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Control of the Morphology and the Size of Complex Coacervate Microcapsules During Scale-up

C. Y. G. Lemetter
Unilever Asia Pvt Ltd, 103 Penang Road, #04-01 Vision Crest, Singapore 238467

F. M. Meeuse and N. J. Zuidam
Unilever R&D, Unilever Food & Health Research Institute, Olivier Van Noortlaan 120, 3133 AT Vlaardingen, The Netherlands

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Scale-up of complex coacervation, a fat encapsulation technology, is not trivial since the microcapsules morphology and size are highly affected by the processing conditions. So far it has been achieved empirically (trial and error approach). The goal of this study was to produce at various scale capsules with a single-oil droplet as the core material and small enough to be below sensory threshold. The turbulence level was identified as the main scale-up criterium and a master-curve could be drafted showing the capsule mean diameter as function of the Reynolds number, independent of the level of production scale. From a parent emulsion with specific oil droplets size (12–15 μm), the Reynolds number had to be maintained above a critical value (15,000) to avoid capsules agglomeration with multiple oil cores and large particle sizes. To avoid aggregation, this turbulence level had to be kept until the temperature dropped below a critical value (14°C for a cooling rate of 35°C/2 h). Applying these learning led to a successful scale-up from bench (2 L) to a pilot plant scale of 50 L. © 2009 American Institute of Chemical Engineers *AIChE J*, 55: 1487–1496, 2009
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Introduction

An increasing number of food products contain actives providing either added value (e.g., taste with flavour or aroma, etc.), and/or nutritional and health benefits (e.g., vitamins, omega 3 oils, etc.). Controlling their delivery at the right place and time is usually a key property influencing their functionality and/or bioavailability.[1] As a result, since the last 20 years food businesses have been investigating more and more controlled release technologies, with a special focus on microencapsulation ones, which may deliver novel ingredients with unique properties.

Complex coacervation between oppositely charged proteins and polysaccharides is a well-known oil encapsulation technology. This aggregative phase separation phenomenon was first discovered in 1911, and was then more systematically studied for the polymer system "gelatin/gum-arabic" between the 20s and the 40s. However, its applications to the food industry and particularly to the formation of oil-filled microcapsules started in the 50s and more extensive studies only emerged two decades ago. Most of these studies were mainly focussing on the identification of new polymer systems (other than gum arabic/gelatin) suitable for the food industry requirements (milk/plant proteins, fish gelatin, and so on), on the effects of the system chemical properties

Correspondence concerning this article should be addressed to C. Y. G. Lemetter at cedric.lemetter@unilever.com

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(such as pH, ionic strength, polymer ratio or concentration[2,3,4]), on the coacervate phase properties (such as rheology, yield[2]), and on the encapsulation of specific compounds.[2,5–9] However, only few studies have been published to describe the effect of processing parameters (shear, temperature, residence time, and so on) on the final product characteristics.[6,9,10] As a result, scaling-up was almost always achieved empirically (trial and errors setup) at the expense of cost and resources. There is consequently a need to determine more precisely the effect of the various processing parameters (shear, temperature, cooling rate, and so on), and to study scale-up in a more systematic approach. The focus of the current work was to design a process to manufacture oil-encapsulates, with enough understanding to have an accurate control on the final product quality and size at various production scales (from laboratory to pilot plant and larger scales). The work was carried out with the most-described system in the available literature, the well-known polymer system ''gelatin/gum-arabic'' in a 1 to 1 weight-ratio. At bench scale (600 mL scale), mononucleated capsules with a diameter up to 10 μm were previously obtained. However, trials done at larger scale (2 L and more) often resulted in aggregation and/or formation of polynucleated capsules, resulting in a hardly controllable capsule size. The objectives of the current study are:

- To identify the main processing parameters affecting the product quality (size, nucleation type and aggregation), and the process operation (yield, etc.).
- To understand the effect of those process parameters on the capsule formation.
- To setup a reliable and reproducible pilot plant process (at a 50 L scale) to produce ''mononucleated'' oil-filled microcapsules with a defined size distribution.

Theory and Expectations

The complex coacervation reaction, an aggregative phase separation

In an aqueous solution of one or more hydrocolloids, coacervation is the reaction leading to the separation of the initial solution into two aqueous phases; a hydrocolloid-rich phase (coacervate), and a hydrocolloid lean phase. The coacervate phase appears as amorphous liquid droplets, which can coalesce and separate out from the water by gravity into a translucent homogeneous colloid-rich layer. According to the number of hydrocolloid(s) present, the reaction can be identified as simple or complex coacervation, the later one referring to systems with two or more dispersed hydrophilic colloids. This study focuses on the complex coacervation reaction, which will be simply referred from now as ''coacervation''. The neutralization of the overall positive charges of one of the hydrocolloids by the negative charge of the other, resulting in a polymer complex formation that can then precipitate (due to electrostatic attractive forces), is used to bring about the phase separation based on the Voorn-Overbeek theory.[4,11]

Only mixtures with quite low-polymer concentrations are able to undergo this phase separation transition. For example, with the polymer system ''gelatin/gum-arabic'', the concentration of both polymers has to be below 8.0% (w/w) for coacervation to occur.[12] At higher-polymer concentrations, the polymer sol remains stable without any phase separation occurring. At even higher-polymer concentrations, segregative phase separation can occur, resulting in the formation of two incompatible sols, one being rich in one colloid and the other one in the second colloid.

Usually an amphoteric polymer is used to induce coacervation. Starting from a solution with two polymers carrying similar charges, the reaction can then be induced by pH adjustment, bringing both polymers to carry net opposite charge. For example with the system ''gelatin/gum-arabic'', type A gelatin is negatively charged above its isoelectric point (ca. 9), and positively charged below, while the arabic gum is always negatively charged in solution with a pH above 2. Therefore, starting with a stable solution of hydrated gelatin and gum arabic at neutral pH (pH above 7), an acidification step until a pH low enough to have enough opposite electrical charges (usually, until a pH of 4.3) will lead to the phase separation.

The microencapsulation process via complex coacervation

As schematically represented in Figure 1, the production of microencapsulated oil droplets via the coacervation process is a 5-step process.

1. *Polymer dissolution and hydration*

Creation of the carrier solution (at a pH at which coacervation cannot occur, and above the gelling temperature of the polymer).

2. *Emulsification (formation of the core)*

An emulsion of oil droplets in the water phase is created. The emulsion is stabilized by the two polymers.

3. *Coacervation (formation of the shell)*

By lowering the pH under agitating, the polymers interact via electrostatic interactions and form a polymer complex. This coacervate phase formed is fluid and distributes over the entire oil surface. A uniform coating of liquid coacervate phase is then formed around the oil droplets.

By lowering the temperature of the mixture below the gelation temperature of the gelatine, a gel is created that will lead to a solidified polymer shell.

4. *Hardening*

A crosslinking agent like glutaraldehyde can be added to strengthen the wall material.

5. *Washing/Separation stage*

Any additional crosslinking agent can now be washed out. Afterwards the capsules can be concentrated and/or separated from the solution.

The scope of this article is limited to the first three steps.

Specifications of the microcapsule dispersion

The goal of this study was to setup a reliable and reproducible process at pilot plant scale providing ''mononucleated'' microcapsules, meaning capsules with a single oil droplet as core material and small enough to be below sensory threshold. However, as mentioned before, during some initial trials, two other coacervate types were produced as well (see also Figure 2):

- ''Polynucleated capsules'', which are made of several oil droplets included in a single polymer shell.

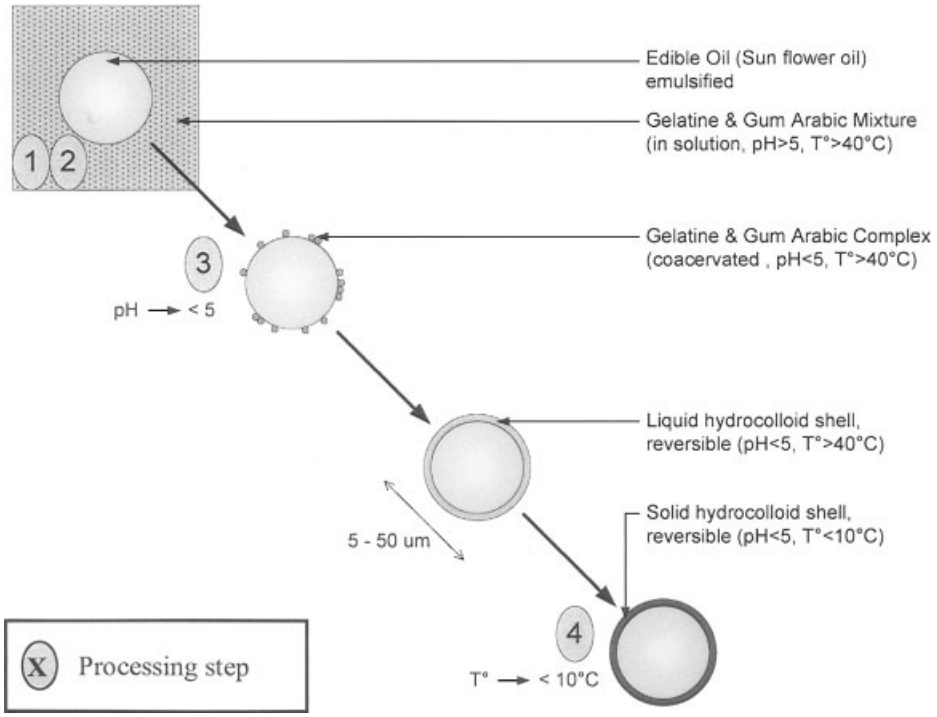

Figure 1. Schematic representation of the complex coacervation process.

• "Grape coacervates" that are aggregates of mononucleated capsules.

Because the microcapsules have to be below sensory threshold, an accurate control of the size was required as well. The capsule size distribution is specified as follows: 95% above 1 μm, 95% below 50 μm and 99% below 100 μm, which correspond to a D3,2 between 10 to 25 μm.

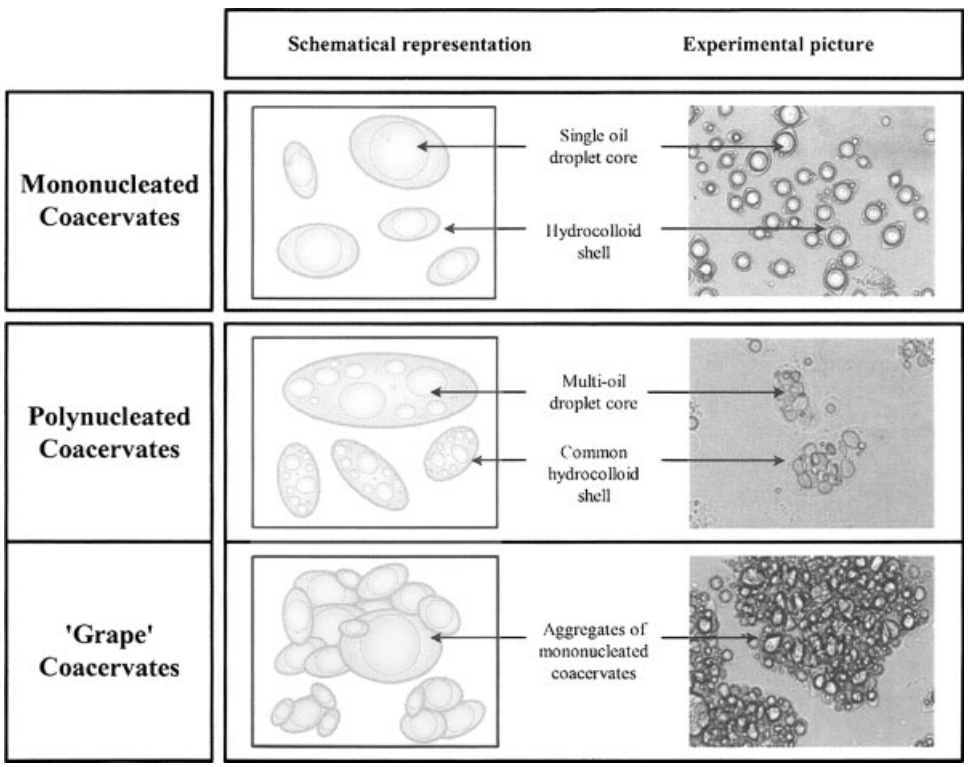

Figure 2. Representation of three coacervate types.

Expectations and Experiments Setup

Emulsification and control over the oil droplet-size distribution

The size of mononucleated capsules is determined by a combination of the parent oil droplet diameter and shell thickness. The target of the emulsification step was then to produce a parent o/w emulsion with monomodal size distribution as narrow as possible and a $D_{3,2}$ between 5 and 20 μm, which would allow, after the coverage of the shell to obtain microcapsules with a $D_{3,2}$ between 10 and 25 μm. A first set of experiments was designed to select a suitable emulsification device and the appropriate process-parameters values (shear, residence time, etc.).

Polynucleation: Level of shear required during the capsule formation

The second step to be studied was the coacervation step. Bench scale experiments showed that capsules with a right diameter (10–25 μm) could be obtained. However, previous experience showed that scale-up from bench scale to 2 L scale often resulted in aggregation and/or polynucleation. Polynucleation was believed to be due to a too low-shear rate during the shell formation. Therefore, a second set of experiments was designed to determine the effect of the impeller rotation speed (and, thus, the effect of the level of shear) on mononucleated and/or polynucleated capsule formation and on the corresponding capsule-size distribution.

Aggregation: importance of the stirring time during cooling

Finally, a third and last set of experiments was organized to investigate the effect of stirring time on aggregation and the formation of "grape coacervates". Gelation of gelatin is greatly affected by the cooling rate and by the low-temperature residence time (time required for the gelatine to order and form networks). Aggregation was believed to be due to an uncompleted gelation of the capsule shell when stirring was stopped. The aim of the following experiments was to determine at what stage (temperature and time) the stirring could be stopped, and stable capsules dispersion had been formed.

Materials and Methods

Materials

Porkskin gelatin powder (type A, bloom value 250, pI $\sim$ 9.0 as measured by the Zetasizer, Nanoseries ZS equipped with MPT-2 multipurpose Titrator, Malvern Instruments, U.K.) was purchased from Gelita (Sweden) under the trade name of Geltec (UG-719N). Two distinct gum arabic powders were used. The first one, used specifically for laboratory trials, was an analytical grade gum purchased from Sigma-Aldrich (gum arabic from acacia tree, G9752-1kg, pI 2.1 as measured by the Zetasizer). The second gum, used for both laboratory and pilot plant tests, was purchased from Valmar under the trade name of Valspray F (pI 2.1 as measured by the Zetasizer). The encapsulated material was edible sunflower oil (at intermediate scale: Zonnebloem olie 1L, purchased from Albert Heijn, a Dutch supermarket and at pilot-plant scale: sunflower oil, supplied by Unilever sourcing unit of Nassaukade on June 2005). Finally, analytical grade 0.5 M hydrochloric acid and 0.1 M sodium hydroxide, both supplied by Merck, were used for all pH adjustments.

Methods

Emulsification experiments: Control over the oil droplets-size distribution

Emulsification at Intermediate Scale (2 L). Four different emulsification devices were tested on a 2 L scale. Stock solutions of gelatin and gum arabic with a concentration of 4% (w/w) were prepared separately in 950 g of hot tap water. The mixture was stirred for 1 h at 60°C to ensure full polymer dissolution and hydration. The two polymer solutions were then poured together in a premix vessel a (standardized 2 L vessel with a Rushton turbine), and 180 g of sunflower oil, which was preheated at 60°C, was added. This resulted in concentrations of sunflower oil, gelatin and gum arabic of 10, 2 and 2% (w/w), respectively (corresponding to a ratio of 5:1:1 w/w). The solution was then pre-emulsified in the vessel (5 min, 350 rpm), and finally sent to the studied emulsification device. The four following emulsification device tested were:

1. Stirred vessel with standard Rushton turbine (2 L vessel, baffled, with Stirrer RW 27W Ika-Werk Janke & Kunkel). Process parameters: Impeller rotation speed, range studied: 0 to 5,000 rpm.
2. Colloid Mill (PM 30, WO: 2930, 1331/A/2, O Krieger, Muttenz CH, Mastinen-und Metallban AG, 1976). Process parameter: Rotation speed, range studied: 0 to 3600 rpm. Gap width, range studied: 0 to 640 μm.
3. High-shear mixer - Silverson type (L4RT-A, 14835, Silverson machine ltd, England). Process parameter: Rotation speed, range studied: 0 to 4,000 rpm.
4. High-pressure homogenizer (HPH, NS-1001-L, NS-3605, Niro Soavi, 1998). Process parameter: Homogenizing pressure, range studied: 0 to 450 bars.

From these first observations, the most appropriate device(s) was selected for further investigation at pilot plant scale (50 L scale).

Emulsification at Pilot Plant Scale (50 L). Following the previous intermediate scale (2 L scale) experiments, 2 emulsification devices were selected and tested at pilot plant scale: a colloid mill and a high-shear mixer (Silverson type). The previously described protocol was performed up to a 50 L scale using the following equipment:

- For polymer dissolution: 4-blades impeller stirrer was used (Ika Werk RW 28W, Janke & Kunkel, equipped with an impeller).
- For Pre-emulsification: Premix vessel (baffled & jacketed 100-L premix tank, Terlet Zutphen Holland, temperature set at 60°C - standard Rushton turbine).
- Condition: 30 min, $\sim$ 50 rpm.
- For Emulsification:

1. In-line colloid mill (MZ80/D, M13048, Fryma Maschinen AG, CH, 1987, 50 Hz, coupled with a frequency

converter). Process parameters: Rotation speed, range studied: 0 to 3600 rpm. Gap width, range studied: 0 to 640 μm.

2. High-shear mixer – In-line Silverson (150250, 50250M043, Waterside Chesham, Bucks, U.K.). Process parameter: Rotation speed, range studied: 0 to 4,000 rpm.

Processing parameters of the selected devices were then set to produce an emulsion with a mean diameter ($D_{3,2}$) as close as possible to 12 μm (experimentally: between 10 and 15 μm).

Coacervation experiments: Control over the polynucleation reaction

The coacervation was first studied at intermediate scale (2 L) before investigation at a larger scale. For all experiments, the concentrations of sunflower oil, gelatin and gum arabic were 10, 2 and 2% (w/w), respectively (in a ratio 5:1:1 w/w).

Coacervation at Intermediate Scale (2 L). An o/w emulsion was produced according to the emulsification protocol described above at a pH >5, poured in a standardized 2 L vessel and stirred with a Rushton turbine. Acid was then added until the pH reached 4.3 and the dispersion was cooled to 5°C by surrounding the beaker with ice and/or chilled water). For these set of experiments the stirring speed was varied between 150 and 450 rpm. All other process parameters were kept constant.

Coacervation at Pilot Plant Scale (50 L). The same emulsification protocol as described previously was carried out at a 50 L scale. The o/w emulsion with a pH >5 was then sent to a jacketed standardized 80 L tank kept at 45°C using hot water as heating medium. Stirring was started and acid was added until the pH reached 4.3. The dispersion was then cooled to 5°C by allowing the cooling medium to flow within the double jacket. For these set of experiments the stirring speed was varied between 200 and 500 rpm. All other process parameters were kept constant.

Coacervation Experiments: Control over Aggregation and Stability of the Capsules

For the last set of experiments, a set of process conditions that lead to mononucleated coacervation (final pH of 4.3 and a rotation speed of 350 rpm) was selected. During the cooling step (from 40 to 5°C), samples were taken every 2°C - decrease and were placed immediately at 4°C without stirring. Particle-size analysis was performed on all the samples. Consequently, with this protocol all samples followed the same cooling profile (from 40 to 5°C within 2 h), but the temperature/time at which the stirring was stopped varied from 40°C (0 min) to 5°C (120 min).

Microscopic observations and size distribution analysis

The size distribution of the emulsions and the final capsule solutions were assessed in duplicate using a Mie light-scattering instrument (Mastersizer 2000, Malvern Instruments, UK). The following mean diameters were recorded: $D_{3,2}$, $D_{4,3}$, $D_{0.1}$ and $D_{0.9}$. Two indicators were used for comparison of the results, the $D_{3,2}$ or Sauter diameter and the dispersion ratio (RDisp). This dispersion ratio is defined as the difference between $D_{0.1}$ and $D_{0.9}$.

Experimental Results

Emulsification experiments and equipment selection

Four different emulsification devices were tested at a 2 L scale to produce 5-20 μm emulsions composed of 10% (w/w) sunflower oil, 2% (w/w) gelatin and 2% (w/w) gum arabic at 60°C. As the aim of these emulsion experiments was first to screen the achievable oil-droplet size distributions for several emulsification devices and then to determine the appropriate process parameter value for the selected device(s), the underlying mechanisms and working principles of each device are not presented later. Moreover, only a general overview of the range of size distribution achievable for each device, valid for this particular polymer system (gelatin/gum-arabic) with specific conditions (given concentration, ratio, temperature, etc.) is provided. Figure 3 shows the achievable ranges of particle sizes for the various emulsification devices.

Based on these results, the two rotor-stator type devices were selected to produce o/w emulsion with the desired specifications (average mean diameter between 5 and 20 μm). The following procedure was selected to produce o/w emulsion with an oil droplet mean diameter of approx 12 μm: a pre-emulsion, formed in a stirred vessel (fully baffled system, Rushton impeller, 5 min, 400 rpm) was sent at 45°C to a colloid mill (at a 2 L scale: 6,000 rpm, 2 min, gap: 80 μm; at pilot-plant scale: in-line, 3,300 rpm, gap: 160 μm).

Effect of the shear rate during the capsule formation

The emulsions prepared as described previously were used to prepare complex coacervates by adjusting the pH from over 5.0 to 4.3 under turbulent conditions at 45°C, followed by cooling the dispersion to 5°C. For a given oil-droplet diameter ($D_{3,2}$ between 10 to 15 μm), the size distribution of the final dispersion of oil-filled capsule depends on the impeller rotation speed applied during this coacervation step (acidification and cooling). As the rotation speed increased, the mean diameter of capsule size decreased until reaching the value of 18 μm at 350 rpm (Figure 4a). Correspondingly, the width of the size distribution decreased with increasing stirring speed until RDisp = 23 μm at 350 rpm (Figure 4b). Furthermore, it was observed that capsules formed at different stirring speeds had different shapes (Figure 4c): at 150 rpm, large polynucleated capsules (diameter above 50 μm) with many oils droplets enclosed in one shell (more than 30) were formed, and almost no mononucleated capsules could be detected. On the contrary, at 350 and 450 rpm, much more mononucleated capsules were present in the dispersion and the remaining polynucleated capsules were quite small (<20 μm), and just contained few oil droplets (<10). Moreover, at larger scale, similar observations were made. At a 50 L scale, the capsule mean diameter decreased with increasing rotation speed, reaching a value of 15 μm at 500 rpm (Figure 5a). Moreover, no polynucleated capsules

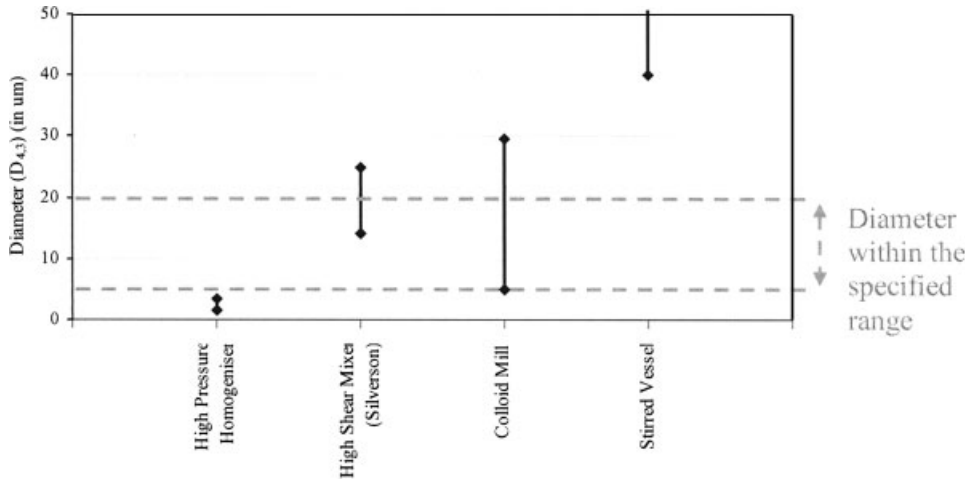

Figure 3. Mean diameter range achievable with four emulsifying devices (using 10% oil, 2% gelatin and 2% gum arabic in water at 60°C).

could be found at 500 rpm, whereas at 200 rpm, the capsule size was larger and few polynucleated capsules were present (containing less than 10 oil droplets) (Figure 5b).

Finally, it was also found with the 2 L scale setup, that foaming occurred at high-rotation speed. Above 350 rpm, the impeller created a vortex, thereby incorporating air in the dispersion. This resulted in the formation of a foam layer (up to half of the vessel volume at 450 rpm). This foaming affected dramatically the capsule formation and increases in the mean diameter, and in the wide of the capsule-size distribution were observed (Figure 4 a and b). A particle size analysis revealed that, foaming resulted in a bimodal capsule-size distribution with a first peak at the expected diameter (~20 μm), and a second at much larger particle size (~200 μm), indicating aggregates.

A minimum level of shear to prevent polynucleation

Increasing the shear rate leads to smaller capsule sizes due to a reduced chance of having polynucleation. Above a certain shear rate the capsule size cannot be reduced any

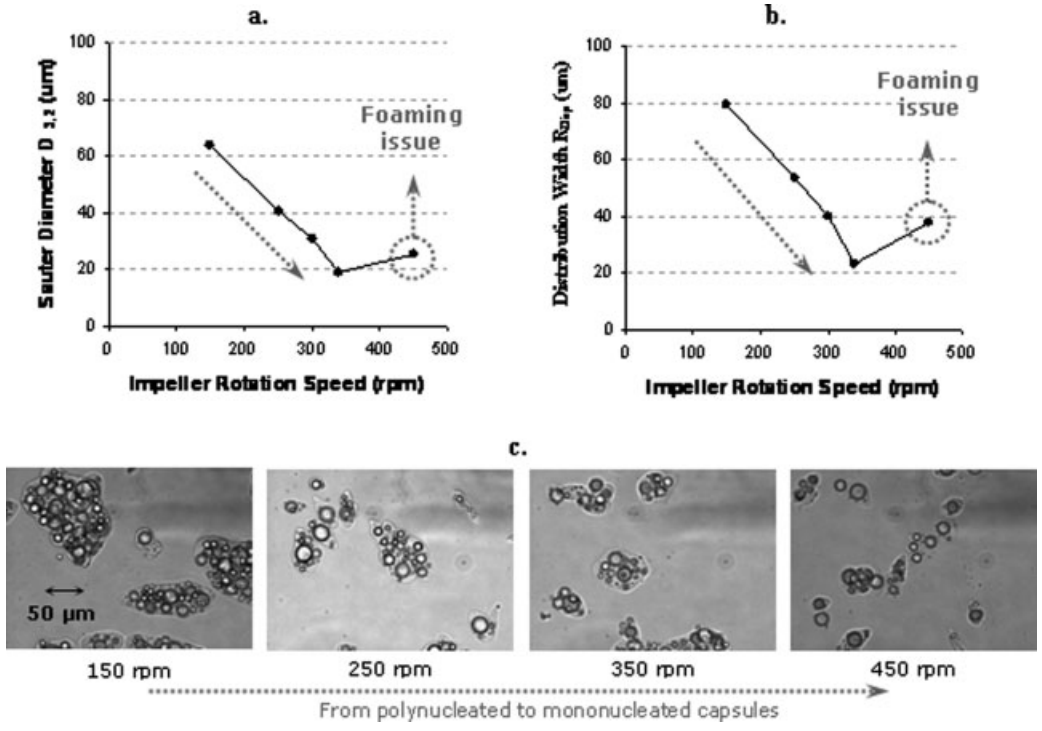

Figure 4. Effect of stirring speed on the size distribution (a), the width of the size distribution (b), and the morphology as seen by light microscopy (c) of the microcapsules (2 L scale).

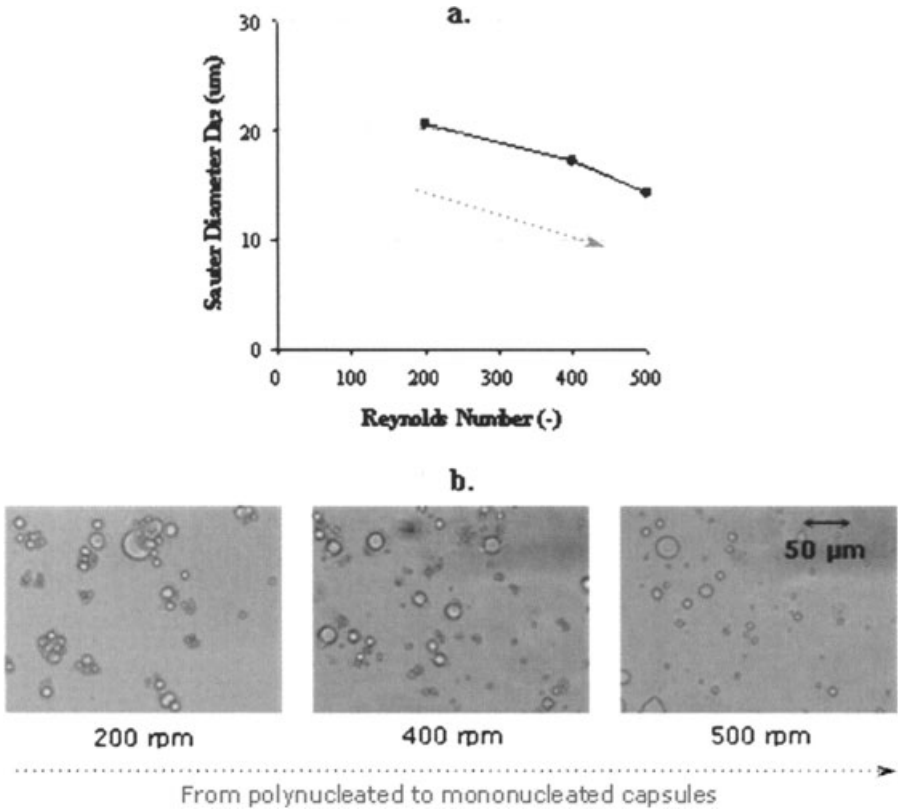

Figure 5. Effect of stirring speed on the size distribution (a), and the morphology as seen by light microscopy (b) of the microcapsules (50 L scale).

further without affecting the capsule integrity (with 12 μm oil droplets, capsule were expected to have an average diameter around 15 μm). Consequently, the polynucleation phenomenon can be controlled by having a certain minimum shear rate during the capsule formation.

A maximum level of shear to prevent foaming

Increasing the shear rate too much leads to foaming. In this foam layer much less shear is exerted on the dispersion and capsules aggregate together. Consequently, there is a limit to the shear rate that can be applied on the dispersion during the capsule formation. This limit depends on the system properties (vessel and impeller geometry, and so on). Under similar conditions, foaming did not occur with the 50 L scale setup at 500 rpm. This was most likely due to the fact that at a higher scale, the height of liquid above the impeller was larger (95 mm at the 2 L scale vs. 280 mm at the 50 L scale). Above this limit, air was incorporated in the system, creating a foam layer, in which capsules were no longer subjected to shear.

Effect of stirring time during the shell gelation

After capsule formation at a 50 L scale, the dispersion was cooled down from 40 to 5°C within 2 h while stirring. Three distinct behaviors were observed depending on the time stirring was maintained:

- When stirring was stopped below 14°C (after 77 min cooling), no aggregation was observed and a stable mononucleated capsule dispersion was obtained.
- When the stirring was stopped between 24 and 14°C (after 40 to 77 min of cooling), aggregation occurred. As a result the mean diameter increases.
- When the stirring was stopped above 24°C (before 40 min of cooling), severe capsule aggregation occurred resulting in flocculation. These flocculates had a particle size above the maximal detection limit of the equipment used.

The evolution of size as a function of stirring time is presented in Figure 6. This experiment was repeated three

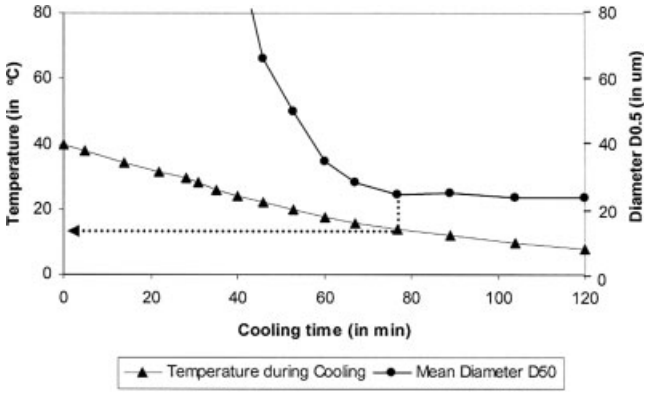

Figure 6. Evolution of temperature during the cooling step and aggregation limit identification.

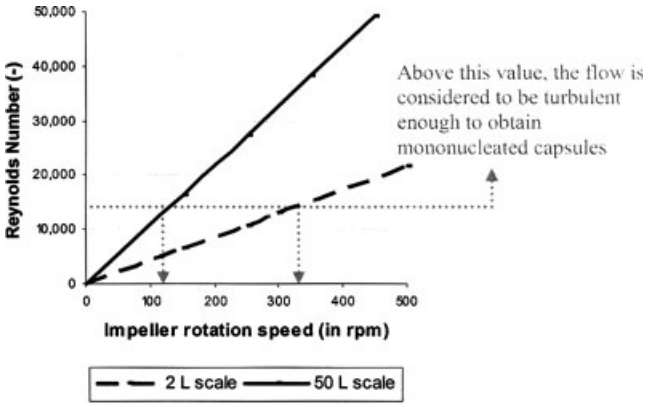

Figure 7. Reynolds number as a function of impeller rotation speed for 2 L and 50 L scale setup's.

times, and all results were in agreement. For this given cooling rate (from 40 to 5°C within 120 min), stirring had to be maintained until the temperature of the dispersion dropped under 13 to 15°C to obtain stable mononucleated capsules.

A minimum stirring time is required to prevent aggregation

The final temperature of the cooling step is of crucial importance. For the system studies by us the temperature should be below 14°C before stopping to apply shear on the system. Otherwise, capsule aggregation could happen due to insufficient gelation of the gelatine, leading to the so-called grape coacervates.

Discussion

Selection of the appropriate emulsification devices

As mentioned previously, to produce an emulsion with the specific properties (10–15 μm), four different emulsification devices were tested. With the condition selected, only the two rotor-stator type devices tested (i.e., the colloid mill and the Silverson high-shear mixer) provided the desirable range and size distribution. We chose the colloid mill as the emulsion device for further studies.

Flow Regime and Explanation of the Polynucleation Mechanism

Difference between the 2 L and 50 L scales

After emulsification, the complex coacervates were prepared by adjusting the pH from above 5.0 to 4.3 under turbulent conditions at >45°C, followed by cooling to 5°C. As shown in Figures 4 and 5, a certain degree of mixing was required during the coacervation process to prevent polynucleation. The capsule size decreases with increasing impeller rotation speed. With two setups of different scale, 2 L and 50 L, mononucleated capsules were not obtained at similar rotation speed values despite a similar geometry of the stirred vessels. Actually the Reynolds number is a better variable than the steering speed. For an impeller system the Reynolds number is defined as:[13]

$$N_{\mathrm{Re}} = \frac{D_a^2 N \rho}{\mu} \tag{1}$$

With N as the rotational speed (in rps), D_a as the impeller diameter (in m), ρ as the fluid density (in kg m^{-3}), and μ as the fluid viscosity (in Pa.s).

The flow in the tank will be laminar if the Reynolds number is smaller than 10 and fully turbulent if the Reynolds number is larger than 10,000. In the transition region the flow will be turbulent close to the impeller and laminar in the remote parts of the vessel. Moreover, when using turbine impellers, it is common to use baffled vessel at Reynolds numbers greater than 2,000 to increase the turbulence zone. With Eq. 1 one can calculate that turbulent flow takes place in the whole vessel for rotation speed above 250 rpm at the 2 L scale, and from 100 rpm at pilot-plant scale (see Figure 7). This confirmed that at equivalent rotation speed, the flow regime in the pilot-plant mixing tank was more turbulent than the flow regime in the 2 L vessel, and generating mononucleated capsules should, therefore, be possible at lower rotation speed at the 50 L scale than at the 2 L scale. When the capsule mean diameter was plotted as a function of the Reynolds number value (see Figure 8), both the 2 L and 50 L scales showed consistent results. So, we propose that the Reynolds number is the right parameter to consider for scalability of the coacervation process.

Polynucleation process: a coalescence-like process

We believe that the preparation of complex coacervates is quite similar to a coalescence process and a theory describing what is happening was elaborated. Right after the acidification step, the shell is formed by covering the oil droplets with a layer of coacervate phase, which finally distributed uniformly around them. However, as long as the temperature of the dispersion does not drop below the gelling temperature of gelatin, this polymer shell remains fluid. If two oil droplets coated with this fluid layer come in contact with each other for a sufficient amount of time, the two fluid polymer shells will merge together and form a bridge between two capsules. Due to capillary forces, the two

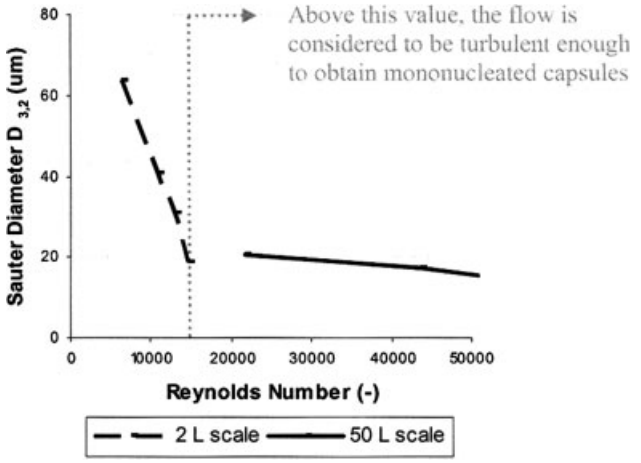

Figure 8. Capsule Mean diameter as a function of the Reynolds number (for 2 L and 50 L scales).

capsules will then get closer and closer to each other and will finally form a more spherical structure enclosing two oil droplets. This process can be repeated and so capsules with more than two oil droplets can be formed. The time that two capsules remain in close contact with each other is determined by the level of turbulence in the vessel. The higher the turbulence level, the smaller the contact time between two capsules will be. This theory can therefore explain why a minimum Reynolds number was needed to avoid polynucleation and to obtain mononucleated capsules (see Figure 8).

Critical stirring time during the cooling step

After capsule formation at a 50 L scale, the dispersion was cooled down from 40 to 5°C within 2 h while stirring. When the stirring was stopped below 14°C, stable mononucleated capsules were obtained. However, above this temperature aggregation occurred (see Figure 6). These results do not fit with the expectation that aggregation could only occur above the gelation temperature of gelatine, which was believed to be between 25 and 35°C. Three reasons might explain the experimental results:

- First, gelatin is a complex combination of several polypeptides and so it is likely that the gelling point of the mixture used was not sharply at 25–35°C, but more widely distributed. This was confirmed by DSC measurements (results not showed).
- Moreover, gelatin, the only compound able to gel in the system, was not in a pure state but was complexed with the gum arabic (which is unable to gel). This almost certainly affected the gelation properties, and it is likely that the gelling point of the complex is lower and wider than the one of pure gelatine.
- Finally, gelling of polymer is not only a function of the final temperature but it also depends on the cooling rate to reach this temperature.

More studies would be required to fully understand the gelation of the polymer shell and to determine exactly when the stirring can be stopped during the cooling stage.

Conclusions and Recommendations

In this article, we have shown how to control the morphology and the size of oil droplets encapsulated in a polymer shell during the complex coacervation process at various production scales. In case of mononucleated capsules, the particle size of the capsule is mainly determined by the parent oil droplet size, which is control by the settings of the emulsification step.

Capsule agglomeration (polynucleation and/or aggregation) could occur during the capsule formation (coacervation step), which led to uncontrolled final capsule size and a size large enough to be sensed (out of specifications). A ''master'' curve could be described showing the capsule mean diameter as function of the Reynolds number during the pH adjustment. For formation of complex coacervates of 12–15 μm in size, the Reynolds number had to be above 15,000 to avoid polynucleation. Below that value, liquid capsules could fuse together resulting in the formation of polynucleated capsules. This reaction, a coalescence-like process, is probably mainly governed by the time two capsules with a fluid shell remain in close contact with each other. Further studies would be required to validate and complete the ''master'' curve (extension to other set-up, other parent oil droplet distribution size, and so on). Moreover, in order to achieve sufficient shell gelation and avoid capsules aggregation during the cooling step, the critical level of turbulence had to be maintained until the temperature of the dispersion dropped below 14°C (for a cooling rate of 35°C in 2 h). More studies are required to fully estimate which cooling rate is optimal under different experimental conditions to achieve full gelation of the capsule shell. From these understandings, it was possible to successfully scale-up the process from bench scale (600 mL) first to intermediate scale (2 L), and finally to pilot plant scale (50 L).

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Notation

o/w = oil in water
w/w = weight by weight
$D_{3,2}$ = Sauter diameter (or surface weighted mean diameter)
$D_{4,3}$ = volume weighted mean diameter
$D_{0.X}$ = percentiles of the (cumulative) volume distribution
$D_{0,1}$, $D_{0,5}$ and $D_{0,9}$, respectively, 10, 50 & 90% of the volume of the particles is contained in particles with a diameter smaller than $D_{0,1}$, $D_{0,5}$ and $D_{0,9}$
N_{Re} = Reynolds number
N = rotational speed, in rps
Da = impeller diameter, in m
ρ = fluid density, in kg.m^{-3}
μ = fluid viscosity, in Pa.s
pI = isoelectric point

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