A Novel Method of Preparing PLGA Microcapsules Utilizing Methylethyl Ketone

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Purpose. To substitute dichloromethane with a safer solvent, a solvent extraction process using methylethyl ketone (MEK) was developed to prepare poly(*d,l*-lactide-*co*-glycolide) microcapsules.

Methods. The MEK dispersed phase containing PLGA and progesterone was emulsified in the MEK-saturated aqueous phase (W_1) to make a transient oil-in-water (O/W_1) emulsion. It was then transferred to a sufficient amount of water (W_2) so that MEK residing in polymeric droplets could be extracted effectively into the continuous phase.

Results. This solvent extraction process provided the encapsulation efficiency for progesterone ranging from 77 to 60%. The amount of MEK predissolved in W_1, as well as the degree of progesterone payload, influenced the encapsulation efficiency. The leaching profile of MEK analyzed by GC substantiated that, upon dispersion of O/W_1 to W_2, MEK quickly diffused into the continuous phase. Such a rapid diffusion of MEK from and the ingression of water into polymeric droplets produced hollow microcapsules, as evidenced by their SEM micrographs.

Conclusions. When solvent extraction/evaporation techniques are employed for preparing PLGA microcapsules, water-immiscibility of a dispersed phase is not an absolute prerequisite to the successful microencapsulation. Adjustment of an initial extraction rate of MEK and formation of a primary transient O/W_1 emulsion are found to be very crucial not only for the success of microencapsulation but also for the determination of microcapsule morphology.

KEY WORDS: methylethyl ketone; poly(*d,l*-lactide-*co*-glycolide); microcapsules; morphology; solvent extraction/evaporation.

INTRODUCTION

Lactic/glycolic acid copolymers are extensively used in the microencapsulation of various drugs due to their excellent biocompatibility, biodegradability and versatile ability to display sustained drug release over a wide range of periods. Many hydrophobic compounds have been encapsulated into microparticles composed of these biodegradable polymers via a traditional oil-in-water emulsion technique. The conventional technique usually consists of three major steps: (a) emulsification of a water-immiscible organic solution with an aqueous phase containing surfactants; (b) removal of the solvent by extraction and/or evaporation; and (c) isolation of microparticles by filtration or centrifugation. Selection of the dispersed and the continuous phases is important in successful microparticle formation and efficient entrapment of drug. In general, it is believed that the dispersed phase should be immiscible with the continuous phase and possess a boiling point lower than that of the continuous phase (1). Methylene chloride and water have been used as the two liquid phases in the majority of solvent extraction/evaporation techniques. The reasons for the widespread use of methylene chloride are: (a) it has a high volatility so that it can be removed easily by evaporation; and (b) it is a good solvent for a wide range of polymers. However, a major concern has emerged recently because of its potential toxicity. Chlorinated solvents are now considered hazardous to environmental safety and undesirable for use in manufacturing processes.

Despite many microencapsulation-related publications over recent years, there are comparatively few reports on the issue of solvent selection. Bodmeier and McGinity considered the influence of various solvents on the mechanism of microsphere formation, but did not consider toxicological aspects of the selected solvents (2). It has been reported recently that water-miscible solvents, such as N-methyl-2-pyrrolidone and dimethylsulfoxide, can be used for *in situ* formation of poly(*d,l*-lactide-*co*-glycolide) spheres (3). In other techniques termed as "precipitation", "desolvation", "salting-out" and "quasi-emulsion solvent diffusion", micro- and nano-particles have been produced with either acetone or ethanol as the solvent (4,5).

Substitution of methylene chloride with nonchlorinated solvents may be a challenging work to accomplish. The objective of this study was to investigate the potential of methylethyl ketone (MEK) as a solvent for the preparation of PLGA microcapsules. Key process parameters affecting the formation and the morphology of microcapsules were also identified.

MATERIALS AND METHODS

Materials

Poly(*d,l*-lactide-*co*-glycolide) with a lactide:glycolide ratio 85:15 (inherent viscosity = 0.28 dL/g in chloroform at 30°C) was obtained from Birmingham Polymers Inc. (Birmingham, AL) and noted as PLGA85:15 in the text. Sigma Chemical Co. (St. Louis, MO) was the supplier of progesterone and Polysciences Inc. (Warrington, PA), 88% hydrolyzed poly(vinyl alcohol) (Mw = 25,000).

Preparation of Microcapsules

PLGA85:15 (800 mg) was dissolved in 10 ml of MEK. The initial loading of progesterone in the polymer was changed from 0 to 10, 20, 30 or 43 w/w %. The polymer/drug solution was then poured into 40 ml of a 2% poly(vinyl alcohol) aqueous solution (W_1) in which 0 or 10 ml of MEK was predissolved. During the addition, the W_1 phase was stirred at 400 rpm with a magnetic stirrer (400 HPS/VWR Scientific) to produce an O/W_1 emulsion. After 2 min, the emulsion was transferred into 250 ml of a 0.5% poly(vinyl alcohol) aqueous solution (W_2) and stirred at the same speed for 1 hr. The microcapsules were collected by filtration and transferred into 500 ml of a 0.5% poly(vinyl alcohol) aqueous solution (W_3). They were kept at room temperature with stirring for 3 hr. The microcapsules were then collected by filtration, washed with distilled water and dried under vacuum for at least 2 days. A schematic of the microencapsulation process is illustrated in Figure 1.

Observation of O/W_1 Emulsion via a Light Microscope

The stability of the primary oil-in water (O/W_1) emulsion, without being transferred to W_2, was investigated as a function

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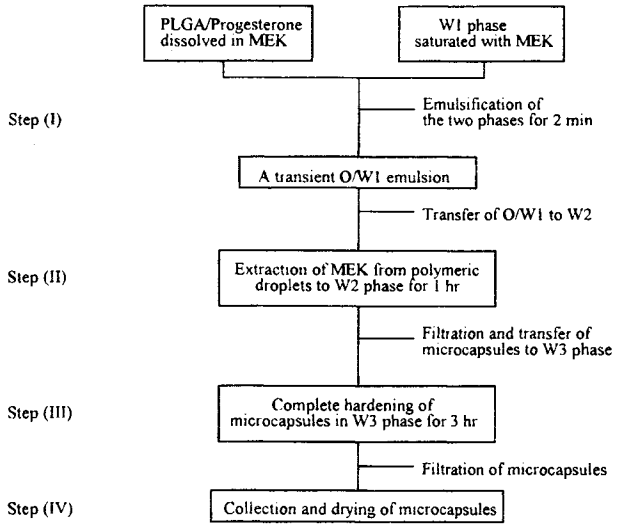

Fig. 1. A schematic illustration of the solvent extraction process consisting of 4 major steps.

of time. After a polymeric solution was emulsified in W_1, aliquots of the emulsion were collected at 2, 10 and 20 min and observed under a Zeiss MC63 light microscope.

Microcapsule Size Distribution

A Leeds & Northrup Microtrac SRA 150 particle size analyzer (Leeds & Northrup Co., FL) utilizing a forward light scattering technique was used to measure the particle size distribution of final dried microcapsules. Samples (200 mg) were suspended in 70 ml of Isopar G solution. An aliquot of the microcapsule suspension was loaded into the Microtrac SRA 150, and the flow rate of automated small volume recirculator was set at 60 ml/sec. To deagglomerate microcapsules, ultrasonic energy was applied at 40 Watts for 90 seconds.

Measurement of MEK in Aqueous Phases and Microcapsules

The level of MEK in both continuous phases and incipient microcapsules was monitored, following the dispersion of O/W_1 emulsion in W_2. At appropriate time intervals, 8 ml of microcapsule suspension was taken and centrifuged quickly. An aliquot (1 ml) of the supernatant was mixed with an acetone internal standard solution and was subjected to GC analysis. Meanwhile, wet microcapsules were collected by filtration, weighed immediately and dissolved in dimethylacetylformamide. The solution was then mixed with the acetone internal standard solution and was analyzed by GC. The residual MEK in dried microcapsules was also determined by the same procedure in which 30 mg of microcapsules was dissolved in 4 ml of dimethylacetylformamide.

A Hewlett Packard 5880A gas chromatograph with a flame ionization detector (FID) was used. The nonpolar bonded HP-1 column (dimethyl polysiloxane phase; 0.32 mm in diameter $\times$ 50 m in length) was employed as a stationary phase, whereas helium was used as a carrier gas (1 ml/min). The initial oven temperature was set at 40°C for 2 min and increased to a final temperature of 170°C at a rate of 10°C/min.

Quantitation of Progesterone

The progesterone in the continuous phases and microcapsules was determined by a modified HPLC method (6). To measure progesterone content in microcapsules, they (ca. 40 mg) were dissolved in methylene chloride (3 ml). The solution was further diluted with methanol to a final volume of 18 ml to precipitate PLGA85:15. The suspension was filtered through an organic membrane and the filtrate was analyzed by HPLC. The Zorbax Rx-C18 (4.6 mm $\times$ 15 cm) was used as an analytical column with a mobile phase consisting of methanol and water (80:20, v/v) at a flow rate of 1 ml/min. The UV detector was set at 254 nm. Encapsulation efficiency was defined as the ratio of the actual drug loading in microcapsules to the theoretical drug loading in polymer.

Scanning Electron Microscopy

Microcapsule morphology was observed by an Amray 1400 scanning electron microscope (Amray Inc., MA). The internal structure was revealed by cross-sectioning microcapsules embedded in epoxy resin. The samples were mounted on aluminum holders and sputter-coated in an argon atmosphere.

RESULTS AND DISCUSSION

When a methylene chloride solution of PLGA85:15 was dispersed into a poly(vinyl alcohol) aqueous solution under stirring, a stable o/w emulsion was formed. The ensuing extraction and evaporation of methylene chloride resulted in the formation of microspheres without complication. However, when MEK was employed for the dispersed phase solvent, the traditional solvent evaporation technique did not give satisfactory results. Not only microcapsules but also large irregular aggregates formed as soon as a polymeric MEK solution was added into a poly(vinyl alcohol) aqueous solution. This caused a great loss in the yield of microcapsules. The high polarity of MEK, relative to methylene chloride, may be partially responsible for this phenomenon. The corresponding dielectric constants of methylene chloride and MEK are 8.9 and 18.3. The solubility of the two solvents in water at 25°C is 1.32 and 26.8 wt%, respectively.

To eliminate the formation of large aggregates and ensure the formation of discrete microcapsules, we found it necessary to first form a transient oil-in-water (O/W_1) emulsion. Such an emulsion was generated by doping a poly(vinyl alcohol) aqueous solution with a sufficient amount of MEK prior to the addition of the organic solution. MEK is not miscible in all proportions with water; 1 part of MEK is soluble in about 4 parts of water. Therefore, when the volume of the continuous phase was fixed, the corresponding amount of MEK needed to saturate W_1 was calculated and added. Once the embryonic oil-in-water (O/W_1) emulsion was established, it was transferred to a 400 ml beaker containing water (W_2, 250 ml) to complete the extraction of MEK from the polymeric phase to the aqueous phase.

Bindschaedler *et al.* reported the preparation of nanoparticles by a salting-out procedure which avoided the use of chlorinated solvents (7). In accordance with this technique, an electrolyte-saturated aqueous solution was used as the continuous phase with acetone as the dispersed phase. The electrolyte-saturated aqueous solution was reported to prevent acetone from mixing with water by a salting-out effect. The later addition of

a sufficient amount of water induces acetone to diffuse into the aqueous phase, thereby forming PLA nanoparticles (8). In this study, an initial oil-in-water emulsion was established temporarily by saturating the W_1 phase with MEK.

In step (I), the emulsification time was fixed at 2 min, which was a sufficient period to break down the polymer-containing dispersed phase into small oil droplets (Figure 1). When the dispersed phase was poured into MEK-free W_1, polymeric droplets hardened so quickly that microcapsules appeared right away. This observation was confirmed by an experiment designed to investigate the stability of the primary O/W_1 emulsion as a function of time. Without the O/W_1 being transferred into W_2, samples were collected at 2, 10 and 20 min to take light microscope photographs (Figure 2A). The use of MEK-free W_1 made it possible to observe hardened microcapsules at any time interval. In this case, irregular aggregates, although not evident in the figure, were formed too. However, when the W_1 phase was pre-saturated with MEK, the results were quite different. The emulsion droplets mounted in a microscope slide coalesced together as shown in Figure 2(B). This indicates that the MEK-saturated W_1 keeps the MEK used as the dispersed phase in polymeric droplets and, without a mechanical stirring, they are prone to coalescence. If summarized, microcapsules are formed and hardened in the step (I) when MEK-free W_1 is used as a continuous phase. In contrast, when W_1 is saturated with MEK, microcapsule hardening does not take place in the step (I).

During the microencapsulation step (II), LM photographs were also taken to observe the status of microcapsule suspensions. The use of MEK-free W_1 led to formation of bigger microcapsules (Figure 3A) as well as large mass of irregular polymer aggregates (Figures 3B and C). In contrast, saturating W_1 with extra MEK before emulsification eliminated the formation of polymeric aggregates and also led to the fabrication of much smaller particles (Figure 3D). The MEK predissolved in W_1 may cause a reduction of interfacial tension between oil and W_1, which makes a contribution to the formation of smaller particles.

Figure 4 is a histogram illustrating a log-normal size distribution of microcapsules prepared using the MEK-saturated W_1 phase. The Mv (volume mean diameter) was found to be 96 μm with a standard deviation of 40 μm. The particle size distribution was observed to be affected by the types of energy devices employed for emulsification as well as the amount of MEK predissolved in W_1. The conventional techniques, using water-miscible solvents such as acetone and ethanol, are usually reported to produce nanoparticles (4).

The termination of a microencapsulation process is based mainly on an empirical approach rather than a process optimization. Detailed information on the profile of solvent removal during microencapsulation will be helpful in controlling microcapsule quality and optimizing its harvest time (9). Therefore, the solvent removal behavior of MEK from the dispersed phase to the aqueous phase was investigated during the microencapsulation step (II). After O/W_1 emulsion was dispersed in W_2, samples were prepared at 5, 20, 40 and 60 min (at that time, the W_1 phase was not saturated with MEK before emulsification). The concentrations of MEK in the aqueous phase and wet microcapsules are illustrated as a function of time in Figure 5. Provided that 100% of MEK diffuses into the aqueous phase, the concentration of MEK will be 33.33 μl per ml of the aqueous phase. As seen in Figure 5A, more than 90% of the used MEK was recovered in the aqueous phase as soon as the O/W_1 was added into the W_2. The level of MEK in the aqueous phase gradually decreased due to evaporation through air/liquid interface. Figure 5B shows the corresponding residual MEK in wet microcapsules at the same time intervals. The concentration of MEK in wet microcapsules (%, w/w) at 5 min was only 7.6($\pm$0.2)%. After 1 hr-hardening, it decreased to 4.8($\pm$0.2)%.

A similar experiment was also performed with saturating the W_1 phase prior to the preparation of O/W_1 emulsion. The equilibrium concentration of MEK in the aqueous phase, after the addition of O/W_1 to W_2, was expected to be 64.52 μl/ml. The measured value ($\pm$SD) at 5 min was 61.31($\pm$0.76) μl/ml and further decreased to 44.58($\pm$0.23) μl/ml at 60 min. Meanwhile, the residual MEK in wet microcapsules (%, w/w) at 5 and 60 min was found to be 6.05($\pm$0.1) and 3.8($\pm$0.4)%, respectively (Figures 6A & B). These results support the contention that, as soon as the O/W_1 emulsion is poured into W_2, hardening of microcapsules takes place immediately. The residual MEK in final dried microcapsules ranged from 0.9 to 1.4%.

It has been previously reported that the size of microcapsules significantly changes during various phases of solvent evaporation process (10–12). At the initial stage, the embryonic semi-solid droplets are very soft and elastic. As evaporation of volatile solvents proceeds, they become smaller due to the inward shrinkage of polymer. However, in the extraction procedure using MEK, such shrinkage was not observed during the microencapsulation step (II). The GC analysis indicates that a rapid solvent exchange precipitates polymer quickly and the hardened microcapsules are no longer subject to shrinkage.

The effect of process parameters, such as the degree of progesterone payload and MEK dissolved in W_1, on the drug encapsulation efficiency was investigated (Figure 7). As the MEK present in W_1 can facilitate drug partitioning from the dispersed to the continuous phases, a loss in drug incorporation is predicted. However, when the theoretical loading of progesterone in polymer was 10% (w/w), the encapsulation efficiency was the same regardless of presence or absence of the MEK predissolved in W_1; when it contained 0 and 10 ml of predissolved MEK, the average encapsulation efficiency ($\pm$SD)% of progesterone into microcapsules was 77.0($\pm$1.3) and 77.1($\pm$1.1)%, respectively. The encapsulation efficiency decreased with increasing theoretical loading of progesterone. When MEK-free W_1 was used and the theoretical loading of progesterone was increased to 20, 30 and 43%, the efficiency decreased to 72.1($\pm$1.7), 69.0($\pm$1.3) and 63.1($\pm$1.4)%, respectively. A similar trend was observed with the microencapsulation procedure using the W_1 phase in which 10 ml of MEK was predissolved. The corresponding efficiency was 67.2($\pm$1.5), 64($\pm$2.1), and 60.0($\pm$0.8)%, respectively.

In all experiments, most of the unentrapped progesterone was found to be present in W_1 and W_2 aqueous phases. The W_3 phase contained less than 2% of total progesterone used, indicating that the hardened polymer membrane prevents residual progesterone in microcapsules from diffusing into the continuous phase during the microencapsulation step (III). It was suggested previously that a successful drug entrapment within microspheres is related to the fast precipitation of the coating polymer from the organic phase (2). Water-miscible MEK allows the fast precipitation of PLGA polymer to fabricate

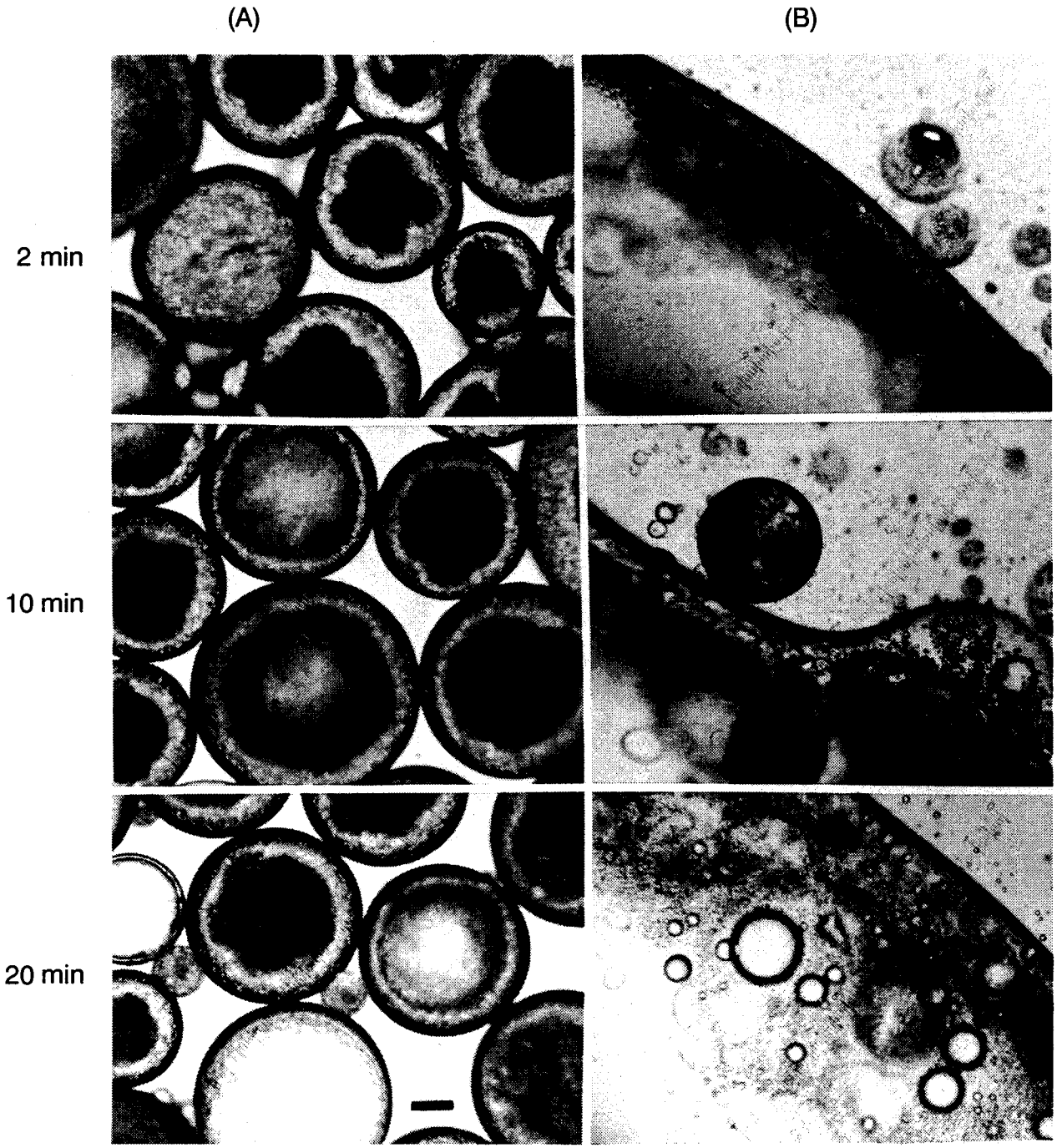

Fig. 2. LM photographs of O/W_1 emulsions sampled at 2, 10 and 20 min. Prior to emulsification with polymeric dispersed phase, 0 ml (A) or 10 ml (B) of MEK was added into W_1. The size of bar is 100 μm.

microcapsules but the flux of MEK toward the aqueous phase is also involved in determining the degree of drug incorporation efficiency. The effect of the extra MEK predissolved in W_1 on drug encapsulation is also found to depend markedly on the degree of drug loading in the polymer. In other previous studies using water-soluble organic solvents to prepare nanoparticles, incorporation yield was usually low and loading capacity was limited to a certain degree (13). For example, the use of acetone for the preparation of poly(*d,l*-lactide) nanoparticles limited the maximal loading of indomethacin below 4.0% (14).

Figure 8 shows the external and internal appearance of progesterone-loaded microcapsules. Microcapsules with a progesterone loading of 8% have a smooth external morphology (Figure 8A). Responding to the increase of the actual progesterone loading to 21%, their surface morphology becomes textured and irregularly shaped (Figure 8B). The cross-sectioned view observed by SEM illustrates that this solvent extraction tech-nique produces interesting hollow microcapsules encased by nonporous polymeric shell layers (Figures 8 C&D).

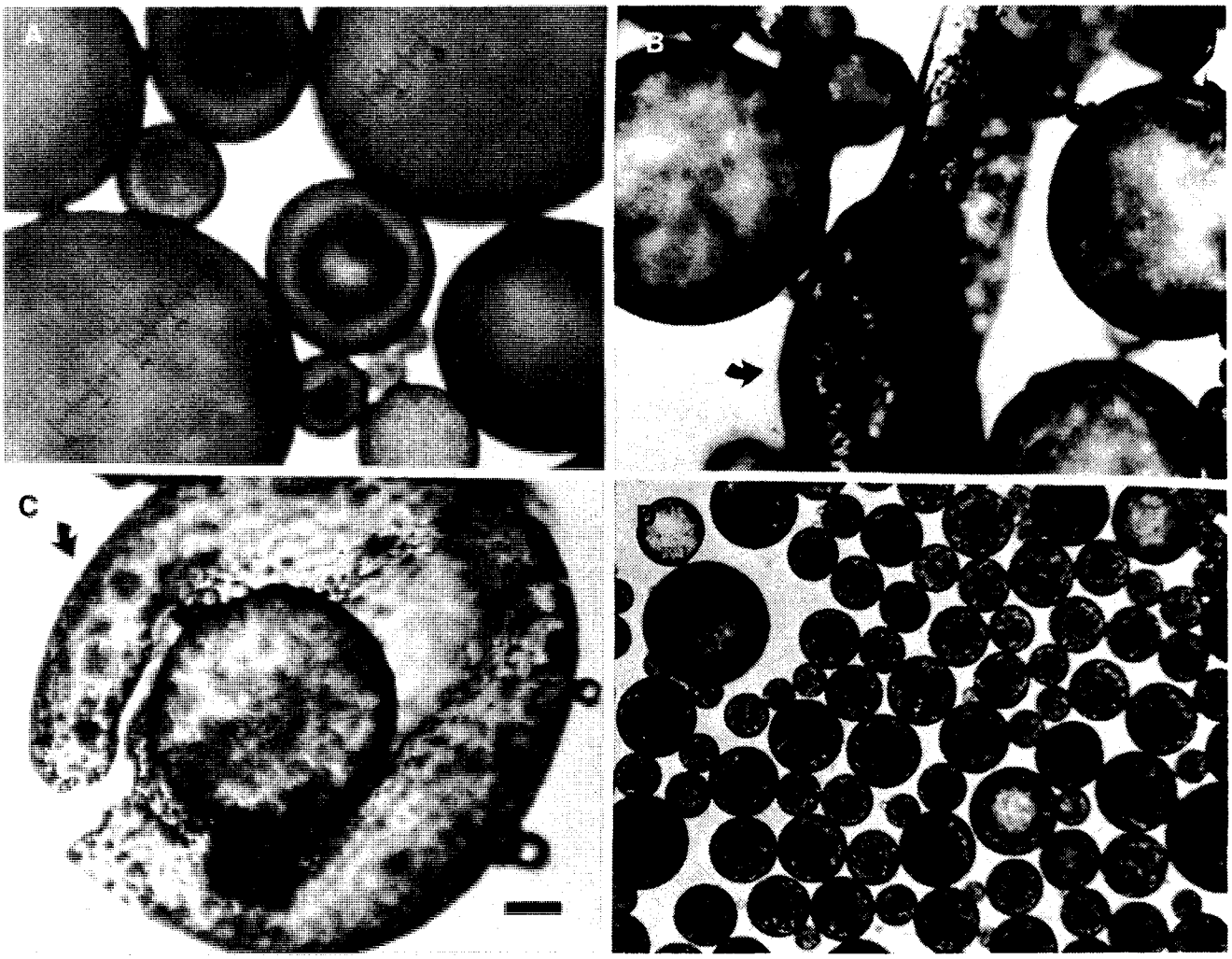

Fig. 3. LM photographs of microcapsules collected at the end of microencapsulation step (II). For preparing microcapsules, either MEK-free (A,B and C) or MEK-saturated (D) W_I was used. The bar size is 100 μm.

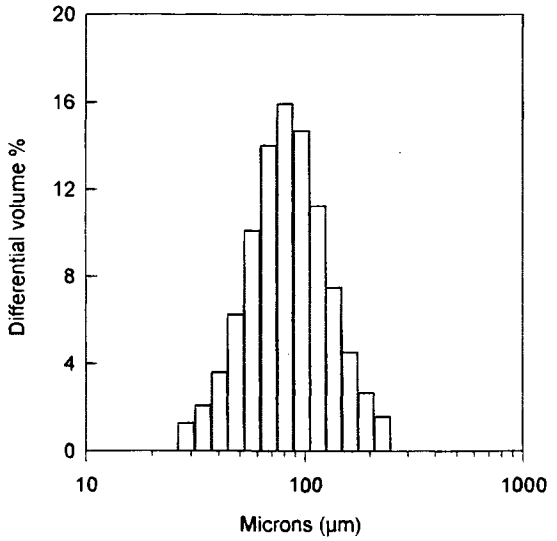

Fig. 4. Log-normal histogram plot illustrating the size distribution of microcapsules prepared using the MEK-saturated W_I.

Three major methods have been proposed to produce hollow microparticles. In the case that a water-in-oil-in-water (w/o/w) multiple emulsion technique is employed for preparing microparticles, a microcapsular structure with a hollow core is generated due to the instability of a primary oil-in-water emulsion (15). Two modified conventional water-in-oil (o/w) emulsion techniques can also fabricate hollow microspheres. The second approach relies on the use of two solvents differing in their leaching rates as well as polymer solubility (16–18). The third way is to manipulate the method and the rate of solvent leaching. A rapid solidification of the external wall of microspheres followed by expansion of residual methylene chloride by thermal means is attributed to the formation of microspheres with a hollow core (9). This hypothesis that the leaching rate of solvents determines microparticle morphology is in good agreement with our speculation on the formation of hollow microcapsules reported in this study; the high miscibility of MEK with water provided its fast leaching from polymeric droplets, which resulted in the generation of a shell structure with hollow cavity. It is also worth suggesting that the counter-diffusion of water into polymeric droplets is also involved in the process PLGA85:15 is precipitated. To back up this

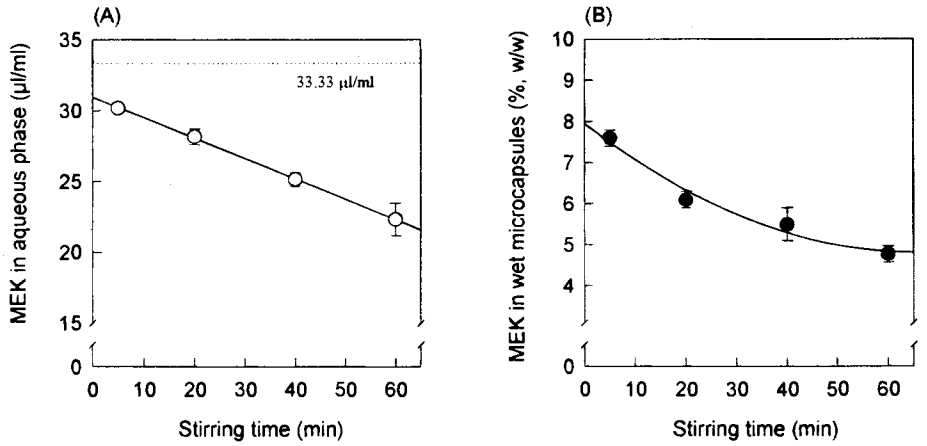

Fig. 5. (A) MEK removal profile from the dispersed to the continuous phases after the transfer of O/W_1 to W_2. The MEK-free W_1 was used. (B) The level of residual MEK in wet microcapsules during microencapsulation step (II).

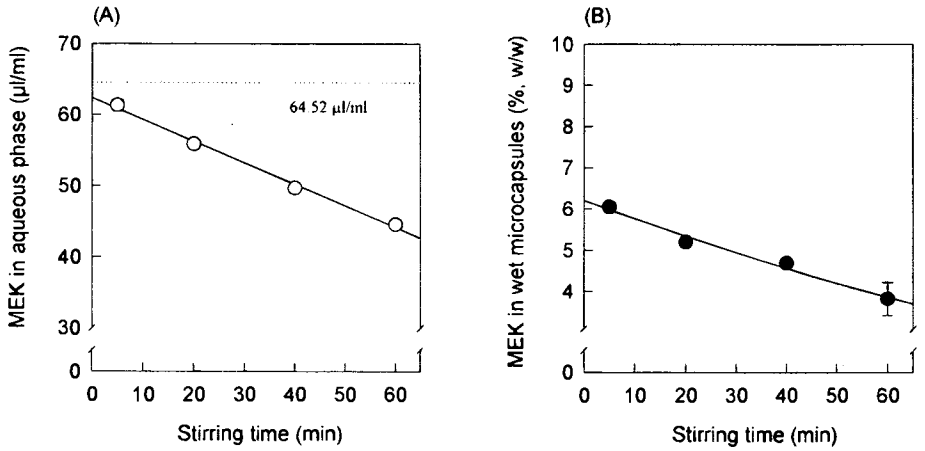

Fig. 6. (A) MEK removal profile from the dispersed to the continuous phases after the transfer of O/W_1 to W_2. Prior to emulsification with the dispersed phase, W_1 was saturated with MEK. (B) The level of residual MEK in wet microcapsules during microencapsulation step (II).

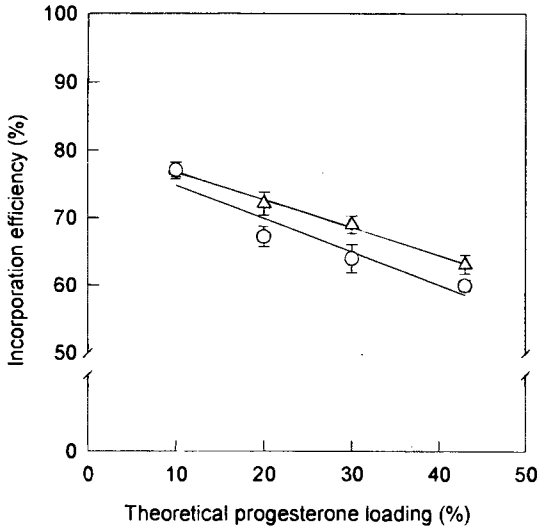

Fig. 7. Effect of the degree of progesterone loading and the amount of MEK in W_1 on drug encapsulation efficiency. Prior to emulsification with polymeric dispersed phase, either 0 ml (△) or 10 ml (○) of MEK was added into W_1.

supposition, the diffusion of water into MEK was observed at room temperature using a Side-Bi-Side™ cell (Model DC-100B, Crown Glass Co.). A nylon membrane (0.22 μm pore size) was installed between donor and receptor reservoirs filled with water and MEK. Then, the amount of water passing through the membrane as a function of time was determined by a moisture meter (Model CA-06, Mitsubishi Kasei Co.). The concentrations of water in MEK measured in 10, 20, and 40 min were 22.9, 36.8, and 48.6 mg/ml, respectively. Therefore, it can be suggested that, during our microencapsulation process, MEK leaching from and water diffusion into polymeric droplets concur to make PLGA85:15 precipitate at the interface between oil and water phases.

On the basis of the experimental results observed in this study, solvent extraction/evaporation methods using methylene chloride and methylethyl ketone are compared to each other as follows. The solubility of methylene chloride in water is 1.32 wt%, whereas water solubility in the solvent is 0.2 wt% at 25°C. As long as the volume ratio of the continuous to the dispersed phases is not sufficiently high, the continuous phase is easily saturated by a fraction of methylene chloride used.

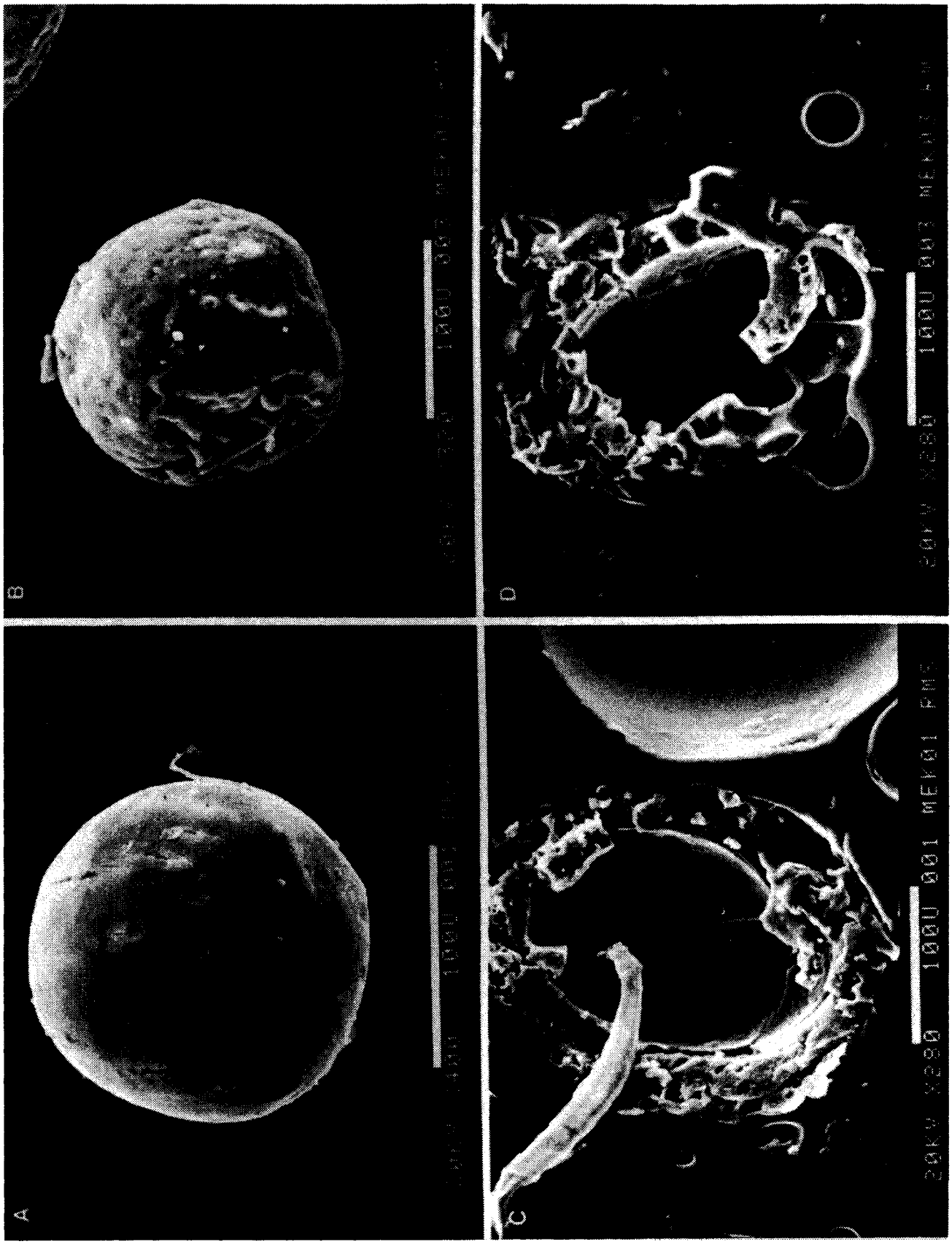

Fig. 8. SEM micrographs of either 8% (A,C) or 21% (B,D) progesterone-loaded microcapsules prepared by solvent extraction microencapsulation process. The bar size is 100 μm.

Therefore, the rate of solvent diffusion from embryonic droplets to the continuous phase is dependent upon that of solvent evaporation from the continuous phase. The consequent loss of the residual solvent in semi-solid and thermodynamically unstable droplets elicits an inward polymer shrinkage, thereby causing a decrease in their size. A series of these events usually fabricates nonporous microspheres with a homogeneous monolithic polymer matrix. By contrast, the solvent extraction technique reported in this study is quite different with regard to the rate of solvent removal from the dispersed to the continuous phases. Upon transferring a transient O/W_1 emulsion into W_2, polymeric droplets solidify instantly due to the almost complete diffusion of MEK from polymeric droplets to the continuous phase. The ensuing evaporation of MEK from the W_2 phase has little effect on microcapsule hardening, and microcapsule shrinkage does not occur as the evaporation proceeds. The fast dissipation of MEK from and the ingression of water into embryonic droplets cause a rapid polymer precipitation, thereby forming hollow microcapsules encased with a shell structure.

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