macromolecules (Figure 4(B), step IV). Membrane thickness was thus
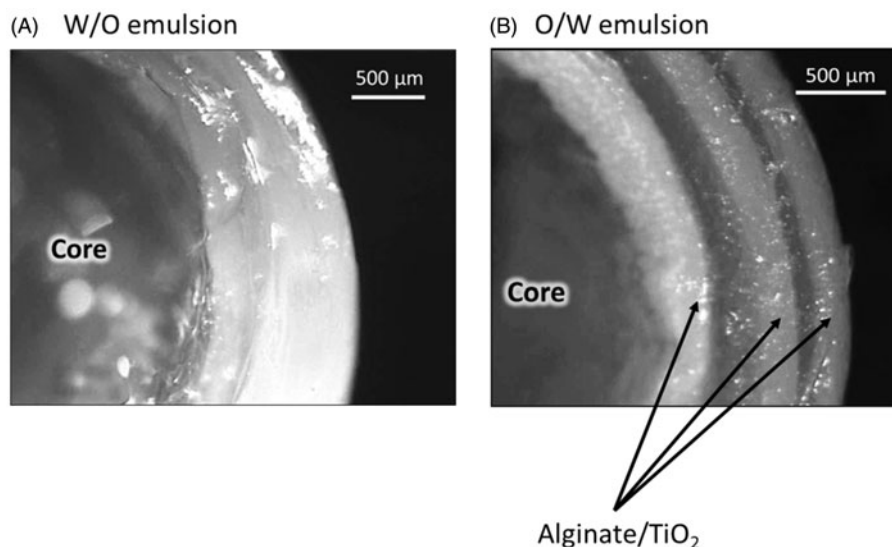
Figure 3. Cross-section of the alginate membrane in capsules produced from (A) W/O emulsion and (B) O/W emulsion. The emulsions were drop wise into alginate bath and after 5 min of curing time and the capsules were transferred into the alginate bath containing titanium dioxide at 1% w/v. The transfer of capsules (from alginate bath to alginate/TiO$_2$ bath and vice-versa) was repeated five times (25 min of curing time). The presence of alginate/TiO$_2$ layers due to the gradual release of Ca^{2+} from the emulsion core was only possible to be observed in capsules produced from O/W emulsion.
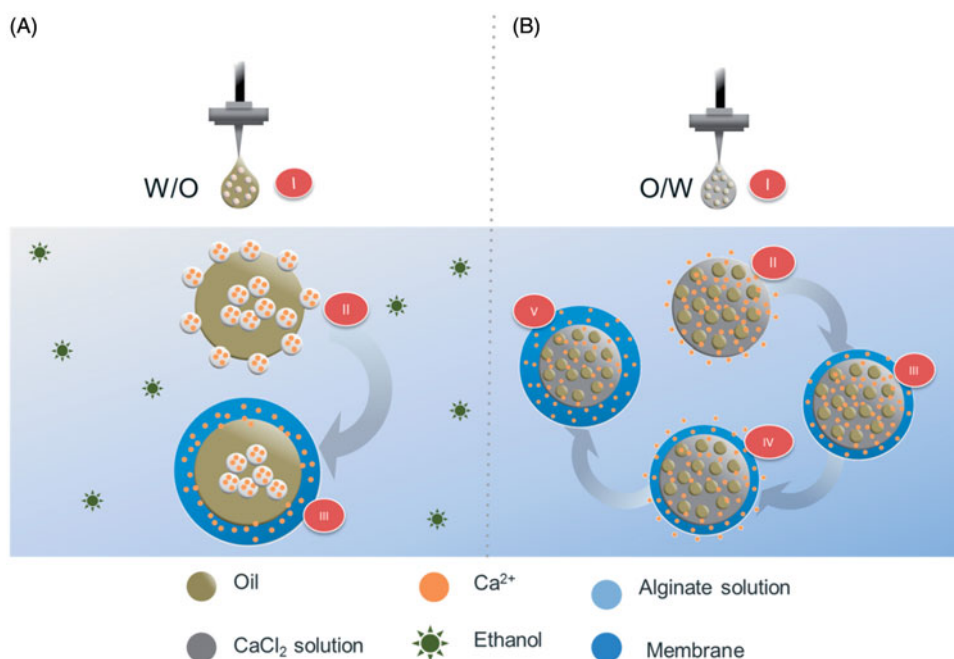

Figure 4. Mechanism of capsule formation by inverse gelation using (A) W/O emulsion or (B) O/W emulsion. (A) The emulsion drop (step I) was made of CaCl$_2$ solution droplets dispersed in the oil phase acting as a membrane that hinders the contact of Ca^{2+} with the alginate solution. To break this barrier, emulsion destabilisers as Tween 20 and ethanol were added to the alginate bath. The CaCl$_2$ droplets close to the oil/alginate interface left the emulsion (step II) and alginate macromolecules were instantaneously cross-linked leading to the membrane formation (step III). (B) The emulsion drop had a hydrophilic surface charged in calcium ions (step I) that diffused outside the emulsion (step II) and cross-linked the alginate macromolecules (step III). By increasing the curing time, more calcium ions diffused outside the emulsion cross-linking additional alginate macromolecules (step IV). When the calcium content in the O/W emulsion was depleted, the membrane formation stopped (step V).

increased through adsorption of Ca^{2+}-alginate complexes at the surface of the O/W emulsion drops (Fu et al., 2014). When the calcium content in the O/W emulsion was depleted, the membrane formation stopped (Figure 4(B), step V).

Capsules characteristics

By comparing the capsule production with both emulsions, it was easier to produce capsules using O/W emulsions due to the higher stability of the emulsion, the easier extrusion and the absence of emulsion destabiliser (ethanol) in the alginate bath.

Although differences existed in the method and in the mechanism of membrane formation, wet and dry capsules produced from W/O and O/W emulsions showed similar diameters and membrane thicknesses

Table 2. Characteristics of wet and dry capsules produced from W/O and O/W emulsions.

Capsules characteristics	W/O emulsion	O/W emulsion
Wet		
Diameter (mm)	7.1 ± 0.3	6.8 ± 0.4
Membrane thickness (mm)	1.1 ± 0.1	1.0 ± 0.1
Oil loading (oil volume in the core/capsule volume)	23%	27%
Diameter (mm)	3.6 ± 0.1	3.2 ± 0.1
Dry		
Membrane thickness (μm)	220 ± 60	53 ± 12
Oil loading (oil volume in the core/capsule volume)	68%	90%

Note: All measurements were assayed in triplicate corresponding to a total of 60 capsules.

(Table 2). Furthermore, the capsules were monodispersed in size with diameter variation smaller than 6%. However, wet capsules produced from W/O emulsions contained a slightly lower percentage of oil, due to the higher aqueous fraction in the emulsions (Table 2).

Nonetheless, dry capsules based on W/O emulsion had a membrane thickness approximately four-fold higher than dry capsules based on O/W emulsions (Table 2; Figure 5). This result would suggest that the membrane produced in presence of ethanol had more alginate macromolecules in its structure.

Although dry capsules produced with both emulsions displayed similar diameters, capsules based on W/O emulsions had a lower oil loading, in agreement with the presence of a thicker membrane (Table 2).

To summarise, capsules produced from both emulsions showed very similar characteristics except for the membrane thickness and oil loading in the case of dry capsules. To determine how the emulsion type affected the capsule properties, the capsules were evaluated in terms of mechanical resistance and release profile of actives.

Mechanical properties of capsules

Capsules produced from emulsions and hydrogel alginate beads were submitted to compression tests. Typical profiles, where compression pressure σ was plotted against deformation ε, were shown in Figure 6.
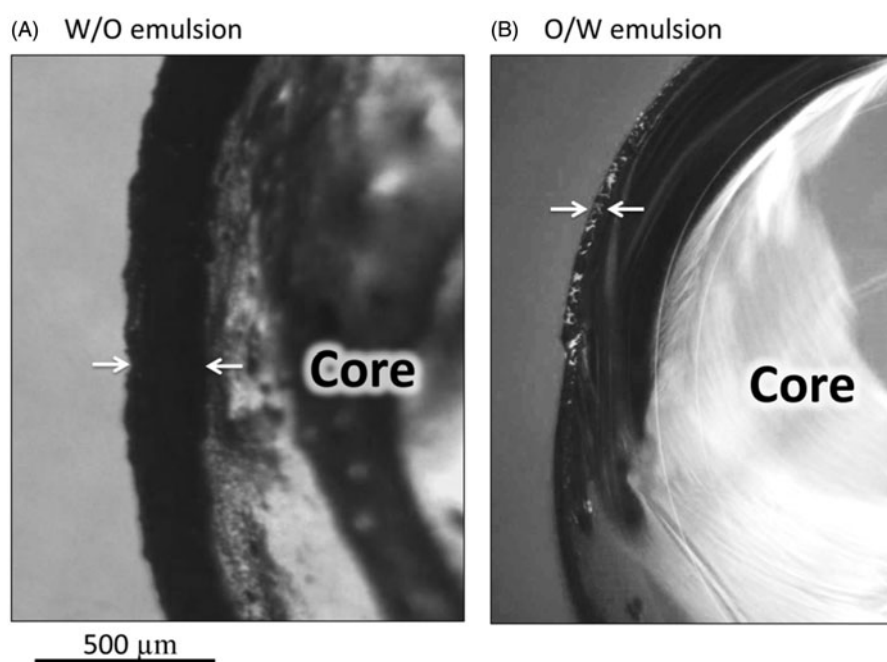

Figure 5. Optical microscopy images of a dry capsule produced from (A) W/O emulsion and (B) O/W emulsion. Double arrows: membrane thickness.
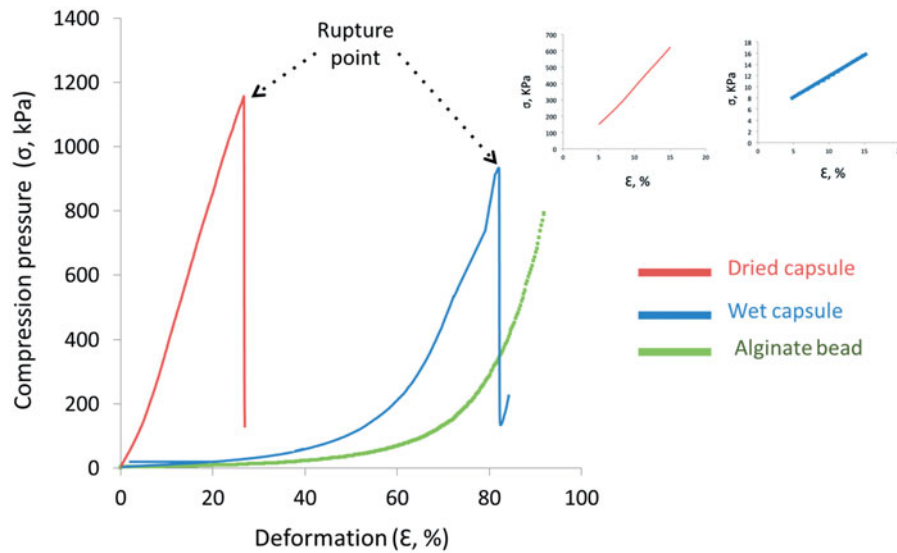
Figure 6. Compression profiles, compression pressure σ vs. deformation ε, of capsules produced by inverse gelation and of alginate bead. The capsules were deformed with the increase of the compression pressure applied until the rupture where a maximal deformation (ε_{max}) was reached. By the contrary, rupture of alginate bead was not observed. Wet capsules displayed a compression profile intermediate between an alginate bead and a dry capsule. Measurements were made on five capsules from three different batches corresponding to a total of 15 capsules.

Table 3. Mechanical properties of wet and dry capsules produced with W/O emulsion or O/W emulsion.

	Wet capsules			Dry capsules		
	Rupture force (N)	ε_{max}%	$E_{membrane}$ (kPa)	Rupture force (N)	ε_{max}%	$E_{capsule}$ (kPa)
Emulsion						
W/O	7.6 ± 0.1^a	84.0 ± 0.3^c	0.13 ± 0.01^e	10.6 ± 1.2^f	27.1 ± 2.8^h	54.1 ± 4.3^j
O/W	21.3 ± 2.4^b	86.7 ± 0.7^d	0.13 ± 0.03^e	37.8 ± 3.6^g	46.0 ± 3.4^i	37.9 ± 2.2^k

Note: Different letters in the same column indicate significant differences with $p < 0.05$.
Measurements were made on five capsules with a diameter of 7.0 ± 0.1 mm from three different batches corresponding to a total of 15 capsules.

The capsules were gradually deformed with the increase of the compression pressure applied until the rupture where a maximal deformation (ε_{max}) was reached (Figure 6, Table 3). By the contrary, rupture of alginate bead was not observed (Figure 6).

Wet capsules displayed a compression profile intermediate between an alginate bead and a dry capsule. For deformations between 0 and 20%, wet capsules similarly behave like the alginate bead (Figure 6). By contrast, for higher deformations, the wet capsules behave like a dry capsule with however, a higher maximal deformation and smaller rupture force (Figure 6, Table 3). From these observations, it was concluded that for weak deformations (0–20%) only the membrane was compressed in the wet capsules. Therefore, the elastic modulus of the membrane of the wet capsules ($E_{membrane}$) could be calculated.

For wet and dry capsules submitted to weak deformations (5–15%), the compression pressure linearly increased with the deformation (insets, Figure 6). For high deformations (>20%), a nonlinear relation of compression pressure versus deformation was observed. According to Degen et al. (2015), for high deformations, the inner fluid (i.e. the core of the capsule) would leak through the alginate pores and would compromise the linearity of the compression pressure versus deformation curve.

The elastic modulus of the dry capsule, $E_{capsule}$, or of the membrane in the case of the wet capsule, $E_{membrane}$, was calculated according to the Hooke's law for deformations between 5 and 15%:

$$E = \frac{\sigma}{\varepsilon} \qquad (7)$$

where σ is the compression pressure (kPa) and ε the deformation (%) applied to the capsule.

Several models of calculations of the Young or elastic modulus of the membrane can be found in the literature (Rachik et al., 2006; Rolland, 2013). However, most of the models were only applied on capsules where the ratio of the membrane thickness/capsule radius was lower than 0.05. These models, however, did not apply to the wet capsules produced by inverse
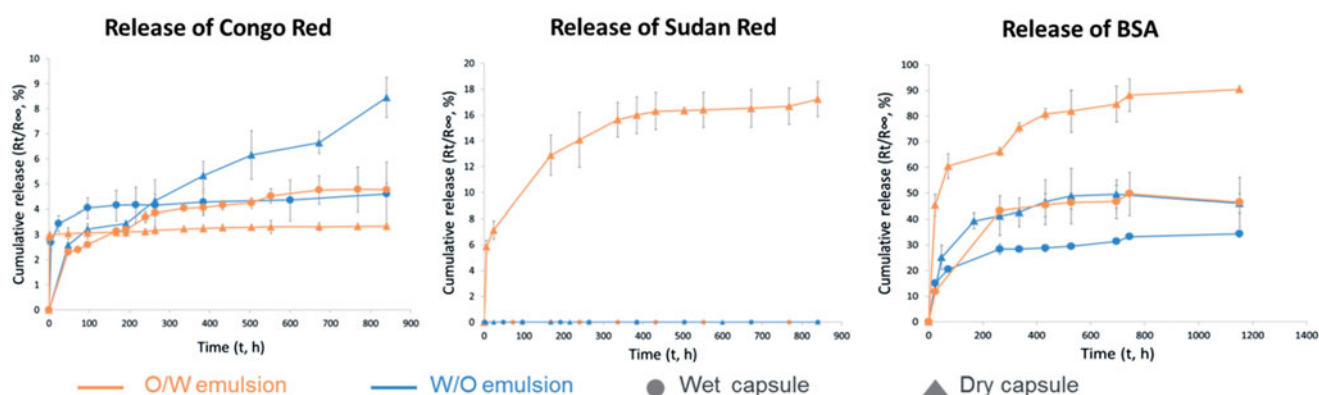
Figure 7. The cumulative release profile of actives from capsules produced from W/O emulsions (blue lines) or O/W emulsions (orange lines). Release profiles from wet capsules (●) and dry capsules (▲). For Congo red, a release lower than 10% was observed for all capsules tested after ~36 days. For the Sudan red, no release was observed on wet capsules, but a low and slow dye release (17% after 21 days) was only observed on dry capsules produced with O/W emulsions. For BSA, capsules produced with W/O emulsions released >34% of active after 46 days. Higher BSA release was achieved on the capsules based on O/W emulsions with 46% for wet capsules and 90% for dry capsules. Release kinetics were made in triplicate at room temperature.

gelation where the ratio was of 0.3; consequently, the Hooke's law was applied in this study to determine both the elastic modulus of the membrane in wet capsule and the elastic modulus of the dry capsule for small deformations ($\varepsilon_{max} = 0.15$).

Table 3 summarised the mechanical properties of capsules produced with both emulsions.

Wet capsules produced from W/O emulsions showed both a lower rupture force and maximal deformation compare to wet capsules produced with O/W emulsions (Table 3). On the other hand, the elastic modulus of the membrane, $E_{membrane}$, was similar in both cases, in agreement with the similar membrane thickness (Table 3).

The elastic modulus of the membrane in wet capsules produced by inverse gelation was similar to those obtained from alginate bead, indicating that the membrane could be ascribed to a hydrogel made of alginate.

However, according to literature, the Young modulus of alginate gel varied between 0.18 and 100 kPa depending on the alginate type, molecular weight, mannuronic/guluronic acid ratio, concentration and cross-linking density (West et al., 2007; Banerjee et al., 2009; Rolland, 2013). In addition, it was demonstrated that $E_{membrane}$ increased non-linearly with the membrane thickness (Rachik et al., 2006).

After drying, the capsules displayed a higher rupture force and a lower maximal deformation, in agreement with the higher rigidity of the capsules (Table 3).

In addition, dry capsules produced from W/O emulsions displayed higher rupture force and maximal deformation values compare to capsules based on O/W emulsions (Table 3). By contrast, capsules made from W/O emulsion had an elastic modulus 1.5-fold

higher (Table 3). The difference of $E_{capsule}$ value obtained between capsules from O/W and W/O emulsions could be explained by the thicker membrane in the dry capsules produced from W/O emulsion (Table 2) and a higher density of crosslinks in the membrane induced by the presence of ethanol. These results were in agreement with those of Li et al. (2015) who demonstrated that films of alginate gels produced with $CaCl_2$ solution and ethanol were more resistant to the mechanical compression in comparison with films produced with a $CaCl_2$ solution only.

To conclude, wet capsules produced using O/W and W/O emulsions showed a similar elastic modulus value (0.13 kPa), in agreement with the similar membrane thickness; in addition, dry capsules produced from W/O emulsion were more resistant to mechanical compression.

Release profile of actives

Three actives were chosen to study their release from wet and dry capsules: a hydrophilic dye (Congo Red) and a protein (BSA) and a lipophilic dye (Sudan Red).

Congo Red is a secondary diazo dye used as a pH indicator (Steensma, 2001; Sawhney and Kumar, 2011). The pH of the samples was therefore constantly monitored during the experiment to avoid any colour variation. In addition, pH has a direct effect on the release kinetics, which can compromise interpretation of results (Polk et al., 1994; Bosio et al., 2014).

For Congo Red, a release lower than 10% was observed for all capsules tested after ~36 days (Figure 7). The weak release of the hydrophilic dye would indicate that most of active interacted with the core components or with the membrane. This hypothesis was

supported by other studies suggesting that Congo Red can interact electrostatically with alginate molecules or can associate with calcium ions into Ca^{2+}-alginate complexes (Radha et al., 2005; Paques et al., 2013; Bosio et al., 2014). Although several works indicated a possible interaction between Congo Red and alginate molecules, the mechanism of this interaction remains unknown.

For the lipophilic Sudan Red, no release was observed on wet capsules (Figure 7), the diffusion through the hydrophilic membrane being inhibited whatever the type of emulsion.

A low and slow dye release (17% after 21 days) was observed on dry capsules produced with O/W emulsions (Figure 7). The absence of release of Sudan Red from dry capsules produced with W/O emulsions indicated that the membrane acted as an impermeable barrier. The thicker membrane (200 µm) of capsules made from W/O emulsions could be responsible for the absence of release of Sudan Red dye with time.

For BSA, a higher release was found compared to the hydrophilic Congo Red dye (Figure 7). For capsules produced with W/O emulsions, 34 and 46% of active was released after 46 days, respectively, from wet and dry capsules (Figure 7). On the other hand, higher BSA release after 46 days was achieved on the capsules based on O/W emulsions with 46% for wet capsules and 90% for dry capsules (Figure 7).

In all cases, however, the actives were not completely released after 46 days indicating that part of them was entrapped in the core or in the membrane, interacted with alginate, or even were adsorbed at the aqueous-oil interface of droplets.

Except in the particular case of Congo Red interacting with the alginate matrix, actives were more readily released from dry capsules (Figure 7). This observation was justified by the fact that dry capsules contained thinner membranes. The actives, therefore, diffused on smaller distances to achieve the external media. In addition, during drying of the capsules, it could be possible that structural variations occurred in the alginate matrix leading to changes in pore diameters of the membrane. Further experiments, and in particular scanning electron microscopy (SEM) or better cryo-SEM, to probe the structure of the membrane after drying, should improve the understanding of the controlled release of lipophilic and hydrophilic actives in capsules produced from inverse gelation method.

For the Sudan Red and BSA release, the actives were more rapidly released from capsules produced with O/W emulsions (Figure 7). During the experiments, it was verified that, contrary to dry capsules produced from W/O emulsion, dry capsules produced from O/W emulsion increased their diameters of near 1 mm over 35 days of assays. The swelling of capsules in time could have facilitated the active release due to an increase in pores size of the alginate matrix. In the case of capsules from W/O emulsions, the ethanol used in capsules production can generate a more rigid structure of alginate gel, which could reduce the swelling of alginate matrix, as suggested by Li et al. (2015).

Conclusion

The method of oil encapsulation by inverse gelation was easier to perform using O/W emulsions. This type of emulsion displayed a high stability allowing its storage in time and a higher flexibility during the capsule production. It was found that the calcium ions release from this type of emulsion was more efficient, requiring lower $CaCl_2$ concentrations and did not necessitate the use of emulsion destabilisers (ethanol). On the other hand, capsules produced from W/O emulsions were more resistant to the compression and showed a slower active release, interesting property for the encapsulation of volatile compounds.

The findings detailed in this study widened the knowledge of the inverse gelation mechanism using W/O or O/W emulsions and gave opportunities to develop new techniques of oil encapsulation.

This work allowed the choice of the method of encapsulation based not only on practical aspects but also on the mechanical and controlled release properties of the capsules.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Oil encapsulation in core–shell alginate capsules by inverse gelation II: comparison between dripping techniques using W/O or O/W emulsions

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