Ammonolysis-Induced Solvent Removal: A Facile Approach for Solidifying Emulsion Droplets into PLGA Microspheres

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An ammonolysis-based microencapsulation technique useful for the preparation of biodegradable microspheres was described in this study. A dispersed phase consisting of poly-D,L-lactide-*co*-glycolide, progesterone, and methyl chloroacetate was emulsified in an aqueous phase. Upon addition of ammonia solution, the emulsion droplets were quickly transformed into poly-D,L-lactide-*co*-glycolide microspheres laden with progesterone. Rapid solvent removal was accompanied by ammonolysis. The chemical reaction converted water-immiscible methyl chloroacetate to water-miscible chloroacetamide and methanol. Chloroacetamide formation was proved by ^{1}H NMR and ESI-MS studies. Thermogravimetric analysis showed that the microspheres contained only small amounts of residual methyl chloroacetate. Incorporation efficiencies of progesterone ranged from 64.3 ± 1.1 to 72.8 ± 0.3%, depending upon microsphere formulations. X-ray powder diffractometry analysis substantiated that no polymorphic transition of progesterone occurred during microencapsulation. To evaluate the feasibility of this new method against the commonly used microencapsulation method, microspheres were also prepared by a typical dichloromethane-based solvent evaporation process. The important attributes of microspheres prepared from both methods were characterized for comparison. The new ammonolysis-based microencapsulation process showed interesting features distinct from those of the solvent evaporation process. The microencapsulation process reported in this study might be applicable in loading pharmaceuticals into various polymeric microspheres.

Introduction

Poly-D,L-lactide-*co*-glycolide (PLGA) polymers are often formulated into biodegradable microspheres to deliver pharmaceuticals over a wide range of periods. Recently, PLGA microspheres have also found applications in the delivery of cells, genes, vaccines, and scaffolds for cell growth.[1–4] So far, a number of microencapsulation techniques have been developed to load various pharmaceuticals into PLGA microspheres.[5–8] Among the various microencapsulation techniques, a solvent evaporation and/or extraction process has been frequently applied to prepare PLGA microspheres. For instance, Lupron Depot delivering leuprolide acetate and Risperdal Consta releasing risperidone are manufactured by the modified solvent evaporation/extraction processes. Microsphere preparation by solvent evaporation/extraction involves the three major steps of emulsification, solvent removal, and microsphere harvesting. The selection of an appropriate dispersed solvent usually depends upon the mechanism of solvent removal. When solvent removal heavily relies on evaporation, volatile methylene chloride (dichloromethane) is a good disperse solvent. When solvent removal is done by extraction, however, ethyl acetate having considerable water miscibility is preferable.[9] One essential requirement for solvent evaporation/extraction is that a dispersed solvent should be more volatile than water, which serves as a common continuous phase. Numerous reports have focused on both controlling the rate of solvent removal and manipulating microsphere characteristics in the solvent extraction/evaporation processes.[10–12]

We have recently proposed a strategy for an efficient microencapsulation technique using methyl dichloroacetate as a dispersed solvent.[13] Application of this new concept to the processing of microsphere solidification led to the fast formation of discrete and free-flowing PLGA microspheres. However, these microspheres showed propensity for aggregation upon long-term storage. There has been a need to improve the practical usefulness of this method. Hence, in the present study, we have reported the newly optimized process that uses methyl chloroacetate as dispersed solvent. In this method, progesterone was used as a model drug to be encapsulated into PLGA microspheres. The dispersed solvent used in this process readily dissolves hydrophobic materials and also easily undergoes ammonolysis to produce water-soluble components. Investigated in this study were the effects of PLGA concentration and the degree of progesterone payload upon microsphere characteristics such as encapsulation efficiency, microsphere morphology, thermal behavior, and physical status. PLGA microspheres were also prepared by a typical dichloromethane-based solvent evaporation process. Major characteristics of the microspheres prepared by ammonolysis-induced solvent removal and solvent evaporation were compared.

Materials and Methods

Materials. PLGA with a lactide:glycolide ratio of 75:25 (inherent viscosity, 0.70 dL/g in $CHCl_3$ at 30 °C) were obtained from Durect Corp. (Alabama). Polyvinyl alcohol (PVA; 88% hydrolyzed, MW = 25000) was purchased from Polysciences, Inc. (Pennsylvania). Ammonia solution (28%), methyl chloroacetate (MCA), and methyl dichloroacetate (MDA) were from Junsei Chemical Co., Ltd. (Tokyo, Japan). Progesterone and phenolphthalein were from Sigma-Aldrich (Missouri). Methanol and water of HPLC grade were purchased from Mallinckrodt Baker, Inc. (New Jersey). Dichloromethane (DCM) and tetrahydrofuran were obtained from Burdick & Jackson (Michigan).

Ammonolysis Reaction. Methyl chloroacetate (4 mL) was emulsified in 40 mL of water using a magnetic hot plate stirrer (model 400

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HPS/VWR Scientific). Because methyl chloroacetate was sparingly soluble in water (water solubility = 46 mg/mL at 25 °C), an oil-in-water (O/W) emulsion was easily formed. After 3 min stirring, 4 mL of ammonia solution was added to the emulsion. The methyl chloroacetate droplets disappeared completely in 10 min, and the emulsion became a clear solution. Presumably, through ammonolysis, water-immiscible methyl chloroacetate was converted into water-miscible chloroacetamide and methanol. To verify this supposition, chloroacetamide was precipitated from the solution via salting-out. The precipitated chloroacetamide was then extracted with ethyl acetate (30 mL × 3 times), and the ethyl acetate solution was dried over anhydrous $MgSO_4$. The suspension was filtered through a pad of Celite, and the filtrate was evaporated under reduced pressure. The resultant white powder was analyzed by proton nuclear magnetic resonance (^{1}H NMR) spectroscopy and mass spectrometry (MS) experiments as described below.

Monitoring of Ammonolysis Reaction. To 44 mL of a solution of water and the ammonia solution (10:1, v/v) was added an aliquot (100 μL) of methanolic phenolphthalein solution (2 mg/mL). Methyl chloroacetate (4 mL) was mixed with the above solution and the mixture was gently agitated. At time intervals, 1 mL aliquots were removed, and the absorbance at 550 nm was recorded by a U-3000 UV/vis spectrophotometer (Shimadzu, Kyoto, Japan). As a reference, the absorbance of 1 mL of the aqueous ammonia/phenolphthalein solution without methyl chloroacetate was monitored as a function of time.

NMR and MS. The white powder obtained above was dissolved in $CDCl_3$, and ^{1}H NMR spectra were recorded using a Unity Inova 400 MHz FT-NMR spectrophotometer (Varian, CA). ^{1}H chemical shifts were referenced to the internal standard tetramethylsilane. In a separate experiment, the samples were introduced into the electrospray ionization (ESI) source of a Q-tof 2 mass spectrometer (Micromass, Massachusetts) and their mass spectra were scanned over the *m*/*z* range of 0 to 340.

Preparation of PLGA Microspheres by an Ammonolysis-Based Process. PLGA (0.25, 0.30, 0.35, or 0.40 g) and progesterone (60, 100, 160, 200, or 250 mg) were dissolved in 4 mL of methyl chloroacetate. The dispersed phase was emulsified in 40 mL of a 0.5% aqueous PVA solution. Emulsification was performed by agitation at 550 rpm by using a magnetic stir plate. After 3 min stirring, the ammonia solution was added at once to the emulsion and stirred for 15 min. After addition of water (40 mL), the microsphere suspension was stirred for an additional 5 min. The resultant suspension was first passed through a 425 μm sieve and isolated by filtration. The microspheres were redispersed and stirred in 80 mL of a 0.1% aqueous PVA solution for 2 h. They were then collected by filtration and dried overnight in a vacuum oven. To analyze batch-to-batch reproducibility, more than three batches were prepared for a given microsphere formulation. Throughout the text, PLGA microspheres prepared by this encapsulation process were abbreviated as MCA microspheres.

Preparation of PLGA Microspheres by a Solvent Evaporation Method. PLGA (0.25 g) and progesterone (60, 100, 160, 200, or 250 mg) were dissolved in 4 mL of dichloromethane. The dispersed phase was emulsified in 40 mL of a 0.5% aqueous PVA solution. Emulsification was performed by agitation at 550 rpm by using a magnetic stir plate (model 400 HPS/VWR Scientific). After 5 h stirring, the microsphere suspension was passed through a 425 μm sieve, collected by filtration, and dried overnight in a vacuum oven. At least three batches were prepared for a given microsphere formulation. PLGA microspheres prepared by this dichloromethane-based microencapsulation technique were abbreviated as DCM microspheres in text.

Microsphere Size Measurements. The median size measurements of various microspheres were carried out by the Mastersizer 2000 (Malvern Instruments Ltd., Worcestershire, UK). The fully automated instrument had the ability to measure particles in the range of 0.02−2000 μm. A volume weighted diameter was reported in text.

Determination of Progesterone Encapsulation Efficiency. Microsphere samples (15.2−25.8 mg) were completely dissolved in 4 mL of tetrahydrofuran. The solution was further diluted with methanol to a final volume of 24 mL and filtered with a nylon syringe filter (0.45 μm pore size) to remove PLGA precipitates. Aliquots of the filtrate (20 μL) were applied to a Waters Symmetry C_{18} 5 μm HPLC column (150 × 4.6 mm), which was eluted isocratically with a methanol and water (8:2, v/v) mobile phase at a flow rate of 0.8 mL/min. The column effluent was monitored at 254 nm. A calibration curve was prepared using a series of progesterone standards of known concentrations. The percentage encapsulation efficiency of progesterone was calculated as follows: % progesterone encapsulated = 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).

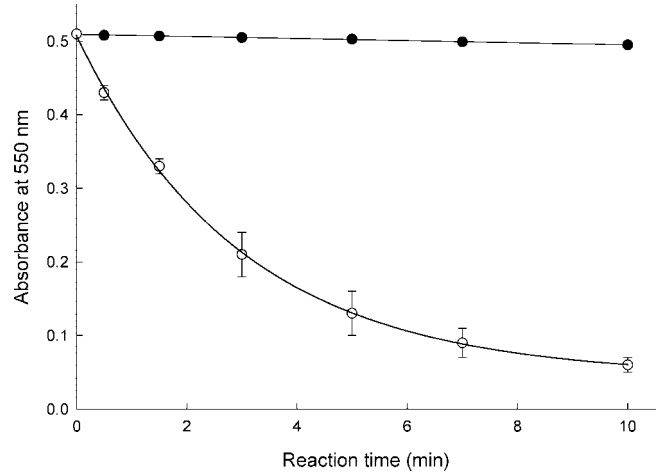

Figure 1. Changes in 550 nm absorbance values of the phenolphthalein-containing ammonia solution in the (●) absence and (○) presence of methyl chloroacetate.

Thermogravimetric Analysis (TGA). The thermogravimetric analyses of DCM and MCA microsphere samples were performed with a TGA 2050 (TA Instruments, Delaware). They were housed inside a platinum pan, and temperature was increased at a rate of 10 °C/min under a dry nitrogen purge (50 mL/min). Weight changes as a function of temperature were automatically recorded.

Scanning Electron Microscopy (SEM). Microsphere samples were placed on a metal stub and sputter-coated with gold under vacuum in an argon atmosphere (model SC7620 sputter coater/VG Microtech, West Sussex, UK). The JSM-5200 microscope (Jeol Inc. Massachusetts) was utilized to observe the morphology of PLGA microspheres. To observe their internal structure, microsphere samples were embedded in an epoxy resin and cross-sectioned by a blade.

X-ray Powder Diffractometry (XRD). Progesterone, blank DCM/MCA microspheres, or DCM/MCA microspheres containing different progesterone payloads were placed into an aluminum sample holder and exposed to Cu Kα radiation (40 kV × 30 mA) in a wide-angle X-ray powder diffractometer (model SWXD Series/Rigaku International Corp., Tokyo, Japan).

Results

To demonstrate the rapid reaction of ammonia with methyl chloroacetate, we performed a presumptive test based on the color change of the indicator dye, phenolphthalein. In basic solutions, phenolphthalein is pink (λ_{max} = 550 nm) but changes to colorless at pH less than 8. In the absence of methyl chloroacetate, no significant changes in absorbance as a function of time occurred to the basic solution (Figure 1). By sharp contrast, upon emulsification with methyl chloroacetate, the absorbance of the aqueous phase declined exponentially. Also, the methyl chloroacetate droplets disappeared within 10 min

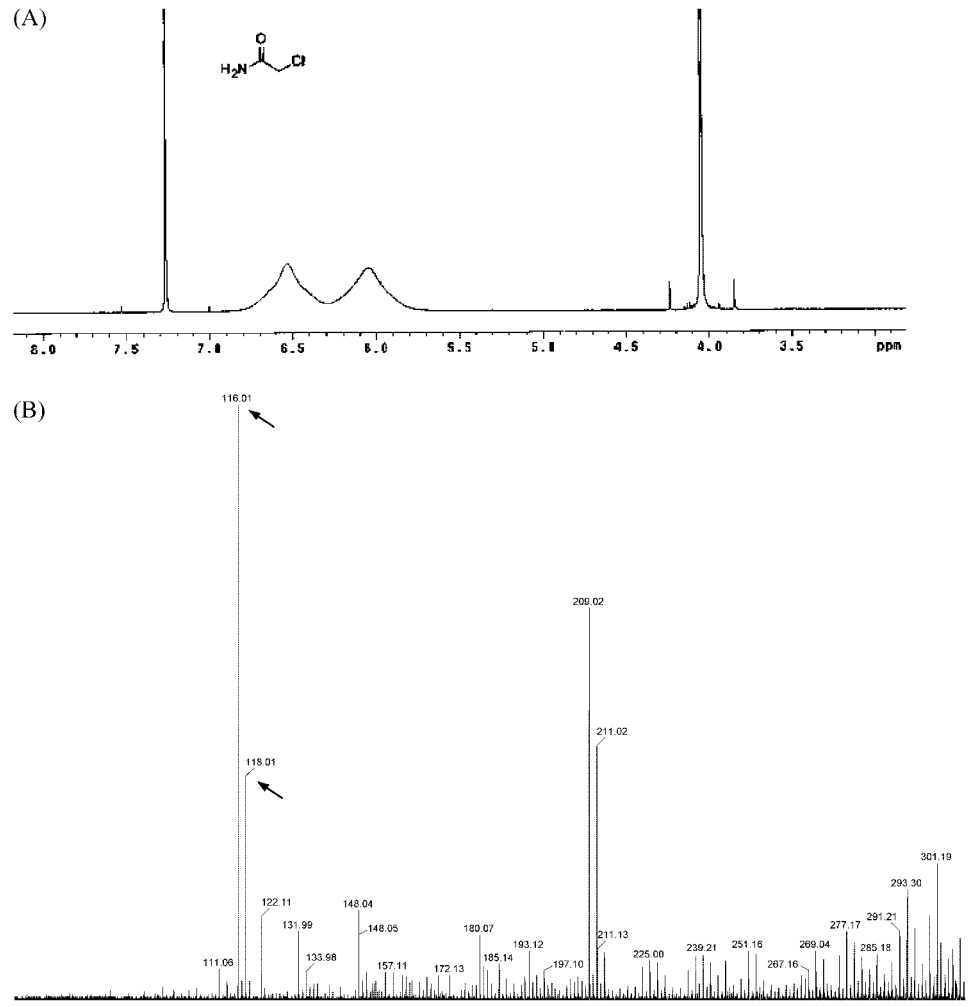

Figure 2. Identification of chloroacetamide (C_2H_4ClNO) by ^1H NMR spectrophotometer and electrospray ionization mass spectrometer. (A) ^1H NMR: $\delta_H = 6.55$ (br s, 1H), 6.04 (br s, 1H), 4.05 (2H, s). (B) ESI-MS: $m/z = 116.01$ [M + Na^+], 118.01 [M + 2 + Na^+].

and the emulsion droplets turned into a colorless, clear solution. This result suggested that methyl chloroacetate completely reacted with ammonia.

The formation of chloroacetamide was confirmed by ^1H NMR and ESI-MS analyses. The ^1H NMR spectrum displays the signals for two amide protons at $\delta_H = 6.55$ and 6.04 (each 1H, br s) (Figure 2A). The signal representing two protons attached to the carbon having one chlorine appeared at $\delta_H = 4.05$ (2H, s). In the ESI-MS spectrum, a peak of [M + Na^+] was detected at $m/z = 116.01$ (Figure 2B). A quite distinctive [M + 2 + Na^+] peak was also noticed at $m/z = 118.01$. The presence and intensity of the two peaks in a 3:1 ratio reflects the natural abundance of ^{37}Cl, which is about 32.5% that of ^{35}Cl. Taken together, the ^1H NMR and ESI-MS data demonstrate the formation of chloroacetamide with the molecular formula of C_2H_4ClNO. From these results, it was evident that an ammonolysis-based process could be successfully utilized for the removal of methyl chloroacetate from emulsion droplets. Conversion of the dispersed solvent into the water-miscible solvents of chloroacetamide and methanol would trigger microsphere solidification in an efficient manner.

The ammonolysis-based microencapsulation process reported in this study produced free-flowing microspheres that did not aggregate upon vacuum drying. Table 1 summarizes the data relevant to progesterone encapsulation efficiencies, microsphere production yields, and median particle sizes of various MCA microsphere batches. Our encapsulation efficiency values are

Table 1. Progesterone Encapsulation Efficiency (EE), Microsphere Production Yield, and Median Microsphere Size (d_{50}) Attained with Various Microsphere Formulations[a]

microsphere formulation (mg)				
PLGA	progesterone	% EE	% production yield	d_{50} (μm)
250	160	68.9 ± 1.4	76.8 ± 6.4	167.2 ± 10.3
300	160	67.1 ± 1.3	81.3 ± 4.4	178.9 ± 19.6
350	160	65.9 ± 1.4	83.6 ± 5.0	181.1 ± 10.6

[a] All values represent mean ± SD ($n \geq 3$).

comparable to those attained with the PLGA microspheres prepared by a solvent extraction process.[14] In all cases, the microencapsulation process provided reproducible progesterone encapsulation efficiencies for a given microsphere formulation, thus assuring the batch-to-batch reproducibility and content uniformity.

MCA microspheres were also prepared by varying the progesterone payload while maintaining the amount of PLGA at 0.25 g. When 60 mg progesterone was used to make microspheres, an encapsulation efficiency of 64.3 ± 1.1% was observed (Figure 3). Increases in the amount of progesterone led to modest improvements in its encapsulation efficiency. For instance, the average encapsulation efficiency observed with the microspheres made of 0.25 g PLGA and 250 mg progesterone was 72.8 ± 0.3%; in this case, the average actual drug payload was determined to be 36.4%. When the solvent evaporation

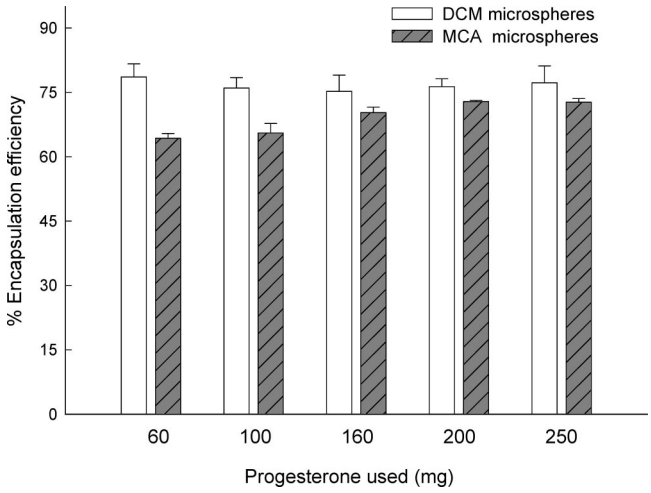

Figure 3. Progesterone encapsulation efficiencies attained with DCM and MCA microspheres. The amount of PLGA used to prepare all microspheres was fixed at 250 mg, but progesterone payload was changed. At least three batches were prepared for a given microsphere formulation.

process was conducted, encapsulation efficiencies of progesterone ranged from 75.2 ± 3.8 to 78.6 ± 3.1%. These encapsulation efficiency values are quite in line with those determined with the PLGA microspheres prepared by a solvent evaporation technique.[15,16]

TGA analysis provides useful information on whether volatile residual solvents are present in polymeric delivery systems. In this study, mass changes of vacuum-dried DCM and MCA microspheres with different progesterone payloads were monitored as a function of temperature. Upon heating to 150 °C, DCM microspheres lost 0.5−2.3% of their original weights due to dichloromethane volatilization (Figure 4A). The values are in good agreements with that reported with a typical solvent evaporation process.[17] In contrast, MCA microspheres showed mass losses ranging from 1.6 to 3.5% of their original weights (Figure 4B). It should be mentioned that no pronounced mass loss was observed around the boiling point (129.5 °C) of methyl chloroacetate. These results indicate that the ammonolysis effectively removes the dispersed solvent out of emulsion droplets. It was also of interest to pinpoint that the amounts of residual solvents in DCM and MCA microspheres were substantially affected by the degree of progesterone payload. In both the cases, more residual solvents are entrapped in the microspheres laden with a lower progesterone payload.

Figure 5 illustrates the versatile surface morphology of DCM microspheres. At 15.2% progesterone loading, the microspheres had a smooth and spherical surface. A few crystals started to appear on the surface of the microspheres with 21.7% progesterone content. Increasing progesterone payloads to ≥29.4% resulted in the formation of numerous crystals on their surface. In addition, they possessed surface areas that were sunken and indented. By and large, a typical solvent removal process often leads to the formation of drug crystals on the surface of microspheres.[18,19] As opposed to DCM microspheres, however, quite distinctive features were observed with MCA microspheres. The surface morphology of MCA microspheres was somewhat affected by the extent of progesterone payload. The microspheres with 12.4% progesterone tended to display imperfections on their surface morphology (Figure 6). Increases in progesterone payload led to formation of spherical microspheres without such imperfections. The development of

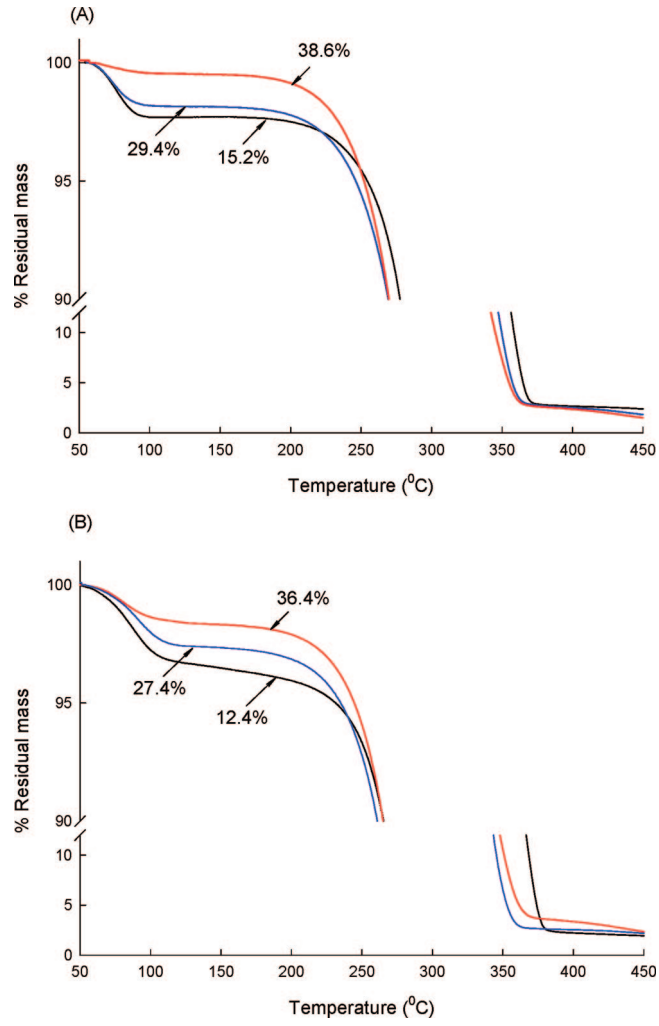

Figure 4. Thermogravimetric analyses of (A) DCM microspheres laden with 15.2, 29.4, and 38.6% progesterone and (B) MCA microspheres containing 12.4, 27.4, and 36.4% progesterone.

progesterone crystals on the surface of microspheres was repressed to greater extents in the case of MCA microspheres as compared to DCM microspheres.

The internal structure of DCM microspheres is illustrated in Figure 7. The magnitude of progesterone payload played a decisive role on the inner morphology of these microspheres. At the actual loading of 15.2%, DCM microspheres had compact and smooth matrices. However, increases in progesterone payload seem to facilitate its crystallization in the polymeric matrices during solvent evaporation. This event is likely to accelerate phase separation between progesterone and the PLGA polymer. Subsequently, the inner matrices of their microspheres severely twisted out of the compact structure at higher progesterone payloads. As far as MCA microspheres are concerned, their matrices have a number of small-sized cavities (Figure 8). However, the effect of progesterone payload upon the internal microsphere morphology was less pronounced compared to the cases of DCM microspheres.

Shown in Figure 9 are the XRD patterns of progesterone, DCM/MCA microspheres containing different progesterone payloads, and their blank microspheres. The XRD pattern of progesterone demonstrates that it is highly crystalline, thereby confirming a previous report.[20] No Bragg diffraction was observed with blank MCA and DCM microspheres, which suggested that they were amorphous. The XRD patterns of MCA and DCM microspheres containing 12.4−15.2% progesterone

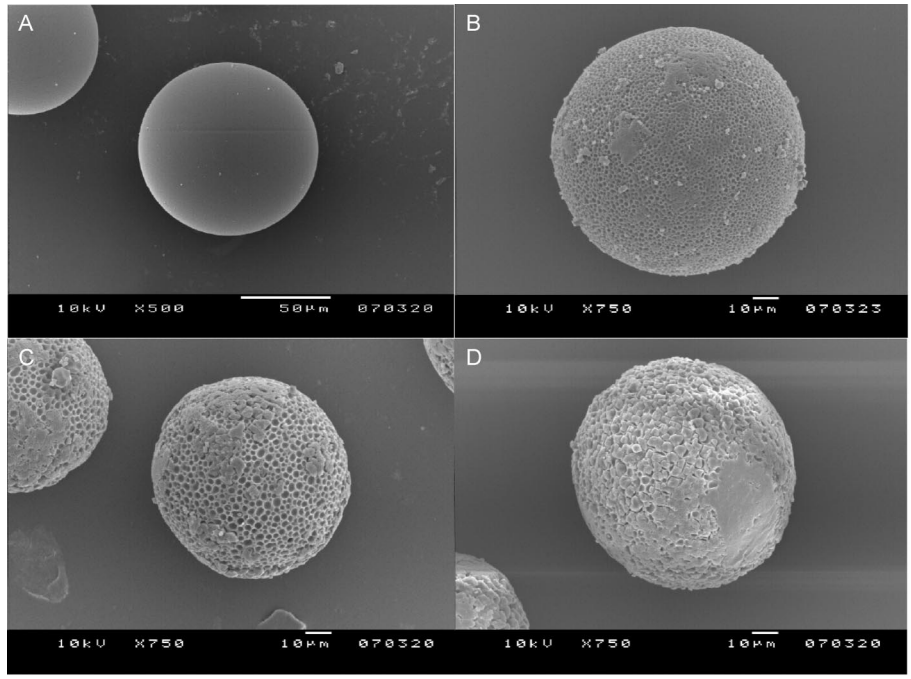

Figure 5. SEM micrographs illustrating the surface morphology of DCM microspheres containing (A) 15.2, (B) 21.7, (C) 29.4, and (D) 38.6 wt % progesterone, respectively. Increasing progesterone payload leads to the formation of drug crystals and the surface deformation of DCM microspheres.

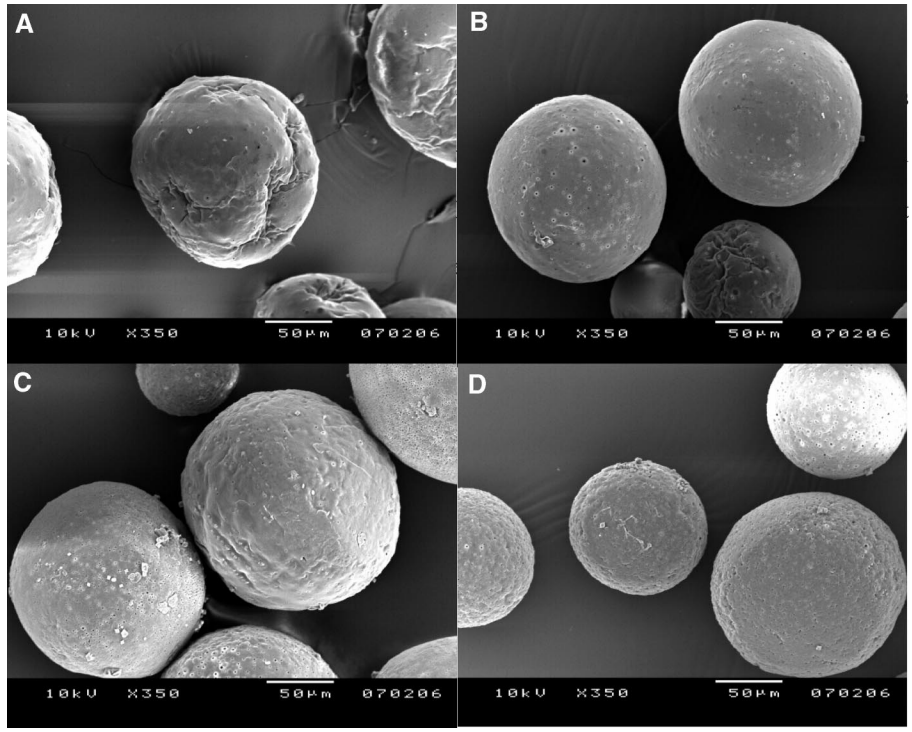

Figure 6. SEM micrographs showing the external morphology of MCA microspheres laden with (A) 12.4, (B) 18.7, (C) 27.4, and (D) 36.4 wt % progesterone, respectively.

also indicated that any significant crystallinity was absent. The results substantiated that progesterone was molecularly dispersed in the polymeric matrices. However, the crystalline form of progesterone appeared in both DCM and MCA microspheres laden with higher progesterone payloads. The amount of crystalline progesterone present in the microspheres increased as a function of its payload, but its XRD pattern remained unchanged before and after microencapsulation.

Discussion

Selecting a dispersed solvent for microencapsulation depends upon a number of factors including polymer/drug solubility, safety, toxicity, and regulatory issues. Particularly, volatility and water miscibility are important attributes for optimization of emulsion-based solvent evaporation and/or extraction microencapsulation. Preferred solvents are dichloromethane and ethyl acetate. The volume of a continuous phase used in a typical

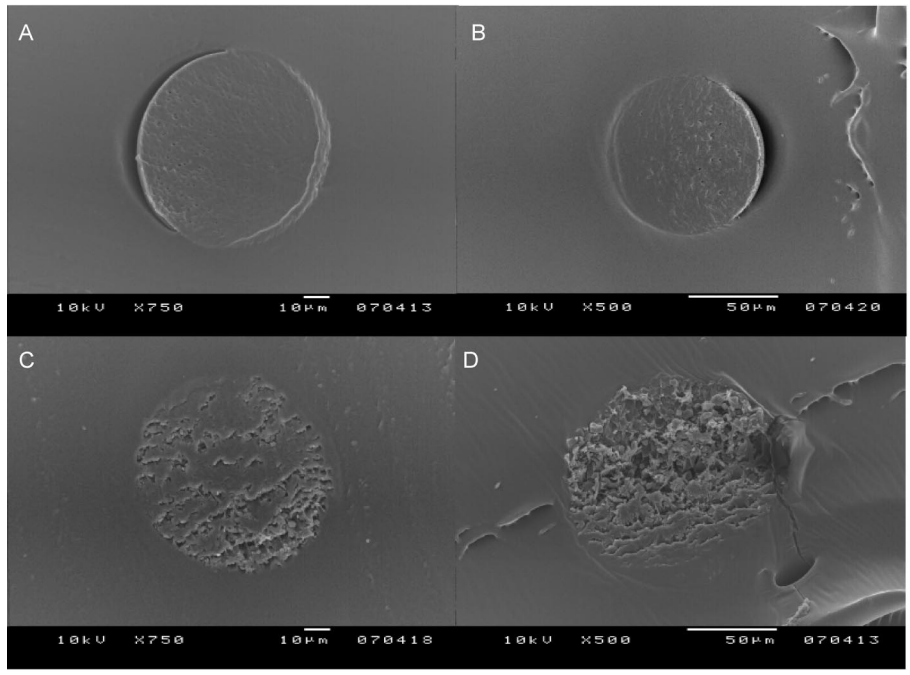

Figure 7. SEM micrographs demonstrating the internal morphology of DCM microspheres laden with (A) 15.2, (B) 21.7, (C) 29.4, and (D) 38.6 wt % progesterone, respectively.

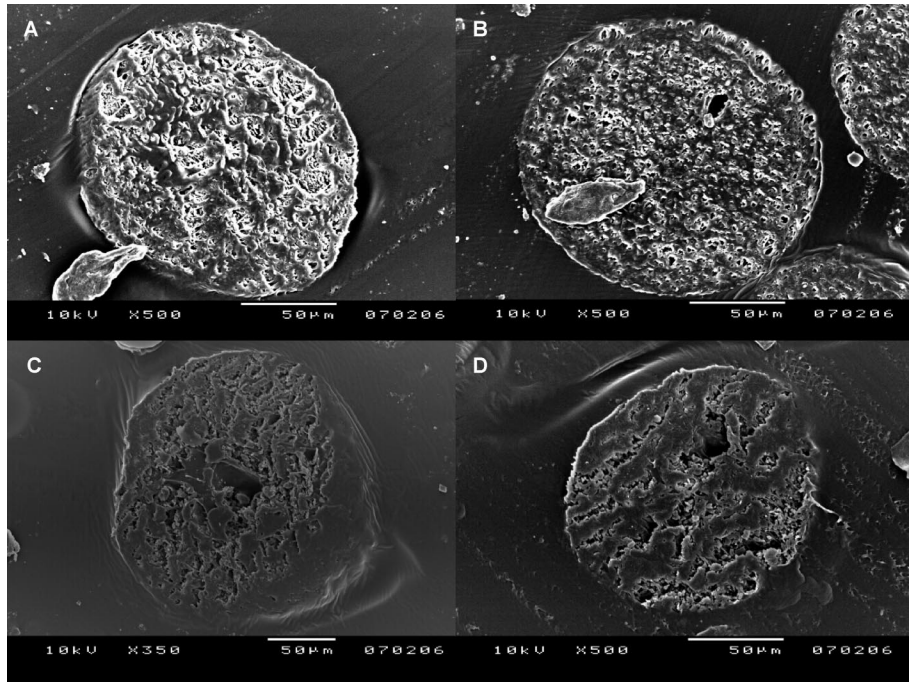

Figure 8. SEM micrographs displaying the internal morphology of MCA microspheres laden with (A) 12.4, (B) 18.7, (C) 27.4, and (D) 36.4 wt % progesterone, respectively.

solvent evaporation process is barely sufficient for solubilizing the entire organic solvent in a dispersed phase. Because evaporation is a prerequisite for the further partitioning of the dispersed solvent molecules into the continuous phase, the processing of microsphere solidification is a lengthy process.[21] Elevating temperature or reducing pressure frequently facilitates the process of solvent evaporation.[22] In the case of solvent extraction, the continuous phase is supplemented with a quench medium to promote extraction of organic solvent molecules from the dispersed phase.[14] However, such extraction techniques generate vast quantities of solvent wastes that impose burdens on the industry. Waste disposal causes significant competitive disadvantage in terms of time and capital expenses. Trends toward reducing waste amount and improving the process efficiency of microsphere solidification are in practice. Relevant approaches have made use of a solvent exchange method, a combination of solvent extraction and evaporation procedures, and a nonaqueous emulsion process.[23–28] In contrast to these approaches, our microencapsulation technique involves two principal processes: preparation of an O/W emulsion and transformation of emulsion droplets into microspheres via ammonolysis. Microsphere solidification takes a comparatively short time and does not generate large volumes of the solvent wastes. Likely, these features are distinguished from those of

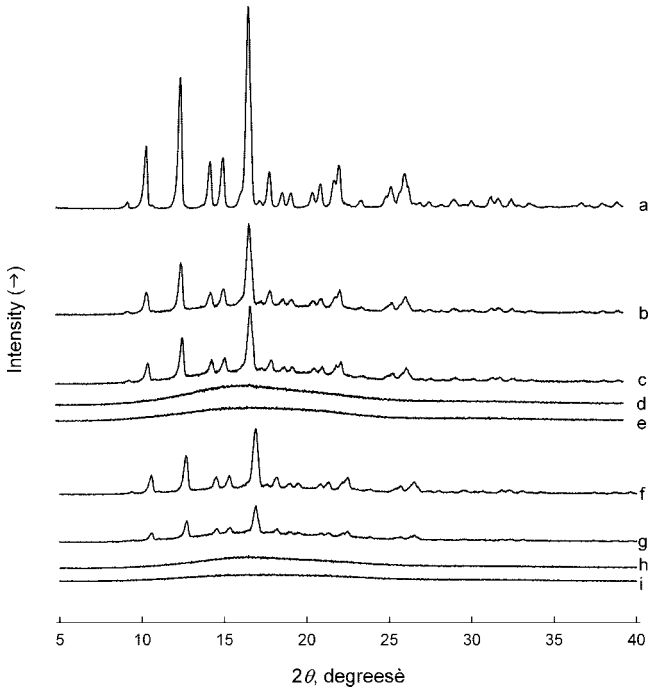

Figure 9. X-ray powder diffraction patterns of (a) progesterone, DCM microspheres laden with (b) 38.6, (c) 29.4, and (d) 15.2 wt % progesterone, (e) progesterone-free DCM microspheres, MCA microspheres laden with (f) 36.4, (g) 27.4, and (h) 12.4 wt % progesterone, and (i) progesterone-free MCA microspheres.

typical solvent evaporation and extraction techniques. It should be mentioned that methyl chloroacetate is a nonvolatile organic solvent and is barely water-soluble. Thus, it is extremely difficult, if not impossible, to form PLGA microspheres by conventional solvent evaporation and extraction techniques. The new strategy outlined in this study, however, leads to the formation of PLGA microspheres in an efficient manner.

Recently, we have proposed the ammonolysis-based microencapsulation concept for an efficient microencapsulation technique using methyl dichloroacetate (MDA) as a dispersed solvent.[13] This process provided free-flowing microspheres after vacuum drying. However, it suffered from a serious drawback that flowability was lost on storage. We attributed this to the traces of residual solvents entrapped in microspheres and centered on solving this problem. The key thrust of the ammonolysis-based concept lies in the fact that ammonia induces the breakdown of an organic solvent into water-soluble components, which should diffuse into an aqueous continuous phase. Hence, to effectively solidify microspheres, we optimized the dispersed solvent to methyl chloroacetate instead of methyl dichloroacetate. Important physical properties of methyl chloroacetate (bp = 129.5 °C, water solubility = 46 g/L) are more favorable for easy removal of the residual solvent as compared to those of methyl dichloroacetate (bp = 142.9 °C, water solubility = 16.6 g/L). In addition, the ammonolysis product of methyl chloroacetate (chloroacetamide, water solubility = 90 g/L, bp = 225 °C) is more water soluble than that of methyl dichloroacetate (dichloroacetamide, water solubility = 71 g/L, bp = 234 °C). It has been contemplated that their better partitioning into the aqueous continuous phase, due to more water solubility, might help to reduce their residual amounts in MCA microspheres. To our delight, even upon long-term storage at room temperature, the MCA microspheres did not undergo aggregation.

To support our contemplation, microspheres were also prepared using 4 mL of methyl dichloroacetate (MDA) as a

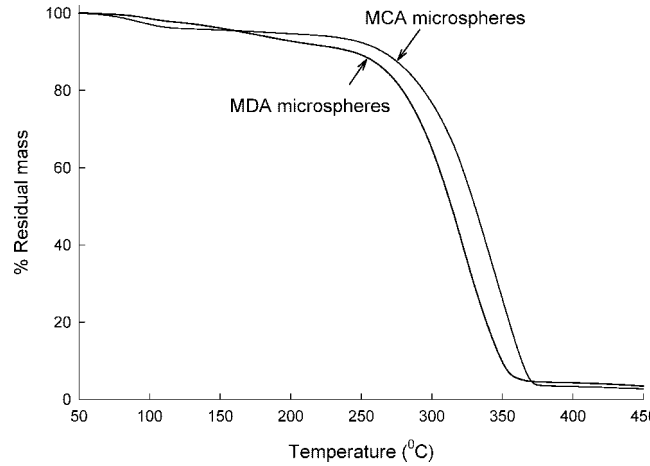

Figure 10. Thermogravimetric analyses of the microspheres prepared using 0.40 g PLGA and 160 mg progesterone. Methyl chloroacetate (MCA) and methyl dichloroacetate (MDA) were used as dispersed solvents.

control (they were made of 0.4 g PLGA and 160 mg progesterone, and microencapsulation was conducted under the same processing conditions). There were no significant differences between the encapsulation efficiencies of MCA and MDA microspheres: the values were in the range of 71.6 ± 1.5 to 72.5 ± 1.4%. However, TGA analysis indicated that more residual solvents accumulated within the MDA microspheres compared to the MCA ones (Figure 10). For instance, the MDA microspheres displayed noticeable mass loss over the range of 200−250 °C, whereas the MCA ones showed negligible mass change over the temperature range. This suggests that the characteristics of a dispersed solvent and its ammonolysis product affect the accumulation of residual solvents inside microspheres. It should be noted that identification of the component and composition of the residual solvents in both microspheres deserves further investigation. Development of a suitable analytical methodology such as gas chromatography–mass spectrometry is underway.

In the practice of a typical solvent evaporation process, slow solidification of emulsion droplets often leads to the formation of PLGA microspheres with a dense inner morphology.[5] Accordingly, the DCM microspheres with lower progesterone payloads possessed dense polymeric matrices (Figure 7A,B). In contrast, it was observed from SEM micrographs that the MCA microspheres with similar progesterone payloads had some pores/cavities inside their matrices (Figure 8A,B). The formation of such porous structures might be due to the rapid solvent removal achieved by ammonolysis. This speculation agrees well with a conclusion that the fast solvent removal during microsphere solidification provokes the formation of pores/cavities with the polymeric matrix.[5,29] Another possible mechanism accounting for the presence of pores/cavities in the MCA microspheres might be related to solvent extraction from polymeric matrices. It has been reported elsewhere that porous microspheres are produced as a variety of porogens leach out of polymeric droplets during solvent evaporation.[30] In the practice of our microsphere solidification process, ammonia molecules diffuse into the emulsion droplets consisting of methyl chloroacetate, PLGA, and progesterone. The following ammonolysis generates water-soluble methanol and chloroacetamide evolving through the polymeric matrices. A series of these successive events are likely to contribute to the formation of pores and cavities in the MCA microspheres.

Physical transformation of pharmaceuticals and excipients may occur during a variety of pharmaceutical processes, thereby altering their stability, dissolution rate, or transport characteristics. In fact, there is a chance that the physical status of progesterone might be affected by microencapsulation. For instance, poly(L-lactide) yielded microsphere matrices of different crystallinities depending upon solvent evaporation conditions.[31] When solvent removal was performed under normal atmospheric conditions, a crystalline matrix was obtained. In contrast, rapid solvent removal at reduced pressure resulted in formation of an amorphous matrix. In our cases, blank DCM and MCA microspheres were demonstrated to be amorphous. In addition, the magnitude of progesterone payload affects its physical status in the microspheres (Figure 9). Depending on the magnitude of progesterone payloads, the drug is molecularly dispersed in the microsphere matrices or forms crystalline domains. The results also suggest that polymorphic transformation of progesterone does not occur during our microencapsulation process.

The apparent success of the ammonolysis-based microencapsulation technique indicates several potential directions for further research. For instance, this process could be extended to loading hydrophilic drugs via an ammonolysis-based water-in-oil-in-water (W/O/W) emulsion process. In particular, a microencapsulation process allowing rapid microsphere solidification might help improve their incorporation efficiency.[32] These issues also merit further investigation.

Conclusion

Progesterone was successfully encapsulated into PLGA microspheres by the new ammonolysis-based solvent removal process. In this practice, microsphere solidification was caused by the chemical reaction converting the water-immiscible dispersed solvent into water-soluble solvents. As a consequence, microsphere solidification did not necessitate a lengthy procedure, nor did it require a vast quantity of a quench medium. Microspheres were also prepared by the typical solvent evaporation process, and their important attributes were compared. There were considerable differences between the microspheres prepared by the two methods in terms of the degree of residual solvents, thermal behavior, microsphere morphology, and the location of drug crystals in the microspheres. Our study demonstrated that progesterone payload, the type of a dispersed solvent, and solvent removal technique were critical and interconnected factors influencing the characteristics of PLGA microspheres. In conclusion, it is likely that the new ammonolysis-based microencapsulation process might find applications for loading pharmaceuticals into various types of polymeric microspheres.

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