Accelerated Degradation of Poly(ϵ-caprolactone) by Organic Amines

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The solid-state degradation of poly(ϵ-caprolactone) catalyzed by primary, secondary and tertiary alkylamines was investigated. The degradation process was monitored by weight loss and molecular weight change measured by gel permeation chromatography. Degradation studies were conducted at 37°C in methanol solutions of the alkylamines. Primary alkylamines caused rapid weight loss (i.e., ~90% weight loss in 30 days) that depended on alkylamine concentration, molar ratio of alkylamine to poly(ϵ-caprolactone) monomer and alkyl chain length. The secondary alkylamines caused less rapid polymer weight loss (i.e., ~90%) weight loss within 80 days). One tertiary alkylamine (*N,N*-diisopropylethylamine) showed little catalytic effect while a bicyclic tertiary alkylamine (quinuclidine) was about as catalytic as the primary alkylamines. The degradation products isolated when primary alkylamines were used include both esters and amides indicating that nucleophilic attack by the alkylamines competed with the amine-catalyzed methanolysis reaction. Only ester moieties could be identified in the products from reactions containing secondary and tertiary alkylamines, which indicated that they acted as nucleophilic catalysts. All of the primary alkylamines reduced poly(ϵ-caprolactone) molecular weight from about 25,000 to 10,000 within 10 days after which the molecular weight of the remaining solid leveled off even though weight loss continued.

KEY WORDS: poly(ϵ-caprolactone); basic alkylamines; accelerated degradation; weight loss; molecular weight; biodegradable poly(ester).

INTRODUCTION

Biodegradable poly(esters) have frequently been used for the preparation of sustained-release drug delivery systems and biomaterials (1–13). The advantage of using biodegradable polymers as drug delivery matrices is that they do not require removal after delivering their dose because they are hydrolyzed to soluble non-toxic oligomers or monomers. Generally poly(esters) degrade in four major stages: (1) polymer hydration causing disruption of the primary and secondary structure due to hydrogen bonding and van der Waals forces, (2) loss of mechanical strength caused by the rupture of covalent bonds forming the polymer backbone, (3) loss of mass integrity resulting in accelerated water absorption, and (4) polymer dissolution and/or phagocytosis (14). The hydrolysis rate depends on the physicochemical properties of the polymers, including crystallinity, hydrophobicity, chemical structure and molecular weight. The degradation and release studies of biodegradable poly(esters) from different manufacturers, prepared by different methods, and in different dosage forms have been investigated (15–22). The methods to prepare these dosage forms include the use of solvents or heat, by solvent extraction/precipitation, rotary evaporation, spray drying and heat compression methods.

The acceleration of poly(D,L-lactide) hydrolysis in the presence of tertiary amine drugs like local anesthetics has been reported (23–25). A similar phenomenum was also observed by Maulding et al. (26) in poly(D,L-lactide) microcapsules containing up to 50% thioridazine free base where the authors proposed that the decomposition of poly(D,L-lactide) proceeded through an *N*-acylammonium ion intermediate. Cha and Pitt (27) suggested that the rapid release of L-methadone from microspheres of poly(D,L-lactide) and its copolymer, resulted from the acceleration of polymer chain hydrolysis in the presence of the basic drug. Pitt et al. (18) found that other tertiary amine drugs including methadone, naltrexone, promethazine and meperidine, also catalyzed poly(ester) chain scission and showed that there was no correlation between catalytic efficiency and the pK_a or partition coefficient of the amine drug. One limitation of this study was the absence of data regarding whether the drug was dissolved or dispersed in the polymer. Thus, the intrinsic catalytic effect of these amine drugs could not be predicted. Most studies involving acceleration by amine drugs have been performed on poly(D,L-lactide) and its copolymers while little information is known about whether amines accelerate the degradation of more stable poly(esters) such as poly(ϵ-caprolactone) (ϵ-PCL).

Poly(ϵ-caprolactone) (ϵ-PCL) is a biodegradable poly(ester) that exhibits certain desirable characteristics: (1) ϵ-PCL is a semicrystalline aliphatic polymer with a low glass transition temperature (T_g) of −60°C and a melting point of about 60°C (22); (2) ϵ-PCL is permeable to low molecular weight drugs (<400 D), thus it can be used in diffusion-controlled delivery systems that biodegrade after drug depletion (19,20); (3) ϵ-PCL's lack of toxicity makes it of interest as a matrix for controlled-release; (4) ϵ-PCL is considerably less expensive than other biodegradable poly(esters), such as poly(glycolide), poly(lactide) and their copolymers. The application of ϵ-PCL for drug delivery has one major drawback; it's high crystallinity and hydrophobicity results in long *in vivo* degradation times ranging from one to two years (21). The purpose of this study is to investigate the catalysis of ϵ-PCL degradation using primary, secondary and tertiary alkylamines. Also, the reaction products formed on amine-catalyzed degradation were characterized to elucidate the possible mechanism of action for these catalysts. Finally, this study is designed to gain insight into how amine-containing drugs may interact with poly(ester) matrices in controlled-release devices.

MATERIALS AND METHODS

Materials

Poly(ϵ-caprolactone) flakes were from Scientific Polymer Products, Inc. (Lot No. 047, MW25400). Polystyrene standards (MW 600-104,000) were from Polysciences, Inc.

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(Warrington, PA). All alkylamines were from Sigma Chemical Company (St. Louis, MO) and used as received. Other chemicals and solvents were reagent grade or better.

In vitro Degradation Studies

Weighed samples of solid ϵ-PCL (~70 mg) were placed in glass screw-capped test tubes containing 10 mL of 0.5 M alkylamine in methanol and then maintained at 37°C in an incubator shaker. Samples were removed periodically, filtered through a 0.2 μm membrane (Millipore, GVWP), with the solid phase and filtrate being collected. The degradation process was monitored gravimetrically for weight loss and molecular weight change by gel permeation chromatography. Fourier transform infrared (FTIR) spectroscopy was used to identify functional groups in the degradation products.

Weight (%) Remaining

The collected solid samples were dried *in vacuo* at room temperature for 3 days, and weighed to obtain the solid dry weight (M_d). The weight (%) remaining was calculated by dividing solid dry weight by the initial weight (M_o), where

$$\text{Weight (\%) remaining} = (M_d/M_o) \times 100\% \quad \text{Eqn.1}$$

Molecular Weight Characterization

Gel permeation chromatography (GPC) was used to determine molecular weight of the initial ϵ-PCL solid and the remaining solid collected during the degradation studies. An Ultrastyragel linear column from Waters Associates (7.8 mm (ID) × 30 cm) was used with chloroform as the eluting solvent at a flow rate of 1 mL/min at 35°C with a refractive index detector (Shimadxu RID-6A, Columbia, MD). The GPC procedure was calibrated using polystyrene standards of different molecular weights. The M_{GPC} is the molecular weight corresponding to the peak in the GPC chromatogram.

FTIR Spectral Analysis

The collected filtrate was adjusted to a pH below 7 using hydrochloric acid, and the reaction products were then extracted into chloroform. The spectrum of extracted reaction products was collected by FTIR (Nicolet 5DXB) using a liquid cell with potassium bromide windows.

Thermal Analysis

The melting point and the enthalpy of fusion of the polymer were determined by differential scanning calorimetry (DSC) at a scan rate of 10°C/min with a Perkin-Elmer DSC 7 (Perkin Elmer, Wilton, CT) in aluminum sample pans.

RESULTS AND DISCUSSION

Characterization of Polymer

The initial average molecular weight (M_{GPC}) of ϵ-PCL determined by GPC was 25,400. The melting point determined by DSC was 56.6°C, and the enthalpy of fusion (ΔH_f) was 104.1 J/g. The enthalpy of fusion for totally crystalline ϵ-PCL was reported to be 139.5 J/g (28), therefore the calculated crystallinity of the original ϵ-PCL was 74.6%.

In vitro Degradation Studies

The weight percent remaining of ϵ-PCL in 0.5 M primary alkylamine methanol solutions at 37°C over a period of 22 days is shown in Figure 1. Ethylamine, propylamine, amylamine, heptylamine and octylamine caused degradation of 50% of ϵ-PCL ranging from 17 days for ethylamine to 8 days for octylamine. Increasing the chain length of the alkylamines from two to eight carbons increased the degradation rate by about two-fold. Because these primary alkylamines have similar basicity, the increasing degradation rate of ϵ-PCL with increasing alkylamine chain length is probably due to their increasing hydrophobicity and consequently their higher partition coefficient into the polymer. The accelerated degradation of ϵ-PCL also depends on the molar ratio of alkylamine to ϵ-PCL monomer and the concentration of the alkylamine as shown in Fig. 2 for butylamine.

The FTIR spectra of reaction products extracted from reaction solutions containing butylamine are shown in Figure 3. Each spectrum represents the reaction product obtained at specific reaction times from 3–22 days. The peak at 1730 cm^{-1} is assigned to the carbonyl group of a methyl ester, which is the methanolysis reaction product. The peak at 1660 cm^{-1} is assigned to the carbonyl group of butylamide, the aminolysis reaction product. This butylamide is the major reaction product formed on the breakdown of the polymer ester linkage in the presence of primary alkylamines, representing the primary cause of polymer weight loss and molecular weight decrease. The presence of both ester and amide moieties in these FTIR spectra indicate that nucleophilic attack by the primary alkylamines competes well with the amine-catalyzed methanolysis.

The change in molecular weight of the remaining ϵ-PCL solid is shown in Figure 4. The initial molecular weight decreased rapidly from 25,400 to ~10,000 during the first 10 days after which it leveled off from day 10 to 22 even though

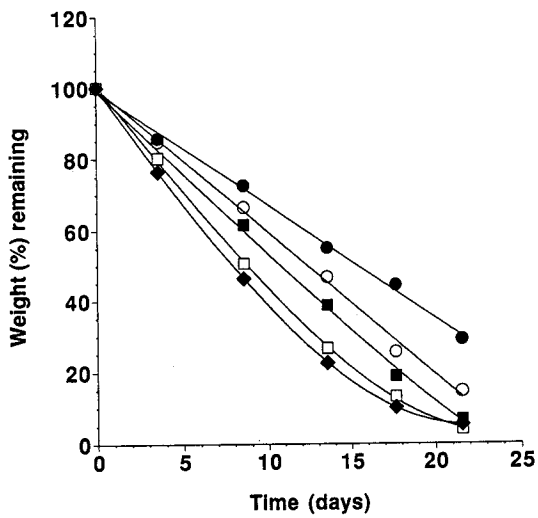

Fig. 1. Weight (%) remaining of ϵ-PCL solid in 0.5 M primary alkylamine methanol solutions at 37°C; (●) ethylamine; (○) propylamine; (■) amylamine; (□) heptylamine; (◆) octylamine.

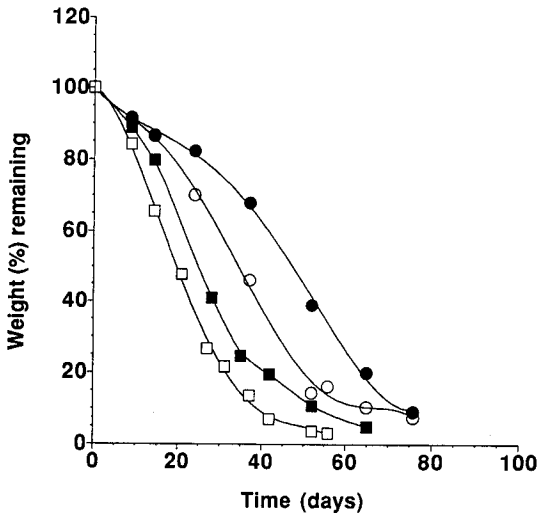

Fig. 2. Weight (%) remaining of ϵ-PCL solid in different molar ratios of butylamine: PCL monomer in methanol solutions at 37°C; (●) 0.2:1; (○) 0.33:1; (■) 0.5:1; (□) 1:1.

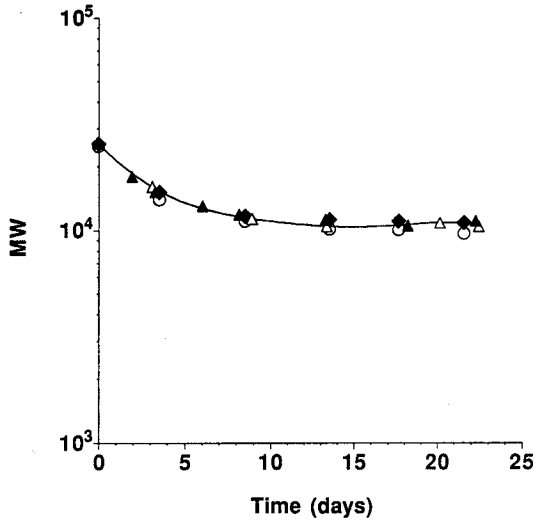

Fig. 4. Molecular weight of ϵ-PCL solid in 0.5 M primary alkylamine methanol solutions at 37°C; (○) propylamine; (♦) octylamine; (△) decylamine; (▲) dodecylamine.

mass loss continued. These results suggest that the partially degraded ϵ-PCL dissolved in methanol when its molecular weight dropped below 10,000.

The weight percent remaining of ϵ-PCL in 0.5 M secondary alkylamine methanol solutions over a period of 90 days is shown in Figure 5. The secondary alkylamines studied were diisopropylamine, dibutylamine and diethylamine. Diisopropylamine was the least catalytic, while diethylamine was the greatest among these secondary alkylamines. This order of catalysis was unexpected, since as the chain length of the secondary alkylamines increases their hydrophobicity increases. The dominant effect here appears to be steric hindrance due to the increased size of the alkyl groups. For example, dibutylamine would have greater steric hindrance than diethylamine for approach of the nitrogen lone pair electrons to the carbonyl of ϵ-PCL ester groups. Similarly, diisopropylamine having two branched alkyl chains shows even greater steric hindrance markedly reducing its catalytic effect.

The FTIR spectra of products extracted from reaction solutions containing diethylamine or diisopropylamine, are shown in Figure 6. Only a single carbonyl band at 1730 cm^{-1} is observed in these FTIR spectra, corresponding to the carbonyl group of an ester. No band at 1660 cm^{-1} corresponding to an amide is present in the reaction products, indicating secondary alkylamines act as nucleophilic catalysis rather than by nucleophilic attack.

The mass loss of ϵ-PCL in methanol containing tertiary alkylamines, which included *N,N*-diisopropylethylamine or quinuclidine, is shown in Figure 7. Quinuclidine, a bicyclic tertiary alkylamine with seven carbons, caused degradation of 90% of the ϵ-PCL solid within 30 days. The catalytic ef-

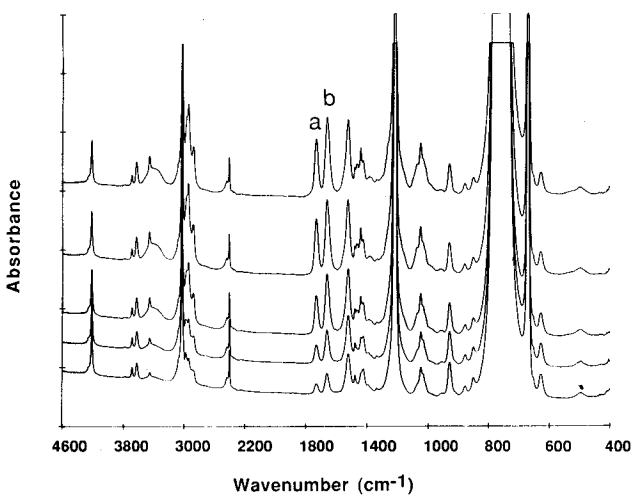

Fig. 3. FTIR spectra of reaction products obtained from ϵ-PCL solid in butylamine methanol solutions at 37°C. The spectra from lowest to highest correspond to reaction times of 3, 8, 13, 18, and 22 days. The band labeled **a** at 1730 cm^{-1} corresponds to an ester carbonyl and the band labeled **b** at 1660 cm^{-1} corresponds to an amide carbonyl.

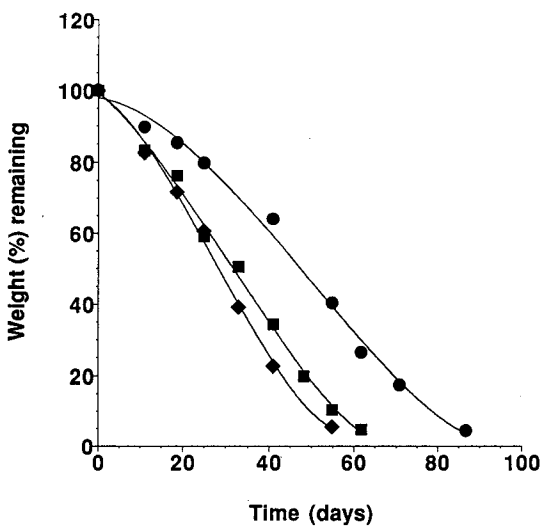

Fig. 5. Weight (%) remaining of ϵ-PCL solid in 0.5 M secondary alkylamine methanol solutions at 37°C; (●) diisopropylamine; (■) dibutylamine; (♦) diethylamine.

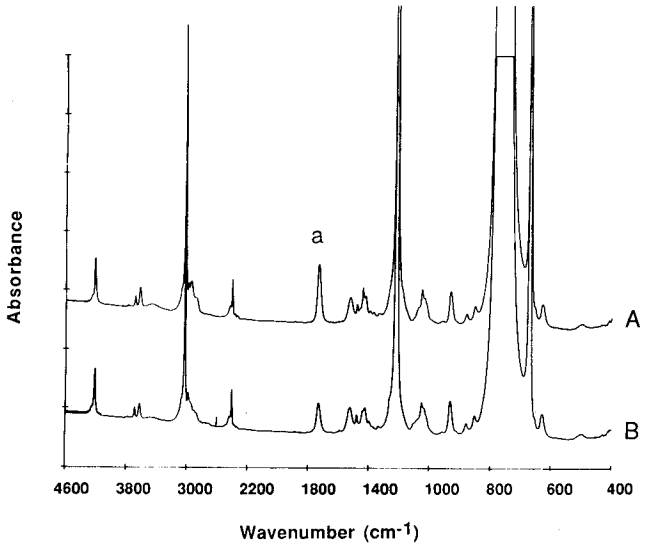

Fig. 6. FTIR spectra of reaction products obtained from (A) ϵ-PCL solid in secondary diethylamine methanol solutions at 37°C after 22 days; (B) ϵ-PCL solid in secondary diisopropylamine methanol solutions at 37°C after 22 days. The ester carbonyl band at 1730 cm^{-1} is labeled as **a**.

fect of this tertiary amine is comparable to that obtained with propylamine. A second tertiary amine, *N,N*-diisopropylethylamine, displayed a much smaller catalytic effect requiring ~90 days to cause the same degree of degradation of ϵ-PCL. The reasons for the large differences in the degradation rate of ϵ-PCL in the presence of these two tertiary alkylamines are not entirely clear. The molecular size of the quinuclidine is smaller than the *N,N*-diisopropylethylamine possibly allowing quinuclidine to be more soluble in ϵ-PCL. Alternatively, quinuclidine's nitrogen may be less sterically shielded by the rest of the molecule making it a better catalyst.

N,N-diisopropylethylamine and quinuclidine resulted in the formation of the same reaction products as were seen using secondary alkylamines. The FTIR spectra of these extracted reaction products are shown in Figure 8. Only ester products were indicated with a peak at 1730 cm^{-1}. These results indicate that both secondary and tertiary alkylamines act to accelerate the degradation of ϵ-PCL by the same mechanism and without direct reaction with the ester group.

Comparison of the degradation rate of ϵ-PCL solid in 0.5 M methanol solutions of primary, secondary and tertiary alkylamines is shown in Figure 9. The amines chosen for comparison, octylamine, dibutylamine and *N,N*-diisopropylethylamine, each had an equivalent number of carbons. This comparison clearly shows the difference in catalytic ability of primary, secondary and tertiary amines. The major difference in the degradation rates of ϵ-PCL by primary, secondary and tertiary alkylamines is probably attributable to differences in degradation mechanism and to differences in steric hindrance by the alkyl side chain.

In this work we have not only demonstrated that simple amines may react with ϵ-PCL or cause its accelerated degradation, but we have also established the degradation process involves steric as well as hydrophobic factors for the catalysts. In other such studies, only the degradation of a poly(ester) was monitored by mass loss and/or MW change with no further investigation to elucidate the products or underlying mechanism (18,23–27). Our results show that primary amines can react with ϵ-PCL and produce amide reaction products which has implications for the delivery of primary amine drugs from such polymers. In particular, protein and peptide drugs have primary amino groups in their side chains that may irreversibly react with ϵ-PCL or other poly(esters) causing loss of protein/peptide over time or producing new immunogenic conjugates. Drugs with secondary and/or tertiary amino functionalities do not react directly with poly(esters) but instead catalytically accelerate their breakdown. Controlling this process will be critical for both the controlled release of drugs and the accelerated biodegradation of ϵ-PCL. Future efforts will be directed at extend-

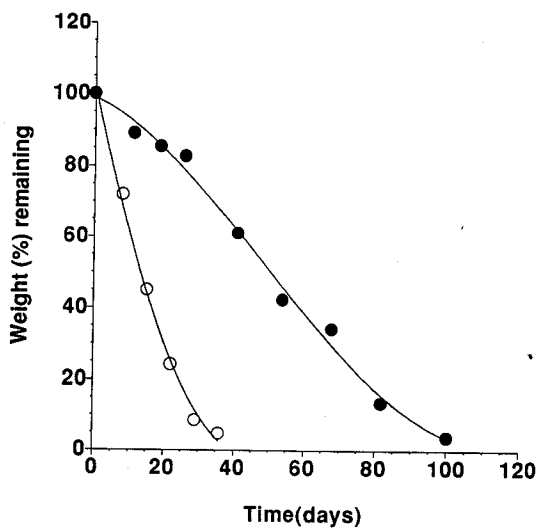

Fig. 7. Weight (%) remaining of ϵ-PCL solid in tertiary alkylamine methanol solutions at 37°C; (●) *N,N*-diisopropylethylamine, 0.5 M; (○) quinuclidine, 0.479 M.

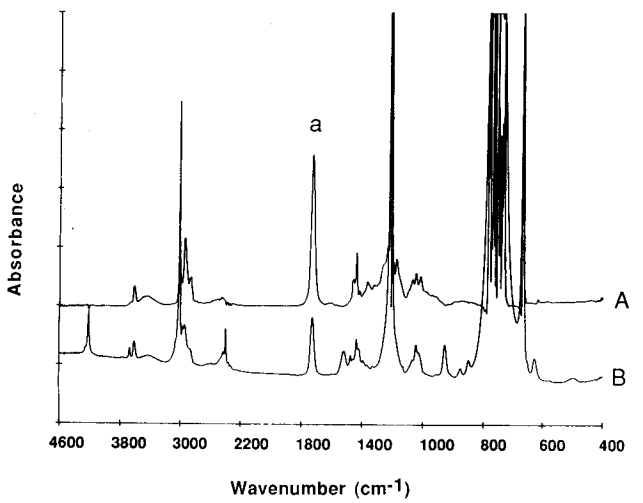

Fig. 8. FTIR spectra of reaction products obtained from (A) ϵ-PCL solid in tertiary quinuclidine methanol solutions at 37°C after 29 days; (B) ϵ-PCL solid in tertiary *N,N*-diisopropylethylamine methanol solutions at 37°C after 63 days. The ester carbonyl band at 1730 cm^{-1} is labeled as **a**.

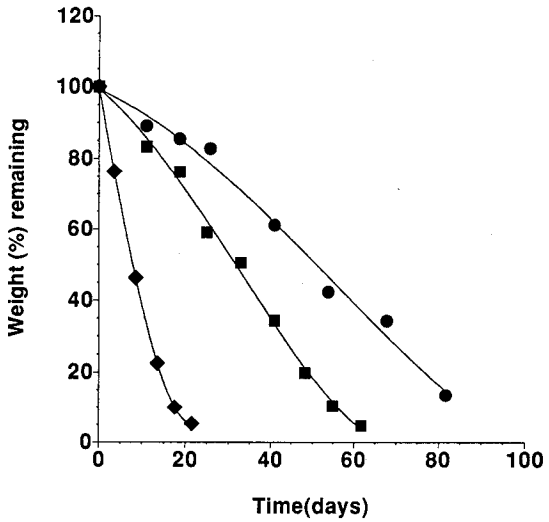

Fig. 9. Weight (%) remaining of poly(ϵ-caprolactone) solid in 0.5 M primary, secondary and tertiary alkylamine methanol solutions at 37°C; (●) *N,N*-diisopropylethylamine(3); (■) dibutylamine(2); (◆) octylamine(1).

ing these findings to aqueous systems and to systems in which the drug is dissolved or dispersed in the poly(ester) matrix.

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