Copolymers of Unsaturated and Saturated Poly(ether ester amide)s: Synthesis, Characterization, and Biodegradation

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ABSTRACT: A series of biodegradable random unsaturated/saturated poly(ether ester amide)s copolymers (USPEEAs) were synthesized by an active solution polycondensation of unsaturated and saturated dicarboxylic acid-based diester monomers with diamine salts of phenylalanine and saturated oligo(ethylene glycol) (OEG). These USPEEA copolymers were obtained with fairly good yields in DMA solvent. The chemical structures of the USPEEA copolymers were confirmed by both IR and NMR spectra. The molecular weights (M_n and M_w) of USPEEAs measured by GPC ranged from 3 to 27 kg/mol with the molecular weight distribution (MWD) ranging from 1.52 to 2.13. USPEEA copolymers obtained had T_g lower than that of the pure UPEEAs but higher than that of pure saturated poly(ether ester amide)s (SPEEA). An increase in the unsaturated component in USPEEAs led to an increase in their T_g. A preliminary *in vitro* biodegradation property of USPEEA copolymers were investigated in both pure PBS buffer and α-chymotrypsin solutions. The USPEEA copolymers showed a pronounced weight loss in enzyme solutions, but a smaller weight loss in a pure PBS. The biodegradation rates of USPEEA copolymers in α-chymotrypsin solution were much slower than those of pure PEEAs. Therefore, upon adjusting monomers feed ratio, USPEEA copolymers could have controlled chemical, physical, and biodegradation properties. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 110: 1858–1869, 2008

Key words: poly(ether ester amide); biodegradable polymers; unsaturated biodegradable polymers; solution polycondensation; enzymatic degradation

INTRODUCTION

Efforts have been directed toward the preparation of synthetic biocompatible and biodegradable polymers as drug carriers or scaffolds for tissue engineering in past decades.[1,2] Poly(ester amide)s (PEAs), especially those derived from amino acids like L-phenylalanine, glycine, L-leucine, or/and L-alanine[3–10] as an emerging class of biodegradable polymers, have been extensively investigated for various biomedical purposes, such as coating for drug-eluting stent,[8,11] cell culture,[5,12] and drug delivery.[13]

Recently, in efforts to expand the scope of amino acid-based PEA biomaterials to provide improved solubility, hydrophilicity, chain flexibility, and biodegradability, a novel family of amino acid-based biomaterials, Poly(ether ester amide) (PEEA), were designed and successfully synthesized by solution polycondensation.[14] Instead of traditional diols like butanediol, oligo(ethylene glycol)s (OEG) were used as one of the building blocks in PEEA, and the resulting PEEAs have well-defined blocks of not only ester and amide linkages as those PEAs from traditional diols but also ether linkage from OEG. Depending on the type and concentration of monomers used, such as α-amino acid, saturated, or unsaturated dicarboxylic acids, different dialcohols and OEG, the synthesized PEEAs could have vastly different thermal, mechanical, and biological properties to meet the needs of a wide range of applications in pharmaceutical, biomedical, and tissue engineering arena. The presence of additional ether linkages in the backbones of these OEG-based PEEAs were found to enhance the hydrophilicity, flexibility, and biodegradability of the polymers when comparing with diol-based PEAs.[14]

In addition to the amino acids-based PEEAs, nonamino acid-based PEEAs have also been reported; most of them are segmented (instead of alternate) copolymers prepared by interfacial polymerization, and their ether linkages are from poly(ε-caprolactone) (PCL),[15,16] poly(L-lactide) (PLLA),[17] poly(ethylene glycol) (PEG),[18] or others.[19] Some of these nonamino acid-based PEEAs have also been studied for different biomedical applications, such as scaffold materials,[20] cell culture,[17] and drug carriers.[16,21,22]

In this article, we reported the syntheses, characterization, and biodegradation of amino acids-based copolymers of saturated/unsaturated poly (ether ester amide) (USPEEAs) by solution

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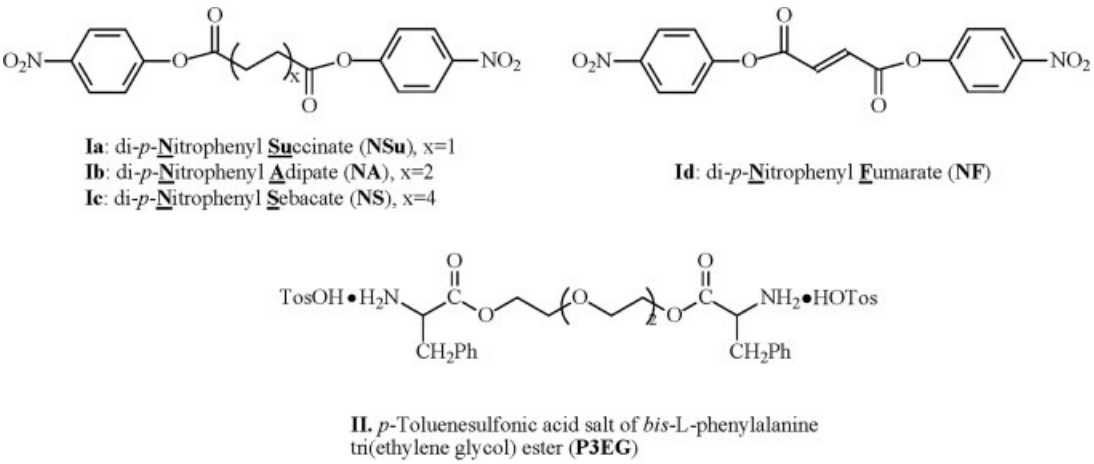


Scheme 1 Chemical structure of monomers for USPEEA Copolymer Synthesis.

polycondensation of unsaturated or saturated diester monomers and saturated OEG-based diamine salts. Such copolymers could combine the merits of both saturated PEEA (SPEEA) and unsaturated PEEA (UPEEA) into a single entity and, by simply controlling the feed ratio of saturated to unsaturated components, we expected to achieve controllable chemical, physical, thermal, and biological properties over a wide range that neither UPEEA nor SPEEA alone could achieve. The effects of different types of monomers and their feed ratios on yield, molecular weight, molecular weight distribution (MWD), thermal property, solubility, and biodegradability (in PBS or enzyme solution) of the resulting USPEEA copolymers were examined and discussed in detail.

EXPERIMENTAL

Materials

L-Phenylalanine (L-Phe), *p*-toluenesulfonic acid monohydrate, succinyl chloride, adipoyl chloride, sebacoyl chloride, fumaryl chloride, triethylene glycol, (Alfa Aesar, Ward Hill, MA) and *p*-nitrophenol (J. T. Baker, Phillipsburg, NJ) were used without further purification. Triethylamine from Fisher Scientific (Fairlawn, NJ) was dried by refluxing with calcium hydride, and then distilled. Other solvents like toluene, formic acid, trifluoroethanol (TFE), tetrahydrofuran (THF), ethyl acetate, methanol (MeOH), chloroform ($CHCl_3$), acetone, acetonitrile, *N,N*-dimethylformamide (DMF), *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 copolymers

USPEEA copolymers were synthesized by the similar procedures reported in our previous study of PEEA homopolymers.[14] In brief, the synthesis of USPEEA copolymers involved the following three basic steps: (1) synthesis of di-*p*-nitrophenyl esters of dicarboxylic acids, including bis-*p*-nitrophenyl succinate (NSu, **Ia**), bis-*p*-nitrophenyl adipate (NA, **Ib**), bis-*p*-nitrophenyl sebacate (NS, **Ic**), and bis-*p*-nitrophenyl fumarate (NF, **Id**), and they served as diester monomers; (2) synthesis of di-*p*-toluenesulfonic acid salts of bis-L-phenylalanine esters (**II**) from triethylene glycol (P3EG, **II**) and served as diamide monomer; and (3) solution polycondensation of the above diester and diamide monomers obtained in Steps (1) and (2).

Synthesis of monomers

There were four different types of di-*p*-nitrophenyl esters of dicarboxylic acids used as monomers to provide the carboxylic ester group of USPEEA in this study. The three saturated bis-*p*-nitrophenyl esters of dicarboxylic acids [bis-*p*-nitrophenyl ester of succinic acid NSu (**Ia**), NA (**Ib),** and NS (**Ic**)], and the unsaturated di-*p*-nitrophenyl esters of dicarboxylic acids [NF (**Id)**] were prepared by reacting the corresponding dicarboxylic acyl chlorides with *p*-nitrophenol as reported previously.[23] The chemical structures of these monomers are shown in Scheme 1.

To control the number of possible combinations between diester and diamine salts monomers (**I** and **II**), *p*-Toluenesulfonic acid salts of L-phenylalanine tri(ethylene glycol) monomer (P3EG, **II**) was the only diamine salt monomer used in this work and prepared by the procedures reported previously.[14]

Solution polycondensation of Monomers I and II

Copolymers of USPEEA and PEEAs were prepared by the solution polycondensation of bis-*p*-toluenesulfonic acid salt (P3EG) with a mixture of saturated

TABLE I
The monomer combinations for the USPEEAs syntheses

Monomer feed ratio				
I		II		
NF (unsaturated, mol%)	NSu, NA, or NS (saturated, mol %)	PB (mol %)	Polymer denomination	Expected saturation level (%)
0	100	100	(Su, A, or S)P3EG	100
30	70	100	F(Su, A, or S)P3EG37	70
50	50	100	F(Su, A, or S)P3EG55	50
70	30	100	F(Su, A, or S)P3EG73	30
100	0	100	FP3EG	0

di-*p*-nitrophenyl ester (NSu, NA, or NS) and unsaturated bis-*p*-nitrophenyl ester (NF) at a predetermined feed ratio. The combinations tried in this study were summarized in Table I and illustrated in Scheme 2.

An example of the synthesis of FAP3EG37 via solution polycondensation is given later to illustrate the details of the synthesis procedures. Triethylamine (0.31 mL, 2.2 mmol) was added dropwise to the mixture of monomers NA (**Ib**, 0.7 mmol), NF (**Id**, 0.3 mmol), and P3EG (**II**, 1.0 mmol) in 1.5 mL of dry DMA, and the solution was then heated to 60°C with stirring until a complete dissolution of monomers. The reaction vial was then kept at 70°C for 48 h without stirring. The FAP3EG37 copolymer in the solution was precipitated by adding chilled ethyl acetate, and the precipitate was filtered and then extracted by ethyl acetate in a Soxhlet apparatus for 48 h and finally dried *in vacuo* at room temperature.

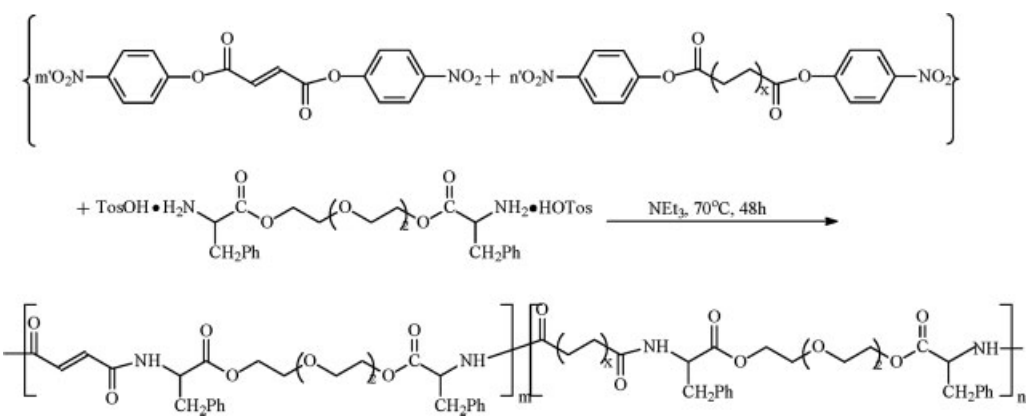
To investigate the difference between the copolymers (USPEEA) and the corresponding homopolymers (UPEEA or SPEEA), FP3EG from NF and P3EG monomers, SP3EG from NS and P3EG monomers, AP3EG from NA and P3EG monomers, and SuP3EG from NSu and P3EG monomers were also synthesized in a similar way.

To study the biodegradability of USPEEA copolymers, the USPEEA55 films were cast onto Teflon molds from 10% (wt/v) chloroform solution, 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 shape pieces (diameter 12.5 mm) for the biodegradation test.

Materials characterization

The chemical structures of USPEEA copolymers synthesized were characterized by standard chemical methods. Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFTS) technique was utilized to collect IR spectra of the copolymers, as DRIFTS provides a faster and simpler sample preparation than traditional FTIR for IR measurement. Samples were ground into powder and filled into the microcup of the diffuse reflectance accessory on a Perkin–Elmer Nicolet Magana 560 FTIR spectrometer (Madison, WI), and IR information of samples was collected and processed with Omnic software.

NMR spectra were recorded by a Varian Unity INOVA-400 400 MHz spectrometer (Palo Alto, CA) operating at 400 MHz and 100 MHz for ^{1}H and ^{13}C



Scheme 2 Syntheses of USPEEA copolymers. (FSuP3EG, $x = 1$; FAP3EG, $x = 2$; FSP3EG, $x = 4$).

NMR, respectively. Deuterated DMSO-d_6 (Cambridge Isotope laboratories) was used as the solvents.

Thermal property of the synthesized copolymers and homopolymers 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 under nitrogen gas at a flow rate of 25 mL/min. TA Universal Analysis™ software was used for thermal data analysis. The melting point (T_m) was determined at the peak maxima of the melting endotherm, and the glass transition temperature (T_g) was taken at the inflection point.

The number and weight average molecular weights (M_n and M_w) and MWD of the synthesized USPEEAs were determined by gel permeation chromatography (Model 510, Waters Associates Inc. Milford, USA) 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 MWD.

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.

In vitro enzymatic biodegradation

Biodegradation of USPEEA copolymers was carried out in a small vial containing a small piece of dry USPEEA film, (ca. 80 mg) and 10 mL of PBS buffer solution (pH = 7.4, 0.1 *M*) consisting of α-chymotrypsin at different concentrations (0, 0.05, 0.1, or 0.2 mg enzyme/mL). The vial was then incubated at 37°C with a constant reciprocal shaking (100 rpm). The incubation media were refreshed daily to maintain enzymatic activity. At predetermined immersion durations, USPEEA film samples were removed from the incubation medium, washed gently with distilled water, and 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 USPEEA film sample based on the following equation:

$$W_t(\%) = \frac{W_o - W_t}{W_o} \times 100$$

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

Scanning electron microscope (SEM) was employed to analyze the effect of biodegradation process on the surface morphology of USPEEA polymers. The copolymer film samples after predetermined periods of immersion were dried, fixed on aluminum stubs, and coated with gold for 60 s for SEM observation by Hitachi S4500 SEM (Mountain View, CA). The molecular weight changes of the USPEEA copolymers upon biodegradation were also monitored by GPC.

The surface hydrophilicity of USPEEA films was determined by IMASS Contact Angle Analyzer in a conditioning room of 65% RH and 21°C. Distilled water was used as the wetting medium, and the contact angles of five randomly chosen surface areas of each USPEEA film were measured, and two USPEEA films of each type were tested.

RESULTS AND DISCUSSION

USPEEA copolymer synthesis

Effect of monomers feed ratio on the properties of USPEEAs

As shown in Scheme 2 and Table I, nine different types of USPEEAs were prepared by a solution polycondensation of different feed ratios of saturated to unsaturated monomers **I**. Excess triethylamine was used as the acid receptor for TosOH during the polymerization to regenerate free amino groups in di-*p*-toluenesulfonic acid salt monomer.[4,14] The reaction conditions, like temperature, duration, and type of solvents, were chosen according to our previous published study.[14] Polymerization took place in a homogeneous phase, and the polymer obtained remained dissolved in the reaction solution.

Table II summarized the fundamental property of USPEEA copolymers synthesized. All the USPEEAs were obtained in fairly good yields (63–89%) with η_{red} ranging from 0.08 to 0.40 dL/g. Among all the nine USPEEA copolymers, four of them cannot dissolve in THF, which has been the designated eluent for the central GPC facility available to us. The other five USPEEA samples had a wide range of molecular weight (M_n = 3.6–27.0 kg/mol) with a relative broad MWD (1.52–2.13). FSP3EG37 and FSP3EG555 had the highest molecular weights (M_n = 27.0 and 20.9 kg/mol, respectively). The large difference among the copolymers' molecular weight and viscosity data suggested that the polycondensation reactivity of the four types of Monomer **I** (NF, NSu, NA, and NS) with Monomer **II** (P3EG) are very different. The molecular weight and viscosity both increased with the order: FSuP3EG < FAP3EG < FSP3EG. For example, when the feed ratios of unsaturated (NF) to

TABLE II
Fundamental property of USPEEA copolymers

Polymer	Yield (%)	M_n (kg/mol)	M_w (kg/mol)	M_w/M_n	η_{red}[a] (g/dL)	T_g (°C)	T_m (°C)
SuP3EG	69					41	103
FSuP3EG 37	89	3.55	5.60	1.52	0.09	48	123
FSuP3EG 55	72	—	—	—	0.12	55	153
FSuP3EG 73	74	—	—	—	0.08	65	153
AP3EG	79					35[b]	92[b]
FAP3EG 37	69	17.9	30.0	1.85	0.20	43	117
FAP3EG 55	80	16.2	25.6	1.58	0.25	49	132
FAP3EG 73	68	—	—	—	0.28	56	143
SP3EG	74					23[b]	67[b]
FSP3EG 37	63	27.0	46.2	1.83	0.40	33	112
FSP3EG 55	80	20.9	44.6	2.13	0.30	39	129
FSP3EG 73	77	—	—	—	0.30	54	150
FP3EG	83					67[b]	180[b]

[a] Measured in DMSO at 25°C, polymer conc = 0.25 g/dL.
[b] Ref. 14.

saturated Monomer **I** (NSu, NA, NS) in the polycondensation solution were 30–70%, the corresponding copolymers obtained had the molecular weight (M_n) order: FSuP3EG37 (3.55 kg/mol) < FAP3EG37 (17.9 kg/mol) < FSP3EG37 (27.0 kg/mol), and viscosity order: FSuP3EG37 (0.09 g/dL) < FAP3EG37 (0.20 g/dL) < FSP3EG37 (0.40 g/dL). The USPEEA copolymers with higher homologues of dicarboxylic acid building blocks, i.e., sebacate (NS) > adipate (NA) > succinate (NSu), showed higher MW, and this relationship is probably due to steric hindrance as a longer methylene group within a dicarboxylic acid could reduce steric hindrance of nitrophenol-based Monomers **I** toward the reaction with *p*-toluenesulfonic acid salts of bis-L-phenylalanine triethylene glycol (P3EG) monomer **II.**

Chemical structure identification

The structures of these USPEEA copolymers were confirmed by both IR and NMR spectra data. The FTIR spectra of all the USPEEA copolymers had the characteristic absorption bands of ester groups ($\sim$ 1740 cm^{-1}, Peak 1), Amide I and II groups ($\sim$ 1650 cm^{-1}, Peak 2; and $\sim$ 1536 cm^{-1}, Peak 3), and trans-unsaturated =CH bonds ($\sim$ 986 cm^{-1}, Peak 4) attributed to fumaryl group (Fig. 1). As the

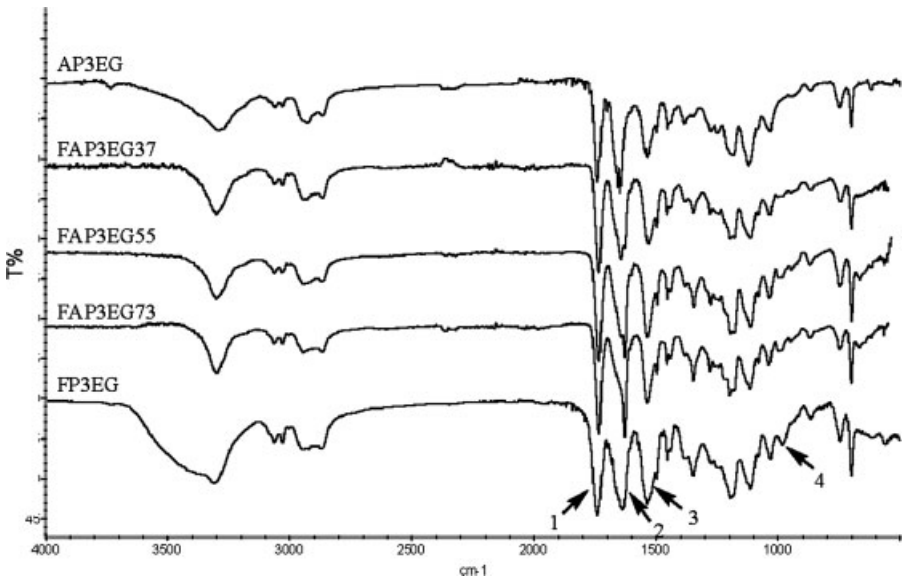


Figure 1 FTIR spectra of USPEEA copolymers. (1) C=O, st; (2) C=O, st, amide I; (3) NH, δ, amide II; (4) trans =CH, δ. (st: stretching vibration; δ: deformation vibration)

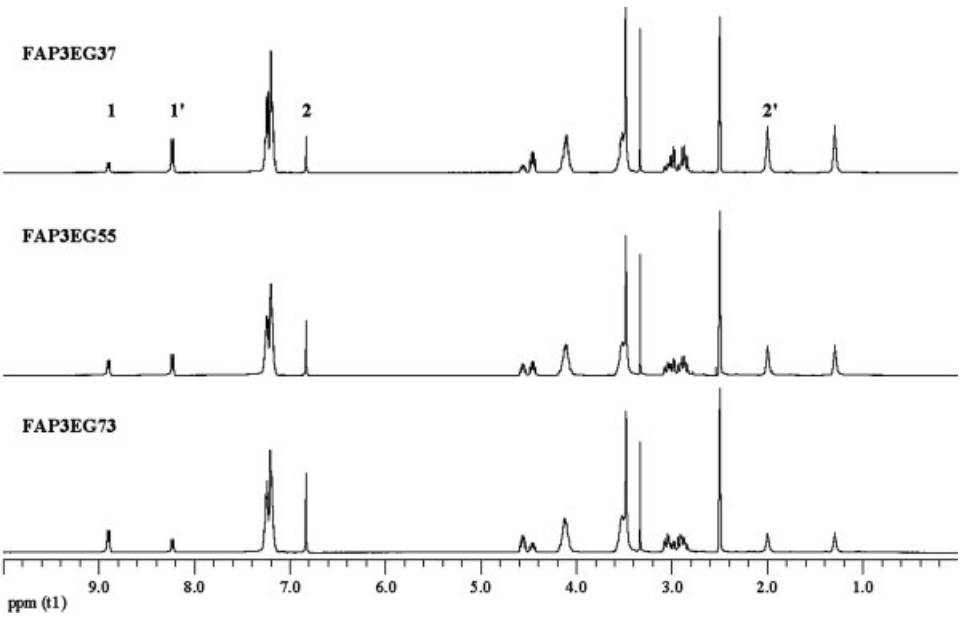


Figure 2 ^{1}H NMR spectra of three USPEEA copolymers (FAP3EG of different feed ratio of saturated NA to unsaturated NF) in DMSO-d_6. [(1) δ = 8.90 ppm, —CH=CH—CO—N**H**—; 1′: δ = 8.23 ppm, —CH_2—CO—N**H**—; (2) δ = 6.83 ppm, = C**H**—CO—; 2′: δ = 2.00 ppm NH—C(O)—C$\mathbf{H}_2$—].

feed ratio of unsaturated NF to saturated Monomer **I** (NS, NA, NSu) increased in the polycondensation solution, the intensity of the unsaturated =CH peak (Peak 4 at ~ 986 cm^{-1}) became stronger, and this unsaturated peak was absent in the spectra of saturated PEEAs like pure AP3EG polymer.

^{1}H NMR spectra of FAP3EG copolymers are shown in Figure 2. All these spectra showed two ^{1}H peaks of the amide bonds (—CO—N**H**—), one of the two ^{1}H peaks (Peak 1, δ = 8.90 ppm) was attributed to the amide bond in the UPEEA block (NF with P3EG segments), whereas the other ^{1}H peaks (Peak 1′, δ = 8.23 ppm) was from SPEEA block (NSu, NA, or NS with P3EG). Also, the fumaryl group signal (Peak 2, δ = 6.83 ppm) and the signal (Peak 2′, δ = 2.00 ppm) of methylene groups (NH—C(O)—C$\mathbf{H}_2$—) from saturated diamide moiety (NSu, NA, or NS) could also be used to assess the actual unsaturated to saturated composition ratio (m/n) of the USPEEA copolymers.

As shown in Table III, the actual USPEEA copolymers composition ratios of unsaturated to saturated moiety as determined from the ^{1}H NMR data (by the integration areas of the characteristic peaks of either —C***H*** or —N***H***) were different from the experimental feed ratios, and the magnitude of difference depended on the type of the copolymers. The difference between the experimental feed and actual composition ratios was minimum for FSuP3EG copolymers and maximum for FSP3EG copolymers.

A further analysis of the data in Table III indicated that the actual UPEEA composition (m block in Scheme 2) was just slightly higher than the experimental feed in FSuP3EG copolymers. On the other hand, in FASP3EG and FSP3EG copolymers, the actual SPEEA compositions (n block in Scheme 2) were both higher than the feed ratios of saturated monomers. This discrepancy could also be attributed to the different reactivity between monomer **II** (P3EG) and variety of monomers **I** (unsaturated NF or saturated NSu, NA, or NS). It can be concluded that the reactivity of NF with P3EG is similar to the case of NSu with P3EG but lower than NA or NS with P3EG.

TABLE III
The feed ratio and composition ratio of USPEEA copolymers

Polymer	Experimental feed ratio (m'/n′)	Actual composition ratio (m/n)		
		By —C**H**	By —N**H**	Avg.
FSuP3EG37	0.43	0.41	0.49	0.45
FSuP3EG55	1.00	1.05	1.14	1.10
FSuP3EG73	2.33	1.84	2.84	2.34
FAP3EG37	0.43	0.30	0.30	0.30
FAP3EG55	1.00	0.76	0.84	0.80
FAP3EG73	2.33	1.91	2.16	2.04
FSP3EG37	0.43	0.21	0.28	0.25
FSP3EG55	1.00	0.77	0.75	0.76
FSP3EG73	2.33	1.95	2.39	2.17

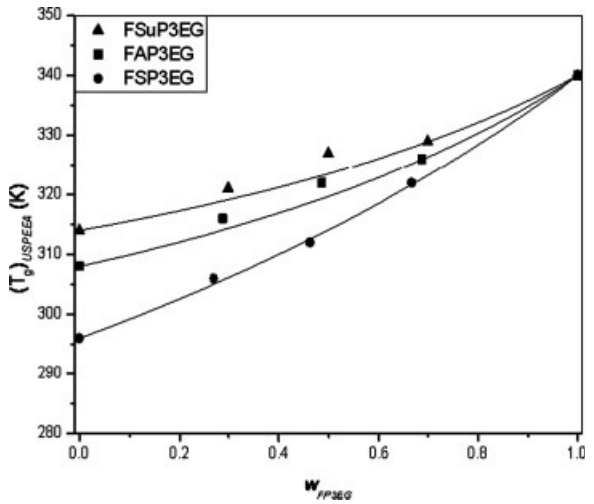


Figure 3 T_g of USPEEA copolymers as a function of the FPB weight fraction in the copolymers. [The curves were plotted according to the Gordon-Taylor-Wood eq. (1)].

Thermal property

The glass transition (T_g) and melting temperatures (T_m) of the USPEEA copolymers obtained by DSC are given in Table II. The T_g ranged from 23 to 67°C, and most USPEEA copolymers had T_g lower than 60°C. As the ratio of fumaryl unsaturated block increased, both T_g and T_m of the copolymers increased as well, which can be attributed to the reduced molecular flexibility due to the presence of unsaturated fumaryl group.

In Figure 3, the T_g data of USPEEA copolymer was shown as a function of the weight fraction of UPEEA, i.e., FP3EG (w_{FP3EG}), in the USPEEA copolymers. The curves were plotted according to the Gordon-Taylor-Wood equation [eq. (1), see later for details]. The T_g of the copolymers was between the T_g's of their corresponding pure SPEEA (SP3EG, 100% saturated,) and pure UPEEA (FP3EG, 100% unsaturated). When the feed ratio of unsaturated monomer (NF) to saturated (NA, NS, or NSu) monomer increased, the corresponding T_g of the USPEEA also increased. This is because the rigidity of the unsaturated block in the USPEEA copolymer made the polymer chain more difficult to rotate, and hence, reduced the flexibility of the polymer chain and increased the difficulty of chain segmental movement, i.e., higher T_g. For example, the order of T_g of FAP3EG copolymer series is FP3EG (67°C, w_{FP3EG} = 1.0) > FAP3EG73 (56°C, w_{FP3EG} = 0.7) > FAP3EG55 (49°C, w_{FP3EG} = 0.5) > FAP3EG37 (43°C, w_{FP3EG} = 0.3) > AP3EG (35°C with w_{FP3EG} = 0).

To better understand the effect of the monomers composition of USPEEA copolymers on their glass transition temperature, the empirical Gordon-Taylor-Wood equation[24,25] was used to study such a relationship, and it can be expressed as follows for the USPEEA copolymers in this work:

$$(T_g)_{USPEEA} = \frac{w_{SPEEA}(T_g)_{SPEEA} + k w_{UPEEA}(T_g)_{UPEEA}}{w_{SPEEA} + k w_{UPEEA}} \quad (1)$$

This equation can also be expressed as the following alternative form:

$$(T_g)_{USPEEA} = (T_g)_{SPEEA} + k\frac{[(T_g)_{UPEEA} - (T_g)_{USPEEA}] \cdot w_{UPEEA}}{1 - w_{UPEEA}} \quad (2)$$

Equation 1 is based on the "free volume" concept and where k is a fitted constant. The experimental results were plotted according to eq. (2) and are shown in Figure 4. The value of k, estimated from the slopes of the linear lines (obtained via linearization by the best fitting method) of the experimental data in Figure 4, yielded k values of 0.58 (FSuP3EG), 0.58 (FAP3EG), and 0.70 (FSP3EG), respectively. These k values were then substituted into eq. (1) to arrive Figure 3; the data in Figure 3 showed that the experimental results followed the Gordon-Taylor-Wood plot pretty well, when the glass transition temperature of the copolymers were plotted against the weight fraction of FP3EG in the USPEEA copolymers.

As shown in Figure 3 and Table II, different types of saturated blocks in the USPEEA at the same feed ratio were also found to affect T_g. The data suggest that a longer methylene group in the saturated block of USPEEAs reduced their T_g because such a longer methylene group is expected to increase polymer chain flexibility and hence, lower their T_g. For

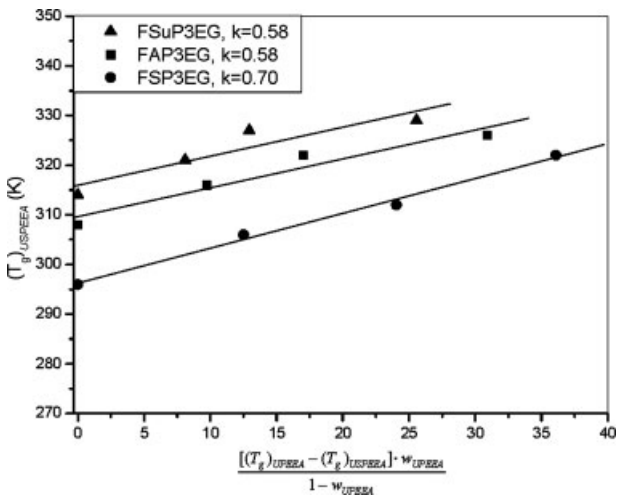


Figure 4 Plots of the glass transition temperature for USPEEA copolymers according to the Gordon-Taylor-Wood.[24,25]

TABLE IV
Solubility of USPEEA at room temperature (25°C). (+): soluble; (−): insoluble; (±): partially soluble or swell

Polymer	H_2O	Formic Acid	TFE	DMF	DMSO	THF	MeOH	Ethyl Acetate	$CHCl_3$	Acetone
FP3EG	−	+	−	+	+	−	−	−	−	−
FSuP3EG73	−	+	−	+	+	−	−	−	±	−
FSuP3EG55	−	+	+	+	+	−	−	−	±	−
FSuP3EG37	−	+	+	+	+	+	−	−	+	−
SuP3EG	−	+	+	+	+	+	−	−	+	−
FAP3EG73	−	+	+	+	+	−	−	−	±	−
FAP3EG55	−	+	+	+	+	+	−	−	+	−
FAP3EG37	−	+	+	+	+	+	−	−	+	−
AP3EG	−	+	+	+	+	+	−	−	+	±
FSP3EG73	−	+	+	+	+	−	−	−	+	−
FSP3EG55	−	+	+	+	+	+	−	−	+	−
FSP3EG37	−	+	+	+	+	+	−	−	+	−
SP3EG	−	+	+	+	+	+	−	−	+	+

instance, the order of length of methylene groups of saturated Monomers **I** (from short to long) is NSu (2) < NA (4) < NS (8). Consequently, the T_g of the corresponding USPEEA copolymers at the same feed ratio decreased from FSuP3EG, FAP3EG to FSP3EG. This relationship between T_g and number of methylene groups in USPEEA is also consistent with the results in our previous reported studies of PEEA[14] and PEA.[23]

Solubility

As shown in Table IV, the solubility of USPEEAs (50 mg) in water and several common organic solvents (1.0 mL) at room temperature (25°C) was evaluated. All the USPEEAs are soluble completely in DMSO, DMF, and formic acid (polar protic solvent) but cannot dissolve in water, methanol, and ethyl acetate. USPEEAs synthesized from feed ratios of NF to saturated Monomer **I** up to 30% can also dissolve in TFE, THF, and $CHCl_3$. These USPEEA having lower NF content behaved more like pure SPEEA. When the feed ratio of NF reached 70%, the USPEEA copolymers obtained had the solubility similar to pure unsaturated PEEA, i.e., FP3EG. When the NF content in the feed ratio was 50%, the USPEA copolymers were found to be able to dissolve in TFE and have a limited solubility in $CHCl_3$, which is attributed to the balance of the unsaturated and saturated components in these particular USPEA copolymers.

In vitro biodegradation of USPEEA films

The biodegradation behaviors of USPEEA copolymers were illustrated by the weight loss of a representative USPEEA sample (FAP3EG55) over 13 days in both PBS and enzyme media of pH 7.4 at 37°C, and the data are shown in Figure 5. Generally, FAP3EG55 has just very little weight loss in PBS buffer (about 6%) during the whole 13-day incubation. The biodegradation data showed that FAP3EG55 was sensitive to an enzymatic biodegradation. In a low concentration of α-chymotrypsin solution (0.05 mg/mL), FAP3EG55 degraded gradually and had 9% of weight loss in the first day and 44% of weight loss after 13 days. As the concentration of α-chymotrypsin increased, the degradation rate of FAP3EG55 also increased; however, all their weight loss kinetics are close to zero-order behaviors, especially after Day 2. It was observed that the FAP3EG55 films can maintain their appearance very well up to 75% of weight loss, while their thickness became thinner with time. This suggests that the USPEEA copolymer films eroded evenly on surface upon enzymatic hydrolysis. This biodegradation

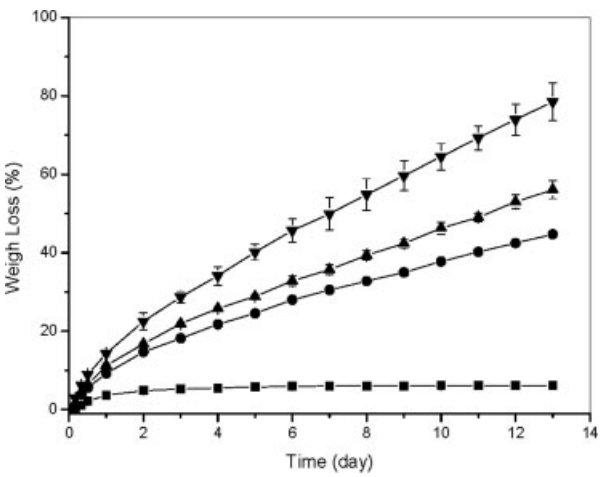


Figure 5 Effect of enzyme (α-chymotrypsin) concentration on the weight-loss kinetics of FAP3EG55 at 37°C. The samples in pH 7.4 PBS buffer served as the control. (■) PBS buffer; (●) [α-chymotrypsin] = 0.05 mg/mL; (▲)[α-chymotrypsin] = 0.10 mg/mL; (▼)[α-chymotrypsin] = 0.20 mg/mL.

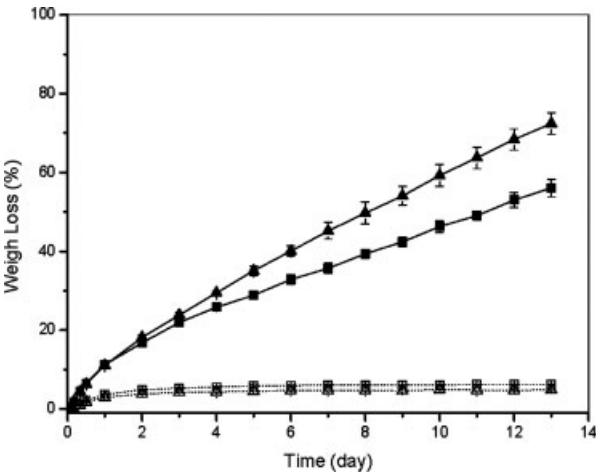


Figure 6 Effect of chemical structure of poly(ether ester amide) copolymers (FAP3EG55 and FSP3EG55) on their weight-loss behavior in PBS or α-chymotrypsin medium (0.1 mg/mL) at 37°C. (□) FAP3EG55 in pure PBS buffer; (-■-) FAP3EG55 in α-chymotrypsin solution; (Δ) FSP3EG55 in pure PBS buffer; (-▲-) FSP3EG55 in α-chymotrypsin solution.

mode is different from the bulk hydrolytic degradation of aliphatic polyesters, like PGA, PLA, and PLGA.[26,27]

The effect of chemical composition of USPEEA copolymers on their biodegradation property in both PBS and α-chymotrypsin media are shown in Figure 6. Two different USPEEA copolymers at a fixed unsaturated to saturated monomer feed ratio (50 to 50), FAP3EG55 ($x = 2$ in Scheme 2), and FSP3EG55 ($x = 4$ in Scheme 2) were used for such an illustration. In PBS buffer media (dotted lines), there were virtually no difference in weight loss between FAP3EG55 and FSP3EG55 samples during 13 days incubation, i.e., 6.0% versus 4.8% of weight loss. However, both copolymer samples showed a pronounced difference in weight loss in a α-chymotrypsin solution (0.10 mg/mL). FSP3EG55, which had eight methylene groups in the diacids segment, reached a weight loss of 72.4% at the end of Day 13, which was 16% more than that of FAP3EG55 that had four methylene groups in the diacids segment. Similar results were also found in our previous study of pure saturated SPEEA.[14] In that study, sebacoyl-based SPEEA had a faster biodegradation rate than that of adipoyl-based SPEEA. The possible reason might be that FSP3EG55, which is more hydrophobic due to the longer methylene group (eight for sebacoyl group) in every repeating unit than FAP3EG55 (four for adipoyl group), could have a higher affinity for α-chymotrypsin and hence, a higher enzymatic hydrolysis than FAP3EG55. The role of hydrophobicity in enzymatic biodegradation of the amino acid-based PEAs was also found in PEAs synthesized from aliphatic diols (e.g., butanediol, hexanediol).[7,9,28] In addition to the hydrophobicity role, the effect of methylene groups on chain mobility may also have an impact on enzyme-catalyzed biodegradation of this class of polymers. As shown in Table II, FSP3EG55 had a T_g near 37°C and was lower than FAP3EG55's T_g of 49°C; thus, the FAP3EG55 chains should be far more rigid than FSP3EG55 at the 37°C biodegradation temperature, and this molecular rigidity may impose some difficulty for enzymes to adapt to perform biodegradation.

The effect of chain rigidity on the level of enzymatic biodegradation is also evident when comparing the weight loss data of our current unsaturated copolymer data with saturated PEEAs reported previously. When the unsaturated fumaryl block was incorporated into the saturated PEEA backbone, the rigidity of the polymer increased and resulted in limited access for enzyme molecules, therefore, the enzymatic degradation rates of the copolymers were much lower (around 11% of weight loss for both FAP3EG55 and FSP3EG55 after Day 1) than those corresponding pure saturated PEEAs like AP3EG and SP3EG, which were reported to have 56 and 81% of weight loss in α-chymotrypsin solution (0.1 mg/mL) at 37°C after Day 1, respectively.[14]

The surface morphology changes of these two USPEEA film samples (FAP3EG55 and FSP3EG55) upon biodegradation are shown in Figures 7 and 8. After 13 days incubation, both FAP3EG55 and FSP3EG55 showed a significant α-chymotrypsin-catalyzed biodegradation as evident by the appearance of rough or moon crater shaped eroded surface with more microscopic pores [Fig. 7(c–e)] when comparing with their counterparts in PBS [Fig. 7(b)]. Neither USPEEA copolymer film samples showed much surface erosion in a PBS solution. In addition, as the enzyme concentration increased from 0.05 [Fig. 7(c)] to 0.20 mg/mL [Fig. 7(e)], the erosion level of the polymer film surface became severer, which were also consistent with the weight loss data.

The molecular weight and contact angle of the remaining part of USPEEA polymer films after incubation were also measured to study their biodegradation property (Table IV). Although the weight-loss data showed a significant biodegradation of both FAP3EG55 (56.1%) and FSP3EG55 (72.4%) samples in a α-chymotrypsin solution (0.1 mg/mL) for 13 days, the GPC data showed very little change in their molecular weights and MWDs; similar observations were also found in a PBS buffer (much less weight loss) and α-chymotrypsin medium of either a lower (0.05 mg/mL, less weight loss) or a higher enzyme concentration (0.20 mg/mL, more weight loss). The decrease of 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

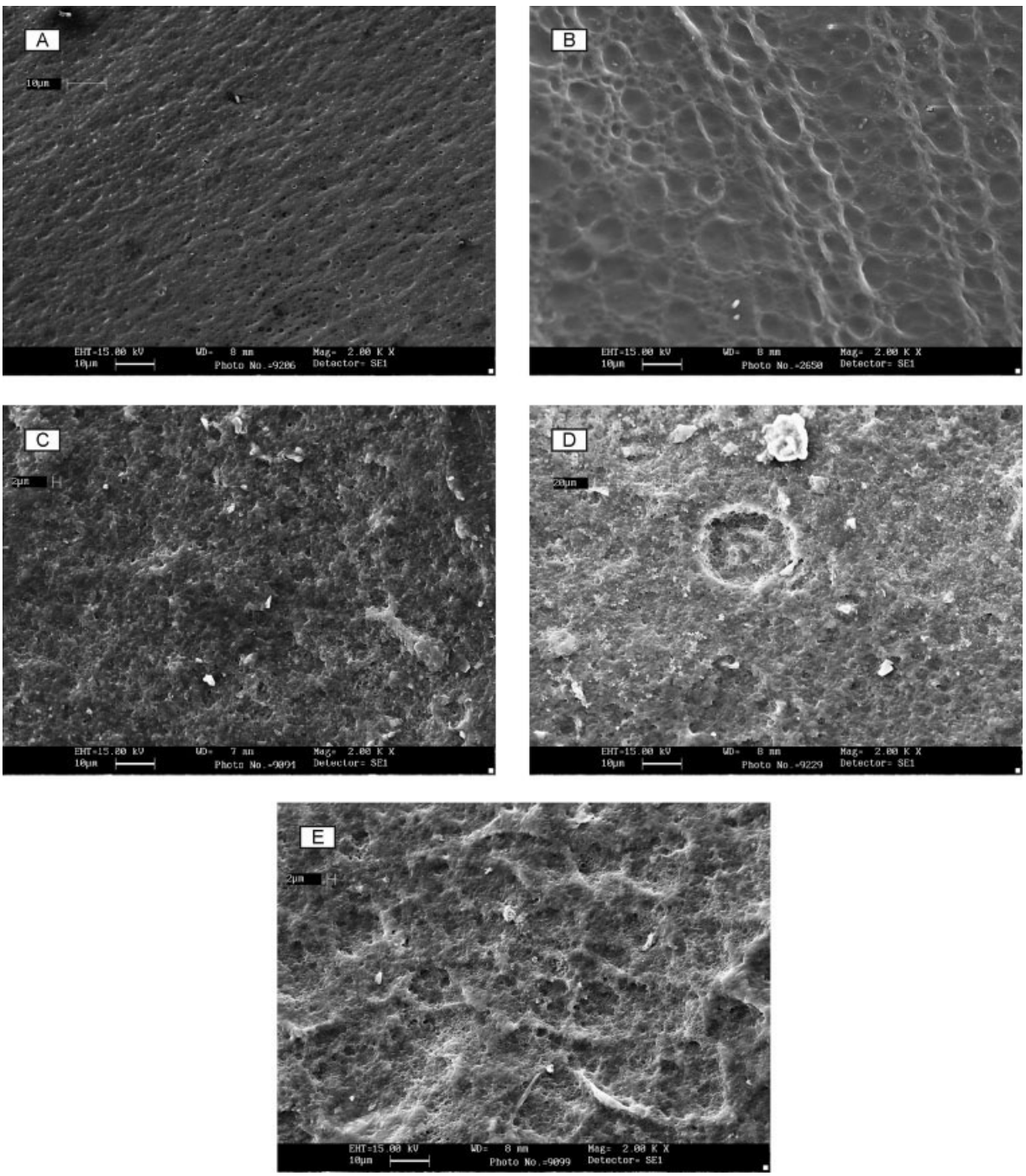


Figure 7 SEM images of FAP3EG55 copolymer after 13 days incubation at 37°C. (a) original film; (b) in PBS; (c) in 0.05 mg/mL α-chymotrypsin; (d) in 0.10 mg/mL α-chymotrypsin; (e) in 0.20 mg/mL α-chymotrypsin.

original FSP3EG55 film was 74° and after 13 days incubation reduced to 65° (4.8% weight loss) in PBS and 62° (72.4% weight loss) in 0.10 mg/mL α-chymotrypsin solution. In case of FAP3EG55 film incubated in 0.20 mg/mL α-chymotrypsin for 13 days, the contact angle of the polymer film could not be measured due to the fast spreading of water on the film sample surface.

Based on mass and molecular weight loss data and contact angle data in Table V, it is suggested again that the enzymatic hydrolysis of USPEEA copolymers occurred mostly on the surface of the USPEEA copolymer films, and the interior of the polymer remained intact during the hydrolysis progress, i.e., surface erosion rather than bulk biodegradation. This mode of biodegradation is consistent

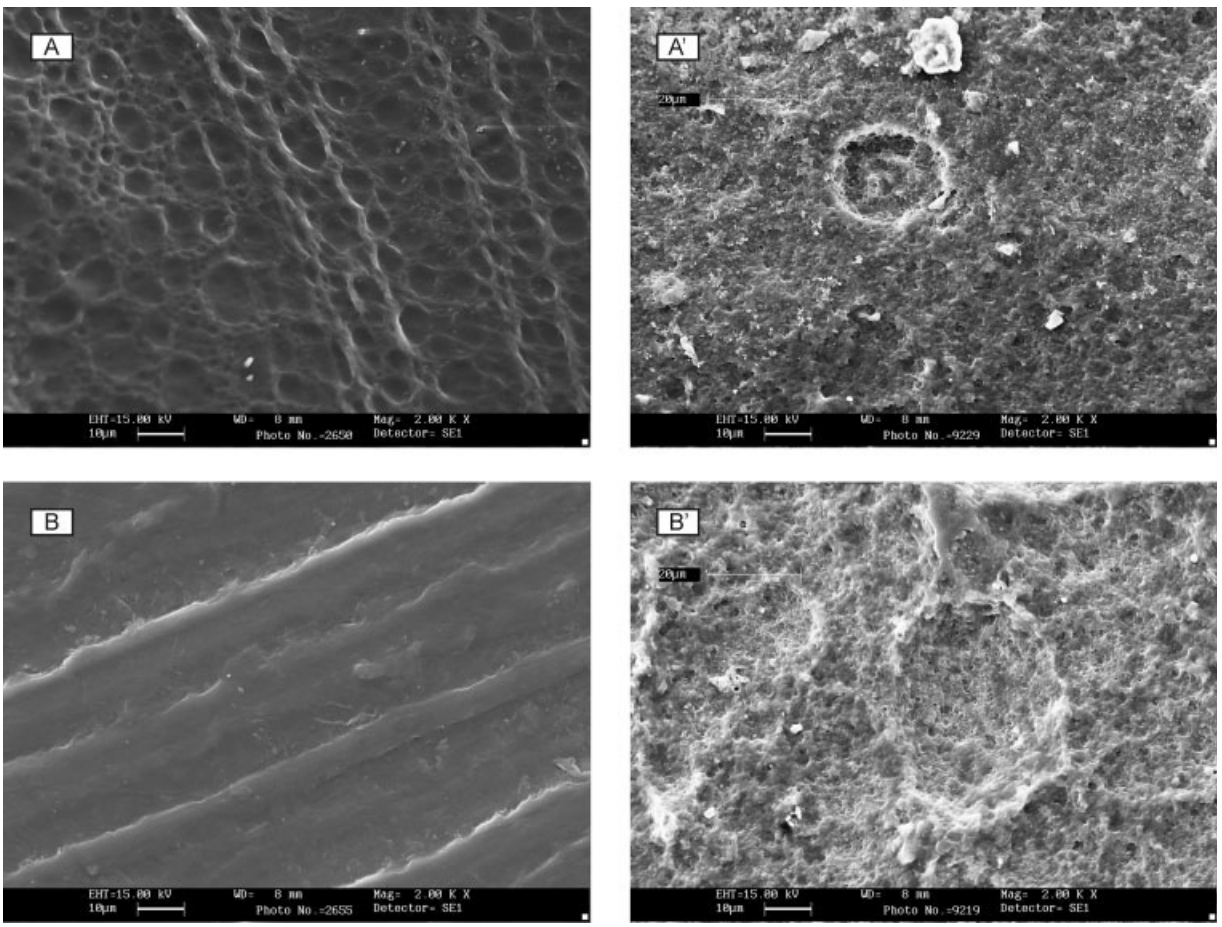


Figure 8 SEM images of FAP3EG55 versus FSP3EG55 polymer after 13 days incubation in pH 7.4 PBS (A and B), and 0.10 mg/mL α-chymotrypsin solution (A′ and B′) at 37°C. A and A′ are FAP3EG55 film, and B and B′ are FSP3EG55 films.

with our previous observations of biodegradation of PEEA and PEA homopolymers.[7,9,14,28]

CONCLUSIONS

A series of random unsaturated/saturated PEEA copolymers were synthesized by solution polycondensation of the unsaturated diester monomer (NF), the saturated diester monomers [NSu, NA, or NS], and the saturated diamine salts, *p*-toluenesulfonic acid salts of L-phenylalanine tri(ethylene glycol) diesters (P3EG). By adjusting the feed ratio of unsaturated to saturated diester monomers, USPEEA copolymers having different unsaturation levels

TABLE V
Weight loss, molecular weight, and contact angle of USPEEA polymers before and after incubation in different media for 13 days

Polymer	Conc. of α-chymotrypsin (mg/mL)	Incubation time (Day)	Weight Loss (%)	M_n (kg/mol)	M_w (kg/mol)	M_w /M_n	Contact Angle (θ°)
FAP3EG55	N/A[a]	0	N/A	17.7	48.1	2.72	66 ± 5
	0 (pure PBS)	13	6.0	17.2	45.2	2.63	66 ± 1
	0.05	13	44.7	16.1	37.9	2.36	69 ± 1
	0.10	13	56.1	14.9	31.1	2.09	61 ± 3
	0.20	13	78.5	15.8	33.3	2.11	N/A[b]
FSP3EG55	N/A[a]	0	N/A	20.9	44.6	2.13	74 ± 5
	0 (pure PBS)	13	4.8	19.2	40.8	2.12	65 ± 1
	0.10	13	72.4	20.2	39.4	1.95	62 ± 6

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

were obtained with the molecular weight (M_n) ranging from 3.6 to 27.0 kg/mol. The thermal, solubility, and biodegradation property of the USPEEA copolymers could also be controllable within a range between pure UPEEA and pure SPEEA. As the unsaturated block in the copolymer increased, the T_g of USPEEA copolymers also became higher. A preliminary *in vitro* biodegradation test showed that the USPEEA copolymers can be enzymatically biodegraded by α-chymotrypsin even at a low enzyme concentration, and their hydrolysis in pure PBS buffer media were very little. However, the biodegradation rates of USPEEA copolymers in α-chymotrypsin solution were much slower than those pure SPEEAs like AP3EG and SP3EG in the same degradation media. It is also found that the biodegradability of USPEEA copolymers were affected by length of methylene groups in the repeating units the same way as PEEA and PEA homopolymers. Therefore, the main advantage of this new family of USPEEA copolymers is that, by simply controlling the feed ratios of unsaturated to saturated monomers, we could achieve a wide range of property of the USPEEA copolymers to meet specific requirements of a wide range of biomedical applications.

References

1. Freier, T. Adv Polym Sci 2006, 1.
2. Okada, M. Prog Polym Sci 2002, 27, 87.
3. Guo, K.; Chu, C. C. J Polym Sci Part A: Polym Chem 2005, 43, 3932.
4. Katsarava, R.; Beridze, V.; Arabuli, N.; Kharadze, D.; Chu, C. C.; Won, C. Y. J Polym Sci Part A: Polym Chem 1999, 37, 391.
5. Paredes, N.; Rodriguez-Galan, A.; Puiggali, J.; Peraire, C. J Appl Polym Sci 1998, 69, 1537.
6. Paredes, N.; Rodriguez-Galan, A.; Puiggali, J. J Polym Sci Part A: Polym Chem 1998, 36, 1271.
7. Jokhadze, G.; Machaidze, M.; Panosyan, H.; Chu, C. C.; Katsarava, R. J Biomater Sci Polym Ed 2007, 18, 411.
8. Guo, K.; Chu, C. C. J Polym Sci Part A: Polym Chem 2007, 45, 1595.
9. Tsitlanadze, G.; Machaidze, M.; Kviria, T.; Djavakhishvili, N.; Chu, C. C.; Katsarava, R. J Biomater Sci Polym Ed 2004, 15, 1.
10. Chu, C. C. In The Biomedical Engineering Handbook; Bronzino, J. D., Ed.; CRC Taylor & Francis: New York, 2006; Chapter 42, p 1.
11. Lee, S. H.; Szinai, I.; Carpenter, K.; Katsarava, R.; Jokhadze, G.; Chu, C. C.; Huang, Y. M.; Verbeken, E.; Bramwell, O.; De Scheerder, I.; Hong, M. K. Coron Artery Dis 2002, 13, 237.
12. Han, S. I.; Kim, B. S.; Kang, S. W.; Shirai, H.; Im, S. S. Biomaterials 2003, 24, 3453.
13. Guo, K.; Chu, C. C. J Biomater Sci Polym Ed 2007, 18, 489.
14. Guo, K.; Chu, C. C. Biomacromolecules 2007, 8, 2851.
15. Maglio, G.; Palumbo, R.; Rachiero, G. P.; Vignola, M. C. Macromol Biosci 2002, 2, 293.
16. Li, M. X.; Zhuo, R. X.; Qu, F. Q. J Polym Sci Part A: Polym Chem 2002, 40, 4550.
17. D'Angelo, S.; Galletti, P.; Maglio, G.; Malinconico, M.; Morelli, P.; Palumbo, R.; Vignola, M. C. Polymer 2001, 42, 3383.
18. Deschamps, A. A.; Grijpma, D. W.; Feijen, J. J Biomater Sci Polym Ed 2002, 13, 1337.
19. Mehdipour-Ataei, S.; Malekimoghaddam, R.; Nami, M. Eur Polym Mater 2004, 40, 2523.
20. Deschamps, A. A.; van Apeldoorn, A. A.; de Bruijn, J D.; Grijpma, D. W.; Feijen, J. Biomaterials 2003, 24, 2643.
21. Bezemer, J. M.; Weme, P. O.; Grijpma, D. W.; Dijkstra, P. J.; van Blitterswijk, C A; Feijen, J. J Biomed Mater Res 2000, 52, 8.
22. Barbato, F.; La Rotonda, M. I.; Maglio, G.; Palumbo, R.; Quaglia, F., Biomaterials 2001, 22, 1371.
23. Guo, K.; Chu, C. C.; Chkhaidze, E.; Katsarava, R. J Polym Sci Part A: Polym Chem 2005, 43, 1463.
24. Gordon, M.; Taylor, J. S. J Appl Chem 1952, 2, 493.
25. Wood, L. A. J Polym Sci 1958, 28, 319.
26. Jain, R. A. Biomaterials 2000, 21, 2475.
27. Chu, C.-C.; Von Fraunhofer, J. A.; Greisler, H. P. Wound Closure Biomaterials and Devices; CRC Press: Boca Raton, Fla, 1997.
28. Tsitlanadze, G.; Kviria, T.; Katsarava, R.; Chu, C. C. J Mater Sci: Mater Med 2004, 15, 185.