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Immobilized Cells: Basics and Applications

Fundamentals of dispersion in encapsulation technology

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Introduction

Bioencapsulation involves gelation of, or membrane formation around aqueous drops containing biocatalysts, leading either to hydrogel beads or microcapsules. Early techniques involved the formation of gel beads by dispensing droplets of the pre-gel suspension containing the immobilizant into a hardening solution.[1]. A finer dispersion was achieved by emulsifying the gel in an oil phase and hardening of the drops by internal gelation [2]. Membrane microencapsulation involved dispensing droplets of an ionically charged gel solution into a polymer solution with opposite charge [3]. Research has also been directed toward encapsulation of cells via interfacial polymerization around dispersed droplets emulsified within a non-aqueous hydrophobic fluid [4]

In all cases, the first step of the bioencapsulation process requires the dispersion of the internal phase within the gel-hardening or membrane forming solution, followed by gelation or membrane formation. Two methods of dispersion are usually applied in cell immobilization, extrusion and emulsification. In the extrusion technique, solutions are extruded through a small orifice or needle, permitting the formed droplets to freely fall into a hardening solution. In the emulsification technique, solutions are mixed and dispersed in a non-miscible phase. When the dispersion equilibrium is reached, hardening or membrane formation are initiated by cooling or addition of a chemical to the emulsion.

Industrial production not only implies scale-up, but also the need to control the mean size and size dispersion of the droplets. Both gelification or membrane formation [5] and final properties of the capsules (resistance, mass transfer [6]) are correlated by the initial dispersion quality. The selection of mean size (large or small) is a function of the applications (continuous flow bioreactor or cell implantion). In all cases, size dispersion will constitute an hindrance to the process control.

Cell encapsulation is intended to provide protection from shear forces prevailing in the bioreactor or during handling procedures. However, bead or microcapsule formulation itself involves dispersive forces. Equipment for the generation of liquid droplets must then be designed to minimize the stress applied to the cells, while achieving a homogeneous distribution of the cells within the carriers.

Most published studies involving biocatalyst encapsulation have been restricted to a small scale, often to a few milliliters. Actual industrial encapsulation processes (drugs, pesticides...), presently conducted on large scale, involve atomization, batch emulsification and centlifugal techniques, and are often not suitable for live cell encapsulation due to shear effects.

The present contribution will review the different aspects of the dispersion process, and parameters will be proposed for selecting one dispersion alternative as a function of the specific objectives.

Extrusion method

The extrusion methods, applicable in biocatalyst encapsulation, may be divided in three classes : drop method, capillary jet breakup and spinning vibrated disk.

The drop method

The simplest method for dispensing individual liquid droplets is to permit the liquid to fall from the tip under the gravity force. The droplet diameter, d, may be computed by equalizing the gravity force with the product of the interfacial tension, γ, and the perimeter of the tip, π de (Tate's law):

$$\frac{\pi}{6} d^3 \rho_d g = \pi d_e \gamma \Phi^3 \text{ or } d = \left(\frac{6 d_e \gamma \Phi^3}{g \rho_d} \right)^{1/3} \quad \{1\}$$

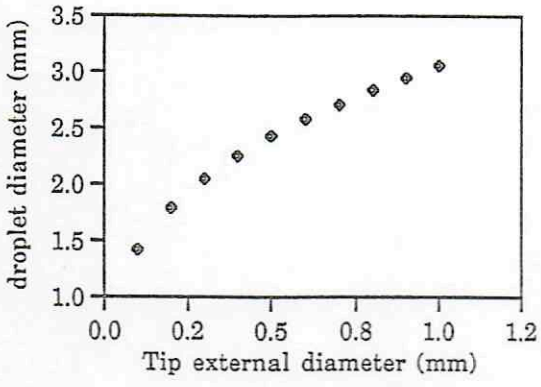
where ρ_d is the density of the dispersed phase, g is the gravity constant, d_e is the external tip diameter and γ is the liquid surface tension. The factor Φ ($\approx$0.85) takes into account that the drop stretches out before breakage leaving a portion of the pendant drop behind [7].

Figure 1 illustrates the droplet size, d, computed as a function of the needle diameter. The diameter of droplets obtained by extrusion under gravity is still larger than 1 mm, even with very small needle diameters.

Lane [8] proposed in 1947, the application of an air jet around a needle to increase the force available to break a nascent drop from a suspending tip. In the case of a coaxial air jet system, the droplet diameter may be obtained by equalizing the sum

of the viscous drag force (laminar effect of the fluid), the kinetic energy dissipation term (impact of the turbulence on drag), gravity forces with the surface tension force [9] :

$$3\pi\mu u d + 0.055\rho u^2 d^2 + \frac{\pi}{6} d^3 \rho_d = 2\pi d_e \gamma \Phi^3 \qquad \{2\}$$



Figurel. Diameter of droplets formed by extrusion under gravity

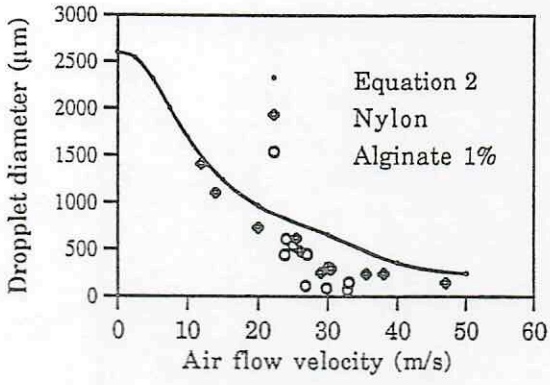


Figure 2. Droplet formation by extrusion under air jet

Experimental data obtained from air jet produced nylon microcapsules [10] and alginate beads [11] (Figure 2) agree for a large part with Equation 3. However, simple assumptions (drop diameter larger than tip, spherical nascent drop, no lateral fluid effects), used to build Equation 3, are not competely correct with increased turbulence. The droplets size dispersion increases and small droplets called satellites are formed (up to 10 %v of the beads) (Figure 3).

Goosen [12] applied high static potential between the capillary and the collecting solution to get droplet size reduction. The process of drop formation is not completely defined and involves electric field forces but also probably the repulsive forces due to the charge accumulated at the drop surface which counteract the surface tension. The standard deviation is generally lower than with air jet but satellite droplets are also formed [13].

With all the drop methods, the liquid exits from the tube as a jet if the liquid in the capillary tube exceeds a certain velocity, u_j :

$$u_j \geq 2\sqrt{\frac{\gamma}{\rho\, d_j}} \quad \text{and} \quad Q = \frac{\pi}{4} d_j^2 \qquad \{3a,b\}$$

where d_j is the jet diameter, Q is the maximum volumetric droplet flow. For all

drop diameter values (Figure 4), the production rate remains quite small. The limited production rate constitute is the largest limitation for the use of the drop technique in a large scale process. Moreover, quasy monodispersion is reached only for large beads (>1 mm).

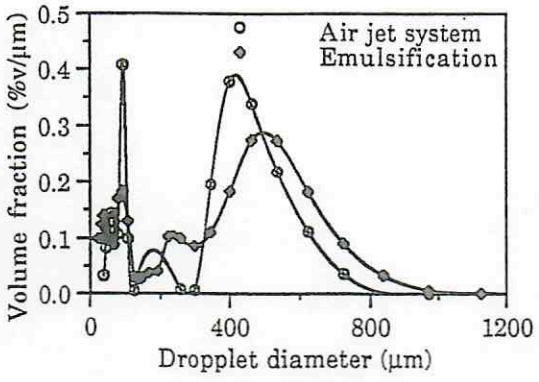


Figure 3. Bead size distribution

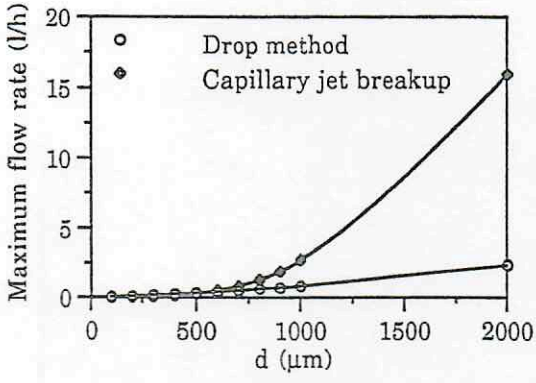


Figure 4. Maximum production rate

Breakup of Capillary Jet

When capillary jets are formed at a tip, they show instablility and break easily forming small droplets. Rayleigh [14] vibrated the jet with sonic waves, which at an optimum frequency, f, lead to a stream of uniform droplets. The optimum condition for jet breakup may be written as :

$$\lambda = 4.058\ d_j \quad \text{with} \quad \lambda = \frac{u_j}{f} \quad \text{and} \quad d = 1.89\ d_j \qquad \{4a,b,c\}$$

The diameter, d, of the resulting drop is determined by assuming that the volume of the drop equals the cylinder having a diameter equal to the jet diameter, d_j, and a height equal to the wavelength, λ. The drop size is thus directly correlated with the jet diameter.

The jet velocity may be freely selected until the vibration frequency is adapted to maintain the wavelength at the optimum value. However, the jet velocity must be lower than the terminal velocity of the droplets, to avoid collision and coalescence between drops. The maximum flow rate in function of the drop diameter is then obtained by equating the jet velocity with drop terminal velocity (figure 4). For large beads, the production rate may be significantly larger (up to 30 l/h) than with the drop technique. Large scale production may be obtained with the capillary jet breakup by multiplying the number of nozzles (to reach hundred

liters per hours for a few nozzles).

For small beads (under 800 µm), the number of nozzles to reach large production rates would be too large to constitute a simple solution. Moreover, the pressure required to insure the jet minimum velocity becomes high (several atmospheres) when the orifice diameter decreases and constitute a limitation for fragile cell encapsulation. Satellite beads are also difficult to avoid.

Spinning vibrating disk

When a liquid flows on a spinning disk, the liquid may leave the disk as small jets or ligaments (Figure 5). These ligaments have a behaviour similar to jets escaping a classical orifice [15]. By applying a well designed wave on the liquid flowing on the spinning disk, the jets are broken in small and very uniform droplets (standard deviation lower than 5 %). Under certain conditions (correct wave amplitude), formation of satellite particles is avoided.

Equations directing the process are more complex than with classical jet rupture. Physcal properties of the liquid (density, surface tension, viscosity), design of the rotating disk (size, rotating speed) and wave (frequency and amplitude) must be adjusted to reach correct particle size and optimum conditions for low dispersion [16]. Typically, on a disk of one centimeter turning at 2000 rpm, around 60 ligaments are formed. Drops of 300 to 400 µm are produced at a flow rate of 6 to 8 l/h. In optimum condition, the droplet diameter is proportional to the spinning disk diameter and the production rate islinked to 5/3 power of the droplet diameter.

Such a droplet generator would offer a solution for large scale production of microdroplets (less 800 µm). In this condition, the production rate is increased by two orders of magnitudes with regard to the simple vibrating orifice technique. In addition, high pressure is not required to cause the liquid to flow on the disk.

Emulsification process

If hydrogel beads are generally formed by the drop method, membrane encapsulation procedures are usually based on emulsification of the core material in a non-miscible phase. Membrane is formed by either interfacial coacervation or polymerisation. Due to scale-up problems with the drop methods, several authors have also considered emulsification as a potential technique for bead formation [17]

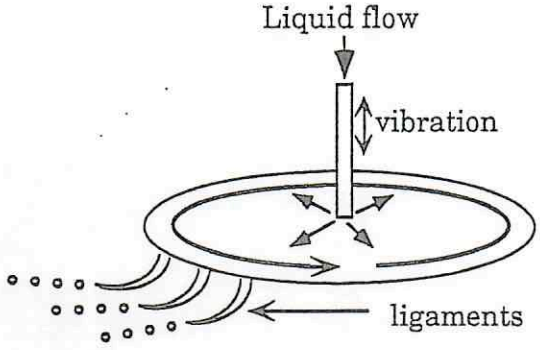


Figure 3. spinning rotating disk device

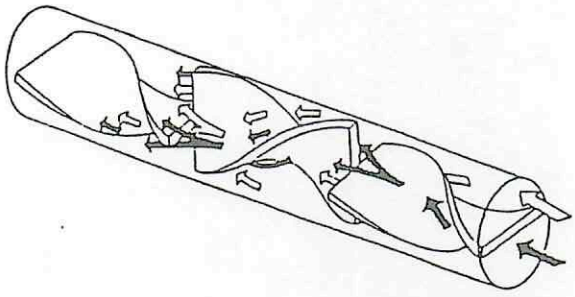


Figure 4. Static mixer (Kenics)

In emulsification, droplets are not formed one by one, but rather in terms of millions by millions. The equations are thus built on statistical basis, partially from mechanistic models and partially from empirical correlations. The fundamental analyses are principally based on the work of Kolmorogov [18] and Hinze [19]. These authors stated that the energy dissipated in a turbulent flow creates a viscous stress or a dynamic pressure which tends to break the drop. The surface tension force and the internal drop viscosity counteract these deformations .

$$\frac{d}{D} = k \ We^{-0.65} Re^{-0.2} \left(\frac{\mu_d}{\mu} \right)^{0.5} \quad \text{with} \quad We = \frac{D \rho u^2}{\gamma} \quad \text{and} \quad Re = \frac{D \rho u}{u} \quad \{5a,b,c\}$$

where D is the hydraulic diameter of the dispersion device (impeller diameter for the turbine reactor, internal diameter for the static mixer, see below), k, a constant function of the design, μd, the dispersed phase viscosity. We and Re are the Weber and Reynold's numbers.

Equation 5 assumes that the parameters defining the final drop size are essentially the rotational speed of the impeller, the viscosity of both continuous and dispersed phases and the interfacial tension. However, fitting of data on this equation has not been successful. Equation 5 was designed for low viscous phases and Newtonian fluids. Gel and pre-encapsulating solutions may behave in a non-ideal manner. The swelling or shrinkage of droplets during gelification and/or polymerization of the membrane constituents may lead to smaller or larger diameters than expected.

The theoritical size dispersion with emulsification is a log normal distribution with a standard deviation of around 35 %. In real cases, the standard deviation may be larger (up to 50 %) and a satellite peak appears that may represent up to 10 % of the microcapsules volume. Ideally, gelification should take place rapidly, but only after emulsion equilibrium is reached. In other cases, the main peak is divided in several peaks. The mean size is also no more correlated with Equation 5.

The reactors used for emulsification are usually cylindrical vessels, mixed by means of various impellers (turbine, marine-style impeller or grid device) [2]. In such devices, shear, energy dissipation, and dynamic pressure are not homogeneously distributed. Dead volumes or stagnant zones may be present, as the vessel volume increases or when mixing viscous fluids such as gels.

One alternative to minimize these problems may be provided by static mixers. These devices consist of a series of stationary elements mounted lengthwise in a pipe (Figure 6). The elements form intersecting channels that split, rearrange and recombine component streams into smaller and smaller layers. Mean diameters of the dispersed phase ranging from a few microns to 1 mm may be produced and the droplet size defined by an equation similar to that of Equation 5 derived from mechanistic models [20]. As homogeneous shear is applied to the whole liquid, dispersion will then lead to narrower size distribution. Moreover, as high shear is not applied near the impeller, the static mixer would be more suitable to encapsulate fragile cells.

A prelimary study of static mixers to produce carrageenan beads [21] show that beads of 500 ~m may be produced at 10 l/h with a 13 mm static mixer. Mean size may be easily controled by the linear velocity in the tube. The scale-up is simply realized by increasing the static mixer diameter. The production rate is correlated with the square of the mixer diameter (150 l/h for 5 cm static mixer).

Emulsion technology has several drawbacks. The size dispersion is generally larger than with the extrusion techniques. The resulting beads or microcapsules need to be transfered from organic solvents (generally vegetal oil) and washed. Prediction of the size is more complex and experiments are needed to design the device. However, when large production is required, such as cubic meters, emulsification appears as the best or presently the one solution.

Conclusions

The need for a dispersion system may be divided into four categories :
- in laboratory scale, capillary jet breakup allows production of small but very

uniform dropplet batchs,
- at larger scale (up to hundred liters) and for large beads (1 to 3 mm), the capillary jet breakup constitue also a simple solution,
- for similar production but lower size, the spinning rotating disk represent a more promising solution,
- finally, if largest production is required, or involving an interfacial process, the engineer may consider emulsification process ~ith static mixer technology.

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