The Production of Uniformly Sized Polymer Microspheres

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INTRODUCTION

As drug carriers, degradable microspheres have the advantages of providing a large surface area, being easily injected, and not requiring removal after completion of drug release. When used as an injectable drug delivery device, it has been found that drug release rate (1) and microsphere interaction with cells (2) is strongly dependent on the size distribution of the microspheres. Numerous methods have been employed to prepare uniformly-sized polymer microspheres (1,3–13). However, when continuous production of uniformly sized biocompatible, biodegradable, drug-loaded microspheres and the use of volatile solvents is necessary, these methods have definite disadvantages (14).

A new method of preparing microspheres of controllable size and having a narrow size distribution will be presented in this article. The method involves the injection of a solution to be emulsified, and eventually formed into microspheres, into a stabilizing solution flowing past the injection point. The mechanism of microsphere formation will also be discussed. To discern the important parameters governing microsphere formation, the process was modeled via dimensional analysis.

MATERIALS AND METHODS

Poly(lactide-co-glycolide) 85:15 (M_w = 88,000) (PLGA) was purchased from Birmingham Polymers Inc., USA, dichloromethane (DCM) was purchased from BDH, Canada, poly(vinyl alcohol) (PVA) (M_w = 13,000 − 23,000) was purchased from Aldrich, USA, and blunt ended needles were obtained from Chromatographic Specialties, Canada.

Microspheres were formed as follows. A solution of PLGA in DCM was pumped through a stainless steel, blunt ended needle and into a PVA in water solution flowing perpendicularly past the needle tip. The droplets formed flow in the stabilizing solution through tubing and are collected in separate tank. The PVA velocity was varied from 16.5 cm/s to 164.7 cm/s, two PLGA concentrations (5 w/v%, 10 w/v%) and two PVA concentrations (1w/v% and 2 w/v%) were used, and two needle gauges (31 and 26, outside diameters of 0.027 and 0.045 μm respectively) were tried. Nascent microspheres were prepared using a constant PLGA volumetric flowrate of 0.5 ml/min for 5 minutes. The nascent microspheres were hardened by decanting excess PVA solution from the collection flask so that only 50 ml remained. To the collection flask was then added 450 ml reverse osmosis water (i.e., water purified by membrane filtration). The microsphere suspension was slowly stirred overnight in a fumehood, using an overhead stirrer.

The solid-liquid interfacial tension between the needle and the PLGA solution, which is a measure of the force of attraction between the PLGA solution to the needle, was determined by measuring the diameter of the droplet forming on the needle tip just prior to it detaching from the needle, within a PVA solution. This measurement (done in quadruplicate) was accomplished by capturing the process of droplet formation using a slow PLGA solution velocity and an image analysis system. The interfacial tension was calculated by equating the forces acting on the droplet under these conditions, i.e., buoyancy ($\Delta\rho\pi d^3 g/6$) and interfacial tension ($\gamma\pi D$). Equating these two forces and resolving for interfacial tension leads to,

$$\gamma = \frac{\pi d^3 \Delta\rho g}{6D} \quad (1)$$

in which g is the gravitational constant, D is the outside diameter of the needle, d is the diameter of the droplet just before falling from the needle, and $\Delta\rho$ is the density difference between the PLGA solution and the PVA solution. $\Delta\rho$ was determined by accurately weighing 1 ml of each polymer solution, kept in an enclosed environment, on an analytical balance 5 times and then using the average of these measurements. The viscosity of the PVA solution used was determined using a Cannon-Fenske capillary viscometer. The reflux time through the capillary for each PVA solution was measured in triplicate. The viscosity of the solution was then determined by correlation of these reflux times to that of water. The size distribution of the hardened microspheres was determined by placing a number of the microspheres on a glass microscope slide and measuring, using a microscale, the diameters of greater than 300 microspheres. The mean diameter of the microspheres is reported as a volume-moment average and the standard deviation is reported as the geometric standard deviation.

RESULTS AND DISCUSSION

The mean diameters and standard deviations obtained, and the operating conditions used to obtain them, are listed in Table I. Examination of the results indicates that smaller microspheres result from increasing the PVA solution velocity, reducing the interfacial tension between the PLGA solution and the needle tip, and, at low PVA solution velocity, reducing the needle outside diameter. The viscosity of the PVA solution alone does not appear to play a large role in determining the microsphere mean diameter. To determine the manner in which these parameters influence the size of the microspheres the process of microsphere formation was analyzed and modeled.

Mechanism of Droplet Formation

Polymer solution droplets are drawn off the needle tip in a regular and periodic fashion under the action of the PVA solution flow. The size and size distribution of the droplets is

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Table I. Experimentally Determined Mean Hardened Microsphere Diameter, d_f, Standard Deviation, s, Π_5, and Operating Conditions[a]

D (μm)	μ_s (g/(cm · s))	γ (dyne/cm)	v_s (cm/s)	d_f (μm)[b]	s	Π_5
254	0.015	71	16.5	295	13.8	286.9
254	0.015	71	28.8	228.2	23.3	164.4
254	0.015	71	41.2	196.6	68.7	114.9
254	0.015	71	82.4	130.7	29.5	57.4
254	0.015	71	123.5	80.7	19.8	38.3
254	0.015	71	164.7	67.7	19.7	28.7
254	0.015	71	20.6	290	19.9	229.8
254	0.015	42.6	41.2	156.4	44.1	68.9
254	0.015	42.6	82.4	79.9	12.4	34.5
254	0.015	42.6	123.5	71.1	12.4	23.0
457	0.025	35.5	16.5	268	33.7	86.1
457	0.025	35.5	41.2	143.3	21.8	34.5
457	0.025	35.5	82.4	100.6	8	17.2
457	0.015	42.6	41.2	244.4	16.1	68.9
457	0.015	42.6	82.4	120	19.1	34.5
457	0.015	42.6	123.5	76.2	16.2	23.0

[a] D is the outside diameter of the needle, μ_s is the viscosity of the PVA solution, and γ is the interfacial tension at the needle-PVA solution interface.
[b] Measured from a single lot.

controlled by the physical properties of the stabilizing solution, the needle outside diameter, and the surface tension between the polymer solution and the needle tip. At the needle tip the forming droplet experiences a number of forces (Fig. 1). There is the force of gravity drawing the droplet downwards, F_g, the surface tension at the polymer solution and needle interface, F_γ, the inertial force of the stabilizing solution contacting the cross-sectional area of the droplet, F_i, and the viscous shearing force of the stabilizing fluid on the droplet, F_v. These forces can be expressed a follows,

$$F_g \propto \Delta\rho d^3 \tag{2}$$

$$F_\gamma \propto \gamma D \tag{3}$$

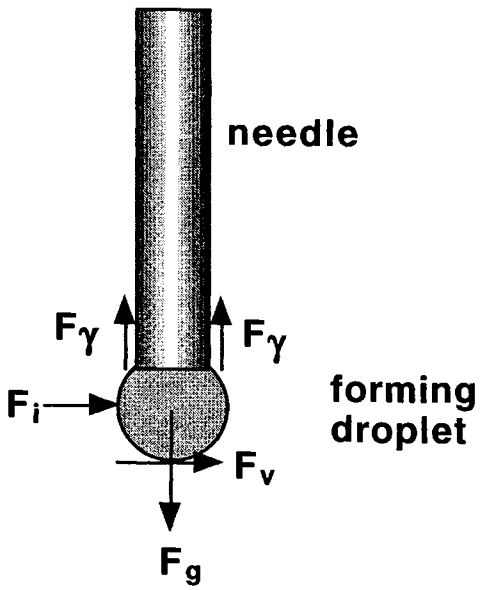

Fig. 1. Forces acting on forming polymer solution droplet.

$$F_i \propto \rho_s v_s^2 d^2 \tag{4}$$

$$F_v \propto \mu_s v_s d \tag{5}$$

in which Δρ is the density difference between the two solutions, ρ_s is the density of the PVA solution, γ is the interfacial surface tension, v_s is the velocity of the PVA solution, and μ_s is the viscosity of the PVA solution.

Due to the complexity of the situation, an analytical relationship between these forces would be extremely difficult to derive and would entail numerous, difficult to validate, assumptions. For these reasons, a dimensional analysis method will be applied. Dimensional analysis is a useful engineering tool often used to derive relationships between variables in complex problems (15). The method involves converting the important independent variables of the problem into the minimum number of dimensionless groups. These dimensionless groups are functionally interdependent, and so a correlation between the groups can be obtained by performing experiments in which all the independent variables are varied.

An examination of Eq. (2) to (5) reveals the following set of necessary independent variables $\{\Delta\rho, \rho_s, \gamma, \mu_s, D, d, v_s\}$. By using the three fundamental dimensions of time, length, and mass expressed in the subset of variables $\{\rho_s, v_s, d\}$, the following four dimensionless groups were derived,

$$\Pi_1 = \frac{d}{D} \tag{6}$$

$$\Pi_2 = \frac{\rho_s v_s d}{\mu_s} \tag{7}$$

$$\Pi_3 = \frac{\gamma}{\rho_s v_s^2 d} \tag{8}$$

$$\Pi_4 = \frac{\Delta\rho}{\rho_s} \tag{9}$$

The densities of the solutions remained essentially constant over the range of concentrations studied and so Π_4 was removed from consideration. Π_2 is the ratio of inertial forces to viscous forces and is known as the Reynold's number, while Π_3 relates the surface tension force to the inertial force and is known as the Weber number (15). As the groups are all dimensionless and functionally interdependent, the diameter of the forming droplet can be expressed as,

$$\frac{d}{D} = f(\Pi_2, \Pi_3) \tag{10}$$

The functional dependence of Equation (10) was determined by varying the variables in each dimensionless group and fitting an expression to the results. The microsphere diameter used in this correlation was that of the final hardened microspheres and not that of the nascent microspheres. It was assumed that the hardening process for each group of microspheres prepared was identical. This assumption is reasonable because each group of microspheres were hardened using the same procedure.

By plotting the mean diameter as a function of the product of the dimensionless groups it was determined that the mean diameter was a logarithmic function of $\Pi_2\Pi_3$ (Fig. 2). Curve fitting the data yielded the following equation,

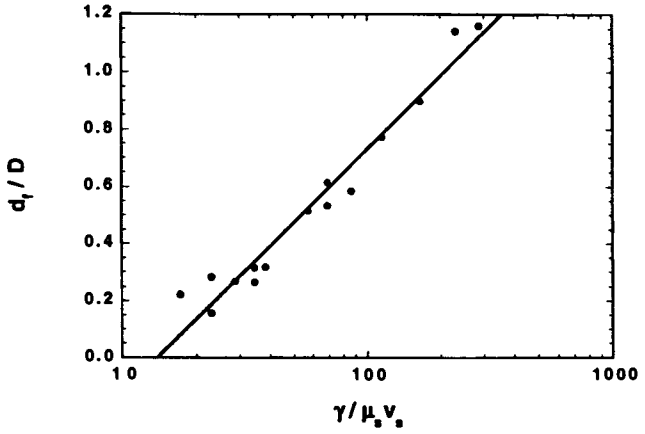

Fig. 2. Relationship between microsphere diameter and dimensionless groups.

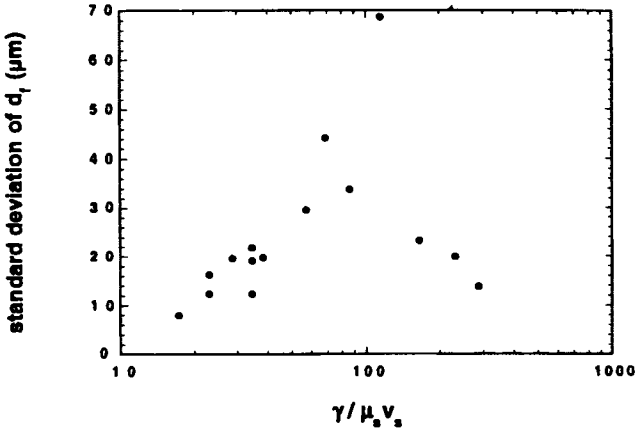

Fig. 3. Dependence of microsphere size distribution as indicated by population standard deviation on the dimensionless group $\gamma/(\mu_s v_s)$.

$$\frac{d_f}{D} = 0.37 \ln(0.072\ \Pi_2\Pi_3) \tag{11}$$

which provided a squared correlation coefficient of 0.96 (d_f = diameter of microsphere). Recognizing that the $\Pi_2\Pi_3$ product is simply another dimensionless group, the product was re-defined as,

$$\Pi_5 = \Pi_2\Pi_3 = \frac{\gamma}{\mu_s v_s} \tag{12}$$

This new dimensionless group relates the interfacial tension force to the viscous force at the needle tip, and is known as the Capillary number (15). Now Eq. (11) can be re-written as,

$$\frac{d_f}{D} = 0.37 \ln\left(0.072 \frac{\gamma}{\mu_s v_s}\right) \tag{13}$$

The finding that the diameter is only a function of Π_5 indicates that inertial forces do not play a significant role in determining the size of the microspheres formed. The size of the microsphere is determined by a balance between shearing action at the needle tip and the force holding the PLGA solution to the needle. To minimize microsphere mean diameter it is thus necessary to minimize the interfacial tension and/or maximize the stabilizing solution viscosity or the stabilizing solution velocity and/or minimize the outside diameter of the needle used. The logarithmic nature of Eq. (13) also indicates that there will be a point of negligible return in the manipulation of each of these variables in reducing the mean microsphere diameter.

Examination of Table I shows that, under the right conditions, it is possible to obtain narrow size distributions, and that these conditions are also determined by Π_5. The relationship between Π_5 and the microsphere population standard deviation is shown in Fig. 3. The figure demonstrates that small standard deviations (about 25 μm) and thus narrow size distributions are obtained when $\Pi_5 \geq 150$ and when $\Pi_5 \leq 40$. In other words, narrow distributions arise when either of the significant forces dominate. For Π_5 less than 40, the viscous shearing forces dominate while for Π_5 greater than 150 interfacial forces dominate.

CONCLUSIONS

A process for preparing microsphere having a narrow diameter distribution was described. This process involves injecting a polymer solution through a needle and into a stabilizing solution flowing past the needle tip. The diameter of the microspheres formed using this new process was found to be a logarithmic function of the stabilizing solution viscosity, μ_s, and velocity, v_s, and the interfacial tension between the polymer solution and the needle, γ. The spread of the microsphere diameter distribution was narrow if the value of $\gamma/(\mu_s v_s)$ was greater than 150 or less than 40. The size of the microspheres formed could be minimized by minimizing the outer diameter of the needle and by minimizing the value of $\gamma/(\mu_s v_s)$.

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