The Effect of Process Variables on the Morphology and Release Characteristics of Protein-Loaded PLGA Particles

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ABSTRACT: Poly(lactide-*co*-glycolide) (PLGA 75 : 25), IV 0.94 dL/g was chosen as the matrix of the microparticles. Bovine serum albumin (BSA) (Fraction V) as the model drug was incorporated in the microparticles by a W/O/W emulsification and solvent evaporation technique. The effect of the various preparation parameters on particle morphology, drug loading efficiency, and drug release profiles of the resultant microparticles were examined. Particle size varied from 5 to 60 μm. The final morphology of the microparticles varied dramatically with preparation variables such as equipment used to produce the primary emulsion (W1/O) and the water-to-oil ratio (W1/O) in the primary emulsion. In general, the viscosity of the primary emulsion had a significant effect on the porosity of particles produced. The release of BSA showed a strong relationship with the preparation parameters of microparticles, partly due to the morphological effects. For example, microparticles made from the vortex mixer that was used to disperse inner aqueous phase (W1) to oil phase (O) showed a lower burst effect than that made from the homogenizer because of its better surface morphology. W1/O ratio, speed of dispersing the primary emulsion into W2, PLGA concentration, and different matrix materials also affected the drug release profiles. In all the samples studied here, only diffusion-controlled release was observed. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 101: 3053–3061, 2006

Key words: drug delivery systems; biodegradable; morphology; processing; microencapsulation

INTRODUCTION

Controlled release of therapeutic agents from biodegradable polymeric microparticles has been extensively studied. The increasing availability of recombinant proteins and synthetic peptides has created the need for new and improved treatments. Recent advances in recombinant DNA technology have led to potential therapies using protein drugs such as vaccines, hormones, growth factors, and cytokines to treat various diseases. However, oral administration of peptides and proteins is impractical due to degradation in the gastrointestinal tract. In addition, administration by the parenteral route may require daily injections.

Controlled drug delivery combines well-characterized, reproducible dosage-form design with clinical pharmacology, in particular, steady-state pharmacology. The most promising approach is the encapsulation of the protein drug within injectable microparticles composed of biodegradable and biocompatible polymers, especially poly(L-lactic acid) and its copolymers with D-lactic acid and/or glycolic acid. By careful selection of the appropriate polymer for microparticle preparation with a defined rate of degradation and morphology, it is possible to prepare a drug delivery system that releases the entrapped active agents for an extended period following administration.

The first injectable formulation of peptide microspheres to be marketed, under the trade name of Decapeptyl® by Ipsen Biotech (France) delivered a LHRH agonist, [D-Trp^6]-LHRH.[1] With a single injection, about 3.75 mg of drug is delivered, and released over a 1-month period. It has also been shown that LHRH analogs can be formulated into microspheres that release LHRH for periods of up to 3 or 6 months by mixing microsphere formulations.[2]

Usually, a water-in-oil-in-water (W/O/W) multiemulsion method is adopted to incorporate water soluble drug in the microparticles. The organic phase acts as a barrier between the two aqueous compartments, preventing the diffusion of the active material toward the external aqueous phase. Basically, an aqueous solution of the active compound is emulsified into an organic solution of the coating polymer (matrix). The primary W1/O emulsion is then dispersed in a second aqueous phase W2, with formation of a double water-in-oil-water (W1/O/W2) emulsion. Evaporation of the organic solvent leads to the hardening of the microparticles.[3–6] Ogawa et al. and Okada were the first to use the double emulsion technique to prepare an injectable poly(lactide-*co*-glycolide) micropar-

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ticle dosage form for the sustained release of leuprolide acetate.[7–9] They investigated the formulation factors affecting the drug-loading efficiency and the microsphere size, and they concluded that the viscosity of the inner aqueous phase influences the drug-loading efficiency: the higher the viscosity, the higher the drug-loading efficiency is under their encapsulation conditions. Jeffery et al.[3] prepared ovalbumin microparticles of poly(lactic-*co*-glycolic acid) using a W/O/W method and evaluated the effect of formulation parameters on the microparticle characteristics. It was found that smooth, spherical microparticles 1–2 μm in diameter containing up to 10% (w/w) OVA could be produced. Yan et al.[6] studied the FITC-BSA encapsulated PLG microparticles produced by a modified W/O/W solvent extraction procedure. One hundred milliliters of 5% isopropanol was used to extract the organic solvent. Three agitation methods: vortex mixing, homogenization and sonication, were used to make the W1/O primary emulsion and second W/O/W emulsion. High encapsulation efficiency (form 60 to 94%) and drug loading (around 10% wt) were obtained. This study revealed that when a sonicator was used to make the first inner W1/O emulsion, microparticles with homogeneous drug distribution were obtained and showed a very low protein burst (7%) and slow release. However, when a vortex was used to make the first inner W1/O emulsion, microparticles with heterogeneous drug distribution were obtained and showed a high burst (63%) due to their large porous structure. Bodmeier and coworkers[10–12] studied mechanisms of microparticle formation from a double emulsion. They found that some microparticle characteristics, such as drug loading, porosity, and surface morphology, are strongly dependent on the way the coating polymer is precipitated. Nihant and coworkers[13] and Schugens and coworkers[14] studied the mechanism of microparticle formation from a double emulsion in more detail. They found that the stability of the primary emulsion has a large effect on the morphology and properties of polylactide microparticles loaded with bovine serum albumin (BSA) used as a model drug. Also these studies found that microparticles of semicrystalline poly L-lactide had proved to be not suitable for the sustained drug release whereas poly(DL-lactide), used as the amorphous counterpart, met the criteria for an efficient microencapsulation, particularly when the primary emulsion is stabilized by gelatin.

In this article, smaller microparticles (less than 100 μm) loaded with BSA have been studied, using a poly(lactide-*co*-glycolide) polymer. Preparation parameters were altered systematically to investigate their effects on drug loading efficiency, particle size, morphology, and drug release profiles of the poly(DL-lactide-*co*-glycolide) microparticles.

EXPERIMENTAL PART

Materials

- Poly(D,L-lactic-*co*-glycolic acid) (PLGA) 75 : 25 was purchased from Purac Biochem., Holland. It has a IV of 0.94 dL/g.
- Bovine Serum Albumin (BSA), Fraction V. minimum 98% was purchased from Sigma Aldrich, Singapore.
- Poly(vinyl alcohol) (PVA), 87–89% hydrolyzed, M_w = 13,000–23,000 was purchased from Sigma Aldrich, Singapore.
- Gelatin, from Aldrich Chemical, USA. 225 bloom, from calf skin.
- Dichloromethane (DCM), from EM Science, Germany. HPLC grade.
- Buffer solution (potassium dihydrogen phosphate), pH = 7.0., from Merck, Germany.
- Distilled water, lab-made.

Methods

PLGA with a ratio of lactide to glycolide 75 : 25 was used as the matrix material for microparticles. BSA (model protein) in distilled water with/without gelatin (inner water phase, W1) was prepared. Water with emulsifier was used as outer water phase (W2) in the W/O/W multiemulsion method.

The inner water phase, W1 and the oil phase were mixed using a vortex mixer (Autovortex SA6, Stuart Scientific, UK, speed up to 11,000 rpm) or a homogenizer (Ultra-Turrax T8, Hand-held disperser for volumes from 0.5–50 mL upwards. Dispersing element, S8*N*-8G) for several minutes at selected speeds. The resulting W1/O microemulsion was poured into a beaker filled with 0.5% PVA aqueous solution. Applying a strong homogenizing force (Silverson laboratory mixer SL2, capacity of homogenizing up to 9 L, maximum speed of 9000 rpm) to the mixture for 30 min, a W/O/W emulsion was produced. The resultant emulsion was then transferred to a round-bottle flask and vacuumed to evaporate the DCM from the emulsion at room temperature. The whole evaporation lasted for 1.5–2 h. The emulsion was centrifuged (Centrifuge, C3i, Jouan, France. Speed up to 12,000 rpm) after solvent evaporation, at 4000 rpm for 10 min. The particles were washed three times with distilled water. The washed microparticles were finally vacuum-dried for 2 days and collected for later analysis.

Unless otherwise stated, the following preparation parameters were kept constant:

- Polymer (PLGA) concentration in DCM was always kept at 7.5% (w/v)
- BSA was always dissolved in 1 mL of a 5% gelatin aqueous solution as the W1 phase

- Volume of PLGA DCM solution was always kept at 3.2 mL as the oil phase
- Volume of outer aqueous phase (W2): 100 mL 0.5% PVA solution
- Vortex mixer speed to produce primary emulsion (W1/O): 11,000 rpm
- Homogenizing speed to produce primary emulsion (W1/O): 10,000 rpm
- Homogenizing speed to disperse primary emulsion to outer aqueous phase: 1000 rpm

The experiments were designed as follows to study the influence of all parameters used in the W1/O/W2 multiemulsion on the particle size, drug loading efficiency, surface morphology and drug release properties.

- Gelatin as an excipient/viscosity-enhancer
- Different equipment used to produce primary emulsion (W1/O) Ultra-Turrax T8 homogenizer or Autovortex SA6 vortex mixer were used to produce the primary emulsion separately.
- Different ratios of W1/O used in the primary emulsion Small ratio (0.3 mL W1/7.0 mL oil) and large ratio (1.0 mL W1/3.2 mL oil) were used to produce the primary emulsion by small homogenizer (Ultra-Turrax T8).
- Different speeds in dispersing the (W1/O) in outer water phase (W2)
- Different PLGA concentrations 4%, 7.5%, and 15.0% (w/v) of PLGA DCM solution (oil phase) were used as the matrices.

The following methods were used to analyze the microparticles:

The particle size was measured by SEM (field emission scanning electron microscope (FESEM), JSM-6340 F, JEOL) under different magnification and estimated the largest population of microspheres as the final particle size. Carefully dried microparticles were cut with a razor blade and the cross sections viewed by SEM.

The drug loading was measured as follows: 10 mg of dried microparticles was dissolved in 10 mL DCM and BSA in the solution was then extracted by 100 mL distilled water. The extraction was analyzed by UV spectrometer (UV-2501 PC, Shimadzu) to detect the BSA content in the microparticles. Drug loading (DL) is defined as

$$DL = [\text{amount of drug (mg)} / \text{total weight of particles}] \times 100$$

It is also expressed as the drug amount in the microparticles inclusive the drug (unit in μg drug/mg microparticle).

TABLE I
The Effect of Addition of Gelatin on Particle Properties

Code	DL (%)/(μg/mg)	DLE (%)	Size (μm)	Morphology
VSWOG	3.3/33	6.6	5–20	Smooth surface with very few pores
VS	6.4/64	12.8	20–50	Smooth surface with a few pores

VSWOG implies that VS W/O gelatin in W1.
VS implies that the Vortex mixer was used for primary emulsion and Standard sample.

Drug loading efficiency (DLE) is defined as

$$DLE = [\text{amount of drug in microparticles} / \text{total amount of drug added}] \times 100$$

i.e., it reflects the percentage of the drug that is successfully incorporated into the microparticles from all the drug that is added in W1.

In vitro release tests were carried out in triplicate at 37°C in oven. One-hundred milligrams of dried microparticles was suspended in a 15 mL PP centrifuge tube containing 10 mL buffer. The supernatant from each tube was periodically removed after centrifugation for 10 min at 4000 rpm. The BSA content of the supernatant was analyzed using UV spectrometer. The protein release results were the average of the parallel triple analysis.

RESULTS AND DISCUSSION

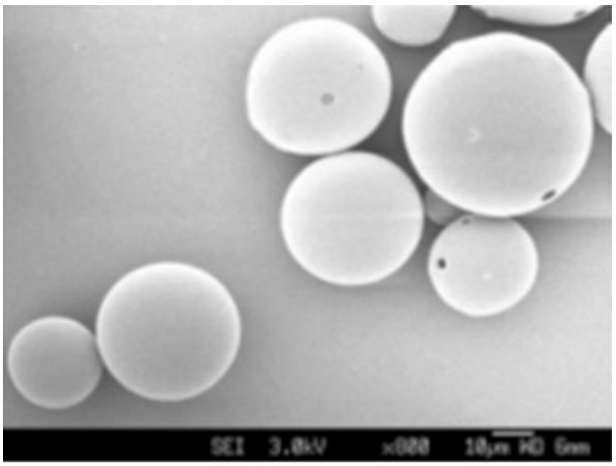
The various effects are discussed separately below.

Gelatin as an excipient/viscosity-enhancer

During the formation of the primary emulsion W1/O, gelatin was added as a viscosity-enhancer together with the model drug in the W1. This is highly recommended in the study done by Ogawa et al.[7] where the drug loading is comparatively high that fall in the range of 10–20%. Table I shows the effect of gelatin participation in W1 on particle size, morphology, drug loading (DL), and drug loading efficiency (DLE) of the microparticles.

It can be seen that when gelatin was added as a viscosity enhancer, the DLE of VS (with added gelatin) is almost twice as high as that of VSWOG (no gelatin). Gelatin acted as an efficient barrier that prevented BSA from partitioning out to the outer aqueous phase W2.

Figure 1 shows the morphology of microparticles produced with and without gelatin. Clearly, the use of gelatin in W1 leads to a fairly smooth particle surface.



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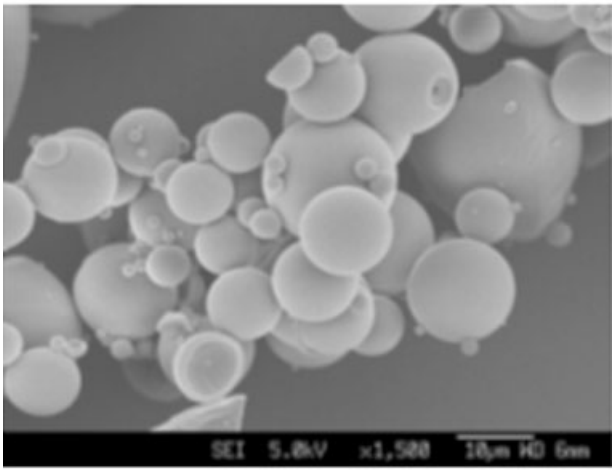


(b)

Figure 1 Morphology of VS (a) and VSWOG (b); VS denotes particles prepared with a vortex mixer, while VSWOG refers to the same preparation conditions but without added gelatin.

Figure 2 shows the drug release character of microparticles produced with and without gelatin in W1 as viscosity-enhancer. Microparticles produced without gelatin exhibited a large burst effect and faster initial release. It released 30% of the total amount of BSA within the first day. By 1 week, 40% of the total amount of BSA has been released. Subsequently, the release slows. In contrast, the particles produced in the presence of gelatin show a very slow and sustained release over the same time period. The difference is due to the difference in particle size. The smaller the particle size, the larger the surface area, and hence faster the release. The incomplete release (only 45% in 8 weeks) may be due either to the fact these particles had not yet entered the degradation phase, or that some of the protein BSA is adsorbed on the particle surface, and could not be detached. Other researchers have also reported incomplete release of BSA. Schwendeman et al.[15] found that the continuous release of BSA from the millicylinders only lasted about 4 days and no significant amount of BSA was further released during the 28-day study. They attributed this phenomenon to the formation of insoluble aggregates, which was confirmed by the aggregation kinetics. Grotts and Park[16] also found that in many cases, protein-loaded microspheres possess an inconsistent release profile with a significant burst and incomplete release even though the PLGA polymers degrade sufficiently. This was also due mainly to protein adsorption on to the microparticles.

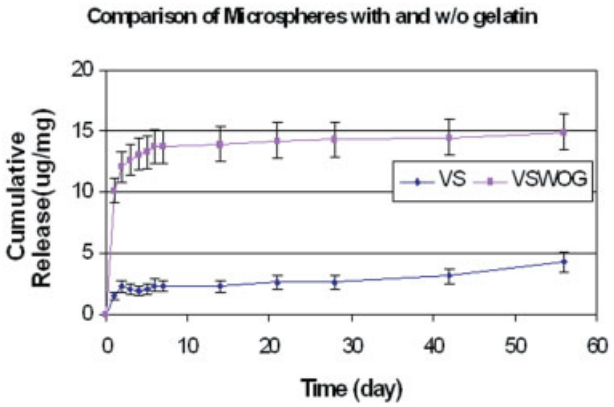


Figure 2 Effect of gelatin on drug release. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Different equipment used to produce primary emulsion (W1/O)

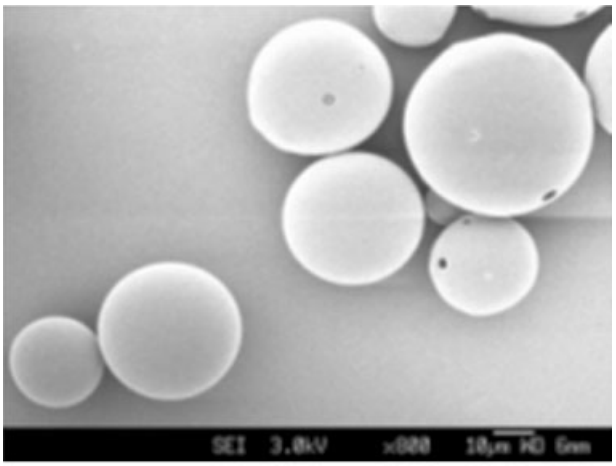
In this experiment, Ultra-Turrax T8 homogenizer or Autovortex SA6 vortex mixer were used to produce the primary emulsion separately. Microparticles produced show dramatic difference in morphology and drug loading. Table II shows the effect of different equipment on particle size, morphology, drug loading (DL) and drug loading efficiency (DLE) of the micro-

TABLE II
The Effect of Using Different Mixing Equipment on Particle Properties

Code	DL (%)/(μg/mg)	DLE (%)	Size (μm)	Morphology
HS	5.4/54	10.7	30–60	Porous surface and porous throughout matrix
VS	6.4/64	12.8	20–50	Smooth surface with a few holes

HS implies that the Homogenizer was used for primary emulsion and Standard sample.

VS implies that the Vortex mixer was used for primary emulsion and Standard sample.



(a)

(b)

Figure 3 Morphology of VS (a) and HS (b); HS in this case refers to the primary emulsion prepared with a homogenizer instead of a vortex mixer.

particles. Morphologies of the microparticles produced by different equipment are shown in Figure 3.

The process of microencapsulation of proteins by double emulsion/evaporation in a matrix of PLGA can be divided into three successive steps. First, an aqueous solution of the active compound is emulsified into an organic solution of the hydrophobic coating polymer. Second, this primary water-in-oil emulsion (W1/O) is dispersed in water with formation of a double water-in-oil-in-water emulsion (W/O/W). Finally, the organic solvent is removed with formation of solid microparticles. The stability of the primary emulsion is a prerequisite for the successful stabilization of a multiple emulsion and the loading of a large amount of drug within the microparticles. When we use the vortex mixer to produce the primary emulsion, microparticles with smooth surface are obtained. When a small homogenizer is used, microparticles show a porous surface and porosity throughout the matrix. The difference between the small homogenizer and the vortex mixer lies in the shear force. The homogenizer generates a higher shear force compared to the vortex mixer. As a result, smaller droplets are generated in the primary emulsion using the homogenizer. For a given overall amount of water in the primary emulsion, the viscosity increases with decreasing particle size.[17] In other words, the homogenizer yields an emulsion with smaller droplets, leading to an inner W/O emulsion of higher viscosity as compared to the vortex mixer. Because of this, the diffusion of water droplets through the primary emulsion to the outer aqueous phase is slower, and occurs after most of the oil phase has evaporated, thus leading to porous structures. A similar explanation accounts for the effect of the higher water-to-oil ratio, see below.

Table II shows that VS has a slightly higher DLE and smaller particle size than HS (W1/O made with the Homogenizer). This may be a consequence of the differences in morphology.

Figure 4 shows the drug release character of microparticles produced from vortex mixer and homogenizer. Microparticles produced from vortex mixer exhibit very little burst effect. The microparticles produced by homogenizer, on the other hand, show a 45% burst effect in the first day of drug release, followed by 48% release after 1 week. Totally, about 60% of the BSA was released in 8 weeks. This difference is clearly due to the porous nature of particles made with the homogenizer. The release of BSA occurs largely by the diffusional process through porous aqueous channels. Diffusion through a preexisting pore network in the formulated microparticles and subsequent enhanced diffusion via erosion-induced pore enlargement and evolution have been regarded as a predominant drug release mechanism.[18]

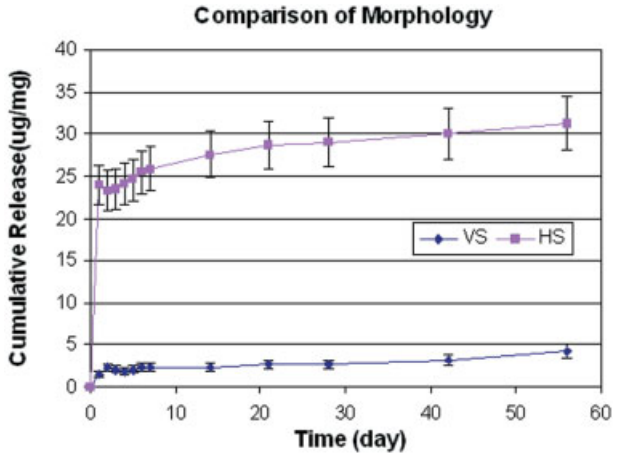


Figure 4 Effect of different equipment used in the preparation of the primary emulsion (W1/O) on drug release. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE III
The Effect of Different Oil-to-Water Ratios on Particle Properties

Code	W1/O ratio	DL (%)/ (μg/mg)	DLE (%)	Size (μm)	Morphology
HS	1.0:3.2	5.4/54	10.7	30–60	Porous surface and porous throughout matrix
H1	0.7:3.0	2.6/26	37.7	20–50	Smooth surface with a few holes

H1 stands for Homogenizer used for primary emulsion and nonstandard sample.

A similar phenomenon has been observed by Wang.[19] They concluded that most of the protein was released by a diffusional mechanism before the loss of polymer mass. Siegel and Langer[20] depict protein domains, connecting channels, and the random walk path of a diffusion protein molecule. The model explains the rapid release of proteins from microspheres as follows: after the polymer matrix takes up water and swells, the protein domains are connected by aqueous micropores in the polymer matrix. These aqueous micopores form tortuous narrow channels that connect protein domains to the surface of a microsphere. Protein molecules can then diffuse out of a microsphere through these connecting channels by a random walk mechanism. After protein molecules diffuse away from the domains, the protein domains themselves become aqueous pores in the polymer matrix, which further facilitates the release of protein molecules.

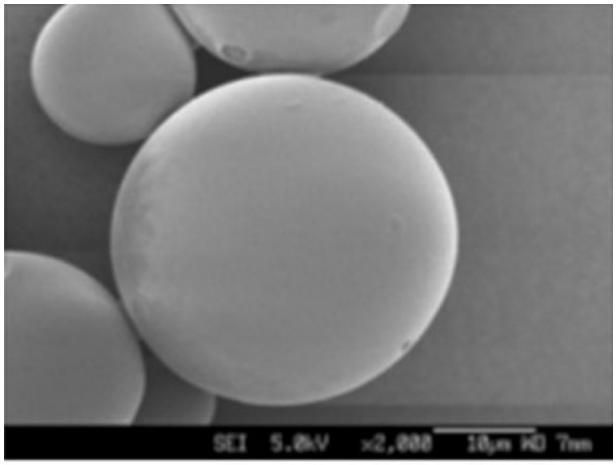
Different ratios of W1/O used in the primary emulsion by homogenizer

Small ratio (0.3 : 7.0 W1/O) and large ratio (1.0 : 3.2 W1/O) were used to produce the primary emulsion by small homogenizer (Ultra-Turrax T8). Table III showed the effect of different ratios of W1 to oil phase on particle size, morphology, drug loading (DL), and drug loading efficiency (DLE) of the microparticles. Morphologies of the microparticles produced using different ratios are shown in Figure 5.

Figure 5 shows that when the ratio of W1/O was reduced dramatically (from 1.0 : 3.2 to 0.3 : 7.0), even if the homogenizer is used to disperse the primary emulsion, smooth surfaces are produced. In contrast, when a higher ratio is used (as in our "standard" or reference condition, where the W1/O ratio is 1.0 : 3.2), the morphology of the microparticles is quite different in that it showed porosity throughout the matrix. This phenomenon is caused by the differences in the viscosity of the primary emulsion caused by the water to oil ratio. The viscosity is lower when the primary emulsion is produced from low W1/O ratio, primarily due to a lowering of the volume fraction of the dispersed phase, as per the Einstein-Stokes equation:

$$\eta = \eta_0(1 + 2.5\phi)$$

Following the argument proposed in "Different equipment used to produce primary emulsion (W1/O)", the lowered viscosity enables a quicker diffusion of water droplets to the outer aqueous phase, thus leading to formation of fewer pores. More surprisingly, the use of a higher water/oil ratio led to a substantial increase in DLE (38%), while the actual drug loading is lower (2.6%). This increased efficiency of drug incorporation (or minimization of drug loss) is most likely due to the better emulsification during the primary emulsion step, that leads to a better-defined oil-water interface during the second emulsification step. Sah[21] also



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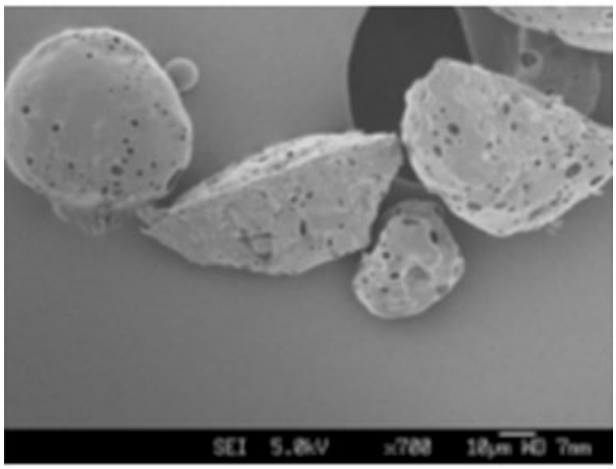


(b)

Figure 5 Morphology of H1 (a) and HS (b); H1 refers to a low water-to-oil ratio, whereas HS refers to a higher water-to-oil ratio.

TABLE IV
The Effect of Mixing Speeds (for Vortex Mixer) on Particle Properties

Code	DL (%)/(μg/mg)	DLE (%)	Size (μm)	Morphology
VS	6.4/64	12.8	20–50	Smooth surface with a few holes
V3000	5.0/50	10.0	10–30	Smooth surface with a few holes
V5000	3.1/31	6.2	5–20	Smooth surface without holes

V3000/5000 stand for Vortex mixer used for primary emulsion and nonstandard sample.

found that at the same W1/O ratio (0.3 : 7.0), when homogenizer was applied at 23 krpm (high shear speed) for the primary emulsion, microparticles had a smooth surface. No burst effect was observed of these microparticles. In further work the drug release on the microparticles should be studied to verify if both low burst and sustained release can be obtained since smooth surface is good for sustained drug release with lower/no burst effect.

Different speeds in dispersing the (W1/O) into outer water phase (W2)

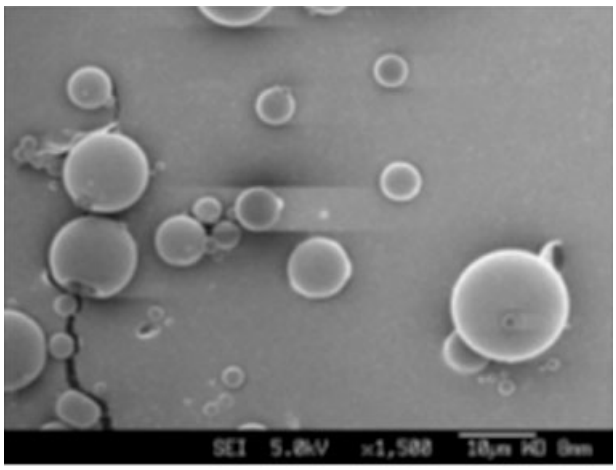
Homogenizing speeds of 1000, 3000, and 5000 rpm were used to disperse the primary emulsion (W1/O) to 100 mL 0.5% PVA solution, using the Vortex mixer. Table IV shows the effect of different speeds on particle size, morphology, drug loading (DL) and drug loading efficiency (DLE) of the microparticles. Morphologies of the microparticles produced by different speeds are shown in Figure 6.

It is observed in Figure 6 that microparticles with smooth surface are obtained at all the three speeds. In addition, at the highest speed used here (5000 rpm), there are no holes at all in all the microparticles. With an increase in the speed the particle size decreases. Table IV also shows that the DL decreases with the decrease of the particle size (increase of the speed). Rafati et al.[22] also reported a similar result. When the homogenization speed was reduced during secondary emulsion, a substantial (3×) improvement in protein loading was achieved. However, the effect was most pronounced when the PVA loading was high (10%), and much less noticeable when 2.5% PVA stabilizer was used. They attributed the result to the effect of shear stress experienced in the secondary emulsion: a higher stress led to greater protein association with the microparticles.

Figure 7 shows the drug release character of microparticles dispersed at different speeds. The effects are striking. Microparticles produced at higher speed have the fastest release rate. This is due to the smaller particle sizes. For V5000, 27% of BSA is released within 1 day and 40% in 4 days. The high burst effect could be due to some adsorption of the protein on to the relatively small particles. After day 4, BSA was released continuously and 80% of total drug was released within 8 weeks. For V3000, the drug release rate was lower than V5000 but higher than that of VS1000. It has a burst effect of 11% in the first day and 15% was released in 1 week. After that, 17.5% was released within 8 weeks. The V5000 particles are smooth with no porosity. Hence this condition is optimum for drug delivery at reasonable rates over 1–2 month period. However, the burst effect may need to further reduced. Yolles[23] also reported the similar results.

Different PLGA concentrations

4%, 7.5%, and 15.0% (w/v) of PLGA in DCM solution (oil phase) are used as the matrices. Table V shows the effect of different polymer concentrations on particle



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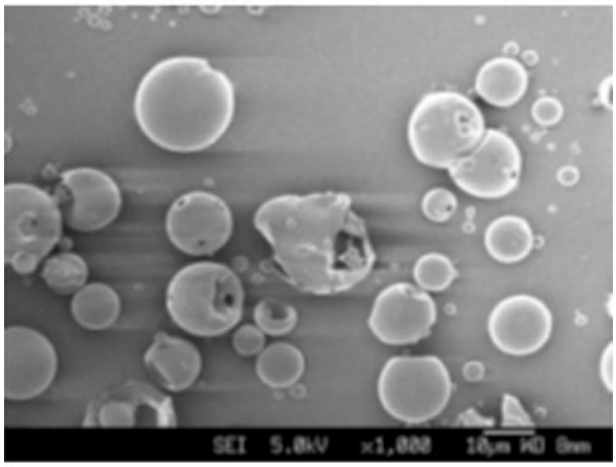


(b)

Figure 6 Morphology of V5000 (a) and V3000 (b).

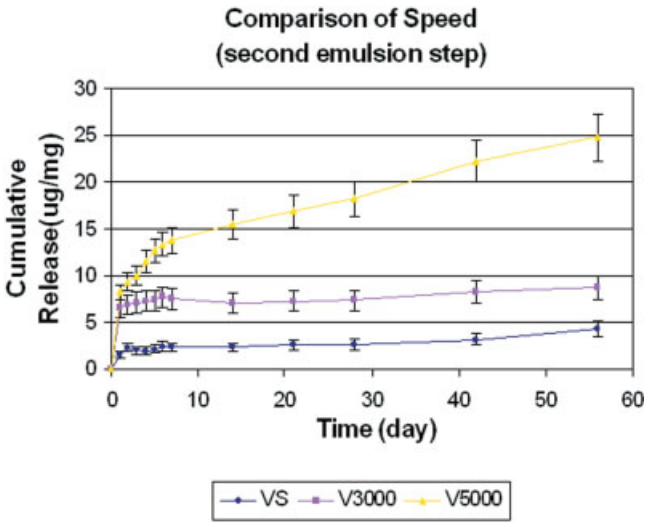


Figure 7 Effect of speed (second emulsion step) on drug release. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

size, morphology, drug loading (DL) and drug loading efficiency (DLE) of the microparticles. Morphologies of the microparticles produced at different PLGA concentrations are shown in Figure 8.

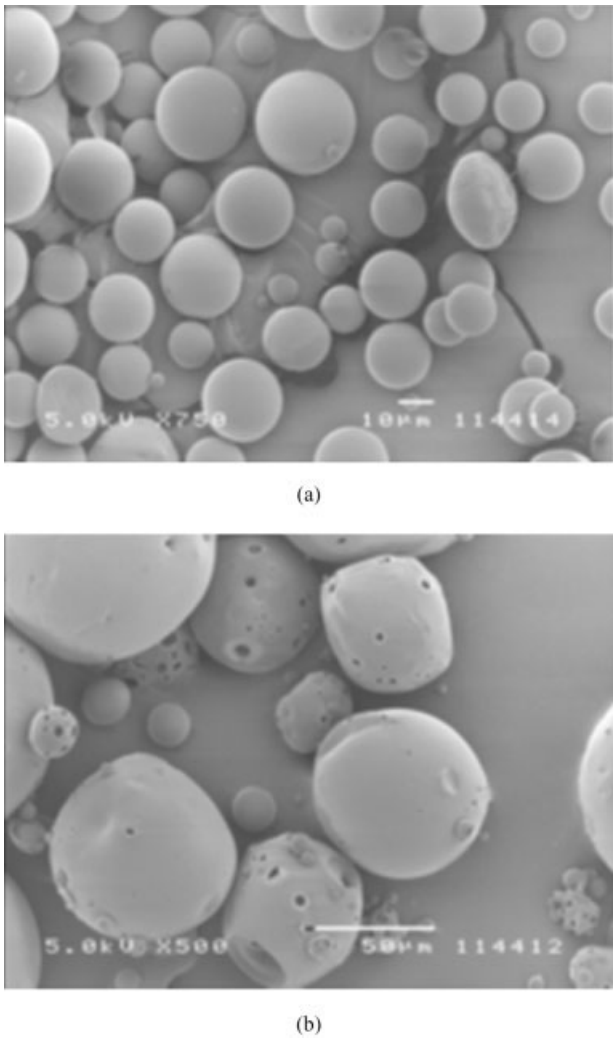
It is observed in Figure 8 that microparticles with smooth surface were obtained at all the three concentrations. When the PLGA concentration is as low as 4%, there are no holes at all on all the surfaces of microparticles. And with the increment of the concentration of PLGA, i.e., 15%, quite a few holes appear on the smooth surface of the microparticles. In this instance, the higher concentration of polymer in the oil phase leads to a shorter time to complete evaporation of the solvent, thus leading to pore formation, as the inner water phase diffuses out more slowly.

Table V also shows that the particle size increases with the increase of the polymer concentration. The DLE increases with the increment of the polymer concentration due to the better barrier effect of oil phase with higher concentration. If the oil phase contains less solvent, it hardens faster. Thus less BSA is allowed to partition out before the hardening of the microparticles.

TABLE V
The Effect of PLGA Concentrations on Particle Properties

Code	DL (%)/(μg/mg)	DLE (%)	Size (μm)	Morphology
V4	8.4/84	9.0	10–20	Smooth surface without holes
VS	6.4/64	12.8	20–50	Smooth surface with a few holes
V15	5.8/58	23.1	20–50	Smooth surface with a lot of holes

V4/15 stand for Vortex mixer used for primary emulsion and nonstandard sample.



(a)

(b)

Figure 8 Morphology of V4 (a) and V15 (b); V4 refers to a 4% PLGA concentration in the oil phase, while V15 refers to a 15% concentration.

Figure 9 shows the drug release character of microparticles produced from different PLGA concentrations. Microparticles produced from the highest concentration have the highest release rate while the other concentrations showed no appreciable difference in release rates. Microparticles produced from 15% PLGA showed an initial burst which is 11% within 1 day. After 8 weeks, a total of 22% of BSA is released. Some other researchers have found the opposite effect, i.e., that microparticles produced form higher polymer concentration gave lower burst effect and slower drug release character.[5,6,24] The difference may be due to the observation of porosity in our 15% PLGA particles, which leads to a higher rate of release. Rationalization of release behavior is closely associated with the observed

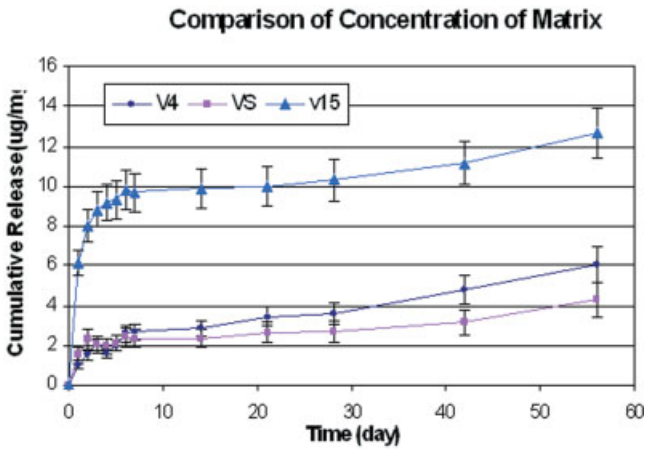


Figure 9 Effect of PLGA concentrations on drug release. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

morphological features. In general, higher porosity leads to quicker release.

CONCLUSIONS

We may divide the findings into two categories: effects related to morphology, and effects related to release rates, recognizing a certain amount of overlap.

1. Particle morphology is dependent on the relative rates of oil evaporation and diffusion of the inner aqueous phase into the outer: if the oil evaporation is faster, then porous particles are obtained and *vice versa*.
2. For production of smooth particles (with little porosity), the Vortex mixer is preferred in the first emulsification step, as it is less sensitive to viscosity differences in the primary emulsion.
3. A lower primary emulsion viscosity is preferred for smooth particle production.
4. A high secondary homogenization speed is preferable for production of smooth particles with no observable surface porosity.
5. The smoothest particles were produced at these conditions: Vortex mixer, 4% PLGA concentration in DCM, with a secondary homogenization speed of 1000 rpm.

Findings related to release rate may be summed up as follows:

1. In general, more porous particles yield a higher burst effect and faster rates of depletion of drug
2. When particles are smooth, the predominant factor governing release is particle size. Smaller sized particles yield faster rates, including a higher burst.
3. In the studies to date, we have been unable to observe degradation-(or erosion)-controlled release of drug. However, in some of the formulations, a second phase of release, attributable to degradation, is starting around day 35–40 (when mass loss becomes measurable).

As far as drug loading is concerned, it is found that the highest drug loading we obtained was about 8%, using a PLGA concentration of 4%. This compares favorably with literature reports of BSA loading in PLGA particles.

References

1. Lacoste, D.; Labrie, F.; Dube, D.; Belanger, A.; Tice, T. R.; Gilley, R. M.; Pledger, K. L. J Steroid Biochem 1989, 33, 1007.
2. Tice, T. R.; Tabibi, S. E. In Treatise on Controlled Drug Delivery; Kydonieus, A., Ed.; Marcel Dekker: New York, 1992; p 315.
3. Jeffery, H.; Davis, S. S.; O'Hagan, D. T. Pharm Res 1993, 10, 362.
4. Herrmann, J.; Bodmeier, R. J Controlled Release 1995, 36, 63.
5. Mehta, R. C.; Thanoo, B. C.; Deluca, P. P. J Controlled Release 1996, 41, 249.
6. Yan, C. H.; Resau, J. H.; Hewetson, J.; West, M.; Rill, W. L.; Kende, M. J Controlled Release 1994, 32, 231.
7. Ogawa, Y.; Yamamoto, M.; Takada, S.; Okada, H.; Shimamoto, T. Chem Pharm Bull 1988, 36, 1502.
8. Ogawa, Y.; Yamamoto, M.; Okada, H.; Yashiki, T.; Shimamoto, T. Chem Pharm Bull 1988, 36, 1095.
9. Okada, H. Proc Int Symp Control Rel Bioact Mater 1989, 16, 12.
10. Alex, R.; Bodmeier, R. J Microencapsul 1990, 7, 347.
11. Bodmeier, R.; Oh, K. H.; Chen, H. Int J Pharm 1989, 51, 1.
12. Bodmeier, R.; MacGinity, J. W. Int J Pharm 1988, 43, 179.
13. Schugens, C.; Laruelle, N.; Nihant, N.; Grandfils, C.; Jerome, R.; Teyssie, P. J Controlled Release 1994, 32, 161.
14. Nihant, N.; Schugens, C.; Grandfils, C.; Jerome, R.; Teyssie, P. Pharm Res 1994, 11, 1479.
15. Schwendeman, S. P.; Shenderova, A.; Zhu, G. Z.; Jiang, W. L. In Handbook of Pharmaceutical Controlled Release Technology; Wise, D. L., Ed.; Marcel Dekker: New York, 2000; p 393.
16. Grotts G.; Park, T. G. J Microencapsul 1998, 15, 699.
17. Sherman, P. In Encyclopaedia of Emulsion Technology; Becher, P., Ed.; Marcel Dekker: New York, 1987; Vol. 1, Chapter 7, p 405.
18. Shah, S. S.; Cha, Y.; Pitt, C. G. J Controlled Release 1992, 18, 261.
19. Wang, H. T. Biomaterials 1990, 11, 679.
20. Siegel, R. A.; Langer, R. S. Pharm Res 1983, 1, 1.
21. Sah, H. K. J Microencapsul 1995, 12, 59.
22. Rafati, H.; Coombes, A. G. A.; Adler, J.; Holland, J.; Davis, S. S. J Controlled Release 1997, 43, 89.
23. Yolles, S. In Polymers Medicine and Surgery; Kronenthal, R. L.; Use, Z.; Martin, E., Eds.; Plenum Press: New York, 1974; Vol. 8, p 245.
24. Yang, Y. Y.; Chung, T. S.; Bai, X. L.; Chan, W. K. Chem Eng Sci 2000, 55, 2223.