

Characteristics of Hydrogel Prepared from Microbial Poly(γ -glutamic acid) by Chemical Crosslinker

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Microbial hydrogel was prepared with poly(γ -glutamic acid) produced from *Bacillus subtilis* BS62 using crosslinking reagent, ethylene glycol diglycidyl ether (EGDE), and its physico-chemical characteristics were examined. Hydrogel which prepared from 10 grams of 10% PGA solution with 600 μ l of EGDE at 50°C for 17 h swelled 4,320 times its dry weight, and time to reach swelling equilibrium in deionized water at 4 to 45°C range was about 20 h. Swollen hydrogel shrunk in ionic solutions, and rate of shrinkage was higher in calcium chloride solution than sodium chloride solution. Swelling rate of hydrogel increased 1.3-fold of initial swelling rate for 30 min at 80°C.

Key words: poly(γ -glutamic acid), chemical crosslinker, EGDE, hydrogel

Natural-based superabsorbent hydrogels made with poly(γ -glutamic acid) (PGA), which is water-soluble, anionic, and edible, have attracted much interest from the viewpoint of improving the tissue tolerance of synthetic polymers and the mechanical properties of natural polymers for applications in pharmaceutical fields of drug delivery systems, foods, wound dressing, and biosensors. Hydrogels, which swell and contract in response to external stimuli such as heat, pH, and salts, have been extensively investigated for potential use in site-specific delivery of low molecular weight protein drugs.^{1,2} Flowry³ described the swelling behavior of charged hydrogel results from a balance between the elastic energy of the network and the osmotic pressure of the ions. In salt-free hydrogels this osmotic pressure is due to the counterions that are confined inside the gel in contact with a water reservoir, whereas, in the presence of salt, is associated with the establishment of a Donnan equilibrium.⁴

Many researchers described a convenient method for the hydrogel formation of PGA by γ -ray irradiation,^{5,6} and the resulting hydrogels could be used as water-sorption materials. The specific water content ranged from 200 to 3,500 times their dry weight in the case of PGA hydrogels. Chemically crosslinked PGA hydrogels produced by chemicals such as dihaloalkane compounds were reported.⁷ The preparation of a hydrogel from a naturally occurring polymer, PGA, is important with respect to biodegradability, biocompatibility, and nontoxicity of the superabsorbing materials.

We previously reported on the production and purification

of microbial PGA⁸ as well as the preparation of the PGA hydrogel by γ -ray irradiation method.⁶ Here, we report on characteristics of the PGA hydrogel synthesized by chemical crosslinker, EGDE.

Materials and Methods

Synthesis of the PGA hydrogel. High-purity PGA hydrogels having diverse structures were synthesized using the crosslinking reagent, EGDE, as follows. Ten grams of 10% PGA aqueous solution was poured into 20 ml flask, in which 200 to 800 μ l EGDE was added, and mixed well. The flasks were kept at 50°C for 17 h. To remove any unreacted molecules of PGA, the hydrogel obtained was swollen in deionized water by exchanging water every day for 1 week at 4°C. Networked and swelled PGA was filtered through an 80-mesh sieve, and lyophilized.⁹ Previously reported methods were employed for quantifying specific water content and hardness of PGA hydrogel.⁶

Swelling and shrinking of hydrogel. PGA hydrogel was placed in various buffer solutions ranging from pH 2.5 to 11 at 4°C, and in NaCl, Na₂SO₄, CaCl₂ or glucose solutions between 0 and 3.0 wt% concentrations to observe the swelling equilibrium. Each 25 mM McIlvaine (citrate/Na₂HPO₄) buffer was prepared at the pH range of 2.5 to 8.0, and 25 mM sodium borate-NaOH buffer was used for the remaining pH values. The gel weight in the equilibrium state was determined by weighing the hydrogel after removing the surface water through an 80-mesh sieve. Degree of swelling was calculated based on the ratio of the remaining gel weight to the initial amount of PGA hydrogel.

Hydrolytic degradation of hydrogel. To estimate the heat

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stability of PGA hydrogel, the hydrogel was placed in a glass bottle with an appropriate amount of deionized water and separately heated at 40 and 80°C. The wet hydrogel sample was removed from the bottle at appropriate intervals and filtered through an 80-mesh sieve. The degree of swelling was calculated by weighing the hydrogel remaining on the sieve to estimate the thermal hydrolytic degradability.

Observation of PGA by scanning electron microscope.

The freeze-dried PGA hydrogel powder was treated by Sputter coater (Model: SCD-005) and observed by a scanning electron microscope (Model: XL 30 ESEM TMP PW 6635) at 15.0 kV.

Results and Discussion

Hydrogel preparation by chemical linker. The hydrogel was prepared by mixing 10% aqueous solution of PGA obtained from *Bacillus subtilis* BS62 and 200~800 μ l chemical linker, EGDE in 20 ml flask, and the mixture was reacted at 50°C for 17 h. The characteristics of the hydrogel were compared with those of the irradiated hydrogel (^{60}Co γ -ray). The specific water content of the hydrogel increased, reaching maximum at 600 μ l EGDE addition, which was 4,320 times its dry weight and 3.2-fold higher compared to hydrogel prepared by γ -ray irradiation method,⁶ in a normal curve pattern (Fig. 1). The hardness of hydrogels formed by EGDE is shown in Fig. 2. Gel strength decreased inversely to below 20 textural units with the addition of 200 to 800 μ l EGDE. On the other hand, the hardness of hydrogel prepared by γ -irradiation increased in proportion to the increasing γ -irradiation dosage.⁶

Effects of temperature. Polyelectrolyte hydrogels such as

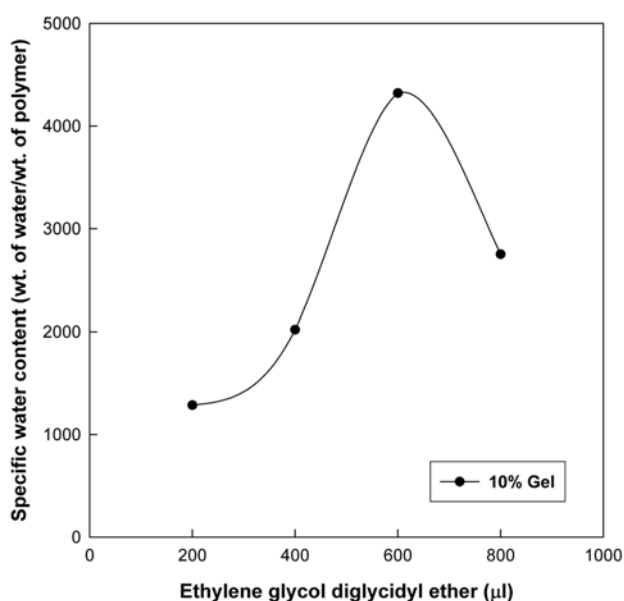


Fig. 1. Changes in specific water contents during the formation of PGA hydrogels with EGDE. Ten percent aqueous solution (w/v) of PGA was employed at 50°C for 17 h.

PGA undergo continuous or discontinuous capacity changes depending on the surrounding environmental parameters such as ions, temperature, and solvents. To compare the changes in swelling according to the temperature, the hydrogel prepared from 10% PGA solution was swollen in deionized water (pH 7.0) and subsequently adjusted to 4, 25, 35, and 45°C. The swelling of the hydrogel reached equilibrium within 20 h at all temperatures examined, and only negligible change was observed thereafter (Fig. 3). The time required to reach the

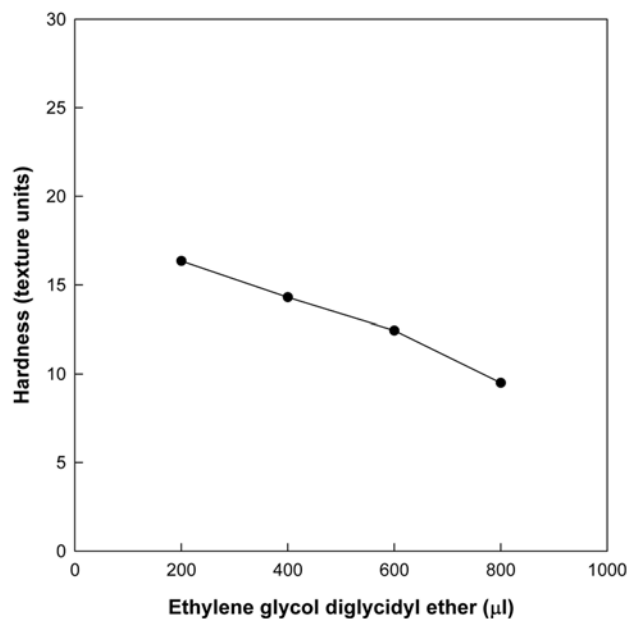


Fig. 2. Change in hardness of PGA hydrogel during the formation of PGA hydrogels by EGDE. Notes refer to Fig. 1.

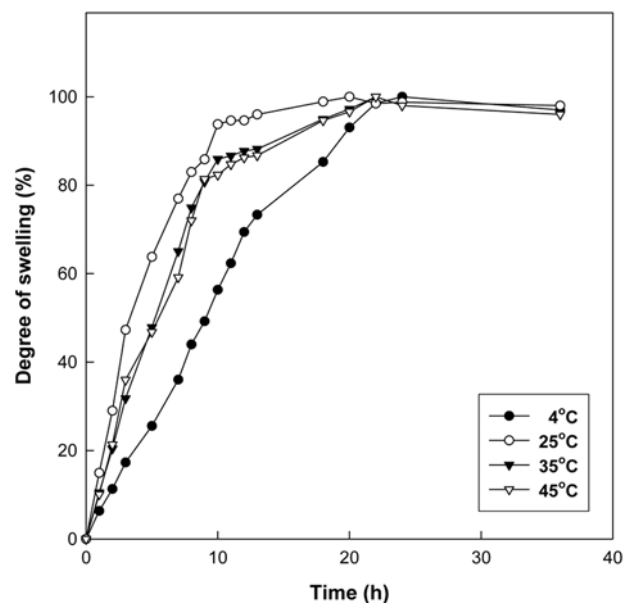


Fig. 3. Changes in degree of swelling of the PGA hydrogels in deionized water as a function of time. The PGA hydrogels prepared from 10 w/v% PGA solution with 600 μ l EGDE were examined.

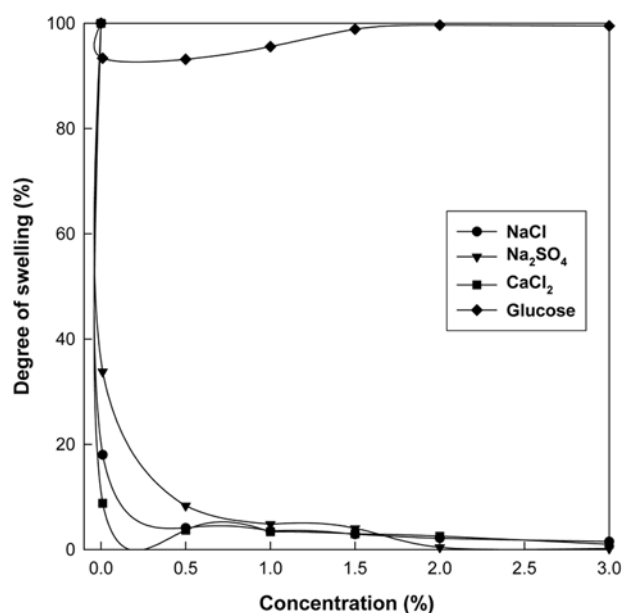


Fig. 4. Changes in degree of swelling of the PGA hydrogels in various solution as a function of ionic strength. Notes refer to Fig. 3. ●, NaCl (1:1 electrolyte); ▼, Na₂SO₄ (2 : 1 electrolyte); ■, CaCl₂ (1 : 2 electrolyte); ◆, glucose (non-electrolyte).

swelling equilibrium in deionized water was the shortest at 25°C and the longest at 4°C. On the other hand, with the hydrogel prepared from γ -ray irradiation method,⁶ the swelling reached equilibrium within 10 h at all temperatures examined, and the swelling equilibrium time was shortest at 4°C.

Effects of salts. The main factors affecting the swelling of hydrogel are the ion strength of the solution and the atomic valence of ions in the solution. Swelling and shrinking patterns of 10% PGA hydrogel synthesized with EGDE were investigated using salts such as NaCl, Na₂SO₄, and CaCl₂, and glucose as a non-electrolyte. PGA hydrogel shrunk in all electrolyte solutions of 0.01% concentration within 30 min, and shrunk to below 10% of the fully swollen hydrogel at 0.5% concentration (Fig. 4). The swelling rate decreased initially and maintained level at salt concentration up to 3.0%. This result, identical to that of the hydrogel by γ -ray irradiation, is known as the Donnan effect,⁴ which is the main driving force in the swelling of PGA hydrogel. These results show increase in the salt concentration outside the gel leads to decreased ionic swelling pressure, and in turn, decreased gel volume.

In general, the shrinking speed is higher than the swelling speed.¹⁰ Shrinking of PGA hydrogel by Ca²⁺ ion was faster than that with Na⁺ ion. The swelling rate decreased to 35, 18, and 10% of the initial swollen gel in 0.01% each of Na₂SO₄, NaCl, and CaCl₂, respectively. Ions such as H⁺, Ca²⁺, and Na⁺ shrunk the hydrogel by connecting each electric charge. Divalent cation is more effective in neutralizing the hydrogel electrically than monovalent cations; therefore, Ca²⁺ ion can

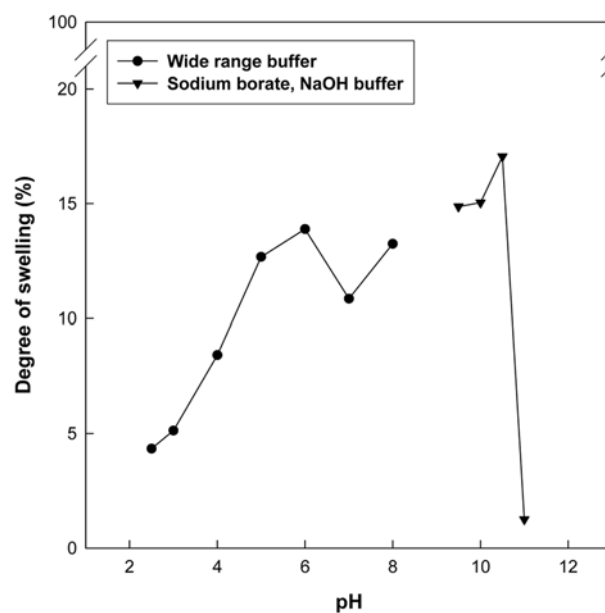


Fig. 5. Changes in degree of swelling of the PGA hydrogel as a function of pH at constant ionic strength (25 mM). Notes refer to Fig. 3. ●, Wide range buffer (pH 2-8); ▼, Sodium borate, NaOH buffer (pH 9.5-11.0).

shrink the hydrogel at half the concentration to that of Na⁺ ion. The shrinking of PGA hydrogel faded in non-electrolyte glucose solution and, therefore, the transition phenomenon did not occur in the network. Likewise, changes in the surrounding environmental condition, PGA hydrogel could also characterize swelling and shrinking, because the properties of the hydrogel change according to type of ions and their concentrations in the solution.⁴

Effect of pH. The swelling-equilibrium of polymer such as PGA hydrogel is affected by the property of network caused by elasticity or osmotic pressure. PGA hydrogel having fixed electrical charges in the structure was shown to be influenced by pH of the surrounding solution due to the systematic property.⁵ Swelling and shrinking of the hydrogel were examined by changing the pH of hydrogels from 2.5 to 11.0. The hydrogel shrunk in both acidic range below pH 6.0 and alkaline range above 10.2 (Fig. 5). In the pH range 6.0 to 10.2, the hydrogel gradually swelled, and the highest swelling rate was found at pH 10.2. Result of Donnan equilibrium⁴ indicates PGA hydrogel in acidic pH range has a low swelling status due to decreased density and osmotic pressure, which continuously fix the electrical charges of the hydrogel. Kunioka and Choi¹¹ reported that the properties of the hydrogel mixed with PGA and ϵ -polylysine (PL) at various pH values and the PL hydrogel swelled due to the ionic repulsion of the protonated amino groups in the PL molecules. The hydrophilic copolymer gels synthesized with N-hydroxypropyl methacrylamide and N,O-dimethacryloylhydroxyl-amine were stable at below pH 5.0, whereas were hydrolyzed at neutral and mild alkaline pH, suggesting that the rate of hydrolysis depends on the pH of the solution and the

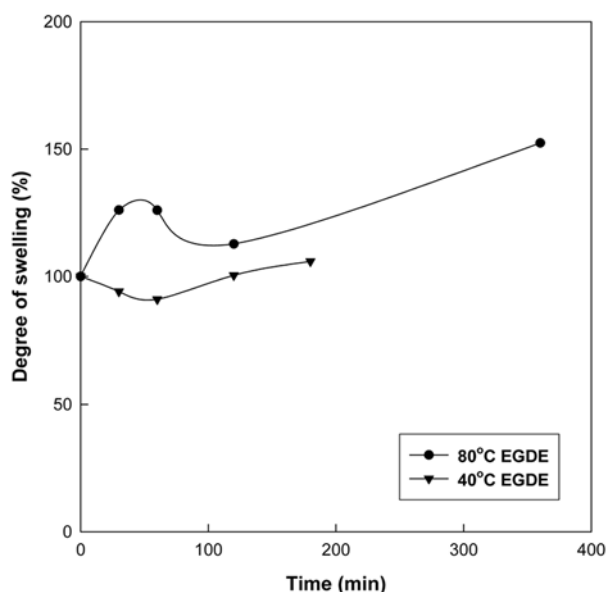


Fig. 6. Changes in degree of swelling of the PGA hydrogels in deionized water as a function of temperature. Notes refer to Fig. 3.

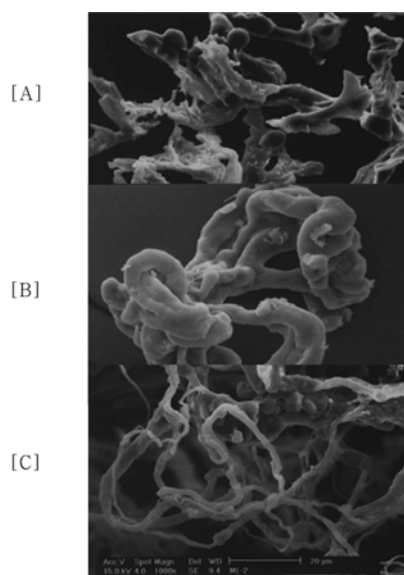


Fig. 7. Morphology of the PGA produced by *Bacillus subtilis* BS62 and its hydrogels. (Bar indicates 20 μm). A, PGA; B, PGA hydrogel by γ -irradiation; C, PGA hydrogel by crosslinking reagent (EGDE).

crosslinking density of the gel.¹²⁾

Thermal hydrolytic degradability. Temperature is an important factor in the swelling of the hydrogel. Kunioka and Choi studied thermal hydrolytic degradability of hydrogels prepared from microbial PGA and ϵ -polylysine using γ -irradiation,¹¹⁾ and the enzymatic degradation of crosslinked PGA hydrogel was reported by Hara *et al.*¹²⁾ Unlike the γ -irradiated hydrogel, which quickly degraded after 1 h at 80°C, the swelling of PGA hydrogel was stable both at 40 and 80°C (Fig. 6). The swelling rate increased to 1.3-fold of the initial swelling rate for 30 min at 80°C, slightly decreased, then

gradually increased again for 6 h. The partial hydrolytic degradation of the hydrogel could have been the result of an increase in the anionic carboxyl group concentration inside the gel.

Structure of PGA hydrogel. To identify the structure of PGA hydrogel, freeze-dried hydrogels obtained from EGDE and γ -ray irradiation treatments were treated with Sputter coater and observed by a scanning electron microscope. PGA showed coarse and irregular surfaces, whereas the hydrogels from both EGDE and γ -ray irradiation had smooth and lumped coil-like shapes (Fig. 7). Coil of the hydrogel prepared by EGDE was fine and long in comparison with that prepared by γ -irradiation, which explains the higher specific water content of the EGDE hydrogel than that prepared by γ -irradiation.

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