Synthesis and Characterization of PEG-Based Ether−Anhydride Terpolymers: Novel Polymers for Controlled Drug Delivery

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ABSTRACT: A series of biodegradable poly(ether−anhydrides) composed of poly(ethylene glycol) (PEG), sebacic acid (SA), and 1,3-bis(carboxyphenoxy)propane (CPP) were synthesized for use in advanced drug delivery applications. PEG (M_n = 8000 Da) was incorporated to reduce polymeric particle clearance rates by the immune system and improve particle resuspension and aerosolization efficiencies. CPP and SA were selected to render the polymer insoluble in water and allow control over polymer degradation and drug release rates. In particular, CPP incorporation caused a significant decrease in polymer degradation rates and release kinetics of model drugs incorporated into poly(ether−anhydride) microparticles. Terpolymers were synthesized with weight-average molecular weights over 65 kDa without catalyst. The first thermal transition in polymers containing ≤10 wt % PEG was ∼80 °C (well above typical storage conditions and body temperature), and there was no evidence of a glass transition (−100 to 200 °C). Several of the polymers were used to produce particles suitable for injection or inhalation; these particles released model drugs, with molecular weights ranging from 443 to 5 143 000 Da, in a continuous fashion for up to 7 days.

Introduction

Biodegradable polymers have been used for many applications in medicine, including controlled release drug delivery systems,[1−3] resorbable bone pins and screws,[4−6] and scaffolds for cells in tissue engineering.[7,8] Systems based on biodegradable polymers obviate the need for surgical removal since their degradation products are absorbed or metabolized by the body. Micron-sized systems made using polymers can be used to deliver precise amounts of drugs, including proteins and genes, over prolonged periods to local tissues or the systemic circulation.[9,10] Of particular interest is the development of drug delivery vehicles that exhibit reduced detection rates by the immune system (e.g., long-circulating carriers for intravenous administration[11−13]) or that can be administered via noninvasive delivery routes (such as inhalation[14−17]). Biodegradable polymers that safely erode in the body, preferably at a rate that closely coincides with the rate of drug delivery,[10,18] are required for these advanced applications.

Despite their wide and growing need in medicine, only two synthetic biodegradable polymers are currently used routinely in humans as carriers for drug delivery: ester copolymers of lactide and glycolide (PLGA family) and anhydride copolymers of sebacic acid (SA) and 1,3-bis-(carboxyphenoxy)propane (CPP). PLGA is the most widely used due to its history of safe use as surgical sutures and in current drug delivery products like the Lupron Depot.[19] While the development of PLGA remains among the most important advances in medical biomaterials, there are some limitations that significantly curtail its use. First, PLGA particles typically take a few weeks to several months to completely degrade in the body, but the device is typically depleted of drug more rapidly.[16] Repeated dosing of such a system leads to an unwanted buildup of drug-depleted polymer in the body. This may preclude the use of PLGA for many applications, especially those that require injection of polymer drug carriers into the blood or, alternatively, their inhalation into the lungs. A second limitation is that PLGA devices undergo bulk erosion, which leads to a variety of undesirable outcomes, including exposure of unreleased drug to a highly acidic environment.[20] Third, it is difficult to release drugs in a continuous manner from PLGA particles owing to the polymers' bulk-erosion mechanism. Instead, special preparation methods[19,21,22] are required with PLGA to avoid the typical intermittent drug release pattern (i.e., burst of drug followed by a period of little or no drug release and then by the onset of a second phase of significant drug release).[23,24] Fourth, the particularly fine PLGA particles needed for intravenous injection or inhalation can agglomerate significantly, making resuspension for injection or aerosolization for inhalation difficult.[16,25] Finally, small, insoluble particles with hydrophobic surfaces, like those made with PLGA, are rapidly removed and destroyed by the immune system (due to fast opsonization).[26−28]

Implants composed of poly(CPP:SA) were approved for use in humans in the late 1990s to deliver chemotherapeutic molecules directly at the site of a resected brain tumor.[29,30] CPP:SA copolymers erode from the surface in (called surface erosion),[31] leading to desirable steady drug delivery rates over time. Proven biocompatibility, current clinical use, and steady drug release profiles make polymers composed of CPP and SA good candidates for new drug delivery applications. However, like PLGA particles, small particles made with poly(CPP:SA) possess hydrophobic surfaces that lead to rapid removal by the immune system and poor resuspension and aerosolization properties.[32]

In this paper, we describe the synthesis of a new family of terpolymers for advanced drug delivery applications that may overcome some important limitations of existing synthetic biodegradable polymers. The polymers are composed of three monomers, poly(ethylene glycol) (PEG), SA, and CPP, polymerized in

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various ratios to develop a series of PEG:SA:CPP poly(ether−anhydrides). These polymers are similar to the FDA-approved poly(CPP:SA) polymers currently in use, except they contain PEG built into their backbone structure. Each monomer is currently used in humans, but they have not yet been polymerized together to produce a useful material.

We previously reported on the synthesis of PEG:SA copolymers that utilized PEG with a lower molecular weight (M_n = 600 Da) and that did not include CPP.[15] In the current paper, we show that larger molecular weight PEG (8000 Da) chains can be incorporated into high molecular weight poly(ether−anhydrides) under optimized synthetic conditions. This is critical since PEG with molecular weights in the range of 2−20 kDa are most effective at protecting particles from removal by the immune system.[26−28] Therefore, the successful incorporation of PEG8000 into the backbone in high amounts is expected to significantly improve the performance of fine nano- and microparticles designed for more advanced drug delivery applications. We also incorporate a third monomer component into the polymer backbone in this study, the hydrophobic monomer 1,3-bis(carboxyphenoxy)propane (CPP), which significantly improves the range of properties of the poly(ether−anhydride) family for drug and gene delivery applications. We use the materials to produce drug-loaded microparticulates capable of injection via small needles or aerosolization as a dry powder, and we demonstrate the utility of this new terpolymer family as controlled release vehicles for drug molecules ranging in molecular weight from 443 to over 5×10^6 Da.

Experimental Section

Materials. Sebacic acid (Sigma) was recrystallized three times from ethanol. Acetic anhydride (Aldrich) was purified by distillation. Toluene (J.T. Baker) and chloroform (Aldrich) were refluxed over and distilled from calcium hydride (Sigma). Hydroxyl-terminated poly(ethylene glycol) (PEG, M_n = 8000) (Sigma) was dried by lyophilization before use. 1,3-Bis(carboxyphenoxy)propane (CPP) was synthesized according to the method described by Conix.[33] Succinic anhydride (Sigma), cadmium acetate (Aldrich), poly(vinyl alcohol) (88 mol % hydrolyzed, 20 kDa MW, Polysciences), pyridine (Aldrich), diethyl ether (J.T. Baker), petroleum ether (Fisher), dimethylformamide (Aldrich), methylene chloride (Fisher), and rhodamine B base (Sigma) were used as received without further purification.

Methods. ^{1}H NMR spectra were recorded in $CDCl_3$ on a Varian UNITY 400 MHz spectrometer. The composition of the poly(ether−anhydrides) was determined by using the ratio of average intensities per proton of each of the monomers. Infrared (IR) spectra were obtained using a Perkin-Elmer 1600 series spectrometer. The samples were ground and pressed into KBr pellets for analysis.

The molecular weight and polydispersity of the polymers were determined by gel permeation chromatography (GPC) analysis (JASCO PU-980 intelligent HPLC pump, 1560 intelligent column thermoset, RI-1530 intelligent refractive index detector). Samples were filtered and eluted in chloroform through a series of Styragel columns (guard, HR4, and HR3 Waters Styragel columns) at a flow rate of 0.3 mL/min. The molecular weights were determined relative to polystyrene standards (Fluka, Milwaukee, WI).

Thermal analysis was performed using a SEKIO DSC220 differential scanning calorimeter. An average sample weight of 5−10 mg was heated from −100 to 200 °C at a rate of 10 °C/min.

Polymer particle morphology was evaluated by scanning electron microscopy (SEM) with an AMRAY 1860 FE microscope. Microparticle samples were attached to SEM mounts using double-sided graphite carbon tape and sputter-coated with gold−palladium using a Hummer VI sputtering system (Bethesda, MD). Populations representative of each microsphere sample were photographed.

Stability studies were performed in solid state and in anhydrous chloroform at 25, 5, −20, and −80 °C under P_2O_5. Polymer molecular weight was followed by GPC with time.

Synthesis of Polyoxyethylene Dicarboxylic Acid Monomer. Hydroxyl-terminated PEG (40.0 g) was dissolved in chloroform (300 mL). Succinic anhydride (5.0 g) and pyridine (5 mL) were added to the chloroform solution, and the mixture was reacted at 60 °C for 72 h. The solution was cooled, filtered, and concentrated to dryness by rotary evaporation. The crude product was dissolved in 30 mL of 1 N HCl, washed with diethyl ether, extracted with chloroform, and dried with anhydrous sodium sulfate. Excess solvents were removed under vacuum. ^{1}H NMR ($CDCl_3$): δ 3.65 (s, OCH_2CH_2), 2.48 (t, CH_2). IR (KBr, cm^{-1}): 1735 (C═O), 1110 (CH_2OCH_2).

Preparation of Acylated Prepolymers. Polyoxyethylene dicarboxylic acid (10.0 g) was refluxed in 200 mL of acetic anhydride for 30 min under N_2 and evaporated to dryness by rotary evaporation. The residue was extracted with anhydrous ether and dried under vacuum. ^{1}H NMR ($CDCl_3$): δ 3.64 (s, OCH_2CH_2), 2.32 (s, CH_3), 2.47 (t, CH_2). IR (KBr, cm^{-1}): 1807, 1743 (C═O anhydride), 1110 (CH_2OCH_2).

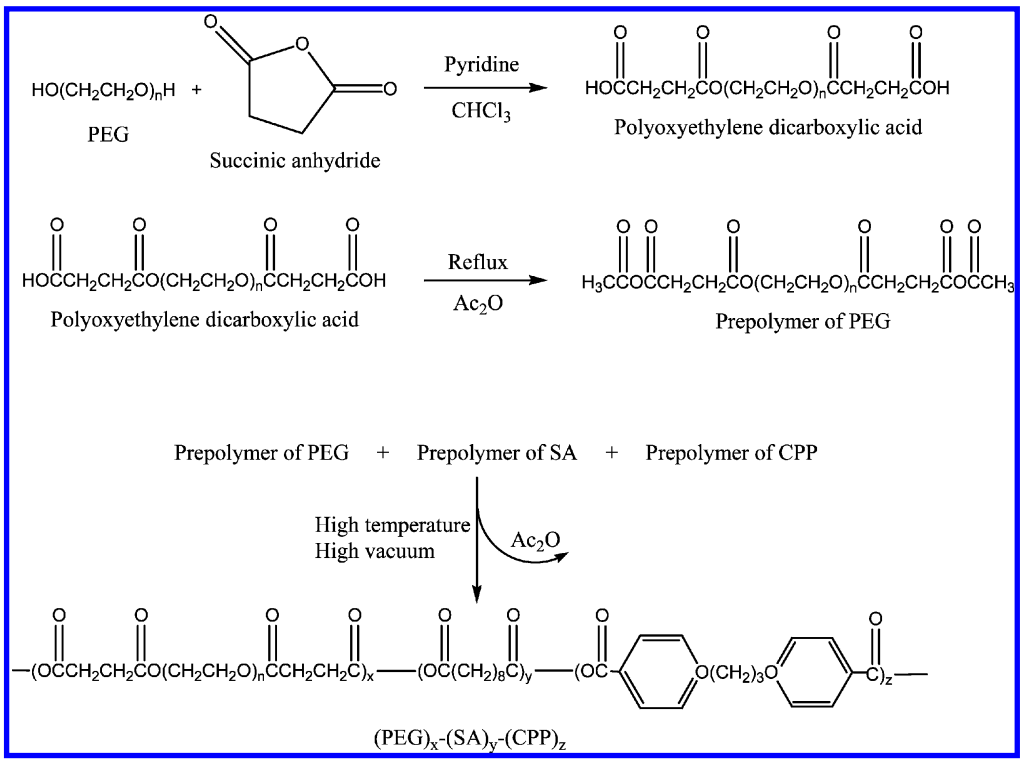
Sebacic acid (10.0 g) was refluxed in 100 mL of acetic anhydride under N_2 for 15 min and evaporated to dryness by rotary evaporation. The crude prepolymer was recrystallized from dried toluene, washed with anhydrous ethyl ether/petroleum ether (1:1), and finally dried under vacuum. ^{1}H NMR ($CDCl_3$): δ 2.45 (t, 4H, CH_2), 2.33 (s, 6H, CH_3), 1.66 (m, 4H, CH_2), 1.33 (m, 8H, CH2). IR (KBr, cm^{-1}): 1813, 1742 (C═O anhydride).

CPP (10.0 g) was refluxed in 200 mL of acetic anhydride for 30 min under N_2, followed by removal of the unreacted diacid by filtration and solvent by evaporation. The residue was recrystallized from dimethylformamide and ethyl ether, then washed with dry ethyl ether, and dried under vacuum. ^{1}H NMR ($CDCl_3$): δ 7.14, 7.99 (d, 4H, ArH), 4.29 (t, 4H, CH_2), 2.38 (s, 6H, CH_3), 2.25 (m, 2H, CH_2). IR (KBr, cm^{-1}): ∼1798, ∼1773, and 1718 (C═O anhydride).

Melt Polymerization. Acyl-PEG, acyl-SA, and acyl-CPP were mixed in a defined ratio (with or without 0.5−2.0% catalyst) in a round-bottom flask with a stopcock adapter. Poly(ether−anhydrides) of eight different compositions were synthesized by melt polycondensation of prepolymers in the bulk under high vacuum.[34] Briefly, the flask was immersed in an oil bath at the selected temperature (150−220 °C), and the monomers were allowed to melt. High vacuum was applied, and the condensation byproduct, acetic anhydride, was collected in a liquid nitrogen trap. Throughout the polymerization, a strong nitrogen sweep was performed for 30 s every 15 min to agitate the reacting melt. At the end of the reaction, the polymers were allowed to cool completely and then dissolved in chloroform. The solution was precipitated dropwise into excess petroleum ether. The precipitate was collected by filtration and dried under vacuum to constant weight. ^{1}H NMR ($CDCl_3$): δ 6.95, 7.98 (d, ArH), 4.25 (s, CH_2), 3.65 (s, OCH_2CH_2O), 2.44 (t, CH_2), 2.33 (m, CH_2), 1.65 (m, CH_2), 1.32 (s, CH_2). IR (KBr, cm^{-1}): ∼1813−1773, ∼1742 (C═O anhydride), 1112 (CH_2OCH_2).

Preparation of Drug-Loaded Poly(PEG:SA:CPP) Particles. Drug-loaded particles were prepared using a double emulsion solvent evaporation method.[15,16] The primary water-in-oil emulsion was created by probe sonication (Sonics and Materials Inc., Newtown, CT) of 100 μL of an aqueous solution (±2 mg/mL DNA) in a 50 mg/mL polymer solution in 4 mL of methylene chloride (±5 mg/mL rhodamine B base). The primary emulsion was then poured into 100 mL of 1% PVA solution and homogenized at 6000 rpm for 1 min to form the double emulsion (Silverson Machines Inc., East Longmeadow, MA). The particles were stirred for 3 h to allow hardening, collected by centrifugation at 3400 rpm (18.5 cm rotor, International Equipment Co., Needham heights, MA), washed twice with deionized water, resuspended in 10 mL of water,

Scheme 1



and freeze-dried. Model drug molecules were added either to the primary aqueous phase (LacZ plasmid DNA; 5148 kDa) or to the organic phase (rhodamine B base; 443 Da) dependent on which phase they were soluble.

Determination of Particle Size and Density. Particle size distribution was determined using a Coulter Multisizer IIe (Beckman-Coulter Inc., Fullerton, CA). Approximately 2 mL of Isoton II electrolyte solution (Beckman-Coulter Inc.) was added to 5−10 mg of microparticles. The solution was briefly vortexed to suspend the microparticles and added dropwise to 100 mL of isoton II solution until the coincidence of particles was between 8% and 10%. Greater than 100 000 particles were sized for each batch of microparticles to determine the mean particle size and size distribution. The bulk density of the particles was determined by tap density. Briefly, particles were loaded into 0.3 mL sections of a 1 mL plastic pipet, capped with NMR tube caps, and tapped approximately 300−500 times until the volume of the powder did not change. The tap density was determined from the difference between the weight of the pipet before and after loading, divided by the volume of powder after tapping.

Degradation of Poly(PEG:SA:CPP) Particles. 10 mg of particles was suspended in 1 mL of phosphate-buffered saline (pH 7.4) and incubated at 37 °C on a rotator. At predetermined time intervals, the remaining particles were collected by centrifugation at 13 000 rpm (7.3 cm rotor, National Labnet Co., Woodbridge, NJ), and the supernatant was removed. Samples were dissolved in chloroform and filtered, and the molecular weight was determined by GPC.

Drug Release from Poly(PEG:SA:CPP) Particles. 10 mg of drug-loaded particles was suspended in 1 mL of phosphate-buffered saline (pH 7.4) and incubated at 37 °C on a rotator. At selected time points, supernatants were removed following centrifugation at 13 000 rpm (7.3 cm rotor, National Labnet Co., Woodbridge, NJ) and were replaced with 1 mL of fresh phosphate buffer. Supernatants were either analyzed for rhodamine B base fluorescence using a TD-700 fluorometer (Turner Designs, Sunnyvale, CA) at excitation and emission wavelengths of 550 and 570−700 nm or assayed for pDNA content using the PicoGreen dye exclusion assay (Molecular Probes, Eugene, OR).

Results and Discussion

We have synthesized a family of poly(ether−anhydrides) in which the ratio of monomers, poly(ethylene glycol) with $M_n = 8000$ Da (PEG), sebacic acid (SA), and 1,3-bis(carboxyphenoxy)propane (CPP), were varied. An outline of the monomer and polymer synthesis is shown in Scheme 1. As discussed in subsequent sections, poly(PEG:SA:CPP) with up to 50 wt % PEG can be used to generate drug-loaded microparticles for inhalation or injection. These particles can efficiently release drugs with molecular weights ranging from 443 to 5 148 000 Da in a continuous fashion for up to 7 days. Importantly, the versatile SA:CPP polymers have been modified to incorporate various percentages of PEG into their backbone, which should significantly enhance their applicability to various advanced drug delivery applications. PEG with M_n between ~2 and 20 kDa is known to render insoluble particles less susceptible to phagocytosis and destruction by macrophages in the bloodstream or the lung,[27,35,36] thereby allowing the particles to release drug for longer periods of time. PEG also reduces the interparticle adhesion forces, which facilitates improved dispersion for injection[36] and enhances particle aerosolization from dry powder inhalers.[32]

Characterization of PEG:SA:CPP Terpolymers. The structure of PEG:SA:CPP terpolymers was confirmed by FT-IR, ^{1}H NMR, and GPC. The ^{1}H NMR (Figure 1) resonance line of the methylene protons of PEG appeared at 3.65 ppm. The three peaks at 2.44, 1.65, and 1.32 ppm were attributed to the methyl protons of SA and peaks at 2.33, 4.25, 6.95, and 7.98 ppm attributed to the protons of CPP. The peak at 7.25 ppm corresponds to the solvent, deuterated chloroform. Importantly, the actual weight percentages of PEG, SA, and CPP in the polymer (estimated by integration and comparison of the corresponding NMR peaks) showed good agreement with the monomer feed ratio. The amount of SA in the copolymer was usually only slightly

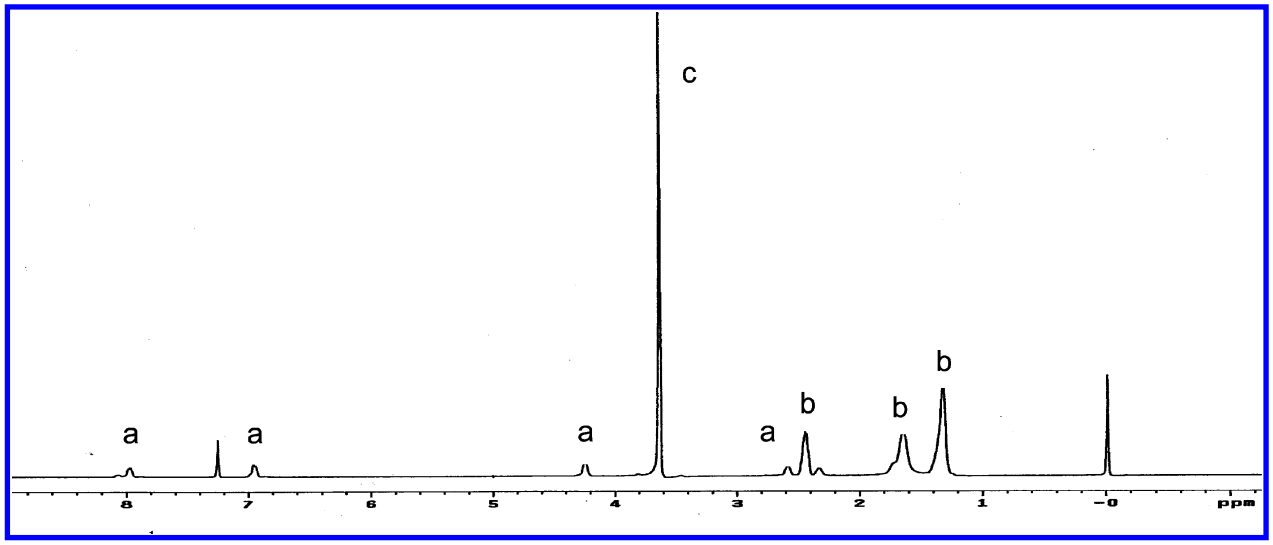


Figure 1. ^{1}H NMR spectra of poly(PEG:SA:CPP) (30:50:20) poly(ether−anhydride): a, peaks attributed to CPP; b, peaks attributed to SA; c, peak attributed to PEG.

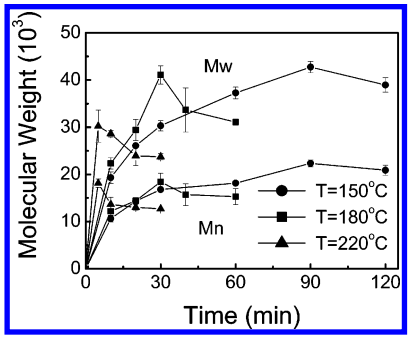


Figure 2. Molecular weight of poly(PEG:SA) (30:70) as a function of reaction time at different temperatures (without catalyst, vacuum ~0.08 Torr).

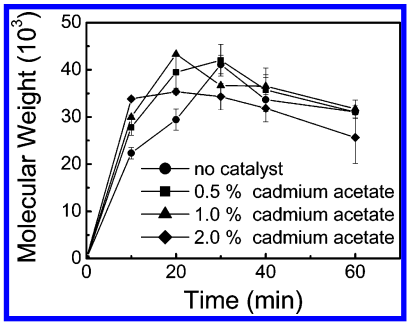


Figure 3. Molecular weight of poly(PEG:SA) (30:70) as a function of reaction time (with catalyst, vacuum ~0.08 Torr). Polymers were melt-polymerized at 180 °C in the presence of cadmium acetate ($CdAc_2$).

higher than the amount fed, while the CPP amount was slightly less than fed. This may be due to the decreased flexibility of CPP owing to steric hindrance. The relatively limited mobility of CPP may cause a decrease in polymer chain interactions with CPP, subsequently decreasing the extent of CPP polymerization. Chromatographs from GPC analysis contained one peak corresponding to the molecular weight of the polymer (not shown). NMR studies combined with GPC data indicated that PEG was successfully copolymerized with SA and CPP.

Polymer Synthesis Optimization. The effects of polymerization time and temperature on poly(ether−anhydride) molecular weight were studied using PEG:SA with a weight feed ratio of 30:70 (Figures 2 and 3). Subsequently, we discovered that a lowered vacuum pressure during polymerization led to significantly enhanced polymer molecular weights at the same reaction temperatures and times (e.g., higher values reported in Table 1).

Figure 2 shows the dependence of polymerization time on polymer weight- and number-average molecular weight. With increased polymerization temperature, a maximum in molecular weight was obtained in a shorter amount of time (90 min at 150 °C to 10 min at 220 °C); however, the maximum molecular weight that could be achieved decreased at the highest temperature. When polymerization was conducted at 180 °C, polymer molecular weight achieved a maximum after polymerization for 30 min. Reaction at 180 °C for 30 min was determined to be the optimal polymerization conditions without catalyst since a high molecular weight polymer could be produced in a relatively short time.

The effect of catalyst on polymer molecular weight was also studied (Figure 3). Cadmium acetate ($CdAc_2$) has previously been shown to be effective in producing high molecular weight polyanhydrides.[34,37] The addition of catalyst led to only slightly higher polymer molecular weights, with the maximum being achieved at 20−30 min. However, since the maximum molecular weight with catalyst (t = 20 min, 1% $CdAc_2$, M_w = 43.3 kDa) was not significantly different than without (t = 30 min, M_w = 42.0 kDa), a catalyst was not deemed necessary. Since it is preferable to avoid the use of potentially toxic catalysts during polymerization, reaction conditions of 180 °C for 30 min (without $CdAc_2$) were used for all further polymerizations.

We used the optimized reaction temperature and time, as determined with PEG:SA:CPP 30:70:0, to produce high molecular weight terpolymers with up to 35 wt % PEG and 45 wt % CPP incorporated into the polymer backbone (Table 1). The molecular weight of polymers generally decreased with increasing amounts of PEG or CPP in the polymer backbone (Table 1) under the same polymerization conditions (180 °C for 30 min). The higher molecular weight polymers (up to 67 kDa for terpolymers with significant percentages of all three monomers) summarized in Table 1 were produced using a slightly lower vacuum pressure than that used to

Table 1. Characterization of Poly(ether−anhydrides) Synthesized with PEG8000[a]

PEG:SA:CPP in the feed[b] (wt %)	yield (%)	PEG:SA:CPP ^{1}H NMR[c] (wt %)	M_w (Da)	M_n (Da)	PDI	T_{m1} (°C)	T_{m2} (°C)
5:95:0	86.4	4.4:95.6:0	80 500	26 000	3.10	*d*	81.3
10:90:0	82.5	9.2:91.8:0	80 800	31 800	2.54	*d*	80.4
30:70:0	85.6	28.6:71.4:0	56 500	25 600	2.21	49.8	79.9
40:60:0	81.4	37.6:62.4:0	49 100	20 600	2.39	*e*	*e*
50:50:0	83.1	46.8:53.2:0	41 600	18 700	2.22	*e*	*e*
30:50:20	81.2	33.1:49.4:17.5	67 000	28 900	2.32	50.9	63.0
30:35:35	78.3	34.7:35.4:29.9	66 500	27 000	2.46	*e*	*e*
30:20:50	77.1	30.7:24.6:44.7	58 100	24 200	2.40	50.5	*d*

[a] No glass transition detected between −100 and 200 °C. [b] Poly(ether−anhydrides) were polymerized at 180 °C, ∼0.04 Torr for 30 min. [c] Estimated from the integral height of hydrogen atoms in the ^{1}H NMR spectra. [d] Not detectable. [e] Not tested.

produce the polymers described in Figures 2 and 3 (∼0.04 Torr compared to ∼0.08 Torr). High molecular weight polymers are necessary to impart mechanical strength to the polymers and to render them water-insoluble (so that devices made from them do not readily dissolve in the body) but should not be so high that processing becomes difficult or impossible. Polyanhydride molecular weights above approximately 15 kDa were sufficient for preparation of microparticles capable of controlled drug delivery. Polymers containing up to 50 wt % PEG or up to 35 wt % CPP all had molecular weights significantly above 15 kDa. Therefore, polymers produced in this study could be processed into microspheres using emulsion techniques, even after molecular weight stabilization during storage, as shown later.

Polymer polydispersities remained fairly constant regardless of the polymer composition, with most polydispersity index (PDI) values between 2.2 and 2.5 (Table 1). PDI values in this range are within the range of both the PLGA and poly(CPP:SA) families of polymers currently used in humans.

Thermal Analysis. Thermal analysis was performed on the poly(ether−anhydrides), as shown in Table 1. Only the SA melting point was observed (∼80 °C) when there was less than 10% PEG in PEG:SA polymers. The melting point of PEG (49.8 °C) and SA (79.9 °C) were both observed when PEG content was increased to 30%. The appearance of two distinct melting points implies that PEG- and SA-rich regions phase separate at high PEG percentages. The SA melt point disappeared, and a new melt point appeared at 60.3 °C, when 20% CPP was introduced into a polymer chain containing 30% PEG. This likely indicates that CPP disrupted SA crystallinity and that CPP and SA phases are colocalized. The addition of 50% CPP caused the SA melting point to be undetectable, leaving only the PEG melting point. Therefore, the CPP:SA-rich phase of the polymer was amorphous, and the CPP and SA monomers are likely distributed uniformly throughout the polymer backbone. No glass transition temperature was observed for any of the polymers (−100 to 200 °C). Additionally, since the melting temperature of the entire family of poly(ether−anhydrides) was 50 °C or higher (∼80 °C for polymers composed of ≤10% PEG), these polymers are not expected to undergo a thermal phase transition under typical storage or use conditions.

Stability of PEG:SA:CPP Terpolymers. The stability of poly(PEG:SA:CPP) (30:50:20), a model terpolymer with significant percentage of all three monomers, was studied in the dry state (as a high surface area powder) and in solution (anhydrous chloroform) at temperatures ranging from −80 to 25 °C (Figure 4). Polymers stored under all conditions showed an initial decrease in molecular weight within a few days and then

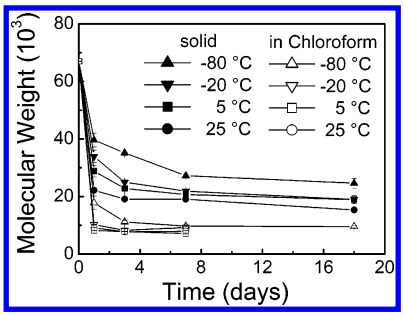


Figure 4. Stability profiles of poly(PEG:SA:CPP) (30:50:20) in the solid state and in anhydrous chloroform at four storage temperatures. Polymerization conditions were 30 min at 180 °C without catalyst.

a stabilization of the molecular weight for at least 18 days (i.e., the duration of the study). A lower storage temperature provided more protection against degradation (∼28 kDa at −80 °C vs ∼20 kDa at 25 °C in the solid state). Additionally, polymers stored in the solid form (∼28 kDa at −80 °C) maintained higher molecular weights than those stored in chloroform (∼15 kDa at −80 °C). The final molecular weights obtained after stabilization was sufficient to allow the formation of microspheres by emulsion techniques and the controlled release of therapeutic drugs from the microspheres for all polymers tested.

Poly(PEG:SA:CPP) Particles for Inhalational Drug Delivery. Large (5−20 μm), low-density dry powder aerosols can be efficiently aerosolized into the deep lungs.[14,16] In particular, a decrease in particle density allows the efficient aerosolization of geometrically large particles (e.g., diameter > 5 μm) since they possess low aerodynamic diameters (e.g., < 3 μm) (see eq 1). Large particle size also reduces particle clearance rates by phagocytic cells, allowing them to remain in the deep lungs and deliver drugs for extended periods of time. We used the poly(ether−anhydrides) to produce geometrically large, but aerodynamically small, particles (owing to high porosity) and found that density decreased as the amount of PEG in the polymer backbone increased (Table 2), from 0.34 g/cm^3 (0% PEG) to 0.06 g/cm^3 (50% PEG). One possible explanation for this significant change in density is that the addition of the hydrophilic PEG monomer increases water uptake by the particles during their preparation, thus causing them to swell. Pores would then be formed as the water is removed during freeze-drying (Figure 5), leading to a decrease in density.

Particle aerodynamic diameter (d_a) was calculated by the following relation:

$$d_a = d\sqrt{\rho/\rho_a}/\gamma \qquad (1)$$

Table 2. Characterization of Poly(ether−anhydride) Microspheres

	geometric diam[a] (μm)	bulk density[a] (g/cm^3)	aerodynamic diam (μm)
PSA	6.26	0.34	3.66
PEG:SA (5:95)	6.05	0.32	3.43
PEG:SA (10:90)	5.97	0.28	3.15
PEG:SA (30:70)	5.66	0.14	2.13
PEG:SA (40:60)	5.86	0.08	1.67
PEG:SA (50:50)	6.45	0.06	1.51
PEG:SA:CPP (10:70:20)	6.54	0.15	2.57
PEG:SA:CPP (30:50:20)	6.28	0.11	2.07

[a] Geometric size and density are the average of three measurements.

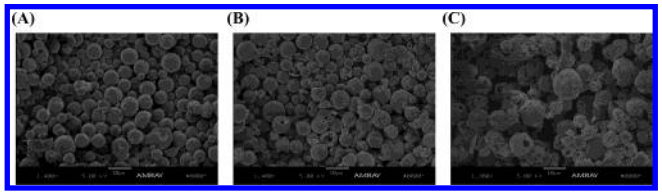


Figure 5. Morphology, determined by scanning electron microscopy, of (A) PSA, (B) poly(PEG:SA) (10:90), and (C) poly(PEG:SA) (30:70) microspheres, showing an increase in the number of visible pores in the microspheres with an increase in the amount of PEG in the polymer backbone.

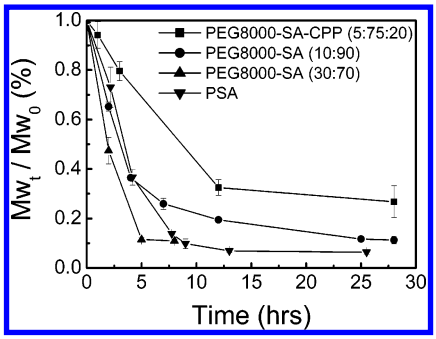


Figure 6. Degradation profiles of poly(ether−anhydride) microspheres in PBS (pH 7.4, 37 °C).

where d = geometric diameter, ρ = particle bulk density (g/cm^3), ρ_a = water mass density (1 g/cm^3), and γ = shape factor = 1 for a sphere. Therefore, an aerosolized particle with a geometric diameter of 1 μm and standard density of 1 g/cm^3 will deposit in the respiratory tract following inhalation as a dry powder in roughly the same manner as a 10 μm particle with a density of 0.01 g/cm^3. The decreased density of particles as PEG content increased resulted in a significant decrease in particle aerodynamic diameter (d_a) from 3.7 (0% PEG) to 1.5 μm (50% PEG) (Table 2). Thus, these particles are within the necessary aerodynamic size range (1−5 μm) for efficient aerosolization into the deep lungs, even though the geometric diameters are significantly larger (6.3−6.5 μm). PEG also aids in the control of particle aggregation that can prevent efficient aerosolization. In particular, we recently showed that the inclusion of PEG in the polymer backbone of ether−anhydrides significantly improves aerosolization of the microspheres.[32]

Degradation of Poly(PEG:SA:CPP) Particles. The degradation rate of poly(PEG:SA:CPP) particles immersed in phosphate buffered saline (PBS) at 37 °C increased with increasing amounts of PEG (Figure 6), likely because the polymer becomes more hydrophilic upon addition of PEG in the backbone. PSA was degraded to 10% its original molecular weight after 13 h, whereas PEG:SA (30:70) was degraded to 10% its original molecular weight after only 5 h. In a related study,[15] we used PEG with a lower molecular weight (600 Da vs 8000 Da used here) and showed that PEG600:SA (30:70) degraded to 20% of its original molecular weight in 2.5 h, compared to 50% for PEG8000:SA (30:70) in the same time period (Figure 6). Thus, an increase in the size of PEG monomer added into the polymer backbone (8000 Da vs 600 Da) caused the polymer to degrade more slowly. The addition of very hydrophobic CPP into the polymer backbone, however, had the most significant effect on decreasing the polymer degradation rate (Figure 6).

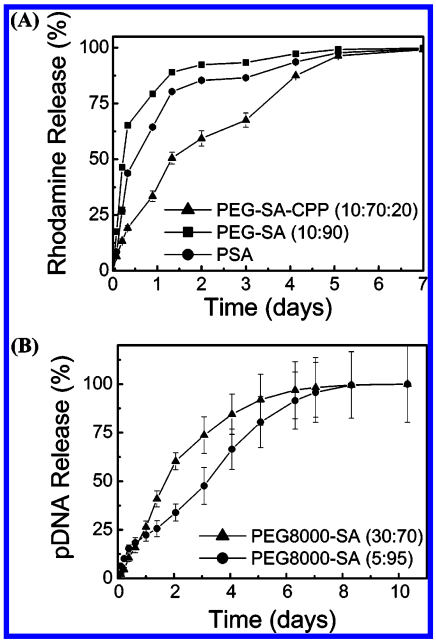


Figure 7. Release of model drugs, (A) rhodamine B base (MW = 443 Da) and (B) LacZ plasmid DNA (MW = 5143 kDa), from poly(ether−anhydride) microspheres.

It is important to note that although the polymer molecular weight decreased significantly in a few hours in PBS at 37 °C, dissolution of the hydrophobic monomers is slow, thus producing steady drug release over longer time periods. Therefore, drug release is controlled mainly by monomer dissolution rates (i.e., particle erosion) rather than polymer degradation rates (see next section). Also, because degradation in the lungs occurs on a thin layer of fluid (~0.2 μm in the alveoli where the majority of oxygen is exchanged with carbon dioxide), the in vivo degradation of the relatively large particles may be significantly slower compared to particles completely immersed in PBS. We are currently developing methods to model the degradation of particles on pulmonary epithelial cells covered by a thin aqueous film.[38]

Drug Release. Varying the monomer composition in the polymer backbone allowed control over the release of rhodamine B base, a small molecular weight hydrophobic fluorescent molecule, from microparticles (Figure 7A). Increasing the hydrophobicity of the particles (by increasing the percentage of SA or CPP relative to PEG) decreased the drug release rate. PEG:SA (10:90) particles released 60% of their drug load in approximately 4 h, whereas PSA particles (more hydrophobic) released 60% in approximately 18 h and PEG:SA:CPP (10:70:20) particles (most hydrophobic) released 60% in about 2.5 days. No initial burst of drug was seen for any of the particles studied.

The release of LacZ plasmid DNA (5148 kDa) from the poly(ether−anhydride) particles (Figure 7B) was similar to that of the smaller molecule, rhodamine B base, and lasted approximately 7 days (compared to 5 days for rhodamine B base). The slightly protracted release time is likely due to the large size of the DNA molecule inhibiting its diffusion through the polymer matrix. Similar to the small molecule release, the composition of the polymer backbone controlled the DNA release rate, with the more hydrophilic polymer (PEG:SA 30:70) releasing DNA faster than the more hydrophobic polymer (PEG:SA 5:95). Again, no initial burst of drug (DNA) was seen for the particles examined.

Conclusions

A new family of poly(ether−anhydrides) containing various ratios of SA, CPP, and PEG was synthesized for use as drug carriers for injection or inhalation. The polymers were designed to enable the creation of particles that erode over time periods roughly equivalent to the rate of drug release, to provide steady controlled release of various drug molecules, and to potentially avoid rapid destruction by the immune system due to the incorporation of PEG8000 into the polymer backbone. These new polymers may have significant advantages compared to those currently used in humans for a variety of applications and are composed of monomers already safely used in humans.

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