

Swelling Behavior of Biodegradable Crosslinked Gel based on Poly(aspartic acid) and PEG-diepoxide

Suk Kee Min and Ji-Heung Kim*

*Department of Chemical Engineering, Polymer Technology Institute,
Sungkyunkwan University 300 Chunchun, Jangan, Suwon, Kyunggi 440-746, Korea*

Dong Jun Chung

*Department of Polymer Science & Engineering, Polymer Technology Institute,
Sungkyunkwan University 300 Chunchun, Jangan, Suwon, Kyunggi 440-746, Korea*

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Abstract : Poly(aspartic acid), PASP, is a biodegradable, water-soluble polymer and offers a biodegradable alternative to polycarboxylates and other non-degradable water-soluble polymers. PASP, one of poly(amino acid)s, possesses carboxylic acid pendant group in its repeating unit, which can be used for various further modification purposes. In this study we prepared high molecular weight polysuccinimide, as the precursor polymer for PASP, by thermal polycondensation of *L*-aspartic acid in the presence of phosphoric acid. The polysuccinimide was hydrolyzed with 0.1 N sodium hydroxide, and then acidified to give PASP. High water-absorbent gels were produced by thermal crosslinking of freeze-dried mixture of partially-neutralized PASP and different amount of low mol. wt. PEG-diepoxide compounds in aqueous medium. The swelling behavior of the prepared gels from different size and composition of crosslinking reagent in different media was investigated and the results were discussed. This PASP-based hydrogel materials possessing inherent biodegradability, potential non-toxicity and biocompatibility, is expected to be used as a substrate for various biomedical applications as well as a general purpose super-absorbent polymer.

Introduction

The importance of polymeric materials possessing biodegradability is being increasingly recognized and extensive studies has been done for their uses in structural supports (e.g. suture), controlled release of drugs, and recently as scaffolds materials in tissue engineering.¹⁻³ In particular, biodegradable and water-soluble polymers are very desirable in terms of the environmental safety because water-soluble polymers are hardly recovered or collected after use. Poly(amino acid)s, which have protein-like linkages, are known to be biodegradable and are desirable to use in place of non-biodegradable, water-soluble polymers such as poly(acrylic acid)s. Among them, poly(aspartic acid) (PASP), one of poly(amino acid)s with free carboxylic pendant, is

a promising water-soluble and biodegradable polymer. PASP can be obtained from the alkaline hydrolysis of poly(succinimide) (PSI) as the precursor polymer.⁴⁻⁷ PSI is prepared by the thermal bulk polycondensation of aspartic acid or the ammonium salts of maleic acid and malic acid.⁸⁻¹² Super-absorbent polymers are used for various kinds of fields such as diapers, hygienic goods, water retaining and gelling agents.¹³ Conventional super-absorbents are crosslinked poly(acrylic acid) and graft polymers of poly(acrylic acid) and starch. However in spite of its various applications, its property which was non-biodegradable in nature brought about environmental pollution problems. The super-absorbency of these polymers are based on partially ionized and lightly crosslinked hydrogel formation. PASP with free carboxylic acid pendants is very interesting as a biodegradable super-absorbent polymer when neutralized and

*e-mail : kimjh@skku.ac.kr

crosslinked. For the crosslinking of PASP, various methods have been introduced, including chemical and radiation processes. Previous researches used difunctional amines, amino acids or their polymeric analogs as the chemical crosslinker, plus physical γ -irradiation to prepare the biodegradable super-absorbent hydrogel.¹³⁻¹⁵

In this work, a relatively high molecular weight PSI, the precursor polymer for PASP, was prepared by the thermal condensation of *L*-aspartic acid. For the preparation of a crosslinked hydrogel, an oligomeric PEG-diepoxy compound was employed and reacted under mild conditions in simple process to produce a biodegradable, and potentially non-toxic superabsorbent gel. The preparation of the materials and results of the swelling behavior relative to the content of different PEG-diepoxy in various conditions are discussed hereafter.

Experimental

Chemicals and Measurements. *L*-Aspartic acid and phosphoric acid were purchased from Aldrich Chemical Co. Poly(ethylene glycol) diglycidyl ethers (PEG200, 400, 600 with average molecular weight of ethylene oxide units, respectively) was purchased from Polysciences, Inc. All the other chemicals purchased were of high quality and used without purification.

The IR spectra were obtained on a Unicam 1000 FT-IR spectrometer. The NMR spectra were taken on a Varian Unity Inova 500 MHz Spectrometer at room temperature. The thermal analysis was carried out on a Perkin Elmer DSC/TGA7 Series thermal analysis system. The solution viscosity was measured in an Ubbelohde capillary viscometer using *N,N*-dimethylformamide as solvent.

Synthesis of Poly(succinimide)(PSI). *L*-Aspartic acid and 85% *o*-phosphoric acid (50:50 wt. ratio) were put into a blender and mixed at a low temperature. The mixture was placed in a 500 mL flask connected to a rotary evaporator, and the flask was immersed in an oil-bath controlled to about 190°C. The mixture was heated for 4.5 h while the system was slowly evacuated from 750 to about 1 mmHg. After the reaction,

DMF was added to dissolve the brown viscous melt completely, then the resulting solution was precipitated into a large amount of methanol to give a light-yellow fibrous powder. The product was washed with methanol and water several times, filtered, and finally dried in vacuum at 80°C for 2 days. FT-IR: 1712 cm^{-1} (C=O), ^1H NMR: δ 2.7, 3.3 (methylene), 5.3 (methine proton) ppm, ^{13}C NMR: 176, 177 (C=O), 36 (-CH₂), 51 (-CH-)

Preparation of Hydrogel.

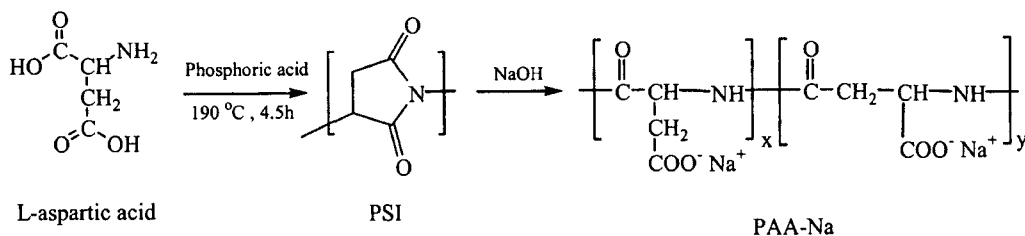
Hydrolysis of PSI to Poly(aspartic acid) (PASP): PSI, previously prepared, was suspended in distilled water and hydrolyzed by adding 0.1 N NaOH aqueous solution. The final clear solution was titrated with dilute HCl and the pH of the solution was adjusted to about 5.5.

Preparation of Mixed Formulation with Crosslinking Agent: The above prepared poly-aspartate aqueous solution was filtered to remove small amounts of insoluble parts, and then mixed with a pre-determined amount of the PEG-diepoxy compound to prepare a formulation with a specific composition (e.g. F40PEG400 designates a mixture containing 40 mol% PEG (400)-diepoxy based on succinimide unit). The aqueous mixture was then freeze-dried for 2 days to obtain an off-white fine powder.

Preparation of Crosslinked Gel: A crosslinked gel was obtained by a curing process of each formulation under different conditions (temperature and time), i.e., a mixed formulation was placed in a vacuum oven, preheated to a certain temperature, for a designated period of time. The cured samples were then kept in dry desiccator before testing their water swelling properties.

Measurement of Water Absorbency. The water absorbency and swelling rate were tested by a simple tea-bag method. A certain amount of dry gel powder (ω_p) was weighed into an empty bag, and put into a deionized water for a designated period of time. The weight of the swollen gel with the bag (ω_1) was measured after 2 h soaking time if not notified, then the weight of the wet tea-bag without powder (ω_2) was subtracted to obtain the pure weight of the swollen gel by the dry polymer. The water absorbency (or swelling ratio) was simply calculated using the equation

Scheme I



below;

$$\text{Swelling ratio (water absorbency)} = (\omega_1 - \omega_2) / \omega_p$$

Results and Discussion

Preparation of PSI and Hydrolysis to Sodium Polyaspartate (PAA-Na). High molecular weight PSI was prepared by the thermal condensation of *L*-aspartic acid in the presence of *o*-phosphoric acid under reduced pressure. The reaction scheme with a following hydrolysis step is shown below (Scheme I). The polymer possessed a reduced viscosity of 0.55 dL/g in DMF. The molecular weight was estimated to be about 180,000 dalton, as calculated from an empirical equation relating the solution viscosity to the molecular weight.¹¹

The resulting PSI fine powder was dispersed in water and titrated with a molar equivalent of a 0.1 N aqueous NaOH solution to provide PAA sodium salt. When the addition of the equivalent amount of NaOH was completed, the clear solution exhibited a pH value of about 10.8. As described in the experimental part, the PAA aqueous solution was then mixed with a predetermined amount of PEG-diepoxide, pH-adjusted to 5.5 by adding HCl, and then lipophilized by freeze-drying to give an off-white bulky powder. By using freeze-dry method, the homogeneity in both mixing and the followed crosslinking reaction in bulk may well be maintained.

The IR spectrum of a typical formulation are obtained to confirm the structure of the mixture. The IR showed strong characteristic bands of both amide and carboxylic groups ($1500\sim 1700\text{ cm}^{-1}$) of the PAA and methylene ether group (1105 cm^{-1}) of the PEG-diepoxide. Typical TGA thermogram

showed that the material remained stable up to about 280°C without a significant weight loss under nitrogen.

Swelling Properties of Cured Polymeric Gel. The freeze-dried mixture of PAA and PEG-diepoxide, the crosslinking agent, was cured under various conditions, and the resulting gels were tested as regards their swelling behavior. The crosslinking process mainly involves the chemistry between (poly)carboxylic acids on the PAA backbone and terminal epoxy groups of crosslinking reagent to form ester linkage. The solid-state curing process was adopted for the advantageous simplicity in manufacturing without any additives, even though an efficient conversion for network formation may be limited to some extent. As reported in our previous communication,¹⁷ an appreciable swelling was observed from the gels containing relatively high contents of PEG-diepoxide in the range of 30~50 mol%. At lower contents it seems that effective network structure is hardly formed, due to insufficient crosslinking reaction and/or possible hydrolysis of epoxide groups. The swelling ratio of the resulting hydrogel was, of course, dependent on the curing temperature and time. A noticeably high swelling was observed for the sample cured above 60°C . The highest swelling was observed at a curing time of around 1 h at 60°C . In the case of F40PEG200 sample, very high swelling ratio up to 1100 g/dry-gel was observed. As the curing time increased over 1 h, the swelling ratio tended to decrease and levelled off. When the curing temperature was raised from 60°C to higher temperatures of 80 or 100°C , the swelling capacities also decreased. These tendency in swelling as curing temperature and time should be resulted from an increased degree of effective crosslinking and the expected

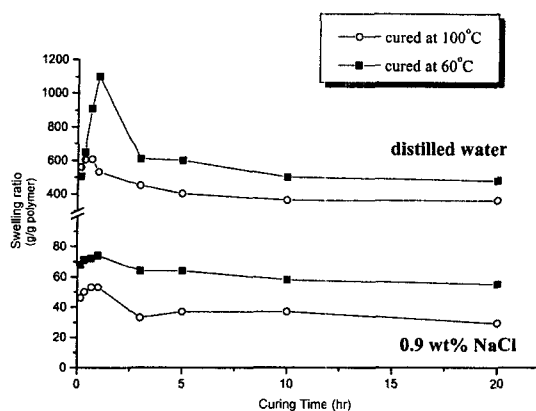


Figure 1. Swelling ratio as a function of curing time in water and 0.9 wt% NaCl solution (sample F40PEG200).

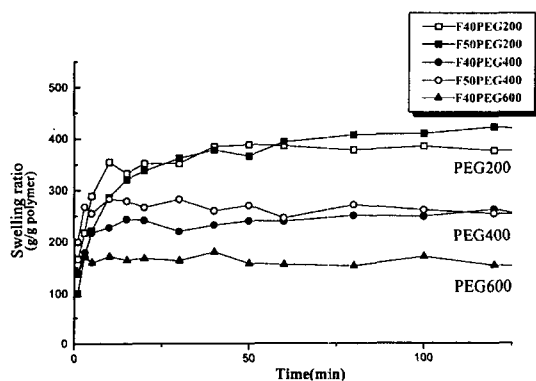


Figure 2. Swelling rate of gels from different molecular weight PEG-diepoxyde.

denser network structure. In contrast to the high swelling capacities of several hundreds in pure water, those in 0.9 wt% NaCl aqueous solution was lower within a range of 40~70 g/dry-gel for the different samples prepared.

Figure 1 shows a typical curves showing the swelling ratios of the prepared gels cured at different conditions both in distilled water and 0.9 wt% NaCl aqueous solution. For the different gels using PEG-diepoxydes with three different molecular weight, cured at the same conditions (1 h, 100°C), the swelling rate in water was measured and plotted as shown in Figure 2. The swelling seemed to levelled off in about 1 h to reach a pseudo-equilibrium state. As the molecular weight or size of crosslinker increased, the swelling degree tended to decrease gradually at the same molar content.

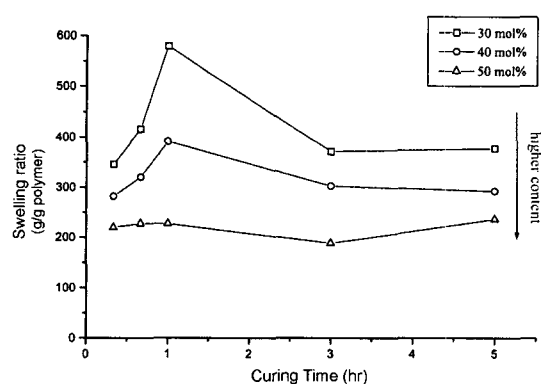


Figure 3. Swelling ratio as a function of content of PEG(600)-diepoxyde (sample cured at 60°C, and the swelling measured at 25°C).

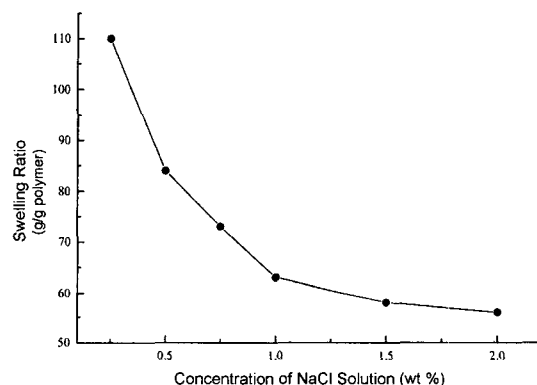


Figure 4. Swelling ratio in aqueous solution as a function of salt concentration (sample F50PEG200, measured at 25°C).

Because of the complexity of this system, simple explanation seems difficult. However, the increase in the length of crosslinker should lower the dissociated ion density within the matrix to decrease the chemical affinity for maximal swelling, due to the nonionic nature of the PEG structure together with the relatively high contents.

Figure 3 shows the dependence of swelling capacity on the amount of crosslinking agent (PEG(600)-diepoxyde in this example). As the content increased, the swelling ratios decreased for all the samples cured at 60°C for the different periods of time.

Relatively low level of swelling degree in salt solution or physiological medium is generally observed in many other super-absorbent polymers

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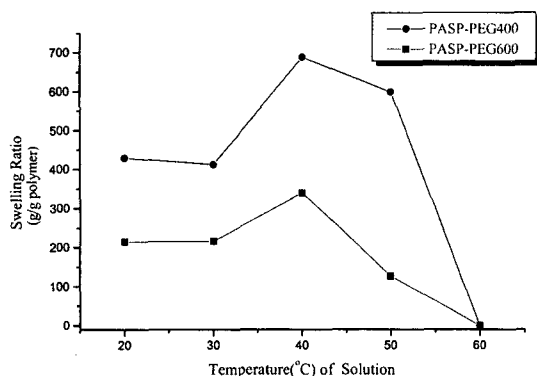


Figure 5. Swelling behavior in aqueous solution at different temperatures (sample F40PEG000, cured at 100 °C for 1 h).

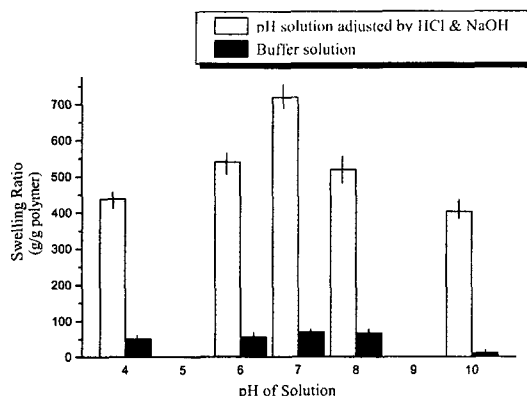


Figure 6. Swelling ratio in aqueous solution as a function of pH (sample F55PEG400, cured at 60 °C for 5 h).

known, which will be resulted from the high ionic strength of medium acting adversely to the equilibrium swelling capacity. The gels prepared in this study showed swelling ratios in the range of 40~70 in 0.9 wt% NaCl solution, varying a little on the compositions and curing conditions. Dependence of swelling on the salt concentration is shown in Figure 4. As expected, the swelling ratios showed monotonous decreasing tendency as the salt concentration increased.

The swelling behavior of hydrogel changes in respond to the environmental conditions. Figure 5 shows apparent swelling degree measured after 2 h immersion in water at the different temperature. As the temperature was increased from ambient temperature to 60 °C by the incremental 10 degree, maximal swelling was observed at 40 °C. A decreased swelling was observed at higher temperature of 50 °C, and no appreciable swelling was noticed at 60 °C. At the elevated temperature, the swelled polymers are expected to decompose, probably due to the hydrolytic cleavage of labile linking group between PASP backbone and PEG. The measured values, accordingly, may be less important because the apparent swelling will depend on the temperature and time as well as the degradation kinetics at a given condition. The chemical nature of gel degradation or decomposition will be discussed again later.

Figure 6 showed the swelling degree in the aqueous solutions of different pH values. When

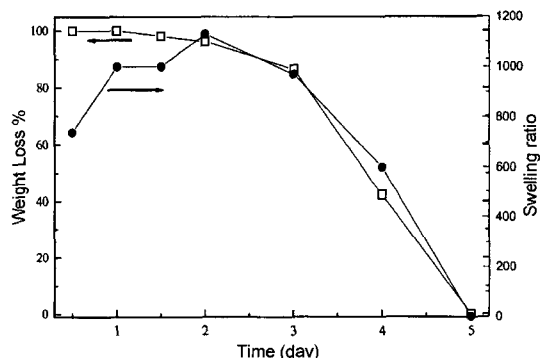


Figure 7. Decomposition of swelled gel in excess water at 25 °C (sample F50PEG200, cured at 80 °C for 1 h).

the pH was adjusted simply by adding HCl and NaOH, the swelling degree was highest in the neutral pH of 7, and lowered in both acidic and basic medium. However, the swelling in different buffer solutions was observed to be quite low, presumably caused by the relatively high ionic strength of the solution containing various electrolytes. In the buffer solution of pH 10, very small swelling was observed, which might be caused by the hydrolytic decomposition of the gel matrices, accelerated in basic condition.

Degradation of the Swelled Gel. The degradation behavior of the swelling gel is interesting and a simple test was carried out in excess water at 25 °C. A series of swelled gel within the tea-bag was placed in aqueous bath at 25 °C and sampled out after a certain time to measure the swelling degree and the weight loss after freeze-drying of

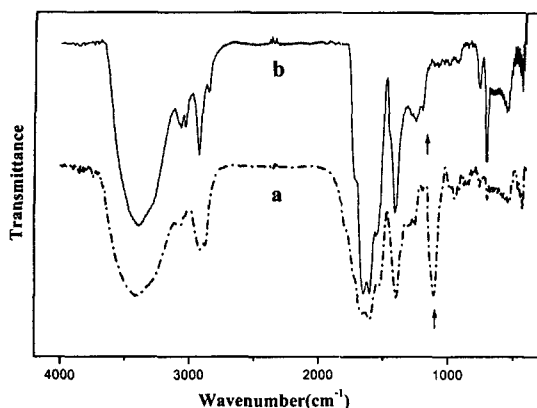


Figure 8. IR spectra of samples before (a) and after (b) gel decomposition (sample F50PEG200, cured at 80 °C for 1 h).

the remained gel. As the results are shown in Figure 7, very small degree of weight loss was observed during the initial 2 days and began to increase significantly thereafter. And it seemed that almost no swelled gel remained in 5 days immersion. On the other hand, the swelling degree of sample continued to increase to show a maximum swelling in 2 days, and then decreased rapidly. This was partly to be expected since upon random hydrolysis of a crosslink the chains that were connected to that crosslink remained intact. Even some part of crosslinks are cleaved by hydrolysis, the network chains remains and the resulting longer chains can expand further to exhibit higher swelling while partial degradation process is undergoing. Complementally to this gel degradation behavior, a swelled gel in excess water was placed in 60 °C bath for some time until the initial gel decomposed completely and no apparent gel part remained. And then the resulting aqueous solution was dialyzed against water for 24 h using tubular membrane (Spectra/Por with MWCO 12000~14000) and subsequently lipophilized by freeze-drying to obtain white powder (about 50% yield). The IR spectrum of this sample was measured and compared with the original dry-gel as shown in Figure 8. The material remained after dialysis was analysed to be polyaspartate without PEG structural units (absorption band at $\sim 1100\text{ cm}^{-1}$ corresponding to C-O-C ether group disappeared), suggesting the release of PEG-containing low molecular

weight fraction, probably generated by gel decomposition. It seems that the loss of gel property is resulted from the gel decomposition via hydrolytic cleavage of linking groups, i.e. the labile ester bonds between polyaspartate backbone and PEG, to provide both water-soluble components. Also above gel decomposition seems to occur relatively fast in an elevated temperature. The gel decomposition accompanied by hydrolysis and its dependence on the temperature and characteristics of medium seems a little complicated and the complementary studies are in progress.

To summarize, polymeric hydrogels based on PASP and PEG-diepoxides were prepared by simple powder process, and the resulting hydrogel exhibited super-absorbency of several hundreds to thousand times water based on dry-polymer. The swelling behavior in different environmental conditions (pH, temperature, salt-concentration), and also preliminary gel degradation phenomena in water was investigated.

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