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RESEARCH ARTICLE

Oil encapsulation in core–shell alginate capsules by inverse gelation. I: dripping methodology

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ABSTRACT

The production of capsules by inverse gelation consists of adding dropwise oil containing calcium dispersion into an alginate bath. A dripping technique to produce capsules from oil-in-water (O/W) emulsions was proposed by Abang. However, little is known about the oil encapsulation using water-in-oil (W/O) emulsions. This work aims to develop a new method of W/O emulsions encapsulation by inverse gelation. The success of the W/O emulsion encapsulation is due to three factors: 1) use of an emulsion with moderate stability (50 min); 2) production of an emulsion with at least 90 g/L of $CaCl_2$ and 3) addition of ethanol (20% v/v) into the alginate bath. Both wet and dry capsules were obtained with a spherical shape with diameters of 7 and 3.6 mm, respectively. All volume of oil was encapsulated and the oil loading in the wet and dry capsules was of 23 and 68% v/v, respectively.

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Alginate; inverse gelation; encapsulation; oil; surfactants

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 (Goula and Adamopoulos, 2012; Sauvant et al., 2012; Soliman et al., 2013).

An efficient strategy to decrease the sensitivity of oils towards environmental conditions consists of its encapsulation in inert polymer matrix using gelation/emulsification technology (Risch and Reineccius, 1988; Jin et al., 2009; Ziani et al., 2012). Some methodologies of oil encapsulation in millimetric alginate capsules have been described in the literature based on external gelation mechanism (Bremond et al., 2010; Whelehan and Marison, 2011, Zhang et al., 2016; Piornos et al., 2017).

For oil encapsulation using extrusion–dripping by external gelation, oil is dispersed into alginate solution and added dropwise into a calcium chloride bath (Zhang et al., 2016; Davarci et al., 2017) (Figure 1(A)). The calcium ions (Ca^{2+}) diffuse from the bath to the alginate drops leading to hydrogel beads (Figure 1(A)). As a result, multi-oil core capsules are produced with oil loading lower than 25% w/w (Soliman et al., 2013). Dried capsules hardly displayed oil loading higher than 65% w/w due to the easy loss of oil through the porous structure of the capsules (Peniche et al., 2004).

In order to increase oil loading in the capsules, an alternative method consists in the use of a coextrusion–dripping device. In this technique, oil and alginate solution are extruded simultaneously through two concentric nozzles and added dropwise into a calcium chloride bath (Whelehan and Marison, 2011) (Figure 1(B)). In this case, core–shell capsules containing substantial oil loading (~43% w/w) were obtained (Hoad et al., 2011).

Another alternative method for oil encapsulation resulting in capsules with high oil loading was proposed in our laboratory based on the inverse gelation mechanism (Andersen et al., 2005; Abang, 2011; Abang et al., 2012). An emulsion of oil and $CaCl_2$ solution is extruded dropwise into an alginate bath (Figure 1(C)). Upon contact, Ca^{2+} ions diffuse to the outer periphery of the droplet and ionotropically cross-link with the alginate polymer chains at the droplet interface. The ionotropic gelation process continues until the free Ca^{2+} ions are depleted. 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. In the dry form, capsules contain more than 95% w/w of oil (Abang, 2011; Abang et al., 2012).

Although production of single core capsules by inverse gelation is easier to perform by the coextrusion–dripping method, some parameters need to be adjusted to guarantee an ideal production (Zukas and Gupta, 2016). As an example, the high viscosity of alginate solution can impede the penetration of the emulsion drops into the bath preventing the formation of capsules or inducing non spherical capsules (Martins et al., 2015; Zukas and Gupta, 2016).

To overcome this drawback, alginates with lower viscosity and the addition of surfactant into polymer solution are recommended to facilitate the penetration of the emulsion drops (Abang, 2011; Davarci et al., 2017). It was also reported that spherical capsules are only obtained when an ideal distance between the dropping tips and the alginate solution surface (80–100 mm) is respected (Abang, 2011). For higher distances, the emulsion drops are deformed on impact whereas, below 80 mm, the drops did not have sufficient kinetic energy to penetrate the alginate solution surface (Martins et al., 2015; Davarci et al., 2017).

All reagents used to produce capsules by inverse gelation are food-grade approved. The core of the capsules can contain both hydrophilic and hydrophobic actives, which is an advantage compared to other methods of oil encapsulation. However, the incorporation of actives into emulsion should be carefully executed to not interfere with its stability. In a previous work, it was verified that emulsions produced with PGPR 90 had their stability little

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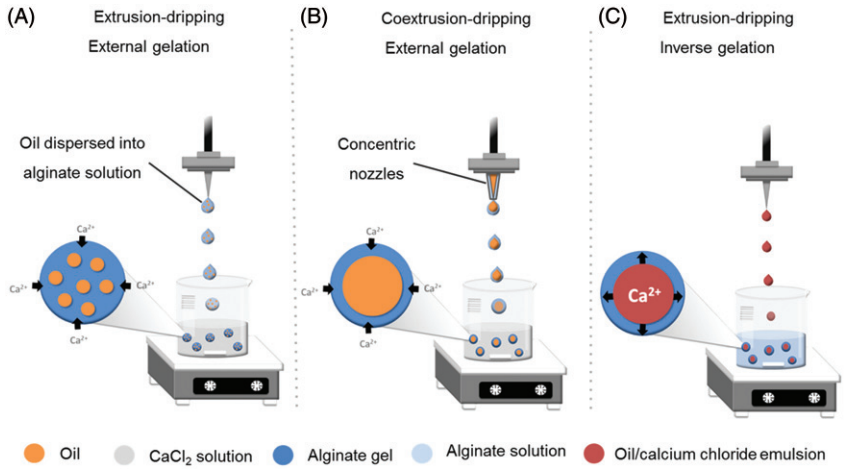


Figure 1. Oil encapsulation by extrusion–dripping techniques. Black arrows: direction of migration of calcium ions (Ca^{2+}).

affected by incorporation of proteins, hydrophobic (Sudan red) or hydrophilic (Congo red) dyes (Martins, 2015). By the contrary, emulsions formulated with Span 85 and/or Tween 85 were prone to destabilisation in presence of the same actives.

Millimetric capsules produced by inverse gelation can therefore find applications in food, home care products, agriculture and cosmetics areas as fragrance diffusers, decorative pearls or for crops protection (i.e. pesticides, herbicides) (Liu et al., 2005; Mezzenga, 2007; Fu et al., 2014). However, only the encapsulation of volatile compounds and enzymes by this method was demonstrated (Abang, 2011; Lopez et al., 2012).

In the work proposed by Abang (2011), the emulsion used in the capsules production was assumed to be a water-in-oil emulsion (W/O emulsion). However, recent investigations revealed that the emulsion was, in fact, an O/W emulsion (Martins et al., 2015).

From literature, production of O/W emulsion based capsules was therefore successful and well documented; nevertheless, no report in the literature describes how W/O emulsions can be used in oil encapsulation processes using inverse gelation.

Considering the lack of information in this area, the objective of this work is to develop and describe step by step a methodology of capsules production from W/O emulsions.

Materials and methods

Materials

Sodium alginate powder Satialginate S 60 NS (Cargill, France) was used to prepare the alginate bath. The ratio of mannuronic (M) to guluronic (G) acid units (M/G = 1.37) and the molar mass of alginate ($M_w = 1.57 \times 10^5$ g/mol) were determined, respectively, by ^{1}H NMR spectroscopy and HPSEC–MALLS (High-Performance Size Exclusion Chromatography–Multi-Angle Laser Light Scattering). Calcium chloride powder ($CaCl_2$. $2H_2O$) (Pancreac Quimica, Spain), sunflower cooking oil (Associated Oil Packers, France), sorbitan trioleate or Span 85 (Sigma Aldrich, France) and polyglycerol polyricinoleate or PGPR 90 (Danisco, France) were used to prepare W/O emulsions.

Preparation of alginate and $CaCl_2$ solutions

Alginate powder was dissolved in demineralised water to get final concentrations of 0.5–2% w/v. 1% v/v of Tween 20 and 0–20% v/v of ethanol were then added and stirred at 300 rpm until complete mixture in the alginate solution.

The calcium chloride solutions were prepared by dissolving $CaCl_2.2H_2O$ powder in demineralised water to get final concentrations varying between 7.5 and 59.8% w/v.

Preparation of W/O emulsions

Span 85 or PGPR90 (0.01 to 1 g), sunflower oil (100 mL) and calcium chloride solution (23–43 mL at 14–59.8% w/v) were dispersed using a high shear mixer (Ultra-Turrax T25, IKA, Germany) at 13,500 rpm during 4 min. The choice of the surfactants was based on the hydrophobic lipophilic balance (HLB) values ($HLB_{SPAN85} = 1.8$; $HLB_{PGPR\ 90} = 1.5$). Surfactants with low HLB values are prone to stabilise W/O emulsions formulations (Al-Sabagh, 2002).

Capsules production

The W/O emulsions were pumped at a flow rate of 30 mL/h using a syringe pump (Harvard Apparatus PHD 2000, France) and extruded through a tapered tip (2 mm of internal diameter). The distance between the end of the tip and the surface of alginate bath was of 10 cm.

Emulsions drops (approximately 5 mm in diameter) were added dropwise into alginate solution. The alginate bath (400 mL) was placed in a beaker of 600 mL (Ø = 95 mm; h = 120 mm) stirred at 300 rpm during 5–25 min with a 80 mm long wedge-shaped magnetic bar.

The wet capsules were sieved, rinsed with demineralised water and stored in calcium chloride solution at 15 g/L (Degen et al., 2015). Dry capsules were obtained by absorption of the water excess using a paper towel followed by air-drying (20 ± 2 °C) during two days.

Characterisation of the alginate solutions

Viscosity

The dynamic viscosities of the alginate solutions were determined using a HAAKE 550 rotational viscometer (Thermo Electro Inc., USA) at a constant temperature of 25 °C. The viscosity was determined at a shear rate of 10 s^{-1}. Measurements were carried out in triplicate.

Surface tension

The surface tension of the alginate solutions was performed with a K12 tensiometer (Krüss GmbH, Germany) using the platinum

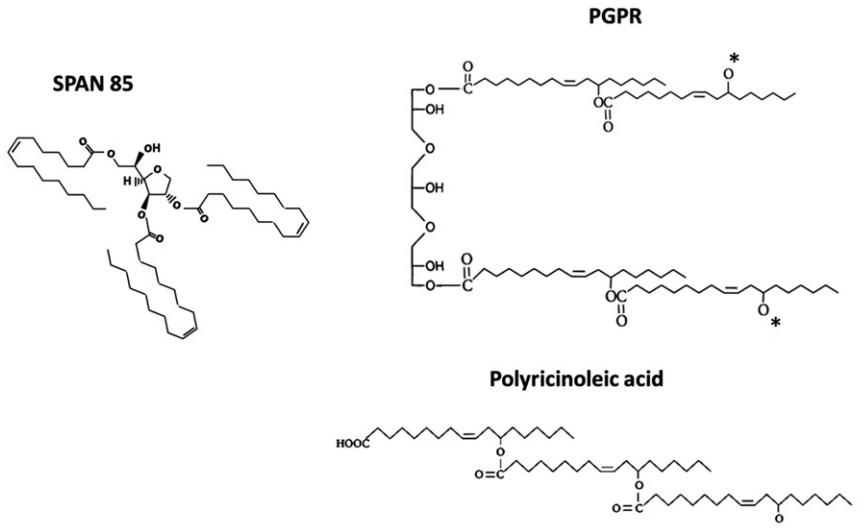


Figure 2. Chemical structure of sorbitan trioleate (Span 85) and polyglycerol polyricinoleate (PGPR). The asterisk in the PGPR structure denotes polyricinoleic acid chains.

plate device at ambient temperature (20 ± 2 °C). Measurements were carried out in triplicate.

Characterisation of the W/O emulsions

Stability of the emulsions

The determination of the W/O emulsion stabilities was performed as described by Martins et al. (2015). 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).

Observation of the emulsions by microscopy

Three different W/O emulsions with similar formulation and freshly prepared were analysed. For each emulsion, nine different areas arbitrarily chosen 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). Nine different zones for each sample were imaged and approximately 100 droplets per zone were measured.

Conductivity of the emulsions

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

Characterisation of the capsules

Measurement of capsule diameter and membrane thickness

Three different samples with twenty wet or dry capsules were observed using a Dino-lite digital microscope pro (Taiwan). The measurements of capsules diameter (*d*) and membrane thickness (*Mt*) were performed using the ImageJ 1.47v freeware (USA).

Statistical analysis

The results were compared using the Student's *t*-test statistical method with a significant difference at p values <0.05.

Results and discussion

Effect of surfactant on the stability of the W/O emulsions

Calcium chloride/oil emulsions were prepared using sunflower oil (100 mL), $CaCl_2$ solution at 240 g/L (23 mL) and the surfactants Span 85 or PGPR 90 at three concentrations (0.08, 0.8 and 8 g/L). Span 85 is a non-ionic sorbitan trioleate where OH groups in the molecule are esterified forming tri-esters. On the other hand, PGPR 90, also known as polyglycerol polyricinoleate, is a polymer of glycerol partially esterified with polyricinoleic acids chains (Graber, 2010). The presence of free carboxyl groups in the polyricinoleic acids chains is responsible for the anionic character of PGPR. The chemical structures of Span 85 and PGPR are displayed in Figure 2.

To verify the W/O structure of each emulsion, an electrical conductivity measurement was performed. The absence of electrical conductivity confirmed the signature of W/O emulsions.

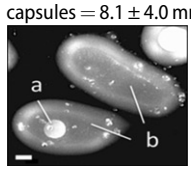
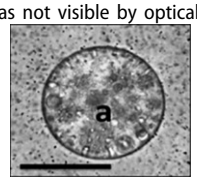
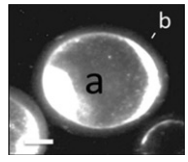
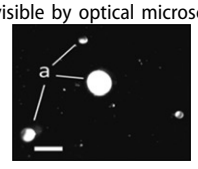
Using concentrations of Span 85 smaller or equal to 0.8 g/L, W/O emulsions low time stability (<20 min) were obtained (Table 1).

Emulsion stability has been characterised by macroscopic variables such as the hydrophilic–lipophilic balance (HLB), with the aim being to predict the surfactant properties of molecules. Nevertheless, this parameter does not take the topology of the molecule into account. In the case of Span 85, the presence of three hydrophobic branches and only one hydroxyl group able to interact via hydrogen bonding with the calcium ions present in the water droplets of W/O emulsion led to weak interactions with the aqueous phase and less surface coverage (Taylor, 2011; Pawlik, 2012). These structural constraints could therefore be prone to emulsion destabilisation at low surfactant concentration.

In contrast, Span 85 at 8 g/L resulted in an emulsion with high stability (15 days; Table 1). More investigations revealed that this last emulsion conducted electricity, proving that it was an oil-in-water (O/W) emulsion (Table 1).

Span 85 shows a critical micellar concentration (CMC) of approximately 4.3 g/L and, for $C > C_{cmc}$, as those used in this study (8 g/L; Table 1), surfactant molecules tend to form reverse micelles (Guo, 2012). This variation of behaviour can be responsible of the change of the emulsion structure, from W/O to O/W emulsion, at high Span 85 concentration. Other works also suggested that the inversion of the emulsion structure could be facilitated by the

Table 1. Characteristics of the oil/calcium chloride emulsions and the capsules produced by extrusion–dripping.

Surfactant	Concentration of the surfactant (g/L)	Conductivity (μS/cm)	Size of the $CaCl_2$ solution droplets in the emulsion (μm)	Stability	Capsules produced
SPAN 85					
	0.08	0	18.8 + 7.0	15 min	Elongated capsules containing or not cores Length of the capsules = 8.1 ± 4.0 mm 
	0.8	0	14.3 ± 5.1	18 min	Particles with *d* = 200 + 120 nm The presence of the membrane was not visible by optical microscopy 
	8	2.6 + 0.2	–	15 days	The emulsion was discarded from the study (O/W emulsion)
PGPR 90					
	0.08	0	15.3 ± 5.6	50 min	Production of two populations of capsules: • d = 5.3 + 1.8 mm and Mt = 0.4 + 0.1 mm • *d* = 0.5 + 0.3 mm and *Mt* = 100 + 50 μm 
	0.8	0	7.0 ± 0.8	1 day	Capsules with *d* = 1.2 ± 0.7 mm Membrane not visible by optical microscopy 
	8	0	0.7 ± 0.1	5 days	No capsules production

a: oil core; b: membrane.
White bar: 1 mm; Black bar: 200 μm.

high stirring rate applied during the emulsification process (Bastida-Rodríguez, 2013; Züge et al., 2013).

As the objective of this work was to produce capsules from W/O emulsions, the O/W emulsion produced with Span 85 at 8 g/L was discarded from the study.

By the electrical conductivity measurements, all calcium chloride/oil emulsions produced with PGPR 90 were of W/O type (Table 1). Furthermore, the increase in the PGPR 90 concentration resulted in emulsions more stable containing smaller $CaCl_2$ solution droplets (Table 1). These results were in accordance with those of Su et al. (2006) and Züge et al. (2013) who demonstrated that more stable W/O emulsions were made of smaller aqueous phase droplets.

The decrease of droplets size is accompanied by an increase of the Laplace pressure within them, facilitating the coalescence process (Cheng and Wang, 2013). By decreasing the interfacial tension with addition of emulsifier, the Laplace pressure is also reduced ensuring the formation of smaller droplets (Taylor, 2011). The results (Table 1) suggested that PGPR 90 was more effective to reduce the interfacial tension in comparison with Span 85. In agreement with our findings, Taylor (2011) and Pawlik (2012) demonstrated that PGPR 90 shows higher interfacial tension reduction and better surface coverage compared to Span 85, explaining the difference of behaviour of emulsions produced with the two kinds of surfactants.

Moreover, the higher emulsion stability showed by PGPR 90 can be related to synergistic effect between PGPR 90 and calcium chloride ions present in the emulsion (Park et al., 2003). Some works suggested that calcium salts increase the adsorption of PGPR 90 at the oil–water interface resulting in more stable W/O emulsions (Paunovic and Schlesinger, 2006; Márquez et al., 2010).

According to Márquez et al. (2010), calcium salt could affect the interactions between PGPR molecules at the oil–water interface by promoting electrostatic interactions with the free carboxyl groups in the polyricinoleic acids chains of PGPR. These electrostatic interactions could origin from better stabilisation properties of emulsions with PGPR by substantially decreasing interfacial tension (Pawlik, 2012).

By comparing the W/O emulsions produced with the two surfactants, it was easier to control the stability of the emulsions by the addition of PGPR 90. Moreover, O/W emulsion was produced with Span 85 due to a phase inversion phenomenon.

Production of capsules by extrusion–dripping

To define how the emulsion stability influences capsule production, the W/O emulsions described in the last section were added dropwise into stirred alginate bath.

Emulsions containing low concentration of Span 85 (0.08 g/L) resulted in elongated capsules containing or not a core (Table 1). During the extrusion of this emulsion, a phase separation (oil and $CaCl_2$ solution) was observed within the tip. The high Ca^{2+} release from the emulsion allowed the production of capsules with thick membranes; however, the procedure was hardly controlled and not reproducible (Table 1). Similar finding was observed by Martins et al. (2017) who reported that W/O emulsions with lower stability tends to be prone to phase separation during extrusion.

In preliminary studies, we checked that the association of Tween 20 and ethanol was more effective to destabilise W/O emulsions than ethanol or Tween 20 alone (Martins et al., 2017).

By increasing the concentration of Span 85 (0.8 g/L), no phase separation was observed in the tip during the extrusion–dripping process. However, instead of millimetric capsules, microscopic particles were recovered by filtration (Table 1). In this case, the emulsion drop fragmented after penetration into the bath and was completely dispersed in the alginate solution, maybe forming microcapsules. Indeed, by optical microscopy, it was not possible to check the presence of a membrane around the emulsion droplets (Table 1).

For all W/O emulsions produced with PGPR 90, no phase separation was observed during the extrusion–dripping process. For the greatest PGPR 90 concentration (8 g/L), the emulsion was completely dispersed into alginate bath and no capsules were recovered by filtration (Table 1). It was supposed that the high emulsion stability prevented the release of Ca^{2+} ions and therefore the membrane formation. In millifluidic devices, it was also evidenced that high PGPR 90 concentrations can prevent the release of Ca^{2+} ions from W/O emulsions (Martins et al., 2017).

Using 0.8 g/L of PGPR 90, millimetric capsules were obtained (Table 1). However, the capsules were polydisperse in size and lower than the diameter of the emulsion drop ($\approx$ 5 mm). During the experiments, it was observed that the emulsion drops fragmented in smaller drops during the contact with the alginate solution and therefore explained the presence of capsules smaller than 5 mm.

With low PGPR 90 concentration (0.08 g/L), spherical capsules (d = 5 mm) with shell-core structure were obtained (Table 1). Nonetheless, the presence of smaller capsules (d = 0.5 mm) was also found (Table 1). The presence of two types of capsules would come from two phenomena:

1. Emulsion drops where membrane was immediately formed after contact with alginate solution (d = 5 mm);
2. Emulsion drops that were fragmented before to form a membrane (d = 0.5 mm).

In a different approach, Paques et al. (2013) produced a dispersion of particles of $CaCl_2$ in oil and put it in contact with alginate solution. $CaCl_2$ migrated from the oil phase toward the alginate solution promoting alginate gelation. This principle was adapted to inverse gelation by addition of oil drops containing $CaCl_2$ particles into alginate solution. It was checked that oil drops broke when in contact with alginate bath and no capsules were formed. It seemed that calcium ions diffusion from oil to alginate phase was not efficient to promote membrane formation under dripping conditions. For this reason, this method was discarded and experiments were pursued using W/O emulsions.

Results in Table 1 showed that by using PGPR 90 at 0.08 g/L, millimetric capsules with well-defined core–shell structure were obtained. Although this result was the most promising, two drawbacks still persisted: the production of millimetric capsules with thin membranes (Mt = 0.4 ± 0.1 mm) and the formation of small capsules (d = 0.5 mm). These drawbacks would indicate that the calcium ions were not efficiently released during the membrane formation (low concentration and/or slow release).

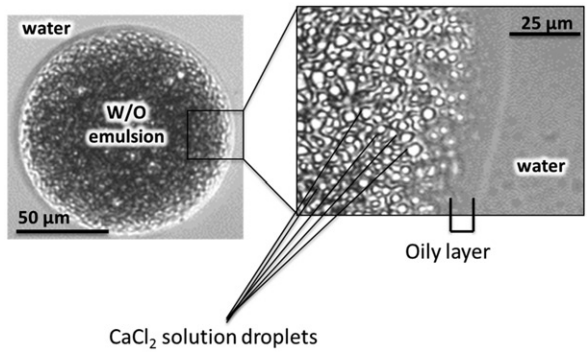


Figure 3. Optical microscopy of W/O emulsion droplet dispersed in water (with W/O emulsion stabilised by 0.08 g/L of PGPR 90).

Improvement of calcium ions release from the emulsion drop

To overcome the inefficacy of the calcium ions release, the mechanism of Ca^{2+} ions release from the emulsion drop needs to be understood. For that, the emulsion structure was thoroughly studied by optical microscopy (Figure 3) and, in order to facilitate the visualisation, the W/O emulsion was previously diluted in water to form droplets.

The emulsion was formed by an oil continuous phase and a $CaCl_2$ solution droplets corresponding to the dispersed phase (Figure 3). It was possible to verify that the emulsion drop surface was surrounded by an oily layer that limited the contact of the $CaCl_2$ solution droplets with the external media (Figure 3). It was supposed that the external oil layer could act as a barrier hindering the release of aqueous $CaCl_2$ droplets during the capsule production.

Based on this observation, it was supposed that the capsule formation using PGPR 90 at 0.08 g/L would occur in three steps:

1. The emulsion drop contacted the alginate solution;
2. The $CaCl_2$ solution droplets near the oil–alginate solution interface were able to migrate from the emulsion to the alginate bath;
3. The Ca^{2+} ions cross-linked the alginate chains resulting in the formation of a membrane.

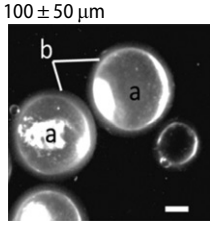
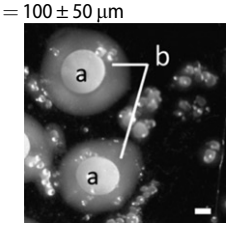
If the $CaCl_2$ droplets release was fast, capsules with similar diameter to the emulsion drop (d = 5 mm) were formed. Otherwise, the emulsion drop was fragmented and smaller capsules were obtained.

In the next sections, strategies to increase Ca^{2+} release were investigated using W/O emulsions stabilised by 0.08 g/L of PGPR 90.

Effect of aqueous phase volume fraction (Φ_{water}) on the membrane thickness

It was expected that by increasing the volume of $CaCl_2$ solution in the emulsion, an increase of the number of the $CaCl_2$ solution droplets at the oil–alginate interface would occur. To test this hypothesis, W/O emulsions with increasing aqueous phase volume fraction ($\Phi_{water} = \frac{V_{CaCl_2}}{V_{emulsion}}$) were produced and added dropwise into alginate bath (Table 2).

Table 2. Characteristics of the W/O emulsions containing 45 g/L of $CaCl_2$ and 0.08 g/L of PGPR 90.

Φ_{water}	Volume of $CaCl_2$ solution added in 100 mL of oil (mL)	Concentration of the $CaCl_2$ solution used to prepare the emulsion (g/L)	Mass of PGPR 90 added to the emulsion (mg)	Emulsion Volume (mL)	Capsules produced
0.20	23	240	9.8	123	Production of two populations of capsules: • $d = 5.1 \pm 1.0$ mm $Mt = 0.4 \pm 0.1$ mm • $d = 0.5 \pm 0.3$ mm $Mt = 100 \pm 50$ µm 
0.25	33	180	10.6	133	
0.30	43	140	11.2	143	Production of two populations of capsules: • $d = 6.2 \pm 1.1$ mm $Mt = 1.0 \pm 0.2$ mm; • $d = 0.5 \pm 0.3$ $Mt = 100 \pm 50$ µm 

Description of the capsules produced by extrusion–dripping.
a: oil core; b: membrane.
White bar: 1 mm.

No difference ($p < 0.05$) between the capsules produced with the emulsions containing $\Phi_{water} = 0.2$ and 0.25 was observed (Table 2). Capsules with thin membranes ($d = 5.1$ mm and $Mt = 0.4$ mm) and small capsules ($d = 0.5$ mm and $Mt = 0.1$ mm) were recovered.

In contrast, capsules with thick membranes ($d = 6.2$ mm and $Mt = 1.0$ mm) were produced from emulsion containing $\Phi_{water} = 0.30$ (Table 2). However, the production of small capsules ($d = 0.5$ mm) was still present (Table 2).

The results showed that if Φ_{water} was increased, capsules with thicker membranes were produced. Nonetheless, the increase of Φ_{water} is associated with a decrease of the oil loading in the capsules. For this reason, Φ_{water} of 0.3 was considered to be a good compromise to get a satisfactory membrane thickness and oil loading.

Effect of ethanol on the capsules production

In the previous studies using unstable W/O emulsions (emulsion containing 0.08 g/L of Span 85, Table 1), a high release of Ca^{2+} ions from the emulsion drops was observed. This indicated that, to overcome the slow release of calcium ions, unstable emulsions needed to be formulated. On the other hand, unstable emulsions are susceptible to phase separation during the extrusion–dripping process rendering the capsule production very unstable.

To guarantee an ideal Ca^{2+} ions release and at the same time a control of the capsules production, the emulsion should be unstable inside the alginate bath.

According to Andersen et al. (2005), surfactants with high HLB value such as Tween 20 (HLB =16.7) and/or organic solvent such as ethanol can act as W/O emulsion destabilizers. In order to destabilise the emulsions and increase the release of Ca^{2+} ions, Tween 20 and ethanol were added to alginate solution.

Tween 20 (0.5–2% v/v) did not affect the release of Ca^{2+} ions from the emulsion drops. The addition of surfactant only improved the emulsion drop penetration reducing its deformation and fragmentation at the bath surface. Abang (2011) also did similar observations and demonstrated that this phenomenon occurred because of the reduction of the surface tension of the alginate bath caused by the addition of surfactant.

By adding Tween 20 at 1% (v/v) in the alginate bath, it was found that the surface tension of the bath decreased from 55 to 33 mN/m. In further experiments, Tween 20 at 1% (v/v) was added into alginate bath in order to improve the emulsion drop penetration.

As Tween 20 was not effective to destabilise the emulsion drop, ethanol was added, drop by drop, into alginate bath (10 g/L) to obtain polysaccharide solutions with increasing concentrations of organic solvent.

For alginate solutions containing between 0 and 10% v/v of ethanol, slightly elongated capsules with $d = 5.6$ mm and smaller capsules with $d = 0.5$ mm were produced.

Using alginate added with 20% v/v of ethanol, only capsules with $d = 6.2$ mm were produced. The capsules had therefore a tear-shaped morphology but no small capsules were produced, giving strong confidence to the destabilisation of the W/O emulsion by adding 20% v/v ethanol.

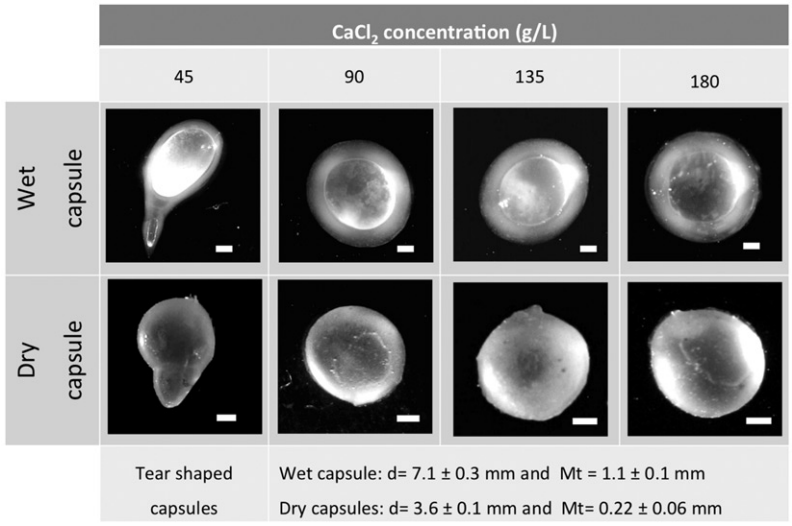


Figure 4. Characteristics of the capsules produced by extrusion–dripping process with increasing concentrations of $CaCl_2$ in the W/O emulsions. White bar: 1 mm.

In preliminary studies, we verified that the association of Tween 20 and ethanol was more effective to destabilise W/O emulsions than ethanol or Tween 20 alone (Martins et al., 2017). Probably, ethanol can also increase the Tween 20 solubility in the oil phase allowing deeper migration of the surfactant inside the emulsion structure. Tween 20 would be more efficient to destabilise the PGPR 90 films that cover the $CaCl_2$ solution droplets increasing the Ca^{2+} release.

The synergistic interaction of Tween 20 with ethanol was supposed to be the main factor controlling the Ca^{2+} release during the capsules production.

Although the problem of small capsule was solved by adding 20% v/v ethanol, the capsules showed a tear shape. The tear shape was associated to the drag forces applied on the capsules during the stirring of the alginate bath. In addition, it was supposed that the capsule deformation simultaneously occurred during the formation of the membrane. In other words, the membrane was formed rapidly to avoid the emulsion drop fragmentation but the process was not sufficiently fast to avoid the capsules deformation. Increasing ethanol concentration however did not speed up the process as alginate precipitated in the bath.

Effect of $CaCl_2$ concentration on the membrane thickness

As the amount of ethanol in the alginate bath cannot be increased, the release of Ca^{2+} ions was tentatively improved by using emulsions more concentrated in $CaCl_2$. Emulsions containing increasing concentrations of $CaCl_2$ (22.5–180 g/L) were thus produced and added dropwise into alginate bath added with Tween 20 and ethanol (20% v/v).

For the emulsions containing $CaCl_2$ concentration <45 g/L, capsules were obtained. However, they broke during the rinsing step due to the presence of thin and fragile membranes.

For $CaCl_2$ concentrations <90 g/L, tear-shaped capsules were obtained. On the other hand, spherical capsules ($d \approx 7$ mm) with 23% v/v of oil loading (oil volume in the core/capsule volume) were produced for $CaCl_2$ concentrations ≥ 90 g/L (Figure 4).

The dry capsules, obtained after a drying step of 48 h at ambient temperature, showed diameters 50% lower than the wet capsules (Figure 4). Analyses by optical microscopy of the core content in dry capsules revealed the presence of a single phase (oil). We measured after prolonged heating at $T = 60\,^{\circ}C$ the weight of a W/O emulsion and we found that 95% w/w of water was removed after drying. We made the hypothesis that similar drying occurred in the encapsulated emulsion. It was therefore estimated that the dried capsules, where water was present in membrane and oil core, contained less than 5% w/w of water. In addition, the cores of the wet capsules ($d \approx 5$ mm) were reduced to approximately 3 mm after drying meaning that the dry capsules contained up to 70% v/v of oil.

No changes ($p < 0.05$) were observed for the diameters and the membrane thicknesses of the wet or dry capsules produced with $CaCl_2$ concentrations ≥ 90 g/L, suggesting that $CaCl_2$ concentration had no or little effect on the membrane thickness, but had a direct impact on the shape of the capsules.

Effect of the curing time on the membrane thickness

W/O emulsions containing 90 g/L of $CaCl_2$ were then added dropwise into alginate bath added of Tween 20 and ethanol. The emulsion drops were kept in contact with the alginate bath by increasing curing time.

No difference ($p < 0.05$) was observed on the membrane thickness of the capsules after only 5 min of curing time ($Mt \sim 1$ mm). This result indicated that all Ca^{2+} ions used in the membrane formation were released during the first minutes of contact between the emulsion drop and the alginate bath. In agreement with this result, a recent study using droplets millifluidic demonstrated that the membrane achieved the maximal thickness with a curing time of approximately 8 s (Martins et al., 2017). It corroborates with the idea that Ca^{2+} ions were instantaneously released after contact with alginate solution.

Based on these findings, we may wonder whether only part or all Ca^{2+} content was released from the core to induce membrane formation. A capsule was therefore half-cut and the core was visualised by light microscopy. The micrograph revealed the presence of $CaCl_2$ droplets indicating that only part of Ca^{2+} content was released from the core.

Considering the core perfectly spherical, the amount of Ca^{2+} ions in the core can be described by Equation (1) (Martins, 2015):

$$N_{Ca,core} = [Ca]_{core} \frac{\pi}{6} d_{core}^3 \quad (1)$$

where $N_{Ca,core}$ is the number of Ca^{2+} ions in the core (mol), $[Ca]_{core}$ is the Ca^{2+} ions concentration in the core (0.6 mol/L) and d_{core} is the core diameter (5×10^{-2} dm).

Table 3. Characteristics of the alginate solutions and the capsules produced.

Alginate concentration (g/L)	Density (kg/m^3)	Viscosity (n_{alg}) mPa s	Surface tension (mN/m) Without Tween 20	Surface tension (mN/m) With Tween 20 (1% v/v)	Description of the capsules produced
5	997 ± 9^a	31 ± 2^e	52 ± 1^h	34 ± 1^l	Capsules with $d = 7.1 \pm 0.3$ mm and
10	1000 ± 10^b	86 ± 12^f	55 ± 1^i	33 ± 1^l	$Mt = 1.1 \pm 0.1$ mm
15	1002 ± 9^c	222 ± 61^g	59 ± 1^j	32 ± 1^l	Capsules with $d = 6.4 \pm 0.3$ mm and $Mt = 1.0 \pm 0.1$ mm.
20	1004 ± 9^d	257 ± 34^g	50 ± 1^h	34 ± 1^l	No capsules production

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

Assuming that all calcium released is used to form the membrane and that the capsules are perfectly spherical, the amount of Ca^{2+} linked to the membrane ($N_{Ca,m}$, in mol) can be estimated by Equation (2):

$$N_{Ca,m} = [Ca]_m \frac{\pi}{6}\left(d_{cap}^3 - d_{core}^3\right) \quad (2)$$

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 core φ can be estimated by Equation (3):

$$\varphi = \frac{N_{Ca,m}}{N_{Ca,core}} \quad (3)$$

More details about the demonstration of Equations (1), (2) and (3) can be found in Martins (2015).

Using Equation (3), it was calculated that only 3% of the calcium content in the core was released. This low calcium content was however sufficient to induce the alginate membrane formation.

Effect of the alginate concentration on the capsules production

To evaluate the effect of alginate concentration on the capsules production, emulsion was added dropwise into alginate bath containing Tween 20 (1% v/v) and ethanol (20% v/v).

While the viscosity of the alginate solutions increased with concentration (Table 3), the surface tensions of the alginate solutions added of Tween 20 remained however constant whatever the alginate concentration (Table 3).

Using diluted alginate solutions (<5 g/L), capsules were obtained but they broke during the rinsing step with distilled water. Probably, the alginate concentration was insufficient to produce cohesive membranes.

No difference ($p < 0.05$) between the diameters of capsules produced with alginate at 5 and 10 g/L was observed (Table 3). For alginate solution at 15 g/L, capsules slightly deformed (with an ellipsoidal shape) were obtained. Scheele and Meister (1968) demonstrated that the drag force that fluid 1 exerts on fluid 2 is proportional to the viscosity of fluid 1. The deformation of the capsules in this case was mainly associated to the high drag force of the alginate solution (Table 3).

For alginate solutions at 20 g/L, the addition of ethanol led to alginate precipitation and the impossibility to produce capsules.

To summarise, alginate solutions with concentrations between 5 and 10 g/L were more favourable for the capsules production. Low alginate concentrations (<5 g/L) led to fragile capsules while high alginate concentrations (15 g/L) compromised their spherical shape.

Conclusions

A technique of oil encapsulation by inverse gelation using W/O emulsion was demonstrated and optimised. It was found that the production of capsules by the extrusion–dripping method required an emulsion with an appropriate aqueous fraction ($\Phi_{water} = 0.3$) and $CaCl_2$ concentration (90 g/L) and moderate stability (~50 min). By increasing both $CaCl_2$ concentration in the emulsion and curing time, no changes in the membrane thickness were observed. It was also evidenced that the addition of ethanol at 20% v/v into the alginate bath was essential to increase Ca^{2+} ions release from the emulsion drop and prevent its fragmentation. Wet capsules with 7 mm of diameter and 23% v/v of oil loading were therefore produced after optimisation of the extrusion–dripping method parameters.

The optimal protocol for capsules production using W/O emulsions consisted in:

- Prepare a W/O emulsion containing 100 mL of sunflower oil, 43 mL of $CaCl_2$ solution (300 g/L) and 11.2 mg of PGPR 90;
- Dropwise emulsion into 400 mL of a stirred alginate bath (10 g/L) containing 4 mL of Tween 20 and 80 mL of ethanol 99%;
- Keep the emulsion drops in contact with the alginate solution at least 5 min.

At a flow rate of 30 mL/h, the present dripping–extrusion setup produced 460 capsules/h. However, a high production (> 3700 capsules/h) can be expected by using a multi-nozzle dispensing disc (see Abang, 2011).

The findings detailed in this study widened the knowledge of the inverse gelation mechanism using W/O emulsions and future investigations will compare methodologies of capsules production using W/O and O/W emulsions in order to highlight the advantages and drawbacks of each method. Furthermore, the capsules produced with the two types of emulsions will be compared in terms of mechanical resistance and release profile of hydrophilic and lipophilic actives.

Disclosure statement

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

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