Synthesis, Characterization, and Biodegradation of Novel Poly(ether ester amide)s Based on L-Phenylalanine and Oligoethylene Glycol

Kai Guo and C. C. Chu*

Department of Fiber Science and Apparel, and Biomedical Engineering Program, Cornell University, Ithaca, New York 14853-4401

Received February 6, 2007; Revised Manuscript Received May 11, 2007

A new family of novel biodegradable poly(ether ester amide)s (PEEAs) consisting of three building blocks (L-phenylalanine, oligoethylene glycol, and aliphatic acid dichloride) were synthesized by solution polycondensation. Using *N*,*N*-dimethylacetamide as the solvent, these PEEA polymers were obtained with fairly good yields with reduced viscosity (η_{red}) ranging from 0.13 to 0.61 dL/g. The chemical structures of the PEEAs were confirmed by IR, NMR spectra, and elemental analysis. The PEEAs had T_g values lower than that of the saturated poly(ester amide)s (PEAs) of similar structures due to the incorporation of ether bonds in the backbones. An increase in the number of ether bonds in PEEA resulted in a lower T_g value. The solubility of the PEEA polymers in a wide range of common organic solvents was significantly improved when compared with unsaturated PEAs. The preliminary in vitro biodegradation behaviors of PEEA polymers were investigated in both pure PBS buffer and α-chymotrypsin solution of different concentrations. The polymers showed a significantly faster weight loss in an enzyme solution (α-chymotrypsin) but a very slow biodegradation rate in pure PBS buffer. The enzymatic hydrolysis rates of PEEAs (in terms of weight loss) were found to be much faster than those of saturated and unsaturated polyesteramides reported in previous studies. The zero-order-like biodegradation kinetics and molecular weight data also suggested surface erosion biodegradation mechanisms for these PEEAs.

Introduction

Synthetic biodegradable polymers nowadays have been found as promising biomaterials with increasing applications in pharmaceutical, biomedical, and tissue engineering.[1−15] There are two basic types of aliphatic poly(ester amide)s (PEAs): those derived from non-amino acids like aliphatic diamine and those derived from amino acids like L-phenylalanine, L-leucine, and/or L-lysine.[16−26] Those amino acid derived PEAs appear to have better biocompatibility than those from aliphatic diamines and have formed a new family of biodegradable polymers that combine the favorable properties of both aliphatic polyesters and polyamides into a single entity[25] (i.e., biodegradability and desirable mechanical, physical, and thermal properties).

In our laboratory, amino acid derived saturated PEA (SPEA)[21,27] and unsaturated PEA (UPEA)[24] and copolymers of SPEA and UPEA (USPEA)[26] have been designed, successfully synthesized, characterized, and studied. The major unique characteristics of SPEA designed in our lab are the elastomeric mechanical property and the availability of a pendant free −COOH group, which could be used for the attachment of biological active agents like nitroxyl radicals.[27,28] Because of the relatively lower glass transition temperature, T_g, the second generation PEA, UPEA, was developed and synthesized.[26] This UPEA family has the advantages of not only having a relatively higher T_g than SPEA but also having functional C═C double bonds built into the PEA backbone. These double bonds are photosensitive and can serve as reactive sites for synthesizing additional PEA based derivatives, such as hydrogels for the controlled release of anticancer drugs.[29,30] Because of the C═C double bonds in their backbone, UPEA's solubility became less desirable than SPEA. As a result, the third generation PEA, USPEA, was designed and successfully synthesized.[26] USPEA integrated the merits of both SPEA and UPEA into a single entity, and the properties of USPEA can be easily controlled by simply adjusting the feed ratio of SPEA to UPEA during the polymerization process.

One of the three building blocks in all of the three amino acid based PEA generations has been simple aliphatic diols like butanediol and/or butenediol. In this work, three types of oligoethylene glycol (OEG) were introduced into the PEA macromolecular backbone as the replacement for aliphatic diols. Thus, this fourth generation PEA family, poly(ether ester amide) (PEEA), would have ether bonds incorporated in addition to the ester and amide linkages common to all PEAs. As a result, the hydrophilicity and biodegradability of these OEG based PEEAs were found to be both enhanced when compared to the PEA polymers and copolymers designed with conventional aliphatic diols. In this study, a series of saturated and unsaturated PEEAs were synthesized by solution polycondensation of unsaturated or saturated diester monomers and saturated OEG based diamine salts. The chemical structures of these PEEAs were confirmed by FTIR, NMR spectra, and elemental analysis. The molecular weight, molecular weight distribution (MWD), thermal property, solubility, and biodegradability of the resulting PEEA polymers were studied.

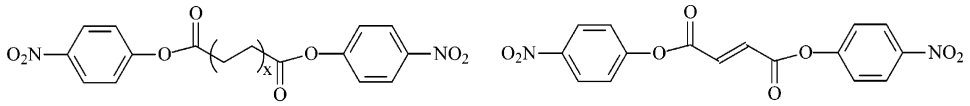
Experimental Procedures

Materials. L-Phenylalanine (L-Phe), *p*-toluenesulfonic acid monohydrate (TosOH·H_2O), sebacoyl chloride, adipoyl chloride, fumaryl chloride, diethylene glycol, triethylene glycol and tetraethylene glycol (Alfa Aesar, Ward Hill, MA), and *p*-nitrophenol (J. T. Baker, Phillipsburg, NJ) were used without further purification. Triethylamine

* Author to whom correspondence should be addressed. Fax: (607) 255-1093. E-mail: cc62@cornell.edu.

10.1021/bm070158c CCC: $37.00 © 2007 American Chemical Society
Published on Web 08/02/2007

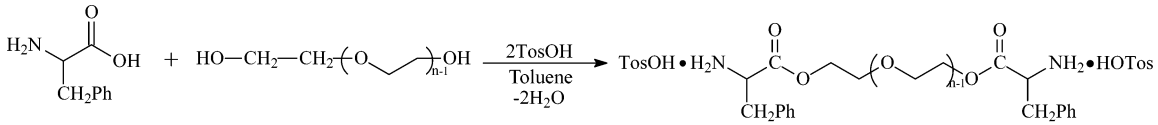
Scheme 1. Di-*p*-nitrophenyl Esters of Dicarboxylic Acids



Ia: di-*p*-**N**itrophenyl **A**dipate (**NA**), x=2
Ib: di-*p*-**N**itrophenyl **S**ebacate (**NS**), x=4

Ic: di-*p*-**N**itrophenyl **F**umarate (**NF**)

Scheme 2. Synthesis of Di-*p*-toluenesulfonic Acid Salts of Bis-L-phenylalanine Esters from Oligoethylene Glycols and L-Phenylalanine



IIa: *p*-Toluenesulfonic acid salt of L-phenylalanine diethylene glycoldiester (**P2EG**), n=2
IIb: *p*-Toluenesulfonic acid salt of L-phenylalanine triethylene glycoldiester (**P3EG**), n=3
IIc: *p*-Toluenesulfonic acid salt of L-phenylalanine tetraethylene glycoldiester (**P4EG**), n=4

from Fisher Scientific (Fairlawn, NJ) was dried by refluxing with calcium hydride and then distilled. *N*,*N*-Dimethylforamide (DMF) from Aldrich Chemical Co. (Milwaukee, WI) was dried over calcium hydride and distilled. Other solvents such as toluene, trifluoroethanol (TFE), tetrahydrofuran (THF), ethyl acetate, acetone, acetonitrile, *N*,*N*-dimethylacetamide (DMA), and dimethyl sulfoxide (DMSO) were purchased from VWR Scientific (West Chester, PA) and were purified by standard methods before use.

Synthesis of Monomers and Polymers. The synthesis of PEEAs involved the following three basic steps: (1) synthesis of three di-*p*-nitrophenyl esters of dicarboxylic acids (I), one of which was unsaturated and the other two saturated; (2) synthesis of three di-*p*-toluenesulfonic acid salts of bis-L-phenylalanine esters (II) from di-, tri-, and tetraethylene glycol; and (3) solution polycondensation of the monomers I and II, obtained in steps 1 and 2.

Synthesis of Di-*p*-nitrophenyl Esters of Dicarboxylic Acids (I). Three di-*p*-nitrophenyl esters of dicarboxylic acids (Ia, Ib, and Ic, Scheme 1) were prepared by reacting the corresponding dicarboxylic acyl chlorides with *p*-nitrophenol as described previously.[24]

Briefly, a solution of triethylamine (0.0603 mol) and *p*-nitrophenol (0.0603 mol) in 100 mL of acetone was prepared at room temperature, and this solution was kept at −78 °C by dry ice and acetone. Fumaryl chloride (0.03 mol, 3.2 mL) in 40 mL of acetone was then added into the previously chilled solution dropwise upon stirring for 2 h at −78 °C and then stirring at room temperature overnight. After that, the mixture was poured into 800 mL of distilled water to precipitate the product, di-*p*-nitrophenyl fumarate (NF), which was filtered, washed thoroughly with distilled water, dried in vacuo at 50 °C, and finally purified by recrystallization from acetonitrile 3 times.

Synthesis of Di-*p*-toluenesulfonic Acid Salts of Bis-L-phenylalanine Esters (II). Di-*p*-toluenesulfonic acid salts of bis-L-phenylalanine esters were prepared by the modified procedures of our previous published study[24] as shown in Scheme 2. Typically, L-Phe (0.176 mol), *p*-toluenesulfonic acid monohydrate (0.176 mol), and di-, tri-, or tetraethylene glycol (0.08 mol) in 300 mL of toluene were placed in a flask equipped with a Dean−Stark apparatus, a $CaCl_2$ drying tube, and a magnetic stirrer. The solid−liquid reaction mixture was heated (ca. 140 °C) to reflux for 16 h until 6.1 mL (0.34 mol) of water evolved. The reaction mixture was then cooled to room temperature. After the solvent was removed by rotary evaporation, the mixture was dried in vacuo overnight and finally purified by recrystallization 3 times. Depending on the type of di-*p*-toluenesulfonic acid salts of bis-L-phenylalanine esters synthesized, different solvents were used for recrystallization. For example, water and 2-propanol were used as the recrystallization medium for the di-*p*-toluenesulfonic acid salt of L-phenylalanine diethylene glycol diester (P2EG, IIa) and L-phenylalanine triethylene glycol diester (P3EG, IIb), respectively.

Di-*p*-toluenesulfonic acid salt of L-phenylalanine diethylene glycol diester (P2EG, IIa): recrystallized from water. mp: 229 °C. IR (cm^{-1}): 1736 [−C(O)−], 1177 (−O−), 1127 (−CH_2−O−CH_2−). ^{1}H NMR (DMSO-d_6, ppm, δ): 2.29 (6H, **H**$_3$C−Ph−SO_3−), 3.05, 3.10 (4H, PhC**H**$_2$-), 3.50 [4H, −(O)C−O−CH_2−C**H**$_2$−], 4.19 [2H, $^+$H$_3$N−C**H**(CH_2Ph)−], 4.31 [4H, −(O)C−O−C**H**$_2$$CH_2$−], 7.11 to ∼7.49 [18H, Ph], 8.39 [6H, $^+$**H**$_3$N−CH(CH_2Ph)−]. ^{13}C NMR (DMSO-d_6, ppm, δ): 20.79 (H$_3$**C**−Ph−SO_3−), 35.94 (Ph**C**H_2-), 53.20 [$^+$H$_3$N−**C**H(CH_2Ph)−], 64.71 [−(O)C−O−**C**H_2−], 67.70 [−(O)C−O−CH_2**C**H_2−], 125.48, 127.28, 128.17, 128.56, 129.43, 134.45, 138.01, 145.08 (**Ph**), 169.00 [−**C**(O)−].

Di-*p*-toluenesulfonic acid salt of L-phenylalanine triethylene glycol diester (P3EG, IIb): recrystallized from 2-propanol. mp: 203 °C. IR (cm^{-1}): 1737 [−C(O)−], 1169 (−O−), 1124 (−CH_2−O−CH_2−). ^{1}H NMR (DMSO-d_6, ppm, δ): 2.29 (6H, **H**$_3$C−Ph−SO_3−), 3.06, 3.10 (4H, PhC**H**$_2$−), 3.48 [4H, −(O)C−O−(CH_2)$_2$−O−C**H**$_2$−], 3.55 [4H, −(O)C−O−CH_2−C**H**$_2$−], 4.19 [2H, $^+$H$_3$N−C**H**(CH_2Ph)−], 4.33 [4H, −(O)C−O−C**H**$_2$−], 7.10 to ∼7.49 [18H, Ph], 8.39 [6H, $^+$**H**$_3$N−CH(CH_2Ph)−]. ^{13}C NMR (DMSO-d_6, ppm, δ): 20.75 (H$_3$**C**−Ph−SO_3−), 35.90 [Ph**C**H_2−], 53.17 [$^+$H$_3$N−**C**H(CH_2Ph)−], 64.78 [−(O)C−O−**C**H_2−], 67.77 [−(O)C−O−CH_2**C**H_2−], 69.58 [−(O)C−O−(CH_2)$_2$−O−**C**H_2−], 125.45, 127.23, 128.12, 129.51, 129.42, 134.42, 137.92, 145.12 (**Ph**), 168.95 [−**C**(O)−].

Di-*p*-toluenesulfonic acid salt of L-phenylalanine tetraethylene glycol diester (P4EG, IIc): recrystallized from 2-propanol. mp: 149 °C. IR (cm^{-1}): 1746 [−C(O)−], 1219 (−O−), 1124 (−CH_2−O−CH_2−). ^{1}H NMR (DMSO-d_6, ppm, δ): 2.29 [6H, **H**$_3$C−Ph−SO_3−], 3.07, 3.11 (4H, PhC**H**$_2$−), 3.49 [8H, −(O)C−O−(CH_2)$_2$−O−C**H**$_2$−C**H**$_2$−], 3.54 [4H, −(O)C−O−CH_2−C**H**$_2$−], 4.19 [2H, $^+$H$_3$N−C**H**(CH_2Ph)−], 4.34 [4H, −(O)C−O−C**H**$_2$−], 7.10 to ∼7.49 [18H, Ph], 8.39 [6H, $^+$**H**$_3$N−CH(CH_2Ph)−]. ^{13}C NMR (DMSO-d_6, ppm, δ): 20.73 (H$_3$**C**−Ph−SO_3−), 35.90 [Ph**C**H_2-), 53.18 [$^+$H$_3$N−**C**H(CH_2Ph)−], 64.77 [−(O)C−O−**C**H_2−], 67.73 [−(O)C−O−CH_2**C**H_2−], 69.64 [−(O)C−O−(CH_2)$_2$−O−**C**H_2−], 69.65 [−(O)C−O−(CH_2)$_2$−O−CH_2−**C**H_2−], 125.44, 127.21, 128.10, 129.50, 129.41, 134.42, 137.92, 145.11 (**Ph**), 168.92 [−**C**(O)−].

Solution Polycondensation of Monomers I and II. PEEAs were prepared by solution polycondensation of one di-*p*-toluenesulfonic acid diester salt (P2EG, P3EG, or P4EG) with one di-*p*-nitrophenyl ester (NA, NS, or NF). The combinations attempted in this work and their name designation are summarized in Table 1 and shown in Scheme 3. In Table 1, all those designations of PEEAs starting with F such as FP3EG were unsaturated PEEAs in the diamide segment, and the rest of the PEEA designations were saturated.

An example of the synthesis of AP2EG via solution polycondensation is given next to illustrate the details of the synthesis procedures. Ten millimoles (1.42 mL) of triethylamine was added dropwise to the

Table 1. Monomer Combinations for PEEA Synthesis

		monomer II		
		P2EG	P3EG	P4EG
monomer I	NF (unsaturated)	FP2EG	FP3EG	FP4EG
	NA	AP2EG	AP3EG	AP4EG
	NS	SP2EG	SP3EG	SP4EG

mixture of monomers NA (Ia, 4.0 mmol) and P2EG (IIa, 4.0 mmol) in 3 mL of dry DMA, and the solution was heated to 60 °C with stirring until complete dissolution of monomers. The reaction vial was then kept under 70 °C for 48 h without stirring. The resulting viscous solution (from light yellow to dark brown depending on the type of monomers used) was divided into two groups and precipitated by different solvents. For the fumaryl based polymer (FP2EG, FP3EG, and FP4EG), chilled acetone was added into the viscous solution to precipitate the product. The polymer was then washed by warm acetone twice, filtered, and finally dried in vacuo for 48 h before further study. For the rest of the polymers (non-fumurate based), chilled ethyl acetate was used as the precipitation solvent, and then the polymer products were filtered and extracted by ethyl acetate in a Soxhlet apparatus for 48 h and finally dried in vacuo for 48 h.

AP2EG: yield 84%. IR (cm^{-1}), 1741 [−**C(O)**−O−], 1643, 1536 [−**C(O)**−NH−], 1127 (−CH_2−O−CH_2−), 3311 [−C(O)−**NH**−]. ^{1}H NMR (DMSO-d_6, ppm, δ): 1.29 [4H, −NH−(O)C−CH_2−C**H**$_2$−], 1.99 [4H, −NH−(O)C−C**H**$_2$−], 2.84 to ∼3.02 [4H, PhC**H**$_2$−], 3.46 to ∼3.57 [4H, −(O)C−O−CH_2−C**H**$_2$−], 4.11 [4H, −(O)C−O−C**H**$_2$−], 4.46 [2H, −HN−C**H**(CH_2Ph)−], 7.15 to ∼7.25 [10H, Ph], 8.24 [2H, −**H**N−CH(CH_2Ph)−]. ^{13}C NMR (DMSO-d_6, ppm, δ): 24.55 [−NH−(O)C−CH_2−**C**H$_2$−], 34.63 [−NH−(O)C−**C**H$_2$−], 36.67 [Ph**C**H$_2$−], 53.41 [−HN−**C**H(CH_2Ph)−], 63.69 [−(O)C−O−**C**H$_2$−], 68.05 [−(O)C−O−CH_2−**C**H$_2$−], 126.42, 128.12, 128.99, 137.12 [Ph], 171.65 [−O**C**(O)−], 172.09 [−**C**(O)−NH−].

AP3EG: yield 79%. IR (cm^{-1}), 1742 [−**C(O)**−O−], 1646, 1536 [−**C(O)**−NH−], 1127 (−CH_2−O−CH_2−), 3293 [−C(O)−**NH**−]. ^{1}H NMR (DMSO-d_6, ppm, δ): 1.29 [4H, −NH−(O)C−CH_2−C**H**$_2$−], 2.00 [4H, −NH−(O)C−C**H**$_2$−], 2.83 to ∼3.02 [4H, PhC**H**$_2$−], 3.47 to ∼3.57 [8H, −(O)C−O−CH_2−C**H**$_2$−O−C**H**$_2$−], 4.10 [4H, −(O)C−O−C**H**$_2$−], 4.46 [2H, −HN−C**H**(CH_2Ph)−], 7.15 to ∼7.26 [10H, Ph], 8.24 [2H, −**H**N−CH(CH_2Ph)−]. ^{13}C NMR (DMSO-d_6, ppm, δ): 24.55 [−NH−(O)C−CH_2−**C**H$_2$−], 34.63 [−NH−(O)C−**C**H$_2$−], 36.68 [Ph**C**H$_2$−], 53.40 [−HN−**C**H(CH_2Ph)−], 63.72 [−(O)C−O−**C**H$_2$−], 68.08 [−(O)C−O−CH_2−**C**H$_2$−], 69.67 [−(O)C−O−$(CH_2)_2$−O−**C**H$_2$−], 126.42, 128.13, 128.98, 137.14 [Ph], 171.66 [−O**C**(O)−], 172.07 [−**C**(O)−NH−].

AP4EG: yield 74%. IR (cm^{-1}), 1742 [−**C(O)**−O−], 1658, 1547 [−**C(O)**−NH−], 1117 (−CH_2−O−CH_2−), 3299 [−C(O)−**NH**−]. ^{1}H NMR (DMSO-d_6, ppm, δ): 1.29 [4H, −NH−(O)C−CH_2−C**H**$_2$−], 2.00 [4H, −NH−(O)C−C**H**$_2$−], 2.84 to ∼3.02 [4H, PhC**H**$_2$−], 3.45 to ∼3.58 [12H, −(O)C−O−CH_2−C**H**$_2$−O−(C**H**$_2$)$_2$−], 4.11 [4H, −(O)C−O−C**H**$_2$−], 4.47 [2H, −HN−C**H**(CH_2Ph)−], 7.16 to ∼7.27 [10H, Ph], 8.24 [2H, −**H**N−CH(CH_2Ph)−]. ^{13}C NMR (DMSO-d_6, ppm, δ): 24.56 [−NH−(O)C−CH_2−**C**H$_2$−], 34.64 [−NH−(O)C−**C**H$_2$−], 36.69 [Ph**C**H$_2$−], 53.42 [−HN−**C**H(CH_2Ph)−], 63.70 [−(O)C−O−**C**H$_2$−], 68.06 [−(O)C−O−CH_2−**C**H$_2$−], 69.70 [−(O)C−O−$(CH_2)_2$−O−**C**H$_2$−], 69.72 [−(O)C−O−$(CH_2)_2$−O−CH_2−**C**H$_2$−], 126.44, 128.14, 129.00, 137.17 [Ph], 171.67 [−O**C**(O)−], 172.09 [−**C**(O)−NH−].

FP2EG: yield 76%. IR (cm^{-1}), 1736 [−**C(O)**−O−], 1630, 1542 [−**C(O)**−NH−], 1138 (−CH_2−O−CH_2−), 3299 [−C(O)−**NH**−]. ^{1}H NMR (DMSO-d_6, ppm, δ): 2.88 to ∼3.08 [4H, PhC**H**$_2$−], 3.48 to ∼3.60 [4H, −(O)C−O−CH_2−C**H**$_2$−], 4.14 [4H, −(O)C−O−C**H**$_2$−], 4.56 [2H, −HN−C**H**(CH_2Ph)−], 6.84 [2H, −C(O)−C**H**═], 7.18 to ∼7.24 [10H, Ph], 8.90 [2H, −**H**N−CH(CH_2Ph)−]. ^{13}C NMR (DMSO-d_6, ppm, δ): 36.58 [Ph**C**H$_2$-], 53.82 [−HN−**C**H(CH_2Ph)−], 63.91 [−(O)C−O−**C**H$_2$−], 68.06 [−(O)C−O−CH_2−**C**H$_2$−], 126.57, 128.21, 128.99, 136.83 [Ph], 132.50 [−C(O)−**C**H═], 163.51 [−**C**(O)−NH−], 171.10 [−O**C**(O)−].

FP3EG: yield 83%. IR (cm^{-1}), 1741 [−**C(O)**−O−], 1638, 1536 [−**C(O)**−NH−], 1115 (−CH_2−O−CH_2−), 3311 [−C(O)−**NH**−]. ^{1}H NMR (DMSO-d_6, ppm, δ): 2.90 to ∼3.07 [4H, PhC**H**$_2$-], 3.48 to ∼3.60 [8H, −(O)C−O−CH_2−C**H**$_2$−O−C**H**$_2$−], 4.12 [4H, −(O)C−O−C**H**$_2$−], 4.56 [2H, −HN−C**H**(CH_2Ph)−], 6.83 [2H, −C(O)−C**H**═], 7.19 to ∼7.25 [10H, Ph], 8.90 [2H, −**H**N−CH(CH_2Ph)−]. ^{13}C NMR (DMSO-d_6, ppm, δ): 36.56 [Ph**C**H$_2$-], 53.80 [−HN−**C**H(CH_2Ph)−], 63.96 [−(O)C−O−**C**H$_2$−], 68.06 [−(O)C−O−CH_2−**C**H$_2$−], 69.69 [−(O)C−O−$(CH_2)_2$−O−**C**H$_2$−], 126.56, 128.21, 128.98, 136.86 [Ph], 132.49 [−C(O)−**C**H═], 163.49 [−**C**(O)−NH−], 171.10 [−O**C**(O)−].

FP4EG: yield 69%. IR (cm^{-1}), 1738 [−**C(O)**−O−], 1630, 1537 [−**C(O)**−NH−], 1114 (−CH_2−O−CH_2−), 3309 [−C(O)−**NH**−]. ^{1}H NMR (DMSO-d_6, ppm, δ): 2.88 to ∼3.09 [4H, PhC**H**$_2$-], 3.47 to ∼3.60 [12H, −(O)C−O−CH_2−C**H**$_2$−O−(C**H**$_2$)$_2$−], 4.14 [4H, −(O)C−O−C**H**$_2$−], 4.56 [2H, −HN−C**H**(CH_2Ph)−], 6.83 [2H, −C(O)−C**H**═], 7.18 to ∼7.27 [10H, Ph], 8.90 [2H, −**H**N−CH(CH_2Ph)−]. ^{13}C NMR (DMSO-d_6, ppm, δ): 36.59 [Ph**C**H$_2$-], 53.83 [−HN−**C**H(CH_2Ph)−], 63.98 [−(O)C−O−**C**H$_2$−], 68.03 [−(O)C−O−CH_2−**C**H$_2$−], 69.70 [−(O)C−O−$(CH_2)_2$−O−**C**H$_2$−], 69.73 [−(O)C−O−$(CH_2)_2$−O−CH_2−**C**H$_2$−], 126.59, 128.23, 129.00, 136.89 [Ph], 132.51 [−C(O)−**C**H═], 163.51 [−**C**(O)−NH−], 171.13 [−O**C**(O)−].

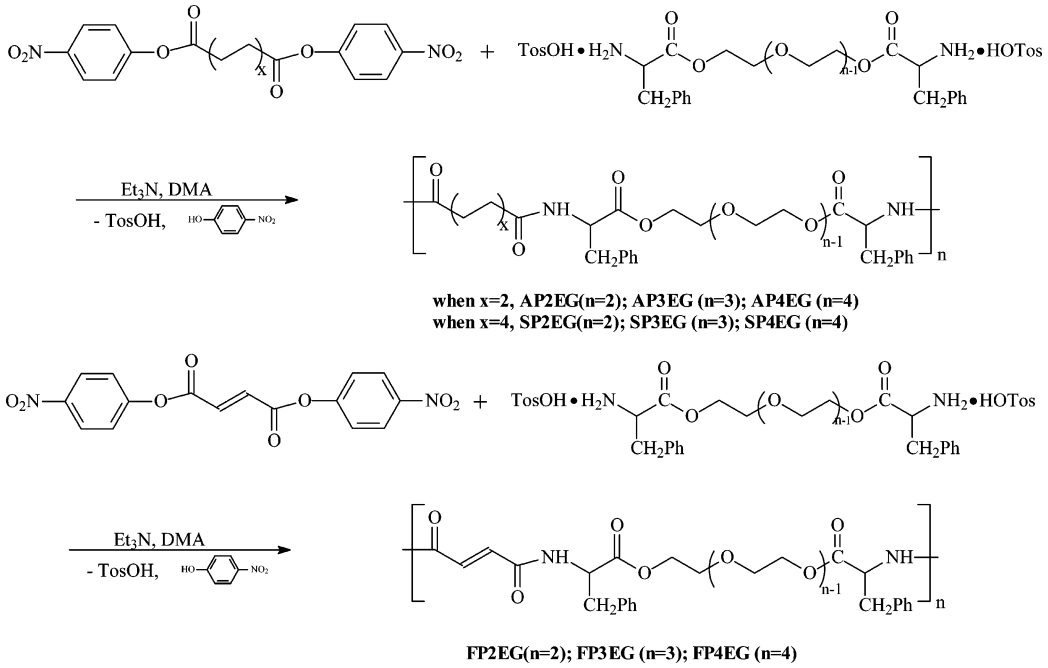
SP2EG: yield 62%. IR (cm^{-1}), 1740 [−**C(O)**−O−], 1648, 1541 [−**C(O)**−NH−], 1126 (−CH_2−O−CH_2−), 3309 [−C(O)−**NH**−]. ^{1}H NMR (DMSO-d_6, ppm, δ): 1.10 [8H, −NH−(O)C−$(CH_2)_2$−(C**H**$_2$)$_2$−] 1.36 [4H, −NH−(O)C−CH_2−C**H**$_2$−], 2.01 [4H, −NH−(O)C−C**H**$_2$−], 2.83 to ∼3.03 [4H, PhC**H**$_2$-], 3.46 to ∼3.59 [4H, −(O)C−O−CH_2−C**H**$_2$−], 4.12 [4H, −(O)C−O−C**H**$_2$−], 4.48 [2H, −HN−C**H**(CH_2Ph)−], 7.18 to ∼7.26 [10H, Ph], 8.24 [2H, −**H**N−CH(CH_2Ph)−]. ^{13}C NMR (DMSO-d_6, ppm, δ): 25.11 [−NH−(O)C−CH_2−**C**H$_2$−], 28.41 [−NH−(O)C−$(CH_2)_2$−**C**H$_2$−], 28.64 [−NH−(O)C−$(CH_2)_3$−**C**H$_2$−], 34.92 [−NH−(O)C−**C**H$_2$−], 36.62 [Ph**C**H$_2$-], 53.35 [−HN−**C**H(CH_2Ph)−], 63.69 [−(O)C−O−**C**H$_2$−], 68.07 [−(O)C−O−CH_2−**C**H$_2$−], 126.38, 128.07, 128.98, 137.20 [Ph], 171.67 [−O**C**(O)−], 172.27 [−**C**(O)−NH−].

SP3EG: yield 74%. IR (cm^{-1}), 1743 [−**C(O)**−O−], 1648, 1541 [−**C(O)**−NH−], 1126 (−CH_2−O−CH_2−), 3296 [−C(O)−N**H**−]. ^{1}H NMR (DMSO-d_6, ppm, δ): 1.11 [8H, −NH−(O)C−$(CH_2)_2$−(C**H**$_2$)$_2$−] 1.37 [4H, −NH−(O)C−CH_2−C**H**$_2$−], 2.03 [4H, −NH−(O)C−C**H**$_2$−], 2.83 to ∼3.03 [4H, PhC**H**$_2$−], 3.45 to ∼3.62 [8H, −(O)C−O−CH_2−C**H**$_2$−O−C**H**$_2$−], 4.11 [4H, −(O)C−O−C**H**$_2$−], 4.47 [2H, −HN−C**H**(CH_2Ph)−], 7.19 to ∼7.25 [10H, Ph], 8.24 [2H, −**H**N−CH(CH_2Ph)−]. ^{13}C NMR (DMSO-d_6, ppm, δ): 25.09 [−NH−(O)C−CH_2−**C**H$_2$−], 28.41 [−NH−(O)C−$(CH_2)_2$−**C**H$_2$−], 28.64 [−NH−(O)C−$(CH_2)_3$−**C**H$_2$−], 34.91 [−NH−(O)C−**C**H$_2$−], 36.63 [Ph**C**H$_2$−], 53.32 [−HN−**C**H(CH_2Ph)−], 63.72 [−(O)C−O−**C**H$_2$−], 68.11 [−(O)C−O−CH_2−**C**H$_2$−], 69.69 [−(O)C−O−$(CH_2)_2$−O−**C**H$_2$−], 126.38, 128.07, 128.97, 137.20 [Ph], 171.68 [−O**C**(O)−], 172.23 [−**C**(O)−NH−].

SP4EG: yield 63%. IR (cm^{-1}), 1741 [−**C(O)**−O−], 1643, 1536 [−**C(O)**−NH−], 1118 (−CH_2−O−CH_2−), 3317 [−C(O)−**NH**−]. ^{1}H NMR (DMSO-d_6, ppm, δ): 1.11 [8H, −NH−(O)C−$(CH_2)_2$−(C**H**$_2$)$_2$−] 1.36 [4H, −NH−(O)C−CH_2−C**H**$_2$−], 2.03 [4H, −NH−(O)C−C**H**$_2$−], 2.83 to ∼3.04 [4H, PhC**H**$_2$-], 3.41 to ∼3.60 [12H, −(O)C−O−CH_2−C**H**$_2$−O−(C**H**$_2$)$_2$−], 4.11 [4H, −(O)C−O−C**H**$_2$−], 4.47 [2H, −HN−C**H**(CH_2Ph)−], 7.18 to ∼7.25 [10H, Ph], 8.24 [2H, −**H**N−CH(CH_2Ph)−]. ^{13}C NMR (DMSO-d_6, ppm, δ): 25.11 [−NH−(O)C−CH_2−**C**H$_2$−], 28.43 [−NH−(O)C−$(CH_2)_2$−**C**H$_2$−], 28.66 [−NH−(O)C−$(CH_2)_3$−**C**H$_2$−], 34.93 [−NH−(O)C−**C**H$_2$−], 36.65 [Ph**C**H$_2$−], 53.35 [−HN−**C**H(CH_2Ph)−], 63.74 [−(O)C−O−**C**H$_2$−], 68.08 [−(O)C−O−CH_2−**C**H$_2$−], 69.71 [−(O)C−O−$(CH_2)_2$−O−**C**H$_2$−], 69.73 [−(O)C−O−$(CH_2)_2$−O−CH_2−**C**H$_2$−], 126.40, 128.10, 128.99, 137.22 [Ph], 171.70 [−O**C**(O)−], 172.25 [−**C**(O)−NH−].

To study the biodegradability of PEEA polymers, the PEEA films were cast from a 10% (w/v) chloroform solution onto smooth Teflon Petri dishes, and the solvent was allowed to evaporate completely at room temperature. The films were further dried in vacuo at room temperature overnight and finally punched into small disc shaped pieces (diameter 12.5 mm) for the biodegradation test.

Scheme 3. Synthesis of Saturated and Unsaturated L-Phenylalanine Based PEEAs



Materials Characterization. For Fourier transform infrared (FTIR) characterization, samples were ground into powder and mixed with KBr at a sample/KBr ratio of 1:10 w/w. FTIR spectra were then obtained from a PerkinElmer Nicolet Magana 560 (Madison, WI) FTIR spectrometer with Omnic software for data acquisition and analysis.

NMR spectra were recorded by a Varian Unity INOVA-400 400 MHz spectrometer (Palo Alto, CA) operating at 400 and 100 MHz for ^{1}H and ^{13}C NMR, respectively. Deuterated dimethyl sulfoxide (DMSO-d_6, Cambridge Isotope Laboratories, Cambridge, U.K.) was used as the solvent. Elemental analyses of the polymers synthesized were performed with a PE 2400 CHN elemental analyzer by Atlantic Microlab (Norcross, GA).

Thermal properties of synthesized monomers and polymers were characterized by a DSC 2920 (TA Instruments, New Castle, DE). The measurement was carried out from 0 to 300 °C at a scanning rate of 10 °C/min and nitrogen gas flow rate of 25 mL/min. TA Universal Analysis software was used for thermal data analysis, such as the determination of the glass transition temperature. The melting point was determined at the onset of the melting endotherm.

The number and weight averaged molecular weights (M_n and M_w) and MWD of synthesized PEEAs were determined using a Model 510 gel permeation chromatographer (Waters Associates Inc. Milford, MA) equipped with a high-pressure liquid chromatographic pump, a Waters 486 UV detector, and a Waters 2410 different refractive index detector. THF was used as the eluent (1.0 mL/min). The columns were calibrated with polystyrene standards having a narrow molecular weight distribution. The reduced viscosity (η_{red}) of the polymers synthesized was determined by a Cannon-Ubbelhode viscometer in DMSO solution at a concentration of 0.25 g/dL at 25 °C.

An in vitro biodegradation test of OEG based PEEAs was carried out in a small vial containing a dry PEEA disc film (diameter 12.5 mm) and 10 mL of pure PBS or α-chymotrypsin in PBS buffer (pH 7.4, 0.1 M) of different concentrations (0, 0.05, 0.1, or 0.2 mg/mL). Two representatives OEG based PEEA polymers (AP3EG and SP3EG) were used for this preliminary biodegradation study. There was no unsaturated PEEA sample for this study because it does not dissolve in $CHCl_3$, and no quality film could be formed in DMA solvent either. The vial was then incubated at 37 °C with constant reciprocal shaking (100 rpm). At predetermined intervals, the PEEA film samples were taken out of the incubation medium and washed gently with distilled water, and the surface water was blotted by filter paper and weighed until no further weight change. The degree of biodegradation was estimated from the weight loss of the PEEA film sample based on the following equation:

$$W_l\,(\%) = \left[\frac{W_o - W_t}{W_o}\right] \times 100$$

where W_o is the original weight of the dry PEEA film sample before immersion, and W_t is the dry PEEA film sample weight after incubation for t h (with or without enzyme). The weight loss average of the three specimens was recorded.

SEM was employed to analyze the surface morphology of the PEEA polymers during the biodegradation process. After being taken out of the incubation media, the polymer film sample was dried, fixed on aluminum stubs, and coated with gold for 60 s for SEM observation by a Hitachi S4500 scanning electron microscope (Mountain View, CA). The molecular weight changes of the PEEA polymers were also monitored by GPC.

The surface hydrophilicity of the PEEA films was determined using an IMASS contact angle analyzer in a conditioning room of 65% RH and 21 °C. Distilled water was used as the spreading liquid, the contact angles of five randomly chosen surface areas of each PEEA film were measured, and two PEEA films of each type were tested.

Results and Discussion

Synthesis of Di-*p*-nitrophenyl Esters of Dicarboxylic Acids. Three different types of di-*p*-nitrophenyl esters of dicarboxylic acids, NA, NS, and NF, were used as monomers to provide the carboxylic ester segment of PEEA. NA and NS have a saturated methylene structure, while NF has unsaturated C═C double bonds in the methylene structure. These three monomers were reported in our previous work.[24]

Synthesis of Di-*p*-toluenesulfonic Acid Salts of Bis-L-phenylalanine Esters. Three different types of di-*p*-toluenesulfonic acid salts of bis-L-phenylalanine diesters were synthesized the first time and used as the monomers to provide the amino acid segment of PEEA. These three salts had oligomers of ethylene glycol with ether bonds in the segment.

The structures of the di-*p*-toluenesulfonic acid salt monomers, P2EG, P3EG, and P4EG, were all confirmed by FTIR and NMR

CDV

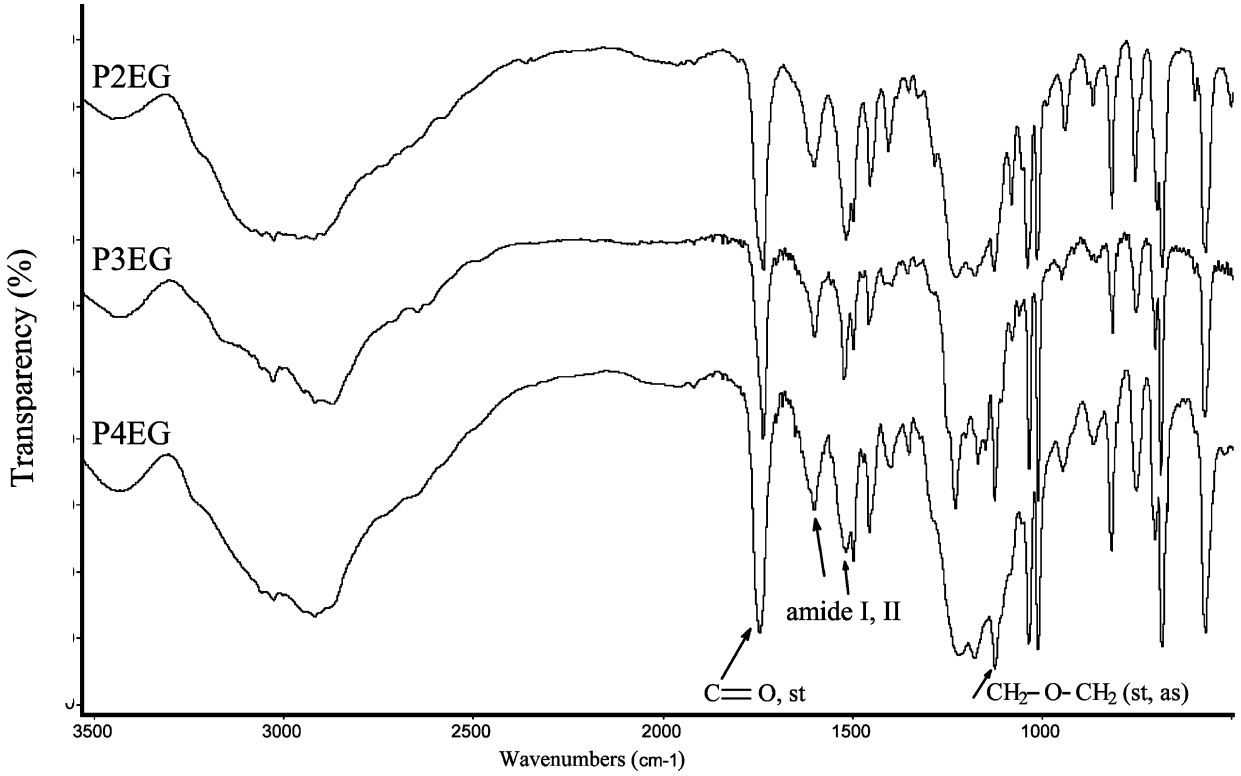


Figure 1. FTIR spectra of the di-*p*-toluenesulfonic acid salts of bis-L-phenylalanine diester monomers: P2EG, P3EG, and P4EG. (st: stretching vibration and as: asymmetry vibration).

spectra. The FTIR spectra are shown in Figure 1, and their main absorption bands, such as ester groups (1736 to ~1750 cm^{-1}) and ether groups (~1127 cm^{-1}), were assigned. The broad absorption between 2500 and 3300 cm^{-1} could be attributed to both primary amine salt and aliphatic hydrocarbon structure existing in those monomers. The ^{1}H and ^{13}C NMR data of P2EG, P3EG, and P4EG also showed characteristic signals of $-CH_2-O-CH_2-$ (^{1}H: δ ~3.50 ppm). All three monomers were obtained as white powders, and the yield of P3EG was about 86%, higher than those of P2EG (66%) and P4EG (60%).

Polymer Synthesis. As shown in Scheme 3, nine different types of new PEEAs were synthesized by solution polycondensation of different combinations of monomer I (Ia, Ib, or Ic) and monomer II (IIa, IIb, or IIc). Excess triethylamine was used as the acid receptor for TosOH during the polymerization to regenerate free amino groups in the di-*p*-toluenesulfonic acid salt monomer.[21,24,31] Polymerization took place in the homogeneous phase, and the polymer obtained remained dissolved, but the reaction solution became more viscous.

The structures of these PEEAs were confirmed by both IR and NMR spectra data. The FTIR spectra of all the fumaryl based PEEAs (Figure 2) had characteristic absorption bands of ester groups (~1740 cm^{-1}), ether groups (~1115 cm^{-1}), amide groups (~1640 and ~1530 cm^{-1}), and unsaturated H—C═ bonds (~983 cm^{-1}). The broad absorption around 2900 cm^{-1} could be attributed to the aliphatic hydrocarbon structure existing in the polymers.

The NMR spectra (^{1}H and ^{13}C) of three typical PEEAs based on tri-ethylene glycol are shown in Figures 3 and 4 (see the following discussion for detailed spectral band assignments of all polymers). The spectral data were fully in agreement with the anticipated chemical structure of the PEEA polymers shown in Scheme 3. All the PEEAs showed ^{1}H peaks of the -NH— bonds of amides (8.90 or 8.24), the ether CH_2-O-CH_2 bonds in the diester unit (~3.50), and the —HC═ bonds in the amide unit (6.83) for fumaryl based PEEAs. The ^{13}C spectra contained all the peaks for every magnetically different carbon presented in the repeating unit of the polymer. The data from elemental analysis (Table 2) were also consistent with the composition calculated.

.

Table 3 summarizes the fundamental properties of the PEEAs synthesized. All nine PEEAs were obtained in fairly good yields (59 to~83%), with η_{red} ranging from 0.13 to 0.61 dL/g. The three fumaryl based unsaturated PEEAs (FP2EG, FP3EG, and FP4EG) could not dissolve in THF, which was the designated eluent for the central GPC facility available to us. Among the adipoyl and sebacoyl based PEEAs, AP3EG and SP3EG showed the highest M_n, M_w, and η_{red} in their series, respectively. FP3EG showed the highest η_{red} among the fumaryl based PEEAs. Irrespective of saturated or unsaturated PEEAs, the data in Table 3 demonstrated that, among the three monomers II (P2EG, P3EG, and P4EG), P3EG had the highest reactivity toward monomers I (NA, NS, and NF) because the PEEAs from P3EG consistently resulted in the highest η_{red}.

The T_g and T_m of the synthesized PEEAs were measured by DSC and are listed in Table 3. The PEEAs obtained had lower T_g values than the corresponding saturated and unsaturated PEAs and USPEA based on aliphatic dialcohols.[21,24,26] For example, AP2EG had a T_g value 9% lower than APB (49 vs 54 °C), FP2EG had a T_g value 20% lower than FPB (82 vs 103 °C), and SP2EG had a T_g value 20% lower than SPB (32 vs 40 °C). Within each PEEA series, an increase in the ether bonds (from diethylene glycol and triethylene glycol to tetraethylene glycol) led to a reduction in T_g as well. For example, in the unsaturated fumaryl based PEEAs, their T_g value decreased from 82 °C (FP2EG with two ether bonds per repeating unit) to 67 °C (FP3EG with three ether bonds per repeating unit) and 54 °C (FP4EG with four ether bonds per repeating unit). The reduction in T_g appeared to be more severe in the saturated PEEA series

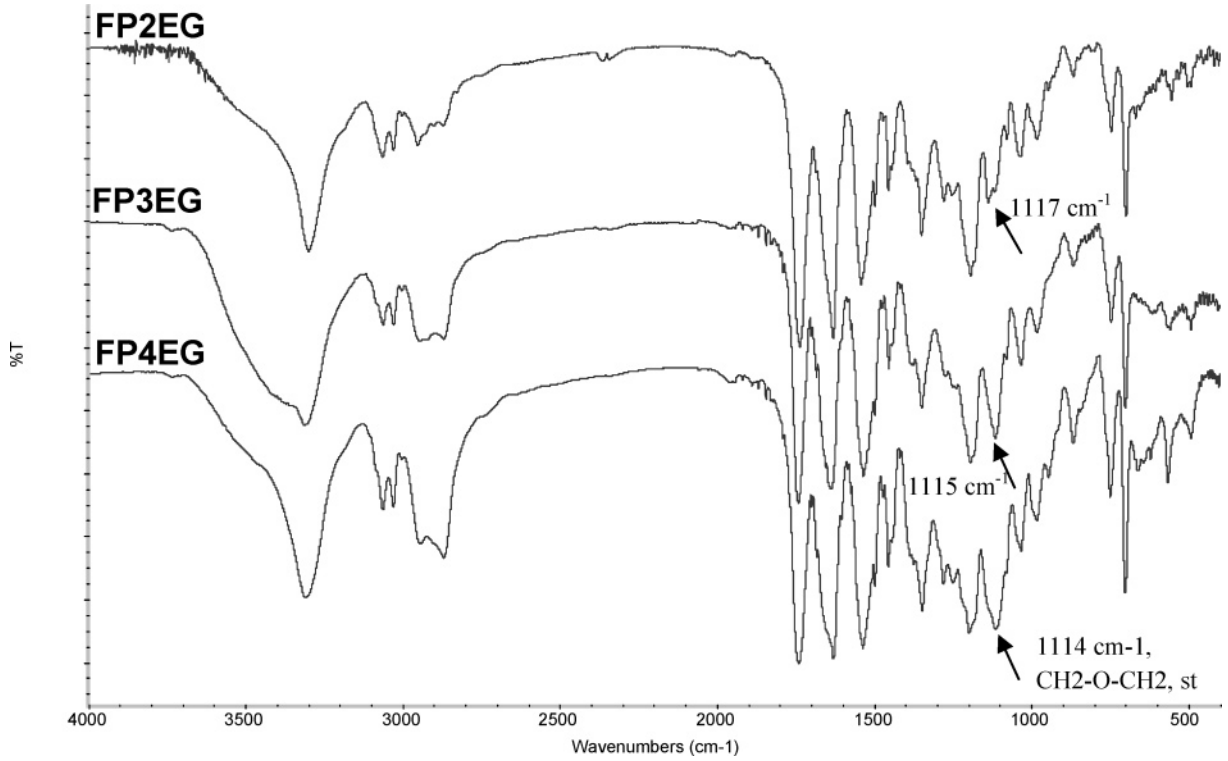


Figure 2. FTIR spectra of fumaryl based poly(ether ester amide)s: FP2EG, FP3EG, and FP4EG. (st: stretching vibration).

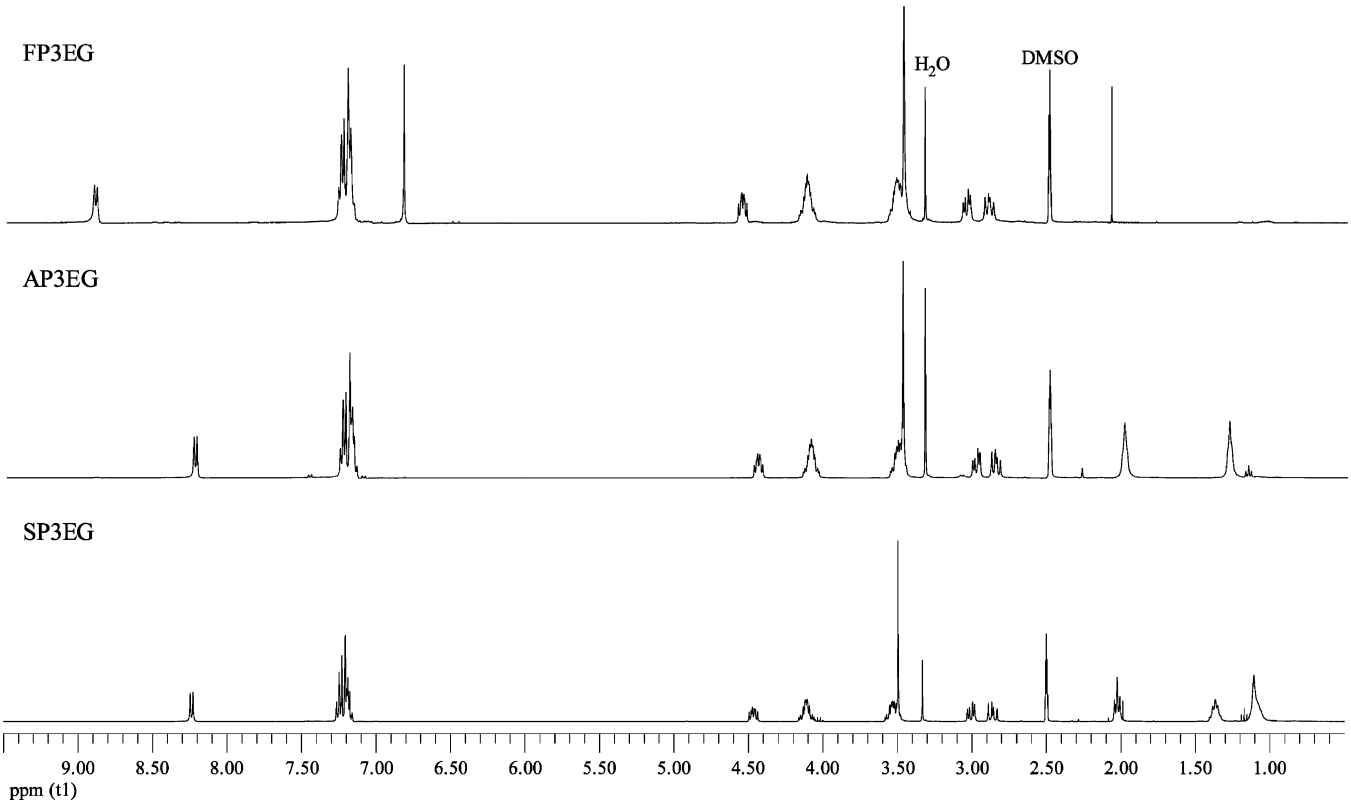


Figure 3. ^{1}H NMR spectra of three poly(ether ester amide)s in DMSO solvent: FP3EG, AP3EG, and SP3EG.

than in the unsaturated PEEA series. For example, there was a 71% reduction in T_g from saturated AP2EG to AP4EG, while only 34% reduction was found from FP2EG to FP4EG. The lower T_g of PEEAs was attributed to the presence of one or more ether bonds in the repeating units of the PEEA macromolecules, and it is well-known that the ether bond is a quite flexible bond for free rotation (i.e., increasing the polymer chain flexibility and promoting the chain segmental movement and hence a lower T_g value).

As shown in Table 4, the solubility of PEEAs (50 mg) in common organic solvents (1.0 mL) at room temperature (25 °C) was evaluated. All PEEAs are completely soluble in DMSO, DMF, and formic acid (except FP2EG) but cannot dissolve in water, methanol, and ethyl acetate. Saturated PEEAs can also

CDV

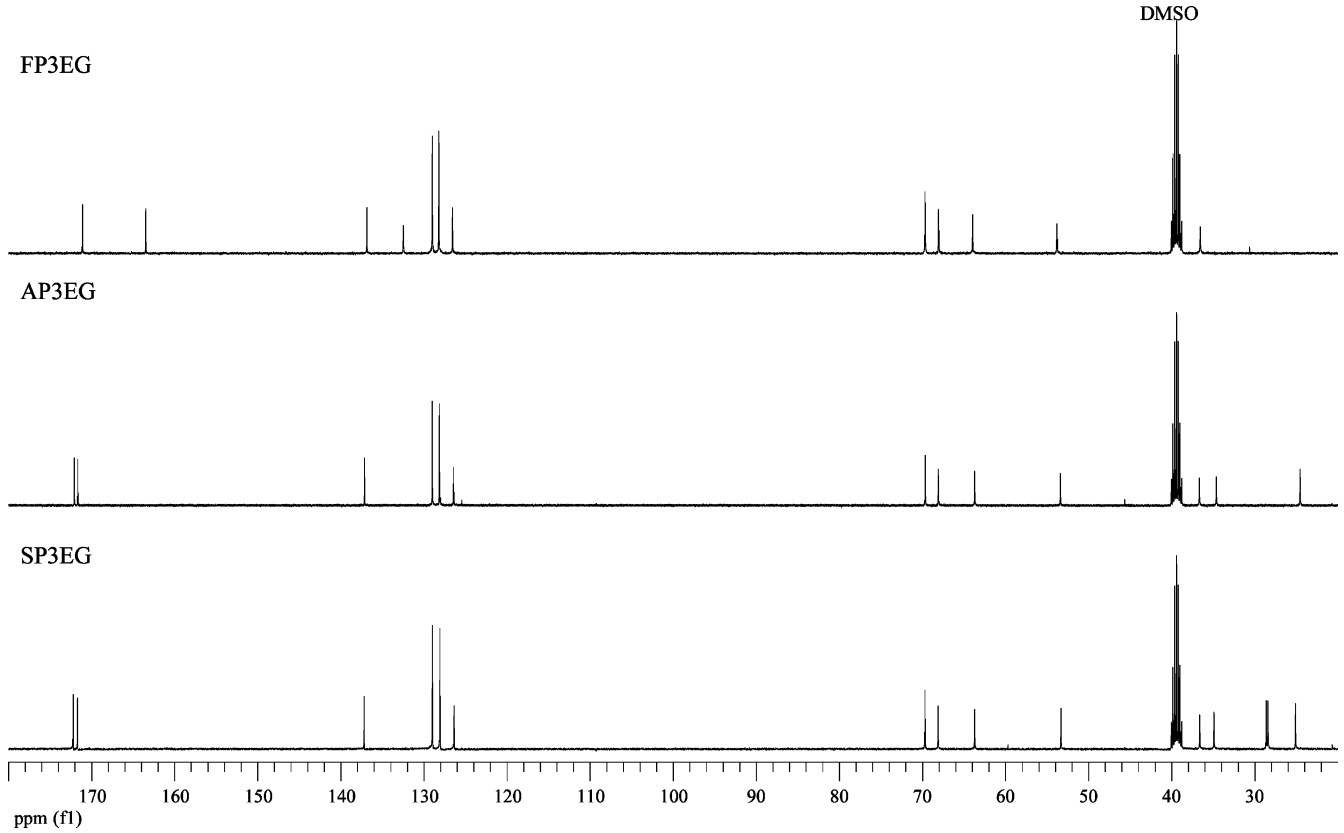


Figure 4. ^{13}C NMR spectra of three poly(ether ester amide)s in DMSO solvent: FP3EG, AP3EG, and SP3EG.

Table 2. Elemental Analysis Results of PEEAs

			calcd (%)			exptl (%)		
sample	formula	*fw* (g/mol)	C	H	N	C	H	N
FP2EG	$(C_{26}H_{28}N_2O_7)_n$	480.52*n*	64.99	5.87	5.83	64.74	5.75	5.82
FP3EG	$(C_{28}H_{32}N_2O_8)_n$	524.57*n*	64.11	6.15	5.34	63.93	6.17	5.36
FP4EG	$(C_{30}H_{36}N_2O_9)_n$	568.62*n*	63.37	6.38	4.93	62.82	6.34	4.99
AP2EG	$(C_{28}H_{34}N_2O_7)_n$	510.59*n*	65.87	6.71	5.49	65.64	6.81	5.46
AP3EG	$(C_{30}H_{38}N_2O_8)_n$	554.64*n*	64.97	6.91	5.05	64.37	6.98	5.03
AP4EG	$(C_{32}H_{42}N_2O_9)_n$	598.69*n*	64.20	7.07	4.68	63.07	7.24	4.74
SP2EG	$(C_{32}H_{44}N_2O_7)_n$	568.71*n*	67.58	7.80	4.93	66.92	7.33	4.94
SP3EG	$(C_{34}H_{46}N_2O_8)_n$	610.75*n*	66.86	7.59	4.59	66.64	7.62	4.67
SP4EG	$(C_{36}H_{50}N_2O_9)_n$	654.80*n*	66.03	7.70	4.28	66.03	7.69	4.40

Table 3. Fundamental Properties of PEEAs[a]

	yield (%)	η_{red} (dL/g)[b]	M_n (kg/mol)	M_w (kg/mol)	M_w/M_n	T_g (°C)	T_m (°C)
FP2EG[c]	76	0.13 ± 0.01				82	233
FP3EG[c]	83	0.49 ± 0.01				67	180
FP4EG[c]	69	0.27 ± 0.00				54	138
AP2EG	59	0.13 ± 0.01	2.6	4.6	1.76	49	101
AP3EG	79	0.61 ± 0.03	17.4	26.0	1.49	35	92
AP4EG	74	0.24 ± 0.01	9.0	14.6	1.63	14	63
SP2EG	62	0.16 ± 0.01	6.4	10.2	1.59	32	90
SP3EG	74	0.54 ± 0.00	27.3	41.1	1.51	23	67
SP4EG	63	0.17 ± 0.00	7.3	18.5	2.55	12	58

[a] Synthesis conditions: $C = 0.90$ mol/L, $T = 70$ °C, DMA as the solvent. [b] Measured in DMSO at 25 °C, $C = 0.25$ g/dL. [c] Molecular weight data not available because fumaryl based PEEA cannot be dissolved in THF, which is the solvent for the central GPC facility available to us.

dissolve in trifluoroethanol, THF, and chloroform. However, the three unsaturated PEEAs (FP2EG, FP3EG, and FP4EG) had poor solubility in those regular organic solvents because of the extra conjugation effect between C═C double bonds and carbonyl groups in their structure, which did not exist in the adipoyl and sebacoyl based saturated PEEAs. When comparing

Table 4. Solubility of PEEA at Room Temperature (25 °C)[a]

	H_2O	formic acid	TFE	DMF	DMSO	THF	MeOH	ethyl acetate	$CHCl_3$	acetone
AP2EG	−	+	+	+	+	+	−	−	+	±
AP3EG	−	+	+	+	+	+	−	−	+	±
AP4EG	−	+	+	+	+	+	−	−	+	±
SP2EG	−	+	+	+	+	+	−	−	+	±
SP3EG	−	+	+	+	+	+	−	−	+	+
SP4EG	−	+	+	+	+	+	−	−	+	+
FP2EG	−	±	−	+	+	−	−	−	−	−
FP3EG	−	+	−	+	+	−	−	−	−	−
FP4EG	−	+	−	+	+	−	−	−	±	−

[a] +: Soluble; −: insoluble; and ±: partially soluble or swelling.

to SPEA,[21] UPEA,[24] and USPEA[26] that were derived from aliphatic dialcohols, the new OEG based PEEA, especially unsaturated PEEA, showed a significant improvement in solubility. For example, SP3EG and SP4EG can both dissolve in acetone, while SPB (1,4-butanediol based) and SPH (1,6-hexanediol based) cannot. All three unsaturated PEEAs (fumaryl based) can dissolved in DMF and DMSO, while FPB (1,4-butanediol based) and FPH (1,6-hexanediol based) can only partially dissolve.

In Vitro Biodegradation Test of PEEA Polymers. The biodegradation kinetics of two representative OEG based PEEA polymers (AP3EG and SP3EG) in either pH 7.4 PBS buffer or α-chymotrypsin solution at 37 °C is illustrated in Figures 5 and 6. The data show that both polymers exhibit much faster and more severe weight loss in the α-chymotrypsin solution than in a PBS buffer and that the level of biodegradability of these PEEA polymers in an enzyme solution depends on their chemical structures and enzyme concentration.

The biodegradation data showed that AP3EG was very sensitive to enzymatic biodegradation (Figure 5). Even in a very low concentration of α-chymotrypsin solution (0.05 mg/mL), AP3EG was biodegraded quickly and had a 13% weight loss

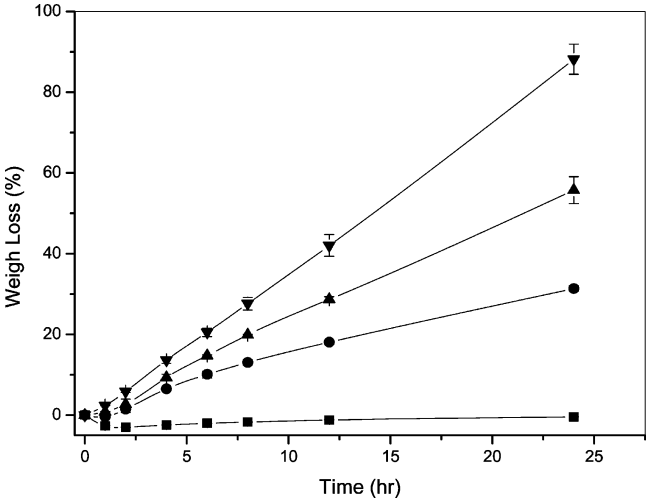


Figure 5. Effect of enzyme (α-chymotrypsin) concentration on weight loss kinetics of AP3EG at 37 °C; pH 7.4 PBS buffer serves as the control. (-■-) PBS buffer; (-●-) α-chymotrypsin at 0.05 mg/mL; (-▲-) α-chymotrypsin at 0.10 mg/mL; and (-▼-) α-chymotrypsin at 0.20 mg/mL.

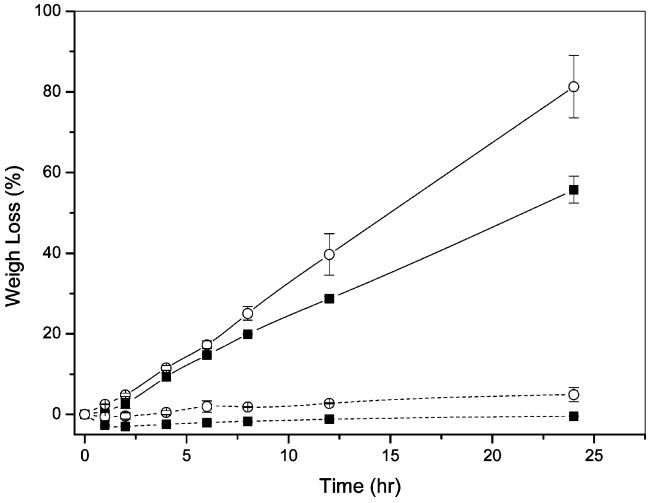


Figure 6. Effect of chemical structure of poly(ether ester amide)s on their weight loss behavior in PBS or α-chymotrypsin medium (0.1 mg/mL) at 37 °C. Solid line: enzyme medium and dashed line: PBS medium. (···■···) AP3EG in pure PBS buffer; (-■-) AP3EG in α-chymotrypsin solution; (···○···) SP3EG in pure PBS buffer; and (-○-) SP3EG in α-chymotrypsin solution.

in the first 8 h and 31% weight loss after 1 day. The biodegradation profiles of AP3EG showed that its hydrolysis rate increased with a higher α-chymotrypsin concentration. Irrespective of different α-chymotrypsin concentrations, the weight loss kinetics was very close to zero-order. It was also observed that the AP3EG films can maintain their constant surface area very well up to 80% weight loss, while their thickness became thinner and thinner. This suggests that the polymer films eroded evenly on the surface upon enzymatic hydrolysis, which is contrary to aliphatic polyesters' bulk biodegradation.

The effect of different types of monomer I on biodegradability is shown in Figure 6. The data concluded that the tendency of AP3EG (from monomer Ia: di-*p*-nitrophenyl adipate with four methylene groups, NA) to undergo α-chymotrypsin catalyzed hydrolysis is lower than that of SP3EG (from monomer Ib: di-*p*-nitrophenyl sebacate with eight methylene groups, NS). In a 0.10 mg/mL α-chymotrypsin solution, SP3EG also showed biodegradation kinetics close to zero-order and an even faster biodegradation rate than that of AP3EG, 81 versus 56% weight loss within 24 h. Similar results can be found in our previous work on 1,4-hexanediol based saturated PEA.[32] 8-Phe-6 synthesized from NS (monomer Ib) also showed a faster hydrolysis than 4-Phe-6 from NA (monomer Ia) in the α-chymotrypsin solution. The possible reason might be that a higher hydrophobic PEEA-like SP3EG could have a higher affinity for α-chymotrypsin and hence a higher enzymatic hydrolysis than AP3EG.

These OEG based PEEAs also showed a much higher tendency than those PEAs derived from conventional aliphatic diols toward α-chymotrypsin catalyzed biodegradation. For example, within 24 h, SP3EG showed a weight loss of 81.3%, while SPBe (from 2-butene-1,4-diol) had 13.8% and SPB (from 1,4-butanediol) had 6.4% only.[33] Therefore, the incorporation of ether linkages into the amino acid derived PEA backbone also promotes enzymatic biodegradation when compared to those PEAs from aliphatic diols.

The surface morphology changes of PEEA polymer films upon biodegradation are shown in Figures 7 and 8. After 18 h of incubation, both AP3EG and SP3EG PEEAs showed a significant α-chymotrypsin catalyzed biodegradation as evident by the appearance of a more severe moon crater shaped eroded surface with more pores when compared to their counterparts in PBS (Figure 8). Both of these PEEA film samples did not show visible surface erosion in a PBS solution. In addition, as the enzyme concentration increased, the erosion level of the polymer film surface became more severe, especially from 0.05 to 0.10 mg/mL (Figure 7). These SEM data are consistent with the weight loss data.

The GPC and contact angle data of the insoluble part of the PEEA polymer films in the incubation media were also measured to study their biodegradations (Table 5). Although the weight loss showed significant biodegradation of AP3EG (34.9%) and SP3EG (64.8%) samples in 0.1 mg/mL α-chymotrypsin solution for 18 h, the GPC data showed very little change in their molecular weights and molecular weight distributions, and a similar phenomenon also occurred in lower (0.05 mg/mL, less weight loss) and higher (0.20 mg/mL, more weight loss) α-chymotrypsin concentrations or PBS buffer (almost no weight loss). The contact angle data indicated that the polymer surface became slightly more hydrophilic as the weight loss of the polymer increased. For example, the contact angle of the original SP3EG film was 88° and, after 18 h of incubation, was reduced to 78° (3.4% weight loss) in PBS and 49° (64.8% weight loss) in the 0.10 mg/mL α-chymotrypsin solution. In the case of the AP3EG film incubated in 0.20 mg/mL α-chymotrypsin for 18 h, the contact angle of the polymer film could not be measured due to the fast spreading of water on the film sample surface. The much higher contact angle of the original SP3EG (88°) than AP3EG (62°) also confirmed that SP3EG was more hydrophobic than AP3EG because of a longer methylene chain length (eight vs four $-CH_2-$ groups).

On the basis of both GPC and contact angle data, it is also suggested that the enzymatic hydrolysis occurred mostly at the surface of the PEEA films and that the interior of the polymer remained intact during the hydrolysis progress. Similar findings were reported by Fan et al.[34,35] that L- and D-phenylalanine based PEA particles underwent surface hydrolysis in α-chymotrypsin medium. Fan et al. also suggested that the rate and extent of the weight loss of the polymers by the enzymatic hydrolysis depended strongly on the solubility of the hydrolysis products because the polymer surface could be covered by unreactive biodegradation products that would retard the further biodegradation of the polymers if the biodegradation products were

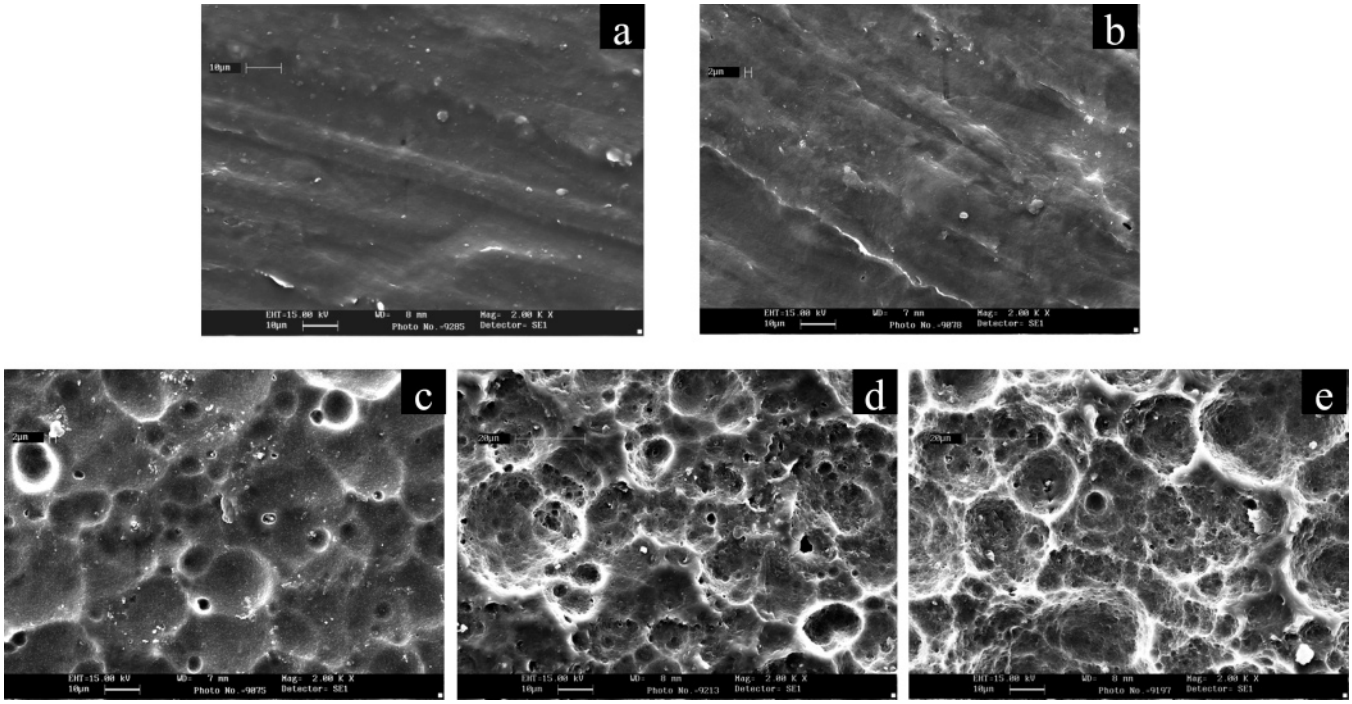


Figure 7. SEM images of AP3EG polymer after 18 h incubation at 37 °C. (a) Original film; (b) in PBS; (c) in 0.05 mg/mL α-chymotrypsin; (d) in 0.10 mg/mL α-chymotrypsin; and (e) in 0.20 mg/mL α-chymotrypsin.

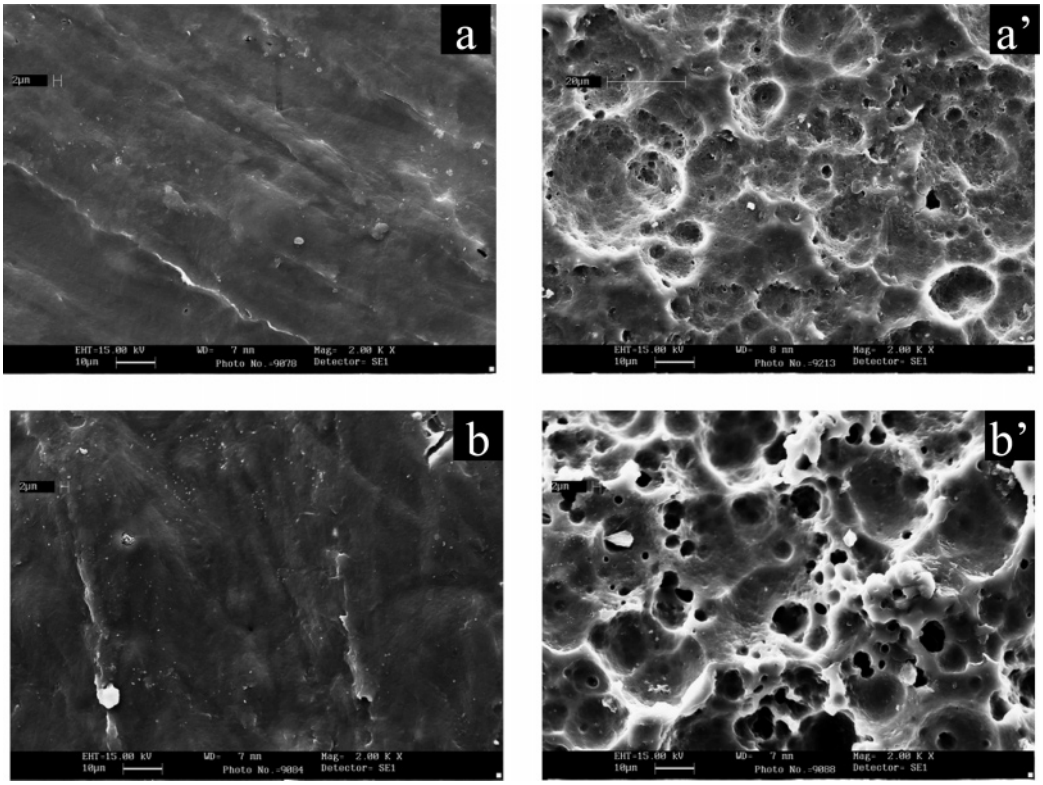


Figure 8. SEM images of AP3EG and SP3EG polymers after 18 h incubation in pH 7.4 PBS (a and b) and 0.10 mg/mL α-chymotrypsin solution (a′ and b′) at 37 °C. Panels a and a′ are AP3EG film, and panels b and b′ are SP3EG films.

insoluble. In addition, He et al.[36] reported that the melt spun fibers based on non-amino acid aliphatic PEA copolymers (from 6-aminocaproic acid and ϵ-caprolactone monomers at a 50:50 molar feed ratio) exhibited a typical surface erosion in a concentrated alkaline solution (1.0 M NaOH) because their molecular weight and chemical structure did not change significantly during degradation.

However, Bezemer et al.[37] reported bulk degradation of amphiphilic PEEA multiblock copolymers synthesized by melt polycondensation of poly(ethylene glycol) (PEG), 1,4-dihydroxybutane, and short bis-ester−bis-amide blocks in PBS because the inherent viscosity of their PEEA decreased significantly as the mass loss increased with time, and the degradation did not result in significant changes in the surface morphology of their PEEA films.

In Chu et al., previously published research on the biodegradation of phenylalanine based saturated PEA,[32] which has similar structures to the phenylalanine based PEEA in this study,

Table 5. Weight Loss, Molecular Weight, and Contact Angle of PEEA Polymers Before and After Incubation in Different Media for 18 h

polymer	concn of α-chymotrypsin (mg/mL)	incubation time (h)	weight loss (%)	M_n (kg/mol)	M_w (kg/mol)	M_w/M_n	contact angle (θ)
AP3EG	N/A[a]	0	0	17.4	26.0	1.49	62 ± 5
	0 (pure PBS)	18	−0.6	15.3	24.7	1.61	70 ± 13
	0.05	18	25.1	17.4	25.5	1.46	47 ± 17
	0.10	18	34.9	18.2	26.2	1.44	N/A[b]
	0.20	18	62.3	17.6	25.7	1.46	N/A[b]
SP3EG	N/A[a]	0	0	27.3	41.1	1.51	88 ± 5
	0 (pure PBS)	18	3.4	28.3	43.5	1.54	78 ± 4
	0.10	18	64.8	29.0	44.2	1.53	49 ± 5

[a] Original sample as the control. [b] Water drop spreads on the sample surface.

was suggested that hydrolysis cleavage of the ester bonds was the mechanism of degradation and that the degradation products were also confirmed by FTIR and UV spectra as the anticipated *N*,*N*′-adipoyl-(bis-L-phenylalanine) species. The biodegradation products of PEEAs synthesized in the current study, due to the similarity of their chemical structures to PEA's, may also follow a similar hydrolysis route of ester bond cleavage. A separated detailed study of the exact chemical nature of the biodegradation products of PEEAs would be needed and will be initiated in the near future.

Conclusion

A series of novel biodegradable unsaturated and saturated PEEAs based on oligoethylene glycol (OEG) and L-phenylalanine were successfully synthesized by solution polycondensation. The polymers can be obtained with fairly good yields (59 to ~83%) at 70 °C for 48 h in DMA solvent. The molecular weights (M_n and M_w) measured by GPC could be as high as 27.3 kg/mol and with a MWD of 1.51 for SP3EG. The chemical structures of all the nine types of PEEAs were confirmed by IR, NMR spectra, and elemental analysis. The unsaturated PEEAs have higher T_g values (>138 °C) than the saturated PEEAs (<101 °C) of a similar backbone structure. The solubility of the polymers was poor in water, methanol, and ethyl acetate and better in formic acid, DMA, and DMSO. A preliminary biodegradation study showed that the L-phenylalanine based PEEAs had a much higher tendency toward the α-chymotrypsin catalyzed biodegradation than the corresponding SPEA or UPEA based on aliphatic dialcohols. On the basis of the analysis of the data of weight loss, and the changes in dimension, contact angle, and molecular weight of AP3EG and SP3EG samples upon biodegradation, it is suggested that the L-phenylalanine based PEEAs eroded evenly on the surface upon enzymatic hydrolysis and were subject to surface, rather than bulk, biodegradation.

References and Notes

(1) Behravesh, E.; Jo, S.; Zygourakis, K.; Mikos, A. G. Synthesis of in situ cross-linkable macroporous biodegradable poly(propylene fumarate-*co*-ethylene glycol) hydrogels. *Biomacromolecules* **2002**, *3*, 374−381.

(2) Salem, A. K.; Cannizzaro, S. M.; Davies, M. C.; Tendler, S. J. B.; Roberts, C. J.; Williams, P. M.; Shakesheff, K. M. Synthesis and characterization of a degradable poly(lactic acid)-poly(ethylene glycol) copolymer with biotinylated end groups. *Biomacromolecules* **2001**, *2*, 575−580.

(3) Deschamps, A. A.; van Apeldoorn, A. A.; de Bruijn, J. D.; Grijpma, D. W.; Feijen, J. Poly(ether ester amide)s for tissue engineering. *Biomaterials* **2003**, *24*, 2643−2652.

(4) Lee, K. H.; Chu, C. C.; Quimby, F.; Klaessig, S. Molecular design of biologically active biodegradable polymers for biomedical applications. *Macromol. Symp.* **1998**, *130*, 71−80.

(5) Won, C. Y.; Chu, C. C.; Lee, J. D. Novel biodegradable copolymers containing pendant amine functional groups based on aspartic acid and poly(ethylene glycol). *Polymer* **1998**, *39*, 6677−6681.

(6) Jeong, B.; Bae, Y. H.; Lee, D. S.; Kim, S. W. Biodegradable block copolymers as injectable drug-delivery systems. *Nature* **1997**, *388*, 860−862.

(7) Kumar, N.; Ravikumar, M. N. V.; Domb, A. J. Biodegradable block copolymers. *Adv. Drug Delivery Rev.* **2001**, *53*, 23−44.

(8) Chu, C. C. Biodegradable polymeric biomaterials: An updated overview. In *The Biomedical Engineering Handbook*, 3rd ed.; Bronzino, J. D., Ed.; Taylor and Francis: New York, 2006; pp 421−422.

(9) Bastioli, C. *Handbook of Biodegradable Polymers*; Woodhead Publishing Ltd.: Cambridge, 2005.

(10) Chiellini, E.; Meisel, I. *Recent Advances in Biodegradable Polymers and Plastics*; John Wiley and Sons: New York, 2003.

(11) Hollinger, J. *Biomedical Applications of Synthetic Biodegradable Polymers*; CRC Press: Boca Raton, FL, 1995.

(12) Kimura, Y. Biodegradable Polymers. In *Biomedical Applications of Polymeric Material*; Tsuruta, T., Hayashi, T., Kataoka, K., Ishihara, K., Kimura, Y., Eds.; CRC Press: Boca Raton, FL, 1993; pp 164−190.

(13) Shalaby, S. W. *Biomedical Polymers: Designed-to-Degrade Systems*; Hanser Publishers: New York, 1994.

(14) Smith, R. *Biodegradable Polymers for Industrial Application*; Woodhead Publishing Ltd.: Cambridge, 2005.

(15) Vert, M.; Feijen, J.; Albertsson, A.; Scott, G.; Chiellini, E. *Biodegradable Polymers and Plastic*; Royal Society of Chemistry: Cambridge, 1992.

(16) Barrows, T. H.; Grussing, D. M.; Hegdahl, D. W. Poly(ester amides): A new class of synthetic absorbable polymers. *Trans. Soc. Biomater.* **1983**, 109.

(17) Veld, P. J. A. I.; Dijkstra, P. J.; Feijen, J. Synthesis of biodegradable polyesteramides with pendant functional groups. *Macromol. Chem. Phys.* **1992**, *193*, 2713−2730.

(18) Grigat, E.; Koch, R.; Timmermann, R. BAK 1095 and BAK 2195: Completely biodegradable synthetic thermoplastics. *Polym. Degrad. Stab.* **1998**, *59*, 223−226.

(19) Paredes, N.; Rodriguez-Galan, A.; Puiggali, J. Synthesis and characterization of a family of biodegradable poly(ester amide)s derived from glycine. *J. Polym. Sci., Part A: Polym. Chem* **1998**, *36*, 1271−1282.

(20) Puiggali, J.; Aceituno, J. E.; Paredes, N.; Rodriguez-Galan, A.; Pelfort, M.; Subirana, J. A. Preliminary structural data on new biodegradable poly(esteramide)s derived from L- and L,D-alanine. *Abstr. Pap.—Am. Chem. Soc.* **1998**, *216*, 858.

(21) Katsarava, R.; Beridze, V.; Arabuli, N.; Kharadze, D.; Chu, C. C.; Won, C. Y. Amino acid-based bioanalogous polymers. Synthesis and study of regular poly(ester amide)s based on bis(α-amino acid) α,ω-alkylene diesters, and aliphatic dicarboxylic acids. *J. Polym. Sci., Part A: Polym. Chem.* **1999**, *37*, 391−407.

(22) Fan, Y. J.; Kobayashi, M.; Kise, H. Synthesis and biodegradability of new polyesteramides containing peptide linkages. *Polym. J.* **2000**, *32*, 817−822.

(23) Qian, Z. Y.; He, Y.; Zou, Y. B.; Li, S.; Liu, X. B. Structure and property study of degradable polyesteramide fibres: Processing and alkaline degradation behavior. *Polym. Degrad. Stab.* **2004**, *83*, 127−132.

(24) Guo, K.; Chu, C. C.; Chkhaidze, E.; Katsarava, R. Synthesis and characterization of novel biodegradable unsaturated poly(ester amide)s. *J. Polym. Sci., Part A: Polym. Chem.* **2005**, *43*, 1463−1477.

(25) Lips, P.; Dijkstra, P. J. Biodegradable Polyesteramides. In *Biodegradable Polymers for Industrial Applications*; Smith, R., Ed.; Woodhead Publishing Ltd.: Cambridge, 2005; pp 107−139.

(26) Guo, K.; Chu, C. C. Synthesis, characterization, and biodegradation of copolymers of unsaturated and saturated poly(ester amide)s. *J. Polym. Sci., Part A: Polym. Chem.*, in press.

(27) Chu, C. C.; Katsarava, R. Elastomeric functional biodegradable copolyester amides and copolyester urethanes. U.S. Patent 6,503,-538, Jan. 7, 2003.

(28) Jokhadze, G.; Machaidze, M.; Panosyan, H.; Chu, C. C.; Katsarava, R. Synthesis and characterization of functional elastomeric poly(ester amide) copolymers. *J. Biomater. Sci., Polym. Ed.*, in press.

(29) Guo, K.; Chu, C. C. Synthesis and characterization of novel biodegradable unsaturated poly(ester amide)/poly(ethylene glycol) diacrylate hydrogels. *J. Polym. Sci., Part A: Polym. Chem.* **2005**, *43*, 3932−3944.

(30) Guo, K.; Chu, C. C. Controlled release of hydrophobic drugs from biodegradable unsaturated poly(ester amide)s/poly(ethylene glycol) diacrylate hydrogels. *J. Biomater. Sci., Polym. Ed.*, in press.
(31) Villuendas, I.; Iribarren, J. I.; Munoz-Guerra, S. Poly(ester amide)s derived from L-tartaric acid and amino alcohols. 1. Regic polymers. *Macromolecules* **1999**, *32*, 8015−8023.
(32) Tsitlanadze, G.; Machaidze, M.; Kviria, T.; Djavakhishvili, N.; Chu, C. C.; Katsarava, R. Biodegradation of amino acid-based poly(ester amide)s: In vitro weight loss and preliminary in vivo studies. *J. Biomater. Sci., Polym. Ed.* **2004**, *15*, 1−24.
(33) Guo, K.; Chu, C. C. Biodegradation study of biodegradable unsaturated poly(ester amide)s and their hydrogels. *Biomaterials*, in press.
(34) Fan, Y. J.; Kobayashi, M.; Kise, H. Synthesis and specific biodegradation of novel polyesteramides containing amino acid residues. *J. Polym. Sci., Polym. Chem.* **2001**, *39*, 1318−1328.
(35) Fan, Y. J.; Kobayashi, M.; Kise, H. Synthesis and biodegradation of poly(ester amide)s containing amino acid residues: Effect of the stereoisomeric composition of L- and D-phenylalanines on the enzymatic degradation of the polymers. *J. Polym. Sci., Part A: Polym. Chem.* **2002**, *40*, 385−392.
(36) He, Y.; Qian, Z. Y.; Zhang, H. L.; Liu, X. B. Alkaline degradation behavior of polyesteramide fibers: Surface erosion. *Colloid Polym. Sci.* **2004**, *282*, 972−978.
(37) Bezemer, J. M.; Weme, P. O.; Grijpma, D. W.; Dijkstra, P. J.; van Blitterswijk, C. A.; Feijen, J. Amphiphilic poly(ether ester amide) multiblock copolymers as biodegradable matrices for the controlled release of proteins. *J. Biomed. Mater. Res.* **2000**, *52*, 8−17.

BM070158C