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How to circumvent untoward drug crystallization during emulsion-templated microencapsulation process

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ABSTRACT: Unwanted drug crystals often form on the surface of PLGA microspheres or in an aqueous phase when a hydrophobic drug undergoes emulsion-templated microencapsulation processes. In our study, over 70% of progesterone crystallizes in the aqueous phase when microencapsulation proceeds with a typical oil-in-water solvent evaporation process. During filtration employed for microsphere recovery, unentrapped drug crystals are collected alongside with progesterone-containing microspheres. This phenomenon accompanies unfavorable consequences on the microsphere quality. In contrast, when microspheres are prepared with a new solvent extraction-evaporation hybrid process, it is possible to completely avoid drug crystallization. Consequently, the new microencapsulation technique yields high drug encapsulation efficiencies of $\geq$90.8%, and the resultant microspheres show a homogeneous size distribution pattern. Also, the microsphere surface is free of drug crystals. For loading hydrophobic drugs into PLGA microspheres, the new microencapsulation process reported in this study has distinct advantages over commonly used emulsion-templated solvent evaporation processes. © 2016 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2016**, *133*, 43768.

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INTRODUCTION

Poly-*d,l*-lactide-*co*-glycolide (PLGA) microspheres are actively used in long-acting depot systems of various pharmaceuticals, chemoembolizing agents for the treatment of unresectable tumors, and cosmetic fillers for the correction of nasolabial folds.[1] Nowadays, PLGA microspheres are also widely used as biodegradable scaffolds in tissue engineering and regenerative medicine.[2,3] Compared to other three-dimensional scaffolds that have fixed forms, microparticulate scaffolds make it possible to target defects and fill cavities simply through injection without surgical intervention. At the same time, they also serve as delivery systems for drugs such as growth factors. While there are multiple available microencapsulation techniques, an emulsion-templated solvent evaporation process is commonly employed to encapsulate hydrophobic drugs into PLGA microspheres. This microencapsulation process includes producing an oil-in-water (o/w) emulsion through the emulsification of a polymeric dispersed phase in an aqueous continuous phase. Solvent evaporation serves as a driving force toward the continuous diffusion of the organic solvent from emulsion droplets to the aqueous phase. Emulsion droplets are eventually transformed into microspheres through repetition of this solvent removal step. An oil (e.g., liquid paraffin) is sometimes used instead of water as an continuous phase, in which case the process is called oil-in-oil (o/o) emulsion solvent evaporation.[4,5]

An o/w emulsion-based microencapsulation process can also be conducted under reduced pressure or at an elevated temperature to accelerate solvent removal. In addition, there are variations such as purging the emulsion with gas, adding an excessive amount of a quenching liquid in the emulsion, using membrane separation, changing the composition of an aqueous continuous phase, or hybridizing solvent evaporation with solvent extraction. Among them, solvent extraction techniques particularly focus on adding a large amount of a quench liquid (e.g., water) to harden emulsion droplets into microspheres. Many studies describe how formulation-related and process-related parameters affect the morphology of microspheres, their size distribution, drug encapsulation, drug release rate, and the level of residual organic solvents.[6,7] Typical variables that are studied are organic solvent, PLGA, mixing device, emulsifier, phase volume, temperature, and solvent removal method.

An interesting fact is that hydrophobic drug crystals often exist on the surface of PLGA microspheres prepared from solvent evaporation processes.[8–13] Estradiol, clonazepam, testosterone, progesterone, ibuprofen, and piroxicam are several examples. There are also reports that drug crystals form on the surface of microspheres when carrying out solvent evaporation processes

on hydrophobic polymers other than PLGA.[14–19] Similarly, drug crystals are observed on the surface of microspheres prepared by o/o emulsion solvent evaporation processes.[20] Surprisingly, it is hard to find in-depth literature related to drug crystallization that can occur during emulsification and microsphere hardening. One reason might be a perception that hydrophobic drugs are easily encapsulated into PLGA microspheres, stemming from the fact that they usually do not diffuse into the aqueous continuous phase. Also, there is a tendency to regard a portion of the drug to become loaded on the microsphere surface during microencapsulation.

In reality, there are a number of parameters that affect drug crystallization that occurs on the microsphere surface and in the aqueous continuous phase. This present study is aimed to shed light on the phenomenon of drug crystallization that happens during emulsion-templated microencapsulation and develop a strategy to overcome this problem. Two microencapsulation processes have been developed to fulfill our goals. Most widely used dispersed organic solvents for emulsion-templated microencapsulation processes are methylene chloride, chloroform, and ethyl acetate. In our recent study, non-halogenated methyl propionate was suggested as a new substitution for the organic solvents listed above.[21] Therefore, our first microencapsulation process attempted to use methyl propionate as a dispersed solvent to encapsulate progesterone into PLGA microspheres through solvent evaporation. For our second process, after producing an o/w emulsion, primary solvent removal was achieved by using water as a quenching liquid to extract methyl propionate residing in emulsion droplets. After then, microspheres were completely hardened through evaporation. Therefore, this method was named as the solvent extraction-evaporation hybrid process in text. The effects of the two microencapsulation techniques on major quality attributes of microspheres and drug crystallization were reported, and relevant discussions were made in this study.

EXPERIMENTAL

Materials

PLGA with a lactide:glycolide ratio of 50:50 (lot no. LP1042) was obtained from Evonik Degussa Corporation (Mobile, AL). The polymer had inherent viscosity of 0.43 dL/g in chloroform at 30 °C, and it was abbreviated as PLGA in our text. Polysciences, Inc. (Warrington, PA) was the supplier of polyvinyl alcohol (PVA; 88% hydrolyzed, Mw = 25,000 g/mol; lot no. 652279). Progesterone (lot no. 2P72J-AJ) and methyl propionate (lot no. 10184004) were procured from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan) and Alfa Aesar (Ward Hill, MA), respectively. All other reagents and solvents were of analytical grade.

Preparation of Microspheres by Solvent Evaporation

PLGA (0.30 g) and progesterone (30, 60, 90, or 120 mg) were dissolved in 4 mL of methyl propionate. A composition ratio of progesterone to PLGA was referred to as an initial progesterone payload in text. This meant that an initial progesterone payload varied from 10 to 20, 30, and 40% under our experimental conditions. This dispersed phase was poured onto 40 mL of a 0.5% aqueous PVA solution being stirred at 450 rpm. This led to the formation of an o/w emulsion, which was subsequently subjected to overnight stirring for solvent removal. The resultant microsphere suspension underwent two different treatments. In the first method, the microsphere suspension was filtered through a Whatman grade 1 filter paper. The particulate substances staying on the filter were collected and vacuum dried overnight. In the second method, wet sieving was performed to separate progesterone-loaded microspheres from unentrapped progesterone crystals present in the microsphere suspension. To do so, the microsphere suspension was first passed through a 25 μm stainless steel sieve mesh. The filtered particles were then taken off the sieve and dried overnight under vacuum.

Preparation of Microspheres by Solvent Extraction-Evaporation Hybrid

Progesterone was dissolved in 4 mL of methyl propionate in which 0.3 g of PLGA was predissolved. The amount of progesterone used for microencapsulation was changed from 30 to 60, 90, and 120 mg. As described earlier, the resultant dispersed phase was emulsified in 40 mL of the aqueous PVA solution. After 20 min-stirring, the emulsion was dumped onto 200 mL a 0.1% PVA aqueous solution preheated to 30 °C. This step was performed to extract methyl propionate from emulsion droplets into the aqueous phase. The resultant embryonic microsphere suspension was stirred for additional 4 h, to complete solvent removal via evaporation. Microspheres loaded with progesterone were then collected by filtration and subject to vacuum drying.

Observation of Emulsion and Microsphere Suspension by Light Microscopy

In the practice of both microencapsulation processes described above, aliquots of the o/w emulsions were taken out at predetermined time intervals. The physical status of emulsion droplets and the onset of progesterone crystallization in the aqueous phase were monitored under a light microscope (model S16C; MICro Scopes, Inc., St. Louis, MO).

Particle-Size Analysis

After completion of solvent removal, the size distribution pattern of a microsphere suspension was determined with the Cilas particle size analyzer (model Granulomètre laser 1090; Orleans, France). In addition, $d_{10\%}$ (10% of the volume distribution was below this value), $d_{50\%}$ (the volume mean diameter), and $d_{90\%}$ (90% of the volume distribution was below this value) were determined to calculate the span index of each microsphere suspension.

$$\text{Span index} = \frac{(d_{90\%} - d_{10\%})}{d_{50\%}} \quad (1)$$

Scanning Electron Microscopy

The morphology of microspheres was observed by a JSM-5200 SEM (Jeol Inc., Tokyo, Japan). Microsphere samples were sprayed on a double-sided adhesive tape mounted on a metal specimen stub and were sputter-coated in an argon atmosphere by a SC7620 sputter coater (VG Microtech, West Sussex, UK).

Encapsulation Efficiency of Progesterone

Microsphere samples were accurately weighed and dissolved in 4 mL of tetrahydrofuran. The sample solution was diluted 10 times with a methanol-water mixture (8:2, by v/v). The appearing PLGA precipitates were removed by filtration through a

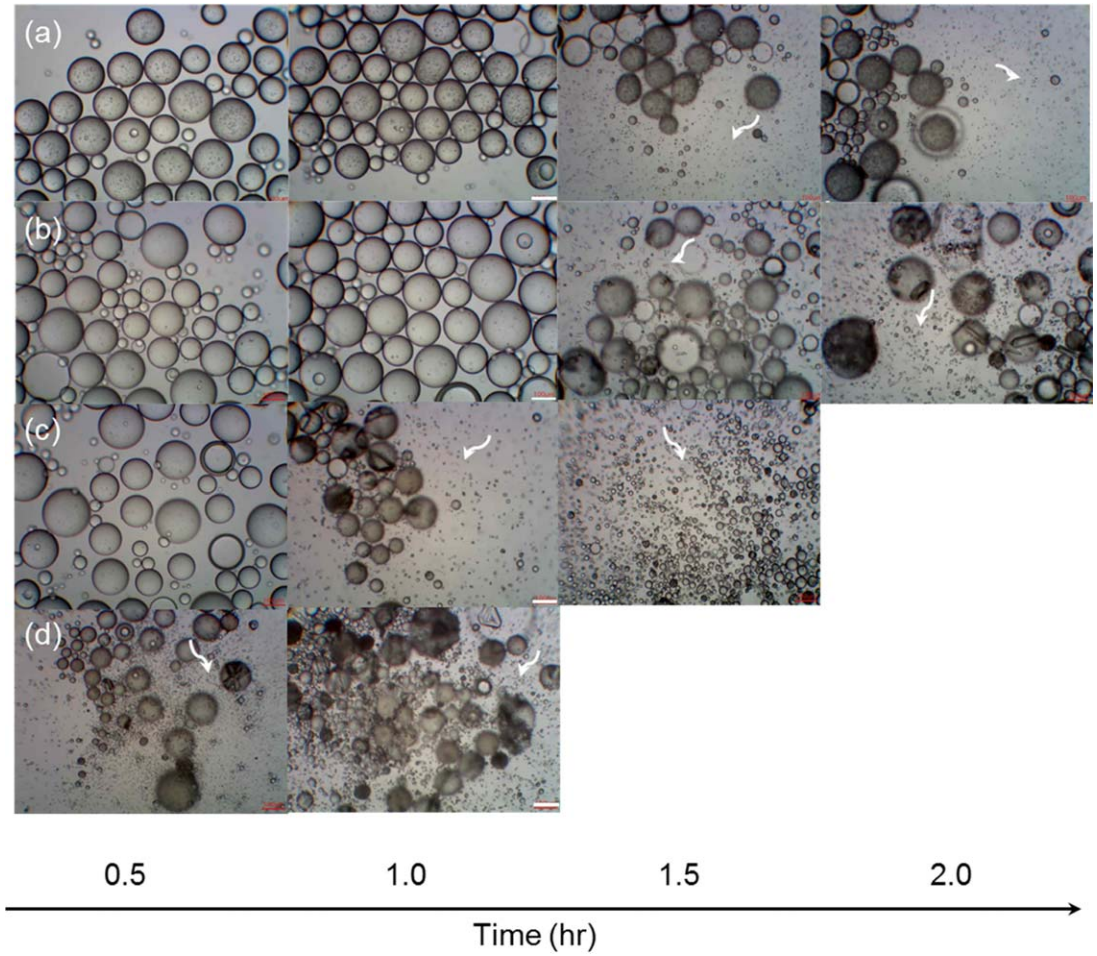

Figure 1. Monitoring dynamic changes in the status of emulsion droplets as a function of stirring time during the solvent evaporation process. An initial progesterone payload in the dispersed phase varied from (a) 10 to (b) 20, (c) 30, and (d) 40%. Arrows indicate progesterone crystals dispersed in the aqueous continuous phase. The bar size is 100 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

0.45 μm nylon filter. The concentration of progesterone in the resultant filtrate was analyzed by HPLC (Shimadzu LC-20AD). The Luna 5 μm C18(2) was used as an analytical column, while the methanol–water mixture was employed as a mobile phase at the flow rate of 1 mL /min. Progesterone eluting out of the analytical column was detected by a UV detector at 254 nm. Drug EE% was calculated as follows.[21]

$$\text{EE\%} = \frac{\text{actual loading}}{\text{theoretical loading}} \times 100 \quad (2)$$

where actual loading = (progesterone weight found in microspheres)/(microsphere sample weight) and theoretical loading = (progesterone weight used for microencapsulation)/(combined weight of PLGA and progesterone used for microencapsulation)

Differential Scanning Calorimetry

A Q2000 DSC (TA Instruments, New Castle, DE) was used to monitor thermal behavior of PLGA as received, progesterone powders, and PLGA microspheres laden with various amounts of progesterone. A Differential Scanning Calorimetry (DSC) sample was put into a Tzero aluminum pan and closed with a Tzero non-hermetic lid. The sample was heated from 20 to 160 °C at a rate of 10 °C/min. The balance chamber and the sample cell were purged with nitrogen gas at flow rates of 40 and 60 mL /min, respectively. The glass-transition temperature (T_g) of microsphere samples and the melting point of progesterone were evaluated with the Universal Analysis software program of TA Instruments.

RESULTS AND DISCUSSION

Drug Crystallization and Its Prevention

The state of emulsion droplets was observed as a function of time during the solvent evaporation process (Figure 1). When an initial progesterone payload was 10%, translucent emulsion droplets were observed at early stages of stirring. However, after 1.5 h, tiny drug particles appeared in the aqueous continuous phase. When an initial progesterone payload was increased to 20, 30, and 40%, larger amounts of unentrapped drug crystals were observed in a relatively shorter period of time. Based on the hydrophobic nature of progesterone, it was expected that it would exist in the dispersed phase of the emulsion, that is, emulsion droplets and/or embryonic microspheres. However, to our surprise, Figure 1 shows that considerable amounts of free drug crystals are dispersed in the aqueous continuous phase. It

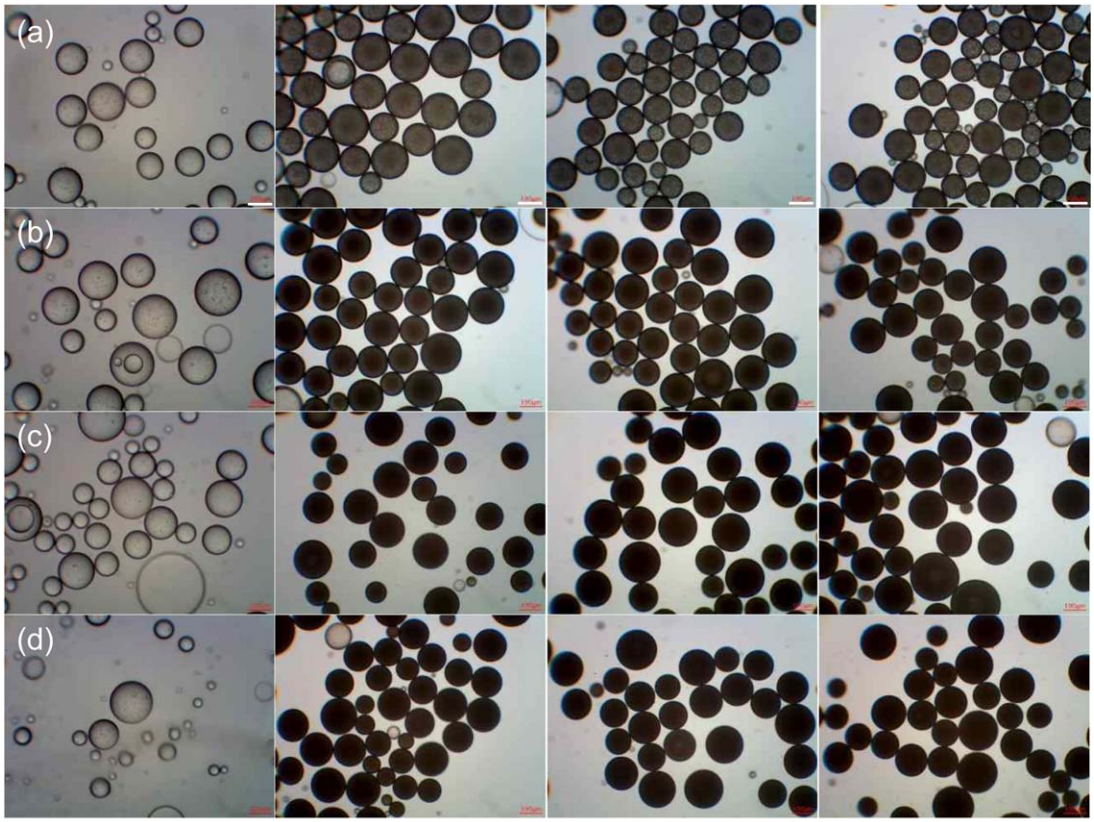

Figure 2. The status of emulsion droplets observed at 20 min, 1-, 2-, and 4-h stirring time during the solvent extraction-evaporation hybrid process. An initial progesterone payload varied from (a) 10 to (b) 20, (c) 30, and (d) 40%. The bar size is 100 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

was clear that the timing and amount of drug crystals appearing in the aqueous continuous phase were largely influenced by an initial progesterone payload.

When microspheres were prepared with the solvent extraction-evaporation hybrid process, completely different results were obtained (Figure 2). Emulsion droplets observed at 20 min were present as translucent droplets under an optical microscope. However, when a quenching liquid (i.e., water) was added into the emulsion, emulsion droplets were quickly hardened into microspheres, resulting in an opaque image. This meant that our solvent extraction step was quite effective in removing a sufficient amount of methyl propionate from emulsion droplets. Even more intriguingly, when an initial progesterone payload was increased up to 40%, unentrapped free drug crystals were not observed in the aqueous continuous phase.

In the practice of solvent evaporation, emulsion droplets experience dynamic changes such as breakups, the Ostwald ripening, solvent diffusion, and water influx. Since our dispersed solvent, methyl propionate, has the water solubility of 6.4%, it diffuses from emulsion droplets to the aqueous continuous phase.[21] Therefore, the aqueous continuous phase contains a small amount of dissolved methyl propionate. Under this condition, progesterone diffuses from emulsion droplets into the aqueous phase and reaches a saturation concentration. As solvent evaporation proceeds, this equilibrium state is broken. It is speculated that, as the removal of dissolved methyl propionate facilitates the formation of progesterone nuclei, drug crystallization takes place in the aqueous continuous phase. When an initial progesterone payload was 10%, drug crystallization occurred after approximately 1.5 h. The flux of progesterone from emulsion droplets to the aqueous phase would be proportional to its initial payload used for microencapsulation. Therefore, as shown in Figure 1, incrementing an initial drug payload would result in an increase in the amount of drug crystals with a faster rate of crystallization. Conversely, in the solvent extraction-evaporation hybrid process, emulsion droplets are free from undergoing dynamic changes: emulsion droplets are quickly hardened into microspheres before the above series of the events occur. At the same time, PLGA precipitation brings about the inhibition of drug diffusion. Therefore, as Figure 2 demonstrates, the solvent extraction-evaporation hybrid process seems to be able to fundamentally block progesterone crystallization in the aqueous continuous phase.

After hardening of microspheres, they were collected through filtration and subjected to vacuum drying. Because progesterone crystals always appeared in the aqueous phase in the practice of solvent evaporation, the microspheres showed a large amount

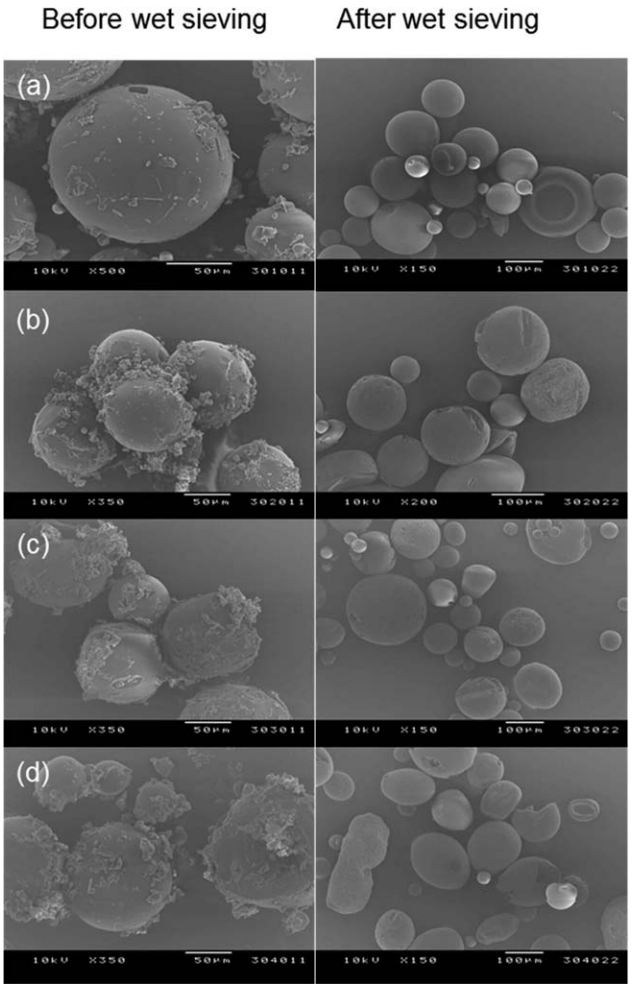

Figure 3. SEM micrographs of progesterone-loaded PLGA microspheres prepared by solvent evaporation. An initial progesterone payload was changed from (a) 10 to (b) 20, (c) 30, and (d) 40%. Prior to vacuum drying, microspheres were subject to either filtration alone (left micrographs) or wet sieving−filtration (right micrographs). The left and right bar sizes are 50 and 100 μm, respectively.

of free drug crystals on their surface (Figure 3). A greater initial drug payload resulted in a much larger amount of unentrapped drug crystals. The data in Figures 1 and 3 suggested that a considerable amount of drug crystals was collected alongside with microspheres during filtration employed for microsphere recovery. To remove free progesterone crystals from progesterone-loaded microspheres, the microsphere suspension was passed through a sieve with 25 μm pore size right before filtration. The microspheres remaining on the sieve were collected, filtered and dried under vacuum. Figure 3 illustrates the Scanning Electron Microscopy (SEM) micrographs of such microspheres, which demonstrates that wet sieving is able to separate microspheres from unentrapped drug crystals. As mentioned previously, many SEM micrographs reported in literature illustrated that numerous drug crystals were present on the surface of microspheres. As seen in Figures 1 and 3, it might be a phenomenon that arose because there was no additional step of refinement to separate microspheres from unentrapped drug crystals existing in a continuous phase. In fact, drug crystals might have not formed on the surface of microspheres.

Figure 4 illustrates SEM micrographs of the microspheres prepared by the solvent extraction-evaporation hybrid process. Favorably, even though wet sieving was not performed, unentrapped drug crystals were not observed on the surface of microspheres laden with various amounts of progesterone. Results from Figures 2 and 4 suggested that the majority of progesterone used for microencapsulation was effectively loaded into the inner matrices of microspheres. Recently, Kastellorizios *et al.* have presented an excellent research result where they uniformly distributed dexamethasone in PLGA microsphere matrices and suppressed the formation of dexamethasone crystals at the same time.[22] After dissolving dexamethasone and PLGA in a methylene chloride-dimethyl sulfoxide mixture, they produced PLGA microspheres through a solvent evaporation and extraction process. To prevent dexamethasone crystallization in the aqueous phase, they optimized microencapsulation by making dexamethasone precipitate in the dispersed polymeric phase first and then achieving solvent extraction to allow PLGA to precipitate. They pointed out that not only the volume ratio of the dispersed solvents but also the concentration of dimethyl sulfoxide predissolved in the aqueous phase was critical parameters. In our study, a very simple solvent extraction-evaporation hybrid technique using methyl propionate as a dispersed solvent made it possible to overcome a commonly observed phenomenon of drug crystallization.

Influence of Microencapsulation Techniques on Microsphere Quality

Figure 3 indicated that when solvent removal was done through solvent evaporation, a microsphere suspension contains both microspheres and free drug crystals. Its particle-size distribution was measured to support this claim. Due to unentrapped progesterone crystals being dispersed in the aqueous phase rather than being encapsulated in microspheres, the size distribution pattern showed wide variations (Figure 5). The particle-size distribution pattern was largely affected by an initial drug payload: increasing an initial drug payload accompanied a larger amount of differently-sized drug crystals. When an initial progesterone payload varied from 10 to 20, 30, and 40%, $d_{10\%}$ values of the microsphere suspensions were 9, 5, 3.8, and 3 μm, respectively. At the same microsphere preparation conditions, the corresponding percentages of particles less than 10 μm were 11.1, 22.7, 29.0, and 35.2%. It can be inferred from Figure 3 that these particles are free drug crystals rather than microspheres. The span index is a commonly used measure of the width of particle-size distribution. The smaller its value is, the narrower the particle-size distribution is. When an initial progesterone payload was 10%, the span index of the microsphere suspension was measured as 1.7. When its initial payload was increased from 20, 30, and 40%, the span indices of the resultant microsphere suspensions were between 2.9 and 6.1. These values show that their particulate sizes are very heterogeneous due to the coexistence of drug crystals and microspheres.

Figures 2 and 4 suggest that when microspheres are prepared from the solvent extraction-evaporation hybrid process, the

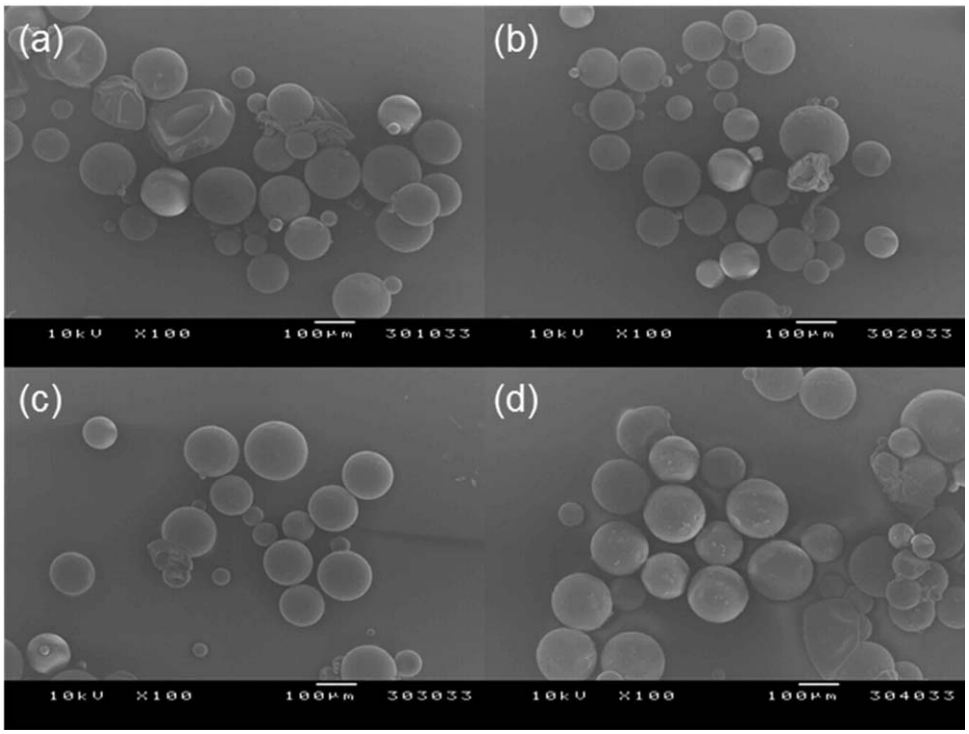

Figure 4. SEM micrographs of progesterone-loaded PLGA microspheres prepared following the solvent extraction-evaporation hybrid process. An initial progesterone payload varied from (a) 10 to (b) 20, (c) 30, and (d) 40%.

microsphere suspension is mostly composed of progesterone-loaded microspheres. When its particle-size distribution was measured, as expected, a single modal distribution was shown at all initial progesterone payloads (Figure 6). The frequency of particles under the size of 10 μm was less than 1%. When an initial progesterone payload was changed from 10 to 20, 30, and 40%, $d_{10\%}$ values of the resultant microsphere suspensions were measured as 45, 66, 71, and 75 μm respectively. The span indices of the microsphere suspensions showed values between 1.0 and 1.3, reflecting uniform microsphere populations.

Also investigated in this study was the amount of progesterone encapsulated inside PLGA microspheres prepared by solvent evaporation. Depending on an initial drug payload, drug Encapsulation Efficiency (EE) measured with the microspheres that did not go through wet sieving were 55.5 ± 6.0 to $71.8 \pm 8.6\%$ (Figure 7). However, as evidenced by Figure 3, the samples used for EE analysis are the mixtures of drug-containing microspheres and unentrapped drug crystals. Therefore, the EE data would not truly represent correct information. When free drug crystals were removed through wet sieving, the real EE values were determined to be 20.9 ± 2.5 to $29.4 \pm 6.7\%$. These poor EE data arise from the fact that considerable amounts of progesterone are not encapsulated but dispersed as free drug crystals in the aqueous continuous phase. Using methylene chloride for solvent evaporation to encapsulate piroxicam and glycyrrhetinic acid into PLGA microspheres also led to poor EE values ranging from 15.1 to 33.6%.[12,23] These data are in accordance with our results demonstrating that the preparation of microspheres through solvent evaporation has a tendency to show a poor EE value due to drug crystallization in a continuous phase.

When microspheres were prepared with the solvent extraction-evaporation hybrid process, unentrapped drug crystals were not observed not only in the aqueous continuous phase but also on the surface of microspheres (Figures 2 and 4). Based on this fact, it was able to predict that most of progesterone used for microencapsulation would be loaded inside PLGA microspheres. In fact, when an initial drug payload was set between 10 and 40%, EE values were determined to be 90.3 ± 2.7 to $93.8 \pm 5.0\%$ (Figure 8).

Drug EE can be measured in many different ways, and one approach is to centrifuge a hardened microsphere suspension and measure a drug concentration in the supernatant to indirectly calculate an EE value.[24] If the supernatant does not offer a sink condition for the hydrophobic drug, its crystals will undergo pelletization along with microspheres. Therefore, this method of measuring EE is not able to provide the true EE. Microspheres collected through other methods, such as filtration, would also be contaminated by unentrapped drug crystals. The results shown in Figure 7 support this argument. Benelli *et al.* also reported a similar problem that drug crystals always formed outside microspheres when clonazepam followed microencapsulation by a methylene chloride-based o/w solvent evaporation technique.[8] Unentrapped drug crystals existed with microspheres even when they lowered the drug:PLGA weight ratio to 5:95. Therefore, they concluded that clonazepam EE could not be measured and it was undesirable to encapsulate clonazepam into microspheres with an o/w emulsion solvent evaporation process. In our study, unentrapped drug crystals could be separated from microspheres through wet sieving, and an accurate EE would be obtained only through using drug crystals-free microspheres.

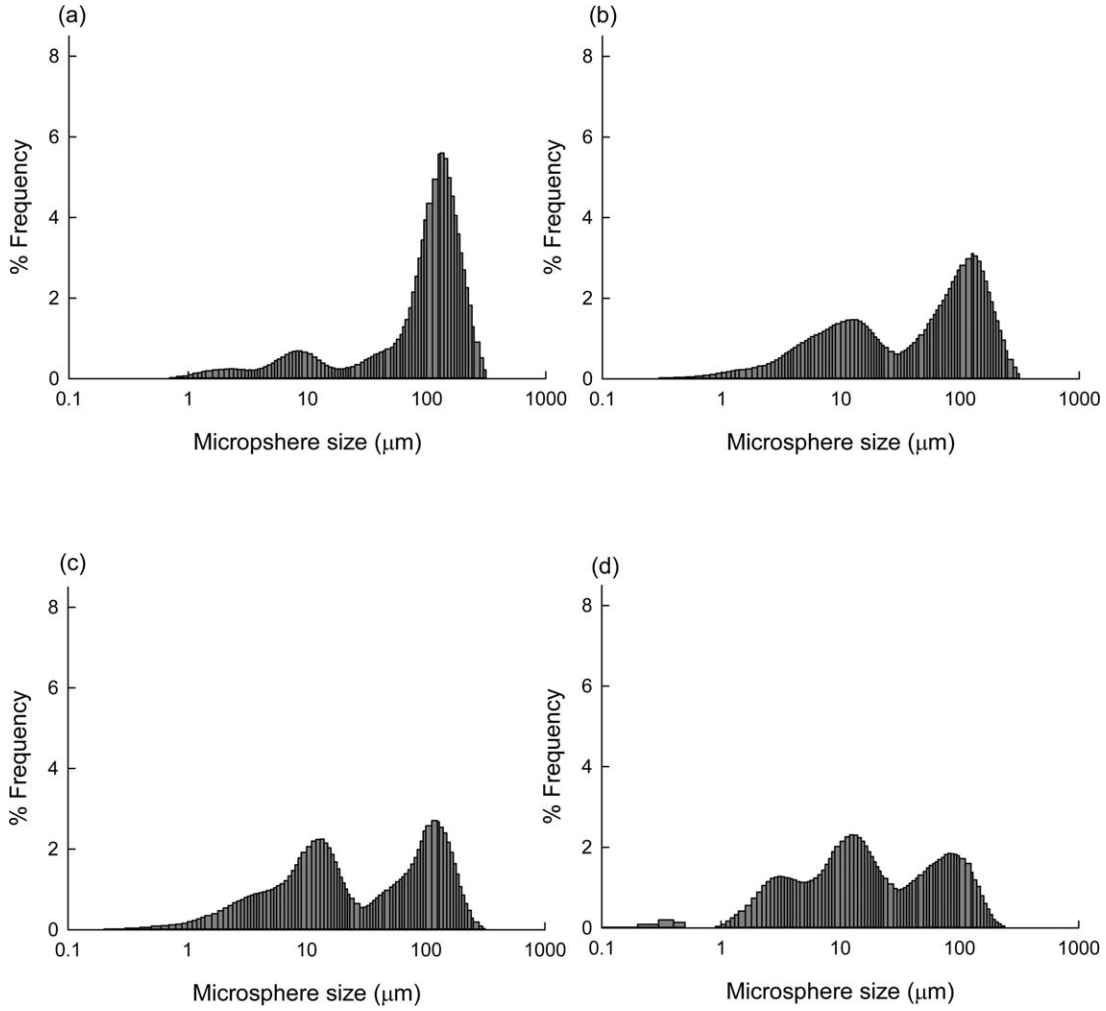

Figure 5. The size distribution patterns of various microsphere suspensions prepared by the solvent evaporation process. An initial progesterone payload was changed from (a) 10 to (b) 20, (c) 30, and (d) 40%.

Other than our method of using wet sieving, there would be different ways to remove unentrapped drug crystals. For example, Leo *et al.* reported that a considerable amount of ibuprofen existed as crystals on the surface PLGA microspheres prepared by solvent evaporation.[13] To remove ibuprofen crystals, they used a method of stirring the hardened microsphere suspension in a 0.1% sodium carbonate solution at pH 11. After then, microspheres were collected by centrifugation. This method took advantage of the increased aqueous solubility of ibuprofen in an alkaline solution. When they measured ibuprofen EE toward the microspheres that did not undergo the sodium carbonate solution treatment, the result was around $90.2 \pm 2.3\%$. By sharp contrast, when the microspheres were treated with the sodium carbonate solution, the drug EE was calculated to be $58.5 \pm 0.3\%$. This indirectly showed that the alkaline solution effectively removed ibuprofen on the surface of microspheres. Similarly, there was also a case where piroxicam crystals existing on the microsphere surface was removed by washing it with sodium bicarbonate.[12] In the case of theophylline with aqueous solubility increasing under pH 3, its crystals on the surface of microspheres would be able to be removed by an acidic solution wash.[17] Nevertheless, there exists the possibility that a portion of the encapsulated drug inside the microspheres would be removed as well. Therefore, it should be cautioned that the characteristics such as matrix porosity or drug release rate could be influenced after these treatments.

Origins of Drug Crystals on Microsphere Surface

The mechanism of drug crystal formation on the surface of microspheres has been explained in several ways. When a very high amount of a drug is dissolved in a dispersed solvent, the wall of microspheres might be perforated by drug crystals/needles during the solvent removal process.[7] Birnbaum *et al.* reported that drug encapsulation occurred on the outer side of microspheres and thus surface-embedded drug crystals were observed.[9] It was an argument that drug crystals formed in the dispersed phase did not diffuse to the aqueous continuous phase and therefore existed at the organic solvent/water interface (i.e., phase boundary). As the emulsion droplets hardened into microspheres, drug crystals were claimed to be loaded on the microsphere surface. Ré and Biscans suggested that, as an organic solvent diffused quickly into the aqueous continuous

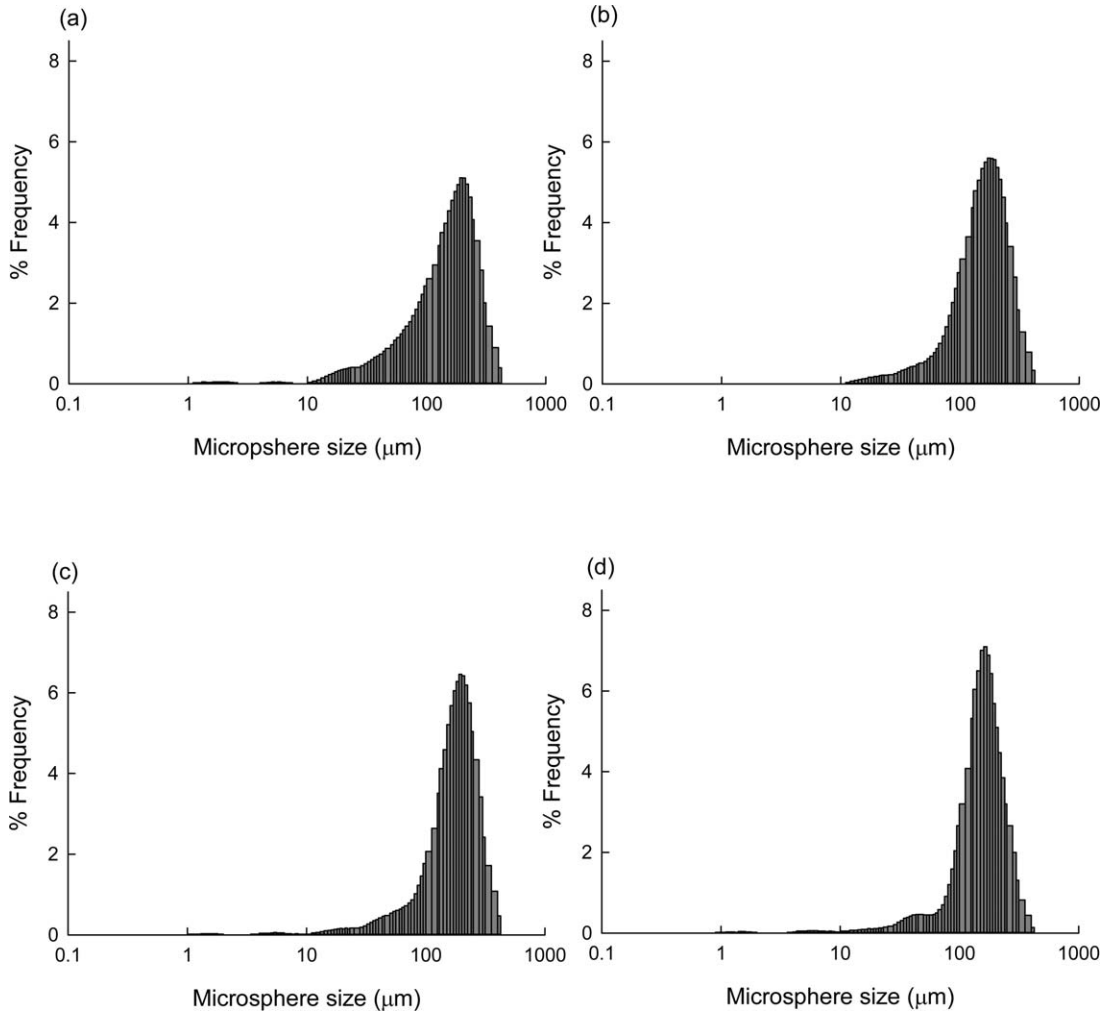

Figure 6. The size distribution patterns observed with various microsphere suspensions. The solvent extraction-evaporation hybrid process was used to prepare microspheres. An initial progesterone payload was changed from (a) 10 to (b) 20, (c) 30, and (d) 40%.

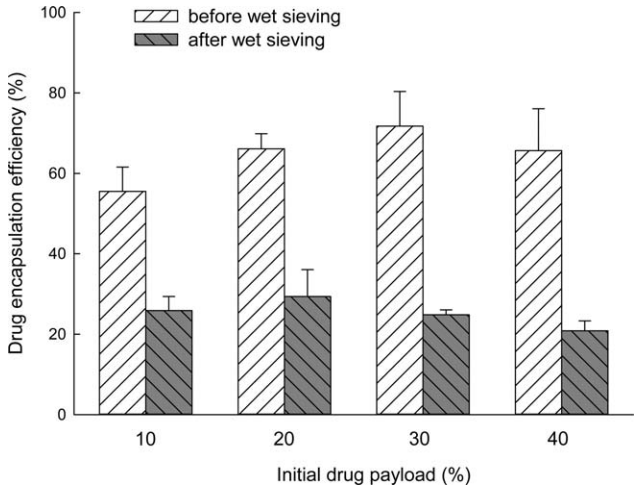

Figure 7. Progesterone EE observed with PLGA microspheres prepared by solvent evaporation. Prior to vacuum drying, microspheres were subject to either wet sieving−filtration or filtration alone.

phase, a film-like polymeric membrane was formed around an emulsion droplet.[18] Subsequently, a hydrophobic drug underwent crystallization when it contacted with the aqueous phase. This led to the formation of drug crystals on the surface of microspheres. Similarly, Dubernet *et al.* hypothesized that crystal nuclei formed in the non-stirred layer surrounding emulsion droplets and ultimately drug crystals deposited on the surface of microspheres as solvent evaporation neared completion.[16] In this case, the nature of an emulsifier layer that encloses the periphery of emulsion droplets would have a considerable effect on drug crystallization. In our study, it has been demonstrated that progesterone crystals on the surface of dried microspheres prepared by solvent evaporation is not produced by the mechanisms listed above, such as being encapsulated on the surface of microspheres or bursting out from the inner matrix of microspheres. Our results prove that, during the microsphere recovery step, progesterone-loaded microspheres are contaminated by unentrapped drug crystals present in the aqueous continuous phase. This claim agrees with the report stating that the quick diffusion of drugs from emulsion droplets to an aqueous continuous phase during solvent evaporation is responsible for

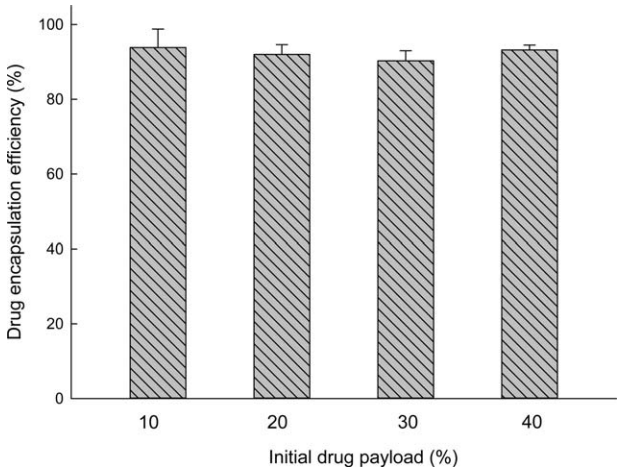

Figure 8. Progesterone EE observed with PLGA microspheres prepared by the solvent extraction-evaporation hybrid process.

their crystallization in the aqueous continuous phase.[8] In case of the solvent extraction-evaporation hybrid process reported in our study, microsphere hardening occurs quickly because solvent removal is achieved efficiently by a quenching liquid. Under this condition, progesterone is well encapsulated into the microspheres, thereby preventing drug crystallization both on the microsphere surface and in the aqueous continuous phase.

Thermal Behavior of Microspheres Laden with Progesterone

DSC was conducted to investigate the physical status of progesterone encapsulated in the microspheres. Figure 9 illustrates the thermograms of progesterone, PLGA as-received, and progesterone-loaded microspheres prepared by solvent evaporation and wet sieving. Progesterone exists mainly as α and β polymorphs with corresponding melting points of 128–133 and 120–122 °C. Before microencapsulation, progesterone had a melting point of 130 °C, showing that it was the α polymorph. T_g of PLGA raw powders was observed as 50 °C. When an initial progesterone payload was 10%, the actual drug loading of the microspheres was 2.4%. The characteristic melting point of progesterone and T_g of PLGA were not observed with these microspheres. This can be interpreted as progesterone behaving as a plasticizer by becoming molecularly dispersed in the microsphere matrix. When microspheres had actual drug loadings of $\geq$ 4.9%, the characteristic T_g of PLGA was marginally observed. These results suggested that the amount and physical status of progesterone in microspheres affected the amplitude of chain mobility of PLGA. In addition, the β polymorph was observed around 120 °C. These results indicate that a polymorphic transition of progesterone took place during microencapsulation.

The amount of molecularly dispersed progesterone in microsphere matrices was affected depending on the manufacturing process used. Interestingly, when the solvent extraction-evaporation hybrid process was used rather than the solvent evaporation process, a much larger amount of progesterone was molecularly dispersed in microsphere matrices (Figure 10). The microspheres containing 8.5 to 20.8% progesterone did not exhibit its characteristic melting point. The characteristic T_g of PLGA polymers was not observed as well. For microspheres containing 26.6% progesterone, its β polymorph was observed. Drug crystallization occurring in microsphere matrices is a complex phenomenon that depends on organic solvent type, solvent evaporation rate, drug type and payload, drug-polymer molecular interaction, and the ratio of drug to polymer.[14] In our methyl propionate-based microencapsulation processes, as Figures 9 and 10

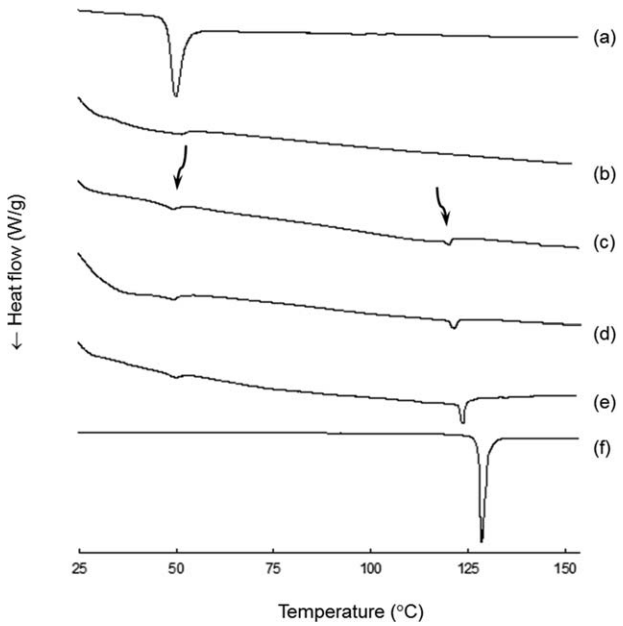

Figure 9. DSC thermograms of (a) raw PLGA powders as-received, (b–e) progesterone-loaded microspheres, and (f) progesterone. Microspheres were prepared by solvent evaporation. The actual progesterone loads in microspheres were (b) 2.4 (c) 4.9, (d) 5.7, and (e) 6.0%. Arrows indicate T_g or the melting point of progesterone.

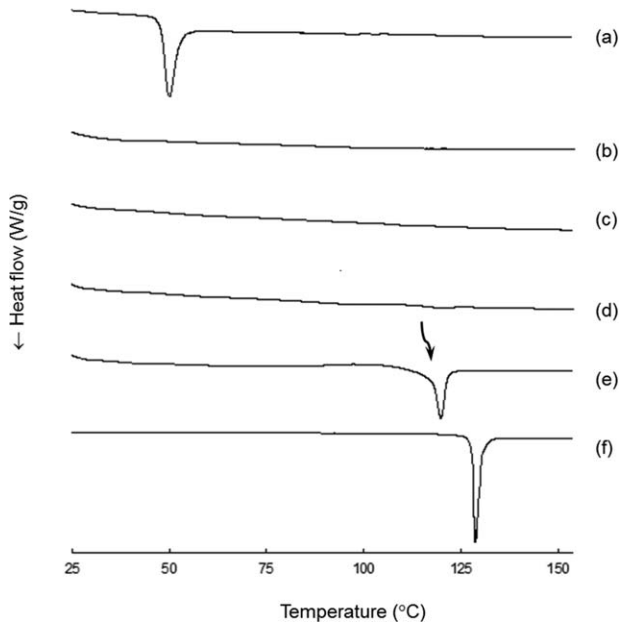

Figure 10. DSC thermograms of (a) raw PLGA powders as-received, (b–e) progesterone-loaded microspheres, and (f) progesterone. Microspheres were prepared by the solvent extraction-evaporation hybrid process. The actual progesterone loads in microspheres were (b) 8.5 (c) 15.3, (d) 20.8, and (e) 26.6%.

show, the solvent removal rate is demonstrated to be a significant factor that decides the physical status of progesterone in microspheres. The solvent extraction-evaporation hybrid process that provides a faster solvent removal is able to encapsulate a much higher amount of progesterone in an amorphous form into microsphere matrices, compared to the solvent evaporation process where the solvent removal rate is slow. It is generally perceived that drug crystals existing in microspheres can act as a core to make the crystal size larger, or delay drug dissolution. Therefore, an amorphous form of a drug is favored when one attempts to regulate drug dissolution or release rate. Considering all these aspects, when encapsulating a hydrophobic drug into microspheres, it would be desirable to use the solvent extraction-evaporation hybrid process rather than the solvent evaporation process.

CONCLUSIONS

When using non-halogenated methyl propionate as a dispersed solvent to produce microspheres through solvent evaporation, there is a tendency of progesterone to crystallize in the aqueous continuous phase. The time required for the formation of drug crystals and their amount depend on an initial progesterone payload. When drug crystals are not removed, the microsphere samples are a mixture of drug-containing microspheres and free drug crystals. Such microsphere products can raise serious problems on the quality and efficacy of microspheres. Through the solvent extraction-evaporation hybrid process achieving quick solvent removal, it is possible to circumvent drug crystallization in the aqueous phase and on the microsphere surface. Therefore, it is not necessary to develop any additional step to remove drug crystals. The resultant microspheres are uniform in populations, and the microencapsulation process helps maximize the drug EE. Therefore, when encapsulating a hydrophobic drug into PLGA microspheres, the solvent extraction-evaporation hybrid process has distinct advantages over commonly used solvent evaporation processes.

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REFERENCES

1. Prajapati, V. D.; Jani, G. K.; Kapadia, J. R. *Expert Opin. Drug Deliv.* **2015**, *12*, 1283.
2. Li, Z.; Shen, H.; Shuai, K.; Hu, X. *J. Appl. Polym. Sci.* **2016**, *133*, DOI: 10.1002/app.42861.
3. Qutachi, O.; Vetsch, J. R.; Gill, D.; Cox, H.; Scurr, D. J.; Hofmann, S.; Muller, R.; Quirk, R. A.; ShakeSheff, K. M.; Rahman, C. V. *Acta Biomater.* **2014**, *10*, 5090.
4. Xie, X.; Lin, W.; Xing, C.; Yang, Y.; Chi, Q.; Zhang, H.; Li, Y.; Li, Z.; Yang, Y.; Yang, Z.; Li, M. *PLoS One* **2015**, *10*, e0125953.
5. Hamishehkar, H.; Emami, J.; Najafabadi, A. R.; Gilani, K.; Minaiyan, M.; Mahdavi, H.; Nokhodchi, A. *Colloids Surf. B Biointerfaces* **2009**, *74*, 340.
6. Shelke, N. B.; Rokhade, A. P.; Aminabhavi, T. M. *J. Appl. Polym. Sci.* **2010**, *116*, 366.
7. Wischke, C.; Schwendeman, S. P. *Int. J. Pharm.* **2008**, *364*, 298.
8. Benelli, P.; Conti, B.; Genta, I.; Costantini, M.; Montanari, L. *J. Microencapsul.* **1998**, *15*, 431.
9. Birnbaum, D. T.; Kosmala, J. D.; Henthorn, D. B.; Brannon-Peppas, L. *J. Control. Release* **2000**, *65*, 375.
10. Benita, S.; Benoit, J.-P.; Puisieux, F.; Thies, C. *J. Pharm. Sci.* **1984**, *73*, 1721.
11. Kobayashi, D.; Tsubuku, S.; Yamanaka, H.; Asano, M.; Miyajima, M.; Yoshida, M. *Drug Dev. Ind. Pharm.* **1998**, *24*, 819.
12. Lalla, J. K.; Sapna, K. J. *Microencapsul.* **1993**, *10*, 449.
13. Leo, E.; Forni, F.; Bernabei, M. T. *Int. J. Pharm.* **2000**, *196*, 1.
14. Alhnan, M. A.; Basit, A. W. *J. Pharm. Sci.* **2011**, *100*, 3284.
15. Bodmeier, R.; Chen, H. *J. Control. Release* **1989**, *10*, 167.
16. Dubernet, C.; Benoit, J. P.; Puisieux, F. *Eur. J. Pharm. Biopharm.* **1991**, *37*, 49.
17. Obeidat, W. M.; Price, J. C. *J. Microencapsul.* **2004**, *21*, 47.
18. Ré, M. I.; Biscans, B. *Powder Technol.* **1999**, *101*, 120.
19. Rajkumar, M.; Bhise, S. B. *J. Young Pharm.* **2010**, *2*, 7.
20. Mateović-Rojnik, T.; Frlan, R.; Bogataj, M.; Bukovec, P.; Mrhar, A. *Chem. Pharm. Bull.* **2005**, *53*, 143.
21. Kang, J.; Sah, E.; Sah, H. *J. Microencapsul.* **2014**, *31*, 323.
22. Kastellorizios, M.; Tipnis, N.; Papadimitrakopoulos, F.; Burgess, D. J. *Mol. Pharm.* **2015**, *12*, 3332.
23. Zhong, D.; Liu, Z.; Xie, S.; Zhang, W.; Zhang, Y.; Xue, W. *J. Appl. Polym. Sci.* **2013**, *129*, 767.
24. Gupta, A.; Nagar, M.; Sharma, P. *Int. J. Pharm. Life Sci.* **2012**, *3*, 1437.

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