Journal of Macromolecular Science, Part A
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597274

Novel Temperature- and pH-Responsive Linear Polymers and Crosslinked Hydrogels Comprised of Acidic L-α-Amino Acid Derivatives

Kaori Kurata[a]; Akira Dobashi[a]
[a] School of Pharmacy, Tokyo University of Pharmacy and Life Science, Tokyo, Japan

Online publication date: 28 January 2004

To cite this Article Kurata, Kaori and Dobashi, Akira(2004) 'Novel Temperature- and pH-Responsive Linear Polymers and Crosslinked Hydrogels Comprised of Acidic L-α-Amino Acid Derivatives', Journal of Macromolecular Science, Part A, 41: 2, 143 — 164
To link to this Article: DOI: 10.1081/MA-120027300
URL: http://dx.doi.org/10.1081/MA-120027300

JOURNAL OF MACROMOLECULAR SCIENCE®
Part A—Pure and Applied Chemistry
Vol. A41, No. 2, pp. 143–164, 2004

Novel Temperature- and pH-Responsive Linear Polymers and Crosslinked Hydrogels Comprised of Acidic L-α-Amino Acid Derivatives

Kaori Kurata* and Akira Dobashi

School of Pharmacy, Tokyo University of Pharmacy and Life Science,
Horinouchi, Hachioji, Tokyo, Japan

ABSTRACT

This paper presents the preparation and characterization of novel temperature- and pH-responsive linear polymers and hydrogels produced from *N*-acryloyl-*N*′-alkylamide derivatives of both L-glutamic acid and L-aspartic acid, which have the potential to act as intelligent drug carriers. The hydrophilicity–hydrophobicity balance of the linear polymers was controlled through the appropriate selection of *N*-alkyl groups at C-terminal amide sites to induce reversible transformation between water-soluble and insoluble state with pH or temperature changes. The lower critical solution temperature (LCST) required for this transformation increased with increased pH in the solution of linear polymers. This was due to the dissociation of residual carboxyl groups, which makes the chains hydrophilic and enhances the electrostatic repulsion between their anionic residues. Low crosslinked hydrogels comprised of *N*-acryloyl-L-glutamic acid *N*′-propylamide showed reversible swelling and shrinking with temperature changes at a particular pH. These hydrogels experienced greater swelling due to local dissolution of the interior polymer chains at temperatures lower than the LCST of their corresponding linear polymers. The hydrophobic surface was also formed to restrict to

*Correspondence: Kaori Kurata, School of Pharmacy, Tokyo University of Pharmacy and Life Science, 1432-1, Horinouchi, Hachioji, Tokyo 192-0392, Japan; E-mail: kurata@ps.toyaku.ac.jp.

DOI: 10.1081/MA-120027300
1060-1325 (Print); 1520-5738 (Online)

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

release absorbed water into the polymers toward their outer phase when shrinking at temperatures higher than the corresponding LCST.

Key Words: L-Glutamic acid; Temperature responsive polymer; pH responsive polymer; Water soluble-insoluble transformation; Lower critical solution temperature; Volume phase transition; Telomerization.

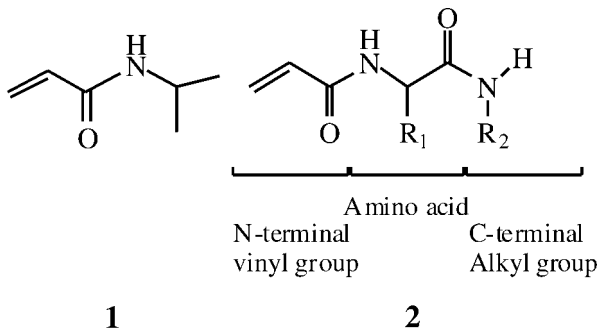
INTRODUCTION

Considerable research has been directed toward the development of intelligent materials that respond to chemical and physical stimuli. Polymers with this ability have great potential for use in drug delivery systems (DDS),[1–3] cell cultures,[4,5] and separative analysis.[6–9] Temperature is one of the most important physical stimuli and pH is one of the most important chemical stimuli.

Poly(*N*-isopropylacrylamide) (PNIPAAm) undergoes a structural change in response to temperature fluctuations in aqueous solution. Poly(*N*-isopropylacrylamide) exists as an aggregated structure that precipitates at temperatures exceeding 32°C, or as an extended structure that dissolves at temperatures under 32°C in water.[10] These features are due to changes in the hydrogen-bonding between its amide sites and water molecules and are also due to the hydrophobic nature of its *N*-alkyl substituents in bulk water.

The temperature at which the solubility transition begins is defined as the lower critical solution temperature (LCST). Lower critical solution temperature depends on the hydrogen bonding nature of the residues linked to the hydrocarbon backbones of polymers. Control of the LCST has been attempted through the co-polymerization of NIPAAm with various hydrophilic monomers. A co-polymer derived from NIPAAm and acrylamide was shown to be more hydrophilic than NIPAAm alone. Consequently, LCST of the co-polymer[11] was greater than that of PNIPAAm.

Amino acid diamide polymers prepared from *N*-acryloyl-*N*′-alkylamide derivatives of either L-alanine or L-valine were found to be effective in controlling the LCST without co-polymerization, as noted in our previous study.[12] Structure **1** shows the structures of NIPAAm (**1**) and *N*-acryloyl-L-amino acid *N*′-alkylamide monomers (**2**).



Structure 1. *N*-isopropylacrylamide (**1**) and *N*-acryloyl-L-amino acid-*N*′-alkylamide (**2**).

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

The latter monomers possess two amide sites. This is where the intermolecular hydrogen-bond network was initially formed along the linear polymer backbone when dehydration was brought about by an increase in temperature. The hydrophilic–hydrophobic balance of the polymers was then controlled through the selection of the appropriate amino acids, such as alanine and valine, and *N*-alkyl groups such as methyl and dimethyl group, so as to ensure a transition in state between water solubility and insolubility upon temperature change.

This study was conducted to investigate how temperature-sensitivity changes by pH. Two different acidic amino acids, L-aspartic and L-glutamic acid, were used as precursors to prepare *N*-acryloyl-L-amino acid *N*′-alkylamide monomers. These derivatives underwent telomerization[13,14] to produce polymers having a narrower distribution of molecular weights. Telomerization is carried out with 3-mercaptpropionic acid as thiol-telogen to obtain carboxyl-terminated linear polymer. Modification of the alkyl substituents of the C-terminal amide, and the amino acid selection, were examined in order to obtain the desired hydrophilicity–hydrophobicity balance within the linear polymers. The introduction of carboxyl groups to the amino acid side chains of diamide moieties, can make polymers sensitive to pH. Terminal carboxyl groups in the residues would undergo dissociation in the buffer solution at a certain pH and the LCST would also change as a result. This was confirmed by measuring transmittance during a shift in temperature.

Polymers crosslinked with a small amount of methylene-*bis*-acrylamide (MBAAm) were expected to swell reversibly in accordance with changes in pH and temperature due to local solubility transitions within the polymer chains. We also expected to observe this with the linear polymers comprised of acidic L-amino acid diamides. The reversible transition between swelling and shrinking was assessed for each of the prepared hydrogels on the basis of the weight of water absorbed. We assessed whether the hydrogels would have sufficient capacity to retain pharmaceuticals within their three dimensional network, as compared to their corresponding linear polymers. This capacity would lend them the ability to achieve controlled-release.

EXPERIMENTAL

NMR was conducted using a Varian Gemini-300 (300 MHz NMR). The internal standard for ^{1}H-NMR was chloroform (7.26 ppm) in deuteriochloroform or tetramethylsilane, and that for ^{13}C-NMR was deuteriochloroform (77.01 ppm).

Molecular weight (M_w) and its distribution were made by means of gel permeation chromatographic (GPC) analysis. They were estimated on polystylene resin column [TOSOH TSK-GEL GMHHR-M; 0.78 (i.d.) × 30 cm], with the refluctive index detector using 0.5 mL/min dimethylformamide (DMF) as mobile phase at 40°C. Average M_w was computed from the calibration curve obtained by using 12 TSK standard polystylenes (TOSOH; Mw ranged from 5.0×10^2 to 1.06×10^6).

IR measurement was made using JASCO FT/IR-410 with KBr tablets. Removal of the *tert*-butyl protecting group of *ω*-carboxylic acid from the polymers was confirmed by IR measurement.

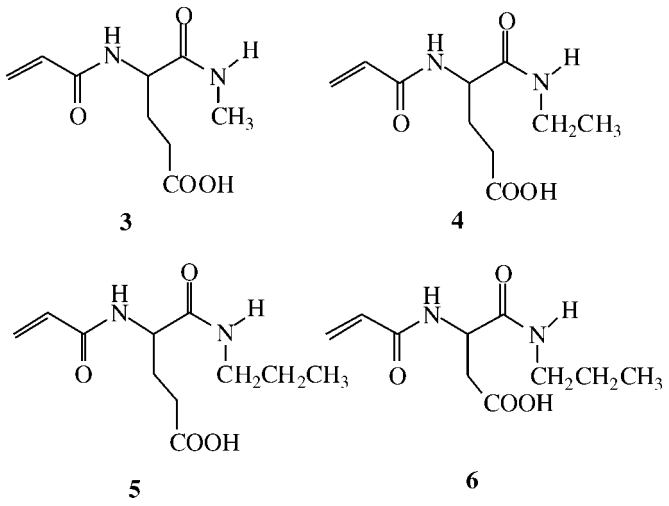
MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Preparation of *N*-Acryloyl-L-amino-ω-*tert*-butyl Ester *N′*-Alkylamide Derivatives

Vinyl group-terminated acidic L-amino acid diamide monomers (**3**, **4**, **5**, and **6** in Struc. **2**) were synthesized according to a previously reported procedure[12] (Fig. 1). The *N*-hydroxysuccinimide esters of both *N*-benzyloxycarbonyl(Z)-L-aspartic acid *ω-tert*-butyl ester and Z-L-glutamic acid *ω-tert*-butyl ester were reacted with methylamine, ethylamine, and propylamine hydrochlorides, under the presence of triethylamine. After removal of the Z group by hydrogenation with palladium–carbon from the *N′*-methylamide, ethylamide, and propylamide derivatives, *N*-acryloylation with acryloyl chloride was carried out in order to obtain the corresponding *N*-acryloyl derivatives. The following four monomers were obtained:

N-Acryloyl-L-glutamic acid-γ-*tert*-butyl ester *N′*-methylamide (**3**): mp 158.0–158.5°C (recrystallized from ethyl acetate–hexane); ^{1}H-NMR ($CDCl_3$)δ 1.45 (*s*, 9H), 1.98–2.58 (*m*, 4H), 2.82 (*d*, 2H, $J = 4.9$ Hz), 4.46 (*d.t*, 1H, $J = 5.7$, 7.5 Hz), 5.68 (*d.d*, 1H, $J = 1.5$, 10.2 Hz), 6.12 (*d.d*, 1H, $J = 10.2$, 17.0 Hz), 6.30 (*d.d*, 1H, $J = 1.5$, 17.0 Hz), 6.52–6.54 (br *s*, 1H), 6.75–6.78 (br *s*, 1H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 26.28, 27.56, 28.04, 31.87, 52.75, 81.14, 127.14, 130.38, 165.61, 171.62, 173.31; Anal. Calcd. for $C_{13}H_{22}N_2O_4$: C, 57.76; H, 8.20; N, 10.36. Found: C, 57.88; H, 8.20; N, 10.65; $[\alpha]_{21}^{D} = -22.50$ ($c = 1.00$, MeOH).

N-Acryloyl-L-glutamic acid-γ-*tert*-butyl ester *N′*-ethylamide (**4**): mp 153–155°C (recrystallized from ethyl acetate–hexane); ^{1}H-NMR ($CDCl_3$) δ 1.14 (*t*, 3H, $J = 7.3$ Hz), 1.44 (*s*, 9H), 1.92–2.54 (*m*, 4H), 3.29 (*q*, 2H, $J = 6.8$ Hz), 4.43–4.50 (*m*, 1H), 5.67 (*d.d*, 1H, $J = 1.6$, 10.2 Hz), 6.13 (*d.d*, 1H, $J = 10.1$, 17.0 Hz), 6.29 (*d.d*, 1H, $J = 1.6$, 17.0 Hz), 6.50–6.52 (br *s*, 1H), 6.74–6.77 (br *s*, 1H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 14.65, 27.83, 28.03, 31.81, 34.43, 52.64, 81.02, 127.00, 130.45, 165.52, 170.84, 173.12; Anal.



Structure 2. *N*-acryloyl-L-glutamic acid-*N′*-alkylamide (**3, 4,** and **5**) and *N*-acryloyl-L-aspartic acid-*N′*-alkylamide (**6**).

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

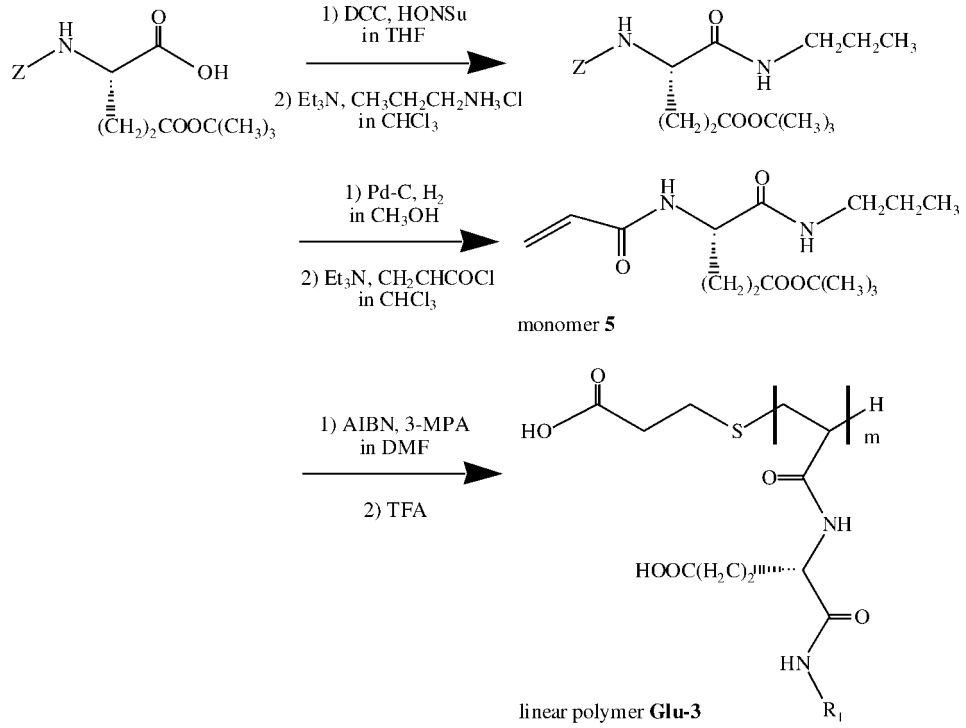


Figure 1. Preparation of pH/temperature-responsive polymer.

Calcd. for $C_{14}H_{24}N_2O_4$: C, 59.13; H, 8.51; N, 9.85. Found: C, 58.87; H, 8.49; N, 9.93; $[\alpha]_{21}^{D} = -24.00$ ($c = 1.00$, MeOH).

N-Acryloyl-L-glutamic acid-γ-*tert*-butyl ester *N*′-propylamide (**5**): mp 152–153.5°C (recrystallized from ethyl acetate–hexane); ^{1}H-NMR ($CDCl_3$) δ 0.92 (*t*, 3H, $J = 7.4$ Hz), 1.45 (*s*, 9H), 1.53 (*s*, 2H, $J = 7.3$ Hz), 1.93–2.58 (*m*, 4H), 5.12 (*s*, 2H), 3.22 (*q*, 2H, $J = 6.7$ Hz), 4.46 (*d.t*, 1H, $J = 5.6$, 7.3 Hz), 5.67 (*d.d*, 1H, $J = 1.2$, 10.2 Hz), 6.12 (*d.d*, 1H, $J = 10.1$, 10.7 Hz), 6.30 (*d.d*, 1H, $J = 1.0$, 17.0 Hz), 6.52–6.53 (br *s*, 1H), 6.71–6.74 (br *s*, 1 h); ^{13}C-NMR (75 MHz, CD_3OD) δ 11.31, 22.68, 27.88, 28.04, 31.87, 41.27, 52.71, 81.4, 126.93, 130.50, 165.54, 170.99, 173.17; Anal. Calcd. for $C_{15}H_{26}N_2O_4$: C, 60.38; H, 8.78; N, 9.39. Found: C, 60.39; H, 8.70; N, 9.45; $[\alpha]_{21}^{D} = -30.40$ ($c = 1.00$, MeOH).

For L-aspartic acid only the following *N*-propylamide derivative was made: *N*-acryloyl-L-aspartic acid-γ-*tert*-butyl ester *N*′-propylamide (**6**): mp 111–111.5°C (recrystallized from acetone–hexane); ^{1}H-NMR ($CDCl_3$) δ 0.89 (*t*, 3H, $J = 7.4$ Hz), 1.46 (*s*, 9H), 1. 50 (*s*, 2H, $J = 7.3$ Hz), 2.55 (*d.d*, 1H, $J = 7.4$, 17.0 Hz), 2.91 (*d.d*, 1H, $J = 4.0$, 17.0 Hz), 3.11–3.29 (*m*, 2H), 4.79 (*d.t*, 1H, $J = 4.0$, 7.6 Hz), 5.70 (*d.d*, 1H, $J = 1.4$, 10.2 Hz), 6.14 (*d.d*, 1H), 7.02–7.05 (br *s*, 1H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 11.23, 22.63, 28.01, 36.90, 41.28, 49.30, 81.86, 127.36, 130.27, 165.24, 170.17, 171.71; Anal. Calcd. for $C_{14}H_{24}N_2O_4$: C, 59.13; H, 8.51; N, 9.85. Found: C, 59.32; H, 8.51; N, 9.89; $[\alpha]_{21}^{D} = -50.50$ ($c = 1.00$, MeOH).

Preparation of the Linear Polymers

Linear polymers Glu-1, Glu-2, Glu-3, and Asp-1, were obtained by telomerization of the monomer **3**, **4**, **5**, and **6**, initiated with 2,2′-azo-*bis*(isobutyronitrile) (AIBN) at 80°C. 1/60 molar of 3-MPA against monomer was used as telogen.

To a solution of monomer **5** (1.78 g) in 10 mL of DMF were added 8.7 μL 3-MPA and 8.25 mg AIBN. After degassing by argon bubbling for 20 min, the solution was heated at 80°C for 18 h under argon. The residue was purified by precipitation with methanol-diisopropyl ether solution to get 1.65 g of poly(*N*-acryloyl-L-glutamic acid-γ-*tert*-butyl ester *N*′-propylamide). The materials and molecular weights are summarized in Table 1.

The typical procedure used to cleave the protecting γ-*tert*-butyl ester group of the terminal carboxyl groups is as follows. A solution of 1.01 g of this polymer in 3 mL of trifluoroacetic acid (TFA) was stirred at room temperature for 2 h. The residues were evaporated to dryness under vacuum and the residual mass was dissolved in methanol and precipitated with diisopropyl ether to give 0.92 mg of white powder of poly(*N*-acryloyl-L-glutamic acid *N*′-propylamide) (Glu-3). The completion of deprotection was assessed based on IR and NMR spectra.

One co-polymer (Glu/NIPAAm) was prepared from monomer **5** and NIPAAm (**1**) in an equimolar ratio.

Average M_ws and molecular weight distribution (M_w/M_n) for the protected polymers and the unprotected polymers were determined by GPC analysis as follows:

Poly(*N*-acryloyl-L-glutamic acid-γ-*tert*-butyl ester *N*′-methylamide): M_w, 2.7×10^5; M_w/M_n, 24; IR (KBr) cm^{-1} 1732, 1645, 1558, 1255, 1156.

Poly(*N*-acryloyl-L-glutamic acid *N*′-methylamide) (Glu-1): M_w, 9.9×10^5; M_w/M_n, 2.9; IR (KBr) cm^{-1} 1719, 1647, 1559.

Poly(*N*-acryloyl-L-glutamic acid-γ-*tert*-butyl ester *N*′-ethylamide): M_w, 3.3×10^5; M_w/M_n, 24; IR (KBr) cm^{-1} 1729, 1648, 1542, 1255, 1156.

Poly(*N*-acryloyl-L-glutamic acid *N*′-ethylamide) (Glu-2): M_w, 1.5×10^6; M_w/M_n, 1.70; IR (KBr) cm^{-1} 1721, 1646, 1544.

Table 1. Raw materials and molecular weights and molecular distributions of acidic amino acid diamide polymers.

Polymer	Monomer	Monomer (g)	3-MPA (μL)	AIBN (mg)	Mw[a] (10^6)	(M_w/M_n)[a]
Glu-1	**3**	1.68	8.7	8.2	1.0	2.9
Glu-2	**4**	0.99	5.1	5.0	1.5	1.7
Glu-3	**5**	1.78	8.5	8.5	1.8	1.9
Asp-1	**6**	1.40	5.6	5.6	1.6	1.6
Glu/NIPAAm	**5**	0.99/0.39	9.8	9.8	1.6	2.5

[a]Average molecular weights (M_w) and polydispersity (M_w/M_n) of polymer determined by GPC, with TSK-GEL GMHHR-M and eluent DMF at 40°C.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Poly(*N*-acryloyl-L-glutamic acid-γ-*tert*-butyl ester *N*′-propylamide): M_w, 4.3×10^5; M_w/M_n, 32; IR (KBr) cm^{-1} 1731, 1649, 1544, 1255, 1156.

Poly(*N*-acryloyl-L-glutamic acid *N*′-propylamide) (Glu-3): M_w, 1.8×10^6; M_w/M_n, 1.9; IR (KBr) cm^{-1} 1718, 1647, 1545.

Poly(*N*-acryloyl-L-aspartic acid-β-*tert*-butyl ester *N*′-propylamide): M_w, 7.8×10^5; M_w/M_n, 23; IR (KBr) cm^{-1} 1727, 1654, 1543, 1254, 1157.

Poly(*N*-acryloyl-L-aspartic acid *N*′-propylamide) (Asp-1): M_w, 1.6×10^6; M_w/M_n, 1.6; IR (KBr) cm^{-1} 1719, 1654, 1543.

Poly(*N*-acryloyl-L-glutamic acid-γ-*tert*-butyl ester *N*′-propylamide-co-*N*-isopropylacrylamide): M_w, 2.8×10^5; M_w/M_n, 26; IR (KBr) cm^{-1} 1737, 1644, 1548, 1255, 1156.

Poly(*N*-acryloyl-L-glutamic acid *N*′-propylamide-co-*N*-isopropylacrylamide) (Glu/NIPAAm): M_w, 1.6×10^6; M_w/M_n, 2.5; IR (KBr) cm^{-1} 1718, 1647, 1544, 1173.

Lower Critical Solution Temperatures Measurement of Synthesized Linear Polymers

Lower critical solution temperatures for linear polymers were determined from the transmittance of a 1.0 w/v% polymer solution in 0.1 M ammonium acetate buffer at 500 nm using a spectrophotometer (JASCO, Ubest-50). A solution temperature was maintained with a Peltier thermostatically controlled cell holder (JASCO, ECT-505). The transmittance was measured 5 min later after a temperature change. The measurement was made at intervals of 2°C from 0°C to 60°C. The LCST was defined as the temperature at which transmittance started to decline with increasing temperature.

Preparation of the Crosslinked Hydrogels

The crosslinked hydrogels were synthesized from NIPAAm (**1**), monomer **5**, and an equimolar ratio of **1** and **5**. The monomer's total weight was kept at 1.0 g, and it was dissolved in 10 mL of 10 v/v% methanol–distilled water. Methylene-*bis*-acrylamide was added to the solution as the crosslinker, and ammonium persulfate (APS) as the initiator. Argon gas was bubbled into the solution for 20 min for degassing. After the addition of 40 μL of *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TMEDA) as an accelerator, the solution was injected between two glass plates separated by a silicon gasket (1.0 mm thick) and up righted. Degassed hexane of 0.3 mL was then laid on the solution. This was followed by polymerization at room temperature over a period of 9 h (Table 2). The polymerized hydrogel membrane was then immersed in methanol at room temperature and the methanol was changed every 24 h to remove the unreacted reagent. The membrane was soaked in a methanol–distilled water mixture (1 : 1 in volume) for 24 h, followed by water for another 24 h. The swollen gel membrane was cut into disks with a cork borer (20 mm diameter) and dried at ambient temperature for 24 h and then under vacuum for an additional 24 h at 50°C. The monomer **5**-containing gel disk was treated with 3 mL of TFA

Table 2. Raw materials and experimental conditions of crosslinked hydrogel preparation.

Hydrogels	Crosslinker (mol%)	Monomer **1** (mg)	Monomer **5** (mg)	MBAAm (mg)	APS (mg)
NIP	1	1001	0	13.5	4.23
	2	996	0	27.2	4.21
Glu/NIP	1	275	725	7.50	4.10
	2	275	725	15.0	3.98
Glu-3	1	0	1000	5.54	4.01
	2	0	1000	10.5	4.06

Note: TMEDA 40 μL, H_2O 0.5 mL, CH_3OH 4.5 mL, hexane 0.3 mL, temperature 23°C, and reaction time over 9 h.

to remove the protecting group in the same manner as was done for the linear polymer. The completion of deprotection was assessed based on the IR spectrum. The absorption bands observed for the 2 mol% crosslinked hydrogel were as follows:

Poly(*N*-isopropyl acrylamide) gel (NIP gel): IR (KBr) cm^{-1} 1637 (−CONH−), 1543 (−CONH−), 1388 (−$CH(CH_3)_2$), 1366 (−$CH(CH_3)_2$), 1173 (−$CH(CH_3)_2$).

Poly(*N*-acryloyl-L-glutamic acid *N*′-propylamide-co-*N*-isopropyl acrylamide) gel (Glu/NIP gel): IR (KBr) cm^{-1} 1719 (−COOH), 1638 (−CONH−), 1542 (−CONH−), 1387 (−$CH(CH_3)_2$), 1368 (−$CH(CH_3)_2$).

Poly(*N*-acryloyl-L-glutamic acid *N*′-propylamide) gel (Glu-3 gel): IR (KBr) cm^{-1} 1719 (−COOH), 1655 (−CONH−), 1647 (−CONH−), 1638 (−CONH−), 1561 (−CONH−), 1543 (−CONH−).

Measurement of Swelling Ratios by Weight

The swelling ratio of each hydrogel (W_{H_2O}/W_p) was defined as the weight of water that was penetrated and absorbed into the dried hydrogel network (W_{H_2O}) over the initial weight of the dried gel (W_p). W_{H_2O} was determined by subtracting the initial weight of the dried gel (W_p) from the weight of the swollen gel (W_s). This was performed as follows:

$$\frac{W_{H_2O}}{W_p} = \frac{W_s - W_p}{W_p} \tag{1}$$

where W_s is the weight of the hydrogel at equilibrium in 100 mL of 0.1 M ammonium acetate buffer (pH in the range of 4.0–4.9). The weight of the hydrogel swollen under a particular pH condition was measured gravimetrically after wiping off any excess water from the gel surface with a filter paper at 10°C less than, or 30°C in excess of, the LCST of each hydrogel.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Swelling Kinetics of the Hydrogels

The swelling kinetics of the hydrogels were analyzed by measuring the degree of swelling. This was defined as the relative swelling ratio, that is, weight of water absorbed over 24 h ($W_{H_2O}(24)$) vs. the weight of water ($W_{H_2O}(t)$) remaining after a specified interval of time (t h). The W_{H_2O} was measured in the same manner as the swelling ratio at 10°C.

RESULTS AND DISCUSSION

Preparation of the Linear Polymers Containing Acidic L-Amino Acid Diamide Moieties

The linear polymers were produced from monomer **3**, **4**, **5**, and **6** by telomerization with 3-MPA as the chain transfer agent, and AIBN as the radical initiator. Methyl, ethyl, and *n*-propyl groups were used as *N*-alkyl substitutents on the C-terminal amide sites of the glutamic acid derivatives (**3**, **4**, and **5**), and *n*-propyl groups were used for the aspartic acid derivatives (**6**). The polymers were treated with TFA to remove the *tert*-butyl protecting groups of the *ω*-carboxylic acids placed on the residues. After this treatment, the absorption bands to the ester carbonyl groups, 1729–1737 cm^{-1} were eliminated and that of the carboxyl groups, 1718–1721 cm^{-1} appeared. The average M_ws of the deprotected polymers measured with GPC exceeded expectations based on the molar ratio of the vinyl group-terminated monomer and 3-MPA. The polydispersity (M_w/M_n) was sufficiently low to indicate that monomer elongation was controlled by telomerization.

N-Acryloyl-L-glutamic acid *N′*-*n*-propylamide *γ*-*tert*-butyl ester (**5**), which had the longest *N*-alkyl substituent, was also co-polymerized with NIPAAm (**1**) using an equimolar mixture for telomerization. The same procedure was used as outlined above for the preparation of the four homopolymers. The peak area of the two methyl groups in the isopropyl residue (1.1 ppm) and the terminal methyl groups in the *N*-*n*-propyl residue (0.9 ppm) were 1.8 and 1.1. The actual molar ratio of **5** against **1** was, then, calculated to be 0.84.

Temperature Response as a Function of pH in the Linear Polymers

Figure 2 shows the temperature dependence of the transmittance of Glu-3 in 0.1 M ammonium acetate buffer solution (pH 4.6). The polymer concentration was kept at 1.0 w/v% for all transmittance experiments. Transmittance for this solution started gradually decreasing at 18°C when the temperature was raised from 0°C to 60°C, and it reached about zero at 30°C. In contrast, when the temperature was lowered from 60°C to 0°C, zero transmittance was observed before 12°C, at which point it increased sharply.

This hysteresis phenomenon observed for Glu-3 may arise from electrostatic interactions between the carboxyl residues within the network structure because hysteresis was not noted for the ordinary temperature-responsive polymers such as PNIPAAm. Dehydration of the amide site follows the sequential formation of intra-molecular hydrogen bonds between the residues, as well as the formation of inter-molecular hydrogen bonds between the polymers. Such polymers thus form three-dimensional

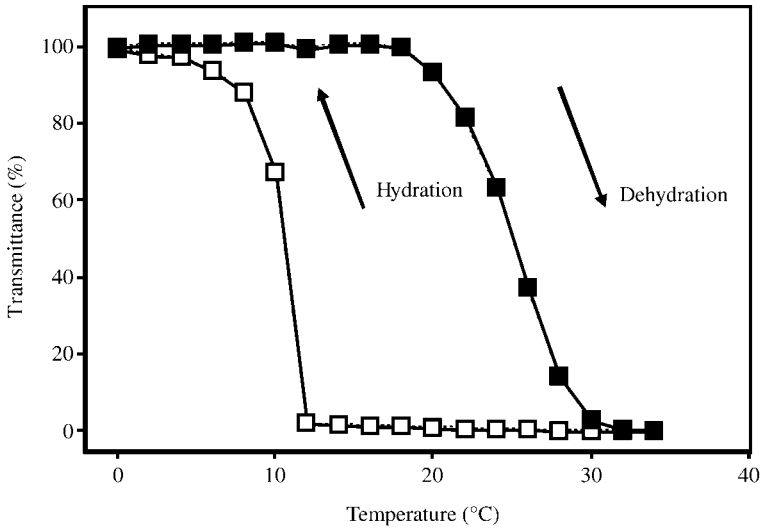


Figure 2. Temperature dependence of the transmittance of a 1.0 w/v% ammonium acetate buffer solution of Glu-3: temperature increase (■); LCST, 18°C, and decrease (□); LCST, 12°C.

networks caused by these hydrogen bonds. Glu-3 might form a stronger three-dimensional network during aggregation due to this additional interaction and therefore, the network might resist collapse. A rate-determining step of hydration at the polar residues would be different from that of dehydration. This hysteresis phenomenon was also noted in the Glu/NIPAAm and Asp-1 solutions. In this study, the temperature and pH dependence of the transmittance of all polymers is thus considered only a function of a rise in temperature.

Figure 3 shows the LCSTs for Glu-3, Asp-1, and Glu/NIPAAm in 0.1 M ammonium acetate buffer solutions prepared at various pH. The PNIPAAm was obtained the same LCST as that observed in the aqueous solution. Glu-3 gave temperature-sensitivity within a pH range of 4.3 to 4.75, when the temperature was raised from 0°C to 60°C, but not at pHs exceeding 4.9. The LCST was determined to be 2°C, 10°C, 18°C, and 30°C, at pH 4.30, 4.45, 4.60, and 4.75, respectively, thus showing that the LCST increases with pH. Both Glu-1, which has a *N*-methyl group, and Glu-2, which has a *N*-ethyl group, at their C-terminal amide sites, showed no solubility transition at temperatures ranging from 0°C to 60°C and pHs ranging from 1 to 9. Substitution of these groups with an *N*-*n*-propyl group gave a sufficient hydrophilicity–hydrophobicity balance to affect the LCST. This structural difference makes the hydrophobicity of Glu-1 and Glu-2 less than that of Glu-3, which is indicated by a disappearance of LCST at any given pH.

Asp-1 showed different pH range from Glu-3. The 10°C of LCST for Asp-1 was found at pH 3.70. The LCST of Asp-1 was increased up to 18°C and 24°C when the pH increased to 3.85 and 4.00, respectively. Asp-1 has a methylene unit less hydrophobic than an ethylene unit of Glu-3 in the alkylene spacer between an asymmetric carbon and a carboxylic acid group. This structural difference makes the hydrophobicity of Asp-1 less than that of Glu-3, which is indicated by its higher LCST at pH 4.3. The LCST of Asp-1 observed at pH 4.3 was 56°C and that of Glu-3 was 2°C.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

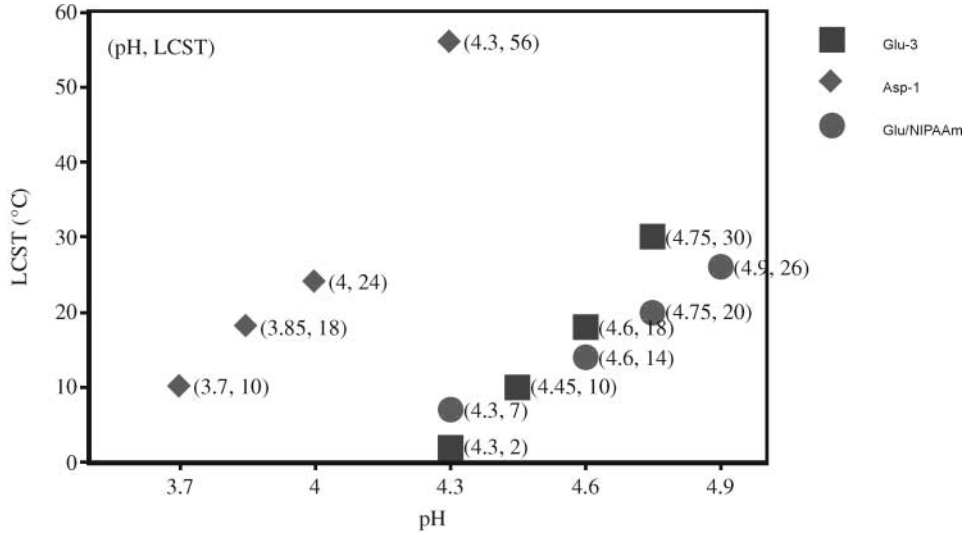


Figure 3. Lower critical solution temperatures of pH/temperature responsive polymers in 1.0 w/v% of a 0.1 M ammonium acetate buffer solution.

Glu/NIPAAm co-polymer had an LCST of 26°C, even at pH 4.9, under which Glu-3 showed no solubility transition in the temperature range examined.

Figure 4 showed the temperature dependence for transmittance of Glu-3 solution as a function of pH. The temperature range over which complete precipitation was noted ranged from 8°C at pH 4.30 and 20°C at pH 4.75. The temperature dependence of PNIPAAm transmittance in the buffer solution at pH 4.6 is also presented in Fig. 4 for

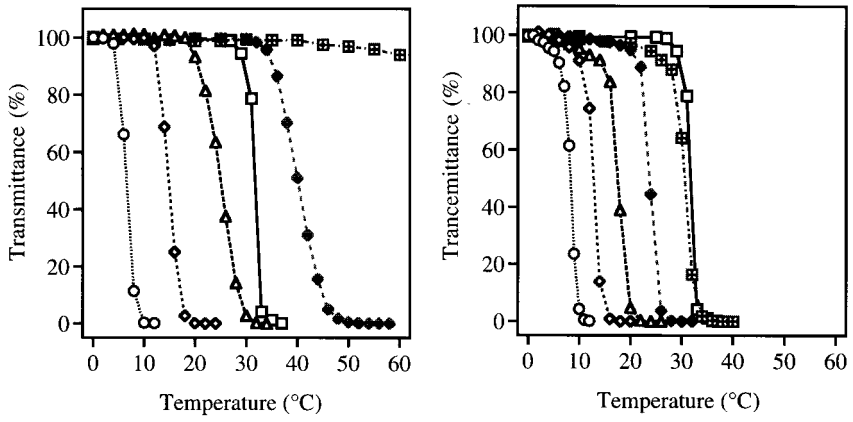


Figure 4. Temperature dependence of the transmittance of a 1.0 w/v% 0.1 M ammonium acetate buffer solution of Glu-3 (left), Glu/NIPAAm (right), and PNIPAAm. *Key:* ○, pH 4.30; ◇, pH 4.45; △, pH 4.60; ◆, pH 4.75; ⊞, pH 4.90; □, PNIPAAm.

comparison. The temperature range over which the complete precipitation of Glu-3 was examined had a slightly greater range than that of PNIPAAm (8°C) at the experimental pH conditions. As for Asp-1, the temperature range over which complete precipitation was noted was 6°C at all experimental pH conditions. This indicates that the solubility transition of Asp-1 occurs quickly more than that of Glu-3. The temperature range over which the complete precipitation of Glu/NIPAAm was observed, was narrower than that observed for Glu-3 under comparable pH conditions: 5°C for the co-polymer and 20°C for Glu-3 at pH 4.30 (Fig. 4).

The p*K*a values (20°C) of the γ-terminal carboxyl group of glutamic acid and β-terminal carboxyl group of aspartic acid were 4.07 and 3.91, respectively. The actual p*K*a values of the polymers may be slightly higher than their values because the polymers α-carboxyl groups were converted to C-terminal alkyl amides. Those p*K*a values, however, permit a rough calculation of the degree to which the terminal carboxyl group of the residue will dissociate under the pH conditions examined in this study. At least 63% of the carboxyl groups of Glu-3 were expected to exist in the dissociated state, even at pH 4.3, which is the lowest pH condition used in the transmittance studies. Temperature control of the state of a polymer may thus be controlled by electrostatic repulsion between dissociated carboxyl groups. Thus given that the polymer has sufficient hydrophobicity to precipitate completely in water upon dehydration of the residue as depicted in Fig. 5.

Co-polymerization of monomer **5** and NIPAAm (**1**) lessens electrostatic repulsion between the dissociated carboxyl groups of the glutamic acid *N*-propyl amide residues. This promotes aggregation of the co-polymer and consequently decreases the LCST. Glu-3 had the highest sensitivity to pH changes, that is, it experienced the greatest increase in its LCST for a given increase in pH.

The temperature dependence of transmittance of Glu/NIPAAm was biphasic. A slow decrease in transmittance was observed during the early aggregating stage, and a rapid decrease after the transmittance fell below 90%. This may be due to interactions between the *N*-isopropyl amide of NIPAAm and other residues, since this feature became apparent after the NIPAAm residues were introduced to Glu-3. An increase in pH made this biphasic pattern less distinct as shown in Fig. 4.

Preparation of Poly(*N*-Acryloyl-L-glutamic Acid *N*′-Propyl Amide) Crosslinked with MBAAm

The absorption bands assigned to the ester carbonyl groups, 1737 cm^{-1} for the protected Glu-3 gel, and 1734 cm^{-1} for the protected Glu/NIP gel, were eliminated upon TFA treatment. NIP gels crosslinked with either 1 or 2 mol% MBAAm were transparent and had sufficient mechanical strength to be used as membranes. However, the Glu-3 hydrogel with 1 mol% MBAAm was fragile and could not be handled. Crosslinking of 2 mol% provided stronger membranes which could be used to examine pH/temperature response, that is, the effects of temperature and pH on the state of the polymers. Co-polymerization of equimolar amounts of NIPAAm (**1**) and monomer **5** in the presence of either 1 or 2 mol% MBAAm yielded hydrogels that were stronger than the Glu-3 hydrogel. Of the two different concentrations of MBAAm used, the 2 mol% crosslinked hydrogel was not as transparent but had more mechanical strength than the 1 mol%

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

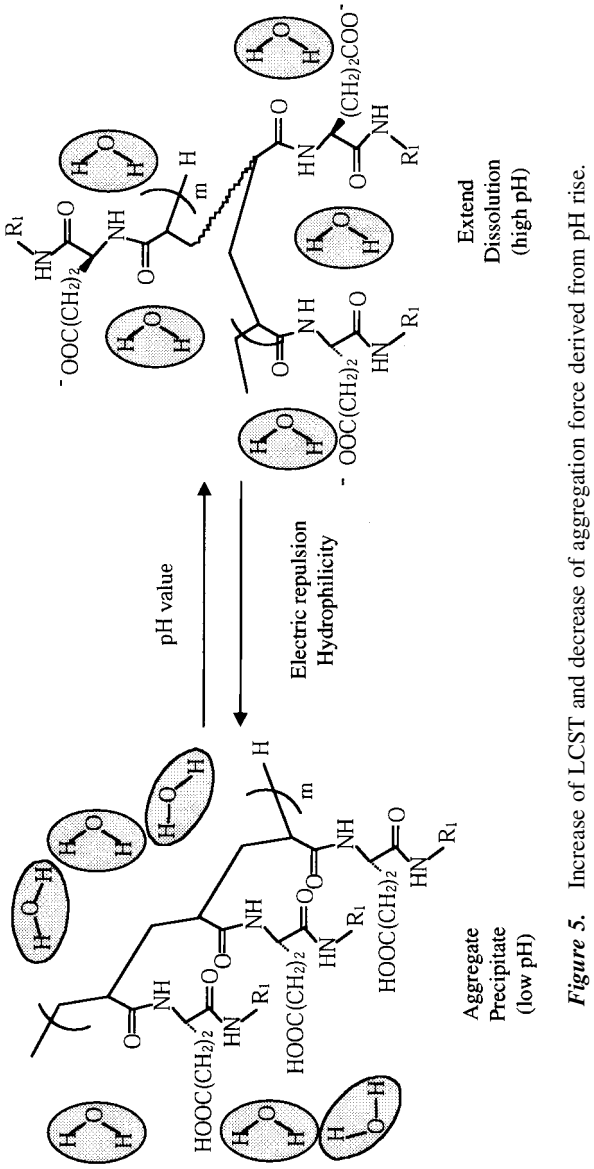


Figure 5. Increase of LCST and decrease of aggregation force derived from pH rise.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

crosslinked hydrogel. In this study, 2 mol% crosslinked hydrogels were thus used to assess pH/temperature response of the polymers.

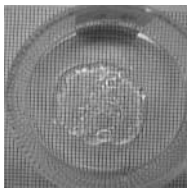
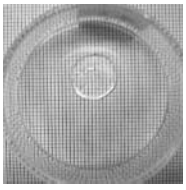
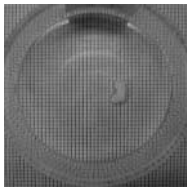
Glu-3 gels were more resistant to being covered with water than NIP gels, even after the bulky and hydrophobic *tert*-butyl groups were removed from the terminal carboxyl groups.

Temperature Response of Hydrogels as Determined by Swelling Ratio Measurements

Hydrogels prepared from monomer **5** and a mixture of monomer **5** and NIPAAm (**1**) were found to be insoluble and had pH/temperature sensitivity similar to that of the NIP gel which has been described as a temperature-responsive hydrogen.[15] This was indicated by the reversible volume transition that occurred between the shrunken and swollen states of each hydrogel. This volume transition was measured by determining the swelling ratio, defined as the weight of the absorbed water when the gel swollen vs. that of the dried gel.

Table 3 illustrates 2 mol% crosslinked NIP, Glu-3 and Glu/NIP hydrogels equilibrated in a 0.1 M ammonium acetate buffer (pH 4.6) at both 10°C and 30°C. The temperature at which the volume transition occurred in each hydrogel can be reasonably assumed to correspond to the LCST of their corresponding linear polymers (containing the same monomer as the hydrogels) because swelling of the hydrogel is due to a local dissolution of the interior polymer network. All hydrogels were thus assumed to be swollen at 10°C because the LCSTs of their corresponding linear polymers were 32°C, 18°C, and 14°C, for the NIP, Glu-3, and Glu/NIP gels, respectively. Furthermore, at 30°C, two kinds of monomer **5**-containing hydrogels was assumed to be shrunken, but not for NIP gel.

Table 3. Temperature sensitivity of swelling ratio of cross-linked hydrogels at pH 4.6.

Gel	NIP	Glu/NIP	Glu-3
Swelling ratio (10°C)	14.5 ± 0.56	22.0 ± 0.51	74.3 ± 3.22
			
Swelling ratio (30°C)	5.31 ± 0.47	2.26 ± 0.34	4.99 ± 1.44
			

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

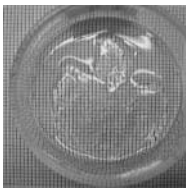
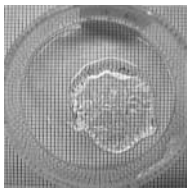
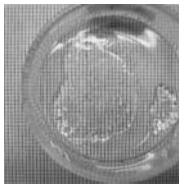
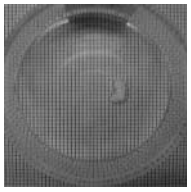
The amount of water that was absorbed into the gel interior from the bulk aqueous phase, was dependent on both temperature and pH in hydrogels containing monomer **5**. All three hydrogels swelled at 10°C, in comparison with the degree of swelling observed at 30°C, but the gels differed in their swelling ratios. Hydrogels containing larger amounts of monomer **5** showed more swelling. A swelling ratio of 15 has been reported for NIP gels crosslinked with 1 mol% ethylenedimethacrylate (EDMA) and NIP gels crosslinked with 2 mol% MBAAm.[16] Moreover, the introduction of a second monomer, hydrophilic acrylic acid, to these gels yields a swelling ratio of 30. Glu/NIP gel is similar to an acrylic acid and NIPAAm-containing co-polymer and was found to have a swelling ratio of 22 at pH 4.6. This indicates that the glutamic acid *N*-propylamide residue is a hydrophilic residue comparable to acrylic acid. The Glu-3 gel had the highest swelling ratio (greater than 70), which can be attributed to dissolution of its terminal carboxylic acid, followed by a high extent of water penetration.

To assess the pH response of the Glu-3 gel, its swelling ratio was measured at various pH: 4.0, 4.6, and 4.9 (Table 4). At pH 4.0, the Glu-3 gel remained in a shrunken state and its size and transparency did not change despite a change in temperature. Under the pH 4.0 conditions, the Glu-3 linear polymer was found to be insoluble at both 10°C and 30°C. This is because its LCST was only 2°C at pH 4.3. At pH 4.9, the hydrogel was markedly swollen at both 10°C and 30°C. However, it was more swollen at 10°C than at 30°C.

Reversible Transformation Between the Shrunken and Swollen States of the Glu/NIP and Glu-3 Gels

The NIP gel disk underwent little change in size and transparency after the temperature was raised from 10°C to 30°C, as can be seen in the pictures in Table 3.

Table 4. pH sensitivity of swelling ratio of Glu-3 gels.

pH	4.9	4.6	4.0
Swelling ratio (10°C)	121 ± 15.89	74.3 ± 3.22	1.97 ± 1.03
			
Swelling ratio (30°C)	95.7 ± 4.32	4.99 ± 1.44	3.08 ± 0.61
			

No apparent phase transition took place indicating that the interior polymer residues did not aggregate together to form a turbid membrane surface.

In contrast, both the Glu-3 and Glu/NIP gels, in which glutamic acid *N*-propyl amide residues were grafted instead of the NIPAAm isopropyl group, exhibited remarkable changes in size and transparency following an increase in temperature. Figure 6 shows pictures of the Glu/NIP and Glu-3 gels. These pictures demonstrate the time-course governing the reversible transformation between their shrunken and swollen states as the temperature was raised from 10°C to 30°C. The two gels were swollen at 10°C and both had a clear surface (photo 1). An increase in temperature caused the gels to shrink and their surface to blister (photo 2). At 30°C, the gels were compact and their surface was cloudy, however, the blisters could no longer be observed (photo 3). The blisters were brought about by the formation of a hydrophobic surface due to an aggregation of polymer chains on the gel surface, which prevented water from being released from the interior of the gels. Yoshida et al. found that these blisters frequently form when either butyl or pentyl esters of methacrylic acid are introduced into the NIP gel.[16] Monomer **5** has 5 carbons between its side-chain and C-terminal *N*-*n*-propyl group. This seemed to contribute to the hydrophobic environment created by aggregation. The Glu-3 gel re-swelled after the temperature was decreased to 10°C (photo 6). A swelling front,[17] which refers to observed differences in the transparency of a gel at different depths, was observed in the hydrogel over a 24 h period of swelling. The swelling front moved from the transparent outer surface of the gel to the cloudy interior as water penetrated the interior of the gel, thus hydrating the inner polymer chains (photo 5). A reversible change was also observed in the time course governing gel swelling ratios as the temperature was altered. This is illustrated in Fig. 7. The Glu-3 and Glu/NIP gels shrunk within 2 h due to dehydration of the polar grafted moieties on their backbone, as depicted in the second picture of Fig. 6. In contrast, it took longer for the gels to swell as a result of hydration. This is shown in the fourth and fifth pictures of Fig. 6. It took the Glu-3 gel at least 12 h to stabilize at a constant weight. It took the Glu/NIP gel under 8 h to swell completely. The slow rate of swelling of the Glu-3 gel corresponded to the slow rate at which the Glu-3 linear polymer changed from its compact insoluble state to its expanded soluble state as opposed to the reverse process which occurs more quickly.

Pulsatile Change in Swelling Ratios with Change in Temperature

For the Glu-3 gel, the pulsatile change observed during its volume transition with a variation in temperature was examined at pH 4.6. Dependence of the swelling ratio on temperature was found to be reversible. Unfortunately, this experiment could not be carried out at pH 4.9 because the gel was too fragile under this pH condition (Fig. 8).

Swelling Behavior of the Hydrogel Determined Relative Swelling Ratio

The swelling of each gel was consistent with the relaxation of its polymer macromolecular chain network and the simple diffusion of water molecules into the gel

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Figure 6. Transformation between the shrunken and swollen states of crosslinked hydrogels. (A) Glu/NIP gel; (B) Glu-3 gel.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

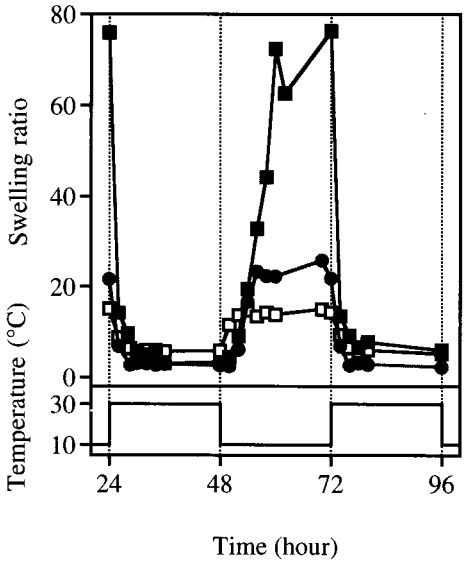


Figure 7. Time course of the swelling ratio with temperature change in 1.0 M ammonium acetate buffer (pH 4.6). *Key:* □, NIP gel; ●, Glu/NIP gel; ■, Glu-3 gel.

interior.[18–21] Penetration of a gel interior with water is described by the Fick's equation for simple diffusion:

$W_{H_2O}(t)/W_{H_2O}(\infty) = 4(Dt/\pi l^2)^{1/2}$ for $0 \leq W_{H_2O}(t)/W_{H_2O}(\infty) \leq 0.6$, where D is the diffusion coefficient for water, and l is the membrane thickness of the slab. The relative swelling ratio ($W_{H_2O}(t)/W_{H_2O}(\infty)$) is proportional to the square root of time as indicated by Fick's equation. Therefore, a linear relationship between swelling and the square root of time, is expected to be observed during the initial stages of water absorption into the hydrogels. This should taper off with time as the swelling hits a maximum. The relative swelling ratio is thus taken as the degree of swelling noted at a given time vs. the degree of swelling observed at T_{max} (the time at which maximal swelling is observed). Relative swelling ratios were plotted against this time axis. Figure 9 illustrates the time course governing increases in the relative swelling ratios of the NIP, Glu/NIP, and Glu-3 gels. Swelling started from the time the hydrogel reached equilibrium at 30°C and was placed in a 10°C ammonium acetate buffer solution (pH 4.6). A linear realationship was observed between swelling and time for the NIP gel, but not for either the Glu-3 or Glu/NIP gels when the relative swelling ratios were plotted against the square root of time. This indicates that relaxation of the polymer chains is the rate-determining process in the swelling of Glu-3 and Glu/NIP because swelling was slower than the time required for the simple diffusion of water toward the interior regions of their hydrogels. The swelling front moved toward the gel center at a constant rate in accordance with the penetration and absorption of water. Water absorption should accelerate upon elimination of the swelling front if relaxation of the polymer chains is the rate-determining process as indicated above. Thus, in the presence of a swelling front, the rate of water absorption to the gel is not proportional to the square root of time. The hydrogels containing monomer **5** underwent the phase transition from their shrunken to their swollen state between 10 and 30°C.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

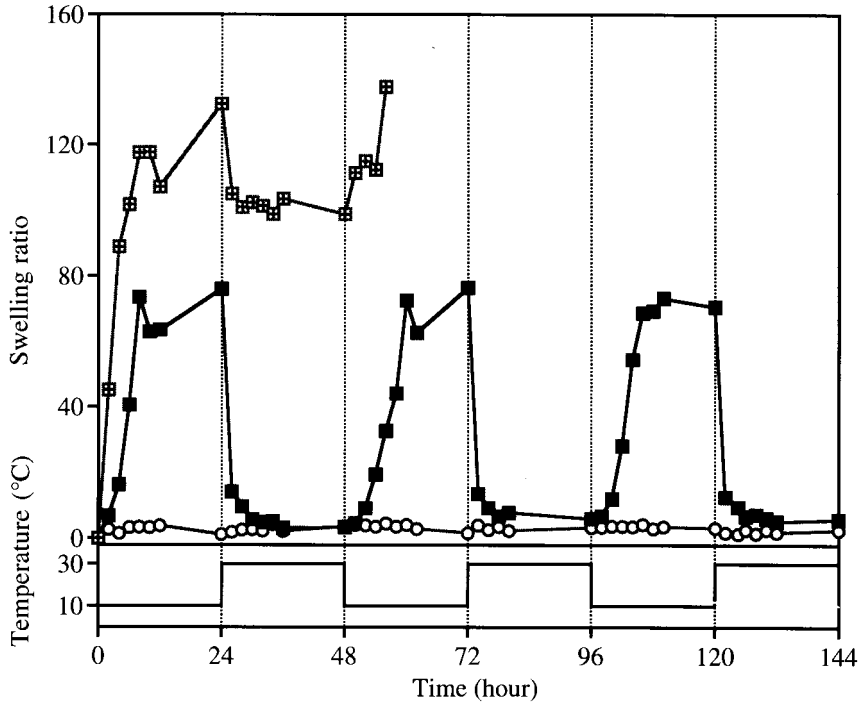


Figure 8. Pulsatile change in the swelling ratio of Glu-3 gel in 1.0 M ammonium acetate buffer when temperature is changed between 30°C and 10°C. *Key:* ○, pH 4.0; ■, pH 4.6; ⊞, pH 4.9.

In Fig. 9 changes in the relative swelling ratios of both the Glu-3 and the Glu/NIP gel followed a sigmoidal pattern over time. This behavior may indicate that water penetration was delayed by the formation of a swelling front and that it accelerated once the swelling front was eliminated.

Since the polymer chains were hydrated more slowly than diffusion of water into the hydrogen bonding network of their diamide residues, swelling fronts were formed into the Glu-3 and Glu/NIP gels. The formation of a swelling front inside polymer networks can make the water-soluble transition of swelling polymer chains slower at lower temperatures. In contrast, the water-insoluble transition of shrinking hydrogels occurs faster at higher temperatures. This is illustrated in Fig. 8. The formation of a swelling front can partly explain the hysteresis phenomenon that was observed for the Glu-3 linear polymer depicted in Fig. 2 because it led to a delay in water penetration.

Linear polymers derived from *N*-acryloyl-*N*′-*n*-propylamide derivatives of L-glutamic and aspartic acids showed reversible transformations between their water soluble and insoluble states with temperature changes in buffer solutions with a pH around the p*K*a of *ω*-carboxylic acid in free acidic amino acids. The structural backbones of the linear polymers were similar to that of PNIPAAm which is known as a temperature-responsive polymer. When carboxyl groups in the linear polymers dissociated in buffer solutions of higher pH, the LCSTs of their linear polymers increased. Substitution of their *N*-alkyl groups and the methylene spacers with suitable hydrophobicity on their C-terminal amide

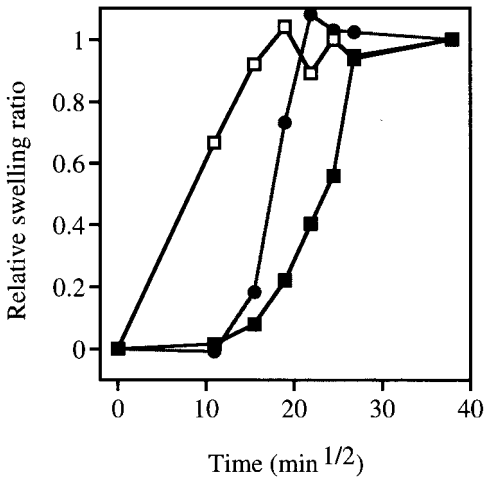


Figure 9. Time course of the relative swelling ratio with gel hydration in 1.0 M ammonium acetate buffer (pH 4.6) from 30°C to 10°C. *Key:* □, NIP gel; ●, Glu/NIP gel; ■, Glu-3 gel.

sites and amino acid side chains was a determining factor in their pH/temperature response.

Poly(*N*-acryloyl-L-glutamic acid *N*′-propyl amide) cross-linked with MBAAm (Glu-3 gel) altered the amount of water absorbed at different temperatures and pHs. Swelling of this hydrogel was observed at 10°C in ammonium acetate buffer (pH 4.6) and its rate of swelling was determined by the relaxation of its macromolecular network accompanied by hydration of its local chain structure. The absorption rate of water inside the hydrogel was thus not proportional with the square root of time because a swelling front formed. Before the hydrogel shrunk at 30°C, polymer chains aggregated on the hydrogel surface to form a hydrophobic environment. This environment would be a skin layer which restricts water penetration into the gel.[22]

REFERENCES

1. Ramkissoon, G.C.; Baudys, F.L.M.; Kim, S. Temperature- and pH-responsive polymers for controlled polypeptide drug delivery. W. ACS Symp. Ser. **1999**, *726*, 322–335.
2. Chung, J.E.; Yokoyama, M.; Yamato, M.; Aoyagi, T.; Sakurai, Y.; Okano, T. Thermo-responsive drug delivery from polymeric micelles constructed using block copolymers of poly(*N*-isopropylacrylamide) and poly(butylmethacrylate). J. Control. Release **1999**, *62* (1–2), 115–127.
3. Bulmus, V.; Ding, Z.; Long, C.J.; Stayton, P.S.; Hoffman, A.S. Site-specific polymer-streptavidin bioconjugate for pH-controlled binding and triggered release of biotin. Bioconjugate Chem. **2000**, *11* (1), 78–83.
4. Takezawa, T.; Mori, Y.; Yoshizato, K. Cell culture on a thermo-responsive polymer surface. Bio/technology **1990**, *8* (6), 854–856.

5. Kushida, A.; Yamato, M.; Konno, C.; Kikuchi, A.; Sakurai, Y.; Okano, T. Temperature-responsive culture dishes allow nonenzymatic harvest of differentiated Madin–Darby canine kidney (MDCK) cell sheets. J. Biomed. Mater. Res. **2000**, *51* (2), 216–223.
6. Saitoh, T.; Ohyama, T.; Takamura, K.; Sakurai, T.; Kise, T.; Matsubara, C. Extraction of iron(II)-tris(1,10-phenanthrolinato) chelate into the polymer phase formed from an aqueous solution of poly(*N*-isopropylacrylamide). Anal. Sci. **1997**, *13* (1), 1–4.
7. Su, P.; Wang, Y.C.; Zhang, X.X.; Sun, L.; Chang, W.B. Thermally reversible hydrogel modified capillary electrophoretic immunoassay of estradiol with laser-induced fluorescence detection. Anal. Chim. Acta **2000**, *418* (2), 137–143.
8. Choi, Y.J.; Yamaguchi, T.; Nakao, S. A novel separation system using porous thermosensitive membranes. Ind. Eng. Chem. Res. **2000**, *39* (7), 2491–2495.
9. Kanazawa, H.; Sunamoto, T.; Matsushima, Y. Temperature-responsive chromatographic separation of amino acid phenylthiohydantoins using aqueous media as the mobile phase. Anal. Chem. **2000**, *72* (24), 5961–5966.
10. Guillet, J.E.; Heskins, M. Solution properties of poly(*N*-isopropylacrylamide). J. Macromol. Sci., Chem. **1968**, *A2* (8), 1441–1455.
11. Kaneko, Y.; Yoshida, R.; Sakai, K.; Sakurai, Y.; Okano, T. Temperature-responsive shrinking kinetics of poly(*N*-isopropylacrylamide) copolymer gels with hydrophilic and hydrophobic comonomers. J. Membr. Sci. **1995**, *101* (1–2), 13–22.
12. Dobashi, A.; Kurata, K.; Senoo, M. Control of the solubility transition in novel temperature-responsive linear polymers comprising α-amino acid diamide derivatives. Anal. Sci. **2000**, *16* (8), 829–835.
13. Okano, T.; Katayama, M.; Shinohara, I. The influence of hydrophilic and hydrophobic domains on the water wettability of 2-hydroxyethyl methacrylate-styrene copolymers. J. Appl. Polym. Sci. **1978**, *22* (2), 369–377.
14. Takei, Y.G.; Aoki, T.; Sanui, K.; Ogata, N.; Okano, T.; Sakurai, Y. Temperature-responsive bioconjugates. 2. Molecular design for temperature-modulated bioseparations. Bioconjugate Chem. **1993**, *4* (5), 42–46.
15. Bae, Y.H.; Okano, T.; Kim, S.W. Temperature dependence of swelling of crosslinked poly(*N*,*N*-alkyl substituted acrylamides) in water. J. Polym. Sci. B **1990**, *28* (6), 923–936.
16. Yoshida, R.; Okuyama, Y.; Sakai, K.; Okano, T.; Sakurai, Y. Temperature-responsive shrinking kinetics of poly(*N*-isopropylacrylamide) copolymer gels with hydrophilic and hydrophobic comonomers. J. Biomater. Sci. Polym. Ed. **1994**, *6* (6), 585–598.
17. Okuyama, Y.; Yoshida, R.; Sakai, K.; Okano, T.; Sakurai, Y. Temperature-responsive shrinking kinetics of poly(*N*-isopropylacrylamide) copolymer gels with hydrophilic and hydrophobic comonomers. J. Biomater. Sci. Polym. Ed. **1993**, *4* (5), 545–556.
18. Yoshida, R.; Okuyama, Y.; Sakai, K.; Okano, T.; Sakurai, Y. Sigmoidal swelling profiles for temperature-responsive poly(*N*-isopropylacrylamide-co-butyl methacrylate) hydrogels. J. Membr. Sci. **1994**, *89* (3), 267–277.
19. Peppas, N.A. Analysis of Fickian and non-Fickian drug release from polymers. Pharm. Acta. Helv. **1985**, *60* (4), 110–111.
20. Ritger, P.L.; Pepps, N.A. A simple equation for description of solute release. I. Fickian and non-Fickian release from non-swellable devices in the form of slabs, spheres, cylinders or disks. J. Control. Release **1987**, *5* (1), 23–26.

21. Ritger, P.L.; Pepps, N.A. A simple equation for description of solute release. II. Fickian and anomalous release from swellable devices. J. Control. Release **1987**, *5* (1), 37–42.
22. Kaneko, Y.; Yoshida, R.; Sakai, K.; Sakurai, Y.; Okano, T. Temperature-responsive shrinking kinetics of poly(*N*-isopropylacrylamide) copolymer gels with hydrophilic and hydrophobic comonomers. J. Membr. Sci. **1995**, *101* (1–2), 13–22.

Received April 2003
Accepted September 2003

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016