Preparation of Hemolysate-filled Hexamethylene Sebacamide Microcapsules with Controlled Diameter

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Hemolysate-filled hexamethylene sebacamide microcapsules (nylon-6,10 membranes) were prepared by interfacial polymerization. Sheet lattice, frame lattice, turbine and marine impellers were tested to control the microcapsule mean diameter and size distribution parameters. Mean diameters ranging from 40 to 200 μm were obtained depending on the impeller type, rotational speed and emulsifier concentration. The size dispersion of microcapsule preparations was best controlled with a frame lattice impeller, achieving standard deviation values less than 40 % of the mean diameter. The wire diameter and mesh dimensions of the impeller lattice had little effect on the distribution parameters.

Des microcapsules de sébacamide d'hexaméthylène remplies d'hémolysat (membranes de nylon de 6,10) ont été préparées par polymérisation interfaciale. On a testé des treillis en feuilles, des treilles encadré, des turbines et des hélices marines pour contrôler le diamètre moyen des microcapsules et les paramètres de distribution de taille. Des diamètres moyens compris entre 40 et 200 μm ont été obtenus en fonction du type de mobile, de la vitesse de rotation et de la concentration d'émulsifiant. La dispersion de taille de préparations de microcapsules était mieux contrôlée avec un mobile à treillis encadré, les valeurs d'écart-type obtenues étant inférieures à 40 % du diamètre moyen. Le diamètre du fil et les dimensions de la maille du treillis du mobile avait peu d'effet sur les paramètres de distribution.

Keywords: hemolysate-filled microcapsules diameter control, size distribution control, microencapsulation, interfacial polymerization.

Enzymes, living cells and biologically active materials may be advantageously immobilized and retained within ultrathin, semipermeable membranes by the process of microencapsulation. The biocatalyst is thus protected from wash-out, shear damage or harmful environmental effects (Tramper, 1985). The microencapsulation process must provide capsules with a uniform and optimum size using techniques and equipment compatible with industrial scale.

Medical applications frequently require small microcapsule diameters, ranging from less than 10 μm for artificial red blood cells (Arakawa and Kondo, 1975 and 1980) to 50 μm for uses in controlled release of drugs (Chang, 1977). For immobilization of plant cells with diameter up to 200 μm (Jack and Zajic, 1977), larger microcapsules or beads (200 to 500 μm or more) are obviously required.

The performance of a process involving biocatalyst immobilized within spherical matrices depends on the mean size but also on the size distribution. Characterization and control of processes such as fluidization (Dueck et al., 1986), mass transfer (Poncelet et al., 1988), and shear breakage (Poncelet and Neufeld, 1989) requires microcapsules with a narrow distribution.

One technique for preparing microcapsules (Chang et al., 1966) consists in first dispersing the enzyme or drug solution, or the suspension of microorganisms into an organic phase. Following emulsification, membrane formation at the interface results from a polycondensation reaction due to the addition of reactive monomers in the aqueous and organic phases. Nylon-6,10 membranes are formed by reacting sebacoyl chloride dissolved in a water-immiscible solvent, with a aqueous solution of 1,6 hexamethylene diamine, resulting in a poly-hexamethylene-sebacamide polyamide film at the interface. The non-aqueous solvent is then removed leaving intact membrane bound microcapsules. The emulsification procedure is the primary determinant step in establishing the size distribution of microcapsules (Koishi et al., 1969). This step may be influenced by reactor and mixer geometries, in addition to variations in physicochemical properties, such as chemical composition, interfacial tension, respective viscosities, dielectric constants, specific gravities, and pH of the two phases in contact, leading to variations in size distribution (Shigeri et al., 1970) of the dispersed material.

Kondo and fellow workers (Koishi et al., 1969; Shiba et al., 1970; Shigeri et al., 1970) studied the effect of polymerization conditions on the size of polyamide microcapsules having a mean diameter ranging from approximately 2 to 10 μm. Several authors (Chang et al., 1966; Mori et al., 1972; Rambourg et al., 1982) published data demonstrating a relationship between diameter of nylon microcapsules and stirrer speed or concentration of emulsifying agent. Chang et al. (1966) operated with a Jumbo magnetic stirrer, and concluded that at the higher speeds, diameters of collodion and nylon microcapsules tended to be smaller and more uniform, this effect being most marked with nylon. Increasing the concentration of emulsifier above 5.0 % V/V had little effect on microcapsule size. Mori et al. (1972), using an Omuni mixer (Ivan Sorval Inc., USA) at a stirring rate from 600 to 2000 rpm, prepared microcapsules ranging from 500 μm to less than 10 μm, the Span 85 concentration varying from 0.5 to 5.0 % V/V. Rambourg et al. (1982), testing a stirring motor (Heidolph type RZR II, adaptation system type RK6) and a three bladed screw, reported that increasing the concentration of surfactant from 1.0 to 2.0 % V/V had little effect on the average size (87–73 μm) but resulted in a more confined diameter range. When the stirring speed was raised from 450 to 1200 rpm, the size of the capsules decreased regularly from 110 to 63 μm and the distribution of their diameter became more and more homogeneous. The volume of material prepared in all the studies noted above was less than 3 ml, and the conditions under which the studies were performed were not suitable for scale-up consideration if large volumes of microcapsules were desired.

In the present study, the microcapsulation of hemolysate within nylon membrane microcapsules was studied. The influence of the emulsification conditions for the purpose of controlling the diameter and distribution parameters of the

TABLE 1
Geometry and Characteristic Dimensions of Impellers and Vessel

Name	Blade number	D (mm)	H (mm)	W (mm)	Wire diameter (mm)	Mesh size (mm)
Sheet Lattice-1	4	45	55	—	0.2	1
Sheet Lattice-2	4	45	55	—	0.4	1
Frame Lattice-1	2	45	55	10	0.2	1
Frame Lattice-2	4	45	55	10	0.2	1
Turbine	6	38	5	7	—	—
Marine	3	34	5	16	—	—
Vessel		50	95			

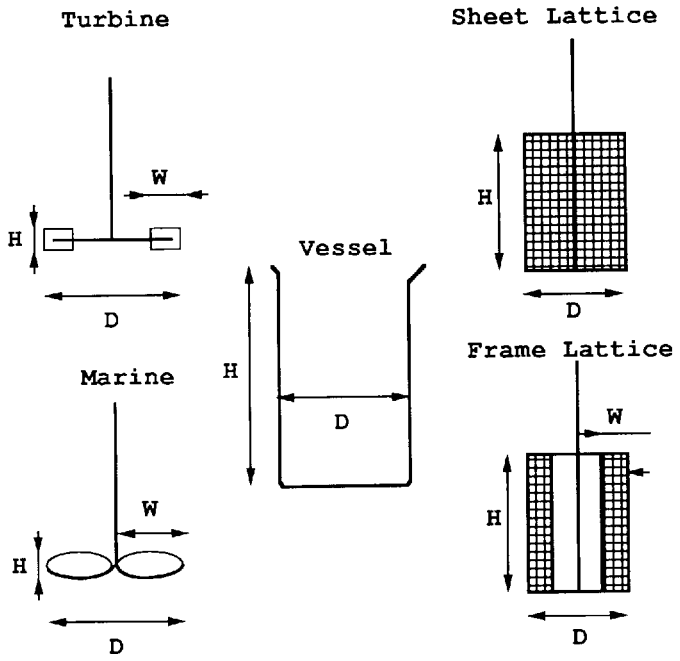

Figure 1 — Geometry and characteristic dimensions of impellers and vessel.

encapsulated preparations was examined to establish optimum equipment configuration and design parameters suitable for scale-up considerations.

Materials and methods

Hemolysate-filled microcapsules were prepared as outlined by Chang et al. (1966). Equal volumes of hemolysate and 1, 6-hexamethylene diamine solution were dispersed in a chloroform/cyclohexane 1/4 V/V organic phase, with the aid of an emulsifier (Span 85), to yield a water-in-oil emulsion. A polymerization reaction was initiated at the interface by introducing sebacoyl chloride to the continuous organic phase. Organic solvent was then removed with a surfactant (Tween 20), and the aqueous microcapsule suspension washed several times with water and sampled for size distribution determination.

A few modifications were introduced to the emulsification procedure, involving use of a Corning 1060, 200 ml beaker. Several impeller geometries: turbine, marine, and sheet or frame lattice impeller, were used in the mixing studies. Characteristic dimensions of the impellers and the vessel are given in Table 1 and Figure 1. The frame was constructed with a similar height and diameter to that of the reactor. The turbine and the marine impeller dimensions and

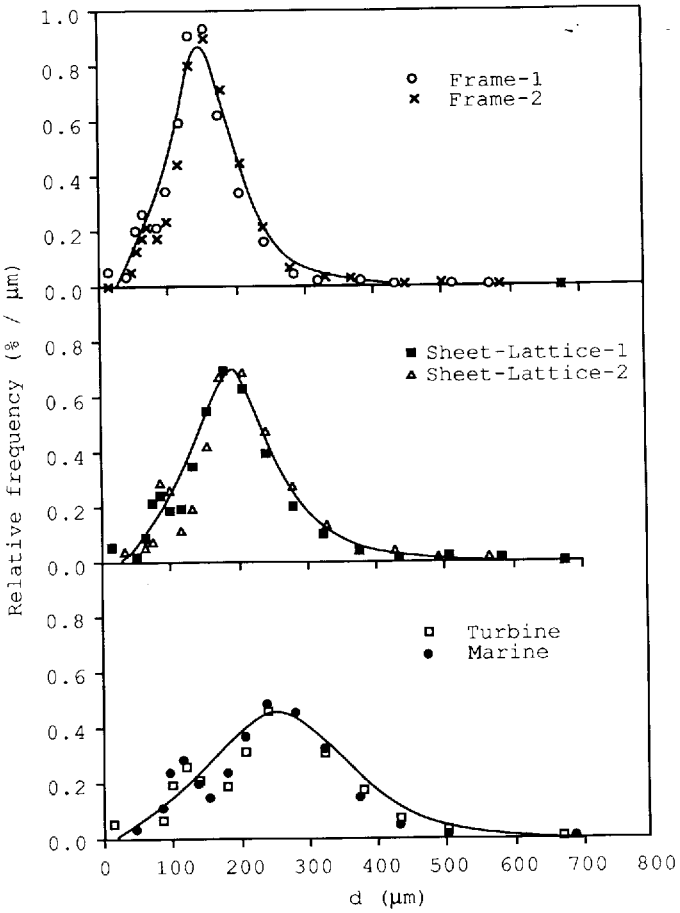

Figure 2 — Influence of impeller type on the size distribution curve of nylon microcapsules prepared at a mixing rate of 300 rpm.

position relative to the beaker dimensions were selected in accordance with Rushton et al. (1950).

Volume distributions (volume of microcapsules in each diameter class) were obtained by use of a Malvern 2605-Lc Particle Size Analyser, according to the log-normal distribution model (Poncelet De Smet et al., 1989). As most of the microcapsule size distribution data published in the literature are number distribution values, both volume and number distribution parameters are reported in the Tables. From each distribution, mean geometric diameter, $\bar{d}_g$, and standard deviation, σ, were computed. The size dispersion was also estimated through the span, s, which defined the centered diameter range containing 80% of the microcapsule volume (microcapsules in number distribution) divided by the mean diameter. Results ($\bar{d}_g$, σ and s) presented in this paper are average values of different means and standard deviations obtained from replicated preparations of microcapsules.

Results

Size distribution curves obtained for nylon-6,10 microcapsules prepared by use of turbine, marine, sheet and frame lattice type impellers operating at 300 rpm are compared in Figure 2. The volume distribution curves are symmetrical in logarithmic scale. The mean geometric diameter, the median (diameter dividing the sample in two equal fractions) and the mode (diameter corresponding to the maximum relative frequency) coincide. Turbine and marine type impellers resulted in similar distributions with a broad diameter range (90 % of microcapsule volume corresponding to a diameter

TABLE 2
Distribution Parameters of Nylon Microcapsules Prepared Using Various Impeller Geometries

Impeller type	Volume distribution $\bar{d}_g$ (μm)	Volume distribution σ (μm)	Volume distribution s (−)	Number distribution $\bar{d}_g$ (μm)	Number distribution σ (μm)	Number distribution s (−)
Turbine	250	111	1.11	155	72	1.10
Marine	250	111	1.11	155	72	1.10
Sheet-1	189	98	1.09	115	52	1.10
Sheet-2	200	93	0.99	133	57	1.00
Frame-1	150	58	0.99	98	34	1.00
Frame-2	158	63	0.96	106	43	1.00

t = 2.5 min, N = 300 rpm, [emulsifier] = 2.0 % V/V, phase ratio = 20 % V/V

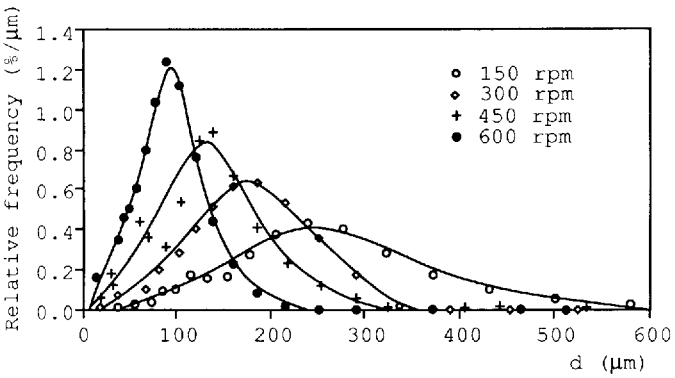

Figure 3 — Evolution of the size distribution curve of nylon microcapsules with increasing sheet lattice impeller rotational speeds.

between 100 and 450 μm), a mean geometric diameter of 250 μm and a standard deviation of 111 μm. Sheet lattice type impellers operating at the same rotational speed resulted in a reduced range of size distribution (90 % between 80 and 325 μm). The mean geometric diameter and standard deviation values were 195 μm and 95μm respectively, based on a volume distribution. A reduction in the lattice wire diameter and mesh size did not affect the microcapsule size distribution. The narrowest diameter distribution was obtained with the frame type lattice impeller (70 to 260 μm), with lowest values for the mean geometric diameter (155 μm) and standard deviation (60 μm).

Volume and number distribution parameters are summarized in Table 2. The values obtained for the mean geometric diameter and standard deviation in the number distribution are 155 μm and 72 μm, respectively, for both turbine and marine type impellers, 125 μm and 55 μm with the sheet lattice type, and 100 μm and 39 μm with the frame impeller design.

Size distribution curves obtained with a sheet lattice operating at different rotational speed are illustrated in Figure 3. Distribution parameters are summarized in Table 3. As the rotational speed was increased from 150 to 600 rpm, the diameter range in volume distribution became progressively more confined (90 % of microcapsules having a diameter ranging between 110 and 500 μm at 150 rpm, and between 40 and 140 μm at 600 rpm); the distribution curves are narrower (standard deviation decreases from 176 to 50) and symmetrical about the mean geometric diameter, which gradually decreases from 296 μm at 150 rpm to 91 μm at 600 rpm. At a rotational speed lower than 150 rpm, the mechanical energy is not sufficient to disperse the aqueous phase, and at a speed higher than 600 rpm, a vortex occurs.

TABLE 3
Effect of Sheet Lattice Rotational Speed, N, on the Distribution Parameters of Nylon Microcapsules

Rotational speed N (rpm)	Volume distribution $\bar{d}_g$ (μm)	Volume distribution σ (μm)	Volume distribution s (−)	Number distribution $\bar{d}_g$ (μm)	Number distribution σ (μm)	Number distribution s (−)
150 rpm	296	176	1.42	143	79	1.40
300 rpm	184	87	1.08	114	51	1.10
450 rpm	132	61	1.09	79	36	1.10
600 rpm	91	51	1.18	51	26	1.20

t = 1.5 min, [emulsifier] = 1.0 % V/V, phase ratio = 20 % V/V

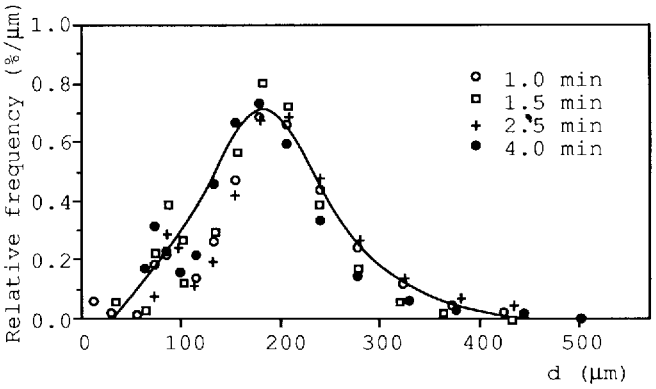

Figure 4 — Size distribution curves following emulsification at 300 rpm with the sheet lattice type impeller for increasing periods of time.

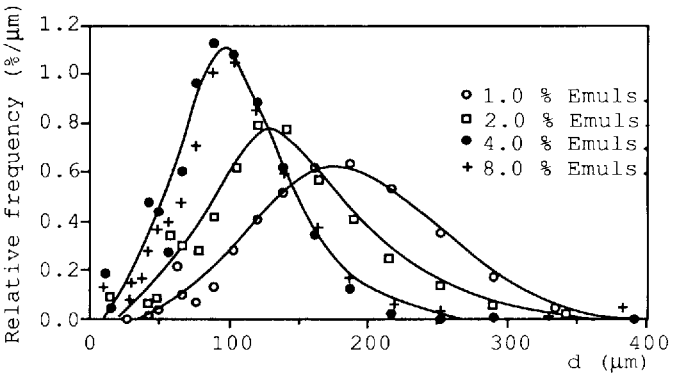

Figure 5 — Influence of the emulsifier concentration on the size distribution curve of nylon microcapsules prepared at 300 rpm, with the sheet lattice type impeller.

A comparison of size distribution curves obtained with emulsification duration from 1.0 to 4.0 min is presented in Figure 4. It appears that prolonged mixing does not affect the microcapsule size distribution.

Varying the emulsifier at low (150 rpm) to moderate (300 rpm) mixing rates provides a control of the mean diameter, its value decreasing with an increase of emulsifier concentration from 1.0 to 4.0 % V/V. The distribution curves of microcapsules obtained at a 300 rpm mixing rate are presented in Figure 5 and the distribution parameters are tabulated for 150 rpm in Table 4, and for 300 rpm in Table 5. When more than 4.0 % V/V emulsifying agent is added to the system, the total volume of recovered microcapsules decreases drastically, while distribution parameters remain relatively unchanged. At higher rotational speed (> 450 rpm), the distribution curves and parameters are unaffected by the emulsifier concentration (Figure 6).

TABLE 4
Distribution Parameters at Low (150 rpm) Sheet-Lattice Impeller Rotational Speed and Varying Emulsifier Concentration

Emulsifier concentration (% V/V)	Volume distribution $\bar{d}_g$ (μm)	σ (μm)	s (−)	Number distribution $\bar{d}_g$ (μm)	σ (μm)	s (−)
1.0	296	176	1.42	143	79	1.67
2.0	265	164	1.33	127	78	1.35
4.0	220	130	1.39	101	61	1.45

t = 1.5 min, phase ratio = 20 % V/V, N = 150 rpm

TABLE 5
Distribution parameters at moderate (300 rpm) sheet-lattice impeller rotational speed and varying emulsifier concentration

Emulsifier concentration (% V/V)	Volume distribution $\bar{d}_g$ (μm)	σ (μm)	s (−)	Number distribution $\bar{d}_g$ (μm)	σ (μm)	s (−)
1.0	184	87	1.08	114	51	1.10
2.0	164	79	1.14	98	35	1.15
4.0	103	57	1.18	59	29	1.20
8.0	107	72	1.50	47	31	1.65

t = 1.5 min, phase ratio = 20 % V/V, N = 300 rpm

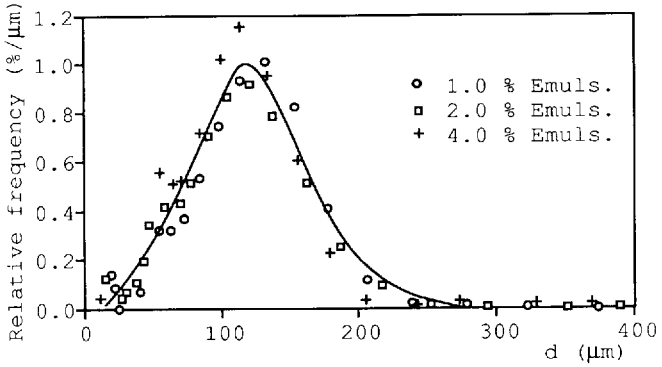

Figure 6 — Effect of the emulsifier concentration on the size distribution curve of nylon microcapsules prepared at 450 rpm, with the sheet lattice type impeller.

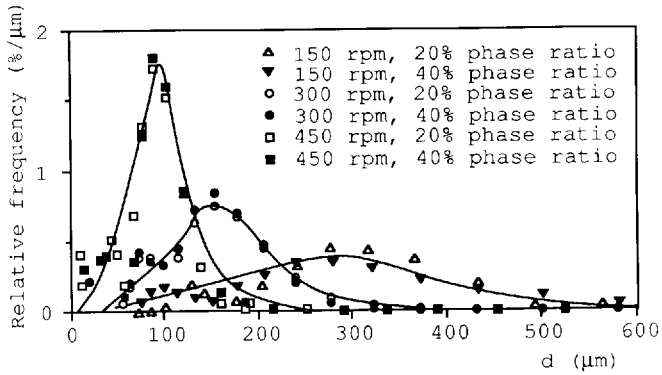

Figure 7 — Influence of the internal/external phase ratio on the size distribution curve of nylon microcapsules prepared with the sheet lattice type impeller.

Distribution curves obtained at three different rates of mixing illustrate (Figure 7) that the size distribution is unaffected by the phase ratio (20 or 40 % aqueous/organic phase).

Discussion

In biotechnological applications involving microencapsulated material, the performance of the biochemical process is often dependent on the volume of microcapsules, or on the specific surface area if there are mass transfer limitations. However, most published data are based on numeric size distributions, which lead to consistently lower estimates of the mean diameter than with the corresponding volumetric distribution (Tables 2 to 5). Significant error in the establishment of size-dependent characteristics such as diffusion constants may arise from using number distribution data (Poncelet et al., 1988). Volume distribution data are generally more appropriate to describe the microcapsule size.

Span is relatively independent of the error associated with the low frequency of measurement at small and large diameters. Thus it is more constant than the standard deviation through replicated sets of measurements on the same sample or from different samples obtained under identical conditions. Its value better reflects changes observed in the size distribution curves facilitating the interpretation of its dependence on various parameters.

In a previous study, the formulation of collodion (cellulose nitrate) bound microcapsules was examined (Poncelet De Smet et al., 1989). The collodion membranes were formed through coacervation while nylon membranes are formed by means of interfacial polycondensation. In addition, important differences in the physicochemical conditions were observed during the polymerization reactions. The viscosity and density of the respective phases were more similar in the nylon system, while the solvent phase was less dense and viscous than the aqueous phase used in collodion membrane formation.

Mechanical emulsification produces a non-uniform droplet size, the diameter of microcapsules varying widely from a few microns to 500 μm or more. Smaller microcapsules (nanometer sized) may be prepared by using mixing systems developing greater shear forces such as the "Virtis 45" type homogenizer (Chang, 1972), or high mixing rates (N > 600 rpm) in baffled reactors (Koishi et al., 1969); Shiba et al., 1970; Shigeri et al., 1970). Large diameter microcapsules (d > 1 mm) distributed on a narrow size range (Okahata et al., 1983) can be formed by other techniques such as droplet formation by syringe extrusion.

A narrower size dispersion of the nylon microcapsules was obtained by forming the emulsion with a frame lattice type impeller rather than a sheet lattice, turbine or marine impeller (Figure 2). This effect may be related to a better distribution of the mixing energy through the reactor. Chang (1972) reported typical standard deviation values in number distribution, of 50 % of the mean diameter of nylon microcapsules resulting from emulsification with a laboratory stir-bar. The standard deviation of microcapsules prepared with the frame lattice impeller in the present study was less than 40 % of the mean diameter (Table 2). Wire diameter and mesh dimensions of the lattice have little effect on the distribution parameters, other operating conditions being maintained constant. Similar results were obtained with collodion microcapsules (Poncelet De Smet et al., 1988) produced with the frame or sheet lattice types of stirrer.

The mean diameter of nylon bound microcapsules may be controlled within a range of 40 to 200 μm (log-normal number distribution data) depending on the impeller rotational speed (150 to 600 rpm, Table 3), resulting from an increased shear rate in the reactor. Similar control of diameter

may be obtained by varying the emulsifier concentration (1.0 to 4.0 % V/V, Tables 4 and 5), resulting in decreasing energy required to break the drops. Similar results were observed with collodion microcapsules.

The decrease of microcapsule yield observed when more than 4.0 % V/V emulsifier was added was also observed by Rambourg et al., (1982). This may be explained by an inhibitory effect on the polymerization reaction due to the formation of a layer of emulsifier at the aqueous droplet surface, preventing the transfer of the hexane diamine from the aqueous to the organic phase. This mass transfer limitation may inhibit the polymerization reaction.

In the case of nylon-6,10 membranes, the emulsification step appears to be much less dependent on physicochemical factors such as duration of emulsification or internal/external phase ratio than was observed in the aqueous/ether system used for formulation of collodion membrane microcapsules. Under the present experimental operating conditions, a mixing time of 1.0 minute was sufficient to achieve a stable distribution, while an emulsification duration of at least 3 min was recommended for a reduction in size dispersion of collodion microcapsules (Poncelet De Smet et al., 1989). Raising the internal/external phase ratio from 20 to 40 % V/V increases the amount of nylon microcapsules produced per batch, but no variation in the size dispersion was observed. In contrast, collodion microcapsule size increased as phase ratio increased, their distribution gradually transforming from a log-normal to a normal distribution profile. Finally, for a given mean diameter, nylon membrane bound microcapsules are generally distributed on a narrower size range than collodion microcapsules, with a resulting lower value for standard deviation and span. These observations may be explained by a greater similarity in physical properties (viscosity, density) of both aqueous and organic phases in the nylon system (water in chloroform/cyclohexane mixture) than in the cellulose nitrate system (water in ether). The equilibrium state between coalescence and dispersion is reached more rapidly with a lower energy requirement for nylon membrane microcapsule formulation.

The objective in scaling emulsification equipment is to achieve as homogeneous a dispersion as possible throughout the entire vessel contents. The frame lattice impellers should accomplish this most effectively since the frame occupies much of the profile of the reactor providing a uniform mixing throughout. Lattice impellers are used in industry but for gentle mixing at low speed. Data are not available to establish scale-up rules under conditions of high shear. As for the turbine or marine style impellers, scale problems may be expected due to changes in mixing régime. Maintaining a constant impeller Reynold's number while scaling based on geometric similarity should avoid a change in mixing régime with scale. Since the impeller tip velocity is proportional to shear, maintaining this parameter constant during scale-up may be an acceptable alternative.

Conclusions

The optimum mean diameter of hexanemethylene sebacamide microcapsules depends on the application and may be obtained with all impellers tested by applying a suitable rotational speed and emulsifier concentration. However, the narrowest size distribution was obtained by use of a frame type impeller. The selection of appropriate impeller design and emulsifier concentration provided a control of size distribution.

Nomenclature

D = impeller (or vessel) diameter (mm)
d = microcapsule diameter (μm)
$\bar{d}_g$ = mean geometric diameter (μm)
H = impeller (or vessel) height (mm)
N = impeller rotational speed (rpm)
s = span (centered range of diameters containing 80% of the microcapsule number or volume/mean diameter)
t = emulsification duration (min)
W = impeller width (mm)
σ = standard deviation (−)

References

Arakawa, M., and T. Kondo, "Flow Properties of Microcapsule Suspension as a Model of Blood", Biorheology **12**, 57–66 (1975).

Arakawa, M., and T. Kondo, "Preparation and properties of poly (N^{α}, N^{ϵ} -*L*-lysinediylterephtaloyl) microcapsules containing hemolysate in the nanometer range", Can. J. Physiol. Pharmacol. **58**, 183–187 (1980).

Chang, T. M. S., F. C. MacIntosh and S. G. Mason, "Semipermeable Aqueous Microcapsules 1. Preparation and Properties", Can. J. Physiol. Pharmacol. **44**, 115–128 (1966).

Chang, T. M. S., "Artificial Cells", Charles C. Thomas, Springfield, Ill., pp. 24–27 (1972).

Chang, T. M. S., "Biochemical Applications of Immobilized Enzymes and Proteins", Vol. 1, Thomas Ming Swi Chang (ed.), Plenum Press, N.Y., pp. 69–90 (1977).

Dueck, C. L., R. J. Neufeld and T. M. S. Chang, "Hydrodynamics of a Fluidized Bed Reactor for Urea Hydrolysis by Microencapsulated Urease", Can. J. Chem. Eng. **64**, 540–546 (1986).

Jack, T. R. and J. E. Zajic, "The Immobilization of Whole Cells", Adv. Biochem. Eng. **5**, 126–145 (1977).

Koishi, M., N. Fukuhara and T. Kondo, "Studies on Microcapsules. II. Preparation of Polyphtalamide Microcapsules", Chem. Pharm. Bull. **17**(4), 804–809 (1969).

Mori, T., T. Sato, Y. Matuo, T. Tosa and I. Chibata, "Preparation and Characteristics of Microcapsules Containing Asparaginase", Biotechnol. Bioeng. **14**, 663–573 (1972).

Okahata, Y., H.-J. Lim, G.-I. Nakamura and S. Hachiya, "A Large Nylon Capsule Coated with a Synthetic Bilayer Membrane. Permeability Control of NaCl by Phase Transition of the Dialkylammonium Bilayer Coating", J. Am. Chem. Soc. **105**(15), 4855–4859 (1983).

Poncelet, D. and R. J. Neufeld; "Shear Breakage of Nylon Membrane Microcapsules in Turbine Reactor"; Biotechnology and Bioengineering **33**: 95–105 (1989).

Poncelet De Smet, B., D. Poncelet and R. J. Neufeld, "Control of Mean Diameter and Size Distribution during Formulation of Microcapsules with Cellulose Nitrate Membranes", Enzyme Microb. Technol. **11**, 27–37 (1989).

Poncelet, D., B. Poncelet De Smet and R. J. Neufeld, "Mass Transfer in Nylon Membrane Bounded Enzyme Microcapsules", 38th Can. Chem. Eng. Conf., Oct. 2–5, Edmonton, Can. (1988).

Rambourg, P., J. Lévy and M.-C. Lévy, "Microencapsulation III: Preparation of Invertase Microcapsules", J. Pharm. Sci. **71**(7), 753–758 (1982).

Rushton, J. H., E. W. Costich and H. J. Everett, "Power Characteristics of Mixing Impellers", Chem. Eng. Prog. **18**(8), 395–404 and (9), 467–476 (1950).

Shiba, M., S. Tomioka, M. Koishi, T. and Kondo, "Studies on Microcapsules. V. Preparation of Polyamide Microcapsules containing Aqueous Protein Solution", Chem. Pharm. Bull. **18**(4), 803–809 (1970).

Shigeri, Y., M. Koishi, T. Kondo, M. Shiba and S. Tomioka, "Studies on Microcapsules. VI. Effect of Variations in Polymerization Condition on Microcapsule Size", Can. J. Chem. **48**, 2047–2051 (1970).

Tramper, J., "Immobilizing Biocatalysts for use in Syntheses", Trends in Biotechnol. **3**(2), 45–50 (1985).

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