Poly(lactide)-poly(ethylene glycol) micelles as a carrier for griseofulvin

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Abstract: In this work, the feasibility to develop micelle carriers of griseofulvin based on PLA-PEG copolymers was investigated. With the use of the dialysis method of micelle formation, the micellization behavior of a range of PLA(*X*)-PEG(5) copolymers was investigated. At copolymer concentrations in the organic solvent 10–20 mg/mL, stable micelles with 100% yield could only be prepared from PLA(*X*)-PEG(5) copolymers with molar composition in the range 50–70% PEG. The copolymers exhibited sufficiently low CMC to provide stable micelles *in vivo*. The loading capacity of PLA(4)-PEG(5) micelles with griseofulvin was 6.5 mg of drug/1 g of copolymer. The release of griseofulvin from the PLA-PEG micelles *in vitro* in phosphate-buffered saline (PBS) was sustained over 30 days. No burst effect was observed. Analysis of the release kinetics suggested that the release was erosion-controlled. The release profile was biphasic. Micelle degradation data in PBS indicated that the second phase of release was induced by copolymer degradation. The PLA-PEG micelles of griseofulvin were stable in simulated gastric and intestinal fluids for a long-enough time for oral application. Overall, the PLA-PEG micelles have suitable properties to be considered as potential oral or topical formulations of griseofulvin, provided that the drug-loading capacity of the micelles is sufficiently improved. © 2005 Wiley Periodicals, Inc. J Biomed Mater Res 75A: 639–647, 2005

Key words: poly(lactide)-poly(ethylene glycol); micelles; griseofulvin; release; degradation

INTRODUCTION

Block copolymer micelles have been proposed for the delivery of hydrophobic drugs with low aqueous solubility. These micelles are formed in aqueous media by amphiphilic block copolymers such as poly(lactide)-poly(ethylene glycol) (PLA-PEG),[1–3] poly(ϵ-caprolactone)-poly(ethylene glycol) (PCL-PEG),[4–6] poly(aspartic acid)-poly(ethylene glycol),[7–9] poly(glutamic acid)-poly(ethylene glycol),[10] and poly(2-ethyl-2-oxazoline)-poly(ϵ-caprolactone).[11] The micelles have a hydrophobic core, which is formed by the association of the hydrophobic moieties and can accommodate the drug load, and a hydrophilic shell, which is formed by the hydrophilic segments (usually PEG) and confers important attributes to the carrier, such as colloidal stability,[12,13] long circulation (stealth) properties when injected intravenously,[14–16] and targeting possibility (by attaching appropriate ligands on PEG).[17,18] Besides the PEG steric barrier, the small size of these micelles also contributes to important micelle characteristics, such as the longevity in blood circulation.

Block copolymer micelles, unlike surfactant micelles, exhibit low values of critical micelle concentration (CMC),[15,19] and are thermodynamically stable even after severe dilution. This is clearly an advantage of the block copolymer micelles with regard to their application as drug carriers because the micelles will be stable and will not dump their drug content upon *in vivo* administration. A further advantage of these micelles is that they can be loaded with drugs during their preparation, using conventional micelle formation methods. A problem of low loading capacity may sometimes arise. However, this might be overcome by changing the copolymer composition,[5,6,11] by modifying the chemical structure of the core-forming polymer,[20] or by conjugating/complexing the drug to the polymer.[7–10] The properties and possible medical applications of block copolymer micelles have been reviewed.[21–24]

Griseofulvin is an important antifungal drug. It remains the drug of choice for tinea capitis in children.[25] Due to its very low aqueous solubility, griseofulvin exhibits a slow, erratic and incomplete absorption following oral administration.[26] Formulation strategies, such as preparation of solid dispersions,[27] association

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with bioadhesive polymers,[28] and incorporation into liposomes[29] have been adopted in an attempt to improve the bioavailability of oral griseofulvin. Formulations of griseofulvin based on *N*-methyl-2-pyrrolidone have been investigated for topical delivery of the drug.[30] This report investigates the feasibility of developing micelle carriers of griseofulvin, which might be useful for oral or topical delivery of the drug. Pegylated nanoparticles have been shown to be capable of prolonging the residence of their drug load in the intestinal tract, increasing the oral bioavailability of drugs.[31] Thus, the incorporation of griseofulvin in PLA-PEG micelles may increase its bioavailability after oral administration. Also, the incorporation of griseofulvin in PLA-PEG micelles may facilitate the preparation of topical formulations of griseofulvin, which is difficult, as griseofulvin dissolves poorly in both water and oil.[30] To this end, PLA-PEG diblock copolymers were prepared and characterized, and their micellization behavior was tested. The copolymer compositions resulting in stable micelles at high yield were used to prepare micelles loaded with griseofulvin. The loading, *in vitro* degradation and *in vitro* drug-release properties of the griseofulvin-loaded micelles were then studied.

MATERIALS AND METHODS

Materials

D,L-lactide (molecular weight: 144) was purchased from Boehringer Ingelheim (Germany). It was recrystallized twice from ethyl acetate and dried under high vacuum at room temperature before use. Monomethoxy-poly(ethylene glycol) (mPEG, several molecular weights) was obtained from Fluka (Switzerland) and dried under high vacuum at room temperature before use. Griseofulvin and stannous octoate were purchased from Sigma (St. Louis, MO). Tetrahydrofuran of HPLC grade and miscellaneous chemical reagents and solvents, all of analytical grade, were obtained from Sigma, Merck, and SDS. Ultrapure water, water purified by a Milli-Q plus System (Waters, Milford, MA), was used in the present work.

Synthesis and characterization of PLA-PEG copolymers

Poly(lactide)-monomethoxy poly(ethylene glycol) (PLA-PEG) copolymers of different composition (PLA/PEG molar ratio) were synthesized by a melt polymerization process under nitrogen, with stannous octoate used as a catalyst. The predetermined amounts of lactide and monomethoxy-poly-(ethylene glycol) were transferred to a three-necked round-bottom flask, which was connected to a continuous supply of dry nitrogen and was immersed in an oil bath (140°C). After monomer melting, the catalyst, at an initiator-to-catalyst molar ratio of 10, was added, and stirring of the reactants begun with the use of a propeller stirrer (IKA-WERK). After polymerization for 2 h, the product was dissolved in dichloromethane and precipitated in excess diethyl ether. The precipitated copolymer was collected by filtration and dried under vacuum.

The synthesized copolymers were characterized with regard to their composition by ^{1}H-NMR (a Bruker AMX-400 instrument was used to obtain the spectra of the samples dissolved in $CDCl_3$), and their molecular weight and molecular weight distribution (PI = M_w/M_n, polydispersity index) by gel-permeation chromatography (GPC). The GPC unit consisted of a Marathon II Rigas Labs pump, a Waters U6k injector, and a 7515A (ERC Inc.) differential refractometer. Three Waters μ-styragel columns (10^4, 10^5, and 10^6 Å) were connected to the apparatus. The mobile phase was tetrahydrofuran at a flow rate of 1 mL/min. The molecular weight of the synthesized copolymers was determined with the use of the universal calibration approach.[32] Polystyrene standards (Shodex SM-105) were used to prepare the calibration curve. Viscosity measurements of the polymers in tetrahydrofuran were carried out with the use of a Shott AVS-300 automated system with Ubbelohde-type viscometer, equipped with an automatic injection system (maximum error ± 0.03%) for *in situ* dilutions. The temperature was controlled with a thermally regulated water bath at 25°C.

The copolymers are referred to in the text as PLA(*X*)-PEG(*Y*), where *X* and *Y* designate the molecular weight (in kilodaltons) of the PLA and PEG block, respectively, as determined by ^{1}H-NMR.

Determination of critical micelle concentration

The critical micelle concentration (CMC) of PLA-PEG was determined by fluorescence and static light scattering (SLS) methods. In fluorescence experiments, the fluorescence emission spectra of pyrene (concentration $7 \times 10^{-7}M$) in the presence of various concentrations of PLA-PEG micelles at 25°C were recorded at an excitation wavelength of 334 nm with the use of a LS5OB (PerkinElmer) fluorometer. At least six measurements were performed at each copolymer concentration. The CMC determination with fluorescence is based on the fact that at CMC the ratio of the intensities at 373 nm (I_1) and 384 nm (I_3) undergoes a substantial decrease.[33] SLS experiments were carried out to measure the inverse light scattering at various PLA-PEG concentrations (0.02–0.6 g/L) in ultrapure water. All the light-scattering experiments were carried out in triplicate with the use of a thermally regulated (±0.1°C) spectrogoniometer, model SEM RD (Sematech), equipped with a He-Ne laser (633 nm). The refractive index increments dn/dc required for the interpretation of the static light scattering measurements were determined with the use of a Chromatic KMX-16 differential refractometer, operating at 633 nm.

Preparation and characterization of PLA-PEG micelles

PLA-PEG micelles loaded with griseofulvin were prepared by the dialysis method.[4] Briefly, PLA-PEG copolymer and griseofulvin were dissolved in tetrahydrofuran and the solution (50 mL) was transferred to a dialysis bag (molecular weight cutoff 3500) and dialyzed against 3 L of ultrapure water for 24 h. The dialysate water was changed after 10, 20, and 40 min; and 1, 2, 4, 6, 9, 12, and 24 h from the beginning of the dialysis. The micellar solutions obtained were filtered (0.45 μ) or centrifuged (5930 g) to remove nonentrapped drug (both procedures gave similar micelle loading results). Blank micelles were produced by the same method without adding griseofulvin at any stage of the preparation.

The hydrodynamic radius R_H of the micelles was determined using dynamic light scattering (DLS). The dynamic light scattering measurements were performed with the use of an RTG correlator (Sematech). The correlation functions were analyzed to the second order by the method of cumulants, the pinhole was 200 nm, and the scattering angle was 90°. The DLS measurements were conducted in ultrapure water at a micelle concentration of 60 mg/mL.

The basic physicochemical characteristics of the micelles were determined by SLS. The light-scattering experiments were carried out in triplicate with the use of a thermally regulated (±0.1°C) spectrogoniometer, model SEM RD (Sematech), equipped with a He-Ne laser (633 nm). Plots of the inverse scattering intensity versus copolymer concentration were used to calculate the molecular weight, the aggregation number, the radius of gyration, and the second virial coefficient of the micelles, as described previously.[34]

The loading of micelles with griseofulvin was determined with the use of fluorescence and UV spectroscopy measurements. The micelle solutions were diluted in tetrahydrofuran (final proportion of micelles:tetrahydrofuran 1:3 by volume) and the drug content was determined by measuring the fluorescence emission intensity at 412 nm with the use of a LS5OB (Perkin Elmer) fluorometer at an excitation wavelength of 338 nm. The drug content in the micelles:tetrahydrofuran (1:3) solutions was also determined by measuring the absorbance at 293 nm with a Hitachi U-2001 spectrophotometer. At 293 nm, griseofulvin exhibited a maximum (peak) and PLA-PEG caused no interference. Three samples of each micelle batch were used in loading determination.

Study of the release of griseofulvin from the PLA-PEG micelles

Micelle samples (2 mL), enclosed in dialysis bags (cellulose membrane, MW cutoff 12400, Sigma), were transferred to a 2-L beaker filled with phosphate-buffered saline (PBS, pH = 7.4), United States Pharmacopeia (USP XXIV) simulated gastric fluid (pH = 1.2), or USP simulated intestinal fluid (pH = 7.5). The bags were kept submerged in the liquid with a stainless-steel wire mesh. The beaker was put into a water bath (37°C).

A constant flow of the release medium in and out of the beaker was maintained by pumping release medium from a 25-L tank to the beaker at a rate of 1 mL/min with the use of an Ismatec (MIDI-vario) pump equipped with silicon tubing. The excess liquid in the beaker flew into the water bath. At predetermined time intervals, bags were withdrawn from the release medium, one milliliter of their content was diluted with tetrahydrofuran (to a final volume ratio 1:3) and analyzed for griseofulvin by measuring the fluorescence emission intensity at 412 nm with the use of an LS5OB (PerkinElmer) fluorometer at an excitation wavelength of 338 nm. Three samples were analyzed at each time interval and the average percent release values were calculated. A control experiment to determine the release behavior of the free drug was also performed. A saturated solution of griseofulvin in PBS (c = 23 μg/L) was prepared, and 2-mL samples of this solution were enclosed in dialysis bags and immersed in 2 L PBS. Then, the procedure described above for the micelle samples was followed.

Determination of the degradation of micelles

The degradation of the PLA-PEG micelles during the drug-release period was evaluated from the reduction of copolymer-specific viscosity with time upon incubation of the micelle samples in PBS (see above in drug-release study). At predetermined time intervals, bags were withdrawn from the release medium and their contents were lyophilized. The copolymer in the lyophilisates was dissolved in dichloromethane. The solution was filtered and transferred to a rotary evaporator, where it was dried. The solid residue was dissolved in tetrahydrofuran, and the viscosity of the resulting solution at 25°C was measured with the use of an automatic Ubbelohde viscometer (apparatus AVS, Schott-Gerate). Three samples were analyzed at each time interval, and the average specific viscosity values were calculated.

Stability of the micelles in simulated gastric and intestinal fluids

The stability of the micelles in simulated gastric and intestinal fluids for a period of 11 days was evaluated from the reduction of copolymer specific viscosity with time upon incubation of the micelle samples in USP simulated gastric (pH = 1.2) or intestinal fluid (pH = 7.5) at 37°C. The reduction of specific viscosity of the samples was measured as described in the previous paragraph.

RESULTS

PLA-PEG copolymer synthesis

The molecular weight of the product did not change after 2 h of reaction time (Fig. 1), indicating that under the experimental conditions applied here the polymerization was completed within a 2-h period. Therefore,

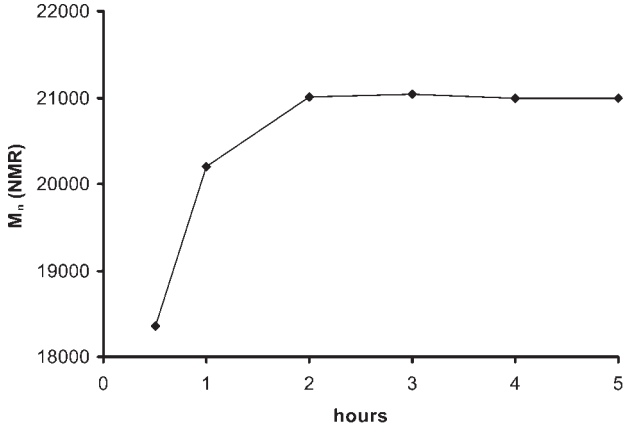

Figure 1. Variation of copolymer molecular weight with polymerization time. Synthesized copolymer composition: PLA(16)-PEG(5).

a 2-h polymerization time was applied in all subsequent syntheses.

Micellization behavior of PLA-PEG

The behavior of PLA(*X*)-PEG(5) during the process of micelle preparation was studied in the range of molar compositions 20–80% PEG (i.e., 20–80% ethylene oxide units in copolymer chains by NMR). At copolymer concentrations in the organic solvent of 10–20 mg/mL, stable micelles and full conversion (without copolymer losses in the form of precipitates) could only be obtained when the PEG content of the copolymer was in the range of about 50–70%. When the PEG content of the copolymer was lower than 50%, solid copolymer precipitates were formed during micelle preparation. On the other hand, unstable micelles were formed when PEG content exceeded 70%. Evidence for the reduced stability of the micelles when PEG content of copolymer exceeded 70% was obtained by fluorescence measurements: the ratio of the intensities at 373 nm (I_1) and 384 nm (I_3) of the pyrene fluorescence was about 1.7 at copolymer concentrations above CMC (Fig. 2), whereas with stable micelles it was between 1.4 and 1.5 [Fig. 3(a)], indicating that in this case (Fig. 2) the probe was not entrapped in a plain hydrophobic environment, as in the case of stable micelles.[33]

The CMC of a PLA(4)-PEG(5) copolymer (M_w = 14,000, M_n = 11,800 and PI = 1.18 by GPC) was determined with the use of both fluorescence [Fig. 3(a)] and SLS [Fig. 3(b)] experiments. The CMC values obtained were 0.07 and 0.09 mg/mL with the fluorescence [Fig. 3(a)] and SLS [Fig. 3(b)] techniques, respectively.

The basic characteristics of the micelles formed by the PLA(4)-PEG(5) copolymer are presented in Table I.

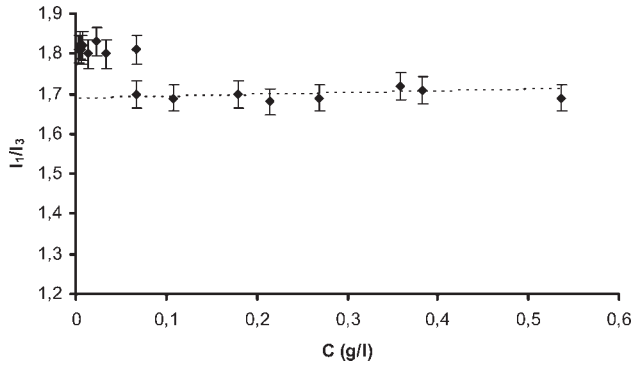

Figure 2. Change of the I_1/I_3 ratio of pyrene fluorescence with the concentration of PLA (1.8)-PEG(5) copolymer (consisting of 83% PEG) in water.

Both the radius of gyration R_g and the hydrodynamic ratio R_H of the micelles were small. Their ratio R_g/R_H = 0.789 was close to the theoretically expected value for spherical micelles of 0.775.[35] These data indicate that the PLA(4)-PEG(5) copolymer formed small and spherical micelles. The negative value of the second virial coefficient $A_2 = -1.32 \times 10^4$ signifies the existence of attractive interaction among the copolymer chains and unfavorable interaction between the copolymer and the solvent (water).[36]

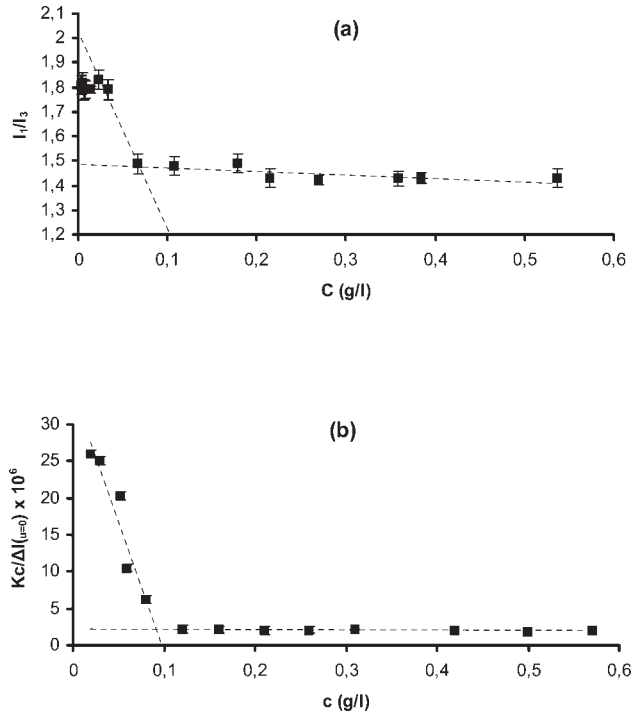

Figure 3. (a) Change of the I_1/I_3 ratio of pyrene fluorescence with the concentration of PLA(4)-PEG(5) copolymer in water, and (b) change of the inverse light scattering intensity with the concentration of PLA(4)-PEG(5) copolymer in water.

TABLE I
Basic Characteristics of the PLA(4)-PEG(5) Micelles

CMC (g/mL)	Molecular Weight	N_{agg}[a]	A_2	R_g (nm)	R_H (nm)
0.075×10^{-2}	$484 \times 10^3 \pm 0.004$	49	$-1.32 \times 10^4 \pm 0.07$	21.3 ± 0.9	26.9 ± 0.8

[a] $N_{agg} = M_w$ (micelle)/M_w (copolymer), M_w (copolymer) = 9800 (by SLS).

Drug loading of micelles

The loading of the PLA(4)-PEG(5) micelles with griseofulvin (milligrams of griseofulvin per 1 g of copolymer) was measured by fluorescence and UV spectroscopy methods. Both methods produced similar loading results (Fig. 4). The drug content of the micelles initially increased when drug input (i.e., the amount of drug added in the feed) was increased, but leveled off at relatively high drug input values. DLS measurements revealed that drug loading did not affect the size of the micelles (data not shown).

During the experiments for the preparation of griseofulvin-loaded micelles, it was observed that the maximum possible concentration of copolymer in tetrahydrofuran, that is, the maximum copolymer concentration not inducing the formation of copolymer precipitates, was higher than that in the preparation of unloaded micelles. For example, the maximum PLA(4)-PEG(5) concentration not inducing copolymer precipitation was 12.5 and 7 mg/mL for the preparation of loaded and unloaded micelles, respectively.

Drug-release properties of micelles

The *in vitro* release of griseofulvin from an aqueous dispersion (control) and from PLA(4)-PEG(5) micelles in PBS is shown in [Fig. 5(a)]. The comparison of the profiles of griseofulvin release from the micelles and from the aqueous dispersion shows that the entrapment of griseofulvin in the nanoparticles could significantly retard its *in vitro* release. The release of nonentrapped griseofulvin was completed within 24 h, whereas the release of micelle-entrapped griseofulvin was completed at 30 days from the beginning of the experiment. The release profile of micelle-entrapped griseofulvin was biphasic, with an abrupt increase of release rate occurring after 20 days from the beginning. During the first phase (0–20 days) 66% of the drug was released. The remaining amount of drug was released during the second phase (20–30 days).

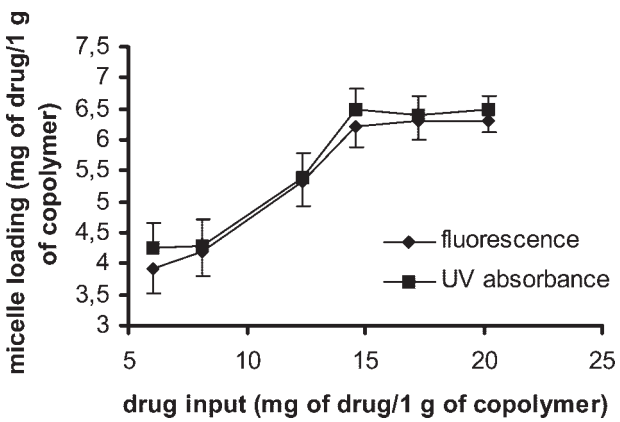

Figure 4. Loading of micelles with griseofulvin as a function of griseofulvin input (loading was measured by fluorescence and UV spectroscopy).

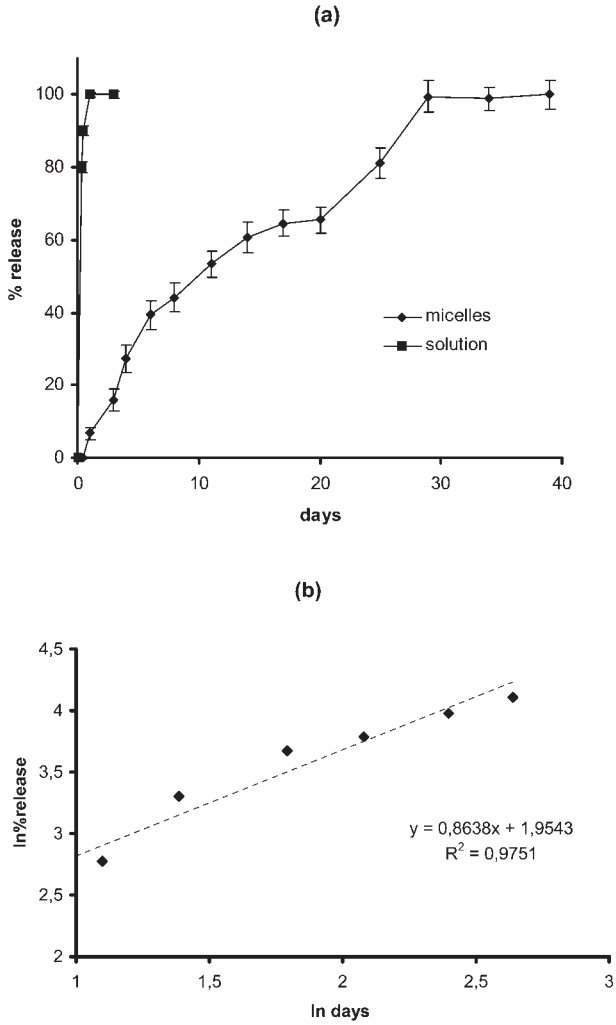

Figure 5. (a) Griseofulvin release from PLA(4)-PEG(5) micelles and from an aqueous solution (control) in phosphate-buffered saline, and (b) fitting of the 0–60% release data to the Korsmeyer-Peppas equation.

No burst release was observed in the initial part of the release profile. In order to investigate the release mechanism, the data within the range 0–60% were fitted to the Korsmeyer-Peppas equation (1):[37]

$$Q = kt^n \quad (1)$$

where Q is the fractional release of the drug, k is a constant incorporating structural and geometric characteristics of the drug carrier (dosage form), and n is the release exponent, indicative of the drug-release mechanism. The exponent n was found to be 0.86 [Fig. 5(b)].

The release profile of griseofulvin from the micelles in the simulated gastric fluid did not differ from that in the simulated intestinal fluid [Fig. 6(a)]. The rate of release was similar in all three media—PBS, simulated gastric fluid, and simulated intestinal fluid—during the first 6 days of incubation [Figs. 5(a) and 6(a)]. Thus, 39.4, 44.2, and 45.2% of drug was released after 6 days of incubation of micelles in PBS, simulated gastric fluid, and simulated intestinal fluid, respectively.

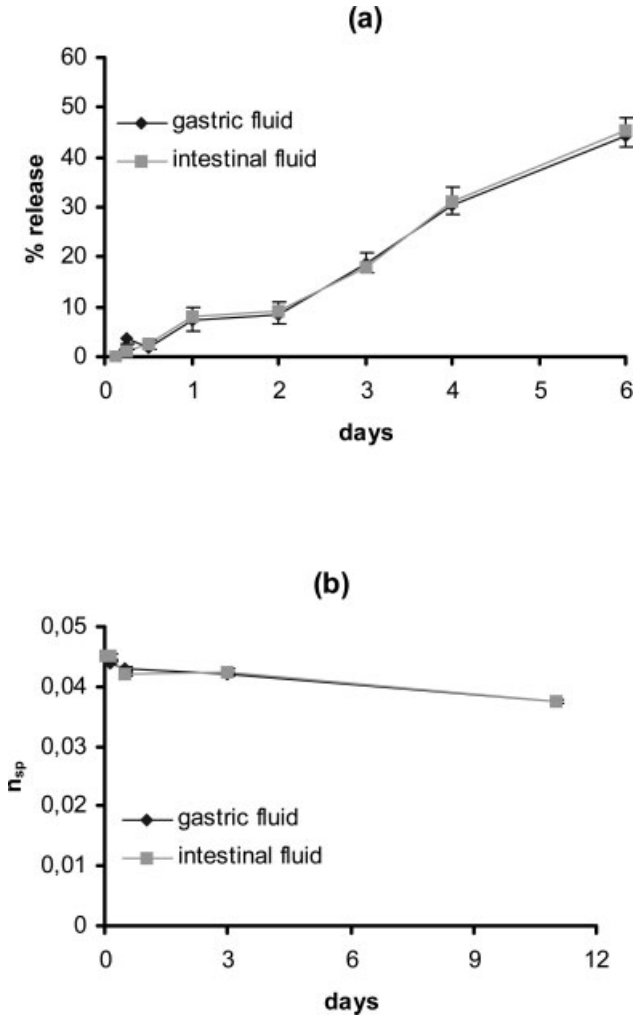

Figure 6. (a) Griseofulvin release from PLA(4)-PEG(5) micelles in simulated gastric and intestinal fluids, and (b) variation of specific viscosity of PLA(4)-PEG(5) copolymer micelles of griseofulvin with incubation time in simulated gastric and intestinal fluids.

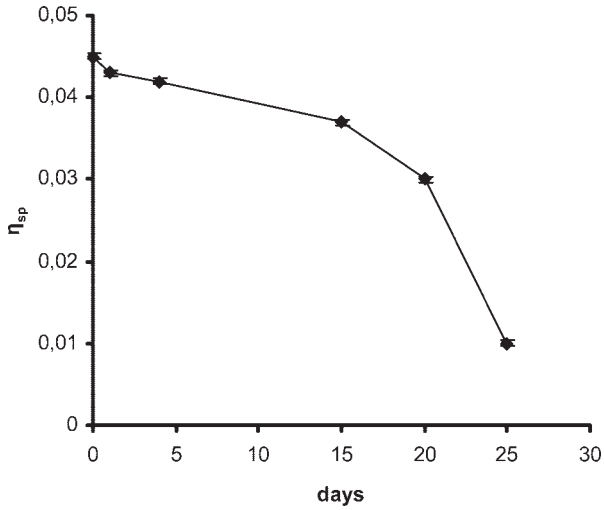

Figure 7. Variation of specific viscosity of PLA(4)-PEG(5) copolymer micelles of griseofulvin with incubation time in phosphate-buffered saline.

Micelle degradation

The specific viscosity of the copolymer decreased slowly with time from Day 0 until Day 15 in PBS, indicating that the copolymer eroded slowly with time during the first 15 days. Then, an abrupt and significant viscosity fall occurred from Day 15 to Day 25, indicating that a significant erosion of the copolymer took place during that period of time (Fig. 7). Provided that similar degradation rate occurs *in vivo*, the relatively fast degradation of micelles observed would be advantageous because it would result in rapid polymer removal from the body, preventing polymer accumulation in cases of multiple administration of micelles *in vivo*.

Stability of micelles in simulated gastric and intestinal fluids

The specific viscosity of the copolymer decreased slowly with time from Day 0 until Day 11, indicating that the copolymer eroded slowly with time during the first 11 days of incubation in simulated gastric or intestinal fluid. The micelles appeared equally stable in these two media for the time period studied [Fig. 6(b)].

DISCUSSION

In this work, PLA-PEG copolymers were synthesized by the ring-opening polymerization of lactide in the presence of methoxy-polyethylene glycol, using stannous octoate as catalyst. With all compositions tested, chain growth proceeded rapidly and polymerization was completed within a 2-h period (Fig. 1). This result is in agreement with a coordination polymerization mechanism proposed by Du et al.[38]

The micellization behavior of the synthesized PLA(X)-PEG(5) copolymers was investigated. At copolymer concentrations in the organic solvent of 10–20 mg/mL (i.e., at concentrations not so low so as to be irrelevant to the application of the resulting micelles as drug carriers), stable micelles with full conversion (100% yield) could only be prepared from copolymers with molar composition in the range 50–70% PEG (% ethylene oxide units in the copolymer by NMR). Micelle formation requires the balance between the attractive interactions of the insoluble PLA moieties and the repulsive interactions of the soluble PEG segments.[39] In effect, PEG moderates the association of PLA-PEG molecules, leading to micelle formation. When the PEG proportion in the copolymer chains is too low, PEG segments cannot moderate the association of the separating PLA-PEG molecules, and macroscopic agglomerates are formed. In line with these observations, Shin et al.[4] reported that micelles could not be formed by a poly(ethylene glycol)/ϵ-caprolactone copolymer with a too-high caprolactone content (70.7% by weight). When the PEG content of PLA-PEG exceeded 70%, the micelles formed were not stable (Fig. 2). In this case, the attractive forces between the relatively small PLA moieties cannot balance the repulsive forces between the relatively large PEG segments, and the micelles formed exhibit reduced stability.

A PLA(4)-PEG(5) copolymer, providing stable micelles, was selected for the preparation of drug-loaded micelles. The CMC of this copolymer was low (0.07/0.09 mg/mL) (Fig. 3). A low CMC is an important feature for the application of these micelles in drug delivery because it assures that micelles will be stable *in vivo*, where considerable dilution takes place. The features of the plot of the inverse scattering intensity versus copolymer concentration [Fig. 3(b)] would indicate that the formation of PLA(4)-PEG(5) micelles followed the closed association model.[40] The size of the micelles formed by the PLA(4)-PEG(5) copolymer (R_H = 26.9 nm, Table I) is consistent with a core-shell micelle architecture,[41] where the condensed PLA core is surrounded by a shell of expanded PEG chains. Experimental evidence for the core-shell structure of the PLA-PEG micellar particles has been provided by ^{1}H-NMR studies.[42,43]

The loading capacity of the PLA(4)-PEG(5) micelles with griseofulvin was about 6.5 mg of drug/1 g of copolymer (Fig. 4). This value is comparable to the loading of PLA(2)-PEG(5) micelles with testosterone (3.4 mg of drug/1 g of copolymer).[15] The loading of the PLA(4)-PEG(5) micelles with griseofulvin found here (~ 6.5 mg of drug/1 g of copolymer) is low and needs improvement in order to obtain griseofulvin-loaded micelles useful for pharmaceutical application, especially if oral griseofulvin administration is considered. The dose of griseofulvin depends on crystal size, and for tinea capitis oral treatment the daily dose of ultramicrocrystalline griseofulvin is 330–375 mg for adults and 82.5–165 mg for children.[44] Given the loading capacity of the PLA(4)-PEG(5) micelles (Fig. 2), prohibitively large amounts of copolymer would be required to administer such doses of griseofulvin in the form of micelles. For topical application, a 1% spray formulation was used for the treatment of experimentally induced fungal infections in healthy volunteers.[45] This 1% drug content is similar to the 0.65% achieved here with the PLA-PEG micelles; thus it may be considered to be a potentially suitable formulation for the topical delivery of griseofulvin. Several factors, such as the composition and molecular weight of the copolymer,[5,6,15] the length of hydrophobic block,[11,46] and the organic solvent used in micelle preparation,[4,11] have been shown to affect the drug incorporation capacity of copolymer micelles. Thus, griseofulvin loading in the PLA-PEG micelles might be improved by optimizing the copolymer composition and the conditions of micelle preparation.

The maximum concentration of copolymer in tetrahydrofuran that could be used in micelle preparation, that is, the maximum copolymer concentration not inducing the formation of macroscopic precipitates, was found to be higher in the case of drug-loaded micelles than in the case of unloaded micelles. It appears that griseofulvin moderates the hydrophobic interactions between the PLA moieties of the copolymer, stabilizing the micellar structures formed and preventing an unlimited growth of micelles into a distinct macroscopic phase. Evidence that the incorporation of hydrophobic compounds into block copolymer micelles may enhance micelle stability was also provided in the study of adriamycin entrapment in poly(ethylene glycol)-poly(aspartic acid) micelles.[9]

Sustained griseofulvin release over 30 days from the PLA-PEG micelles *in vitro* in PBS was observed [Fig. 5(a)]. Provided that similar release characteristics occur *in vivo*, the PLA-PEG micelles have suitable release properties for application as depot griseofulvin formulations. A burst effect could not be seen in the release profile, indicating that the drug was efficiently entrapped within the core of micelles. By fitting the data up to 60% release to the Korsmeyer-Peppas equation (1), an exponent n value of 0.86 was found [Fig.

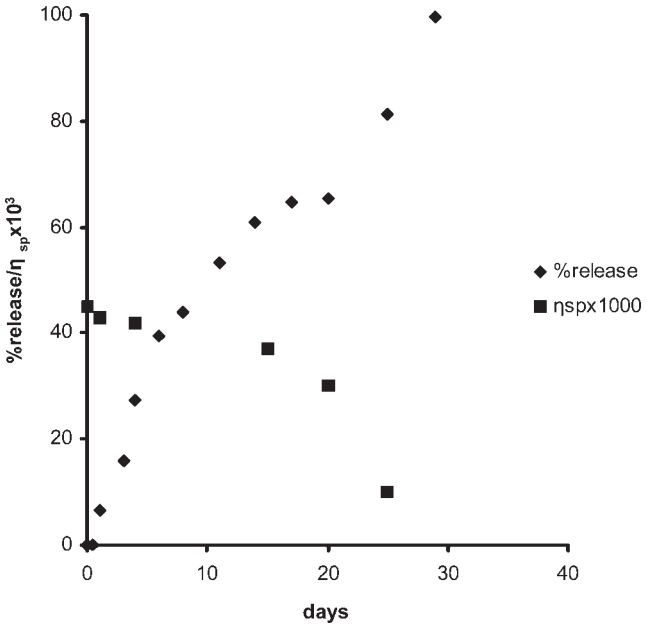

Figure 8. Comparison of griseofulvin release from the PLA(4)-PEG(5) micelles in phosphate-buffered saline with the degradation of the PLA(4)-PEG(5) micelles in phosphate-buffered saline.

5(b)]. This value is in agreement with the theoretically predicted value of 0.85 ± 0.02 for the Case II release from spherical particles,[47] suggesting that griseofulvin release from the micelles was erosion controlled.[47,48] It should be noticed that during the time period in which 60% of the drug was released (15 days) the copolymer exhibited a continuous and slow degradation (Fig. 7). The release profile was biphasic, with an abrupt increase of release rate occurring after the 20th day. At about the same time, significant and rapid copolymer degradation occurred, as evidenced by the abrupt decrease of copolymer specific viscosity from Day 15 to Day 25 (Fig. 7). It appears that the abrupt increase in the rate of release after Day 20 was due to the significant copolymer degradation that took place just before and at that time, and that the second phase of release was induced by the significant copolymer degradation that occurred just before and simultaneously with it (Fig. 8). Overall, the data suggest that polymer degradation (which, of course, would lead to micelle destabilization and, eventually, destruction) was the dominant mechanism of griseofulvin release from the PLA-PEG micelles in PBS. Besides, if griseofulvin release had been diffusion controlled, a much faster release profile should have been observed due to the micelle characteristics (small size and more dynamic structure than conventional polymeric nanoparticles[43,49]).

The PLA-PEG micelles of griseofulvin were stable in simulated gastric and intestinal fluids for a long enough time for oral application [Fig. 6(b)]. Thus, from a stability perspective, these micelles appear to be suitable candidates as an oral dosage form of griseofulvin. The release of griseofulvin from the PLA-PEG micelles in the simulated gastric and intestinal fluids is sustained [Fig. 6(a)]. Assuming that similar sustained release also occurs *in vivo*, this would provide the micelles the necessary time required to exert their influence on drug bioavailability.

CONCLUSION

The PLA-PEG micelles of griseofulvin exhibited sustained release properties and adequate stability in PBS and in simulated gastric and intestinal fluids. These characteristics of PLA-PEG micelles are important with regard to their application as griseofulvin carriers for oral or topical delivery. However, the drug-loading capacity of the micelles was low and needs improvement in order to become pharmaceutically useful, especially if the application of the micelles as an oral dosage form of griseofulvin is envisaged.

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