Emulsification and microencapsulation: State of art

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Abstract

Encapsulation processes generally involve dispersing a liquid and solidifying the droplet. The contribution describes the different microencapsulation methods based on emulsification. It looks to provide some information on the advantages and limitations of the emulsification for encapsulating biological or biochemical material.

Keywords: emulsion, static mixer, gelation

1 Introduction

Encapsulation was proposed more than 50 years ago to immobilize, protect, release and functionalize diverse types of materials. The process could generally be divided in two steps: liquid core dispersion and encapsulation it-self of the dispersed material (called solidification below). During solidification, the dispersed phase is gelified or a membrane is formed around the droplets of particles. The dispersion could be done in the air (spray, extrusion, grinding) but also inside a non-miscible phase (mainly emulsification). Both methods have advantages and disadvantages. Obviously, emulsification is required when an interfacial reaction between two non-miscible liquids is involved or when encapsulation proceed by coacervation (precipitation of polymers at the droplet surface). Emulsification allows very large production (up to tons per hour) even for very small microcapsules (down to a few micrometers). However, there are some drawbacks with the emulsification methods. The size dispersion is always large. In most cases of bioencapsulation (aqueous core), the continuous phase is generally an oil phase. Washing of the capsules may be a tedious problem and may cost as much as the capsules it-self. The present contribution will try to give an overview of the different encapsulation technologies linked to the emulsification and give some rules along when and how to use them.

2 Dispersion methods

2.1 Batch systems

Emulsification is generally done in a batch reactor equipped with impeller or a rotor-stator system. The most usual system at laboratory and pilot scale is the Rushton's turbine reactor (Figure 1). Such a system optimizes the shear and then the drop breakage. Without baffles, the turbine entrains the liquid and the mixing/dispersion effect is reduced. On the other hand, baffles prevent vortex and foam formation.

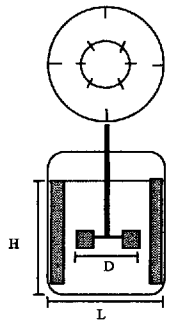

Figure 1:
Rushton type reactor. Reactor description: Cylindrical reactor equipped, D: turbine diameter, L: reactor diameter (D*2), H: liquid height (D*2); Turbine: 6 vertical blades located at H/2, Size D/4*D/4; Baffles: 4 located at distance D/10 of the reactor wall, Width = D /5

At industrial scale, the type of impeller is often different, looking more like a marine impeller. Suppliers generally provide their equipment with characteristic numbers to define the mixing regime.

In bioencapsulation, the continuous phase is generally vegetable or mineral oil. The viscosity ranges from 30 to 60 mPa·s and the interfacial tension with aqueous dispersed phase is quite important ($45 \cdot 10^{-3}$ N/m). A lipophilic emulsifier such as Span 80 (1 %) is often added to the mixture to reduce the interfacial tension and get lower size distribution. For one defined system (reactor, phase composition), the size distribution is mainly determined by the rotational speed of the impeller. Figure 2 presents some data showing the influence of the viscosity of the dispersed phase on the mean size.

The size distribution is generally defined by a log-normal distribution. An interesting parameter to define the size dispersion is the span corresponding to equation 1.

$$span = \frac{d_{90} - d_{10}}{d_{50}} \qquad (1)$$

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where d_{90}, d_{50} and d_{10} are the diameter corresponding to 90, 50 and 10 % of the cumulative size fraction (generally given as volumetric fraction). Span takes into account a large part of the particles and is not sensible to the extremes (very small and very large particles). It is quite reproducible between samples. It could be related to usual (but confusing) standard deviation by dividing it by 2.67.

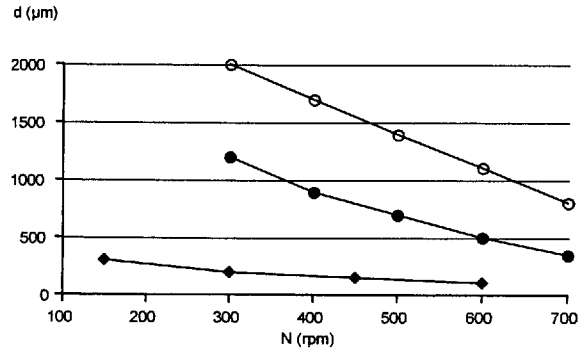

Figure 2:
Mean size of nylon microcapsules (diamond) and κ-carrageenan beads (2 % filled circles, 3 % open circles) from Poncelet et al. 1990a and Audet et al. 1989

The span may range from 67 % to 160 % of the mean (Figure 3). Kolmogoroff's theory (see below) predicts a value of 80 %. The size dispersion is lower while reducing the mean size. It is also improved if the two liquids have more similar physical properties (density, viscosity). Interfacial tension has little influence on the size distribution.

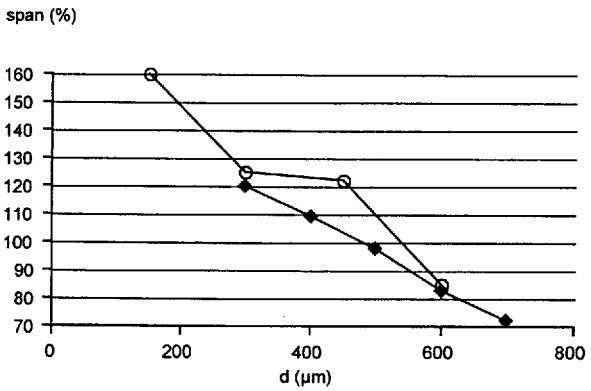

Figure 3:
Standard deviation of nylon microcapsules (open circles) and κ-carrageenan beads (diamonds) in function of the size

2.2 Continuous systems (static mixer)

Several disadvantages of the batch system are:

- Cleaning and filling reactor: these tasks reduce the productivity
- Limitation for scale-up: it is difficult to expect reactors larger than 1 cubic meter.
- Emulsifying viscous liquid in turbine reactor requires 5 to 15 minutes of mixing.
- Good emulsification is linked to high shear that may damage biological cells

Poncelet et al. (1993) then proposed to realize the emulsification using continuous systems based on static mixers (Figure 4). The static mixers consist of a series of stationary elements placed transversely in a tube. These elements form crossed channels that promote division and longitudinal recombination of the liquid flowing through the static mixer. While applied to a two-phase system, emulsion is formed. Figure 4a shows an example of installation using a static mixer. Figure 4b demonstrates the power of the static mixer for emulsification. Passing through 10 elements Kenics mixer (series of single elements), the flow is divided 128 times longitudinally and 128 times transversally. Sulzer mixers (Figure 4 c) are formed by several stationary elements and are even more efficient.

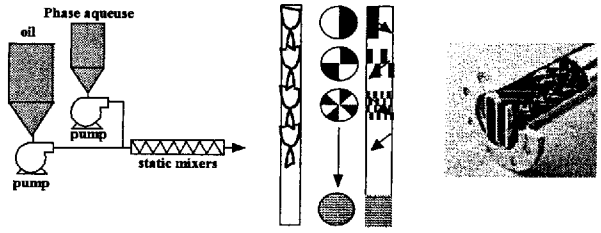

Figure 4:
Static mixers: a) installation, b) emulsification, c) Sulzer mixers

The system could be run continuously, scale-up was realized by simply increasing the tube diameter, even for viscous system, residence or emulsification times could be as low as 0.2 seconds while shear stress was quite low (Poncelet et al, 1993).

With Sulzer mixers, 5 to 10 elements are needed to get fine emulsion. Due to the higher dispersion power of static mixers in regard to turbine mixers, similar mean size could be reached without the use of emulsifier. This constitutes an unexpected advantage of the static mixers.

2.3 Size distribution prediction

Because of the variety of designs, one may be confused by the number of different correlations proposed to describe the dispersion in mixing devices. However, most equations are based on Kolmogorov's theory. The droplet sizes are deduced from the size of

the eddies formed by the agitation. This assumes that the mixing regime is turbulent. The mean size is then given by equation 2.

$$\frac{d_{32}}{D} = A\ W^{-0.6} \qquad (2)$$

where d_{32} is the mean Sauter diameter , D is the reactor diameter, A is a constant function of the system, We the Weber number. The mean Sauter diameter, d_{32}, is defined by equation 3.

$$d_{32} = \frac{\sum_i n_i\ d_i^3}{\sum_i n_i\ d_i^2} \qquad (3)$$

where n_i is the number of droplets having the diameter d_i. The mean Sauter diameter, d_{32}, is equal to the inverse of the specific surface or the surface per unit volume of dispersed phase. The Weber number, We, defines the ratio between inertial force and interfacial surface force and is given by equation 4.

$$We = \frac{\rho_c\ u^2\ D}{2\ \sigma} \qquad (4)$$

where ρ_c is the density of the continuous phase, u the linear liquid-to-blade velocity and σ is the interfacial tension between the two liquids. For turbine, linear liquid-to-blade velocity is given by (π D N), where N is the rotational speed of the impeller. For static mixer, it will be given as the ratio of the flow rate divided by the mixer section.

In fact, one may observe that equation 2 assumes that most of the dispersion energy is linked to increase interfacial surface. However, encapsulation generally involves a viscous liquid (especially for internal phase) and the mixing regime could be laminar or transitional. The viscosity of the continuous phase and the dispersed phase may influence the size distribution. Equation 2 may be rewritten (equation 5), based on both mechanistic analyses and empirical correlations (Hass 1987).

$$\frac{d_{32}}{D} = A\ We^{-0.6}\ \mathrm{Re}^{-0.2} \left(\frac{\mu_d}{\mu_c}\right)^{0.5} \qquad (5)$$

where μ_c and μ_d are the viscosities of the continuous and dispersed phase. The Reynold number, Re, is representative of the ratio between the inertial forces and viscosity forces (equation 6).

$$\mathrm{Re} = \frac{\rho\ u\ D}{\mu_c} \qquad (6)$$

Equation 1 (or 5) described above are valid to calculate the mean diameter for turbine reactor and static mixers. The value of constant A is function of the design of both the impeller, reactor and/or static mixers. For Kenics mixer, one could use 1.2 (Haas 1987) and for SMV Sulzer mixer 0.2 (Streiff 1977). Lower sizes could then obtained with Sulzer mixer. For a more complete review, we advise readers to read the series of papers from Calabrese et al. (1986) for turbines and Legrand et al. (2001) for static mixers.

Referring to the Kolmogoroff's theory, the size distribution of eddies in the reactor determine the size distribution droplets formed by liquid breakage in turbulent system. This leads to a distribution following a lognormal law. The frequency is then defined by equation 7.

$$f(z) = \frac{1}{\sqrt{2\pi}\ \sigma_z} e^{-\frac{(z-\bar{z})^2}{\sigma_z^2}} \qquad (7)$$

where z = log(d) and σ_z is the standard deviation of z. The span corresponds to 80 % of the mean.

3 Encapsulation methods

3.1 Interfacial polymerization and polymer cross-linking

Interfacial polymerization consists of dispersing a solution of one monomer in an immiscible solvent and adding a second monomer soluble in the continuous phase. A typical example is the reaction of a diamine (water-soluble) with a diacid chloride (oil soluble) (Wittbecker 1959). The reaction generally requires a solvent of high polarity (chloroform) and high pH value (> 11). It was then necessary to improve the method to apply it to biocatalyst encapsulation.

Three laboratories was involved in this research: TMS Kondo (Wakamatsu, 1974) in Japan, MC Levy (Guerin 1983) in France and R. Neufeld (Groboillot 1993) in Canada. Their results were quite similar. To avoid drastic conditions, the process must involve low pKa amine (like amino acid) and polyamine (like proteins, polyethyleneimine) (Poncelet 1990b).

The process which then corresponds more to a polymer cross-linking, could be performed at pH as low as 8 and with a solvent like vegetal oil. The weak point remains the use of a strong cross-linker such as diacid chloride, which could react with the encapsulated material near to the surface. Moreover, diacid

chloride frees acid chloride by reaction with water and the polyamine. The pH then drops quickly inside the capsule (Hyndman 1993). Use of thiocyanate as a cross-linker allows avoiding pH drop but capsules are not as strong. Preliminary studies show that diacid anhydride could be a good candidate to replace diacid chloride. At this stage, interfacial polymer cross-linking has been proved successful for enzyme encapsulation (Monshipouri 1992) but still limited for cell encapsulation (Hyndman 1993).

Interfacial polymerization may also be used to form oil loaded microcapsules by dispersing the hydrophobic monomer solution in an aqueous solution and then adding a water soluble monomer. This could be useful for encapsulation of some enzymatic systems (such as lipase for ester synthesis). Another use may be as a carrier of hydrophobic component. Silicone loaded microcapsules have been proved very efficient for transferring oxygen in bioreactor (Poncelet, 1993).

3.2 Coacervation

Coacervation relates to polymer precipitation. In the frame of bioencapsulation, two processes have been mainly developed. Prof. TMS Chang has developed a method (Chang 1966) consisting of dispersing an aqueous phase in an ether solution of cellulose nitrate to yield to a water-in-oil emulsion. The cellulose nitrate is precipitated by addition of a non-solvent (n-butyl benzoate). As only one polymer is involved, this is called simple coacervation. The process is not easy to control. The size distribution is a function of the mixing conditions but also the physico-chemical properties during coacervation (Poncelet 1989). The toxicity of the solvent involved in the process restricts its use to enzyme and biochemical encapsulation.

Complex coacervation involves the precipitation of two or more polymers together. The most well known example is the emulsification of oil in water phase containing arabic gum and gelatin. By dropping the pH, the charge of the gelatin becomes positive and a complex is formed with the negative arabic gum (Madam 1972). This method was extensively used in industry for carbonless copy paper and aroma encapsulation. It could be of interest for enzyme release in consumer products.

3.3 Thermal gelation

Thermal gelation is probably the most usual and simple method of encapsulation by emulsification. A κ-carrageenan, agar or agarose solution is dispersed in oil at 45°C and the temperature is dropped to cause gelification (Scheirer 1984, Tosa 1979). Suppliers now provide low temperature (28-30°C) gelling material which allows encapsulation of even fragile animal or plant cells.

Dropping the hot gelling solution in cold water forms thermal gel beads but emulsification in oil allows larger scale (Audet 1989). The use of static mixers even opens production to a very large scale (Descamp 2002).

3.4 Ionic gelation

Since its introduction (Kierstan, 1977), alginate beads remains the favorite system for cell entrapment. The dropping technologies have been strongly improved and allow now relatively large production. However, for very large scale (cubic meters), especially for small microcapsules (down to 25 μm), an emulsification method is still beneficial. The key point is that alginate gelifies in the presence of calcium ions. The question remains of how to transfer calcium through an oil phase.

Paul Heng (Chan 1990) proposed to add a calcium chloride solution to an alginate solution-in-oil emulsion. However, the transfer of the calcium is critical. The bead size is very inhomogeneous and the shape of the capsules is not spherical. A better alternative is to introduce an insoluble calcium source ($CaCO_3$) into the alginate solution before dispersing it in the oil. By addition of a small volume of acetic acid (soluble both in oil and water), the calcium is released provoking gelation of the dispersed alginate droplets (Poncelet, 1992). The calcium vector must be well selected and in the form of a very fine powder (2 μm). It is possible then to provoke gelation in a very small pH range (dropping from 7.5 to 6).

4 Transfer and washing of capsules

In many applications, traces of organic solvent, even vegetable oil, will be a source of problems. As for example in beer production, even a small amount of hydrophobic liquid could alter the taste and influence foam formation. At lab scale, the transfer and washing of the microcapsules from an organic solvent could be performed using three main methods:

- Removing as much as solvent as possible, transferring the microcapsules to a high concentrate water soluble emulsifier (Tween 20 at 50 %), dilution with water, filtration and resuspension in water
- Transferring the bead-in-oil suspension in a baker containing some water and gently mixing the interface between the phase with a flexible

tube to allow a settling of capsules to the lower aqueous phase.
- Filtrating the bead-in-oil suspension on nylon mesh (40 µm) under vacuum, washing of microcapsules by spraying water on the filter.

The selection of the method depends on the type of microcapsules and solvent. However, while scaling-up the production, the transfer and washing may become a critical point. The volume of water needed to get clean capsules could be important (200 ml for 20 g of capsules) and the cost associated with this step as high as the microcapsule cost it-self. If some data exists at the industrial or pilot scale, they don't seem to have been published.

5 Conclusions

This review is not expected to be complete but to give a good introduction of the potential and limitation of the microencapsulation using emulsification as dispersing techniques. When to use emulsification methods? If one of the extrusion or dropping methods could fit your needs, don't use emulsification. It will allow narrower size dispersion and it will reduce the washing problem. However, extrusion methods don't allow reaching as large scale as emulsification.

On the other hand, sometimes, emulsification setup may be simpler than extrusion (for example during thermal gel bead preparation). Very strong capsules may be obtained by interfacial polymerization. Emulsification is the first method useful for oil-loaded capsules.

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