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Encapsulation of Indomethacin Using Coaxial Ultrasonic Atomization Followed by Solvent Evaporation

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We have encapsulated indomethacin into poly (lactide-co-glycolide) (PLGA) using coaxial ultrasonic atomization technique. The specific aims of this study were to evaluate the effect of drug loading and a change in relative concentration of polymer in the inner and outer layers of coflowing spray liquids on the physicochemical characteristics of the particles. Indomethacin, a non steroidal anti-inflammatory drug, was selected as a model compound. The micro/nanocapsules prepared using a drug free PLGA solution as an outer layer showed higher encapsulation efficiency. Thermal analysis of the formulations indicated that indomethacin was dissolved within the PLGA matrix. The formulations prepared with 25mg indomethacin showed relatively smaller particle size compared with the formulations prepared with 50 mg indomethacin. The particles, in general, showed bi- and tri-modal distribution. Irrespective of the compositions of the liquids 1 and 2, all the particles were smooth and spherical. A cross-section view of the particles revealed the presence of three different internal morphologies. These formulations were a mixture of hollow or solid spheres, and single or multiple spheres encapsulated into a larger sphere. To the best of our knowledge, this is the first study revealing the cross-sectional view of particles prepared with coaxial ultrasonic atomization technique.

Keywords coaxial ultrasonic atomization; microspheres; nanospheres; indomethacin; encapsulation; morphology

INTRODUCTION

Encapsulation of drugs in envelopes of biodegradable polymer has become a well-established technology for controlled release drug delivery (Tamber, Johnson, Merkle, & Gander, 2005). Controlled release biodegradable micro- and nanocapsules of numerous drugs have been developed during the last three decades and several of these formulations have received worldwide marketing approval (Debruyne et al., 1988; Ogawa, Okada, Heya, & Shimamoto, 1989; Okada et al., 1991). One of the advantages of these formulations is that no follow-up surgical removal is required once the drug supply is depleted. The most widely investigated polymers are the aliphatic polyesters based on lactic acid and glycolic acid, poly (lactide-co-glycolide) (PLGA) (Lewis, 1990).

Several methods have been developed for the encapsulation of a wide variety of drugs using PLGA (Graves et al., 2004; Homayoun, Mandal, Landry, & Komiskey, 2003; Mandal, 1998; Pamujula, Graves, Kishore, & Mandal, 2004; Rajeev, 2000). However, the solvent evaporation method has been widely accepted for this purpose (Feng & Huang, 2001; Freitas, Merkle, & Gander, 2005; Prior et al., 2000; Redhead, Davis, & Illum, 2001). These microcapsules are generally smooth, spherical, and provide sustained drug release. However, the analysis of particle size often shows bi- or tri-modal distribution. This heterogeneous distribution of particles occasionally results in a wide variation in drug release characteristics. In an effort to produce uniform particles, several investigators have used atomization technique to spray the drug/polymer solutions (Bittner & Kissel 1999; Cavallari et al., 2005; Felder et al., 2003; Freitas, Merkle, & Gander, 2004; Yasuda et al., 2005;). Recently, coaxial atomization has been used to produce mono disperse micro- and nanocapsules of an aqueous solution coated with DuPont photopolymer Somos® 6120 (Loscertales et al., 2002). In this procedure, two immiscible liquids, an aqueous solution of ethylene glycol and Somos®, were injected at appropriate flow rates through two concentrically located tubes. The aqueous solution was injected through the inner capillary tube; simultaneously a stream of Somos® was forced through a tube surrounding the inner capillary tube. Both the liquids form microthreads at the tip. The microthread of ethylene glycol merged with that of Somos® at the tip to finally form a two-concentric layered micro/nano jet. Based on this principle, reservoir type microcapsules were developed for the encapsulation of drugs (Park & Yeo, 2004; Yeo, Basaran, & Park, 2003; Yeo, Chen, Basaran, & Park, 2004; Yeo & Park, 2004a). The reservoir type microcapsules were formed by midair collision of the two liquids droplets followed by solvent exchange between the polar solvent in the inner liquid containing the drug and the polar solvent in the

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outer liquid containing the polymer. Using this technique, Yeo and Park have successfully encapsulated lysozyme into PLGA without any loss of lysozyme functional integrity (Yeo & Park, 2004b).

We have developed a novel micro-/nanocapsule of indomethacin in PLGA using coaxial atomization. Indomethacin, a non steroidal anti-inflammatory drug, was selected as a model compound. The specific aims of this study were to evaluate the effect of drug loading and a change in relative concentration of polymer in the inner and outer layers of liquids on the physicochemical characteristics of the particles.

MATERIALS AND METHODS

Materials

The copolymer poly (DL-lactic/glycolic acid PLGA 50:50; RG 502; inherent viscosity 0.2 dl/g, MW 14,000) was obtained from Boehringer Ingelheim, Ingelheim (Germany). Indomethacin, polyvinyl alcohol (PVA, MW 30,000–70,000, 98–99% hydrolyzed), dichloromethane, and chloroform were obtained from Sigma Aldrich (St. Louis, MO).

Experimental Design

Eight batches of formulations were prepared using a 2×4 partial factorial design. The effect of drug loading was studied at two levels, 25 and 50 mg, and the effect of polymer concentration (in mg) in the liquid 1/liquid 2 was studied at four levels, 400/0; 300/100; 200/200; 100/300 (Table 1).

Preparation of Micro/Nanocapsules

A schematic diagram of the experimental set up is presented in Figure 1. The ultrasonic atomizer is equipped with a spray drier nozzle, extended length, 60 KHz freq; dual microbore liquid feed; and broad band ultrasonic generator (Sono-Tek, Milton, NY). Nozzle power level was set at 4.8 watts. Liquid 1 was fed through the inner tube and the liquid 2 was fed through the coaxial outer tube of the ultrasonic atomizer. Both liquids were fed simultaneously through a syringe pump at a flow rate of 3 ml/min. Liquid 1 consisted of PLGA solution in dichloromethane containing indomethacin, whereas the liquid 2 consisted of only PLGA solution in dichloromethane. Since both liquids (liquids 1 and 2) consisted of the same solvent, dichloromethane, it is expected that following the atomization process the liquids will mix with each other. However, indomethacin is only present within the internal layer (liquid 1); as a result, following the mixing of the two liquids, the PLGA present within the outer layer (liquid 2) will deposit as a partial coating on the droplets containing the drug. The liquids were sprayed into a 250 ml beaker containing 100 ml of 0.3% PVA solution. The distance between the atomizer tip and the PVA

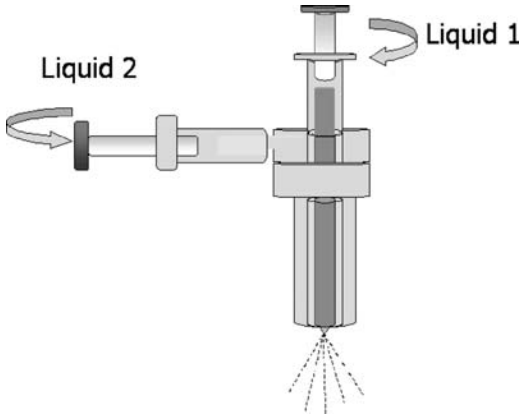

FIGURE 1. Schematic description of coaxial ultrasonic atomizer set up.

TABLE 1
Compositions and Physiochemical Characteristics of Formulations

Formulation	Liquid 1 (10 ml) PLGA (mg)	Liquid 1 (10 ml) Indomethacin (mg)	Liquid 2 (10 ml) PLGA (mg)	Encapsulation Efficiency % (SEM)[a]	Tg (°C) (SEM)	Size (Volume Average) (μm) (80% Confidence)
A_{25}	400	25	—	65.74 (1.09)	36.9 (0.1)	24.8 (0.69–350)
B_{25}	300	25	100	69.61 (1.33)	38.1 (0.5)	6.4 (0.46–76)
C_{25}	200	25	200	77.33 (1.50)	37.4 (0.3)	20.2 (0.63–294)
D_{25}	100	25	300	79.75 (2.29)	37.9 (0.7)	13.7 (0.51–364)
A_{50}	400	50	—	62.23 (0.34)	33.4 (0.6)	87.4 (18.5–526)
B_{50}	300	50	100	91.55 (0.84)	37.0 (0.7)	78.6 (2.5–571)
C_{50}	200	50	200	95.90 (1.10)	39.2 (0.5)	59.1 (0.85–511)
D_{50}	100	50	300	102.16 (1.30)	34.3 (1.1)	37.3 (1.3–430)

[a]Standard Error of Mean ($n = 5$).
Indomethacin melting point: 165°C.

solution was maintained at 3 ± 1 cm. The mixture was stirred magnetically at 500 rpm for 3 hours at room temperature to allow complete evaporation of the solvent. Particles were finally collected by centrifugation at 13,000 rpm and washed four times with deionized water to remove any residual PVA on the surface of the particles. The particles were later freeze-dried (–70°C; 6×10^{-4} mbar) (Labconco, Kansas City, KS) to obtain a free-flowing powder. Each formulation was prepared in triplicate.

Particle Size and Morphology

Particle size and distribution was determined by a Mastersizer 2000 laser scattering device (Malvern Instruments Ltd., Malvern, UK). This technique measures the size of particles dispersed in a medium by the scattering pattern of a laser light shown through the medium. The size calculations assume the presence of spherical particles. Therefore, percent volume distributions assume the volumes of spheres. The samples were analyzed in a water medium and the Frauenhofer method was utilized to calculate the size distributions. For each sample, a background run of deionized water was performed. A sample of particles (5 mg) was added to the deionized water in a small volume sample dispersion unit. After subtraction of the background, the particle size distribution calculation was performed. Each measurement was performed in triplicate.

The samples for scanning electron microscope (SEM) were mounted on metal stubs and the surface and internal morphology of the particles were examined by a Hitachi 3000N variable pressure SEM (Hitachi, Gaithersburg, MD). The analytical parameters included an accelerating voltage of 10 KeV, a working distance of 13.5 mm, and a vacuum 40 Pa in variable pressure mode. Since the samples were analyzed in variable pressure mode, the backscatter detector BSE2 was used. The internal morphology was evaluated following freeze-fracture of dried particles. The dried samples were rapidly frozen at –196°C using liquid nitrogen and immediately sectioned using a surgical blade.

Thermal Analysis

Differential scanning calorimetry of indomethacin, PLGA, and indomethacin-loaded particles were performed using a DSC 2920 (TA Instruments, New Castle, DE) in order to characterize their physical state after encapsulation. About 5 mg of a sample was weighed, crimped into an aluminum pan and analyzed at a scanning rate of 3°C/minute. The glass transition temperature (Tg) was calculated using TA universal analysis software by extrapolating the linear portion of the thermograms above and below the glass transition point and determining the midpoint.

Determination of Total Content

For each formulation, a 5 mg sample was dissolved in 200 µl of dichloromethane. Ten milliliters of methanol was added to the solution followed by ultracentrifugation (35,000 rpm at 15°C) to completely separate the precipitated copolymer. The efficiency of extraction and recovery of indomethacin was measured independently with five different samples. The efficiency of extraction was at least 99%.

Analysis

The analysis of indomethacin in each sample was performed using a rapid and sensitive HPLC method. The chromatographic system consisted of a Waters Model 600 programmable solvent delivery module, Waters Model 717plus auto sampler, and a Waters Model 996 photodiode array detector (Waters, Milford, MA). The chromatography was performed using a Supelcosil C-8 (5 µm, 4.6 × 250 mm; Supelco, Bellefonte, PA) column; the mobile phase consisted of 60% pH 6.0 phosphate buffer with 40% acetonitrile; a flow rate of 1.5 ml/min. The mobile phase was vigorously purged with helium gas for 15 min prior to use. The identity of the eluting peaks was verified using a diode array detector. The concentration of indomethacin in each sample was determined by intrapolating the peak height to the indomethacin standard curve. Each experiment was performed in triplicate.

In Vitro Dissolution Studies

For each formulation, a 10 mg sample of the particles was transferred in a 5 cm long dialysis tube (5.7 mm diameter; mol weight cut off 15,000) (Spectrum Laboratories, Rancho Dominiguez, CA). The tube was filled with 1.5 ml of phosphate buffer (PBS, pH 7.4) and sealed. The sealed tube was immersed in a polypropylene tube containing 40 ml of PBS as the dissolution medium. The polypropylene tubes were incubated in a water bath at 37°C with constant shaking at 20 rpm. The total amount of the dissolution medium (40 ml) was replaced with fresh PBS at preset time intervals. The amount of indomethacin in the dissolution medium was analyzed using HPLC. Dissolution studies of each formulation were repeated three times.

Statistical Analysis

The encapsulation efficiency of indomethacin and the amount of indomethacin released from the particles during the in vitro dissolution study were compared using SigmaStat, version 2.0 software package (SPSS Inc., Chicago, IL). A p value of < 0.05 was considered evidence of a significant difference.

RESULTS AND DISCUSSION

The freeze dried samples were examined for surface and internal morphology. These samples also were evaluated for particle size distribution, efficiency of encapsulation, thermal behavior, and in vitro drug release. The results of these experiments are discussed below.

Irrespective of the composition of the liquid 1 and liquid 2 and the concentration of indomethacin, most of the particles were 500 µm or less with average diameter between 6 and 87 µm

(Table 1; Figure 2A and 2B). The results of the particle size distribution are presented as volume average and the ranges of 80% of the particles. These formulations showed a wide and bi- or tri-modal distribution (data not shown). The formulations prepared with 25 mg indomethacin (A25-D25) showed relatively smaller particle size (smallest size particles were 690 nm or less) compared with the formulations prepared with 50 mg indomethacin (smallest size particles were higher than 850 nm). A comparison of particle size among four formulations containing 25 mg indomethacin also showed significant effect of the compositions of the liquid 1 and liquid 2. The formulation prepared with 100 mg PLGA as an outer layer (i.e., liquid 2; B25) showed relatively smaller particles and these particles also were narrowly distributed compared with the other formulations within the same group (A25, C25, and D25). An increase in the amount of indomethacin to 50 mg showed significant increase in the average particle size (Table 1). Within this set of formulations (A50-D50), the formulation prepared with only one stream of liquid (liquid 1) containing 400 mg PLGA (A50) showed the largest average particle size (87 μm). This particular batch was prepared in the absence of the liquid 2; as a result, 50 mg indomethacin was processed with 400 mg PLGA. Larger particle size of these formulations was a reflection of the high amount of polymer in the solution. As the amount of polymer in the internal liquid layer (liquid 1) decreased, the average particle size also decreased (B50-D50; Table 1). Since the total amount of PLGA was maintained constant, as the amount of PLGA in the internal layer decreased the amount of PLGA in the outer layer increased. Along with the decrease in the amount of PLGA in the liquid 1, drug/polymer ratio in this layer increases and lesser amount of PLGA was available for mixing. Moreover, the content of the liquid 1 did not come in contact with the PLGA available in the outer layer (liquid 2) until the droplets were formed following the atomization of the two liquid layers.

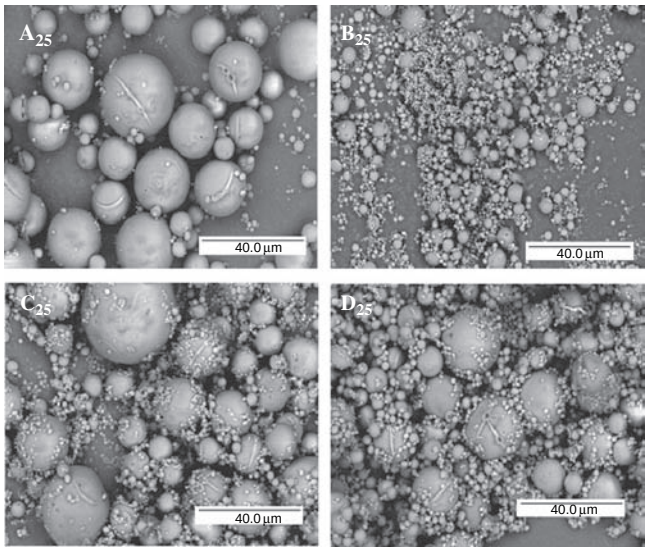

FIGURE 2A. Typical SEM photographs of the formulations prepared with 25 mg indomethacin.

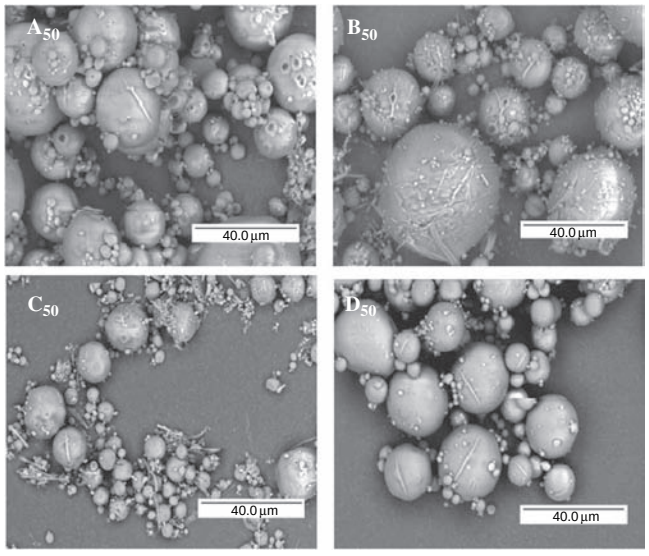

FIGURE 2B. Typical SEM photographs of the formulations prepared with 50 mg indomethacin.

Figures 2A and 2B show the SEM photographs of the particles. A comparison of the SEM photographs also showed that irrespective of the amount of indomethacin and the compositions of the two liquid layers, all the particles were smooth and spherical. A cross sectional view of the particles (Figure 3) revealed the internal morphology of the formulations. Three types of internal structures were observed: (A) solid structure, (B) one or more smooth and spherical particles within a hollow shell, and (C) hollow shell. All three situations were observed in all the formulations. No significant differences were noticed within these formulations. In other words, all of these formulations consisted of a mixture of these three types of particles.

The results of DSC analysis are presented in Table 1 and Figures 4A and 4B. In cases where the drug formed a dispersion in the first stage of encapsulation, we would expect that, at the end of the process, crystalline drug particles would be dispersed in the polymer matrix. In such a system, DSC would display two endotherms, each relating to the melting point of either drug or polymer. In cases where the drug is dissolved in the polymer solution, solvent removal causes the drug to either dissolve in the polymer or crystallize out and form a dispersion. In the later case, DSC would again display two endotherms. In the former case, where the drug forms a solution inside the polymer, no separate event relating to the melting of the drug would occur. Figures 4a and 4b show the DSC thermograms of the formulations. A comparison of the thermograms of indomethacin, PLGA, and the particles showed that there were no significant changes in the glass transition temperature of PLGA in the particles and the characteristic melting thermogram of indomethacin also was absent in all of the formulations

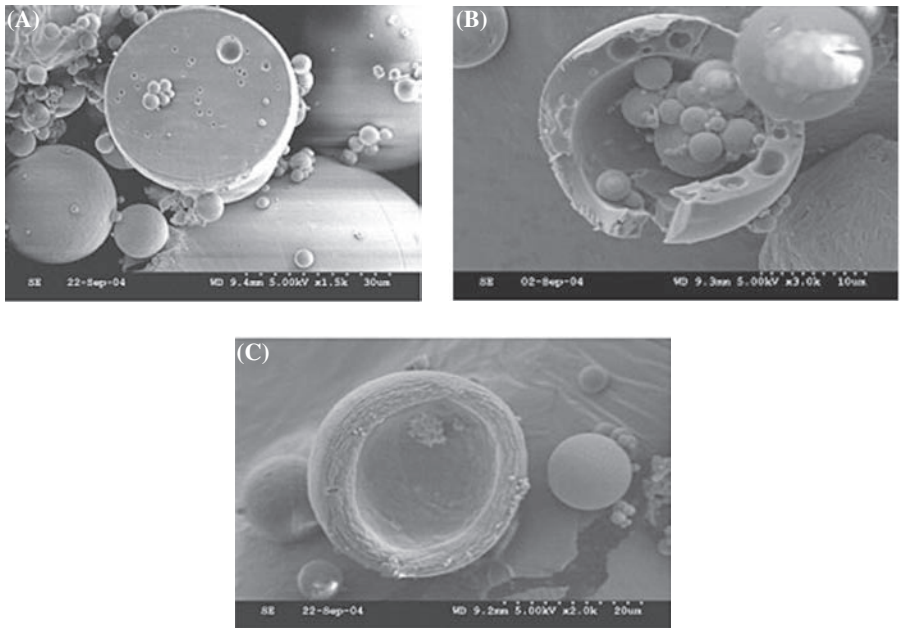

FIGURE 3. Typical cross-sectional view (SEM photographs) of the formulations. Three types of internal structures were observed: (A) solid structure, (B) one or more smooth and spherical particles within a hollow shell, and (C) hollow shell. Only one photograph of each type is included. All three situations were observed in all the formulations. No significant differences were noticed within these formulations. In other words, all of these formulations were consisted of a mixture of these three types of particles.

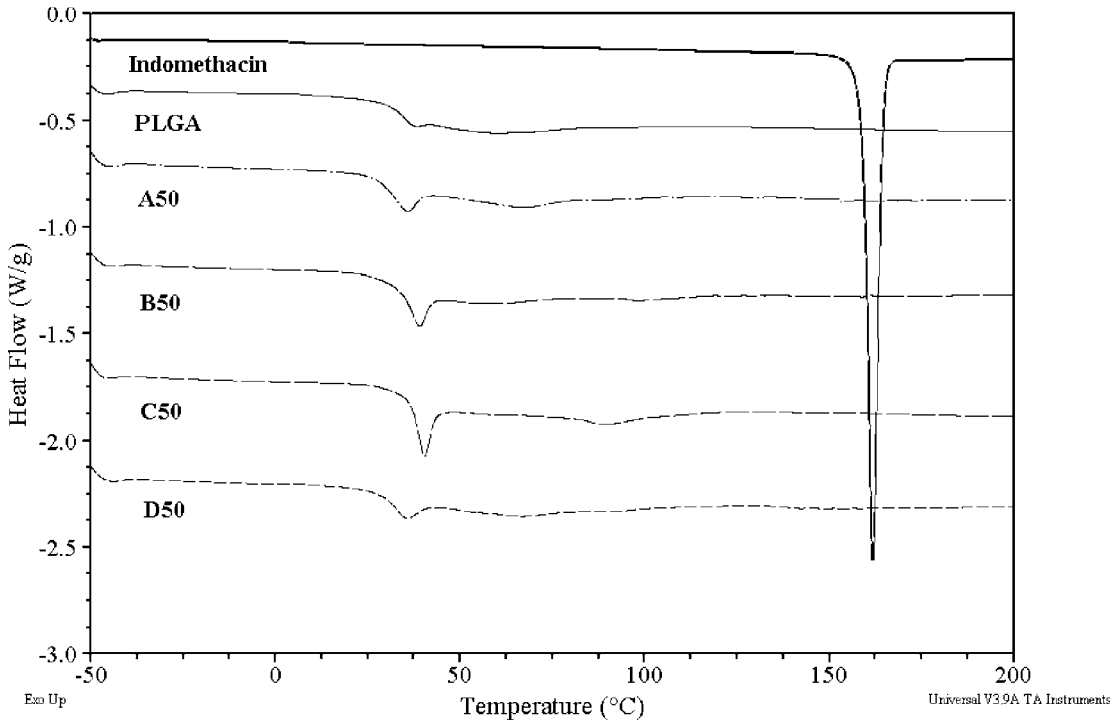

FIGURE 4A. DSC thermograms of indomethacin, PLGA, DSC thermograms of indomethacin, PLGA, and the formulations; Formulations prepared with 25 mg indomethacin.

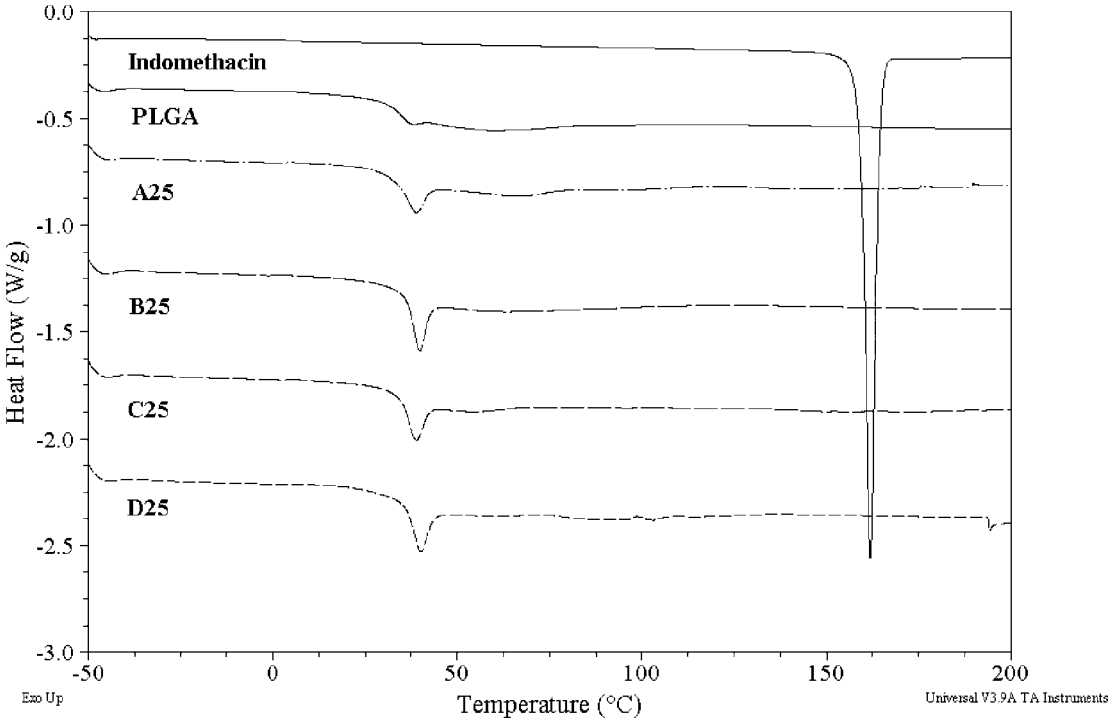

FIGURE 4B. DSC thermograms of indomethacin, PLGA, and the formulations: Formulations prepared with 50 mg indomethacin.

studied. The absence of the indomethacin peak in all of the formulations suggests that the drug was dissolved within the polymer matrix.

Encapsulation efficiency of indomethacin was determined by measuring the total amount of indomethacin present in each 5 mg sample (i.e., core loading experimental), and comparing this value with the expected amount of indomethacin in each of the samples based on the drug loading during the preparation (i.e., core loading theoretical). An analysis of the encapsulation efficiency showed that the presence of a second liquid layer (liquid 2) increased the encapsulation efficiency of indomethacin. Similar observations were noted for both sets of the formulations (25 mg and 50 mg indomethacin, respectively). Formulations prepared with 25 mg indomethacin, showed a gradual increase of the encapsulation efficiency from 66% to 80% as the amount of PLGA was increased in the liquid 2. This increase in encapsulation efficiency was more drastic in case of the formulations prepared with 50 mg indomethacin. Within the later set of the formulations (A50-D50), the presence of a small amount of PLGA (100 mg) as an outer liquid (liquid 2) increased the encapsulation efficiency from 62% to 92% and eventually reached up to 100% as the amount of PLGA in the liquid 2 increased up to 300 mg. The data showing 102% encapsulation efficiency (Table 1) for formulation D50 falls within the 2% experimental error. This observation is in accordance with our postulation that the second PLGA layer will prevent drug loss during the in-water solvent evaporation.

Figures 5A and 5B show the dissolution profiles of the particles. The formulation prepared with 25 mg indomethacin and only one liquid layer (liquid 1) released only 41% of the total drug in 24 hours (A25), whereas the other three formulations in this set (B25, C25, and D25) released 77%, 65%, and 61% drug, respectively, during the same period. These later formulations (B25–D25), showed an inverse rank order correlation ($p < 0.05$) between the amount of PLGA in the outer liquid (liquid 2) and the dissolution of indomethacin. Formulation B25 was prepared with the lowest amount of PLGA (100 mg) in the outer layer and showed the fastest drug release over 4 days (93%), followed by the formulations prepared with 200 mg (88%) and 300 mg (84%), respectively, of PLGA in the outer layer. The formulations prepared with 50 mg indomethacin also confirm the slow release of indomethacin in absence of the outer layer (A50). This particular formulation released only 34% and 72% drug during the first 24 hours and 4 days, respectively. The amount of indomethacin (25 mg or 50 mg) in these two formulations (A25 and A50) came in contact with 400 mg PLGA and had opportunity to dissolve within the matrix. In contrast, three other formulations within these sets (B25, C25, D25; B50, C50, D50), had direct contact only with 300, 200, and 100 mg, PLGA, respectively. Due to this relatively high amount of PLGA within the former formulations (A25 and A50), these sets always showed significantly slower ($p < 0.05$) dissolution. However, the other three formulations within the second set (50 mg indomethacin) did not show any rank order correlation

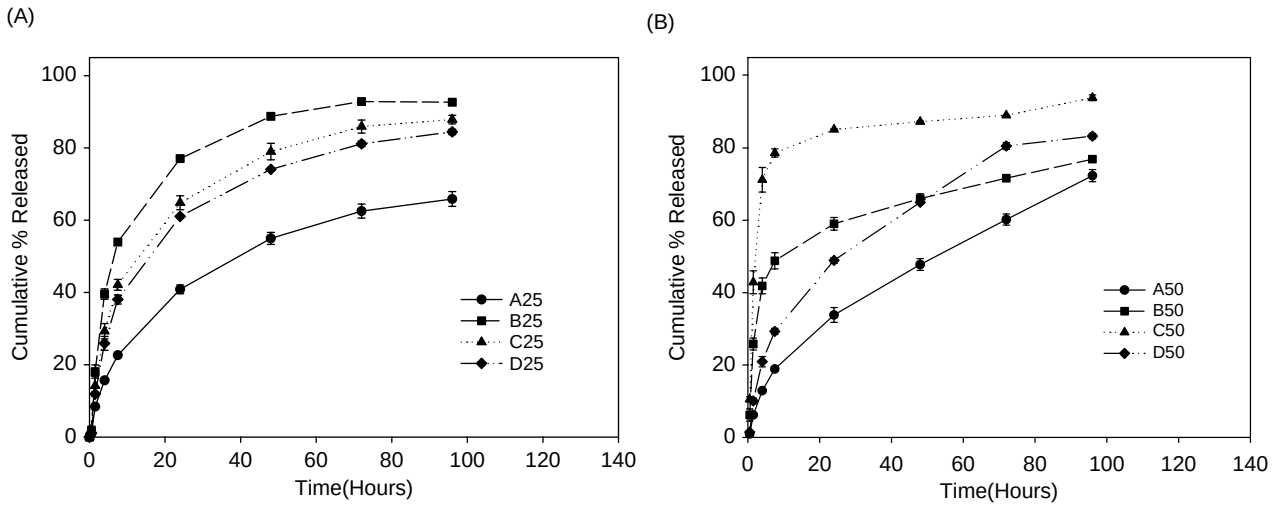

FIGURE 5. Dissolution profiles of the formulations: (A) formulations prepared with 25 mg indomethacin; (B) formulations prepared with 50 mg indomethacin.

($p > 0.05$) and this erratic behavior may be due to the presence of very high amount of drug within these formulations that resulted in initial burst release. These results indicated that the amount of drug loaded within the samples showed significant effect on the overall encapsulation. The dissolution of formulations prepared with 25 mg indomethacin also suggests that the presence of the liquid 2 during the coaxial atomization may have resulted as an outer coat.

CONCLUSION

The micro-/nanoparticles of indomethacin were prepared using coaxial ultrasonic atomization of indomethacin and PLGA dissolved in dichloromethane. The formulations prepared with 25 mg indomethacin showed relatively smaller particle size compared with the formulations prepared with 50 mg indomethacin. The formulations prepared with 25 mg indomethacin also indicated that the presence of a second layer of PLGA solution drastically affected the dissolution profiles despite the use of the same amount of drug and polymer in all four batches. Thermal analysis of the formulations indicated that indomethacin was dissolved within the PLGA matrix. A cross-section view of the particles revealed the presence of three different internal morphologies. These formulations were a mixture of hollow or solid spheres and single or multiple spheres encapsulated into a larger sphere. We were unable to quantitatively measure the true number of each type particle within the mixture because we were able to take cross sectional picture of only a few particles. Recently, coaxial ultrasonic atomizer has been used to prepare micro/nanoparticles of pharmaceuticals, however, to the best of our knowledge, this is the first study revealing the cross sectional view of these particles. This study was conducted using only one flow rate of the liquids. We are now conducting experiments to optimize parameters, mainly flow rate, to obtain either hollow or solid spheres. The primary goal of our future experiments is to study the effect of flow rate of liquids 1 and 2 on the internal morphology of the particles. We believe that development of formulation consisting of hollow particles will have tremendous benefit for the pulmonary delivery of pharmaceuticals due to their smaller aerodynamic diameter.

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