Synthesis of Unsaturated Poly(ether amide)s Based on Amine-Terminated Poly(ethylene glycol)

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ABSTRACT: Novel water-soluble unsaturated poly(ether amide)s (PEAs) were synthesized by low-temperature polycondensation of fumaryl chloride and amine-terminated poly(ethylene glycol) (Jeffamine®). The unsaturated copolymers were further chemically modified with thiols to provide reactive pendant functional groups. Hydrogels based on these copolymers were prepared by copolymerization of the PEA with *N*-vinyl pyrrolidone exposure to ultraviolet (UV) irradiation. The resulting hydrogels exhibited a high swelling ratio, and the magnitude of swelling depended on the molecular weight of Jeffamine®. The swelling ratio and equilibrium water content tended to increase with increasing chain length of the Jeffamine® used in copolymer synthesis. © 1999 John Wiley & Sons, Inc. J Appl Polym Sci 74: 913–920, 1999

Key words: poly(ethylene glycol); fumaryl chloride; hydrogel; Jeffamine®; poly(ether amide)s

INTRODUCTION

Poly(ethylene glycol)s (PEGs) are an important class of polymers that have found many applications in a variety of fields, and, recently, there has been special interest in medicine, biology, and biomedical science. The properties of PEG of significance here include ease of chemical modification, biocompatibility, lack of antigenicity and immunogenicity, a wide range of solubility, and complexing ability with metal ions.[1,2]

Block copolymers of PEG with lactide,[3–11] ε-caprolactone,[12] 1,4-dioxan-2-one,[13] and trimethylene carbonate[14] have been reported by a number of investigators. These block copolymers are important polymeric biomaterials with controllable biodegradation rates. Also, incorporation of amino acids into the backbone of PEG has previously been described for use as drug carriers. ABA- or AB-type block copolymers composed of a poly(amino acid) as the hydrophobic A part and PEG as the hydrophilic B part are obtained via polymerization of *N*-carboxy anhydrides of phenylalanine,[15] γ-benzyl-L-glutamate,[16] N^{ε}-(benzyloxycarbonyl)-L-lysine,[17] β-benzyl-L-aspartate,[18] or proline[19] initiated by primary amino groups on the termini of the PEG chain. Since these amphiphilic block copolymers form polymeric micelles or core-shell-type nanoparticles, they are useful for carrying hydrophobic drugs in drug delivery systems.

Although PEGs have been studied intensively as biomaterials, one of the shortcomings of these linear polymers is that there are only two reactive groups (hydroxyl groups at both chain ends) and no pendant functional groups on the backbone molecules which could be used for covalently bonding of either drugs or biological active agents. Consequently, alternating copolymers of PEG with L-lysine[20] and L-aspartic acid[21,22] have been prepared to improve low reactive groups of PEG. The resulting copoly(PEG-amino acid)s

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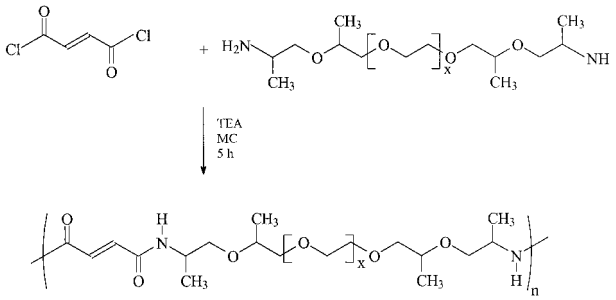


Scheme 1 Synthesis of PEAs.

have multiple pendant functional groups with predetermined intervals along the polymer backbone chain. Also, Koyama et al.[23] prepared new PEG derivatives having pendant amino groups by copolymerization of ally glycidyl ether with ethylene oxide, followed by chemical modification of the double bond side chains.

The present article describes the synthesis of unsaturated poly(ether amide)s (PEA)s based on fumaryl chloride and amine-terminated PEG. In addition, hydrogels were obtained by ultraviolet (UV) photocrosslinking, and the swelling characteristics of these gels were also investigated.

EXPERIMENTAL

Materials

O,O′-bis(2-aminopropyl)polyethylene glycol 130 (Jeffamine D-230®), *O,O′*-bis(2-aminopropyl)polyethylene glycol 500 (Jeffamine ED-600®), and *O,O′*-bis(2-aminopropyl)polyethylene glycol 1900 (Jeffamine ED-2001®) were purchased from Fluka (Ronkonokoma, NY). Fumaryl chloride and triethylamine (TEA) (Aldrich, Milwaukee, WI) were purified by distillation under reduced pressure prior to use. 2,2′-Azobisisobutyronitrile (AIBN) (Aldrich) was purified by recrystallization from methanol. 2-Mercaptoethanol, 3-mercaptopropionic acid, 2-aminoethanethiol hydrochloride, *N*-vinyl pyrrolidone (NVP), and hydroxycyclohexylphenyl ketone (Irgacure 184) were obtained from Aldrich and used as received. All solvents used in this experiment were anhydrous grade and were used without further purification.

Synthesis of Unsaturated Poly(ether amide)s

Water-soluble unsaturated PEAs were prepared by low-temperature polycondensation (Scheme 1). A typical example is as follows: All Jeffamines were dried by azeotropic distillation with toluene prior to reaction. Jeffamine D-230® (5.20 mL, 21.5 mmol) was dissolved in 60 mL of dry toluene, and 30 mL of toluene was removed by distillation. After cooling to room temperature, 30 mL of dry methylene chloride was added to the reaction flask. To an ice-cold reaction mixture were added dropwise freshly distilled anhydrous TEA (7.49 mL, 53.7 mmol) and fumaryl chloride (2.32 mL, 21.5 mmol). After 5 h of stirring at 4°C, the precipitated TEA hydrochloride was removed by filtration, and the filtrate was concentrated. The resulting copolymer was precipitated in 200 mL of cold diethyl ether, and the product was collected by filtration and dried in vacuum at room temperature for 24 h. For further purification, the copolymer was dissolved in deionized water and extracted into methylene chloride. After the organic phase was dried over $MgSO_4$, it was filtered, concentrated, and added to 100 mL of cold diethyl ether to precipitate the product. The copolymer was dried *in vacuo* at room temperature for 24 h.

Derivatization of Poly(ether amide)s with Thiols

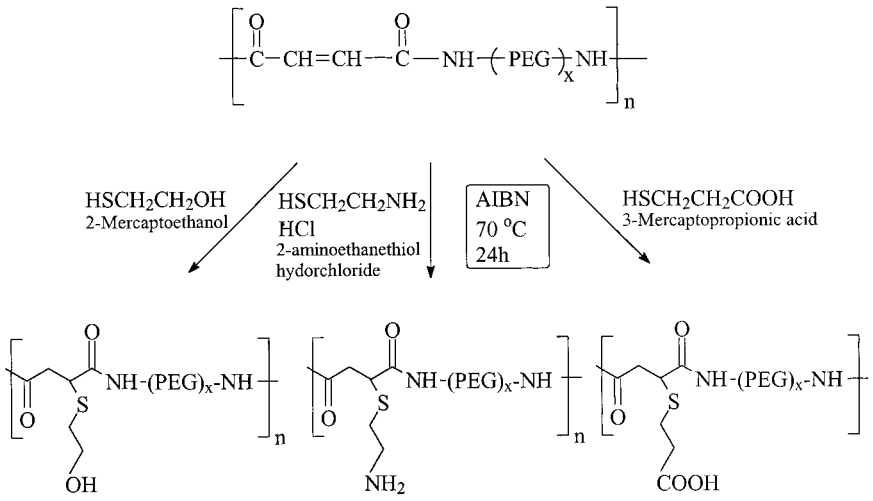
A typical derivatization of PEAs with thiols proceeded as follows (Scheme 2). To a solution of PEA_{2000} (2 g, 0.96 mmol) and AIBN (0.13 g, 0.8 mmol) in 20 mL of dry DMF was added a 10-fold excess of 2-mercaptoethanol (0.68 mL, 9.6 mmol). The reaction mixture was stirred at 70°C for 24 h. The solution was poured into 200 mL of cold diethyl ether to isolate the product. All polymers were purified by reprecipitation using methylene chloride as solvent and diethyl ether as nonsolvent, filtered, and dried *in vacuo* overnight.

Preparation of Photocrosslinked Hydrogel

In a 20 mL vial, the PEA (1 g) and *N*-vinyl pyrrolidone (NVP) (50 mol %) were dissolved in 10 mL of dioxane. Hydroxycyclohexyl phenyl ketone (Irgacure 184) (1 wt %) was added as the photoinitiator to initiate the UV-induced crosslinking reaction. The solution was flushed with nitrogen and poured into a Teflon mold. A 100-W lamp (Black-Ray, model 100A) was positioned above the mold at a height of 18 cm. UV irradiation was continued for 30 min. After irradiation, the gel was dried in a vacuum oven for 48 h to remove solvent.

Swelling Measurements

Dried gels were weighed and placed in buffer solutions of different pHs, pH 2, 7.4, and 10. The



Scheme 2 Derivatization of PEAs with thiols.

hydrogel was removed at predetermined intervals, blotted with tissue paper to remove surface water, and weighed until no further weight change was detected. The swelling ratio (SR) and equilibrium water content (EWC) of hydrogel were calculated from the following equations.

$$\mathrm{SR}\% = \frac{W_s - W_d}{W_d} \times 100$$

$$\mathrm{EWC}\% = \frac{W_s - W_d}{W_s} \times 100$$

where W_s is the weight of a swollen hydrogel and W_d is the weight of a dried hydrogel.

Characterization

Fourier transform infrared (FTIR) spectra were obtained on a Mattson Genesis Series FTIR spectrophotometer with a resolution of 4 cm^{-1} in the region of 4000–500 cm^{-1}. The FTIR spectra of all samples were taken as a cast on KBr discs from tetrahydrofuran (THF) solution. Size exclusion chromatography (SEC) was carried out on a Waters 150-CV apparatus equipped with a differential refractometer and two Phenogel (Phenomenex) columns. The eluent was THF with a flow rate of 1.0 mL/min at 25°C. Calibration with polystyrene standards was used. ^{1}H nuclear magnetic resonance (^{1}H-NMR) (NMR) spectra were recorded with a Bruker 300 MHz spectrometer. ^{13}C-NMR spectra were obtained with a Varian VXR-400 spectrometer. All of the chemical shifts are reported in parts per million (ppm) using tetramethylsilane as an internal standard for both ^{1}H- and ^{13}C-NMR spectra. The sample tube size was 5 mm with a sample concentration of 10 mg/mL in D_2O or DMSO-d_6.

RESULTS AND DISCUSSION

Synthesis of Unsaturated Poly(ether amide)s

Unsaturated, alternating (PEAs) of fumaryl chloride and Jeffamine® [*O,O′*-bis(2-aminopropyl)polyethylene glycol] were prepared by low-temperature solution polycondensation, as shown in Scheme 1. Polycondensation was carried out in the presence of triethylamine as an acid acceptor and in methylene chloride solution at 4°C for 5 h. The resulting copolymers provided multiple carbon–carbon double bonds at strictly controlled, predetermined inter-

Table I Molecular Weights from Size Exclusion Chromatography of Polymers from Low-Temperature Polycondensation of Fumaryl Chloride and Jeffamine®

	SEC			
Polymer[a]	$M_n \cdot 10^{-3}$	$M_w \cdot 10^{-3}$	M_w/M_n	Yield (%)
PEA_{230}	1.2	2.1	1.67	88
PEA_{600}	2.5	5.7	2.28	91
PEA_{2000}	8.1	20.8	2.57	87

[a] Subscripts denote the molecular weight of Jeffamine® used in the synthesis of poly(ether amide)s.

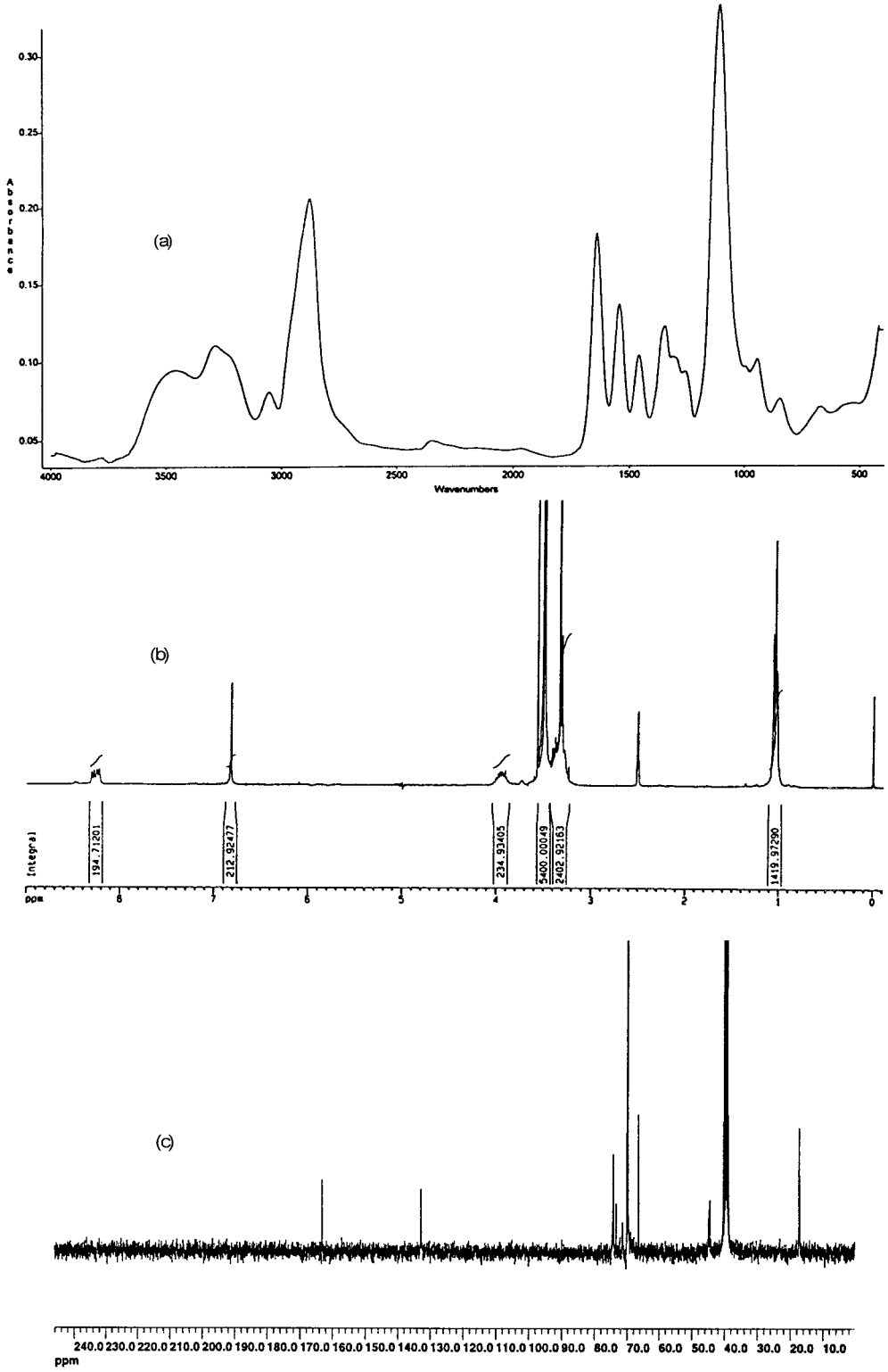


Figure 1 FTIR (a), ^{1}H-NMR (b), and ^{13}C-NMR (c) spectra of PEA_{600}.

vals along the backbone. These double bonds facilitated further chemical derivatization with thiols to provide different types of pendant functional groups and the design of crosslinking reactions for the preparation of hydrogels. Molecular weights from SEC for the copolymers are summarized in Table I.

Table II NMR Spectra, Yields, and Incorporation Ratios for Polymers Prepared from Radical Addition of Thiols to PEA_{3400}.

Thiols	^{1}H-NMR	Yield (%)	Incorporation Ratio (mol %)[a]
2-Mercaptoethanol	—CO—*CH*— (m, 1 H, δ = 4.04); —CO—CH—*CH_2*— (m, 2 H, 2.68–2.75); —S—*CH_2*—CH_2— (m, 2 H, 2.60–2.62); —S—CH_2—*CH_2*— (m, 2 H, 2.77–2.79)	81	42
3-Mercaptopropionic acid	—CO—*CH*— (m, 1 H, δ = 4.12); —CO—CH—*CH_2*— (m, 2 H, 2.72–2.73); —S—*CH_2*—CH_2— (m, 2 H, 2.69–2.71); —S—CH_2—*CH_2*— (m, 2 H, 2.78–2.88)	78	75
2-Aminoethanethiol hydrochloride	—CO—*CH*— (m, 1 H, δ = 3.81); —CO—CH—*CH_2*— (m, 2 H, 2.67–2.69); —S—*CH_2*—CH_2— (m, 2 H, 2.80–2.82); —S—CH_2—*CH_2*— (m, 2 H, 2.93–2.95)	79	92

[a] Measured by ^{1}H-NMR.

SEC analyses showed that the molecular weight distribution of PEAs was the range of 1.67 to 2.57. The molecular weight of the copolymers tended to increase with the increasing chain length of Jeffamine® unit.

The copolymer prepared from PEA_{600} was characterized by FTIR and ^{1}H- and ^{13}C-NMR spectroscopy. The FTIR spectrum of the copolymer is shown in Figure 1(a). The copolymer exhibited the characteristic absorption peaks at 3451 (—NH— stretching), 3053 (—C—H— alkenes), 1641 (amide I band), 1545 (amide II band), and 1105 cm^{-1} (—C—O—C— stretching of polyether chain). The ^{1}H-NMR spectrum [Fig. 1(b)] displayed signals at δ = 6.81 and 6.83, which correspond to the pendant double bond of fumaryl chloride residue of the copolymer. There is also a peak at δ = 3.51 due to the methylene protons in the main backbone chain of the PEG residue and peaks at δ = 8.22–8.30, which are assigned to the amide proton (—CO—NH—). The doublet at 1.05 ppm is due to the methyl groups of the propylene oxide units.

The ^{13}C-NMR spectrum [Fig. 1(c)] displayed the characteristic peaks at δ = 163.09 corresponding to the carbonyl carbon atom of the amide groups. The peaks at δ = 132.68–132.72 are attributed to the carbon atoms of C═C bonds. The signals between δ = 70.00 and 70.28 are due to the methylene carbons of PEG component. This is in agreement with the molecular structure of the copolymer.

Solubility Properties

The PEAs are soluble at room temperature in water as well as THF, methylene chloride, and chloroform but are insoluble in diethyl ether and hexane. Thus, the copolymer retained the favorable solubility characteristics of the parent PEG homopolymer.

Derivatization of Poly(ether amide)s (PEAs) with Thiols

The PEA copolymers have double bonds along the polymer backbone available for radical addition of various thiols to provide a variety of different pendant functional groups (Scheme 2), which could be used for covalently attaching of drugs or biologically active agents. Radical addition of thiols to the double bonds of PEA_{2000} was carried out at 70°C for 24 h using AIBN as a radical initiator in DMF. Addition of thiols to the PEA_{2000} in the absence of initiator was not successful. Crude copolymers were precipitated three times from methylene chloride–diethyl ether to remove excess of reagents. After incorporation of 2-aminoethanethiol hydrochloride to the PEA_{2000}, free primary amine groups were generated by addition of potassium hydroxide solution. The incorporation ratios of thiols were evaluated to be 42–92%, as determined from the integration ratios of the signals from the methylene protons next to sulfur relative to the methylene protons of PEG in the ^{1}H-NMR spec-

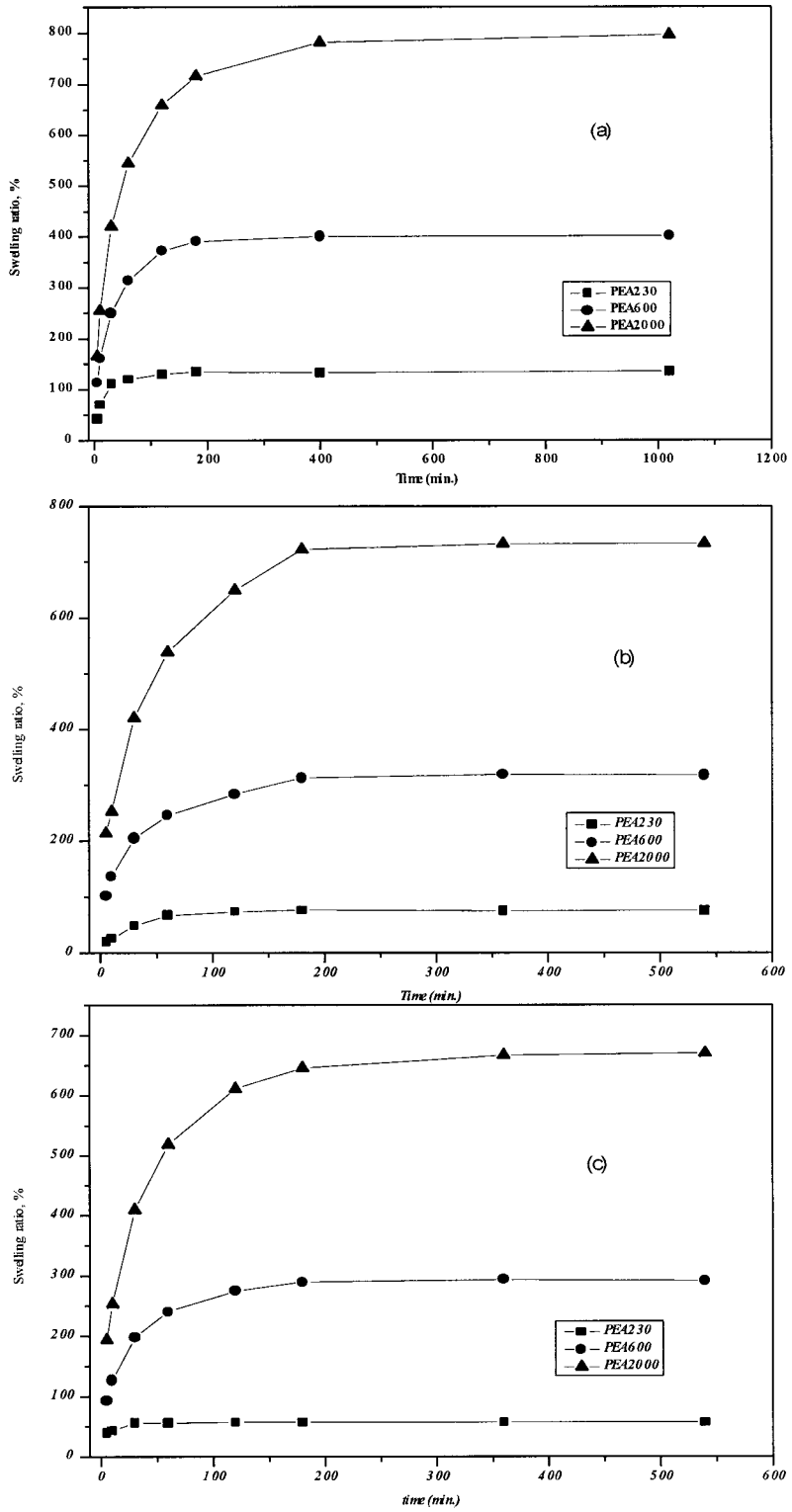


Figure 2 Swelling ratios of PEA–NVP hydrogels at pH (a) 2.0, (b) 7.4, and (c) 10.

tra (not shown). The ^{1}H-NMR spectrum of 2-aminoethanethiol-PEA_{2000} shows almost no residual double-bond peaks, while the products from addition of 3-mercaptopropionic acid and 2-mercaptoethanol do show small double-bond absorptions (Table II).

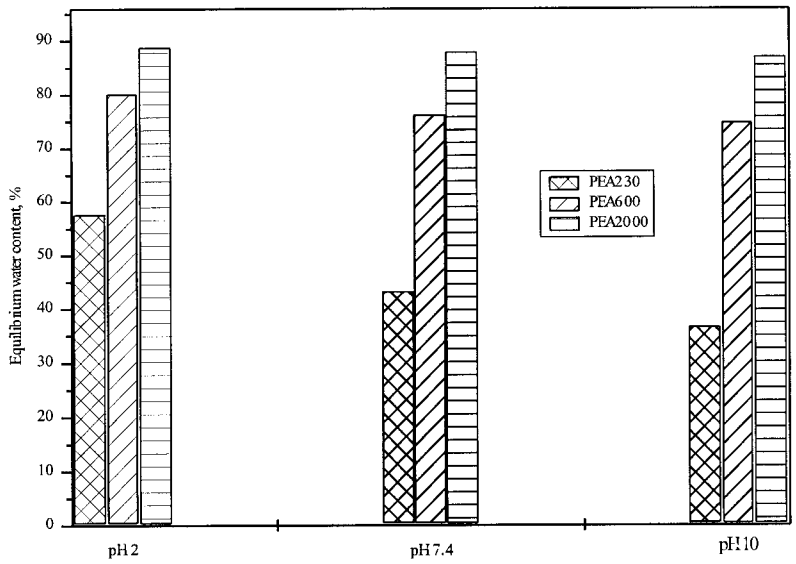


Figure 3 Equilibrium water contents of PEA–NVP hydrogels at pH 2.0, 7.4, and 10.

Preparation of Photocrosslinked Hydrogel

Another attractive feature of the linear, unsaturated PEAs is the ability to copolymerize the double bonds with other vinyl monomers and, thus, to form crosslinked networks that exist in aqueous medium as hydrogels. As an example of this property, we have formed hydrogels by copolymerizing the PEAs with *N*-vinyl pyrrolidone (NVP). In a typical experiment, the PEA and NVP were dissolved in dioxane and exposed to UV radiation in the presence of hydroxycyclohexyl phenyl ketone as a photoinitiator. Gelation occurred within 10 min, and a prolonged (30 min) UV irradiation was done to ensure complete reaction.

Swelling Measurements

The swelling ratios (SR) and equilibrium water contents (EWC) of the PEA–NVP hydrogels are shown in Figures 2 and 3, respectively. As can be seen, swelling appeared to be complete within about 3 h. Also, the networks prepared from the PEA with the longer poly(ethylene glycol) segments showed higher swelling ratios and equilibrium water contents. As would be expected for these essentially neutral polymer networks, the swelling ratios and equilibrium water contents were essentially insensitive to pH (Fig. 2 and 3).

CONCLUSIONS

New unsaturated poly(ether amide)s were synthesized by the low-temperature polycondensation of fumaryl chloride and amine-terminated poly(ethylene glycol) (Jeffamine®). The resulting copolymers contained a reactive double bond on the backbone chain of fumaric acid residue of the copolymer. The radical additions of thiols to the copolymers proceeded in good yields and incorporation ratios. The pendant functional groups of these derivatized copolymers could serve as the sites for immobilizing drugs or biologically active compounds. Also, the copolymers were chemically crosslinked with *N*-vinyl pyrrolidone using UV photocrosslinking method. The resulting hydrogels showed a high swelling ratio and the magnitude of swelling depended on the molecular weight of Jeffamine®.

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