Encapsulation and Sustained Release of a Model Drug, Indomethacin, Using CO_2-Based Microencapsulation

H. Liu, N. Finn, and M. Z. Yates*

Department of Chemical Engineering, University of Rochester, Rochester, New York 14627-0166

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A carbon dioxide (CO_2)-based microencapsulation technique was used to impregnate indomethacin, a model drug, into biodegradable polymer nanoparticles. Compressed CO_2 was emulsified into aqueous suspensions of biodegradable particles. The CO_2 plasticizes the biodegradable polymers, increasing the drug diffusion rate in the particles so that drug loading is enhanced. Four types of biodegradable polymers were investigated, including poly(D,L-lactic acid) (PLA), poly(D,L-lactic acid-*co*-glycolic acid) (PLGA) with two different molar ratios of LA to GA, and a poly(D,L-lactic acid-*b*-ethylene glycol) (PLA-PEG) block copolymer. Biodegradable nanoparticles were prepared from polymer solutions through nonsolvent-induced precipitation in the presence of surfactants. Indomethacin was incorporated into biodegradable nanoparticles with no change of the particle size and morphology. The effects of a variety of experimental variables on the drug loadings were investigated. It was found that the drug loading was the highest for PLA homopolymer and decreased in PLGA copolymers as the fraction of glycolic acid increased. Indomethacin was predicted to have higher solubility in PLA than in PLGA based on the calculated solubility parameters. The drug loading in PLA increased markedly as the temperature for impregnation was increased from 35 to 45 °C. Drug release from the particles is a diffusion-controlled process, and sustained release can be maintained over 10 h. A simple Fickian diffusion model was used to estimate the diffusion coefficients of indomethacin in the biodegradable polymers. The diffusion coefficients are consistent with previous studies, suggesting that the polymer properties are unchanged by supercritical fluid processing. Supercritical CO_2 is nontoxic, easily separated from the polymers, can extract residual organic solvent, and can sterilize biodegradable polymers. The CO_2-based microencapsulation technique is promising for the production of drug delivery devices without the use of harmful solvents.

Introduction

Encapsulation of drugs within colloidal-sized polymer particles can provide sustained release, reduce the side effect of the drugs, and increase their bioavailability.[1] Through accurate control over the particle size and the particle surface properties, nanoparticles may be injected intravenously and directed to specific sites to achieve targeted drug delivery.[2,3] As compared to discovering new drugs, designing novel drug delivery systems for existing effective drugs costs much less money and may be developed in a shorter time.[4] Therefore, microencapsulation processes have attracted a great deal of research attention. Many techniques can be used for drug microencapsulation. Yet most conventional microencapsulation techniques, such as the widely studied emulsification/solvent evaporation method[5] and its modified versions,[6] rely on organic solvents to mix the polymer and the additive to be encapsulated.[5,7,8] Organic solvents are undesirable for drug delivery, and residual solvent must be removed to low levels prior to delivery to avoid harmful health effects. In addition, current techniques typically rely on emulsification to create particles and thus provide limited control over the particle size and size polydispersity.

Recently, we have developed a new microencapsulation technique that we refer to as "CO_2-based microencapsulation".[9] In this process, CO_2 is used as a plasticizer to facilitate mass transport into aqueous polymer colloids. Swelling of the polymer induced by liquid or supercritical CO_2 causes the diffusion rate in the polymer to be enhanced, facilitating the entry of additives into the particles. After impregnation, CO_2 is vented off and the particles return to their original size, entrapping the microencapsulated additive. This technique decouples the particle formation and microencapsulation steps, providing improved control of particle size and polydispersity. Surface modification of the particles with surfactant is an integral part of the process and allows control of the surface functionality of the particles.[10] When compared to traditional microencapsulation approaches, harmful organic solvents are replaced by CO_2, which is nontoxic and easily removed. Our initial studies demonstrated the viability of the process by encapsulating dyes into polystyrene colloids. It is the goal of the present study to investigate the applicability of CO_2-based microencapsulation to biodegradable colloids typically used in drug delivery, such as poly(lactic acid) (PLA) and poly(lactic acid-*co*-glycolic acid) (PLGA). The CO_2-based microencapsulation technique has potential for producing drug delivery devices for targeted delivery and controlled release without the use of harmful solvents.

* Corresponding author. E-mail: myates@che.rochester.edu.

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PLA and PLGA are the most widely used synthetic biodegradable polymers in drug delivery.[11] They are used in implantable devices,[12] and nanoparticle suspensions are used for injection.[6,13] Colloidal suspensions of biodegradable polymers may be formed by emulsification/solvent evaporation, nanoprecipitation, or coacervation.[14] The surfaces of biodegradable particles are often functionalized with poly(ethylene glycol) (PEG) to reduce protein adsorption and increase the circulation time in the body after intravenous administration.[15,16] Surface adsorption of poly(ethylene oxide) (PEO)/polypropylene oxide (PPO) block copolymer surfactants is one convenient and commonly used route to PEG functionalization of particles.[17] The hydrophilic PEG chains can also be covalently attached to biodegradable polymer chains to form amphiphilic block or graft copolymers. The hydrophilic PEG block is attached to a hydrophobic block comprised of a biodegradable polymer such as PLA,[18] PLGA,[19] poly(ϵ-caprolactone) (PCL),[19,20] or polyanhydride.[19] The block copolymers self-assemble into core−shell structured nanoparticles in selective solvents without requiring additional surfactants.[15,21,22] The core−shell structure of PLA-PEG nanoparticles in an aqueous environment has been confirmed using NMR.[23] The inherent core−shell structure is favorable in targeted drug delivery. The hydrophobic biodegradable core acts as a reservoir for the drug. The hydrophilic PEG shell provides steric stabilization and dictates the biodistribution of the carrier.[2,24]

Compressed CO_2 plasticizes a variety of polymers and can enhance diffusion of additives into the polymer phase.[25−27] Plasticization with CO_2 has been used to impregnate biodegradable polymer monoliths with drugs,[28] and to mix proteins into biodegradable foams.[29] In this paper, we applied the CO_2-based microencapsulation technique to impregnate a hydrophobic drug, indomethacin, into biodegradable nanoparticles. Four types of biodegradable polymers were used, including PLA, PLGA with two different molar ratios of lactic acid to glycolic acid, 75/25 and 50/50, respectively, and a PLA-PEG block copolymer. Aqueous suspensions of biodegradable nanoparticles were made by a modified nanoprecipitation technique.[30] Liquid or supercritical CO_2 was emulsified into the aqueous suspension to facilitate diffusion of the drug into the biodegradable polymer particles.

Experimental Section

Materials. Poly(D,L-lactide) (PLA; inherent viscosity 0.63 dL/g, ~91 000 g/mol) and poly(D,L-lactide-*co*-glycolide) (PLGA), with different molar ratios of lactic acid(LA)/glycolic acid(GA) (75/25, inherent viscosity 0.69 dL/g, ~107 000 g/mol and 50/50, inherent viscosity 0.59 dL/g, ~84 000 g/mol, respectively), were purchased from Birmingham Polymers, Inc. Indomethacin (99%) was purchased from Sigma. Poly(ethylene glycol) methyl ether (MePEG), D,L-lactide, Brij 78, and stannous octoate (tin(II) 2-ethylhexanoate) were purchased from Sigma-Aldrich. The poly(ethylene oxide)/polypropylene oxide (PEO/PPO) Pluronic block copolymer (F108, $(PEO)_{129}$-$(PPO)_{58}$-$(PEO)_{129}$) surfactant was donated by BASF. Acetone (HPLC grade) was bought from Mallinckrodt. Dichloromethane (HPLC grade) was purchased from Burdick & Jackson. Ethyl ether (anhydrous), ethyl acetate (HPLC grade), and sodium dodecyl sulfate (SDS) were purchased from J. T. Baker. Carbon dioxide (SFC/SFE grade) was obtained from Air Products and Chemicals, Inc. Deionized water was used in the experiments. The MePEG was purified by extraction from aqueous solution by dichloromethane, followed by precipitation into an excess of diethyl ether. D,L-Lactide was recrystallized twice from dried ethyl acetate. The purified MePEG and D,L-lactide were dried in a vacuum oven and then stored in a desiccator. All other chemicals were used as received.

Preparation of Biodegradable Colloids. Biodegradable nanoparticles were prepared by a modified nanoprecipitation method from bulk biodegradable polymers.[30] Typically, 0.067 g of biodegradable polymer was dissolved in 10 mL of acetone to produce a polymer solution. Next, 0.40 g of surfactant was dissolved in 20 mL of water to make a surfactant solution. For the system of PLA and F108, the molar ratio of F108 to PLA is about 37.2, and the molar ratio of F108 to water is about 0.000025. The polymer solution was added to the surfactant solution at 0.5 mL/min with a syringe pump and stirred gently. After addition, the mixture was stirred vigorously for 4 h to allow for evaporation of the organic solvent. The resulting latex was used directly in microencapsulation.

Microencapsulation. The CO_2-based microencapsulation process is similar to that reported in our earlier study.[9] First, 10 mL of polymer latex made by nanoprecipitation and 0.020 g of drug were added to a stainless steel variable-volume cell (SC Machining, 28 mL maximum volume). The cell was sealed, and then 3.0 g of CO_2 was added via a computer-controlled high-pressure syringe pump (ISCO, Inc., model 260D). The cell was maintained at 310 bar and specified temperature for 24 h under continuous stirring. After slow release of CO_2 over about 5 h, the latex was passed through a filter of 1 μm pore size to remove most of the excess drug. Light-scattering measurement showed that the filtration had no effect on the particle size and size polydispersity. The latex was washed by two redispersion/centrifugation cycles, and 10 mL of water was used each time. After being washed, the particles were dried in a desiccator at 4 °C, followed by drying in a vacuum oven at 50 °C for several hours.

Synthesis of PLA-PEG Block Copolymers. A PLA-PEG diblock copolymer with a PEG chain length of 5 kDa was synthesized by the ring opening polymerization of D,L-lactide using a stannous octoate catalyst.[31] First, 3.0 g of D,L-lactide and 0.15 g of MePEG were added to a dried Schlenk tube, and then 0.0027 g of stannous octoate was added as a 3% w/v solution in toluene. The reactants were dried at 70 °C for 0.5 h under reduced pressure. The tube was sealed under vacuum, and the copolymerization was carried out at 170 °C for 5 h. After polymer-

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ization, the product was dissolved in dichloromethane and precipitated into an excess of methanol. The purified copolymers were dried in a vacuum oven at 50 °C for 2 days and then stored in a desiccator under argon. The weight ratio of PLA to PEG in the copolymer was determined via ^{1}H NMR.

Drug Loading Measurement. Drug loadings were determined by ^{1}H nuclear magnetic resonance spectroscopy (NMR, Bruker Avance-400) in $CDCl_3$. The sample was made by dissolving 8 mg of dried indomethacin loaded polymer particles in about 1 mL of $CDCl_3$. The ^{1}H NMR spectrum of the sample shows three strong singlet peaks from indomethacin (3.85, 3.72, and 2.42), one multiple peak from PLA (5.21), and one multiple peak from PGA (4.83). The drug loading is determined on the basis of their relative integrated peak intensities.

Particle Size and Morphology. The hydrodynamic particle diameter and polydispersity were determined by dynamic light scattering with a 90 plus particle size analyzer (Brookhaven Instruments) using cumulant analysis for polydispersity determination. All measurements were performed at a temperature of 25 °C at a measuring angle of 90° to the incident beam. The morphology of the biodegradable particles was characterized by transmission electron microscopy (TEM) (Hitachi H-7100 electron microscope), following negative staining with phosphotungstic acid solution (2 wt %).

Electrophoretic Measurement. Zeta potential measurements were performed at 25 °C using a phase analysis light-scattering (PALS) zeta potential analyzer (Brookhaven Instruments). The samples were diluted with 0.001 M aqueous KCl solution to maintain a constant ionic strength during zeta potential measurement.

Drug Release Study. The drug release was investigated following a method reported by Peracchia et al.[19] First, 0.060 g of dried biodegradable nanoparticles loaded with indomethacin was dispersed in 5 mL of pH 7.0 phosphate buffer (PB) with a buffer concentration of 0.010 M. The dispersion was added to a dialysis bag, which was placed in 25 mL of the PB at 37 °C. Samples were taken from the outer solution, and then replaced with the same amount (25 mL) of fresh PB each time. The amount of drug released from the polymer was measured with a UV−vis spectrometer (Perkin Elmer Lamda 900 UV−vis spectrometer) at 260 nm.

Degradation of Polymers. The degradation of biodegradable polymer nanoparticles was investigated by using the enzymatic/colorimetric method to measure the amount of released lactic acid. Lactate reagent (Cat. No. 735-10) and lactate standard (Cat. No. 826-10) were purchased from Sigma Diagnostics. Lactic acid causes the reagent to produce a colored dye with an absorption peak at 540 nm. The absorbance at 540 nm is directly proportional to lactic acid concentration in the sample. Thus, the concentration of lactic acid in the sample can be obtained by comparing the absorption of the sample to that of the lactate standard.

All of the experiments were repeated at least twice, and the average values are reported.

Results and Discussion

Biodegradable Nanoparticles. The biodegradable nanoparticles were made by the nanoprecipitation technique.[30] A water-miscible solvent, acetone, was used to dissolve the biodegradable polymers. The resulting polymer solution was added to an aqueous surfactant solution to precipitate particles. Interfacial turbulence created between the organic phase and the aqueous phase due to the spontaneous diffusion of acetone leads to the formation of very small particles.[6] Figure 1A is an electron micrograph of a representative selection of the PLA nanoparticles made by nanoprecipitation. The concentration of PLA solution was 0.67% w/v, and 0.40 g F108 was used to stabilize the particles during nanoprecipitation. The TEM images of PLGA and PLA-PEG nanoparticles are not shown, because the particles appear nearly identical. The long large structures on the TEM images are cracks resulting from drying of the sample on TEM grids. All of the biodegradable polymers form spherical, discrete particles with smooth surfaces after nanoprecipitation into the aqueous media.

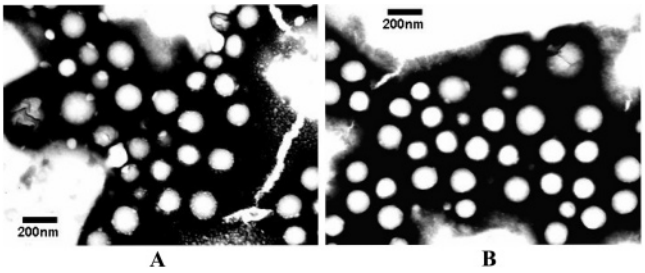

Figure 1. TEM graphs of PLA nanoparticles negatively stained with phosphotungstic acid solution (2 wt %): (A) before microencapsulation, (B) after microencapsulation with indomethacin (2.62 wt %). (The long large structures are cracks resulting from drying of the sample on TEM grids.)

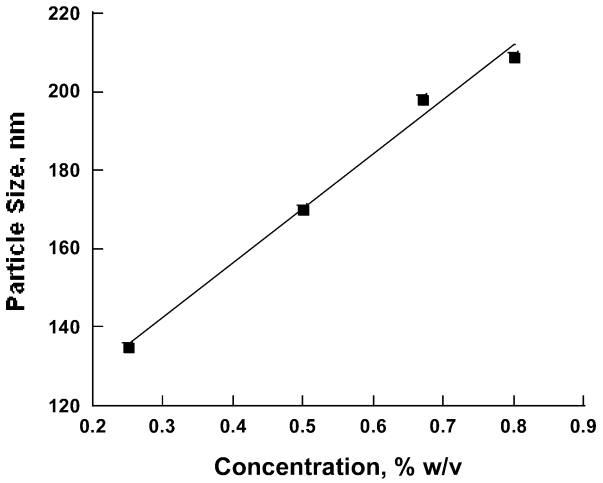

Figure 2. Dependence of the particle size of PLA nanoparticles on the concentration of PLA solution (0.40 g of F108 was used in nanoprecipitation).

The concentration of the polymer solution is the most important factor determining the particle size and size polydispersity. Figure 2 shows the effect of polymer solution concentration on the particle size and size polydispersity of PLA nanoparticles when 0.40 g of the surfactant F108 were used in nanoprecipitation. The particle size increases almost linearly with increasing concentration. The size polydispersity of all of the samples was around 0.070 and did not change significantly in the concentration range used in the experiments. Studies have shown that for nanoparticles administrated intravenously into rats, a diameter of less than 200 nm is required to avoid spleen filtering effects.[32] Therefore, 0.67% w/v concentration was chosen to make biodegradable nanoparticles for microencapsulation.

The amount and type of surfactant also play a significant role in determining particle size and size polydispersity during nanoprecipitation. Three kinds of surfactants, SDS, Brij 78, and F108, were chosen to study the effect of surfactants on the particle size. The results are shown in Table 1. The low molecular weight anionic surfactant, SDS, did not work as effectively as the nonionic surfactants in minimizing particle aggregation during nanoprecipitation. With SDS, the PLA particle size and the size polydispersity are both larger than those obtained with the same amount of nonionic surfactant. SDS molecules can be absorbed on the biodegradable polymer particles

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Table 1. Effect of Surfactants on Particle Size and Drug Loading[a]

no.	surfactant used in nano-precipitation	particle size, nm	poly-dispersity	surfactant added before microen-capsulation	drug loading, wt %
1	SDS, 0.20 g	289	0.112	F108, 0.20 g	0.59
2	F108, 0.10 g	312	0.070	SDS, 0.10 g	0.40
3	F108, 0.20 g	197	0.065	none	1.30
4	F108, 0.20 g	200	0.050	SDS, 0.20 g	1.12
5	F108, 0.40 g	198	0.071	none	2.62
6	Brij 78, 0.20 g	190	0.067	F108, 0.20 g	1.53

[a] Polymer, PLA, 0.67% w/v; indomethacin, 0.020 g; pressure, 310 bar; temperature, 35 °C; time, 24 h.

through hydrophobic interactions between the hydrocarbon chain of SDS and the PLA particle surface. The PLA, PLGA, and PLA-PEG particles have a negative surface charge resulting from the ionized carboxyl end groups of PLA or PGA chains on the particle surface.[33] Adsorption of the anionic surfactant can increase the surface charge density and result in electrostatic stabilization of the PLA particles. However, the repulsion between the negative surface charges and the anionic end groups of SDS may also inhibit adsorption. Low surfactant adsorption during the early stages of nanoprecipitation will allow particle aggregation and result in the larger, more polydisperse particles that were observed.

For the nonionic polymeric surfactant F108, using 0.10 g resulted in PLA particles about 310 nm in diameter. The particle diameter decreased to about 200 nm when the amount of F108 was increased to 0.20 g. Further increasing the F108 to 0.40 g had little effect on the particle size. The size polydispersity was similar for all three samples produced using F108. The low molecular weight nonionic surfactant, Brij 78, resulted in PLA particles with a size and polydispersity similar to that obtained with F108 when 0.20 g of surfactant were used.

CO_2-Based Microencapsulation. The aqueous latex formed by nanoprecipitation was used directly in microencapsulation experiments. To verify the function of CO_2 in aiding drug microencapsulation, two control experiments were conducted using 10 mL of the PLA latex produced using 0.40 g of the surfactant F108. The first experiment was carried out without CO_2 and at atmospheric pressure. The other one was performed in the presence of CO_2 and at 310 bar. In both experiments, 0.020 g of indomethacin was added to the aqueous PLA latex, and the mixture was maintained at 45 °C for 24 h. The drug loading in the absence of CO_2 was 1.11 wt %, and the drug loading in the presence of CO_2 was 6.59 wt %. Even without CO_2, some of the indomethacin can be adsorbed onto the surface of the PLA particles due to hydrophobic interactions. However, the drug loading with CO_2 is much higher, indicating that plasticization of PLA by CO_2 enhances the transport rate of the drug into the interior of the polymer particles. CO_2-induced plasticization of PLA has been employed by others to mix additives into the polymer to create composite foams[34] and microparticles.[35] Our results show that the CO_2 enhances the diffusion of indomethacin into the biodegradable polymer colloids.

Figure 1B shows the PLA particles after microencapsulation of indomethacin using the CO_2-based microencapsulation technique at 310 bar and 35 °C for 24 h. Comparing Figure 1B to Figure 1A shows that the microencapsulation process does not affect the particle morphology or size. Dynamic light-scattering measurements on the aqueous latex give an average diameter before and after microencapsulation of 198 and 201 nm, respectively. The polydispersity of the latex obtained from cumulant analysis before and after microencapsulation was 0.072 and 0.075, respectively. The light-scattering and electron microscopy results indicate no significant change in particle size or polydispersity after microencapsulation. The results confirm that the swelling of the PLA particles with compressed CO_2 is reversible and that colloidal stability of the biodegradable latex is maintained throughout the microencapsulation process. Analysis of the PLA degradation showed that less than 0.1% of the polymer had degraded throughout the entire process, including nanoprecipitation and microencapsulation. Drugs can thus be encapsulated into biodegradable particles using the CO_2-based technique with no significant change in particle size, morphology, or polymer properties.

Effect of Surfactant. Upon exposure to compressed CO_2, the polymer particles become highly plasticized and will coalesce during microencapsulation if colloidal stability is not maintained.[10] The surfactant adsorbed on the particle surface must be effective at maintaining colloidal stability. A surfactant must also be present to stabilize the emulsion of CO_2 in water to increase the interfacial area and enhance the microencapsulation rate.[9] A second surfactant can be added to the latex prior to microencapsulation to stabilize the CO_2-in-water emulsion. Our previous results have shown that, in many cases, a single surfactant can function to both stabilize the polymer colloids and stabilize the CO_2-in-water emulsion, so that two surfactants are not always necessary.[10]

Several different surfactants were examined for encapsulating indomethacin into PLA particles at 35 °C. Table 1 gives the amounts of indomethacin encapsulated into PLA using different surfactants. For entries 3 and 5, no additional surfactant was added after nanoprecipitation, so the surfactant F108 functioned to stabilize both the latex particles and the CO_2-in-water emulsion. In the other experiments, a second surfactant was added to the latex prior to microencapsulation to enhance emulsification of CO_2 into water. During microencapsulation, the emulsion was formed by continuous stirring of the contents in the pressure cell using a magnetically coupled stir bar. Emulsification of CO_2 into water is critical to increase the encapsulation efficiency. For entries 1, 2, 3, and 4 in Table 1, there was an excess CO_2-rich phase visible at the top of the view cell, indicating incomplete emulsification. For entries 5 and 6, there was complete emulsification. The encapsulation efficiency was the highest with complete emulsification of CO_2. The difference in drug loadings between entries 1, 2, 3, and 4 can be partially attributed to the different particle sizes. Our earlier studies have shown that smaller particles have higher drug loadings due to a higher transport rate resulting from larger surface area per unit mass.[9]

The efficacy of the surfactant in stabilizing the CO_2-in-water emulsion is the highest for F108 and decreases in the order F108 > Brij 78 > SDS according to visual observation. When SDS is used alone, very little CO_2 can be emulsified into water, and the resulting emulsion is unstable and rapidly breaks up when stirring is stopped. It was postulated that better encapsulation efficiency could be achieved by using a low molecular weight surfactant such as SDS or Brij 78 to stabilize the particles, and the polymeric surfactant, F108, to stabilize the CO_2-in-water emulsion. If F108 is adsorbed onto the particle surface,

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Table 2. Drug Loading in Different Biodegradable Polymers[a]

polymer	molecular weight	particle size, nm	zeta potential, mV	drug loading @ 25 °C, wt %	drug loading @ 35 °C, wt %	drug loading @ 45 °C, wt %
PLA	91 000	198	−16.37	2.03	2.62	6.59
PLGA (75/25)	107 000	197	−15.96		2.58	4.98
PLGA (50/50)	84 000	192	−16.43		1.52	4.26
PLA-PEG	110 000	170	−12.32			2.43

[a] Indomethacin, 0.020 g; surfactant, F108, 0.40 g; pressure, 310 bar; time, 24 h.

it imparts a thick steric layer that may act as a barrier for drug transport. Lower molecular weight surfactants may provide less of a barrier. However, Table 1 shows that the addition of SDS was detrimental to the encapsulation efficiency. In entry 4, 0.20 g of F108 was used to stabilize particles during nanoprecipitation and 0.20 g of SDS was added to exchange some F108 on the particle surface to reduce the transport barrier. The amount encapsulated in entry 4 was less than that of entry 3, where 0.20 g of F108 was used alone for both particle and emulsion stabilization. Better results were obtained when the low molecular weight nonionic surfactant Brij 78 was used for particle stabilization in conjunction with F108 for emulsification. In entry 6, 0.20 g of Brij 78 was used for nanoprecipitation of PLA particles and 0.20 g of F108 was added to enhance emulsification of CO_2. The indomethacin loading is slightly higher than that obtained using 0.20 g of F108 alone. However, the total amount of surfactant used in entry 6 is 0.40 g, and the drug loading is significantly lower than that when 0.40 g of F108 was used alone (entry 5). It should also be noted that it is not possible to completely separate the functions of the two surfactants when mixtures are used. During microencapsulation, the surfactant used for nanoprecipitation may desorb from the particle surface, and likewise the surfactant added during microencapsulation may adsorb onto the particle surface. For a given total amount of surfactant, the best overall performance was achieved using F108 alone.

For practical use of carbon dioxide-based microencapsulation in drug delivery, the choice of surfactant and the residual surfactant content are important. The surfactants Pluronic F108, Brij 78, and SDS all have low toxicity. F108 and SDS are FDA approved for drug-related applications. The particles were also washed two times with deionized water to remove excess surfactant.

Drug Incorporation into Different Biodegradable Polymers. Table 2 lists the drug loadings for several different biodegradable nanoparticles. The microencapsulation was carried out for 24 h at 310 bar with the initial drug amount of 0.020 g. The results show that indomethacin was encapsulated into all four kinds of biodegradable polymer colloids using the CO_2-based microencapsulation technique. The loading is the highest for PLA particles and decreases in PLGA as the molar ratio of glycolic acid to lactic acid increases. The trend in drug loading is sustained at both 35 and 45 °C. It appears that indomethacin has a higher affinity for lactic acid moiety than glycolic acid.

Solubility parameters can be used to estimate the compatibility of a drug and a polymer.[36] The solubility parameter is defined as the square root of a molecule's cohesive energy density.[37] The compatibility between drug and polymer increases when their solubility parameters get closer. The solubility parameter has three components, δ_d, δ_p, and δ_h, representing contributions from van der Waals dispersion forces, dipole−dipole interactions, and hydrogen bonding, respectively. The partial solubility parameters (δ_d, δ_p, δ_h) can be calculated using the group contribution method.[38] The overall solubility parameter is obtained from the partial solubility parameters using the following equation:

$$\delta = \sqrt{(\delta_d^2 + \delta_p^2 + \delta_h^2)} \quad (1)$$

Table 3. Solubility Parameters for Indomethacin, PLA, and PGA[a] (Mpa$^{1/2}$)

	δ_d	δ_p	δ_h	δ
PLA	17.6	9.7	11.8	23.3
PGA	19.4	14.4	14.3	28.0
indomethacin	21.4	5.8	9.2	24.0

[a] Calculated using the group contribution method.

The solubility parameters of PLA, PGA, and indomethacin were calculated using the group contribution method, and the results are listed in Table 3. The calculated solubility parameters of PLA and PGA are the same as those reported by Liu et al.,[39] while the result for indomethacin is slightly larger than that reported by Forster et al.[40] Both previous studies used the group contribution method. From Table 3, the solubility parameter of indomethacin is closer to the solubility parameter of PLA than to that of PGA, indicating indomethacin has better compatibility with PLA. The calculated solubility parameters are consistent with our experimental result that PLA nanoparticles have higher drug loading than PLGA.

The drug loading is the lowest for the PLA-PEG particles, possibly due to the dense surface coverage of PEG. PLA-PEG is the only one of the selected biodegradable polymers that does not need surfactants added to form a stable latex during nanoprecipitation, because the amphiphilic block copolymers self-assemble in water to form core−shell structured particles. Even though PLA-PEG does not need a surfactant to maintain colloidal stability, some additional surfactant is needed to stabilize the emulsion of CO_2 in water during microencapsulation. To enable direct comparison of microencapsulation with the different biodegradable particles, 0.40 g of the surfactant F108 was used in making all of the biodegradable polymer particles listed in Table 2, including PLA-PEG. The surfactant F108 adsorbs on PLA and PLGA particle surfaces through hydrophobic interaction and provides steric stabilization. The F108 can also adsorb onto PLA patches on the surface of PLA-PEG nanoparticles, giving the PLA-PEG particles a denser PEG surface coverage. Table 2 shows that PLA-PEG particles have a lower zeta potential than PLA and PLGA. As stated before, the negative zeta potentials are due to ionized carboxyl end groups of the PLA or PGA chains on the nanoparticle surface. The lower zeta potential of PLA-PEG nanoparticles is indicative of denser coverage of nonionic PEG chains on the particle surface, which shifts the surface of shear outward and thus lowers the zeta potential. The

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Table 4. Effect of Initial Drug Amount[a] on Drug Loading

initial drug amount, g	drug loading, wt %
0.010	2.28
0.020	2.62
0.040	2.39

[a] Polymer, PLA; surfactant, F108, 0.40 g; pressure, 310 bar; temperature, 35 °C; time, 24 h.

hydrophilic PEG shell can act as a transport barrier for the drug entering the polymer particles. Because the PEG chains on the PLA-PEG particles likely have higher surface coverage, the diffusion rate of the drug entering polymer particles is low, resulting in lower drug loading after the 24-h microencapsulation process.

Effect of Temperature. For all polymers, the drug loading increases as the temperature is increased, as seen in Table 2. Increasing temperature can improve the kinetics of the drug diffusion in the polymer phase because of the temperature dependence of diffusion. Yet the large increase in drug loading from 35 to 45 °C implies that the polymer itself has undergone a significant change. Studies have shown that near ambient temperature (35 °C) and modest pressure (200 bar) are sufficient for CO_2 to plasticize PLA and PLGA.[34] The rheological behavior of a PLA/CO_2 mixture at different temperatures and pressures was investigated by an indirect method, and it was found that the viscosity of the mixture decreased as the temperature and pressure were increased but did not change significantly above 35 °C and 88 bar.[35] CO_2 swelling causes the glass transition temperature to be suppressed. Other researchers have shown that CO_2 can significantly reduce the melting temperature of polymers.[41] If PLA undergoes a transition from a rubber to a liquid between 35 and 45 °C, it would explain the large increase in drug diffusion into the polymer phase. Additional experimental work is needed with bulk polymer samples to verify this hypothesis.

Effect of Initial Amount of Drug. Microencapsulation using initial amounts of indomethacin ranging from 0.010 to 0.040 g resulted in no significant change in the drug loading in PLA particles, as shown in Table 4. Indomethacin is a hydrophobic drug, and its solubility in water is 0.05 mg/mL at 25 °C. 0.010 g of indomethacin is well above the solubility limit in 10 mL of water, even at 35 °C. The driving force for indomethacin to diffuse into the polymer phase is the partition coefficient, or the equilibrium solubility of the drug in the polymer versus the equilibrium solubility in water. Because the water is saturated with drug, even at the lowest concentration studied, the drug loading does not change as the concentration is increased. It is possible that after 24 h the biodegradable nanoparticles are saturated with drug and are in equilibrium with the excess solid drug, but the equilibrium solubility of the drug in the polymer is unknown under the conditions used for microencapsulation.

Drug Release Kinetics. The drug-containing particles obtained by microencapsulation at 45 °C were used for examining drug release in vitro. The drug release profiles are shown in Figure 3. For PLA and PLGA, nearly all of the drug was released within 12 h and their drug release profiles are almost the same. Peracchia et al.[19] studied the degradation of PLGA-PEG nanoparticles and found that less than 25% of the polymer degraded after 16 days. PLA and PLGA should have a lower degradation rate because they are less hydrophilic than PLGA-PEG. The

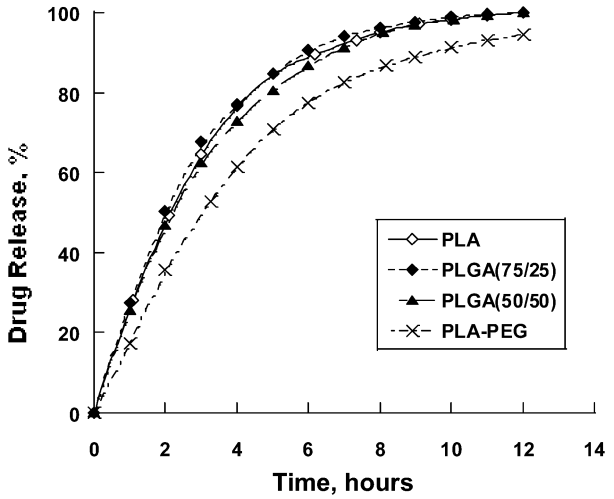

Figure 3. Drug release kinetics of biodegradable nanoparticles.

drug release is a diffusion-controlled process because the release rate is much faster than the degradation rate of the biodegradable polymers. About 50% of the loaded drug was released in 2 h. The initial burst release of drug is not as pronounced as that reported by Peracchia et al.[19] for PLA and PLGA particles. It is possible that the polymeric surfactant adsorbed on the particle surface acts as a barrier to diffusion and suppresses the burst release. The washing of particles after microencapsulation may also have reduced the burst release by removing surface adsorbed drug.

PLA-PEG has a lower drug release rate as compared to PLA and PLGA perhaps due to the denser hydrophilic PEG layer on the particle surface. Peracchia et al. also observed a lower drug release rate in PLA-PEG than in PLA when lidocaine hydrochloride was used as the model drug.[19] Yet the opposite trend was reported by Park et al.[42] when they studied the release of erythromycin estolate from PLA and PLA-PEG microparticles made by the emulsion solvent evaporation technique. The interaction between the drug and the polymer matrix plays a role in the drug release process. Ideally, the initial burst is suppressed and the drug is released at a constant rate. In this sense, hydrophilic coating of the PEG chains on the biodegradable nanoparticles is desirable for drug release. In addition, PEG surface coatings have been shown to increase the blood circulation time of biodegradable nanoparticles by reducing protein adsorption and increasing the stability of the particles in the body.[15,16]

Mathematical Modeling of Drug Release. Because the polymer does not significantly degrade during the time that drug is released, it can be assumed that the drug release is a diffusion-controlled process. The release profile was modeled using Fick's second law of diffusion. The following assumptions were made: (1) spherical particles; (2) constant diffusion coefficients; (3) perfect sink conditions; and (4) a uniform initial drug concentration that is smaller than the solubility of the drug within the system. The following analytical solution of Fick's second law characterizes drug release in this case:[43]

$$\frac{M_t}{M_\infty} = 1 - \frac{6}{\pi^2}\sum_{n=1}^{\infty}\frac{1}{n^2}\exp\left(-\frac{Dn^2\pi^2 t}{r^2}\right) \qquad (2)$$

where M_t and M_∞ represent the cumulative absolute

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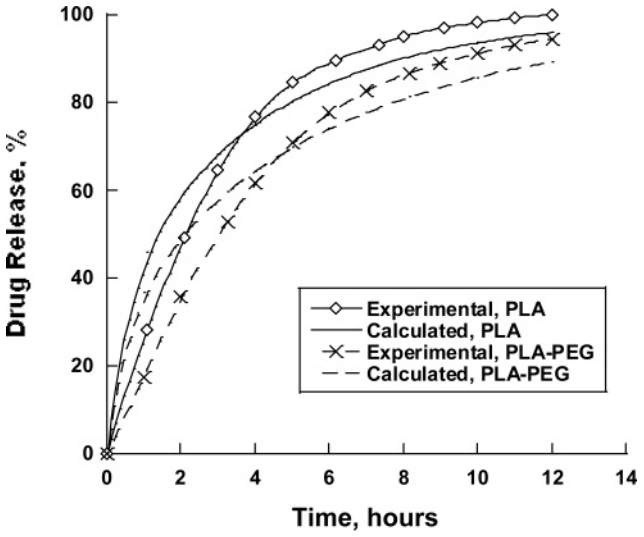

Figure 4. Fit of the mathematical model to the experimentally determined drug release from indomethacin-loaded PLA (solid curves) and PLA-PEG (dashed curves) nanoparticles.

amount of drug released at time t and at infinite time, respectively, D denotes the diffusion coefficient of indomethacin, and r is the radius of the biodegradable nanoparticles.

Equation 2 was fitted to experimentally determined drug release rates from the biodegradable nanoparticles. The results for PLA and PLA-PEG particles are shown in Figure 4. In every case, the theoretical fit overestimates released drug at early times and underestimates the released drug at later times. The polymer particles may be swelling in water and degrading slightly, so that the release rate is accelerating with time. While this simplistic model does not fully capture the release kinetics, the best theoretical fit gives a rough estimate of the diffusion coefficient of indomethacin in the polymers. The estimated diffusion coefficients of indomethacin in PLA, PLGA (75/25), PLGA (50/50), and PLA-PEG particles are 2.25×10^{-16}, 2.35×10^{-16}, 1.98×10^{-16}, and 1.06×10^{-16} m^2/s, respectively. The estimated diffusion coefficients are in reasonable agreement with that reported by Faisant and co-workers.[44] The lower diffusivity in PLA-PEG particles is attributed to the higher transport barrier due to denser hydrophilic PEG coverage on the surface.

Conclusions

The hydrophobic drug, indomethacin, has been successfully incorporated into PLA, PLGA (75/25 and 50/50), and PLA-PEG biodegradable polymer nanoparticles with a CO_2-based microencapsulation technique. Compressed CO_2 plasticizes biodegradable polymers and increases the transport rate of the drug in the polymer phase, resulting in up to 6.6 wt % drug in the polymer particles under the conditions studied. The biodegradable nanoparticles made by nanoprecipitation were spherical, and their particle size and morphology did not change during the microencapsulation process. The technique offers a solvent-free alternative to drug microencapsulation with excellent control of particle size and morphology. Compressed CO_2 has many advantages as a solvent in drug delivery applications: it is nontoxic, is effective at removing residual solvent from biodegradable particles,[45,46] and sterilizes polymers.[47] Exposure to compressed CO_2 may aid in removing the residual solvent remaining in the particles after nanoprecipitation. Supercritical CO_2 also can kill bacteria and sterilize the biodegradable nanoparticles.[47] On the other hand, part of CO_2 dissolves into water and forms carbonic acid, reducing the pH of the CO_2 saturated aqueous solution. At 25 °C and 310 bar, the pH of the CO_2-in-water emulsion is about 3.[48] Indomethacin is stable under these conditions, but the stability in the presence of carbonic acid should be considered when choosing additives for use in the CO_2-based microencapsulation process.

The drug loading in PLA nanoparticles is higher than those in PLGA nanoparticles, probably because the drug indomethacin has better compatibility with PLA than with PGA. Excess surfactant is necessary to protect particles from coagulation during nanoprecipitation, stabilize particles during microencapsulation, and enhance emulsification of CO_2 into water to achieve a high drug loading. The drug loading increases with increasing temperature, with a significant increase between 35 and 45 °C. The drug release from the biodegradable particles is a diffusion-controlled process. The hydrophilic PEO and PEG chains on the particle surface appear to reduce the drug release rate. PLA-PEG diblock copolymer particles have the slowest drug release profile, but the drug loading is also the lowest, likely due to the dense PEG surface coverage.

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