Preparation and Properties of Novel Biodegradable Hydrogel based on Cationic Polyaspartamide Derivative

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Novel copolymers consisting of poly(2-hydroxyethyl aspartamide-co-N,N'-dimethyl-1,3-propane aspartamide) (PHEA-DPA) were prepared from polysuccinimide (PSI), which is the thermal polycondensation product of aspartic acid, via a ring-opening reaction with N,N'-dimethyl-1,3-propane diamine (DPA) and ethanolamine. The prepared water-soluble copolymer was then crosslinked by reacting it with hexamethylene disocyanate to provide the corresponding gel. The swelling behavior and morphology of the crosslinked hydrogels were investigated. The degree of swelling decreased with increasing crosslinking reagent due to the higher crosslinking density. It was also confirmed that the swelling property is affected by pH. At low pH (< pH 4), swelling is increased due to the ionization of DPA with a tertiary amine moiety. In addition, a reversible swelling and de-swelling behavior was demonstrated by adjusting the pH of the solution. The prepared hydrogels showed a well-interconnected microporous structure with regular 5-20 μm sized pores.

Key Words: Polyaspartamide, Graft copolymer, Biodegradable polymer, Hydrogel, Swelling

Introduction

Hydrogels are hydrophilic polymer networks that are capable of absorbing large amounts of water, yet are insoluble due to the presence of physical or chemical cross-links, entanglements, or crystalline regions. Hydrogels can be used in various biomedical applications such as drug delivery systems, biosensors, contact lenses, catheters, and wound dressings. These materials have also been used extensively as matrices in tissue engineering for repairing and regenerating a wide range of tissues and organs on account of their hydrophilic character and potential biocompatibility.1-3 Hydrogels are known as chemical gels when they are covalently-crosslinked networks. Chemical hydrogels can be generated by the crosslinking of water-soluble polymers. In the crosslinked state, the hydrogels reach an equilibrium swelling level in aqueous solutions depending on the crosslink density (estimated by the MW between crosslinks, Mc). Recently, stimuli-responsive polymers have become increasingly attractive for biotechnology and medicines.4 Responsive hydrogels are smart materials that are capable of changing volume in response to specific external stimuli, such as temperature, pH, electric field, etc.

Poly(aspartic acid) (PASP) is a promising water-soluble and biodegradable polymer that can be produced from the hydrolysis of poly(succinimide) (PSI).5-6 PSI, the precursor polymer, is prepared by the thermal bulk polycondensation of aspartic acid or the ammonium salts of maleic and maleic acid. PASP consists of racemic aspartic acid residues with approximately 70 to 75% of α and β linkages, respectively. PASP becomes highly absorbent when neutralized and crosslinked, and is pH and electrolyte sensitive in water and body fluids.7-10 αβPoly(N-2-hydroxyethyl-DL-aspartamide), PHEA, is another important polymer, derived by coupling PSI with ethanolamine, which has potential as a plasma extender and a carrier for macromolecular prodrugs.11-14 The presence of hydroxyl groups in the side chain provides sites for forming a covalent linkage between the polymer and drug molecules or crosslinking reagents. This polymer has been demonstrated to have suitable physico-chemical characteristics for the development of macromolecular prodrugs, including biodegradability, high water solubility, and excellent biocompatibility. In addition, the PSI backbone can be easily modified to form hydrophilic/hydrophobic copolymers. Recently, many groups have reported the hydrophobic modification of PASP by grafting a hydrophobic alkyl chain onto the backbone of PSI polymers using aminolysis procedure. Therefore, long chain alkyl units can interact with each other to form intermolecular and intramolecular hydrophobic microdomains in water as a result of the self-aggregation of these copolymers.15-17

Recently, we examined the preparation and properties of biodegradable copolymers and their crosslinked hydrogels based on poly(aspartic acid) and poly(2-hydroxyethyl aspartamide).18,19,20 This paper reports the preparation of novel copolymers consisting of poly(2-hydroxyethyl aspartamide-co-N,N'-dimethyl-3-amino propyl aspartamide) (PHEA-DPA) from PSI, via a ring-opening reaction with N,N'-dimethyl-1,3-propane diamine (DPA) and ethanolamine followed by a crosslinking reaction with different amounts of hexamethylene disocyanate. The pH-responsive swelling behavior and morphology of the PHEA-DPA crosslinked gels were investigated.
Experimental Section

Materials. L-aspartic acid (98%+), \( \alpha \)-phosphoric acid (98%), \( N,N' \)-dimethyl-1,3-propanediamine (DPA, 99%), ethanolamine (EA, 99%), hexamethylene diisocyanate (HMDI, 98%), dibutylyl diurate (95%), and \( N,N' \)-dimethylformamide (DMF, Anhydrous 99.8%) were purchased from Aldrich Chemical Co. and used as received. Diethylether (99%) was obtained from DaeJung Chemical Co. (Korea) and used after distillation. Buffer pH solutions for swelling measurement were purchased from Aldrich Chemical Co. The dialysis membrane (Spectra/pore4 with MWCO 12,000-14,000) was used to eliminate any unreacted monomer and solvent.

Measurements. \(^1\)H-NMR spectra were recorded on a Bruker AMX-500 spectrometer using D2O as the solvent. The FT-IR spectra were obtained on a PerkinElmer FT-IR spectrometer (Model SPECTRUM 2000). The thermal analyses were carried out on a PerkinElmer DSC/TGA7 Series thermal analysis system at the heating rate of 10 °C/min in nitrogen. The morphology of the prepared gel scaffolds was observed by scanning electron microscopy (ESEM Model XL30 ESEM-FEG, Phillips). A porous gel sample were mounted onto a metal stub with double-sided carbon tape and coated with Pt for 30 s under vacuum (10⁻² Torr) using a plasma sputtering method (Ion sputter coater HC-21).

Synthesis of Polysuccinimide (PSI). L-aspartic acid (20 g) and \( \alpha \)-phosphoric acid (20 g) were charged into a round-bottom flask and stirred under reduced pressure at 200 °C for 5 h. The reaction mixture was then cooled and DMF was added to dissolve the product. The resulting solution was precipitated into 10-fold ethylether. The filtered precipitate of the poly(2-hydroxyethyl aspartamide-co-\( N,N' \)-dimethyl-3-aminopropyl aspartamide) (PHEA-DPA) copolymers were dialyzed using the membrane (MWCO 12,000-14,000) to remove any unreacted monomer and residue solvent. Finally, the dialysis product was freeze-dried to give an “off-white” powder (yield 83%).

Synthesis of PHEA-DPA Crosslinked Hydrogel. The prepared PHEA-DPA copolymer 1 g of PSI was dissolved in 10 mL DMF in a 3-neck round-bottom flask. 70 mol% of \( N,N' \)-dimethyl-1,3-propanediamine (DPA) was then added dropwise at 0 °C. Subsequently, the reaction flask was placed in a water bath controlled at 25 °C and stirred for 24 h. 50 mol% (excess) of ethanolamine (EA) was then slowly added to the solution and stirred for another 24 h. The final solution was then precipitated into a 10-fold ether solvent. The filtered precipitate of the poly(2-hydroxyethyl aspartamide-co-\( N,N' \)-dimethyl-3-aminopropyl aspartamide) (PHEA-DPA) copolymers were dialyzed using the membrane (MWCO 12,000-14,000) to remove any unreacted monomer and residual solvent. Finally, the dialysis product was freeze-dried to give an “off-white” powder (yield 80%).

Measurement of Swelling Capacity. The water absorbance (degree of swelling) of the above-prepared hydrogels was measured by gravimetric analysis. First, the dried samples (ca. 50 mg) were placed in distilled water at 25 °C and removed from the water at regular intervals. The weights of the hydrogels were recorded after wiping off the water on the surfaces of the hydrogels with moistened filter paper. The degree of swelling (swelling ratio) was defined as follows:

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\text{Degree of Swelling} = \frac{W_s}{W_d}
\]

Where, \( W_d \) and \( W_s \) are the weight of the dry and swollen hydrogels at time, \( t \).

Results and Discussion

Poly(\( N,N' \)-dimethyl-3-aminopropyl aspartamide) (PDPA) and Poly(2-hydroxyethyl-co-\( N,N' \)-dimethyl-3-aminopropyl aspartamide) (PHEA-DPA), as novel cationic polyspartamide derivatives, were prepared from polysuccinimide (PSI), which is the thermal polycondensation product of aspartic acid, via an amidolysis reaction with \( N,N' \)-dimethyl-
1,3-propane diamine (DPA) and ethanolamine (EA). The crosslinked gels were prepared from PHEA-DPA via a crosslinking reaction through the hydroxyl moiety (see Scheme 1). Although dialdehyde (ex. glutaldehyde) or diisocyanate compounds are among the most common crosslinking reagents, hexamethylene diisocyanate was used in this study.

Figure 1 shows the $^1$H-NMR spectra of PDP A (A) and PHEA-DPA (B). As shown in Figure 1, the proton peaks C, C', D, and E were assigned to the DPA pendants, and the F and G peaks were related to two methylene protons of the EA pendants. The composition of each group in the PHEA-DPA copolymer was determined from the integration ratio between peaks D and G. A typical copolymer with 20 mol% EA content was used for the next gel preparation.

Figure 2 shows the FT-IR spectra of PDP A (A), PHEA-DPA (B), and PHEA-DPA gel (C).

Figure 2 shows the FT-IR spectra of PDP A (A), PHEA-DPA copolymer (B) and PHEA-DPA crosslinked gel (C). The PDP A copolymer was prepared via an amidolysis reaction between PSI and DPA. As shown in Figure 2, spectrum (A) shows characteristic strong bands at 1649 cm$^{-1}$ (amide I), 1545 cm$^{-1}$ (amide II) and 3305 cm$^{-1}$ (-NH-) corresponding to the aspartamide backbone structure, and the band at 2950 cm$^{-1}$ corresponds to the CH$_2$ stretching band appeared after the amidolysis reaction between PSI and DPA. On the other hand, spectrum (B) shows broad bands at 3400 cm$^{-1}$-3100 cm$^{-1}$ (-OH stretching). Spectrum (C) shows additional characteristic bands that were assigned to urethane groups. New strong bands at 1250 cm$^{-1}$ and 1100 cm$^{-1}$, which were assigned to C-N and C-O stretching, with stronger alkylene absorption band at 2950 cm$^{-1}$ were
observed. FT-IR and 'H-NMR analyses indicated that the PDP A and PHEA-DPA copolymers had been prepared successfully from the aminolysis reaction of PSI.

Thermal analysis of the PDP A and PHEA-DPA under nitrogen showed Tg's of the polymer at 62 and 65 °C respectively, as determined by the mid-point of the baseline change in DSC. These polymers were stable up to ca. 200 °C without any weight loss.

Figures 3 and Figure 4 show the swelling of the prepared gels as a function of the level of HMDI, the crosslinking reagent, in pure water and the phosphate buffered saline (PBS) solution, respectively. The prepared gels were tested to determine their swelling properties in aqueous solutions using the tea-bag method, and the level of water absorbency was measured as a function of time. As shown in Figure 3, the initial fast swelling behavior appeared to level off after 3-5 h in pure water. The swelling ratios were controlled to be in the range of ca. 10-20 by varying the amount of HMDI. The degree of swelling decreased with increasing crosslinking agent due to the higher crosslinking density (estimated by the MW between crosslinks, Mc). The degree of swelling in the PBS (pH 7.4) solution decreased to some extent, as shown in Figure 4. The swelling ratios ranged from 7-14, corresponding to approximately 70% compared to those in pure water. This might be due to the increase in ionic strength of the hydrogels on account of the ionization of DPA with pendant tertiary amine groups. There was a similar dependence of the absorbency in PBS (pH 7.4) on the amount of the crosslinking agent to that observed in water.

Figure 5 shows the water absorbency in the different buffered pH solutions. Due to the presence of basic tertiary amine pendants on the polymer structure, the swelling behavior of the prepared gel can be affected by pH. At pH 2 the degree of swelling increased significantly due to ionization
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of the tertiary amine. The degree of swelling was lower at neutral and alkaline pH. Figure 6 shows the pulsatile swelling behavior of the PHEA-DPA crosslinked gel with pH alternating between pH 2 and pH 10. A reversible swelling and deswelling behavior was observed. As shown in Figure 5, the degree of swelling changed with pH, showing maximum pH 2 and minimum pH 10. After standing overnight at pH 10, a reversible swelling behavior was demonstrated in the same manner.

Figure 7 shows an SEM image of a typical freeze-dried PHEA-DPA crosslinked gel (HMDI 100 mol% sample). A well-interconnected microporous structure with 5-15 μm sized pores was observed. However, there was no appreciable difference in pore size among different gels prepared in this study. Further study on pore size control in this gel system is currently underway. Because of the many advantageous properties of the polyaspartamide polymers including biodegradability and biocompatibility, this PHEA-DPA hydrogel is potentially useful for biomedical applications such as tissue engineering and smart delivery systems.

Conclusion

Novel hydrogels consisting of PHEA-DPA were prepared from PSI via a ring-opening reaction with DPA and EA followed by a crosslinking reaction with HMDI. Hydrogels with a degree of swelling ranging from 10-20 g/g in water were obtained by varying the crosslinking density. It was confirmed that the swelling property was sensitive pH due to the ionization of DPA having a pendant tertiary amine. In addition, a reversible swelling and deswelling behavior of the gels was observed. The prepared hydrogels showed a well-interconnected network structure with regularly spaced micron-size pores.

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References