AAPS PharmSciTech

AAPS PharmSciTech, 2000; 1 (1) article 5
(http://www.pharmscitech.com)

Dynamic Changes in Size Distribution of Emulsion Droplets During Ethyl Acetate–Based Microencapsulation Process

Submitted: January 28, 2000; Accepted: March 20, 2000

Yogita Bahl and Hongkee Sah*

Room 214, 26 S. Dunlap Street, Department of Pharmaceutical Sciences,The University of Tennessee College of Pharmacy, Memphis, TN 38163

ABSTRACT This study investigated the dynamic effect of the emulsification process on emulsion droplet size in manufacturing microspheres using ethyl acetate as an organic solvent. A dispersed phase consisting of poly(lactide-co-glycolide) and ethyl acetate was emulsified in a poly(vinyl alcohol) aqueous solution for a predetermined time ranging from 2 to 9, 16, 23, 30, 40, 50, or 60 minutes. Ethyl acetate was then quickly extracted to transform emulsion droplets into solidified microspheres, and their size distribution was determined. This experimental design allowed quantification of the size distribution of emulsion droplets over the course of emulsification. When emulsification time was extended from 2 to 60 minutes, the emulsion droplets decreased in size from 98.1 to 50.3 μ m and their surface area increased from 0.07 to 0.29 m^2/g. Overall, prolonging emulsification time up to 60 minutes resulted in the progressive evolution of smaller emulsion droplets (1-60 μ m) and the simultaneous disappearance of larger ones (> 81 μ m). Increases in the total number of microspheres and their surface area were caused mainly by continuous fragmentation of emulsion droplets before ethyl acetate extraction. The increase in the smaller microsphere population might also be due in part to shrinkage of microspheres. These results show that the onset of ethyl acetate extraction influenced the kinetics of the breakup and formation of emulsion droplets, thereby affecting to a great extent the size distribution of microspheres.

KEYWORDS: Ethyl Acetate, Emulsion, Microspheres, Microencapsulation, PLGA

***)Corresponding Author:** Hongkee Sah; telephone: (901) 448-5505; facsimile: (901) 448-6092; e-mail: hsah@utmem1.utmem.edu

INTRODUCTION

Poly-*d,l*-lactide-*co* -glycolide (PLGA) polymers are known to exhibit excellent biocompatibility and versatile biodegradability. These features have made it possible to develop a microsphere dosage form that can deliver various drugs over a wide range of time periods. The most frequently used microencapsulation technique is an emulsion-based solvent evaporation/extraction technique [1,2]. The typical manufacturing process to encapsulate a hydrophobic drug consists of three major steps: (1) emulsifying a dispersed phase in an emulsifier-containing aqueous continuous phase; (2) removing a dispersed organic solvent by extraction and/or evaporation to cause PLGA precipitation and subsequent microsphere hardening; and (3) collecting microspheres by filtration or centrifugation. A prime issue in developing a microsphere product is controlling the size distribution of microspheres. The importance of this issue is reflected in designing a reactor, operating it efficiently, and evaluating the reproducibility of a process. Batch-to-batch consistency in microsphere size should also be guaranteed to ensure the same quality of the final microsphere product.

A number of reports have addressed the effects of formulation and process parameters on the size distribution of microspheres. Commonly studied variables include mixer type, mixing intensity, emulsifier concentration and type, the volume ratio of dispersed phase to continuous phase, phase viscosity, and polymer concentration [3– 8]. However, little information is available on the dynamic changes in microsphere size that occur during emulsification. In fact, emulsification is a complicated process that

involves the deformation of an interface between two immiscible liquid phases, the formation of primary emulsion droplets, and their subsequent breakup and coalescence. The complexity of this process has presented an obstacle to studying real changes in droplet size over the course of emulsification.

The current study investigates the dynamic effect of an emulsification process on the size distribution of emulsion droplets. Changes in their size distribution over emulsification time have been correlated with the size distribution of the final hardened microspheres. Ethyl acetate was used in this study as a dispersed solvent to dissolve a PLGA polymer because of its comparatively low toxicity to humans and the environment.

MATERIALS AND METHODS

Materials

PLGA with a lactide:glycolide ratio of 75:25 (PLGA75:25) was obtained from Birmingham Polymers, Inc. (Birmingham, AL). Its inherent viscosity was 0.67 dL/g in chloroform at 30° C. An 88% hydrolyzed poly(vinyl alcohol) with a molecular weight of 25,000 was supplied by Polysciences, Inc (Willmington, DE). Ethyl acetate was of analytical grade.

Preparation of an Ethyl Acetate-in-Water Emulsion

A modified oil-in-water (o/w) emulsion technique was used to prepare an ethyl acetate-in-water emulsion [9]. First, 400 mg of PLGA75:25 was dissolved in 7 mL of ethyl acetate. This dispersed phase was added to 20 mL of a mixture of a 1% poly(vinyl alcohol) aqueous solution and ethyl acetate (18.5:1.5 vol/vol). The presence of 1.5 mL of ethyl acetate in the aqueous phase helped prepare a primary o/w emulsion without causing immediate PLGA75:25 precipitation. During the addition, the continuous phase was stirred at 450 rpm with a magnetic plate stirrer (Model 400 HPS,VWR Scientific; Pittsburgh, PA). The stirring was then carried out for a predetermined time ranging from 2 to 60 minutes.

Transformation of Emulsion Droplets to Hardened Microspheres

To obtain information on the size of emulsion droplets over the course of emulsification, an emulsion stirred for a specific time was quickly transferred into 150 mL of a 1% poly(vinyl alcohol) aqueous solution that was being stirred at 450 rpm. This extraction, the so-called quenching step, allowed the immediate leaching of ethyl acetate in the dispersed phase to the continuous phase, causing PLGA75:25 precipitation. As a result, the emulsion droplets were immediately transformed into hardened, solid microspheres. Observation under a light microscope (Unico™ /World Precision Instruments, Inc., Sarasota, FL) showed that the hardened microspheres did not change their size or coalesce when subjected to mechanical stirring. After 3 hours of stirring, the microspheres were filtered, collected, and resuspended in 20 mL of a 1% poly(vinyl alcohol) aqueous solution to determine their size distribution pattern. (The microsphere suspension had to be concentrated for proper measurement of the size distribution pattern. When filtration occurred right after quenching, it was difficult to resuspend the collected microspheres in 20 mL of the aqueous solution. The residual ethyl acetate in the microspheres seemed to cause microsphere aggregation during filtration. An additional 3 hours of stirring before filtration made it easier to resuspend the collected microspheres in the aqueous solution.)

Determination of the Degree of Solvent Evaporation From an Emulsion

The amount of ethyl acetate evaporating through the air/emulsion interface was determined as a function of stirring time. To mimic the emulsification process described earlier, 8.5 mL of ethyl acetate was added to 18.5 mL of a 1% poly(vinyl alcohol) aqueous solution. Loss in the weight of the emulsion was determined as a function of stirring time to calculate the amount of ethyl acetate that evaporated. A similar experiment was carried out in the presence of 400 mg of PLGA75:25 to evaluate its effect on the rate of ethyl acetate evaporation. In addition, to account for the loss of water from the emulsion, 18.5 mL of 1% poly(vinyl alcohol) aqueous solution alone was stirred and its weight change was monitored as a function of stirring time.

Evaluation of the Viscosity of a Dispersed Organic Phase

Determining ethyl acetate evaporation helped estimate the amount of ethyl acetate residing in the dispersed phase over the course of emulsification. Ongoing emulsification was thought to increase the viscosity of the dispersed phase. To reflect this condition, a series of PLGA75:25-containing ethyl acetate solutions was prepared at concentrations of 57.3, 67.3, 81.8, and 104 mg/mL. The viscosity of each ethyl acetate solution was then determined by a fully automated StessTech Rheometer (ATS RheoSystems, Bordentown, NJ) using a 40-mm parallel plate geometry at ambient temperature.

Analysis of the Size Distribution and Specific Surface Area of Microspheres

A Horiba CAPA-700 particle size analyzer (Horiba, Ltd, Kyoto, Japan) was used to measure the size distribution and specific surface area of microspheres, which represented the same characteristics of emulsion droplets. The instrument measures the intensity of light transmitted through the suspension of microsphere samples. The degree of optical transmission is linked to the Stokes sedimentation law to correlate absorbance with the diameter of microspheres. The distribution (F_i) of the volume-based microsphere diameter (D_i) was then determined by equation (1):

$$F_i = \frac{(\log I_0 - \log I_i)}{\sum_{i=1}^{n}\{(\log I_0 - \log I_i)\times D_i\}} \qquad (1)$$

where I_0 is intensity of light beamed at a microsphere suspension; I_i, intensity of light transmitted through the suspension; and D_i, microsphere diameter. Equation (2) was used to measure the specific surface area of microspheres (S_w, in the unit of m^2/g):

$$S_w = \frac{6}{\rho}\sum_{i=1}^{n}\left(\frac{F_i}{D_i}\right) \qquad (2)$$

where ρ is microsphere density and F_i is the distribution of the volume-based microsphere diameter (D_i).

Microsphere Morphology

After measuring size and surface area, the microspheres were dried overnight under vacuum. Dried microsphere samples were mounted on an aluminum stub and sputter-coated with palladium/gold in an argon atmosphere. Their surface morphology was observed under a scanning electron microscope (Model JSA-840A, Joel Inc, Peabody, MA).

RESULTS

Our ethyl acetate–based emulsion microencapsulation process produced smooth, spherical microspheres. Hardening emulsion droplets at different stirring time intervals during the process did not affect the surface morphology of the resultant microspheres (**Figure 1**).

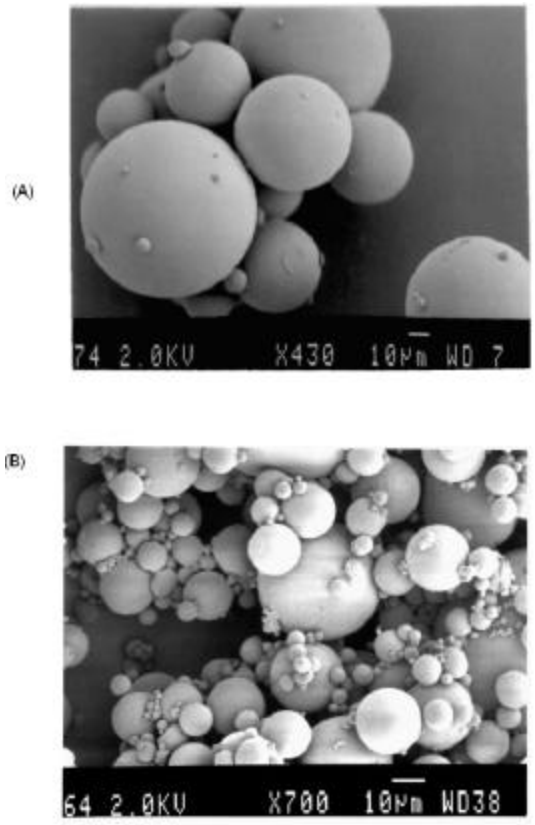


Figure 1. Scanning electron microscope micrographs of the microspheres prepared by quenching after (A) 2-minutes and (B) 30-minutes stirring time. The size of bar is 10 ± m.

However, as seen in **Figure 2**, variations in the onset of ethyl acetate extraction/quenching changed the size distribution of microspheres.

Increases in stirring time were accompanied by the steady, gradual evolution of smaller microspheres and the simultaneous disappearance of larger ones. **Figure 3** reinforces this finding by showing the mean diameters of the microspheres hardened after 2, 9, 16, 23, 30, 40, 50, and 60 minutes of emulsification. For instance, 2-minutes of stirring followed by ethyl acetate quenching led to the formation of microspheres with a mean diameter ± SD of 98.1± 0.9 μ m (determined on three microsphere batches). When an emulsion was stirred for 60 minutes, the microsphere size decreased to 50.3± 5.6 μm.

To determine whether emulsion droplets underwent shrinkage or fragmentation before quenching was executed, the total specific area of microspheres was determined at different emulsification times (**Figure 4**). The specific area of the microspheres prepared by quenching at 2-minutes stirring time was only 0.07± 0.01 m^2/g. In sharp contrast, quenching at 60-minutes stirring time caused their specific surface area to increase more than four times: 0.29± 0.01 m^2/g was the value obtained for these microspheres. These results suggest that emulsion droplets were consistently fragmented into smaller ones to generate greater surface area until quenching hardened them.

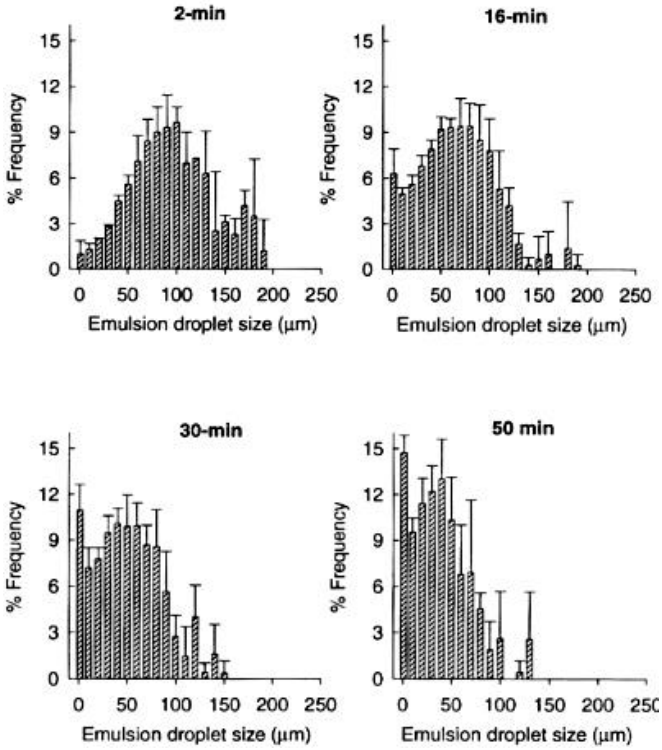


Figure 2. The size distribution patterns of emulsion droplets determined at different emulsification time intervals of 2, 16, 30, and 50 minutes (mean ± SD; n = 3).

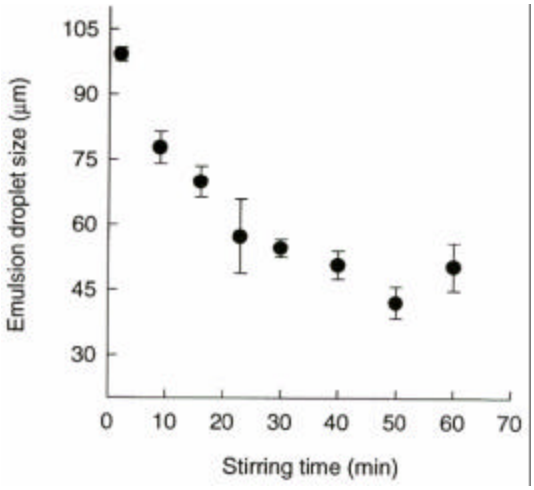


Figure 3. Dynamic changes in the size of emulsion droplets over the course of emulsification (mean ± SD; n = 3). The mean diameter of emulsion droplets was determined as a function of stirring time ranging from 2 to 60 minutes.

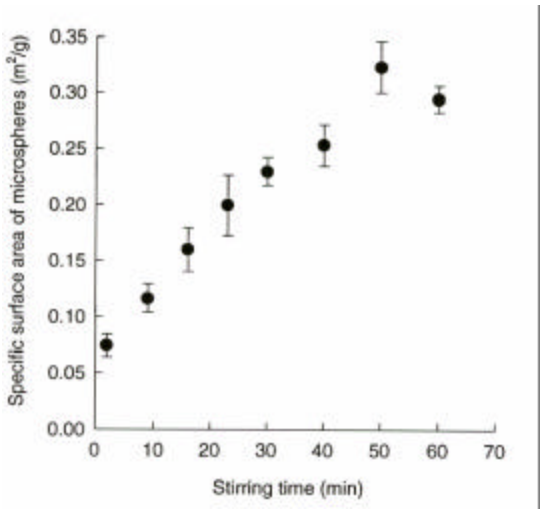


Figure 4. Dynamic changes in the specific surface area of microspheres over the course of emulsification (mean ± SD; n = 3). Their specific surface area was determined as a function of stirring time ranging from 2 to 60 minutes.

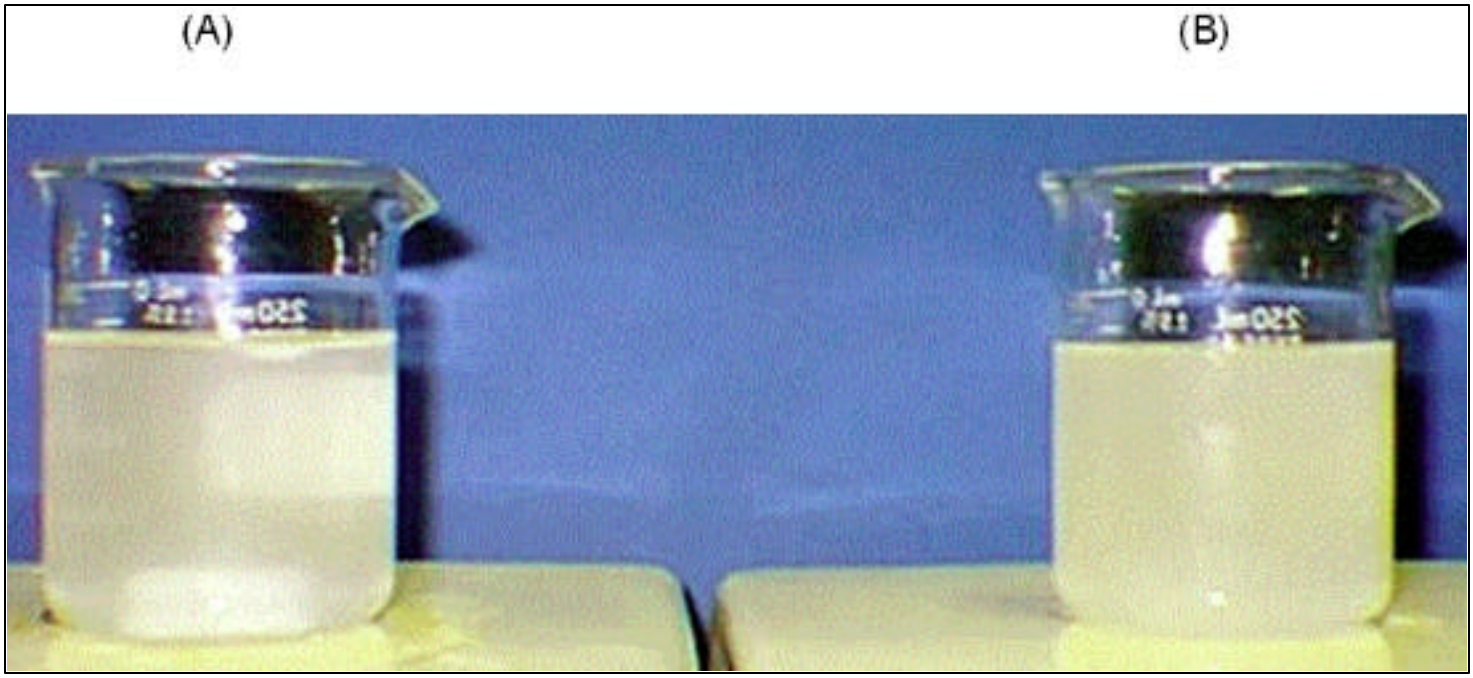
To further investigate this process, the suspensions of the microspheres that had hardened after different stirring periods were inspected visually and under a light microscope. The microsphere suspension that was quenched after a longer stirring time was found to be more turbid than the suspension quenched after a shorter stirring time (**Figure 5**). This increased turbidity was caused by an increase in the total number of microspheres and a reduction in microsphere size, both of which were confirmed by observing microsphere suspensions under a light microscope. These results confirm our finding that when an emulsion is subjected to a longer stirring period, fragmentation of emulsion droplets leads to an increase in the total number of emulsion droplets and hence their surface area.

To further investigate dynamic changes in the size distribution of emulsion droplets, the population of microspheres in a specific size range was monitored at various emulsification intervals. As illustrated in **Figure 6A**, the microsphere population of 1-60 μ m increased steadily as emulsification proceeded. Microspheres of 1-20 μ m showed the sharpest increase: their percentage frequency grew from 1.2± 0.6% to 11.8± 2.9% when emulsification time was extended from 2 to 60 minutes, respectively.

Figure 5. Microsphere suspensions prepared by quenching after (A) 2-min and (B) 30-minutes stirring time. Pictures were taken during stirring conditions.

In comparison with the above results, the microsphere population of 61-80 μm remained relatively unchanged over a 60 minutes of emulsification: at 2- and 60-minutes stirring time their percentage frequency was 7.7± 1.6% and 7.2± 1.3%, respectively. The microsphere population larger than 81 μ m, however, declined with the ongoing emulsification (**Figure 6B**). The percentage populations of the microspheres of 81-100 μ m observed at 2- and 60-minutes stirring time were 9.1± 1.7% and 5.9± 1.0%, respectively. A similar trend was seen with the microspheres of 101-120 μ m and 121-140 μ m. All the data in **Figure 6** strongly support our earlier assertion that larger emulsion droplets were formed in the early stage of emulsification and were continuously broken into smaller ones as emulsification proceeded.

The data in **Figures 3** and **4** indicate that, after 30 minutes of stirring, the reduction in microsphere size and the increase in their surface area were not as sharp as that observed during the initial emulsification stages. A gradual viscosity increase of the dispersed phase was supposed to be one reason for these phenomena. (Continual emulsification would increase the viscosity of the dispersed phase, because the evaporation of ethyl acetate from the aqueous phase would drive its leaching from the dispersed phase to the aqueous phase.) To corroborate this supposition, we investigated the evaporation tendency of ethyl acetate (**Figure 7**).



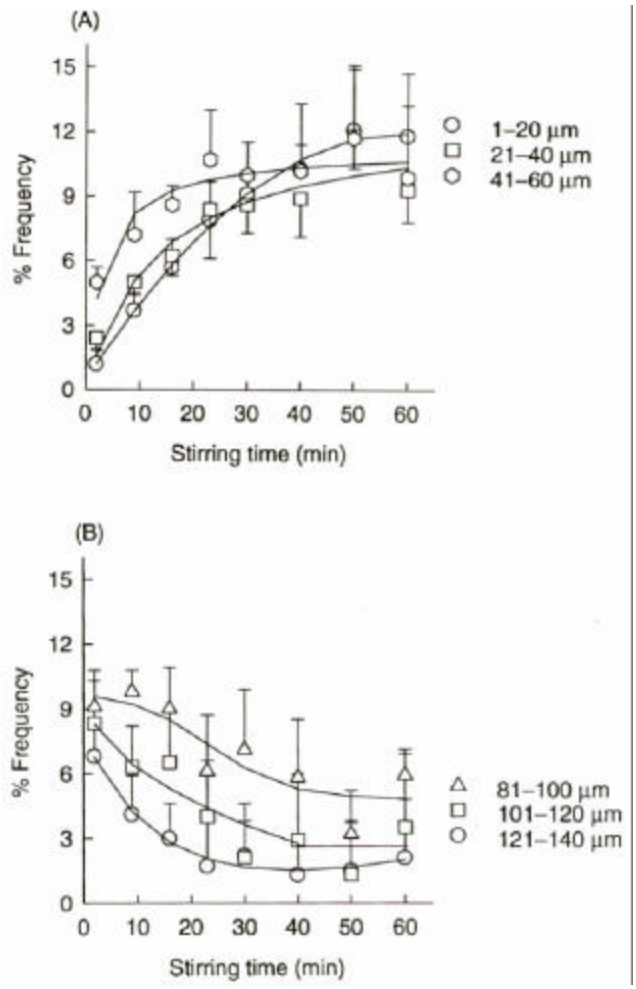


Figure 6. Percentage frequency of the population of microspheres in a specific size range as a function of emulsification time: (A) the steady evolution of 1 ~ 60 μ m emulsion droplets; and (B) the gradual disappearance of emulsion droplets larger than 81 μ m (mean ± SD; n = 3).

There was a linear relationship between stirring time and ethyl acetate evaporation through the air/emulsion interface. When the evaporation continued for 60 minutes under our experimental conditions, 37.4± 1.8% of ethyl acetate evaporated (only 1.5± 0.2% of water evaporated when 18.5 mL of the aqueous solution alone was stirred). In addition, it was confirmed that the presence of 400 mg of PLGA75:25 in the dispersed phase had a negligible effect on the rate of ethyl acetate evaporation: 35.6± 0.5% of ethyl acetate evaporated in 60 minutes.

To simulate the situation of increasing PLGA75:25 concentrations over the course of emulsification, a series of PLGA75:25-containing ethyl acetate solutions was

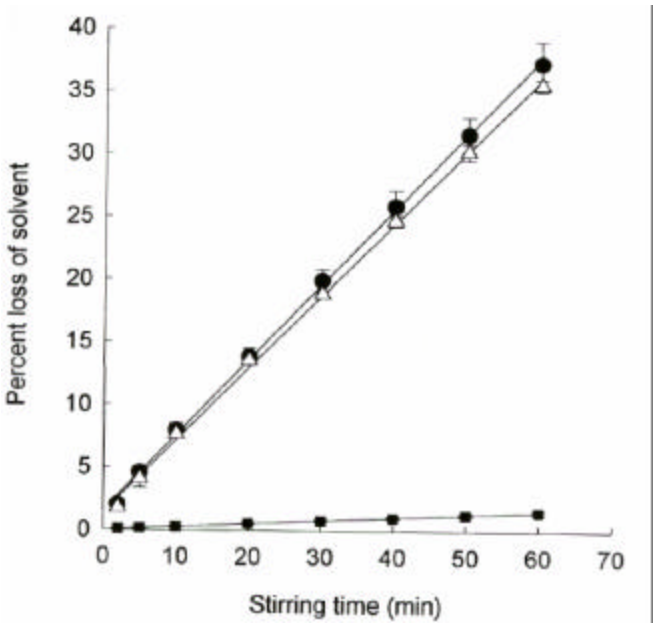


Figure 7. Percentage loss of solvent as a function of stirring time. The dispersed phase consisted of ethyl acetate (● ; mean ± SD; n = 7) or ethyl acetate and PLGA75:25 (Δ ; mean ± SD; n = 3). As a control experiment, water loss was also determined (■ ; mean ± SD; n = 3).

prepared at the 57.3-104 mg/mL concentrations. All the solutions exhibited Newtonian flow: they showed a shear rate–independent viscosity, such that a constant viscosity value was observed when shear rate was changed from 53 to 528 sec^{-1}. As shown in **Figure 8**, a gradual increase in the viscosity of an ethyl acetate solution was observed with increasing PLGA75:25 concentration. For instance, the viscosity of a 104 mg/mL PLGA75:25 solution was 3.4 times as viscous as that of a 57.3 mg/mL PLGA75:25 solution. This increase in viscosity might provide resistance to further breakdown of emulsion droplets at a later phase of emulsification.

DISCUSSION

Once a dispersed phase is placed in a turbulent stream of a continuous phase, large initial droplets appear and turbulent eddies break them up into smaller ones. Under the influence of large shear stress, the droplet size of an emulsion is correlated to

$$d = C_1 \varepsilon^{-2/5} \left(\frac{\sigma}{\rho}\right)^{3/5} f(\phi) \qquad (3)$$

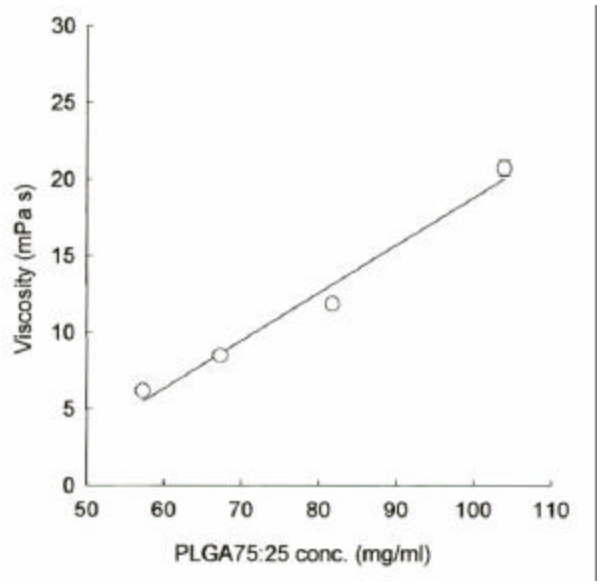


Figure 8. Increase in the viscosity of polymeric ethyl acetate solutions as a function of PLGA75:25 concentrations. Viscosity was measured in shear range from 53 to 528 sec^{-1} and the values obtained were averaged (mean ± SD; n = 15).

where d is the average size of droplets; C_1, a constant; ε, the rate of turbulent energy dissipation per unit mass; σ, the interfacial tension; ρ, the density of a continuous phase; and f (ϕ), a function related to the volume fraction of a dispersed phase [10]. It can be inferred from this equation that reactor and impeller geometry, mixer type, mixing intensity, emulsifier concentration and type, and the phase volume ratio can affect the size of emulsion droplets. A number of researchers have dealt with the subject of controlling the size of emulsion droplets or microspheres by studying these formulation/process variables [3–8]. However, these studies have not addressed the aspect of dynamic changes in the size of emulsion droplets that occur over the course of emulsification.

Our study clearly demonstrates the dynamic effect of emulsification on the size distribution pattern of emulsion droplets. Our data suggest that microsphere hardening does not occur at the ethyl acetate/water interface, as long as the limiting concentration for PLGA75:25 precipitation is never reached over a period of emulsification. (Under our emulsification condition, PLGA75:25 did not precipitate out of the dispersed phase; only after the ethyl acetate quenching step was executed, emulsion droplets transformed into solidified microspheres.) Since equilibrium droplet size is not established during emulsification, the size distribution of emulsion droplets and microspheres are influenced by emulsification timings and the onset of ethyl acetate quenching.

Previously, Maa and Hsu prepared polymeric microspheres by using a typical water-in-oil-in-water (w/o/w) emulsion microencapsulation process [11]. They found that smaller microspheres were consistently obtained when the primary w/o emulsion contained large aqueous droplets. They postulated an interesting "weakening effect" mechanism that accounted for the observed phenomenon: during a secondary emulsification process to form a w/o/w double emulsion, primary emulsion droplets were influenced by turbulent eddies. At that time, the preferred target for their fragmentation was thought to be the domain weakened by the presence of less viscous aqueous droplets. In contrast, our study shows that emulsion droplets that are free of aqueous domains—our droplets consisted of only PLGA75:25 and ethyl acetate—are still subject to an extensive, continual fragmentation over the course of emulsification.

Other groups also reported that the size of microspheres decreased as a function of stirring time during a methylene chloride-based emulsion microencapsulation process [12, 13]. This result was attributed to the shrinkage of microspheres caused by the gradual diffusion of methylene chloride into an aqueous continuous phase [13]. The study, however, did not consider the possibility of the breakup of emulsion droplets as a function of stirring time. In our study, decreases in the microsphere size as a function of emulsification time were accompanied by increases in the total number of microspheres and their surface area (**Figures 4** and **5**). Our results thus indicate that fragmentation of emulsion droplets is a major cause of decreasing microsphere size upon stirring, even though the increase in the smaller microsphere population may be due in part to their shrinkage during solvent extraction and evaporation.

It is of interest to note that the data in **Figures 2** and **6** demonstrate that the fragmentation process of emulsion

droplets is continuous, because the percentage frequency of the microspheres of 1-20 μ m constantly increases with the ongoing emulsification. On the contrary, **Figure 3** demonstrates that after 30 minutes of stirring time, the rate of reduction in the size of emulsion droplets slows down considerably, which suggests that emulsion droplets become stabilized against fragmentation. This supposed contradiction can be explained by the fact that the mean diameter of microspheres reported in this study is volume-based. As a result, the increasing population of the smaller microspheres does not affect the mean diameter of microspheres to a great extent, unless this increase is very sharp. (This was especially true when emulsification was prolonged beyond 30 minutes, because of the slower rate of fragmentation.)

Why does the breakup of emulsion droplets occur to a great extent in the early stage of emulsification and why are they less prone to fragmentation as emulsification time is extended? As mentioned earlier, emulsion droplets increase in viscosity with the ongoing emulsification, which contributes to their ability to withstand shear stress (**Figure 8**). The phenomenon might also be better understood if we consider that the deformation of emulsion droplets by shear stress is opposed by the Laplace pressure (Δ P) that aims to preserve the spherical shape. The Laplace pressure is inversely related to the radius of curvature of droplets and hence to their size ($\Delta P = 2\sigma / R$, where σ is the interfacial tension and R is the radius of an undeformed droplet). Therefore, it is likely that both an increase in the viscosity of emulsion droplets and a decrease in their size, accompanied by prolonging the period of emulsification, present synergistic resistance against movement to break up emulsion droplets.

Finally, it should be pointed out that coalescence also contributes to the dynamic changes in the size of the emulsion droplets, especially when stirring time is prolonged to a great extent. It is likely that during the initial stages of emulsification there is no significant coalescence of emulsion droplets. This supposition is backed up by the data in **Figures 2** and **6**, which clearly show the disappearance of larger emulsion droplets with the simultaneous appearance of smaller ones. When stirring time was extended, interestingly, the coalescence of emulsion droplets seemed to significantly affect the size distribution pattern of microspheres. For instance, 120 minutes of stirring followed by quenching led to a dramatic increase in the number of microspheres bigger than 100 μm: their percentage frequency was 15.4± 3.9% (determined on three microsphere batches). Associated with this was the widening of the size distribution pattern of microspheres. It can be inferred from these results that once PLGA75:25 reaches a critical concentration in the dispersed phase during the ongoing emulsification, emulsion droplets coalesce due to their semisolid, sticky nature. (A detailed study focusing on this issue is still in progress.)

In summary, emulsion droplets are continuously broken into smaller ones during the ethyl acetate–based emulsion microencapsulation process. Thus, prolonging emulsification time causes the disappearance of bigger primary emulsion droplets and the corresponding appearance of smaller ones. The onset of ethyl acetate extraction/quenching significantly influences the kinetics of the breakup and formation of emulsion droplets, thereby affecting the size of microspheres.

ACKNOWLEDGEMENTS

The authors thank Mr. Calvin Lacy for his technical assistance in measuring the size distribution of emulsion droplets and Dr. Rey Chern at Merck & Co., Inc., for his helpful comments on this study. The authors also thank Dr. Peter K.W. Herh at ATS RheoSystems for his kind help in using the StessTech Rheometer. This research was in part supported by the Memphis McNair Program.

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