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Microencapsulation reactor scale-up by dimensional analysis

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A microencapsulation process for preparing protein-loaded microspheres based on a solvent-extraction method was scaled up using continuously stirred tank reactors (CSTR) from 1 L to 100 L in batch size. This study was concerned with developing a quantitative correlation between the size of the microspheres and process parameters. The process parameters considered include operational variables and physical properties associated with both the dispersion and dispersed phases. Dimensional analysis was used to establish such a correlation based on protein-free poly(lactic acid-co-glycolic acid) microspheres in an oil-in-water emulsion system prepared in a 1 L CSTR. This correlation was found to accurately describe the preparation of protein-loaded microspheres in a solid-in-oil-in-water system. Poly(methyl methacrylate) was found to behave similarly to poly(lactic-acid-co-glycolic acid) and could be used as a model polymer for scale-up investigation. This study showed that dimensional analysis can be used to predictably scale the current microencapsulation process up to 100 L to produce particles of defined size.

Keywords: Microencapsulation, reactor scale-up, dimensional analysis, protein, microsphere

Notation

- B: Total baffle area (cm^2)
- c: Polymer concentration (gm/ml)
- C_{PVA}: PVA concentration in (%w/w)
- d_p: Diameter of microsphere (cm)
- $d_{p(calc)}$: Calculated microsphere size (μm)
- $d_{p(exp)}$: Experimentally determined microsphere size (μm)
- D: Diameter of impeller (cm)
- $D\omega$: Velocity (cm/sec)
- g_c: Conversion factor ($ML/F\Theta^2$), equal to 1 in the cgs unit system
- g: Gravity acceleration (cm/s^2)
- h_B: Baffle height (cm)
- H: Height of filled volume in the tank (cm)
- n: Number of baffles
- n_{imp}: Number of impellers
- T: Tank diameter (cm)
- ρ_0: Density of organic phase (gm/ml)
- ρ_α: Density of aqueous phase (gm/ml)

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σ: Interfacial tension between organic and aqueous phases (dyne/cm)
μ_0: Viscosity of organic phase (gm/cm/s)
μ_x: Viscosity of aqueous phase (gm/cm/s)
υ_0: Volume of organic phase (ml)
υ_x: Viscosity of aqueous phase (ml)
ω: Angular velocity of impeller (1/s)
F: Unit of force, *dyne* in cgs system
L: Unit of length, *cm* in cgs system
M: Unit of mass, *g* in cgs system
Θ: Unit of time, *s* in cgs system

Introduction

Microspheres find a variety of uses in biology, medicine and pharmaceutics (Davis *et al.* 1984), especially in the area of drug delivery (Deasy 1984, Baker 1987, Whateley 1992). Research on controlled release of macromolecules, particularly with polypeptides and proteins, is becoming increasingly important (Siegel and Langer 1984, Silveramakrishnan 1990). Many articles describing the methodology of microsphere preparation have been published (Beyger and Nairn 1986, Bindschaedler *et al.* 1988). These studies focus mainly on developing processes that can increase the microsphere's loading efficiency (Iwata and McGinity 1992), minimize initial drug burst, and provide constant, desired release profiles (Rhine *et al.* 1980). Despite its importance, the relationship between the size of the microspheres produced and system parameters has not yet been investigated quantitatively although qualitative observations have been described (Sanghvi and Nairn 1992).

The primary objective of this study was to establish a theoretical basis for the predictable scaling of emulsion encapsulation processes. Process scaling is affected by both equipment design and engineering science. Although scale-up principles are available for some biotechnological processes (Ho and Oldshue 1987, Leng 1991), specific methods for microencapsulation have not been reported. In this study, focus was placed on characterizing particle size and correlating it with system variables and fluid physical properties using the dimensional analysis method. Dimensional analysis is a useful tool for describing the behaviour of complex engineering problems. This method involves converting independent variables into a minimum number of dimensionless groups so that it can reduce the number of experiments to simplify the mathematical manipulations for performance description (Bird *et al.* 1960, Bennett and Myers 1962).

A correlation between particle size and process parameters, in the form of several dimensionless groups, was determined for producing poly(lactic acid-co-glycolic acid) microspheres in an oil-in-water emulsion. The system parameters included the geometric and dynamic variables associated with a 1 L CSTR, the physical properties of a polymer containing methylene chloride phase and poly(vinyl alcohol) containing aqueous phase. This correlation was then used to investigate large scale systems using a less expensive polymer, poly(methylmethanylate). Several different dispersion phases were investigated using carboxymethyl cellulose and poly(vinyl pyrrolidone) as emulsifiers. The utility of the correlation was also investigated for protein-containing microspheres prepared by a solid-in-oil-in-water emulsification method. The batch reactor was scaled from 1 to 100 L and the size of the microspheres produced was compared with that predicted by the correlation.

Materials and methods

Polymers

Poly(D,L-lactic-co-glycolic acid), abbreviated as PLGA in the present study, was provided from two different sources. PLGA of a molecular weight of 12 000 and a lactic acid to glycolic acid with a ratio of 75/25 was obtained from Birmingham Polymers. PLGA of a molecular weight 100 000 with the same monomer ratio was obtained from Medisorb. Poly(methyl methacrylate) and polystyrene, abbreviated as PMMA and PS, respectively, were purchased from Aldrich Chemical. Molecular weight was determined by the supplier to be 45 000 for PS and 25 000 for PMMA.

Emulsifiers

Poly(vinyl alcohol), abbreviated as PVA, was obtained from Air Products under the trade name of Airvol 205. Carboxymethyl cellulose sodium salt, abbreviated as CMC, with a molecular weight of 90 000 was from Sigma. Poly(vinyl pyrrolidone), abbreviated as PVP, with a molecular weight of 40 000 was provided by Eastman Kodak.

Solvent

Analytical-grade methylene chloride, abbreviated as MeCh, was used as the solvent in the oil phase. It was purchased from Baxter.

Protein

Bovine serum albumin (BSA) was used as a model protein. It was provided in a powder form of Cohn fraction V by Interegen. The BSA obtained from Interegen Co. had a broad particle size distribution with a mean diameter of 34 μm. To produce small BSA powders, this material was redissolved in water to a concentration of 10 mg/ml and spray dried using a laboratory scale spray dryer (Buchi, Model B-190). Protein solution was fed at a constant rate of 4·5ml/h with a peristaltic pump to a nozzle (0·5 mm I.D.) where atomization occurred by means of a pressurized air stream. The pressurized air stream was set at a flow rate of 600 l/h measured at 25°C, 1 atmosphere (STP condition). Drying air at 90°C entered into the drying chamber at a rate of 36 000 l/h in the same direction as the spray. The spray-dried BSA powder was collected in a flask attached to a cyclone and the powder was determined to be less than 5 μm in size. Residual moisture content of the powder was determined to be 10% by thermogravimetric analysis (TGA 7, Perkin–Elmer).

Reactors

Four reactor sizes were used: 1, 3, 10 and 100 L. Each reactor was equipped with two stainless steel, 6-blade flat Rashton impellers, baffles, an overhead impeller drive and a stainless steel tube for polymer injection. Dimensions of the impellers, baffles and reactors are given in table 1. The configuration of the reactors is shown in Figure 1. Reactors were geometrically similar in terms of the ratio of impeller diameter (D) and tank diameter (T). The 1 L reactor was obtained from LH Fermentation. The 3, 10 and 100 L reactors were obtained from Applikon. All reactors were designed for fermentation use. For this study each reactor was modified to be loaded with the components described above.

Table 1. Configurations of the reactors and impellers used in the study.

Reactor size (L)	Reactor diameter (cm)	Imp. area (cm^2)†	D/T	H (cm)	$B(n \times hB \times w)$ (cm)‡
1*	10	4 × 0·4	0·40	13	4 × 10 × 1·4
3§	12·5	4·5 × 0·8	0·36	25	3 × 14 × 1·4
10§	21·5	7·5 × 1·5	0·35	27	3 × 23 × 2·2
100§	34·2	13 × 2·0	0·38	108	3 × 62 × 2·2

† Impeller area (diameter × height).
‡ Baffle (number × height × width).
* Flat bottom.
§ Dish bottom.

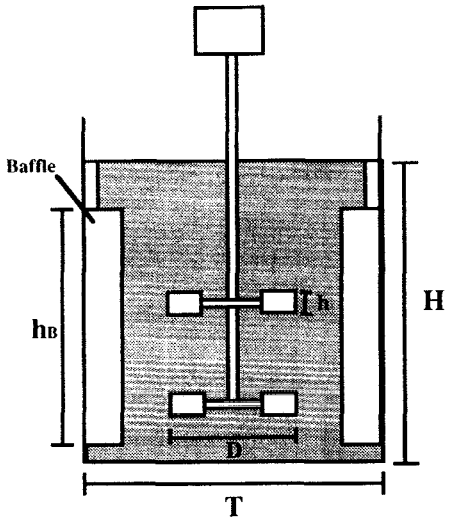

Figure 1. Configurations of the reactors and impellers. Their actual dimensions are listed in Table 1.

Microsphere preparation

Microspheres were prepared by oil-in-water (O/W) emulsification using a solvent extraction method. The two-step process involves an emulsification followed by a hardening process. The following describes a typical microencapsulation procedure. For emulsification, 15 ml of polymer/MeCh solution was injected with a metal syringe into an agitated 1 L reactor containing 900 ml of aqueous PVA solution. For hardening, the microsphere emulsion was diluted ten-fold in water in a 15 L tank after 1 min of emulsification. Microspheres were hardened for about 1 h, and then were allowed to settle and be collected for size analysis. For batches in reactors larger than 1 L, only a portion of the emulsion was transferred to the hardening tank to maintain the same volume ratio as the 1 L system.

Protein-loaded microspheres were prepared by a solid-in-oil-in-water (S/O/W) emulsification method. A three-step process, diagrammed in Figure 2 was used. A pre-weighed protein powder was mixed with 15 ml of polymer solution in a jacketed-glass vessel by homogenization. The homogenization system consisted of an overhead drive, a power controller and a 1 cm homogenization shaft from Virtis

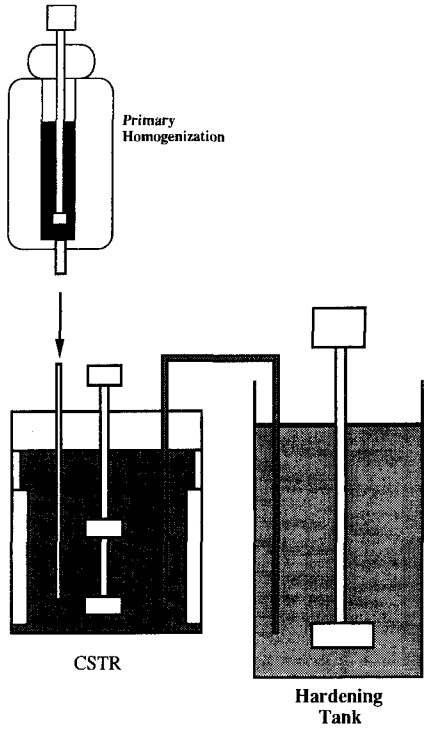

Figure 2. Schematic representation of experimental set-up for the S/O/W emulsification methods.

Table 2. Experimental conditions for microsphere preparation.

Reactor size	1L	3L	10L	100L
Polymer	PLGA/PMMA	PLGA/PMMA	PLGA/PMMA	PMMA
Conc. (g/ml)	0·145–0·58	0·29 and 0·58	0·29 and 0·58	0·29 and 0·58
Viscosity, μ_0 (cps)	4–190	4–190	4–190	4–190
Volume, v_0 (ml)	3–18	36	120	1200
PVA (%)	3–15	6	6	6
Viscosity, μ_α (cps)	4–270	12·4	12·4	12·4
Volume, v_α (L)	0·25–0·9	3·0	10·0	100·0
Agitation, ω (rpm)	500–2000	890–1500	535–870	300–600
BSA powder (%)	10–20	NA	NA	NA

Company. The shaft had a 1 cm rotor/stator tip. The mixture was homogenized at a speed of 10 000 to 15 000 rpm for about 1 min. This solid-in-oil (S/O) emulsion was then transferred to an emulsifying reactor. All experiments were performed at ambient temperature. Table 2 summarizes the experimental conditions and parameter ranges used in the study.

Size analysis

A laser-based particle size analyser (Brinkman, Model 2010) was used to determine the size of the microspheres and BSA powders. The system was capable

of detecting particles ranging in size from 0·5 to 300 μm. The mean diameter of the particles was calculated from the volume-moment average of particle size distribution (i.e. $\Sigma nd^4/\Sigma nd^3$). Each reported value represents the average of at least two determinations. It was observed that the width of particle size distribution increases with increasing particle size. For example, the standard deviation is large for batches producing large microspheres. Therefore, to minimize errors associated with broad size distributions, experiments producing microspheres with a mean size exceeding 100 μm were excluded from data analysis.

Viscosity determination

The viscosities of both polymer and PVA solutions (μ_0 and μ_α) were determined using a Cannon-Fenske capillary viscometer. The viscosity data for aqueous PVA solutions was correlated with concentrations (C_{PVA}) using equation (1):

$$\mu_\alpha(\text{cps}) = 1{\cdot}519 \exp(0{\cdot}151 \times C_{PVA}) \quad (1)$$

The viscosity of low molecular weight PLGA (MW 12 000) in methylene chloride at 25°C as a function of concentration (c) was experimentally determined and expressed in equation (2):

$$\mu_0(\text{cps}) = 2{\cdot}287 \exp(2{\cdot}006 \times c) \quad (2)$$

Only one concentration, 0·145 g/ml, was used for high molecular weight PLGA (MW 100 000). Its viscosity was 35·9 cps at 25°C. The viscosities for other polymer and emulsifying systems are listed in tables 4 and 5.

Determination of interfacial tension

The interfacial tension between the aqueous and organic phases was determined by a tensiometer (Fisher, Model 21) equipped with a 6 cm circumference platinum–iridium ring. A value of 7·7 dyne/cm was determined for the PLGA/MeCh and aqueous PVA phases. This value did not vary over the investigated range of concentration. The interfacial tensions of different systems such as aqueous CMC or PVP with PMMA/MeCh solution are reported in table 5.

Results and discussion

In principle, the microsphere size produced during emulsification is influenced by all the system parameters associated with liquid–liquid emulsification. Given the complexity of this operation, a fundamental theory describing the relationship between particle size and process parameters would be exceedingly difficult to derive. An alternative approach involves deriving a correlation using dimensional analysis. First, we assume that the microsphere size (d_p) is a function of quantities (v_0 and v_α) and physical properties (c, μ_0, μ_α, ρ_0, ρ_α, and σ) of the dispersion and dispersed phases, as well as reactor's configuration (D/T, H, B and n_{imp}), and rotational speed of the impeller (ω):

$$d_p = f(D\omega,\ D/T,\ H,\ B,\ n_{imp},\ g_c,\ g,\ c,\ \mu_0,\ \mu_\alpha,\ \rho_0,\ \rho_\alpha,\ v_0,\ v_\alpha,\ \sigma) \quad (3)$$

All parameters are defined in Notation. Gravity acceleration (g) is included in the equation to relate mass with inertial force. Conversion factor (g_c) represents a constant converting one unit system to another. To minimize the effect of the reactor's configuration, all reactors used in the study had a constant D/T, in the range of 0·36–0·40. Therefore, this parameter was left out from equation (3). H was actually

related to υ_α so that it can be left out from equation (3). Separate experiments using different baffle area, B, suggested that a change in B does not affect the size of the microspheres significantly (data not shown). Therefore, B can be left out from equation (3). In separate experiments, we also found that the number and position of the impellers played a significant role in determining the size of the microspheres (data not shown). To simplify the system, we consistently used double impellers ($n_{imp} = 2$) with the lower one placed close to the bottom of the tank and the other located in the centre of the total emulsion volume.

As far as physical properties of the two fluids are concerned, separate experiments suggested that the volumes of the two phases in a range of our interest played a minor role in affecting the particle size. The nominal volumes in the 1 L system were 15 ml of polymer solution and 900 ml of aqueous PVA solution. Changing the volume of polymer solution in the range of 3–18 ml and that of PVA solution down to 250 ml produced microspheres with sizes about 10% deviated from those produced from the nominal system. Therefore, υ_0 and υ_α was removed from equation (3) in this study. Based on the description above, equation (3) can be rewritten as equation (4):

$$d_p = f(D\omega, g_c, g, c, \mu_0, \mu_\alpha, \rho_0, \rho_\alpha, \sigma) \tag{4}$$

Based on the four fundamental dimensions of time, Θ: length, L; Mass, M; and force, F, six independent dimensionless groups were derived (see appendix for detailed derivation):

$$\Pi_1 = g(\rho_0 - \rho_\alpha)d_p^2/(\sigma)$$
$$\Pi_2 = \rho_\alpha(D\omega)^4/(\sigma g)$$
$$\Pi_3 = g\mu_\alpha^4/(\rho_\alpha\sigma^3)$$
$$\Pi_4 = g\mu_0^4/(\rho_\alpha\sigma^3)$$
$$\Pi_5 = c/\rho_\alpha$$
$$\Pi_6 = \rho_\alpha/\rho_0$$

Densities of the two phases were found to be close to those of the solvents in the two phases, 1·32 g/ml for the polymer/MeCh solution and 1 g/ml for the PVA solution over a wide range of solute concentrations. Though density can be varied by using different solvents, it may completely change the system. Therefore, ρ_α/ρ_0 was considered a constant and Π_6 was removed. As summarized in table 3, dimensionless groups, Π_1–Π_4, can be expressed by one or two well-known dimensionless groups, such as Bond number, Froude number, Galileo number, Weber number, etc. These numbers have been previously used to quantify flow and mixing in complex systems. The Bond number describes the motion of drops (Boucher and Alves 1959). The Galileo number quantifies the circulation of viscous fluid (Boucher and Alves 1959). The Weber and Froude numbers quantify agitation by impellers (Yamaguchi *et al.* 1963). Each number has its own physical meaning in fluid dynamics, representing the relative importance between different physical forces (Bolz and Tuve 1970, Perry and Green 1984). Since microsphere size is determined by the emulsion droplet size, it is expected that the size of hardened particles would depend on the physical forces which influence mixing of the two immiscible phases.

Thus, equation (4) can be expressed in terms of the six dimensionless groups:

$$g(\rho_0 - \rho_\alpha)d_p^2/\sigma = f(\Pi_2, \Pi_3, \Pi_4, \Pi_5) \tag{5}$$

The function on the right hand side of equation (5) may be written in a variety of forms. One of the simplest and most straightforward expressions is to assume that

Table 3. Dimensionless groups and their physical meaning used in this study.

Group	Definition	Significance	Application
Bond number	$(\rho_0 - \rho_x)d_p^2 g/\sigma$	GF/STF	Atomization, motion of drops
Froude number	$D\omega^2/g$	IF/GF	Agitation
Galileo number	$d_p^3 g\rho_x^2/\mu_x^2$	(IF)(GF)/(VF)^2	Circulation of viscous liquid
Weber number	$D^3\omega^2\rho_x/\sigma$	IF/STF	Agitation by impellers
Π_1	Bond number	GF/STF	Motion of dispersed drops
Π_2	(Weber number) × (Froude number)	IF^2/(STF)(GF)	Agitation by impellers
Π_3	(Galileo number)$^{-2}$ × (Bond number)^3	VF^4/(IF^2)(STF^3)	Circulation of dispersed and dispersion phases
Π_4	(Galileo number)$^{-2}$ × (Bond number)^3	VF^4/(IF^2)(STF^3)	Circulation of dispersed and dispersion phases

GF: Gravitational force.
IF: Intertial force.
STF: Surface tension force.
VF: Viscous force.

the functional dependence can be described with a power, product relationship:

$$g(\rho_0 - \rho_x)d_p^2/\sigma = a\Pi_2^b\Pi_3^c\Pi_4^d\Pi_5^e \tag{6}$$

Coefficients in equation (6), i.e. a, b, c, d, and e, could be calculated from experimental data using the method of least squares regression. Equation (6) was tested based on data from protein/excipient-free PLGA microspheres prepared in a 1 L CSTR. Unfortunately, experimental results failed to produce a linear correlation between Π_1 and $\Pi_2^b\Pi_3^c\Pi_4^d\Pi_5^e$ (correlation coefficient $< 0{\cdot}7$). By trial and error, we found that replacing Π_5^e with $a' + b'\Pi_5$ in equation (6) fit the data well:

$$g(\rho_0 - \rho_x)d_p^2/\sigma = \Pi_2^b\Pi_3^c\Pi_4^d(a' + b'\Pi_5) \tag{7}$$

Unlike equation (6) where the coefficients can be computed analytically, the calculation of coefficients in equation (7) requires complex numerical analysis. Alternatively, we used three different polymer concentrations, 0·145, 0·29, and 0·58 g/ml, to obtain three linear correlations between Π_1 and $\Pi_2^b\Pi_3^c\Pi_4^d$. The values for a, b and c were determined to be $-0{\cdot}280$, $-0{\cdot}108$, and 0·056, respectively. Then we calculated $a' + b'\Pi_5$ based on Π_5 and the slope of each linear correlation, which was 0·0221 for 0·58 g/ml, 0·01415 for 0·29 g/ml, and 0·0111 for 0·145 g/ml. We obtained

$$\text{Slope} = 0{\cdot}0255\Pi_5 + 0{\cdot}0071 \tag{8}$$

For polymers used in this study, the suggested concentration range is 0·1–0·6 mg/ml, which corresponds to the viscosity range of 3 to 200 cps. More concentrated solutions were too viscous to be emulsified and more diluted solutions would not provide enough polymer to form microcapsules. With all the coefficients derived above, the effect of process parameters and physical properties on the size of the microspheres can be quantitatively expressed as:

$$g(\rho_0 - \rho_x)d_p^2/\sigma = \Pi_2^{-0{\cdot}280}\Pi_3^{-0{\cdot}108}\Pi_4^{0{\cdot}056}(0{\cdot}0255\Pi_5 + 0{\cdot}0071) \tag{9}$$

Figure 3 shows that 20 data points obtained from 1 L batch size runs using different polymer concentrations were fitted into equation (9) with a correlation coefficient of 0·977. Equation (9) will be used as the correlation equation for data analysis hereafter.

Large-scale production of this microencapsulation process requires a large quantity of polymer, e.g. 700 g in a 100 L scale. A less-expensive polymer was used as a substitute for PLGA to reduce the development cost of system scale-up and other relevant investigations. Criteria for selecting the substitute was that the polymer can form microspheres of a predictable size based on equation (9). PS and PMMA were evaluated in this study. Table 4 summarizes the physical properties of both polymers as well as the calculated and experimentally determined sizes of PMMA and PS microspheres prepared in a 1 L reactor using 6% PVA solution as the dispersion phase agitated at 1000 rpm. The sizes of PMMA microspheres were less than 5% deviated from the calculated sizes but those of PS microspheres were 20–50% off the calculated values. Therefore, PMMA was a better substitute for PLGA than PS. The reason for the difference between these two polymers in liquid–liquid emulsification is not within the scope of this study.

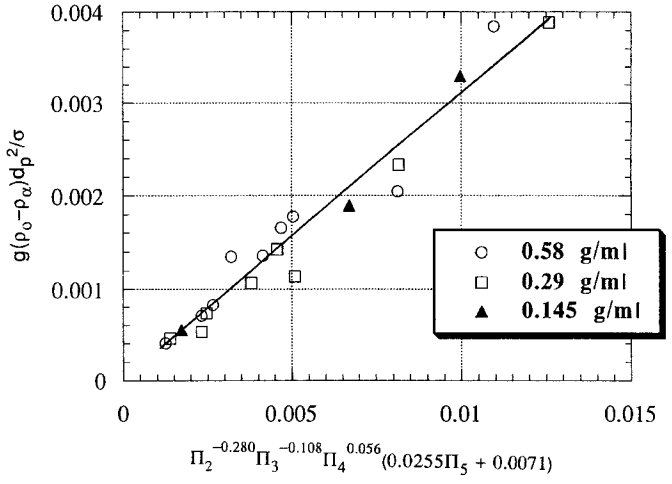

Figure 3. Linear correlations between the size (Π_1) of excipient-free PLGA microspheres prepared in a 1 L CSTR and four dimensionless groups, Π_2, Π_3, Π_4 and Π_5, at polymer concentrations of 0·58 (○), 0·29 (□) and 0·145 (▲) g/ml with the values of ω, μ_0 and μ_α listed in table 2 and $\rho_\alpha = 1$ g/ml, $\rho_0 = 1{\cdot}32$ g/ml, D = 4 cm, g = 980 cm/sec^2 and $\sigma = 7{\cdot}7$ dyne/cm.

Table 4. PMMA and polystyrene (PS) data summary.

Polymeric (conc.) [gm/ml]	μ_0 [cps]	σ[dyne/cm][a]	$d_{p(\text{exp})}$ [μm]	$d_{p(\text{calc})}$ [μm]
PMMA (0·29)	16·6	9·5	52·3	56·8
PMMA (0·58)	190·0	9·5	91·4	91·7
PS (0·29)	9·9	11·0	40·3	59·6
PS (0·58)	57·8	10·2	99·1	84·5

a Interfacial tension with 6% aqueous PVA solution.

Table 5. Results for PMMA microspheres prepared using carboxylmethyl cellulose and polyvinyl pyrrolidone as the dispersion phase.

Dispersion phase	μ_α [cps]	σ[dyne/cm]	$d_{p(\text{exp})}$ [μm]	$d_{p(\text{calc})}$ [μm]
4% CMC	101·5	16·9	48·2	54·5
10% PVP	5·4	11·4	75·3	77·5

Effect of dispersion phases

The break-up of a dispersed liquid drop (dispersed phase) in another immiscible liquid (dispersion phase) is caused by turbulent pressure fluctuations (Mendiboure *et al.* 1991). The pressure acting at the surface of the liquid drop is due to shear forces associated with turbulent eddies in the dispersion phase. Therefore, the dispersion phase plays a significant role in determining the size of the microspheres. Under a similar agitation condition, shear forces intensify as the viscosity of the dispersion phase increases, thereby producing smaller dispersed droplets. On the other hand, interfacial tension between the two liquids is a cohesive force which prevents droplet fragmentation. This phenomenon can be explained by Π_3 in equation (9). This equation suggests that the viscosity of the dispersion phase affects the size of the microspheres more significantly than that of the dispersed phase, $\mu_\alpha^{-0.432}$ versus $\mu_0^{0.224}$. To test the validity of the correlation for predicting the size of the microspheres in different dispersion phases, CMC and PVP were investigated. The selection of CMC and PVP was based on the fact that they are a viscosity agent and an emulsifier.

PMMA microspheres were prepared using 4% CMC and 10% PVP solutions with 0·29 g/ml PMMA in a 1 L CSTR stirred at 1000 rpm. Table 5 summarizes experimentally determined sizes of the microspheres and the calculated sizes based on the measured properties of these two solutions. Both emulsifiers produced microspheres with a size consistent with the calculated size, within 10%, suggesting that contributions from the viscosity of the dispersion phase and interfacial tension to microsphere size can be described by equation (8).

Protein-loaded microspheres

From a pragmatic point of view, equation (9) was further tested for the protein-loaded microspheres. In this study, a S/O/W method was used to encapsulate BSA powder. To achieve protein loadings up to 20%, fine powders were required. Neither jet milling nor lyophilization was able to significantly reduce the size of raw BSA powder to a desired range of 5 μm or less. Spray drying proved to be effective. Spray-dried BSA powders had good dispersity in polymer/MeCh solution so that a fine suspension could be easily achieved by homogenization. The viscosity of the suspension was similar to that of BSA-free polymer solution and the interfacial tension with the PVA solution was assumed to be the same because it was difficult to measure. Both PMMA and PLGA microspheres were prepared with a loading targeted at 10–20% (w/w). Loading efficiency was determined by a weight measurement method to confirm that protein was indeed loaded in the microspheres. The microspheres were dissolved in MeCh and filtered. The collected protein was washed with more solvent, vacuum dried, and weighed. In all batches, BSA loadings were determined to be 80–90% of the targeted loadings. Experimental conditions and results are summarized in table 6. The discrepancy

Table 6. Experimental conditions and the calculated and experimentally determined sizes for BSA-containing microspheres prepared in a 1 L reactor by the S/O/W emulsification method.

Polymer [g/ml]	ω [rpm]	BSA conc.(%)	$D_{p(calc)}$	$D_{p(exp)}$
PMMA (0·58)	2000	10	58·3	62·2
PMMA (0·58)	1500	15	68·5	73·1
PMMA (0·29)	2000	10	47·3	41·5
PMMA (0·29)	1000	20	59·4	56·9
PLGA (0·29)	1000	10	43·2	41·8

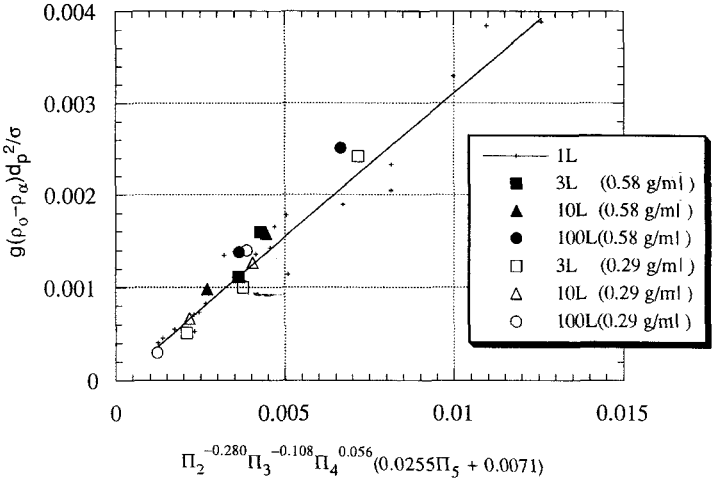

Figure 4. Comparisons of the size of the microspheres prepared in the 3, 10 and 100 L reactors with the correlation equation derived from figure 3; 0·58 g/ml of PMMA and PLGA at 1500 rpm in 3 L (■); 0·58 g/ml of PMMA and PLGA at 870 rpm in 10 L (▲); 0·58 g/ml of PMMA at 350 and 600 rpm in 100 L (●); 0·29 g/ml of PMMA at 500 and 890 rpm in 3 L (□); 0·29 g/ml of PMMA at 535 and 870 rpm in 10 L (△); 0·29 g/ml of PMMA at 300 and 500 rpm in 100 L (○); 1 L CSTR for 0·145, 0·29 and 0·58 g/ml of PLGA (——).

between experimentally determined sizes and the calculated values is mostly within 10%, suggesting that the correlation based on protein-free microspheres could predict the size of the S/O microspheres satisfactorily.

System scale-up

Large-scale production of protein-free microspheres were done in the 3, 10 and 100 L reactors. Four batches of PMMA microspheres were prepared in a 100 L CSTR. Both PMMA and PLGA microspheres were prepared in the 3 and 10 L reactors. In all cases, 6% aqueous PVA solution was used as the dispersion phase. Experimental variations in these batches were polymer concentration, 0·29 or 0·58 mg/ml, and agitation speed. Detailed experimental conditions are listed in table 2, where the range of impeller speed decreased with increasing reactor size. The rotational speed used for the 100 L reactor was in the range of 300–600 rpm because high input energy was required to agitate a large volume of solution at high speeds. Low agitation speeds were avoided because they generated large microspheres with a broad distribution. We plotted the mean size of the microspheres from each batch

into Figure 4. The straight line was calculated based on equation (9) and Figure 3. All the data points are 20% within the linear correlation, suggesting that equation (9) can be used to predict the size of microspheres prepared in reactors up to 100 L. Although the applicability of equation (9) to even larger reactors require more experimental evidence, this study provides system scale-up with a theoretical basis.

Conclusion

The correlation between the size of protein/excipient-free microspheres and all of the relevant process variables was successfully established using dimensional analysis. This empirical correlation was found to be effective in predicting the size of both PLGA and PMMA microspheres in different dispersion phases. When applied to solid protein-loaded microspheres, this correlation was still applicable. More importantly, the same equation offered a theoretical basis for large-scale microencapsulation systems where the size of the microspheres prepared in reactors up to 100 L could be predicted.

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Appendix

We derived dimensionless groups for equation (A1) according to Buckingham theory and Rayleigh's method of indices (Johnstone and Thring 1957). This method is based on the fact that any equation representing the behaviour of the system must be dimensionally homogeneous; therefore, it would be possible to write it in the form of dimensionless groups.

Equation (4) contains 10 variables,

$$d_p = f(D\omega, g_c, g, c, \mu_0, \mu_\alpha, \rho_0, \rho_\alpha, \sigma), \tag{4}$$

so we assume it can be transformed to i dimensionless groups:

$$\Pi_1 = f(\Pi_2, \Pi_3, \ldots, \Pi_i) \tag{A1}$$

where $i = 10 - r$. The quantity r is often equal to the number of fundamental dimensions. In this case we use four dimensions, length (L), time (Θ), mass (M) and force (F); so that there should be six dimensionless groups for equation (A1). In order to find the six groups, it is required to find from equation (4) four variables which will not form a dimensionless group. By trial and error, we find that g_c, g, ρ_α and σ, meet this criteria. That is, for any number of a, b, c and d (except they are all 0s), $g_c^a g^b \rho_\alpha^c \sigma^d$ is not dimensionless.

Then we multiply the above group by a variable from eqn. (4), for example, d_p, to make $g_c^a g^b \rho_\alpha^c \sigma^d$ dimensionless in the form of equation (A2).

$$\Pi_1 = g_c^a g^b \rho_\alpha^c \sigma^d d_p^e. \tag{A2}$$

Substituting each variable with its dimensions in equation (A2) gives

$$1 = (ML/F\Theta^2)^a (L/\Theta^2)^b (M/L^3)^c (F/L)^d (L)^e. \tag{A3}$$

Thus the coefficients of each variable must satisfy the following equations:

For [L] $0 = a + b - 3c - d + e$
For [M] $0 = a + c$
For [Θ] $0 = -2a - 2b$
For [F] $0 = -a + d$

By arbitrarily setting $e = 2$, these four equations can be solved to get $a = -1$, $b = 1$, $c = 1$, and $d = -1$. From these results we obtain equation (A4) from equation (A2)

$$\Pi_1 = g_c^{-1} g^1 \rho_\alpha^1 \sigma^{-1} d_p^2 = g\rho_\alpha d_p^2/(g_c\sigma),$$

or

$$\Pi_1 = g(\rho_\alpha - \rho_0)d_p^2/(g_c\sigma). \tag{A4}$$

For Π_2, $D\omega$ is substituted for d_p as the fifth variable in equation (A2)

$$\Pi_1 = g_c^a g^b \rho_\alpha^c \sigma^d (D\omega)^e, \tag{A5}$$

and

$$1 = (ML/F\Theta^2)^a (L/\Theta^2)^b (M/L^3)^c (F/L)^d (L/\Theta)^e. \tag{A6}$$

Again, the coefficients on each variable should satisfy the following equations:

For [L] $0 = a + b - 3c - d + e$
For [M] $0 = a + c$
For [Θ] $0 = -2a - 2b - e$
For [F] $0 = -a + d$

By arbitrarily setting $e = 4$, these four equations can be solved to get $a = -1$, $b = -1$, $c = 1$, and $d = -1$. From these results we obtain

$$\Pi_2 = \rho_\alpha (D\omega)^4/(g_c\sigma g). \tag{A7}$$

In a similar way the other Πs are obtained by changing the fifth variable in equation (A2) to μ_0, μ_α, c, and ρ_0. The results are

$$\Pi_3 = g\mu_\alpha^4/(\rho_\alpha g_c^3 \sigma^3) \tag{A8}$$

$$\Pi_4 = g\mu_0^4/(\rho_\alpha g_c^3 \sigma^3) \tag{A9}$$

$$\Pi_5 = c/\rho_\alpha \tag{A10}$$

$$\Pi_6 = \rho_0/\rho_\alpha. \tag{A11}$$

Π_6 is not used in this study because ρ_0/ρ_α is a constant.

References

BAKER, R., 1987, *Controlled Release of Biologically Active Agents* (New York: John Wiley) pp. 206–214.

BENNETT, C. O., and MYERS, J. E., 1962, *Momentum, heat, and Mass Transfer*, (New York: McGraw-Hill), pp. 175–192.

BIRD, R. B., STEWARD, W. E., and LIGHTFOOT, E. N., 1960, *Transport Phenomena*, pp. 185–201.

BEYGER, J. W., and NAIRN, J. G., 1986, Some factors affecting the microencapsulation of pharmaceuticals with cellulose acetate phthalate, *Journal of Pharmaceutical Science*, **75**, 573–578.

BINDSCHAEDLER, C., LEONG, K., MATHIOWITZ, E., and LANGER, R., 1988, Polyanhydride Microspheres formation by Solvent Extraction, *Journal of Pharmaceutical Science*, **77,** 696–698.

BOLZ, R. E., and TUVE, G. L., 1970, *Handbook of Tables for Applied Engineering Science* (Boca Raton, Florida: The Chemical Rubber Company) pp. 838–844.

BOUCHER, D. F., and ALVES, G. E., 1959, Dimensionless Numbers. *Chem. eng. Progr.*, **55**(9), 55.

DAVIS, S. S., ILLUM, L., MCVIE, J. G., and TOMLINSON, E., 1984, *Microspheres and Drug Therapy* (Amsterdam, The Netherlands: Elsevier) pp. 32–46.

DEASY, P. B., 1984, *Microencapsulation and Released Drug Processes* (New York: Marcel Dekker), pp. 68–75.

HO, C. S., and OLDSHUE, J. Y., 1987, *Biotechnology processes: Scale-up and mixing* (AIChE, New York, New York), pp. 3–42.

ITAWA, M., and MACGINITY, J. W., 1992, Preparation of multi-phase microspheres of poly(D,L-lactic acid) and poly(D,L-lactic acid-co-glycolic acid) containing a W/O emulsion by a multiple solvent evaporation technique. *Journal of Microencapsulation*, **9,** 201–214.

JOHNSTONE, R. E., and THRING, E. W., 1957, *Pilot Plants Models and Scale-Up Methods*, Chemical Engineering Series (New York: McGraw-Hill).

LENG, D. E., 1991, Succeed at Scale Up. *Chemical Engineering progress*, June, 23–31.

MENDIBOURE, B., GRACIAA, A., LACHAISE, J., MARION, G., BOURREL, M., and SALAGER, J. L., 1991, Influence of the intensity of mixing on the droplet size distribution of emulsion: theory and experiment. *Progress in Colloid Polymer Science*, **84,** 338–341.

PERRY, R. H., and GREEN, D., 1984, Perry's Chemical Engineering handbook, sixth Edition, (New York: McGraw-Hill), Table 5–19, Chapter 5, 62.

RHINE, W. D., HSIEH, D. S. T., and LANGER, R., 1980, Polymers for sustained macromolecule release: procedures to fabricated reproducible delivery systems and control release kinetics. *Journal of Pharmaceutical Science*, **69,** 265–270.

SANGHVI, S. P., and NAIRN, J. G., 1992, Effect of viscosity and interfacial tension on particle size of cellulose acetate timellitate microspheres. *Journal of Microencapsulation*, **9,** 215–227.

SIEGEL, R. A., and LANGER, R., 1984, Controlled release of polypeptides and other macromolecules. *Pharmaceutical Research*, 2–10.

SIVARAMAKRISHNAN, K. N., 1990, Controlled release delivery device for macromolecular proteins, *PCT International Publication Number WO90/11070.*

WHATELEY, T. L., 1992, *Microencapsulation of Drugs*, (Harwood Academic Publishers, Switzerland and UK).

YAMAGUCHI, I., YABUTA, S., and SAGATA, S., 1963, *Chem. eng.* (*Japan*), **27** (8), 576.