

Poly(D, L-lactide-co-glycolide)/Poly(ethylenimine) Blend Matrix System for pH Sensitive Drug Delivery

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ABSTRACT: Poly(D,L-lactide-co-glycolide) (PLGA) and poly(ethylenimine) (PEI) were blended and found to form a homogeneous pH sensitive matrix for drug release. Differential scanning calorimetry (DSC) studies of the PLGA/PEI blends showed a single glass transition temperature at all compositions. Fourier transform infrared spectroscopy (FTIR) demonstrated that the PLGA carbonyl peak at 1760 cm^{-1} shifted to 1666 cm^{-1} as a result of amide bond formation between the two polymers. This was confirmed by ^{13}C nuclear magnetic resonance studies. A PLGA/PEI matrix of 90/10 weight ratio was chosen for evaluation for controlled drug release. Both hydrophobic β -lapachone and hydro-

philic rhodamine B showed pH dependent release profiles with faster release kinetics at lower pH values. The observed pH sensitive drug release was mainly attributed to two factors, pH dependent swelling and protonation of the PEI-PLGA matrix. These results demonstrate utility of a PLGA/PEI matrix and its potential application in pH responsive drug delivery. © 2006 Wiley Periodicals, Inc. *J Appl Polym Sci* 100: 89–96, 2006

Key words: polymer blends; poly(D,L-lactide-co-glycolide); poly(ethylenimine); pH sensitive drug delivery

INTRODUCTION

Drug delivery systems in which drug release rates can be activated by an external stimuli have considerable promise to improve drug efficacy and safety.¹ In particular, pH sensitive drug release is desirable for applications such as insulin delivery^{2–4} and selective drug delivery to the stomach^{5,6} or intestine.⁷ Changes in pH are also known to coincide with the healing response⁸ and the growth of cancerous tumors.⁹ Polymers used for this purpose are usually covalently crosslinked hydrogel networks such as crosslinked graft copolymers of poly(dimethyl aminoethylmethacrylate) and poly(ethylene glycol)⁴ or crosslinked copolymers of poly(*N*-vinyl pyrrolidone-acrylic acid) and poly(ethylene glycol).⁶ However, these hydrogels require custom syntheses and need specialized reagents for their creation. Moreover, most of these hydrogel matrices are not biodegradable and their hydrophilic nature makes them less efficient in deliver-

ing hydrophobic drugs. Here, we report a pH sensitive delivery matrix based on PLGA/poly(ethylenimine) (PEI) blends via the amide formation and subsequent compatibilization of the two polymers.

PLGA is a biocompatible and biodegradable polyester that has been widely used in a variety of biomedical applications such as surgical sutures¹⁰ and drug carriers.^{11–13} The copolymer is composed of glycolic and lactic acid units and its hydrolysis rate can be altered by adjusting the ratio of the two components. These advantages in addition to its relatively high T_g (40–45°C) and mechanical stability make it a favorable material for use in solid implants. The properties of PLGA can be further improved by means of blending it with other polymers such as poly(ethylene glycol)^{14,15}, poly(vinyl alcohol),¹⁶ Chitin,¹⁷ and pluronic.¹⁸ However, complete miscibility has not been observed in these cases. Instead, only a partial miscibility was reported for PLGA and poly[bis(glycine ethyl ester)phosphazene] (PGP).¹⁹ In this case, PLGA and PGP showed partial miscibility as well as hydrogen bonding between the secondary amines of the PGP and the carbonyl groups of the PLGA. Another noteworthy characteristic of PLGA is its chemical activity to amines which has been used to study its degradation.²⁰

On the other hand, hyperbranched PEI is a cationic polymer that has seen promising biomedical applications in the field of gene delivery.²¹ The polymer consists of a mixture of primary, secondary, and tertiary amino groups that give the polymer a pH buff-

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ering capacity²² also known as the "proton sponge effect."²¹ Moreover, the amino groups of PEI have been demonstrated to cause partial miscibility with H—bond accepting polymers such as poly(*p*-vinyl phenol) (PVP).²³ Although miscibility was reported in this case, no pH sensitivity was reported for the blend. Just recently, PEI was blended with PLGA to form particles for use in pulmonary gene delivery.²⁴ Unfortunately, no polymer characterization of the blend material was performed in this case nor was any pH sensitivity reported for the material. A major disadvantage of PEI is that it has poor mechanical properties and needs to be blended with other materials if a solid structure is desired.

In this study, PEI and PLGA were found to form amide linkages upon mixing, which led to creation of a self-compatibilized blend showing miscibility at all compositions. Furthermore, one composition (PLGA/PEI = 90/10) of these blends was found to show pH sensitive swelling and drug release properties. Results from this study establish a bulk material for potential pH sensitive drug delivery applications.

EXPERIMENTAL

Materials

Poly(D,L-lactide-co-glycolide) (PLGA, $M_n = 50$ kDa, inherent viscosity 0.58 dL/g, lactide/glycolide = 50/50) was purchased from Absorbable Polymers (Pelham, AL). Hyperbranched poly(ethylenimine) ($M_w = 1.2$ or 25 kDa), rhodamine B, deuterated DMSO, and chloroform were obtained from Aldrich (St. Louis, MO). β -Lapachone was synthesized via a previously published procedure.²⁵ Citric acid, acetic acid, chloroform, sodium hydroxide, sodium chloride, and potassium chloride were obtained from Fischer Scientific (Fairlawn, NJ). Phosphate buffered saline tablets were obtained from Sigma (St. Louis, MO). All chemicals were used as received.

Blend preparation

PLGA/PEI mixtures were prepared by solvent casting from chloroform. Briefly, 10% solutions of each polymer in chloroform were prepared. The polymer solutions were then mixed by vortex to the desired weight ratios in glass vials. The solutions were then allowed to evaporate at room temperature overnight. The mixtures were then dried in vacuo at room temperature for 3 days. Two types of PLGA/PEI mixtures were prepared using the same PLGA polymer but different hyperbranched PEI with 1.2 or 25 kDa molecular weight. If films were desired, the blend material (~1 g) was pressed between Teflon sheets in a Carver press at 65°C for 5 min to produce polymer films (thickness ~1 mm).

Characterization of PLGA/PEI blends

Differential Scanning Calorimetry (DSC) measurements of pure PLGA, PEI, and PLGA/PEI blends were carried out on a Perkin–Elmer DSC-7 differential scanning calorimeter. Samples weighing 10–20 mg were cooled to –80°C and then heated to 80°C at a heating rate of 10°C/min. T_g was calculated by determining the inflection point of the glass transition by the Pyris software (version 3.81) from Perkin–Elmer. All DSC experiments were performed in triplicate with the error representing the standard deviation of the three trials.

Fourier Transform infrared spectroscopy (FTIR) measurements were performed using a Bio-Rad 575C Fourier infrared spectrometer. Scans were taken at a resolution of 2 cm⁻¹ from 500 to 4000 cm⁻¹. Samples were prepared by placing 5–10 drops of the PLGA/PEI blend solution (10%) in chloroform onto KBr pellets. This was followed by blowing dried nitrogen over the pellet for 5 min to remove the solvent. Samples for pure PLGA and PEI were also prepared similarly to the blends.

¹³C nuclear magnetic resonance (NMR) studies were performed on a 50/50 blend dissolved in deuterated DMSO at a concentration of ~100 mg/mL. NMR was performed on a 200 MHz Varian NMR overnight with 15,000 scans.

Polymer mixtures were also analyzed by gel permeation chromatography (GPC) at 40°C, using 10% acetic acid in chloroform as mobile phase. Measurements were taken using a Series 200 HPLC system (Perkin–Elmer) fit with size exclusion columns (Polymer Labs PLGel Mixed D 5m M columns), Series 200 RI detector, Series 200 UV–vis detector, and Series 200 column oven. Measurements were taken with 20 μ L injections from 50 mg/mL polymer solutions. Molecular weight measurements were calculated versus polystyrene standards (Polymer Laboratories).

Swelling studies

Swelling studies were carried out with PLGA/PEI (PEI $M_w = 1.2$ kD) blends with 90/10 weight ratio used for drug release studies. Segments of blend films (10 \times 3 \times 1 mm³) were placed into 5 mL of buffer solution at 37°C. To evaluate the dependence of swelling on pH, three buffer solutions were used: 10 mM sodium citrate (pH = 3.0), 10 mM sodium acetate (pH = 5.0), and 10 mM sodium phosphate (pH = 7.4). In addition to the buffer ions, we introduced the same concentrations of sodium chloride (137 mM) and potassium chloride (2.7 mM) to balance the osmolarities to that of saline. At time points of 1, 3, 8, 18, 24, 36, and 48 h, the sample was removed from the buffer, dabbed with a Kimwipe to remove any excess water on the surface, weighed, and then placed back into the buffer.

Weight increase was calculated using the following equation:

$$\text{Weight increase} = \left(\frac{W_w - W_0}{W_0} \right) \times 100\%$$

where W_w and W_0 are the weights of the wet and original films, respectively. All swelling experiments were performed six times and the significance of the data was evaluated using a Student's *t*-test (significance was defined as $P < 0.05$). Error bars were presented as the standard deviation from six sample measurements at each time point.

Weight loss studies

Weight loss studies were carried out with PLGA/PEI (90/10) blends in all the three buffers (pH 3.0, 5.0, and 7.4). Segments of polymer films were immersed in each buffer solution. The buffers used were identical to the ones used in the swelling study. At different time points (12, 24, and 48 h), each segment was removed from the buffer, dabbed dry with a Kimwipe, and placed under vacuum for 24 h before weighing. Weight loss was calculated using the following equation:

$$\text{Weight loss} = \left(1 - \frac{W_d}{W_0} \right) \times 100\%$$

where W_d is the weight of the dried polymer blends after buffer exposure. All weight loss experiments were performed six times and significance was evaluated via a Student's *t*-test (significance was defined as $P < 0.05$). Error bars were calculated using the standard deviation of six different trials.

Preparation of drug-loaded PLGA/PEI matrix

PLGA/PEI with a 90/10 weight ratio was used for drug release studies. First, PLGA and PEI were dissolved in chloroform at 10% total polymer concentration (weight ratio PLGA/PEI = 90/10). β -Lapachone or rhodamine B was dissolved in chloroform at 20 mg/mL and 1 mL of drug solution was added to the polymer solution. The solution was first mixed by vortex and then poured into a 100 mL glass beaker and allowed to evaporate overnight. The drug-loaded blends were then dried in vacuo for 3 days at room temperature. The blend was then pressed between Teflon sheets in a Carver press at 65°C for 5 min to yield drug-loaded polymer films of ~1-mm thickness. Films were cut into segments of ~50 mg for the following drug release studies.

Drug release kinetics

Drug release studies were carried out by placing segments ($10 \times 3 \times 1 \text{ mm}^3$) of each film into 5 mL of different buffer solutions in glass vials at 37°C. The buffers used were identical to the ones used in the swelling studies. For all the release studies, the glass vials were placed in an orbital shaker at 100 rpm (New Brunswick Scientific C24 incubator shaker) at 37°C. At specified time points, the buffer (5 mL) was removed and replaced with fresh buffer. Time points were taken every hour for the first 4 h and every 2.5 h for the following 20 h. Drug concentration was analyzed by UV/Vis spectrophotometry, using a Perkin-Elmer Lambda 20 spectrometer. Sample measurement was performed at the maximum absorption wavelengths of 257 and 525 nm for β -lapachone and rhodamine B, respectively. Three replicate samples were measured at each time point for each buffer condition. The films remained intact during the course of the studies.

RESULTS

DSC studies

Figure 1(A) illustrates a series of representative DSC thermograms of PLGA/PEI blends with different compositions. Data showed a single glass transition temperature for all the blend compositions. As both PLGA and PEI are amorphous, no crystallization peaks were observed in any blends. The values of T_g for the pure PLGA and PEI (1.2 kDa) were measured to be 41.5 and -47°C , respectively. The single glass transition temperature indicates mixing of the components at a molecular level and an overall homogeneity of the structure.

The T_g values of the PLGA/PEI mixtures are plotted in Figure 1(B). The glass transition temperatures showed some variations depending on the molecular weight of PEI used. At high PEI content ($>50\%$ PEI, or $<50\%$ PLGA in Fig. 1(B)), blends with 25 kD PEI were found to have a higher glass transition temperature than those with 1.2 kD PEI. At low PEI content ($<50\%$ PEI, or $>50\%$ PLGA), no statistically significant difference was observed between the glass transition temperatures of the blends with the same PEI composition but different PEI sizes (1.2 versus 25 kDa). Since PLGA/PEI blend at 90/10 ratio provides a higher T_g value ($>30^\circ\text{C}$) that ensures the formation of solid films at room temperature, we used this composition in the subsequent swelling and controlled release studies.

FTIR spectroscopy

FTIR spectroscopy was used to characterize the interaction between the polymers. FTIR spectra of different

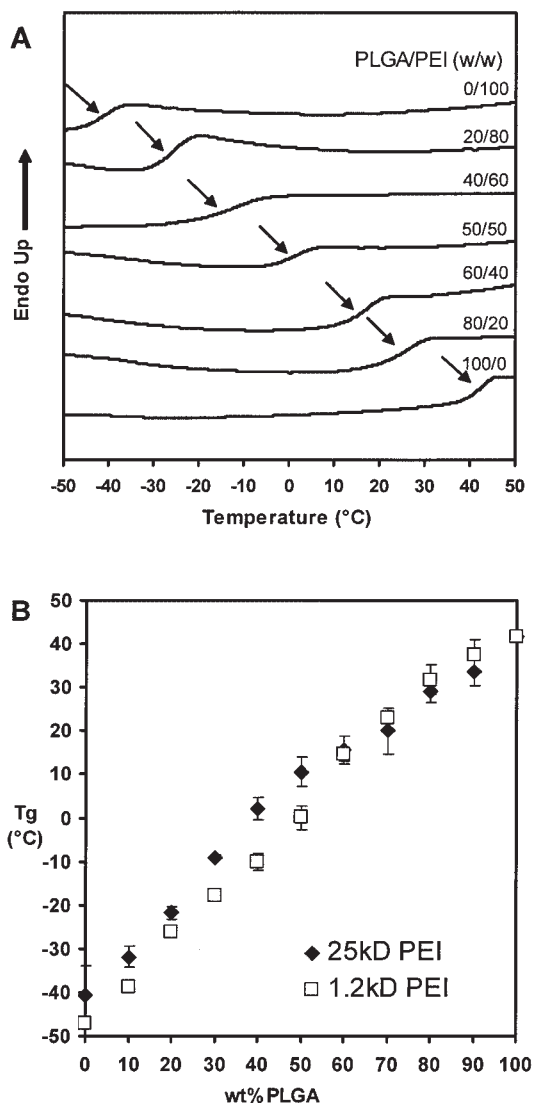


Figure 1 (A) DSC thermograms of PLGA/PEI (1.2 kDa) blends. Blend compositions are provided in weight ratios (w/w) of PLGA/PEI. (B) T_g of PLGA/PEI blends as a function of PLGA composition (wt % PLGA). Two molecular weights of PEI were used in the blends; 25 (◆) and 1.2 kDa (□).

weight ratios of PLGA/1.2kDa PEI blends are shown in Figure 2. Of primary interest was the PLGA ester C=O stretching peak at 1760 cm^{-1} . As the PEI content increased in the blends, the peak at 1760 cm^{-1} was observed to lessen in intensity and a second peak at 1666 cm^{-1} appeared. 1666 cm^{-1} is a characteristic frequency for the C=O stretching of amide bonds.²⁶

Of lesser interest was the C—N stretching band at 1580 cm^{-1} . This peak was present in the pure PEI samples, but disappeared in the blends. Instead, a new peak at 1543 cm^{-1} was observed in the blends. This second peak also showed greater intensity as the PEI content of the blends increased despite the fact that it was not present in the pure PEI.

¹³C-NMR studies

Figure 3 shows the ¹³C-NMR spectra of PLGA and a 50/50 PLGA/PEI cast blend. Most noteworthy are the PLGA ester carbon resonances observed at 166.5 and 167.5 ppm. Upon blending, peaks appear at 174.5, 174, and 172 ppm that are not present in either the PLGA or PEI alone (spectra not shown).

Gel permeation chromatography

GPC was used to determine alterations in the molecular weights of the species present. Figure 4 shows the chromatograms for PLGA, PEI, and a 90/10 PLGA/PEI mixture. The PLGA comes out as a broad peak at 13 min (M_w 47 kDa) while M_w 1200 PEI comes out as a sharp peak at 15.5 min. A 90/10 PLGA/PEI mixture was tested and showed two overlapping peaks, one at 14 (M_w 11 kDa) and the other at 15.5 min.

Swelling and weight loss studies of a 90/10 PLGA/PEI blend

Figure 5(A) shows the swelling behavior of PLGA/PEI blend at different pH values. Swelling was found to increase over time in all cases, with no swelling equilibrium being observed even after 48 h. Pure PLGA films were tested as a control, and they showed less than 5% swelling after 48 h, with no pH dependence over that time period (data not shown). The blend films immersed in pH 3.0 buffer swelled 72% of its initial weight after 48 h. The samples in pH 5.0 and 7.4 buffers swelled 65 and 60% of their initial weights, respectively. At all time points, the swelling was greatest for the film in the pH 3.0 buffer, followed by pH 5.0 buffer, with the pH 7.4 buffer yielding the least amount of swelling. The swelling differences were tested for significance at 3, 24, and 48 h and was found to be significantly different between the pH values tested ($P < 0.05$).

The possibility existed for pH dependent leeching of material from the polymer films. Figure 5(B) shows the weight loss from the films as a function of time and pH. No pH dependence is observed in the weight loss studies.

Drug release of β -lapachone and rhodamine B from 90/10 PLGA/PEI blends

Figure 6 shows the release profiles of β -lapachone [Fig. 6(A)] and rhodamine B [Fig. 6(B)] from PLGA/PEI films. In each figure, the release profiles are depicted as the cumulative percentage of drug released from the film as a function of time.

For the β -lapachone loaded films, the release profiles showed an initial burst followed by a sustained release that is well known for drug impregnated poly-

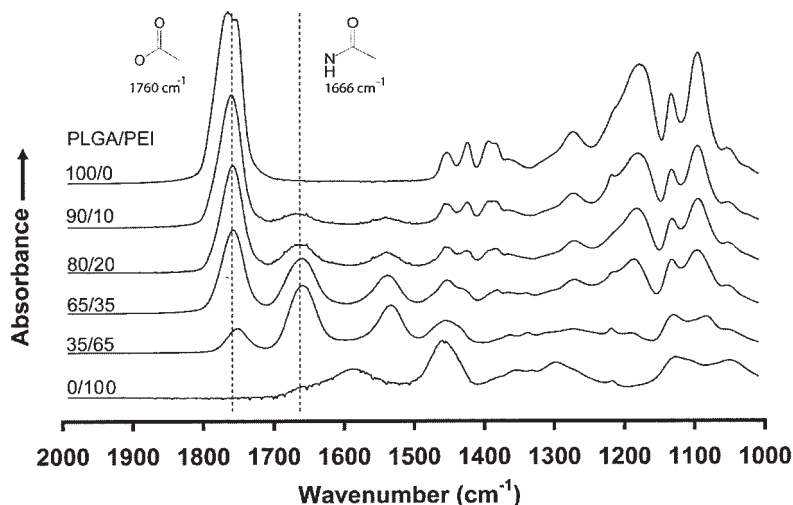


Figure 2 (A) FTIR spectra of PLGA/PEI blends from 1000 to 2000 cm^{-1} . Dotted lines correspond to 1760 cm^{-1} and 1666 cm^{-1} for ester and amide carbonyl stretches, respectively.

mer systems. The films immersed in the pH 3.0, 5.0, and 7.4 buffers had cumulative releases of 19%, 9%, and 7% after 48 h, respectively. Release studies were not followed beyond 48 h due to the loss of the structural integrity of the films. At all time points, the films showed greater cumulative release at pH 3.0 than films at pH 5.0, which was in turn greater than films at pH 7.4. β -Lapachone was also loaded into pure PLGA

films and no pH sensitive drug release was observed within the time course of the study. Less than 5% of the drug was released from the PLGA film after 18 h at all pH values (data not shown).

The rhodamine B loaded films demonstrate different release behaviors from the β -lapachone loaded films [Fig. 6(B)]. The films in pH 7.4 buffer showed very slow release kinetics, with less than 2% of the drug being released after 24 h. The films in pH 5.0 buffer showed an initial burst followed by a slow release, with a cumulative release of 9% after 24 h. The films immersed at pH 3.0 have a cumulative release of 66% after 24 h. Loss of film integrity began to occur at 24 h, at which point the release study was discontinued. Moreover, the release profile of the rhodamine B films at pH 3.0 showed a more linear behavior than the previous films.

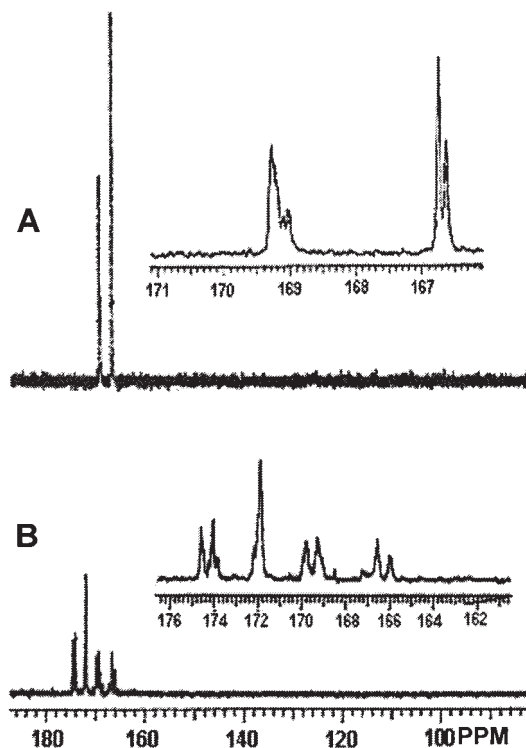


Figure 3 ^{13}C -NMR spectra of (A) PLGA and (B) a 50/50 PLGA/PEI blend. Expansions around the 175–164 ppm regions are showed in insets.

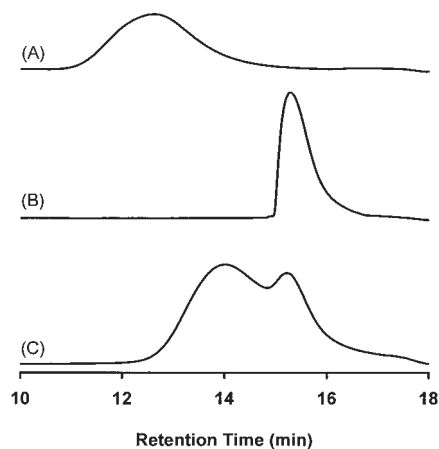


Figure 4 GPC chromatograms of (A) PLGA, (B) PEI, and (C) 90/10 PLGA/PEI in 10% acetic acid/chloroform.

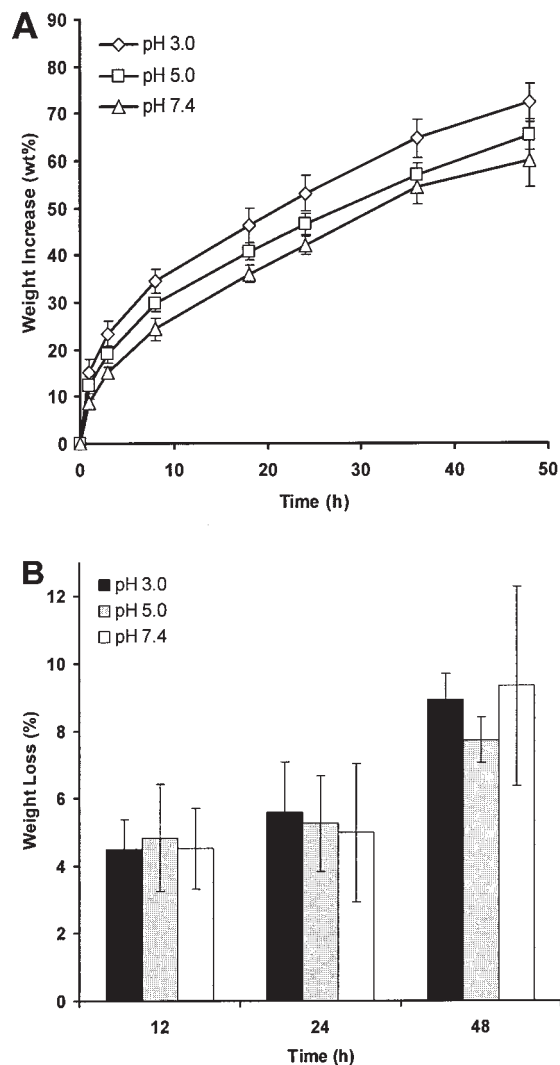


Figure 5 (A) Weight increase percentage of 90/10 PLGA/PEI films as a function of time. (B) Weight loss histograms of 90/10 PLGA/PEI blend films at 12, 24, and 48 h. Three buffer solutions at pH 3.0 (\diamond), 5.0 (\square), and 7.4 (\triangle) were used in this study. The error bars were calculated from six samples for each data point.

DISCUSSION

In this study, PLGA and PEI polymers were able to form a homogeneous blend at all compositions with a single glass transition temperature. Two molecular weights of PEI were tested and found to have slightly different transition temperature-content dependence. It is noteworthy that the 25 kDa PEI blends showed a more traditional T_g dependence than the 1.2 kDa PEI blends at the same compositions. The 25 kDa PEI blends have the expected concave down profile expected for a blend of two interacting polymers, while the 1.2 kDa blends show a much less pronounced curvature and deviate from the 25 kDa blends in the blends of higher PEI content (>50% PEI). The lower T_g being observed in blends using a lower molecular

weight polymer is not unexpected. A lower molecular weight PEI would have less chain entanglements and experience greater chain mobility than a higher molecular weight species. However, the similarities of the T_g in higher PLGA content blends indicate structural similarity between the two types of blends studied. This is consistent with the amide formation creating higher molecular weight PEI species within the structure, leading to greater chain entanglements and higher glass transition temperature.

Amide bond formation between the polymers was demonstrated by the FTIR studies. The peak at 1666 cm^{-1} is from the amide C=O stretch and the peak at 1543 cm^{-1} is from the $\delta(\text{N-H})$ amide vibration.²⁶ Scheme 1 shows the hypothesized mechanism of nucleophilic attack of the PLGA ester groups by the primary amines of the PEI. This amide formation is further confirmed in the NMR studies where amide

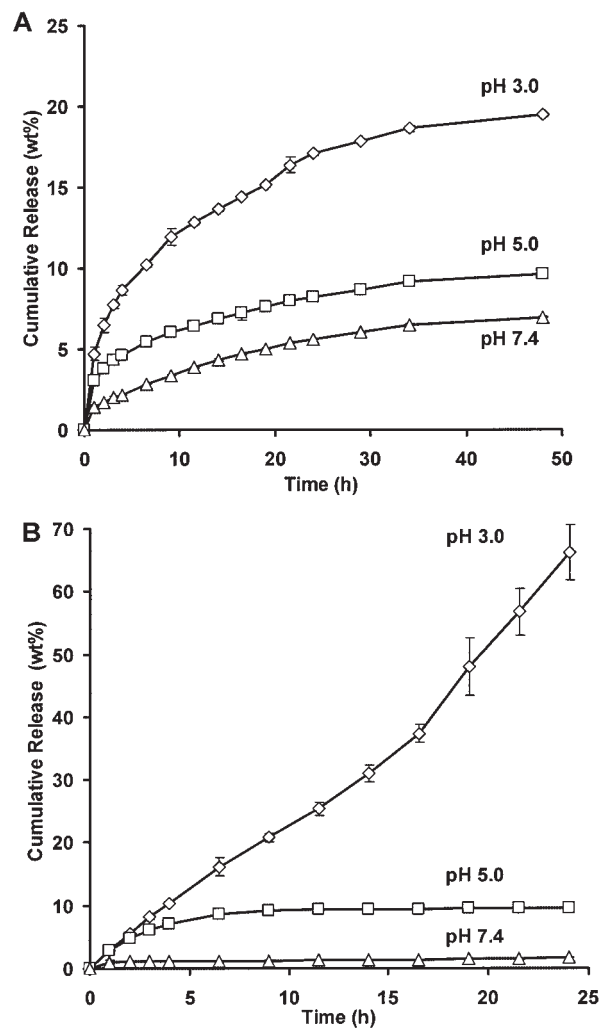
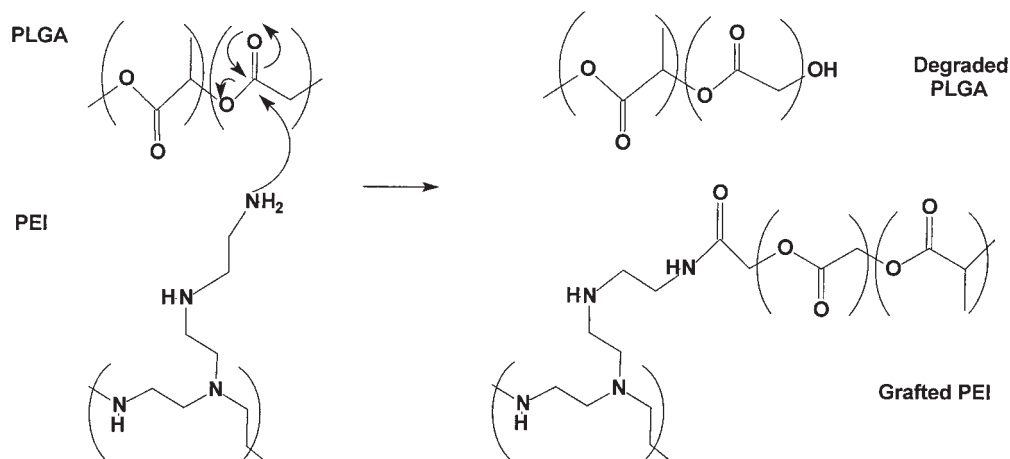


Figure 6 (A) Cumulative release of hydrophobic β -lapachone and (B) hydrophilic rhodamine B from 90/10 PLGA/PEI(1.2 kDa) films. Three buffer solutions at pH 3.0 (224), 5.0 (\square), and 7.4 (\triangle) were used in this study. The error bars were measured from triplicate samples.



Scheme 1 Hypothesized mechanism of formation of amide linkages between PLGA and PEI.

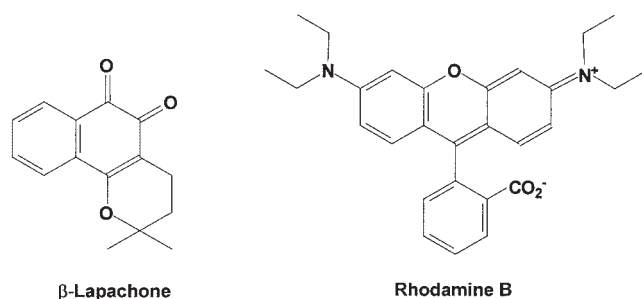
peaks were observed resulting from amide formation from the glycolic and lactic esters. Amide formation would result in scission of the PLGA ester, which is observed in GPC experiments. Indeed, the lower molecular weight peak at 14.2 min results from the PLGA segments that are formed upon reaction. No detectable alteration is observed in the PEI peak due to the hyperbranched nature of PEI. As the PLGA grafts would form on the primary amine branch ends of the PEI, there exists a large possibility of grafting without appreciable alteration in the hydrodynamic radius of the material.

The examination of the 90/10 PLGA/PEI matrix demonstrated pH dependent swelling behavior. This is consistent with the PEI residues protonating at lower pH, increasing the hydrophilicity of the structure. The possibility of this swelling being from the creation of pores in the system caused by pH dependent 'leeching' of material of the structure was tested and no pH dependent weight loss was observed.

This PEI/PLGA blend showed pH sensitive drug release properties for both hydrophobic β -lapachone and hydrophilic rhodamine B. In the case of β -lapachone (structure shown in Scheme 2), a lower pH environment greatly facilitates the drug release from

the blend film. The increased release kinetics correlates well with pH dependent swelling behavior of the PEI/PLGA blend. Theoretically, a more swollen structure provides a larger free volume inside the polymer matrix, which will lead to an increased molecular diffusivity and faster drug release kinetics. The swelling studies of the films [Fig. 5(A)] confirm the earlier mentioned hypothesis for the pH dependent release of the β -lapachone [Fig. 6(A)]. The PEI/PLGA film swelled to 73% at pH 3.0 compared with that of 60% at pH 7.4 after 48 h. Correspondingly, 19% of β -lapachone were released at pH 3.0 compared with that of 7% at pH 7.4 after 48 h. Interestingly, the release properties of rhodamine B (structure shown in Scheme 2) showed a higher pH dependent behavior versus β -lapachone. At neutral pH (7.4), rhodamine B is a zwitterionic dye that does not carry a net charge. This neutrality of structure as well as the large size of the rhodamine leads to a small percentage of release of the dye at pH 7.4 [Fig. 6(B)]. As the pH drops, the benzoic acid moiety of the rhodamine B becomes protonated (pK_a 4–5) and the dye becomes positively charged. Protonation of the PEI molecules in the polymer blend will also lead to a positively charged matrix at the lower pH values. The electrostatic repulsion between the positively charged rhodamine B and the PEI matrix may explain the dramatic increase in release kinetics at pH 3.0. This charge-dependent release mechanism can be exploited further by tailoring the electrostatic properties of drug molecules or polymer matrix to maximize the pH sensitivity of drug release.

In summary, the chemical reaction between PEI and PLGA is a significant finding due to the growing interests in blends and copolymers containing PEI and polyesters.^{24,27} Although amide formation between PEI and polyesters has not been investigated in previous studies, chemical reactions would alter the molecular structure and physical properties of the ex-



Scheme 2 Chemical structures of β -lapachone and rhodamine B.

plored systems. On the other hand, covalent bond formation can be utilized as a viable strategy to induce blend miscibility of PEI with polyesters. This self-compatibilization mechanism leads to the possibility of PEI blends with many other carbonyl group containing polymers such as polycarbonates or polyanhydrides to introduce pH sensitive drug release.

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