The objectives of this study were to apply a base-driven reaction to developing a new microencapsulation technique to prepare progesterone-containing poly-D,L-lactide-co-glycolide microspheres. Nonhalogenated ester solvents such as ethyl acetate and ethyl formate were used as dispersed solvents. After an oil-in-water emulsion was prepared, a sodium hydroxide solution was added to trigger base-catalyzed hydrolysis of organic solvents dissolved in the aqueous phase. Their rapid depletion provided a sink condition and drove the continual diffusion of the organic solvents residing in emulsion droplets into the aqueous phase. These events led to the solidification of emulsion droplets into microspheres over 15–30 min, without the use of a quenching liquid. The rate of the base-driven reaction observed with ethyl formate was 2.3 times faster than that attained with ethyl acetate. The drug encapsulation efficiency was ≥ 93.2%, and solvent residues in the microspheres ranged from 1.87 to 2.69%. GPC and FTIR results demonstrated that the structural integrity of the polymer and progesterone remained unchanged during the base-catalyzed microencapsulation process. This method might serve as a promising alternative for preparing nanoparticles and microspheres.

1. Introduction

Encapsulation of pharmaceuticals into poly-D,L-lactide-co-glycolide (PLGA) microspheres, which exhibit excellent biocompatibility and biodegradability, can provide their controlled release over a wide range of time. Because it is possible to tailor drug release by manipulating the rate of PLGA erosion, biodegradable microspheres draw wide interest as particulate delivery systems.1–3 PLGA microspheres are prepared by a variety of techniques including solvent evaporation/extraction, phase separation, and spray-drying.4,5 Among them, emulsion-based solvent evaporation and extraction techniques have been used frequently to prepare PLGA microspheres.6 For instance, several microsphere products currently available in the marketplace (e.g., Lupron Depot, Vivitrol, and Risperdal Consta) are prepared by the solvent extraction technique. In the typical microencapsulation process, a dispersed organic solvent is chosen to dissolve both PLGA and drug. The dispersed phase is then emulsified in an aqueous phase to make an oil-in-water (o/w) emulsion. Emulsification is carried out with various devices such as stirrer, homogenizer, sonicator, in-line dynamic/static mixer, microporous glass membrane, or multilamination micromixer.7 An excessive amount of a quenching liquid, namely, water, is then added to extract the organic solvent from the emulsion droplets to the aqueous continuous phase. Subsequent solvent removal leads to the solidification of the emulsion droplets into microspheres. Due to this feature, any organic solvent used in this process must have some water miscibility. The volume of water is typically determined by considering the aqueous solubility of the organic solvent and the amount of emulsion produced.

Despite the widespread popularity of solvent extraction technology in the field of microencapsulation, it has several limitations. It may be difficult to produce large amounts of microspheres in a robust and well-controlled manner. During the process of solvent extraction, the starting emulsion droplets tend to undergo dynamic changes in their sizes and disappproportion processes. Solvent extraction also uses relatively large amounts of water, and the resultant waste stream containing organic solvents has to be controlled via recycling or burning. Another issue related to solvent extraction is the optimization of the solvent removal rate.8 Accordingly, there have been demands to develop microencapsulation methods that are economic, safe, and scalable.

Recently, we proposed an ammonolysis-based microencapsulation process to make PLGA microspheres.9,10 Ammonolysis, which mainly occurs in the dispersed phase, converts a water-immiscible solvent in the emulsion droplets into water-soluble components. Their leaching to the aqueous continuous phase causes microsphere hardening. At that time we found that halogenated ester organic solvents such as methyl chloroacetate and ethyl chloroacetate were preferable to nonhalogenated ester organic solvents. Because of the polarization status of their carbonyl groups in ester backbones, the halogenated organic solvents showed greater reactivity toward ammonia. In contrast to these solvents, ethyl acetate, which has a less positively charged ester carbonyl group, displayed little reactivity against ammonolysis. Consequently, ethyl acetate was suggested to be unsuitable for the microencapsulation procedure.

In the present study, we sought a new tactic applicable to the process of microsphere hardening. When an o/w emulsion is prepared under normal conditions, the aqueous phase is easily saturated by a portion of the dispersed organic solvent (Figure 1A). Because the majority of the organic solvent constitutes the dispersed phase, emulsion droplets maintain a liquid status. Thus, the emulsion should be subjected to solvent evaporation, solvent extraction, or a combination of both processes to allow for microsphere hardening. In this study, a novel approach is proposed: If a chemical reaction could quickly change an organic solvent dissolved in the aqueous phase into other water-soluble

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species, it would provide a sink condition for the organic solvent. Such a system would allow continual diffusion of the organic solvent residing in the emulsion droplets into the aqueous phase (Figure 1B). This event would contribute to complete and efficient solvent removal without the use of vast amounts of a quenching liquid. It is anticipated that ester organic solvents with some water solubility would readily react with strong bases. For example, NaOH can be used for the base-catalyzed hydrolysis of ester compounds, and saponification is an example of such reactions. Ethyl acetate and ethyl formate, which have hydrolysis of ester compounds, and saponification is an example for the base-catalyzed hydrolysis was performed on ethyl formate, 100 µL of the filtrates were removed and diluted with 10 mL of butanol containing 0.2 g MgSO4. They were passed through a 0.45 µm nylon syringe filter and used as sample solutions. When ethyl acetate was used as an organic solvent, 700 µL of the filtrates were spiked with 100 µL of isopropanol as an internal standard (IS). When the base-catalyzed hydrolysis was performed on ethyl formate, 100 µL of methanol (IS) was added to 1 mL of the filtrates. All standard solutions of ethanol, ethyl formate, and ethyl acetate were prepared by dissolution in butanol followed by successive dilution with butanol. An aliquot of each sample solution was injected on a GC-2010 gas chromatograph (Shimadzu Corp.; Kyoto, Japan) equipped with a split-injector (split ratio of 15:1) and flame ionization detector. Nitrogen was used as the carrier gas at a column flow rate of 1.3 mL/min, whereas a Zebron ZB-624 column (Phenomenex; CA, U.S.A.) was used as the stationary phase. The temperature of the injector and detector was 250 °C for 3 min, and increased to 180 °C for 6.5 min and increased to 180 °C at a rate of 80 °C/min. A standard calibration curve was constructed by determining the ratios of the peak area of an analyte of interest (ethanol, ethyl acetate, and sodium formate in the presence of NaOH. All of the reaction byproducts are freely soluble in water. The base-catalyzed hydrolysis of ethyl acetate and ethyl formate was monitored by determining dynamic changes in their aqueous concentrations and a common reaction byproduct, ethanol, as a function of time. Various amounts of 10 N NaOH solution (0, 2, 5, or 6 mL) were dissolved in 40 mL of 0.5% polyvinyl alcohol solution. Poured onto the top of the aqueous phase were removed and diluted with 10 mL of butanol followed by successive dilution with butanol. An aliquot of each sample solution was injected on a GC-2010 gas chromatograph (Shimadzu Corp.; Kyoto, Japan) equipped with a split-injector (split ratio of 15:1) and flame ionization detector. Nitrogen was used as the carrier gas at a column flow rate of 1.3 mL/min, whereas a Zebron ZB-624 column (Phenomenex; CA, U.S.A.) was used as the stationary phase. The temperature of the injector and detector was 250 °C for 3 min, and increased to 180 °C at a rate of 80 °C/min. For analysis of ethanol and ethyl formate, the oven temperature was set at 40 °C for 3 min, and increased to 180 °C at a rate of 100 °C/min. A standard calibration curve was constructed by determining the ratios of the peak area of an analyte of interest (ethanol, ethyl acetate,
or ethyl formate) to that of the internal standard. The concentrations of unknown samples were calculated from the standard calibration curves.

**Kinetic Analysis of Base-Driven Hydrolysis of Ethyl Acetate.** A detailed kinetic analysis of the alkaline hydrolysis of ethyl acetate was carried out as follows. Water and ethyl acetate were separately placed overnight inside a 4 °C cold chamber. One part of ethyl acetate was mixed with 4 parts of water, and the mixture was vigorously stirred to saturate the aqueous solution with ethyl acetate. After being left to stand still, the aqueous phase was separated from the ethyl acetate. A total of 5 mL of 10 N NaOH was added to 40 mL of the ethyl acetate-saturated aqueous solution. The admixture was stirred at 550 rpm using the digital hot plate stirrer. At predetermined time intervals, aliquots (200 µL) of the reaction solution were taken out, immediately diluted 500 times with MgSO4-containing butanol, and passed through a 0.45 µm nylon syringe filter. The concentrations of ethyl acetate in the filtrates were then determined by the GC method described earlier.

**Monitoring Emulsion Status by Light Microscopy.** PLGA (0.25 g) and progesterone (0 or 250 mg) were dissolved in 4 mL of ethyl acetate or ethyl formate. Each dispersed phase was emulsified in 40 mL of 0.5% polyvinyl alcohol solution at 550 rpm using the digital hot plate stirrer to make an oil-in-water (o/w) emulsion. After 3 min, 5–6 mL of 10 N NaOH solution was added to the emulsion. The mixture was subject to continual stirring for 30 min to complete the base-catalyzed hydrolysis. After addition of 40 mL of water, stirring continued for another 5 min. The resultant suspension was collected by filtration, redispersed, and stirred in 80 mL of 0.1% polyvinyl alcohol solution for 2 h. The microspheres separated by filtration were left to dry overnight in a vacuum oven at ambient temperature. At least three batches were prepared for a given microsphere formulation. Progest- erone-free microspheres were also prepared by the same procedure.

**Determination of Progesterone Microencapsulation Efficiency.** Microsphere samples, accurately weighed, were dissolved in 4 mL of tetrahydrofuran. One part of the solution was diluted with five parts of methanol to precipitate the PLGA polymer. The suspension was filtered with a 0.45 µm nylon syringe filter to remove the polymeric precipitates. The filtrate was subjected to HPLC analysis as described below. Progesterone peaks in the chromatograms were quantified by peak area measurement using the Shimadzu data processing system. The percentage microencapsulation efficiency of progesterone was calculated as follows: 100 × (actual loading ÷ theoretical loading) = 100 × (wt of progesterone in microspheres ÷ wt of microsphere samples taken for analysis) ÷ (wt of progesterone used ÷ total wt of progesterone and PLGA used to prepare microspheres).

**HPLC Systems.** Chromatographic measurements were performed on a Shimadzu model LC-20A HPLC equipped with a binary pump (LC-20AD), a UV/vis detector (SPD-20A), and an autosampler (SIL-20A). The system was controlled by the Shimadzu LC Solution 1.12 software. A Luna C8 5 µm column (150 × 4.6 mm) was used as an analytical column. A mixture of methanol and water (80:20, by v/v) was used as the mobile phase at a flow rate of 0.8 mL/min. The elution of progesterone was detected at a wavelength of 254 nm. A standard calibration curve was constructed by integrating the peak areas of progesterone standard solutions (16.6, 76.1, 133.1, 199.7, and 266 µg/mL).

**Scanning Electron Microscopy (SEM).** Microsphere morphology was characterized by using a scanning electron microscope (model JSM-5200, Jeol Inc.; MA, U.S.A.). The microspheres were embedded in epoxy resin and cross-sectioned to reveal their internal structure. Prior to observation under the scanning electron microscope, microsphere samples were mounted onto a metal stub with double-sided adhesive tape and sputter-coated under vacuum in an argon atmosphere (model SC7620 sputter coater, VG Microtech; West Sussex, England).

**FTIR Spectrometry.** The infrared absorption spectra of progesterone, blank microspheres, and those laden with 47.1% progesterone were separately recorded by an infrared spectrophotometer (model FTS135, Bio-Rad Laboratories; PA, U.S.A.). The instrument was operated by the IR Mentor Pro 2.0 software. Samples were prepared by the potassium bromide disk method.

**Gel Permeation Chromatography (GPC).** The molecular weights of PLGA before and after fabrication into microspheres were determined by GPC. An Alliance GPCV 2000 (Waters; MA, U.S.A.) equipped with a refractive index detector was used for this experiment. Four Waters Styragel columns (HR1, HR3, HR4, and HR5E) were connected in a series, and the column temperature was maintained at 40 °C. Tetrahydrofuran was used as the mobile phase at a flow rate of 1 mL/min. Raw PLGA powders and PLGA microspheres were separately dissolved in tetrahydrofuran and injected into the GPC system. Polystyrene standards with known molecular weights (Polymer Laboratories Inc.; MA, U.S.A.) were used to construct a standard calibration curve.

**Drug Release Study.** Patterns of progesterone release from various microspheres were investigated by a water-bath method. Briefly, microspheres prepared using different microencapsulation procedures (40.8–44.1 mg) were incubated in 20 mL of 5% sodium dodecyl sulfate solution. The microsphere suspensions were shaken at 37 °C using a water bath (Reciprocal Shaking Bath, Precision Scientific, Inc.; IL, U.S.A.). At periodic intervals, 0.5 mL of the release medium was collected to determine the concentration of progesterone by the HPLC analytical methodology described above.

**Determination of Solvent Residues in Microspheres by GC.** The levels of residual ethyl acetate and ethyl formate in PLGA microspheres were determined by gas chromatographic analyses developed in this study. Microsphere samples (28.10–31.46 mg) were dissolved in 2 mL of methylene chloride and diluted to a final volume of 8–10 mL with butanol. A 0.45 µm nylon syringe filter was used to remove PLGA precipitates, and the filtrates were used as sample solutions. They were prepared in triplicate for each given microsphere formulation. Stock solutions of ethyl acetate and ethyl formate were prepared by dissolving 300 µL of each solvent in butanol to a final volume of 3 mL. These stock solutions were further diluted with butanol to make the standard solutions of ethyl acetate and ethyl formate. For instance, the concentrations of ethyl acetate standard solution ranged from 10.4 to 281.1 µg/mL, whereas those of the ethyl formate standard solution varied from 11.2 to 302 µg/mL. An aliquot of each test solution was analyzed by GC methods similar to those described earlier. In particular, during sample running, the oven temperature was set at 80 °C for 5.1 min and increased to 180 °C at a rate of 200 °C/min. The signal of each solvent from the detector was measured as the peak area by an automatic integration method.

3. Results

To investigate the base-catalyzed hydrolysis of ethyl acetate, the system consisting of 4 mL of ethyl acetate and 40 mL of a 0.5% polyvinyl aqueous solution was stirred gently so that the organic solvent overlaid the aqueous phase. Therefore, at the beginning of the experiment, the system remained as two phases. As stirring proceeded in the absence of NaOH, the concentration
of ethyl acetate dissolved in the aqueous phase increased as a function of stirring time and eventually remained almost the same (Figure 2A). This meant that a portion of ethyl acetate diffused into and saturated the aqueous phase. Under this experimental condition, ethanol was not detected in the aqueous phase, demonstrating the stability of ethyl acetate in water.

When 2 mL of 10 N NaOH was added to the ethyl acetate/water system, ethyl acetate was hardly detected after stirring for 3 and 15 min (Figure 2B). During this period, ethanol began to appear in the aqueous phase. When stirring proceeded further, ethyl acetate was found in the aqueous phase and its concentration increased to a plateau with stirring for 45 min. Ethanol began to appear in the aqueous phase right after NaOH addition. After stirring for 25 min, its concentration remained almost constant. These results substantiated that 2 mL of 10 N NaOH was not enough to hydrolyze 4 mL of ethyl acetate. With 5 mL of 10 N NaOH, however, all the ethyl acetate used in this experiment was completely hydrolyzed: ethyl acetate underwent hydrolysis as soon as it diffused in the aqueous phase. As a result, very little ethyl acetate was found in the aqueous phase, whereas the amount of ethanol in water increased as stirring went on (Figure 2C). Figure 3 illustrates the dynamic changes in the aqueous concentration of ethyl acetate and ethanol under the above experimental conditions.

Figure 2. GC chromatograms reveal dynamic changes in the aqueous concentrations of ethyl acetate and its hydrolysis product (ethanol) as a function of time. Added to the ethyl acetate/water system are (A) 0, (B) 2, and (C) 5 mL of 10 N NaOH. Isopropanol is used as an internal standard for their quantitation.
monitor changes in its aqueous concentration as a function of time. Therefore, ethyl acetate having a reduced degree of reactivity was chosen for investigating reaction kinetics. To further delay the progress of the base-driven hydrolysis, a reaction temperature was set at 4 °C instead of the room temperature. Figure 6A shows that alkaline hydrolysis of ethyl acetate happens within a short time. The plot of time-aqueous ethyl acetate concentration was fitted well by the second order model \(r^2 = 0.99\). The slope shown in Figure 6B corresponds to the second order reaction rate constant \(k = 0.99155\) \text{conc}^{-1} \cdot \text{sec}^{-1}\) for the alkaline hydrolysis of ethyl acetate. The reciprocal of the intercept presents a theoretical initial concentration of ethyl acetate, and the calculated value of 0.119 g/mL is consistent with the real value of 0.111 g/mL. This validates our postulated reaction order. The half-life, the time required for 50% hydrolysis, was calculated to be 9.1 s. All these results substantiate that ethyl acetate rapidly reacts with NaOH to degrade into ethanol and sodium acetate.

The effect of NaOH treatment on the status of o/w emulsions was evaluated by light microscopy. As expected, when an ethyl acetate/water emulsion was stirred for 15 min in the absence of NaOH, solidification of the emulsion droplets did not occur. When placed onto a glass slide for observation under the light microscope, the emulsion droplets coalesced immediately to form polymeric films (Figure 7). This meant that only a portion of ethyl acetate was enough to saturate the aqueous phase and its major proportion still remained in the dispersed phase. Subsequently, the droplets fused together without mechanical stirring. A similar propensity was observed when ethyl acetate was replaced by ethyl formate (Figure 8). In contrast, treating the emulsions with 10 N NaOH led to the quick transformation of the emulsion droplets into microspheres, as shown in Figures 7 and 8. The hydrolysis of ethyl acetate and ethyl formate dissolved in the aqueous phase led to the establishment of sink conditions for the organic solvents. This helped complete the process of solvent removal for microsphere hardening.

When microspheres were prepared for the first time by the microencapsulation procedure described in this study, they were free-flowing powders. When progesterone was used for microencapsulation, excellent encapsulation efficiencies were attained (Table 2). Depending upon the microsphere formulations, the encapsulation efficiencies (mean ± SD) ranged from 93.2 ± 1.4 to 97.9 ± 2.2%. All the microspheres showed spherical geometry, and their surfaces were smooth (Figure 9). Interestingly, when 0.25 g of progesterone was used, some degree of phase separation between progesterone and the PLGA polymer took place inside the microspheres. In addition, our GC analysis demonstrated that the residual ethyl acetate remaining in microspheres prepared using ethyl acetate/0.25 g PLGA/0.25 g progesterone was 2.69 ± 0.13%. When ethyl formate was used as a dispersed solvent, the residue in the microspheres was decreased to 1.87 ± 0.06%. These results indicate that our base-catalyzed hydrolysis effectively removed the solvents over a short period of time.

The compatibility of drug substance-excipient is an important issue in developing a drug product. Therefore, the effect of an excipient on the structural integrity of a drug substance should be evaluated with care. The use of a strong base such as NaOH could trigger drug/polymer degradation and PLGA–drug interactions. To explore this potential issue, the molecular weights of PLGA were evaluated before and after microencapsulation (Figure 10). The molecular weight of the raw PLGA polymer was 88000 g/mol. When fabricated into microspheres by the ethyl acetate-based microencapsulation process, its molecular weight ranged from 87000 to 88000 g/mol. The molecular
weight of PLGA also remained unchanged after microencapsulation after ethyl acetate was substituted with ethyl formate as a dispersed solvent.

IR spectra of progesterone, PLGA, and progesterone-containing microspheres were also determined (Figure 11). PLGA belongs to polyester groups, and the bands around 1760–1790 cm$^{-1}$ are attributed to C=O vibration of its ester bonds. Progesterone contains two carbonyl groups: a vinyl keto group in C3 and an aliphatic methyl keto group in C20. The bands at 1663 and 1699 are attributed to the C=O stretching of C3 and C20, respectively. The bands at 2851 and 1615 cm$^{-1}$ correspond to the stretching of the methylene group (CH$_2$) and C3=C4, respectively. When PLGA microspheres containing 47.1% progesterone were subject to IR spectroscopy, the characteristic bands of progesterone at 1615, 1663, 1699, and 2851 cm$^{-1}$ remained the same. Furthermore, the characteristic bands of the ester carbonyl group of PLGA were observed when the polymer was made into progesterone-containing microspheres. Namely, our base-catalyzed microencapsulation process neither altered the characteristic bands of progesterone and PLGA nor generated new IR bands. These results indicate that no physicochemical reactions occur between prednisolone and PLGA during the base-catalyzed microencapsulation process. These data are in good agreement with the GPC data shown in Figure 10.

Figure 4. GC chromatograms reveal dynamic changes in the aqueous concentrations of ethyl formate and its hydrolysis product (ethanol) as a function of time. Added to the ethyl acetate:water system are (A) 0, (B) 2, and (C) 6 mL of 10 N NaOH.
Our release medium (5% sodium dodecyl sulfate solution) was selected to provide a sink condition during a drug release study (progesterone solubility in the medium = 9.7 mg/mL). All microspheres, when prepared using 60 mg of progesterone and 0.25 g of PLGA, released little amounts of progesterone. Such profiles were unaffected by the type of a dispersed solvent used in our microencapsulation technique (Figure 12). As a control, a drug release study was also carried out with the use of the microspheres prepared following the methylene chloride-based solvent evaporation process reported elsewhere. On the basis of their release profiles, it is supposed that the effect of microencapsulation technique on progesterone release is insignificant: both microencapsulation processes entrap progesterone well into the microsphere matrices and noticeable PLGA degradation does not take place over a 4 day period. When 250 mg progesterone was used for microencapsulation, release profiles of all different microspheres were featured with burst release followed by sustained release. Again, their release profiles were somewhat similar to one another, irrespective of dispersed solvent type and microencapsulation technique. A higher progesterone payload seems to make drug crystals present everywhere: in the inside of the microspheres and on their surface. External progesterone crystals might result in the burst effect. Gradual solubilization and leaching of drug crystals would generate interconnected pores and microchannels that permit the sustained release. In this case, drug release is not controlled by PLGA erosion.

4. Discussion

Ethyl acetate and ethyl formate were used as dispersed solvents in this study. As far as volatility is concerned, their volatility is far less than that of methylene chloride that is a commonly used dispersed solvent (Table 1). Therefore, a quenching liquid (e.g., water) is added to extract the organic solvents in emulsion droplets and to transform them into microspheres. In general, the preferred amount of water is at least 3 to 10 times the minimum volume of water necessary to dissolve the entire volume of a dispersed solvent. For instance, in the emulsion-based manufacturing process of PLGA microspheres, 250 mL of water was used to extract 1 mL of ethyl acetate in emulsion droplets. Consequently, an undesirable feature of solvent extraction is the generation of a vast amount of wastewater in which organic solvents are dissolved. A number of attempts have been made to develop a microencapsulation process that could reduce the amount of a quenching liquid. Relevant examples include the combined practice of solvent evaporation/extraction and intensive flushing of the liquid surface of an emulsion by gas. Elevated temperature or reduced pressures are often applied to facilitate solvent removal. Membrane separation processes are applied to separate organic solvents and water because they have very different polarities and exhibit distinct membrane permeation/adsorption properties. Sometimes, a semipolar organic solvent such as methanol is added to the aqueous phase to accelerate solvent removal from emulsion droplets. A multicom-
ponent mass transfer model is suggested to explain the much stronger extraction of an organic solvent from emulsion droplets into the water–methanol mixture.19

In contrast to these physical methods, we have proposed a chemical method for complete solvent extraction without use of either an excessive amount of water or complicated equipment. With this new microencapsulation technology, only 5–6 mL of 10 N NaOH was used to remove 4 mL of organic solvents. Recently, an ammonolysis-based microencapsulation process was proposed.9,10 During that microencapsulation process, ammonia is added to an o/w emulsion to change ester organic solvents into water-soluble components. The major

Figure 7. LM photographs of o/w emulsions sampled after stirring for 15 min. NaOH treatment leads to quick solidification of emulsion droplets into microspheres. When the emulsions are not subject to the base-driven reaction, emulsion droplets coalesce to form films, as indicated by arrows. Ethyl acetate is used as a dispersed solvent, and the dispersed phase contains (A) 0 or (B) 250 mg of progesterone.

Figure 8. LM photographs of o/w emulsions sampled after stirring for 15 min. The base-driven reaction results in rapid microsphere hardening. Emulsion droplets not subject to NaOH treatment fuse together when mounted on a glass slide. Ethyl formate is used as a dispersed solvent, and the dispersed phase contains (A) 0 and (B) 250 mg of progesterone.
reaction site of ammonolysis is the dispersed phase of the o/w emulsion, not the continuous phase. In an aqueous solution, only a tiny portion of ammonia becomes ammonium ions because the former has a base dissociation constant of $1.8 \times 10^{-5}$. For instance, 1 M ammonia solution has less than 1% of ammonium ions ($\text{NH}_4^+$). Ammonium ions do not partition well into a dispersed phase, but ammonia shows good solubility in various organic solvents. Ammonia partitioning into emulsion droplets rapidly reacts with halogenated ester solvents such as ethyl fluoroacetate. Ammonolysis products, which exhibit substantial water miscibility/solubility, leach well into the aqueous continuous phase, thereby hardening microspheres.

In contrast, the mechanism of microsphere hardening reported in the present study differs from that of the ammonolysis-based process. In fact, it is the aqueous phase in which the base-catalyzed hydrolysis takes place. NaOH is dissolved in water to yield sodium cations and hydroxide anions that are insoluble in nonpolar organic solvents. Hydroxide ions participate in the base-catalyzed hydrolysis of organic solvents dissolved in the aqueous phase. The depletion of aqueous organic solvents establishes sink conditions, thereby driving further leaching of the organic solvents from emulsion droplets to the aqueous phase. This process eventually accomplishes solvent removal, thereby causing microsphere hardening. In the ammonolysis-based microencapsulation process, ethyl acetate was not an ideal dispersed solvent due to its poor reactivity toward ammonolysis. In contrast, ethyl acetate, which has some water miscibility, served as an excellent dispersed solvent in the base-driven microencapsulation process reported in this study.

The present microencapsulation technique employs NaOH, which can potentially react with PLGA and progesterone. In fact, NaOH has often been used to determine the microencapsulation efficiencies of bioactive materials due to its ability to digest any PLGA polymers. It has also been reported that a drug with an amine group catalyzed random chain cleavage of PLGA’s ester bond. However, GPC and IR results shown in Figures 10 and 11 indicate that our microencapsulation procedure does not affect the structural integrity of PLGA and progesterone.

### Table 2. Encapsulation Efficiency of Progesterone Obtained with Various Microsphere Formulations

<table>
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<th>progesterone (mg)</th>
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<th>batch 2</th>
<th>batch 3</th>
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</tr>
<tr>
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<td>250</td>
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<td>92.0</td>
<td>97.7</td>
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</table>

**Figure 9.** SEM micrographs showing the external and internal morphology of various PLGA microspheres. The payloads of progesterone are (a,b) 18.2, (c,d) 47.1, (e,f) 18.0, and (g,h) 48.9%. Ethyl acetate is used as a dispersed solvent to prepare (a–d) microspheres, whereas ethyl formate is used to prepare (e–h) microspheres. The bar represents 50 µm.

**Figure 10.** GPC chromatograms of PLGA before and after microencapsulation: (a) PLGA raw powders; (b) blank microspheres prepared using ethyl acetate; (c) 47.1% progesterone-loaded microspheres prepared using ethyl acetate; (d) blank microspheres prepared using ethyl formate; and (e) 48.9% progesterone-loaded microspheres prepared using ethyl formate.

**Figure 11.** Infrared spectra of (a) progesterone, (b) progesterone-free microspheres, and (c) 47.1% progesterone-loaded microspheres.
coalescence, semisolid adhesion, or engulfment often arise from hardening, complicated setbacks including Ostwald ripening, wastewater, cost constraints, lengthy processing time, and limitations associated with the volume of quenching liquid. Following the alkaline hydrolysis of ethyl formate, the reaction center of the former might be more strongly shielded. Ethyl acetate also has a bulkier alkyl group than that of ethyl formate. Ethyl acetate has a greater reactivity toward NaOH. Even though the two organic solvents used in this study have a greater reactivity than ethyl acetate. Ethyl formate exhibits a greater reactivity than ethyl acetate.

Even though the two organic solvents used in this study showed great reactivity toward NaOH, ethyl formate exhibited a faster reaction rate than ethyl acetate. Figure 13 shows the reaction rates observed with the two organic solvents over the initial period of base-driven hydrolysis. Judging from the rates of ethanol formation, the reaction rate attained with ethyl formate was 2.3 times faster than that of ethyl acetate. Such a difference in their reaction rates can be explained by the electron-donating inductive ability of the alkyl groups: the higher the electron density at the ester carbonyl carbon, the more hindered is the nucleophilic approach to the reaction center. Ethyl acetate also has a bulkier alkyl group than that of ethyl formate, and the reaction center of the former might be more strongly shielded.

**5. Conclusions**

Typical solvent extraction/evaporation techniques have some limitations associated with the volume of quenching liquid, wastewater, cost constraints, lengthy processing time, and solvent toxicity (methylene chloride). During microsphere hardening, complicated setbacks including Ostwald ripening, coalescence, semisolid adhesion, or engulfment often arise from the emulsion instability. In the present study, a chemical strategy is proposed for efficient and complete solvent removal, and its feasibility has been validated. A number of benefits of this new microencapsulation technique stem from the facts that it uses nonhalogenated ICH class 3 organic solvents and does not require a vast amount of quenching liquid or sophisticated equipment. The microencapsulation technique, which is simple and easily scalable, is a promising alternative for preparing microspheres and/or nanoparticles.

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**References and Notes**


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