ELSEVIER

International Journal of Pharmaceutics 195 (2000) 103–113

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

Ethyl formate — alternative dispersed solvent useful in preparing PLGA microspheres

Hongkee Sah *

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

Received 7 July 1999; received in revised form 27 October 1999; accepted 27 October 1999

Abstract

In an effort to substitute methylene chloride with a less toxic solvent, this study was aimed at developing new ethyl formate-based emulsion processes to fabricate poly-D,L-lactide-*co*-glycolide (PLGA) microspheres. To do so, a polymeric dispersed phase was emulsified in a 1% polyvinyl alcohol aqueous solution at an ethyl formate to aqueous volume ratio of 8:20. Microsphere hardening was then achieved by solvent evaporation and quenching techniques. The average encapsulation efficiency of a model drug progesterone amounted to $95.2 \pm 2.7\%$. When the tendency of ethyl formate and methylene chloride to evaporate to air was compared, the evaporation rate of ethyl formate was 2.1 times faster than that of methylene chloride. The ease with which ethyl formate evaporated to air was beneficial in shortening the microsphere hardening step. For the solvent quenching process, only 80 ml of additional water was required to extract ethyl formate to the aqueous phase, due to its considerable water miscibility. In particular, the timing of ethyl formate quenching affected to a great extent dynamic processes of the breakup of elementary microdroplets into smaller ones. Therefore, variations in quenching time affected microsphere characteristics such as the degree of solvation, size distribution, and tendency to aggregate on drying. The results of this study showed that PLGA microspheres were successfully prepared using the new ethyl formate-based processes.

Keywords: Ethyl formate; Microencapsulation; Poly(lactide-*co*-glycolide); Microspheres

1. Introduction

Methylene chloride is the most frequently used dispersed solvent in preparing poly-D,L-lactide-*co*-glycolide (PLGA) microspheres via emulsion-based microencapsulation procedures for several reasons. First, PLGA polymers at different molecular weights and lactide:glycolide ratios are dissolved well in methylene chloride. Second, the solvent, which has a negligible water solubility (1.32 wt.%), fulfills the generally asserted criterion that an emulsion should consist of two immiscible liquids. Third, because of its low boiling point (39.8°C), the solvent molecules leaching from the polymeric dispersed phase to the aqueous contin-

* Tel.: +1-901-448-5505; fax: +1-901-448-6092.
E-mail address: hsah@utmem1.utmem.edu (H. Sah)

0378-5173/00/$ - see front matter
PII: S0378-5173(99)00379-8

uous phase evaporate readily to air. As a result, microspheres become solidified as the solvent evaporation proceeds. Finally, its high volatility also aids in the easy removal of the residual solvent in microspheres on drying. However, methylene chloride is a confirmed carcinogen with experimental carcinogenic and tumorigenic data. It is one of 38 substances found on the Priority List of Hazardous Substances, Agency for Toxic Substances and Disease Registry, Department of Health and Human Services (Federal Register, 1997). Therefore, its routine usage must comply with strict regulations set by regulatory agencies.

In contrast, ethyl formate is a nonchlorinated solvent and is not classified as a carcinogen. The solvent has been used to prepare PLGA microspheres by spray drying (Gander et al., 1996; Johansen et al., 1998). So far, its application in solvent evaporation and/or extraction processes has been hampered by considerable water miscibility (its water solubility at 20°C is 13.6 wt.%). Earlier studies, however, have corroborated that water immiscibility of a dispersed solvent is not an absolute prerequisite for making an emulsion (Sah et al., 1996; Sah, 1997). The objective of this study is to investigate the feasibility of ethyl formate as a dispersed solvent to prepare PLGA microspheres via solvent evaporation and quenching processes. This study also reports on key process parameters that affect the formation and breakdown of an ethyl formate-in-water emulsion. Finally, interesting features observed with the ethyl formate-based microencapsulation processes are discussed in comparison to the methylene chloride-based microencapsulation process. This study represents the author's continual effort to substitute methylene chloride with less toxic, safer solvents for preparing PLGA microspheres. From the perspectives of environmental and human safety issues on chlorinated solvents, this work is of practical importance.

2. Materials and methods

2.1. Materials

PLGA with a lactide:glycolide ratio of 85:15 (inherent viscosity = 0.29 dl/g in chloroform) (PLGA 85:15) was purchased from Birmingham Polymers (Birmingham, AL). Progesterone was supplied from Sigma (St. Louis, MO). Polyvinyl alcohol (PVA) with a molecular weight of 25 000 was obtained from Polysciences (Warrington, PA). Analytic grade ethyl formate was supplied from Aldrich (Milwaukee, WI). HPLC grade methylene chloride and dimethyl formamide were from Fisher Scientific (Malvern, PA). Major properties of ethyl formate and methylene chloride are compared in Table 1.

2.2. Preparation of microspheres

Microspheres were prepared by two different methods. The first method was based on the solvent evaporation technique. To make microspheres, 0.7 g of PLGA 85:15 was first dissolved in 8 ml of ethyl formate. The dispersed phase was

Table 1
Comparison of major properties of ethyl formate (EF) and methylene chloride (MC)[a,b,c]

Solvent	ρ (g/cm^3)	BP (°C)	VP (mm)	Water solubility (wt.%)	IDLH (ppm)	TLV (ppm)
EF	0.924	54.7	200	13.6	8000	100
MC	1.326	39.8	350	1.32	Carcinogen	50, suspected carcinogen

[a] Where ρ is density at 20°C; BP, boiling point; VP, vapor pressure at 20°C; IDLH, immediately dangerous to life or health; and TLV, threshold limit value.

[b] Chapter 11. Properties of groups of solvents. In: A.K. Doolittle (Ed.), The Technology of Solvents and Plasticizers, Wiley, New York, 1954, pp. 352–491.

[c] Compendium of Hazardous Chemicals in Schools and Colleges, J.B. Lippincott Company, Philadelphia, PA, 1990, pp. 408–409 and 575–578.

poured into 20 ml of a 1% PVA aqueous solution. During the addition, the continuous phase was stirred at 450 rpm using a 400 HPS magnetic plate stirrer (VWR Scientific). The oil-in-water (O/W) emulsion was stirred for 220 min at room temperature; all these experiments were performed inside a hood. Microspheres were then collected by filtration, washed with distilled water, and dried overnight under vacuum.

The second method, referred to as the solvent quenching method, used the considerable water miscibility of ethyl formate. The dispersed phase, composed of ethyl formate (8 ml) and PLGA 85:15 (0.7 g), was emulsified in the aqueous continuous phase (20 ml) as described above. After the emulsion was stirred for 2, 7, 11, 15, or 20 min, additional distilled water (80 ml) was added quickly into the initial O/W emulsion to extract ethyl formate into the continuous phase. After continual stirring for total 220 min, microspheres were collected and dried.

2.3. Gravimetric analysis of solvent evaporation through the air/emulsion interface

PLGA 85:15-free ethyl formate (8 ml) was emulsified in a 1% PVA aqueous solution (20 ml) under the same conditions specified in microsphere preparation. The amount of ethyl formate evaporating from the emulsion to air was deduced by monitoring changes in the emulsion weight as a function of stirring time. A similar experiment was repeated after ethyl formate was substituted with methylene chloride, and the evaporation tendency of the two solvents was compared.

2.4. Evaluation of the surface morphology of dried microspheres

Microsphere samples were mounted on an aluminum holder and sputter-coated at a thickness of 120 nm in an argon atmosphere (Hummer VII, Anatech, Alexandria, VA). The surface morphology of microspheres was then investigated using a JSM-840A scanning electron microscope (Joel, Peabody, MA).

2.5. Microencapsulation of progesterone into PLGA 85:15 microspheres

Progesterone (70 mg) and PLGA 85:15 (0.5, 0.6, 0.7, 0.8, or 0.9 g) were dissolved in ethyl formate (8 ml). The dispersed phase was emulsified in a 1% PVA aqueous solution (20 ml). In 20 min, distilled water (80 ml) was added to the emulsion to quench ethyl formate from the dispersed phase. The emulsion was stirred for total 220 min, and microspheres were collected and dried. A known amount of the dried microspheres (40–50 mg) was completely dissolved in 3 ml of dimethyl formamide, and 15 ml of methanol were added to precipitate PLGA 85:15. After the resultant suspension was filtered through a Titan syringe filter membrane (Scientific Resources, Eatontown, NJ), the amount of progesterone in the filtrate was analyzed by HPLC (Sah et al., 1996). Encapsulation efficiency (EE%) was defined as the ratio of the actual to theoretical progesterone loadings, as shown below:

$$\mathrm{EE\%} = \frac{\text{Actual progesterone loading (wt.\%)}}{\text{Theoretical progesterone loading (wt.\%)}} \times 100 \quad (1)$$

2.6. Observation of oil droplets as a function of stirring time

With a light microscope (Model H602; World Precision Instruments, Sarasota, FL), the morphology, size, and stability of polymeric microdroplets dispersed in the aqueous phase were evaluated as a function of stirring time.

2.7. Measurement of the degree of microsphere solvation at the end of manufacturing processes

Microspheres collected by filtration were immediately weighed (M_1) and again after drying to a constant weight (M_2). The percentage microsphere solvation (MS%) was determined by Eq. (2):

$$\mathrm{MS\%} = \frac{(M_1 - M_2)}{M_2} \times 100 \quad (2)$$

2.8. Analysis of the size distribution and specific surface area of microspheres

The Horiba CAPA-700 particle size analyzer (Horiba, Kyoto, Japan) was used to determine the size distribution and specific surface area of microspheres. The particle size analyzer measured the sedimentation velocity of microspheres. On the basis of the proportional relationship between the sedimentation time and particle diameter, the distribution of the volume-based microsphere diameter (F_i) was determined as follows:

$$F_i = \frac{(\log I_0 - \log I_i)}{\sum_{i=1}^{n} \{(\log I_0 - \log I_i) \times D_i\}} \quad (3)$$

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. The specific surface area of microspheres (S_w, in units of m^2/g) was determined by Eq. (4):

$$S_w = \frac{6}{\rho} \sum_{i=1}^{n} \left(\frac{F_i}{D_i} \right) \quad (4)$$

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

2.9. Determination of the residual ethyl formate in wet microspheres

During the two different microencapsulation processes, microspheres were collected at 60, 100, 140, 180, and 220 min. A known quantity of the wet microspheres (25–30 mg) was completely dissolved in 4 ml of dimethyl formamide. The sample solutions were then spiked with an internal standard methanol, and the content of ethyl formate was determined by a Hewlett Packard 5890 gas chromatograph equipped with a flame ionization detector. Helium was used as a carrier gas, whereas the crosslinked methylsiloxane HP-1 column (0.32-mm inner diameter × 30-m length) served as a stationary phase. During sample running, the oven temperature was initially set at 35°C for 7 min and was gradually increased to 200°C at the rate of 25°C/min. The concentrations of ethyl formate in the unknown samples were calculated based on a calibration curve constructed by integrating peak areas of ethyl formate standards of known concentrations.

2.10. Data report

Each set of experiments described so far was repeated at least three times. Results were reported as mean ± standard deviation (S.D.) in text, and statistical analysis was performed using a two-tailed Student's t-test.

3. Results

When 8 ml of PLGA 85:15-containing ethyl formate was emulsified in 20 ml of the aqueous continuous phase, the polymeric phase was well broken into microdroplets. By contrast, irregularly shaped PLGA 85:15 precipitates tended to appear immediately when emulsification was performed at ethyl formate to aqueous volume ratios of 8:50 and 8:80. When solvent evaporation proceeded for only 20 min at the phase volume ratio of 8:20, the microdroplets still consisted of viscous liquids. As a result, they become coalesced to form films without mechanical stirring (Fig. 1). Such coalescence was not observed with the emulsion stirred for 60 min, suggesting that the polymeric microdroplets became solidified between 20 and 60 min. The continual evaporation of ethyl formate present in the aqueous continuous phase accounted for the microsphere hardening: the solvent evaporation from the continuous phase drove the diffusion of ethyl formate from the dispersed phase to the continuous phase, which was subsequently evaporated. Microspheres collected at 220 min and dried were free-flowing and well dispersed, demonstrating that they did not have a propensity to aggregate on drying. The scanning electron microscope (SEM) micrograph revealed that the ethyl formate-based evaporation process led to the formation of spherical microspheres with a smooth surface (Fig. 2).

To investigate the tendency of ethyl formate and methylene chloride to evaporate through the air/emulsion interface, the weight of their emulsions was monitored as a function of stirring time

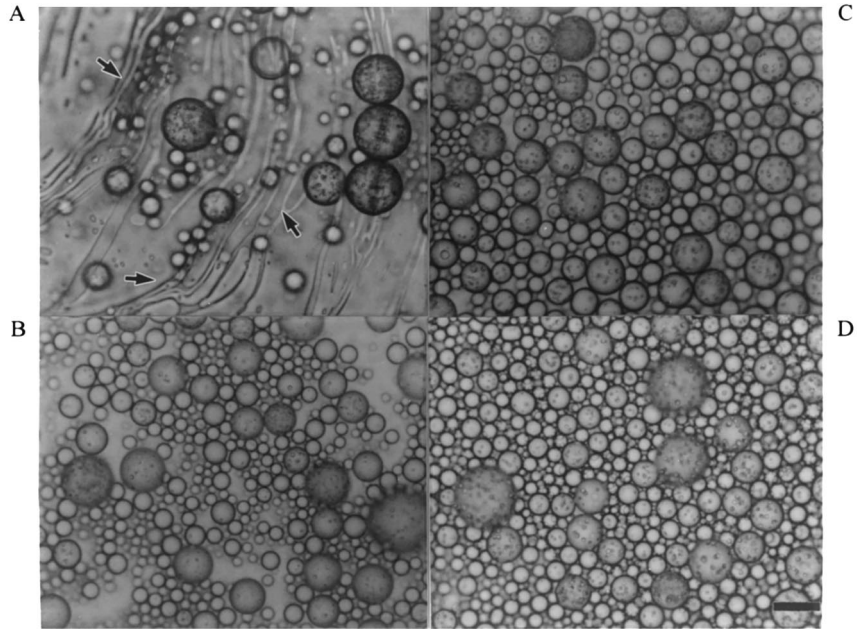

Fig. 1. Light microscope (LM) photographs of oil-in-water (O/W) emulsions sampled at (A) 20, (B) 60, (C) 120, and (D) 220 min during the ethyl formate-based solvent evaporation process. The arrow shows films formed by coalescence of microdroplets of the polymeric phase. The size of bar is 100 μm.

(Fig. 3). The evaporation rate of ethyl formate was 2.1 times faster than that of methylene chloride, although ethyl formate possessed a lower vapor pressure and a higher boiling point than methylene chloride did (Table 1). The percentage of ethyl formate and methylene chloride that evaporated to air over 60 min of stirring was 76.9 ± 5.3 and $35.6 \pm 0.6\%$, respectively.

The solvent quenching process based on the water miscibility of ethyl formate also fabricated free-flowing, spherical microspheres. In this process, only 80 ml of additional distilled water was used to extract ethyl formate from the polymeric phase. A number of different formulations were used to load progesterone into PLGA 85:15 microspheres via the solvent quenching process. Most proportions of progesterone were loaded in microspheres, and similar encapsulation efficiencies were obtained in all measurements (Fig. 4). When 0.5 g of PLGA 85:15 was used, $97.7 \pm 1.8\%$ of progesterone was loaded in microspheres. An increase in the polymer payload to 0.9 g provided a similar encapsulation efficiency of $96.2 \pm 2.8\%$. No significant differences in their encapsulation efficiencies were noticed with changing the polymer payload from 0.5 to 0.9 g ($P = 0.43$).

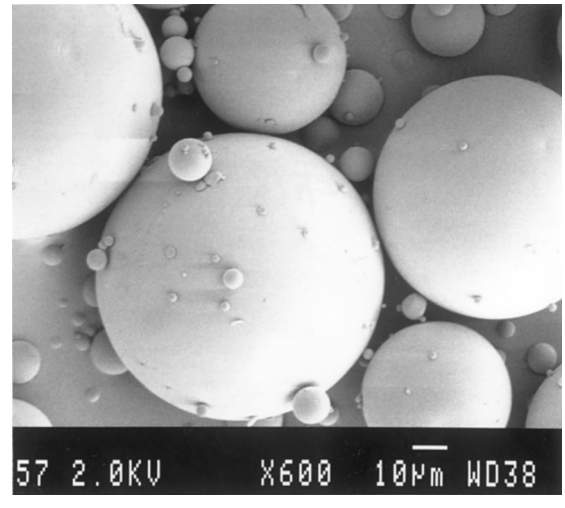

Fig. 2. Scanning electron microscope (SEM) micrograph of dried microspheres prepared by the solvent evaporation process. The size of bar is 10 μm.

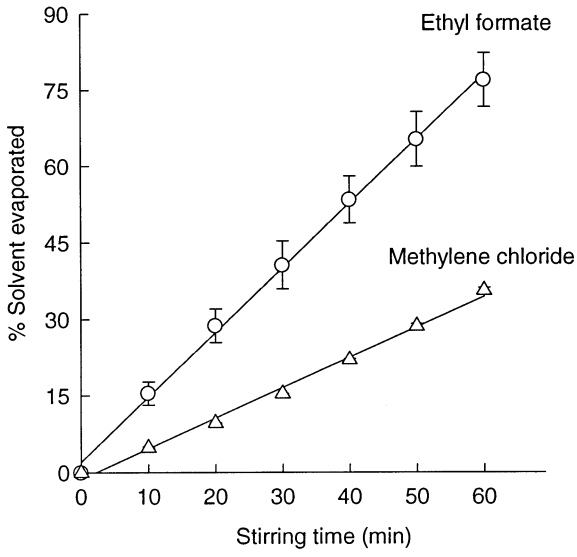

Fig. 3. Comparison of the evaporation rate of ethyl formate with that of methylene chloride. After ethyl formate or methylene chloride was emulsified in a 1% polyvinyl alcohol (PVA) aqueous solution, the amount of the solvents evaporating from the emulsions to air was determined as a function of stirring time (mean ± S.D.; $n = 3$).

During the solvent quenching process, the time to launch the quenching of ethyl formate was identified as a key parameter that affected the characteristics of microspheres including the degree of solvation, tendency to aggregate on drying, and size distribution pattern. In regard to the degree of microsphere solvation, the earliest quenching at 2 min brought about the highest solvated microspheres: their degree of solvation was 117.4 ± 24.2% (Fig. 5). At the same time, they tended to aggregate on drying. As a result, the dried microspheres were not separated into individual particles. On the contrary, retarding the initiation of ethyl formate quenching to 7, 11, 15, or 20 min led to considerable reductions in the percentage of microsphere solvation. In addition, delay in quenching time alleviated the drying-associated microsphere aggregation. For instance, the microspheres prepared by quenching at 20 min were 4.7 times less solvated than those quenched at 2 min, and they were well dispersed into discrete particles after drying.

The size of microspheres was also influenced by quenching time. Fig. 6 shows light microscope

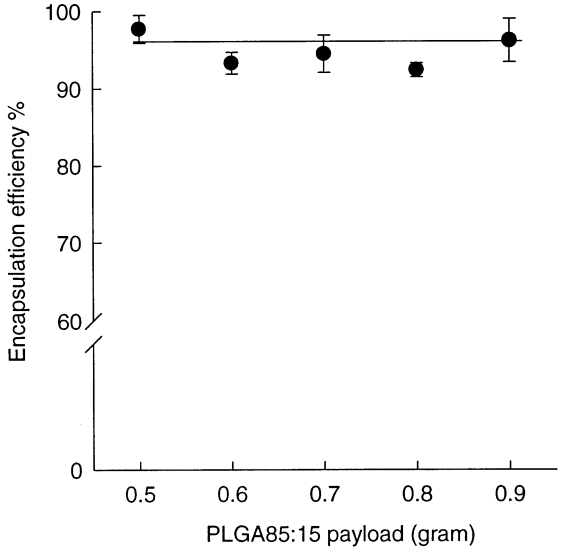

Fig. 4. The effect of poly-D,L-lactide-*co*-glycolide (PLGA) 85:15 payload on the encapsulation efficiency of progesterone (mean ± S.D.; $n = 3 \sim 4$). The polymeric phase used to prepare microspheres contained 70 mg of progesterone and 0.5–0.9 g of PLGA 85:15.

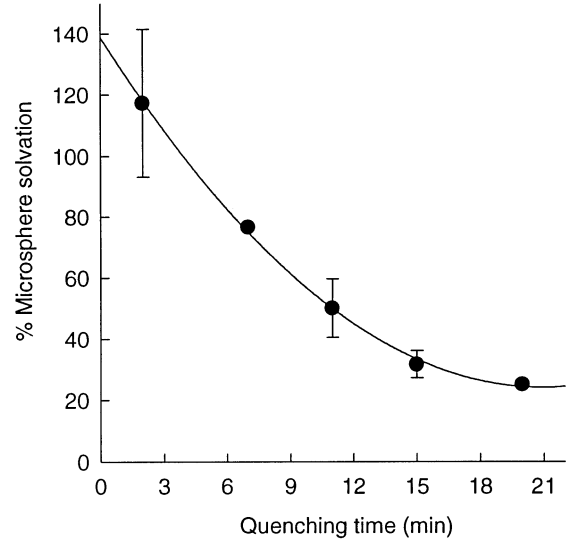

Fig. 5. The effect of variations in the onset of ethyl formate quenching on the degree of microsphere solvation (mean ± S.D.; $n = 3$). Microspheres were prepared by quenching at 2, 7, 11, 15, or 20 min, and their degree of solvation was determined using Eq. (2).

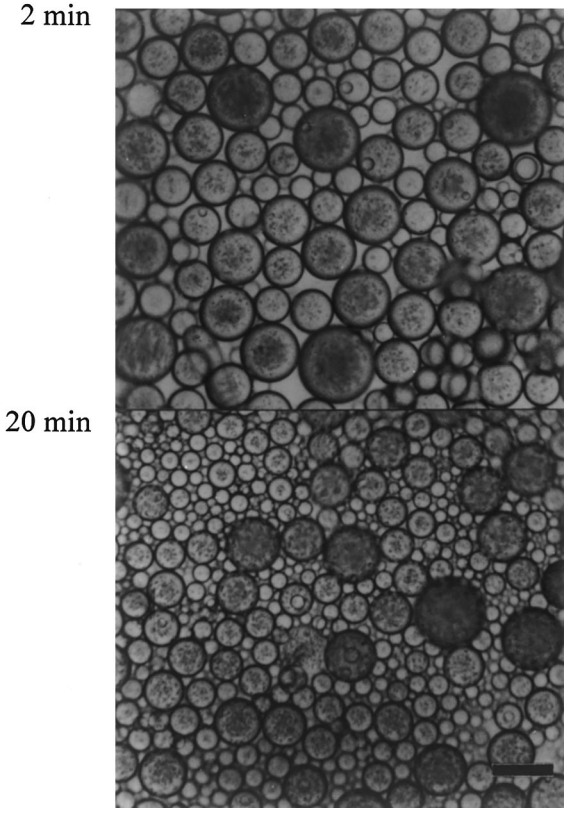

Fig. 6. Light microscope (LM) photographs of microspheres prepared by quenching ethyl formate at 2 and 20 min after the preparation of the initial oil-in-water (O/W) emulsion. The size of bar is 100 μm.

(LM) pictures of the microspheres prepared by quenching ethyl formate at 2 and 20 min after preparing initial O/W emulsions. The photographs provide direct evidence that smaller microspheres were prepared when solvent quenching was delayed. Further elaboration on the effect of quenching time on the microsphere size was done by analyzing the size distribution of microspheres prepared by quenching at 2, 11, and 20 min (Fig. 7). Quenching at 2 min led to the formation of the biggest microspheres of all; their particle size distribution pattern (mean diameter ± S.D.) was 131.0 ± 55.2 μm. In addition, considerable numbers of microspheres bigger than 200 μm were observed in this case. On the contrary, quenching at 11 and 20 min resulted in microspheres with size distribution patterns of 92.7 ± 47.8 and 55.8 ± 39.9 μm, respectively. In addition, very few microsphere with bigger than 200 μm appeared. Based on size distribution data, the specific areas of the three different microspheres were calculated using Eq. (4). The specific areas (mean ± S.D.) of

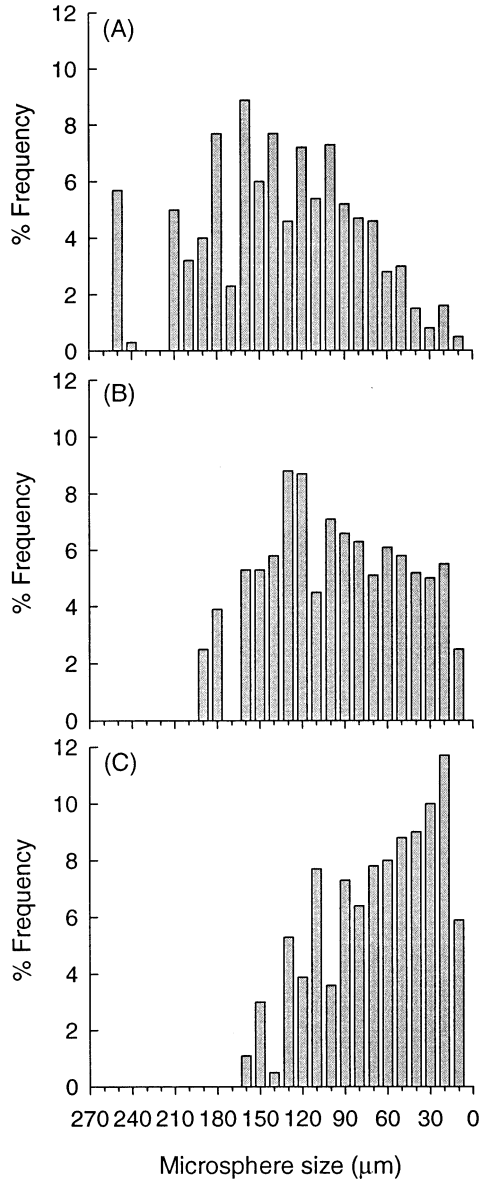

Fig. 7. The effect of quenching time on the size distribution of microspheres. After preparing an initial oil-in-water (O/W) emulsion, 80 ml of distilled water were added to the emulsion at (A) 2, (B) 11, or (C) 20 min.

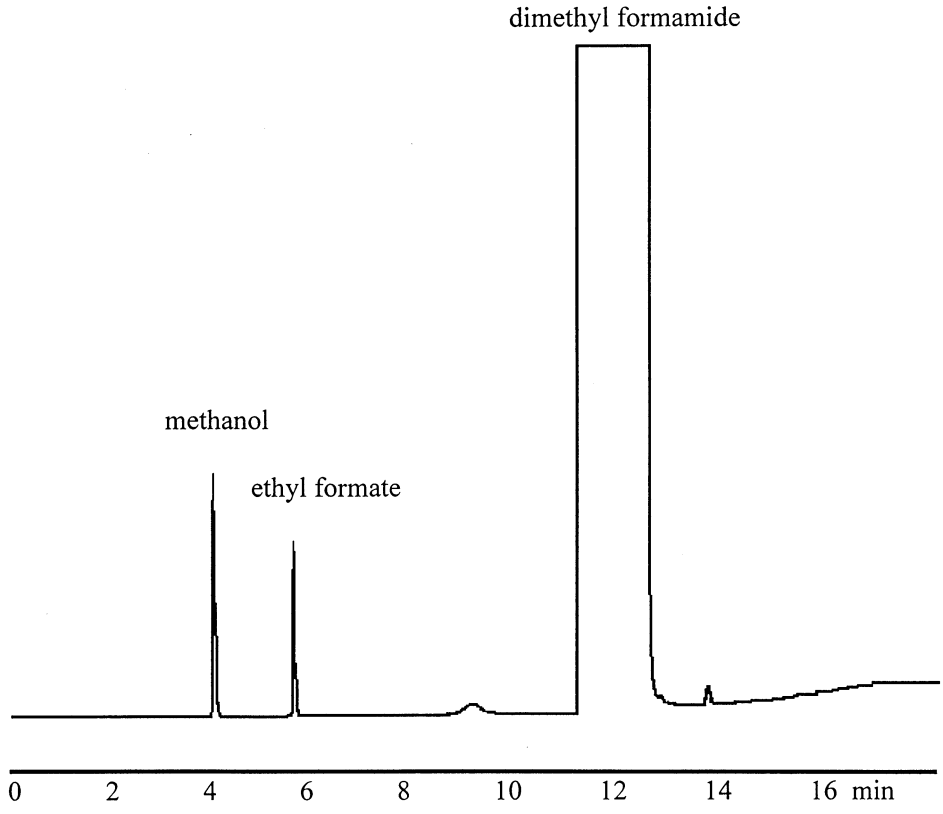

Fig. 8. The gas chromatograms of ethyl formate, methanol, and dimethyl formamide.

the microspheres prepared by quenching at 2, 11, and 20 min were 0.059 ± 0.003, 0.1065 ± 0.002, 0.189 ± 0.016 m^2/g, respectively. As anticipated, there was an inversely proportional relationship between microsphere size and its specific area.

Fig. 8 shows the gas chromatogram of ethyl formate, methanol, and dimethyl formamide detected by the GC analysis. The residual contents of ethyl formate in microspheres at various manufacturing stages are shown in Fig. 9. The microspheres, which were collected at 60 min during the solvent evaporation process, contained $12.1 \pm 1.7\%$ of ethyl formate. Its residual content in microspheres noticeably declined as the process proceeded; after 100 min of stirring, the level of ethyl formate decreased to $6.4 \pm 0.5\%$. The residual solvent content thereafter remained fairly constant, such that microspheres suspended for 220 min contained $4.9 \pm 0.1\%$ of ethyl formate. When microspheres were sampled at 60 min during the solvent quenching process, they were shown to have $6.9 \pm 0.9\%$ of ethyl formate. The solvent content in the microspheres sampled at 220 min was $4.7 \pm 0.8\%$; this solvent level was similar to that noticed with the solvent evaporation process. These data demonstrate that adding 80 ml of distilled water to the initial O/W emulsion effectively contributed to quenching ethyl formate out of the dispersed phase into the aqueous phase. The microspheres dried overnight under vacuum contained 2.8 ± 0.9 wt.% of ethyl formate (seven different microsphere batches were used to determine the residual amount of ethyl formate).

4. Discussion

When a partially water-miscible solvent is used as a dispersed solvent, at least two methods can be used to form the organic solvent-in-water (O/W) emulsion: using a high volume ratio of the dispersed to the continuous phases, or doping the aqueous continuous phase with a sufficient amount of the solvent before emulsification to retard the diffusion of the dispersed solvent to water (Lewis, 1994; Sah, 1997). At an ethyl formate to aqueous volume ratio of 8:20, it was not necessary to predissolve an extra amount of ethyl formate in water before emulsification. This result suggests that when the dispersed phase is emulsified in water, a proportion of ethyl formate leaches to and saturates the water phase. This event contributes to the subsequent breakdown of the dispersed phase into microdroplets in which PLGA 85:15 is still dissolved (Fig. 1). By contrast, increases in the water volume to 50 and 80 ml allow major proportions of ethyl formate to diffuse to the water phase upon emulsification. As a result, PLGA 85:15 starts to lose solubility in the dispersed phase and tends to precipitate right after mixing. Under these conditions, it is necessary to predissolve a sufficient quantity of ethyl formate in water before emulsification to avoid the formation of PLGA 85:15 precipitates and to ensure the formation of embryonic microsphere droplets.

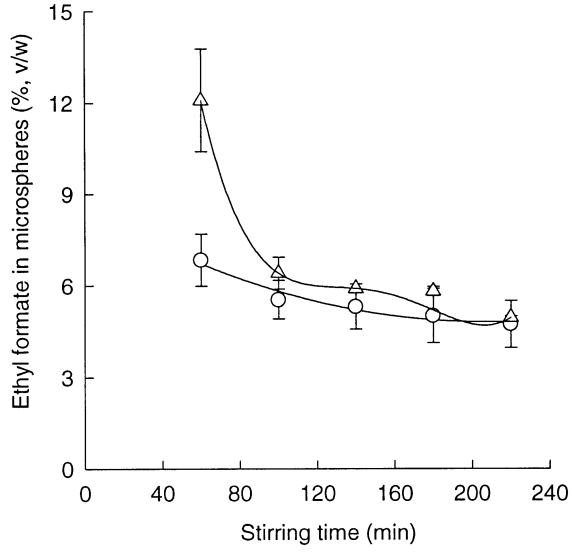

Fig. 9. The level of residual ethyl formate in wet microspheres collected at 60, 100, 140, 180, and 220 min during (△) the solvent evaporation and (○) the solvent quenching processes (mean ± S.D.; $n = 3 \sim 4$).

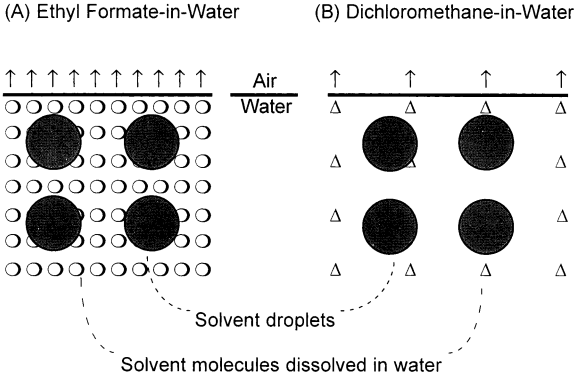

Fig. 10. Schematic illustration comparing the evaporation tendency of ethyl formate and methylene chloride through the air/emulsion interface.

The vapor pressures and boiling points of ethyl formate and methylene chloride shown in Table 1 indicate that methylene chloride is a faster evaporating solvent. Therefore, it was expected that when methylene chloride- and ethyl formate-in-water emulsions were subject to stirring, methylene chloride would evaporate through the air/emulsion interface at a faster rate. In our experiment to monitor the evaporation of the two solvents, however, ethyl formate evaporated faster than methylene chloride did (Fig. 3). This phenomenon is understandable if the number of solvent molecules exposed to the air/emulsion interface is considered. A greater water miscibility of ethyl formate, compared to methylene chloride, permits more ethyl formate molecules to reside in the interface, thereby enhancing the likelihood of evaporation to air (Fig. 10). The ease with which ethyl formate evaporates is an invaluable attribute when microspheres are prepared according to an emulsion-based solvent evaporation process.

So far, some interesting features observed with the ethyl formate-based solvent evaporation process have been discussed. Microspheres have also been successfully prepared by ethyl formate quenching. Previously, a quenching technique was

used to quickly solidify embryonic microsphere droplets that were made of methylene chloride or ethyl acetate (Lewis, 1994; Boisdron-Celle et al., 1995; Cleland et al., 1997; Sah, 1997; Péan et al., 1998). In particular, when methylene chloride was used as a dispersed solvent, 208–2580 ml of water was used to extract 1 ml of methylene chloride in the dispersed phase (Boisdron-Celle et al., 1995; Cleland et al., 1997; Péan et al., 1998). The necessity of a large quantity of water arises partly from the negligible water solubility of methylene chloride. In this regard, the ethyl formate-based solvent quenching process sharply contrasts with the methylene chloride one. Only 100 ml of water — 20 ml as a continuous phase and 80 ml as an extraction medium — was used against 8 ml of ethyl formate. The considerable water miscibility of ethyl formate (13.2 wt.%) minimizes the amount of water required for effective quenching.

It is generally supposed that the size of embryonic microspheres is subject to dynamic changes as a function of emulsification time. For example, it has been described that during a methylene chloride-based evaporation process, the size of PLGA microsphere droplets gradually decreases as the evaporation process proceeds (Cowsar et al., 1985; Crotts and Park, 1995). It is ascribed that the embryonic microsphere droplets generated at the early stage of the microencapsulation process are large and viscous liquids, such that the subsequent solvent diffusion to water and evaporation to air are followed by the continual inward shrinkage of PLGA polymers. A series of these events has been deemed the cause for the observed reduction in microsphere size against solvent evaporation time. In our study, the onset of ethyl formate quenching was altered to discontinue dynamic changes in the size of microspheres at various time intervals (it was felt that microspheres would immediately solidify after a sufficient quantity of water was added to the initial O/W emulsion). Interestingly, smaller microspheres are fabricated when the onset of quenching is delayed (Figs. 6 and 7). However, such a reduction in the microsphere size is not caused by the previously suggested microsphere shrinkage phenomenon. This conclusion is justified on the results of total surface areas of microspheres prepared by quenching at 2, 11, and 20 min. Retardation in launching the solvent quenching from 2 to 11 and 20 min increased total specific area of the resultant microspheres to 1.8 and 3.2 times, respectively. This result suggests that emulsification breaks up the embryonic microsphere droplets continuously into smaller ones to increase their total surface area as a function of stirring time (if microspheres shrank over a period of 2–20 min, delay in quenching time would decrease their surface area). As a consequence, microspheres prepared by quenching at 20 min are smaller and less solvated at the end of the solvent quenching process. The resultant microspheres do not encounter the problem of aggregation on drying. In comparison, quenching at 2 min results in bigger microspheres that are considerably more solvated and that tend to aggregate on drying (Fig. 5). It can be inferred from the results that the less solvated the microspheres are, the easier they dry without forming aggregates.

5. Conclusion

Both the ethyl formate-based evaporation and the solvent quenching procedures lead to successful fabrication of progesterone-loaded PLGA 85:15 microspheres with good qualities. Compared to the methylene chloride-based evaporation and extraction processes, the ethyl formate-based microencapsulation processes have two unique features: the ease with which ethyl formate evaporates through the air/emulsion interface and requirement of a small quantity of water to quench ethyl formate from the dispersed phase. These attributes are invaluable in scaling up the microencapsulation process. Especially, when microspheres are prepared by the solvent quenching process, quenching time is found to affect the dynamic process of the breakup of embryonic microdroplets. As a result, important microsphere characteristics — e.g. the degree of microsphere solvation, size distribution pattern, and tendency to aggregate on drying — are influenced by variations in the onset of ethyl formate quenching.

Acknowledgements

The author thanks Ms Yogita Bahl for her kind help in determining the size distribution of microspheres.

References

Boisdron-Celle, M., Menei, P., Benoit, J.P., 1995. Preparation and characterization of 5-fluorouracil-loaded microparticles as biodegradable anticancer drug carriers. J. Pharm. Pharmacol. 47, 108–114.

Cleland, J.L., Lim, A., Barrón, L., Duenas, E.T., Powell, M.F., 1997. Development of a single-shot subunit vaccine for HIV-1: Part 4. Optimizing microencapsulation and pulsatile release of MN rgp120 from biodegradable microspheres. J. Control. Release 47, 135–150.

Cowsar, D.R., Tice, T.R., Gilley, R.M., English, J.P., 1985. Poly(lactide-co-glycolide) microcapsules for controlled release of steroids. Methods Enzymol. 112, 101–116.

Crotts, G., Park, T.G., 1995. Preparation of porous and nonporous biodegradable polymeric hollow microspheres. J. Control. Release 35, 91–105.

Federal Register, 1997. Notice of the revised priority list of hazardous substances that will be the subject of toxicological profiles, pp. 61332–61335.

Gander, B., Johnasen, P., Nam-Trân, H., Merkle, H.P., 1996. Thermodynamic approach to protein microencapsulation into poly(D,L-lactide) by spray drying. Int. J. Pharm. 129, 51–61.

Johansen, P., Merkle, H.P., Gander, B., 1998. Improving stability and release kinetics of microencapsulated tetanus toxoid by co-encapsulation of additives. Pharm. Res. 15, 1103–1110.

Lewis, D.H., 1994. Growth promoters for animals. US Patent 5 288 496, 22 Feb.

Péan, J.M., Venier-Julienne, M.C., Boury, F., Menei, P., Denizot, B., Benoit, J.P., 1998. NGF release from poly(D,L-lactide-co-glycolide) microspheres. Effects of some formulation parameters on encapsulated NGF stability. J. Control. Release 56, 175–187.

Sah, H., 1997. Microencapsulation techniques using ethyl acetate as a dispersed solvent: effects of its extraction rate on the characteristics of PLGA microspheres. J. Control. Release 47, 233–245.

Sah, H., Smith, M.S., Chern, R.T., 1996. A novel method of preparing PLGA microcapsules utilizing methylethyl ketone. Pharm. Res. 13, 360–367.