

Hydrolyzed Collagen-Based Hydrogel with Salt and pH-Responsiveness Properties

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ABSTRACT: A novel hydrolyzed collagen-based hydrogel has been prepared by grafting the binary mixture of acrylamide and 2-acrylamido-2-methylpropanesulfonic acid onto the collagen backbone in the presence of a cross-linking agent. Its physicochemical properties in aqueous solution were studied. The effect of reaction variables on both gel content and swelling capacity was investigated to achieve a hydrogel with improved absorbency and gel content. The absorbency under load of optimized hydrogel was also investigated by using an absorbency under load

tester at various applied pressures. The swelling ratio in various salt solutions was also determined and additionally, the swelling of hydrogels was measured in solutions with pH ranged 1–13. The synthesized hydrogel exhibited a pH-responsiveness character so that a swelling-collapsing pulsatile behavior was recorded at pH 2 and 8. © 2007 Wiley Periodicals, Inc. *J Appl Polym Sci* 106: 2371–2379, 2007

Key words: hydrogel; superabsorbent; collagen; swelling behavior; gel content

INTRODUCTION

Superabsorbent polymers (SAPs) are three-dimensional hydrophilic networks that can absorb and retain aqueous fluids up to thousands of times of their own weight. Because of their excellent response to changing environment conditions (e.g. temperature, pH, and solvent composition), SAPs have been attracting in many industrial applications such as hygienic products, horticulture, gel actuators, drug-delivery systems, as well as water-blocking tapes, and coal dewatering.^{1–6}

Natural-based SAPs have attracted much attention in medical and pharmaceutical interests because of their nontoxicity, biocompatibility, and biodegradability. Graft polymerization of vinylic monomers onto the natural backbones in the presence of crosslinkers is an efficient approach to synthesis of natural-based SAPs. Free radical graft copolymerization with various monomers can be carried out with different initiator systems. Considerable interest has been focused on employing natural polymers such as cellulose,⁷ starch,⁸ gelatin,⁹ chitosan,¹⁰ carrageenan,¹¹ and protein.^{12,13}

Proteins are widely distributed in nature and are synthesized mainly in animals, i.e. collagen, keratin, gelatin, and etc., and in a few plants such as Soya. In general, proteins are high molecular weight polymers and their solubility in aqueous solutions is dif-

ficult. Two efficient methods for preparation of aqueous soluble proteins are alkaline and enzymatic hydrolysis. According to the literature survey based on Chemical Abstract Service, there are some reports on grafting vinyl monomers, in the absence of crosslinker, onto collagen itself.^{14–16} In the present study, a three-dimensional hydrogel network was synthesized to prepare a superabsorbent. There is no report on grafting of mixture of acrylamide (AAm) and 2-acrylamido-2-methylpropanesulfonic acid (AMPS) onto hydrolyzed collagen, in the presence of MBA as a crosslinker.

Desired features of SAPs are (a) high swelling capacity, (b) high swelling rate, (c) good strength of the swollen gel (high AUL), (d) high swelling capacity in salt solutions, and (e) high gel content. Majority of reported SAPs comprises only the first feature mentioned, i.e. high load-free absorbency. Despite many efforts to synthesize SAPs in recent years, there are few studies for improving other parameters. In the present study, we attempted to synthesize a novel protein-based hydrogel and investigated the effect of reaction variables on both equilibrium swelling (ES) capacity in free state and gel content. As a matter of fact, a good hydrogel is one that has both aforementioned features together, i.e. high swelling capacity and high gel content. "High gel content" means "high efficiency in the synthesis" and, as a consequence, "low residual monomer," that is of prime importance in industrial applications. However, absorbency decreases with the increase of the gel content. Therefore, the preparation of hydrogels with high absorbency and high gel

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content is quite a challenge. Absorbency under load (AUL) was also studied. This is a very important factor that is often reported in the technical data sheets and patent articles. This parameter is of prime importance in many industrial applications, such as baby diapers, female personal care absorbent products, and water release systems in agriculture. The absorbency under load (AUL) of optimized hydrogel was determined by using an AUL tester in various applied pressures.¹⁷ Swelling behavior in various salt solutions was evaluated. Surprisingly, the hydrogel exhibited high water absorbency in 0.15M AlCl₃ solution (38 g/g). Finally, swelling behavior in various pH solutions was also determined. The synthesized hydrogel exhibited a pH-responsiveness character so that a swelling-collapsing pulsatile behavior was recorded at pH 2 and 8. Therefore, this hydrogel may be considered as an excellent candidate for various applications.

EXPERIMENTAL

Materials

Hydrolyzed collagen (from Parvar Novin-E Tehran, Iran) was industrial grade containing nearly 25% insoluble phosphate salts. *N,N'*-methylene bisacrylamide (MBA, from Fluka, Switzerland), ammonium persulfate (APS, from Fluka), acrylamide (AAm, from Merck, Darmstadt, Germany) 2-acrylamido-2-methylpropanesulfonic acid (AMPS, from Fluka) were of analytical grade and used without further purification. All other chemicals were also analytical grade. Double distilled water was used for the hydrogel preparation and swelling measurements.

Preparation of hydrogel

Hydrolyzed collagen (1.33 g) was dissolved in 50 mL distilled water and filtered to remove its insoluble salt. The solution was added to a three-neck reactor equipped with a mechanical stirrer and the reactor was immersed in a thermostated water bath preset at a desired temperature (80°C). Then the initiator solution (0.02–0.1 g APS in 5 mL H₂O) was added to the mixture. After stirring for 5 min, certain weight ratio of AMPS/AAm (0.14 (0.5 g/3.5 g) to 0.6 (1.5 g/2.5 g) in 5 mL H₂O) and MBA (0.02–0.1 g in 5 mL H₂O) were simultaneously added to the reaction mixture. After the completion of the reaction, the produced hydrogel was poured to excess nonsolvent ethanol (200 mL) and remained for 3 h to dewater. Then, ethanol was decanted and the product scissored to small pieces (diameter = 5 mm). Again, 100 mL fresh ethanol was added and the hydrogel was left for 24 h. Finally, the filtered hydrogel is dried in oven at 60°C for 24 h. After

grinding, the powdered superabsorbent was stored away from moisture, heat and light.

Swelling measurements using tea bag method

The tea bag (i.e. a 100-mesh nylon screen) containing an accurately weighed powdered sample (0.5 ± 0.001 g) was immersed entirely in 200 mL distilled water and allowed to soak for 2 h at 25°C. The sample particle sizes were 40–60 meshes (250–400 μm). The tea bag was hung up for 15 min to remove the excess solution. The equilibrium swelling (ES) was calculated according to the following equation:

$$ES(g/g) = \frac{W_s - W_d}{W_d} \quad (1)$$

where W_s and W_d are weights of swollen and dry gel, respectively. The accuracy of the measurements was ±3%.

Measurement of gel content

To determine the gel content values, a weighed sample was dispersed in double distilled water to swell for 72 h. After filtration, the extracted gel was dewatered by nonsolvent ethanol, dried (70°C, 5 h), and reweighed. Gel content (Gel %) was calculated by eq. (2).

$$\text{Gel} = \frac{m_f}{m_i} \times 100 \quad (2)$$

where m_i and m_f stand for initial weight of sample and final weight of sample, respectively.

Swelling in various salt solutions

Absorbency of the optimized sample was evaluated in 0.15M solutions of LiCl, NaCl, KCl, CsCl, MgCl₂, CaCl₂, SrCl₂, and BaCl₂ according to the earlier method described for swelling measurement in distilled water. In addition, swelling capacity of the hydrogel was measured in different concentration of NaCl, CaCl₂, and AlCl₃ salt solutions.

Absorbency at various values of pH

Individual solutions with acidic and basic values of pH were prepared by dilution of NaOH (pH 11.0) and HCl (pH 1.0) solutions to achieve pH ≥ 6.0 and pH < 6.0, respectively. The pH values were precisely checked by a pH-meter (Metrohm/620, accuracy ±0.1). Then, 0.5 (±0.001) g of the dried hydrogel was used for the swelling measurements according to eq. (1).

pH-sensitivity

pH-sensitivity of the optimized sample was investigated in terms of swelling and deswelling of the final product at two basic (pH 8.0) and acidic (pH 2.0) solutions, respectively. Swelling capacity of the hydrogels at each pH was measured according to eq. (1) at consecutive time intervals and for each cycle, a fresh solution was used.

Swelling kinetics

For studying the rate of absorbency of the SAPs, certain amount of samples (0.5 ± 0.001 g) with various particle sizes were poured into numbers of weighed tea bags and immersed in 200 mL distilled water. At consecutive time intervals, the water absorbency of the hydrogels was measured according to the earlier mentioned method.

Absorbency under load

A macroporous sintered glass filter plate (porosity no. 0, $d = 80$ mm, $h = 7$ mm) was placed in a Petri dish ($d = 118$ mm, $h = 12$ mm), and a weighed, dried hydrogel (0.5 ± 0.01 g) was uniformly placed on the surface of a polyester gauze located on the sintered glass. A cylindrical solid weight (Teflon, $d = 60$ mm, variable height) which could slip freely in a glass cyl-

inder ($d = 60$ mm, $h = 50$ mm) was used to apply the desired load (applied pressure 2068, 4137, and 6205 Pa (0.3, 0.6, and 0.9 psi)) to the dry hydrogel sample particles. Then, 0.9% saline solution was added so that the liquid level was equal to the height of the sintered glass filter. Whole of the set was covered to prevent surface evaporation and probable change in the saline concentration. After certain time intervals, the swollen particles were weighed again, and AUL was calculated according to eq. (1).¹⁷

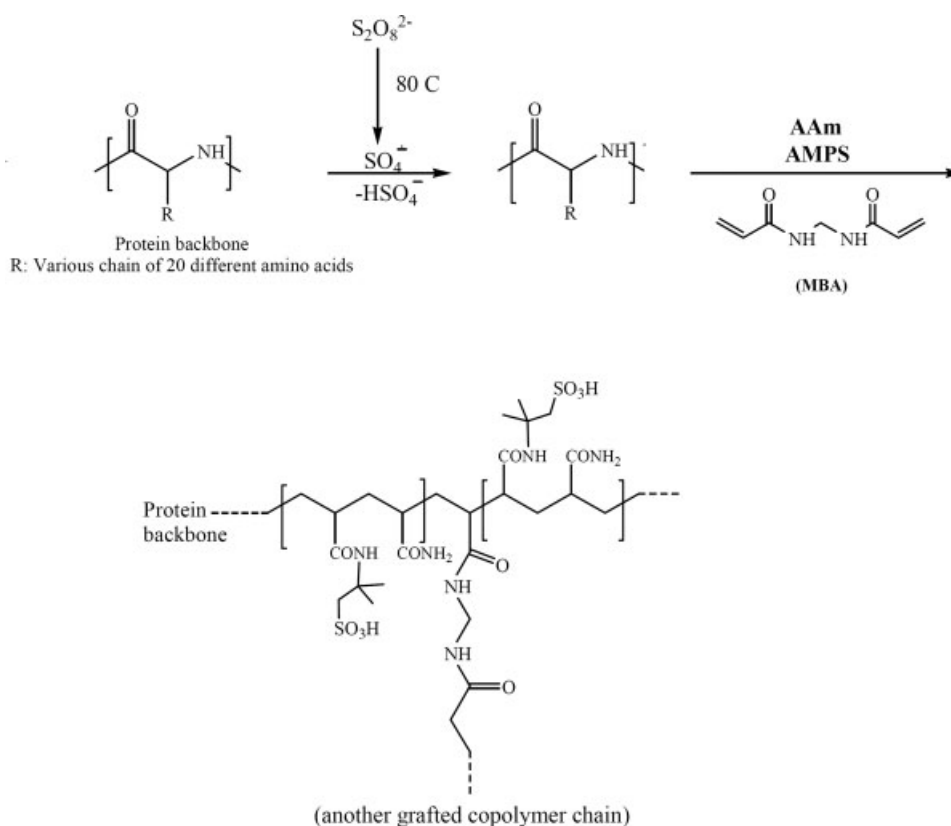
Instrumental analysis

FTIR spectra of samples were taken in KBr pellets using an ABB Bomem MB-100 FTIR spectrophotometer. The surface morphology of the gel was examined using scanning electron microscopy (SEM). Dried superabsorbent powder were coated with a thin layer of gold and imaged in a SEM instrument (Leo, 1455 VP).

RESULTS AND DISCUSSION

Synthesis and spectral characterization

The mechanism for simultaneous crosslinking graft copolymerization of AAm and AMPS onto collagen backbones in the presence of APS and MBA is shown in Scheme 1. At the first step, the thermally



Scheme 1 Proposed mechanistic pathway for synthesis of the hydrolyzed collagen-g-poly(AAm-co-AMPS) hydrogel.

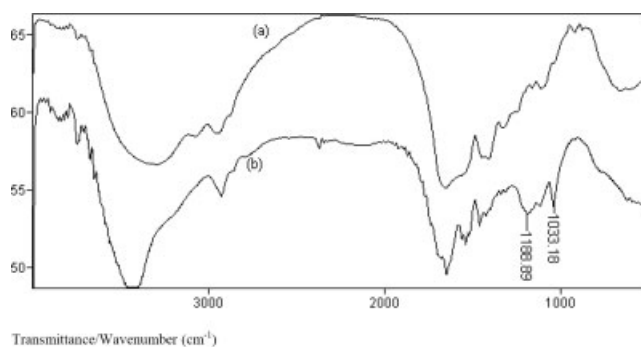


Figure 1 FTIR spectra of the hydrolyzed collagen (a) and hydrolyzed collagen-g-poly(AAm-co-AMPS) hydrogel (b).

dissociating initiator, i.e. APS, is decomposed under heating (80°C) to produce sulfate anion-radical. Then the anion-radical abstracts hydrogen from one of the functional groups in side chains (i.e. COOH, SH, OH, and NH_2) of the substrate to form corresponding radical. These macroradicals, in turn, initiate simultaneous graft copolymerization of AAm and AMPS onto collagen backbone. Since a crosslinking agent, e.g. MBA, is presented in the system, the copolymer comprises a crosslinked structure.

Infrared spectroscopy was carried out to confirm the chemical structure of the hydrogel. Figure 1 shows the FTIR spectra of the hydrolyzed collagen and the synthesized hydrogel. The band observed at 1654 cm^{-1} can be attributed to $\text{C}=\text{O}$ stretching in carboxamide functional groups of substrate backbone [Fig. 1(a)]. The broad band at $3200\text{--}3500\text{ cm}^{-1}$ is due to stretching of hydroxyl groups of the hydrolyzed collagen. The hydrogel comprises a protein backbone with side chains that carry carboxamide and sulfonate groups, which are evidenced by two new characteristic absorption bands at 1033 and 1189 cm^{-1} and a sharper band at $3300\text{--}3500\text{ cm}^{-1}$ [Fig. 1(b)].¹⁸

Furthermore, the resultant polymer was morphologically studied using SEM (Fig. 2). Surface of sample is completely smooth. No phase separation was observed in the SEM micrograph. This morphology confirms the homogeneity of our synthesized superabsorbent hydrogels.

Optimization of the grafting conditions

Different reaction parameters (i.e. AMPS/AAm ratio, APS, and MBA concentration) were systematically varied to achieve superabsorbent with improved water absorbency and gel content.

Effect of MBA concentration

Figure 3 demonstrates the effect of crosslinker concentration on swelling and gel content values of the

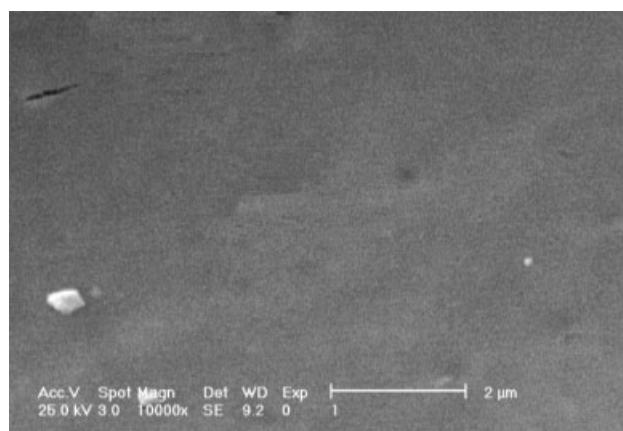


Figure 2 SEM photograph of optimized hydrogel. Surfaces were taken at a magnification of $\times 10,000$ and the scale bar is $2\text{ }\mu\text{m}$.

resultant hydrogel. As shown in Figure 3, a MBA concentration of 5.6 mmol/L provides the best values of swelling (211 g/g) and gel content (71%). At the lower than the latter amount, the gel content is diminished considerably because of the formation of very loosely crosslinked networks, resulting in highly swollen hydrogels with very low gel strength. More crosslinking concentration causes to the higher crosslinking density and decreases the space between the copolymer chains and consequently, the resulted highly crosslinked rigid structure cannot be expanded and hold a large quantity of water. In fact, in all hydrogels a small increase in degree of crosslinking causes an appreciable decrease in swelling

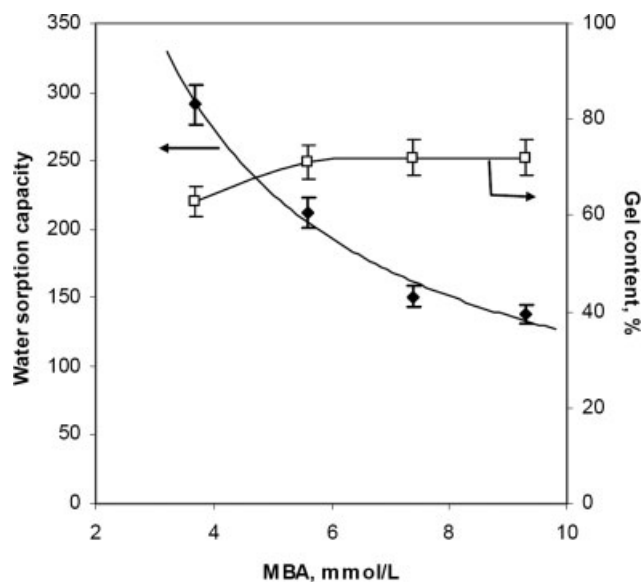


Figure 3 Dependency of swelling and gel content of the hydrolyzed collagen-g-poly(AAm-co-AMPS) hydrogel vs. the crosslinker concentration employed in the polymerization process. Reaction conditions: Collagen 1.33 g , AMPS/AAm ratio 0.33 , APS 6.3 mmol/L , 80°C , 60 min .

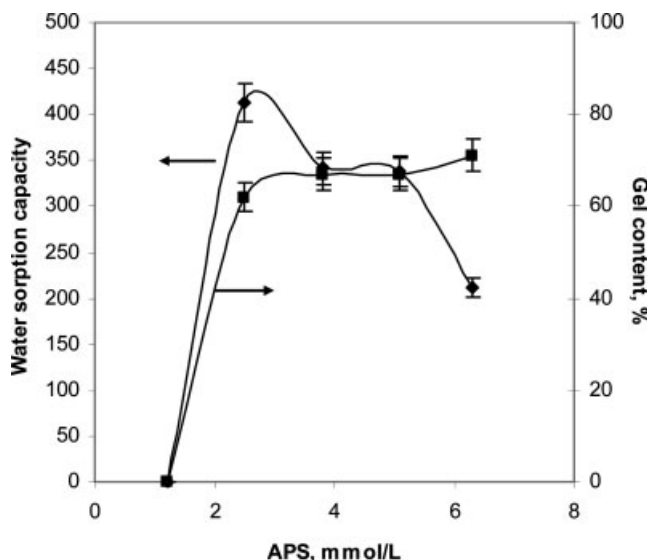


Figure 4 Dependency of swelling and gel content of the hydrolyzed collagen-g-poly(AAm-co-AMPS) hydrogel vs. the initiator concentration employed in the polymerization process. Reaction conditions: Collagen 1.33 g, AMPS/AAm ratio 0.33, MBA 5.6 mmol/L, 80°C, 60 min.

capacity. Such well-known behavior was reported by pioneering scientists.¹⁹

Figure 3 exhibits a power law behavior of swelling-[MBA], with $K = 6.99$ and $n = 0.91$ which is obtained from the curve fitted with eq. (3).

$$\text{Swelling capacity} \approx K[\text{MBA}]^{-n} \quad (3)$$

where K and n are constant values for an individual superabsorbent. The n value represents the extent of the sensitivity of the hydrogel to the crosslinker content, while the K value gives an amount useful for comparing the extent of swelling versus fixed crosslinker content.

Effect of APS concentration

The relationship between the initiator concentration, water absorbency and gel content values were studied by varying the APS concentration from 1.2 to 6.2 mmol/L (Fig. 4). According to Figure 4, the absorbency is decreased with increasing the APS concentrations from 2.5 up to 6.3 mmol/L. When the concentration of initiator is less than 2.5 mmol/L, the resulted polymers are completely soluble. Although, the maximum water absorbency (412 g/g) is achieved at concentration of 2.5 mmol/L of the initiator, its gel content (62%) is less than that of synthesized hydrogel at 3.8 mmol/L of APS (67%), so this concentration of initiator provides the best values of swelling (342 g/g) and gel content (67%). Initial increment in water absorbency may be attributed to increased number of active free radicals on the

protein backbone. Subsequent decrease in swelling is originated from an increase in terminating step reaction via bimolecular collision which, in turn, causes to enhance crosslinking density. This possible phenomenon is referred to as “self-crosslinking” by Chen and Zhao.²⁰ In addition, the free radical degradation of collagen backbones by sulfate radical-anions is an additional reason for swelling-loss at higher APS concentration. A similar observation is recently reported in the case of polysaccharides such as chitosan²¹ and carrageenan.²²

Effect of AMPS/AAm ratio

The swelling capacity and gel content of superabsorbent as a function of the co-monomers ratio is illustrated in Figure 5. As shown in this figure, an AMPS/AAm ratio of 0.33 provides the best values of swelling (342 g/g) and gel content (67%). It is observed that both of absorbency and gel content are substantially increased with increasing in the AMPS/AAm ratio and then they are decreased. The initial increment in swelling values can be attributed to increase in ionic groups existing in copolymer chains along with increase of AMPS in the gel which allows polymer coils to expand more easily. The gel content variation at low AMPS/AAm ratio (Fig. 5) can be attributed to more homopolymerization reaction over graft copolymerization due to higher reaction viscosity. The gel and swelling-loss after the maximum may be originated from reducing the gel strength due to the higher amount of AMPS. Similar conclusions were reported by other investigators.^{23,24}

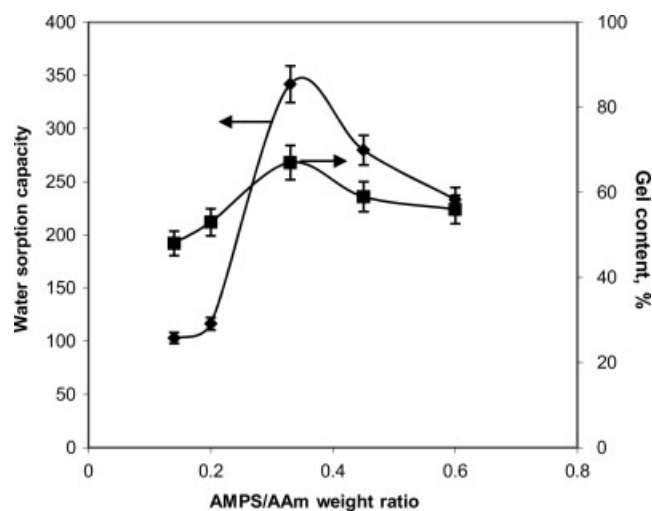


Figure 5 Dependency of swelling and gel content of the hydrolyzed collagen-g-poly(AAm-co-AMPS) hydrogel vs. monomer ratio employed in the polymerization process. Reaction conditions: Collagen 1.33 g, MBA 5.6 mmol/L, APS 3.8 mmol/L, 80°C, 60 min.

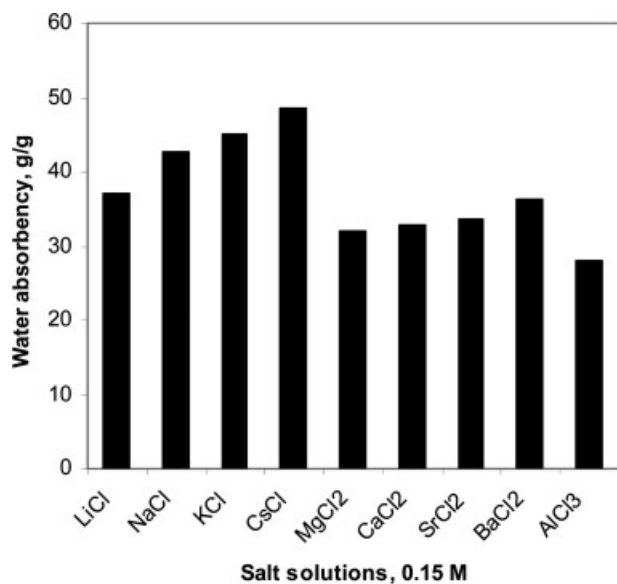


Figure 6 Swelling capacity of the hydrolyzed collagen-g-poly(AAm-co-AMPS) hydrogel in different chloride salt solutions (0.15M).

Swelling in various salt solutions

It is important to know the swelling behavior of superabsorbent hydrogels in salt solutions for many applications, especially agricultural and horticultural ones. Figure 6 shows the effect of the ionic strength of various salt solutions on the water absorbency of the superabsorbent hydrogels. It is well-known that the water absorbency decreased with an increase in the ionic strength of the salt solutions. This result may be attributed to the reduction in the osmotic pressure difference between the superabsorbent hydrogel and the external salt solution with increasing ionic strength. In addition, the screening effect of the additional cations on the anionic group caused a nonperfect anion-anion electrostatic repulsion and reduced the water absorbency.²⁵ Similar results have been reported in similar previous studies.^{26,27}

In this series of experiments, the swelling capacity was measured in various salt solutions (Figs. 6 and 7). It is obvious that swelling decrease is strongly depended on the "type" and "concentration" of salt added to the swelling medium. The effect of cation type (cations with different radius and charge) on swelling behavior is shown in Figure 6. Degree of crosslinking is increased with increasing the charge of cation, and swelling is consequently decreased. Therefore, the absorbency for the hydrogel in the studied salt solutions is in the order of monovalent > divalent > trivalent cations. The effect of cation radius on swelling may also been observed from Figure 6. As reported by Pass et al.,²⁸ anion interacts with small cations, e.g. Li⁺, stronger than with large cations, e.g. Cs⁺. The stronger interactions of anion-

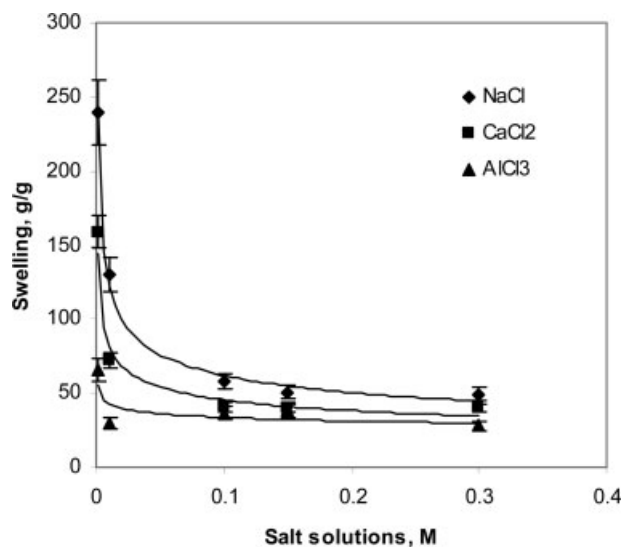


Figure 7 Swelling capacity variation of the hydrolyzed collagen-g-poly(AAm-co-AMPS) hydrogel in saline solutions with various concentrations.

small cation have been observed using measurement of activating coefficients of various cations in several salt solutions. As a result, the absorbency in monovalent and divalent cation salt solutions are in the order of Cs⁺ > K⁺ > Na⁺ > Li⁺ and Ba²⁺ > Sr²⁺ > Ca²⁺ > Mg²⁺, respectively.

Figure 7 illustrates a reverse and power law relationship between concentration of salt solutions (NaCl, CaCl₂, and AlCl₃) and swelling capacity of the hydrogel. Again, charge screening effect and ionic crosslinking are the main explanations for the intense loss of swelling. The known relationship between swelling and concentration of salt solution is stated as following equation¹⁹:

$$\text{Swelling} = k[\text{salt}]^{-n} \quad (4)$$

where k and n are constant values for an individual superabsorbent. The k value is swelling at a high concentration of salt and n value is a measure of salt sensitivity. Figure 7 indicates that changing of the salt concentrations higher than $\sim 0.2M$ has no appreciable influence on superabsorbency of the superabsorbent. As given in Table I, the k values are almost the same (~ 9) for the swelling in various salt solu-

TABLE I
Values k and n (as Obtained from the Curve Fitting, Fig. 7) for the Hydrolyzed Collagen-g-poly(AAm-co-AMPS) Hydrogel

Swelling medium	k	n
NaCl	9.3	0.52
CaCl ₂	9.2	0.65
AlCl ₃	9.4	0.81

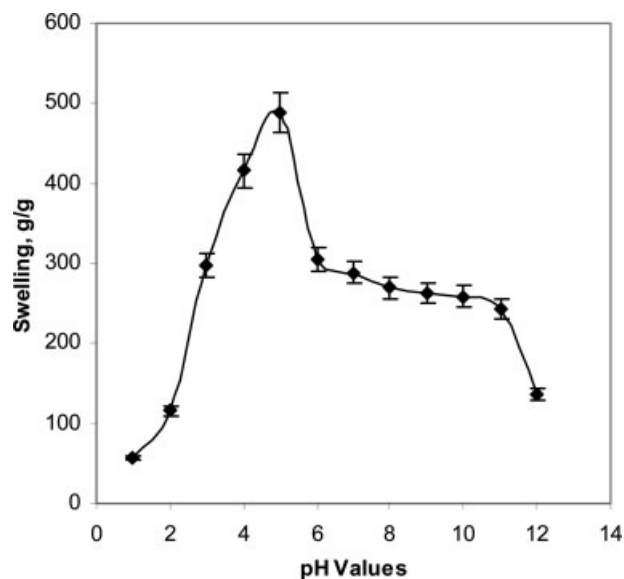


Figure 8 Effect of pH of solutions on swelling capacity of the hydrolyzed collagen-g-poly(AAm-co-AMPS) hydrogel.

tions. The n values are proportionally changes with the cation valency enhancement. Here, the effect of the ionic crosslinking acts as more effective factor against swelling rather than the charge screening effect of the cation.

Effect of pH on ES

Ionic superabsorbent hydrogels exhibit swelling changes at a wide range of pH values. Since the swelling capacity of all "ionic" hydrogels is appreciably decreased by addition of counter ions to the swelling medium, no buffer solutions were used. Therefore, stock NaOH (pH 11.0) and HCl (1.0) solutions were diluted with distilled water to reach desired basic and acidic pH values, respectively. As shown in Figure 8, the absorbency of the optimized sample increased sharply as the pH increased from 2 to 5 and drastically decreased in the pH higher than 12. In addition, the change in the water absorbency was slight from pH 6 to 11.

Maximum swelling (490 g/g) was obtained at pH 5. With increasing the pH, the sulfonic acid groups are neutralized, therefore, the electrostatic repulsion of the chains of the polymer increases, and this leads to an enhancement of the water absorbency. Again, a charge screening effect of the counter ions (cations) limit the swelling at higher basic pH (pH > 11). Similar results have been reported in the case of other hydrogel systems.^{29,30}

pH-responsiveness behavior of the hydrogel

The pH reversibility of these hydrogels was investigated in 0.01M solutions with pH 2 and pH 8

(Fig. 9). Since the pH of the working solutions may be changed by protonation–deprotonation process of superabsorbent hydrogel, a fresh solution was used in each cycle. At pH 8.0, the hydrogel swells up to 370 g/g because of anion–anion repulsive electrostatic forces, while at pH 2.0, it shrinks within a few minutes because of protonation of sulfonate groups. Because of the using the fresh solutions, as shown in Figure 9, the absorbency is increased after each cycle. This sharp swelling–deswelling behavior of the hydrogels makes them as suitable candidate for many probable applications. Such on–off switching behavior as reversible swelling and deswelling has been reported for other ionic hydrogels.^{31,32}

Swelling kinetics

In practical applications, not only a higher swelling capacity is required, but also a higher swelling rate is needed. Buchholz has suggested that the swelling kinetics for the superabsorbents is significantly influenced by factors such as swelling capacity, size distribution of powder particles, specific size area, and composition of polymer.³³ Figure 10 represents the dynamic swelling behavior of the superabsorbent samples with various particle sizes in water. Initially, the rate of water uptake sharply increases and then begins to level off. The time required to reach the ES capacity was achieved after ~ 20 min. A power law behavior is obvious from Figure 10. The data may be well fitted with a Voigt-based equation [eq. (5)]³⁴:

$$S_t = S_e(1 - e^{-t/\tau}) \quad (5)$$

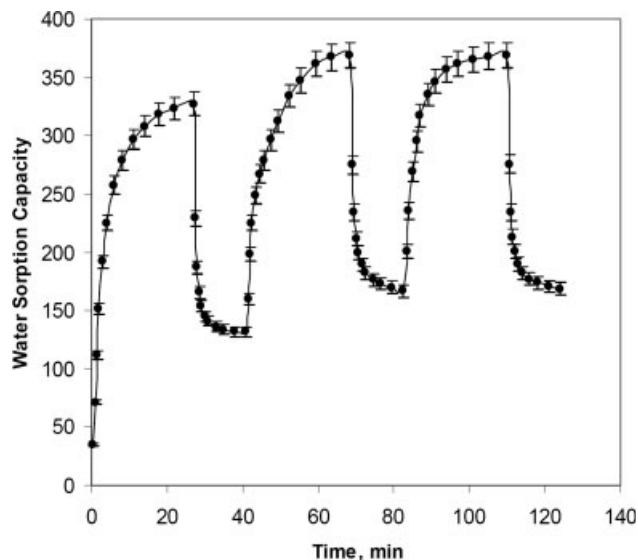


Figure 9 On-off switching behavior as reversible pulsatile swelling (pH 8.0) and deswelling (pH 2.0) of the hydrolyzed collagen-g-poly(AAm-co-AMPS) hydrogel. The time interval between the pH changes was 20 min.

where S_t (g/g) is swelling at time t , S_e is equilibrium swelling (power parameter, g/g); and τ (min) stand for the "rate parameter." The rate parameters for superabsorbent are found to be 4.5, 6.2, and 11.3 min for superabsorbents with particle sizes of 100–250, 250–400, and 400–550 μm , respectively. It is well-known that the swelling kinetics for the SAPs is significantly influenced by particle size of the absorbents.³⁵ With a lower the particle size, a higher rate of water uptake is observed. An increase in the rate of absorption would be expected from the increase in surface area with decreasing particle size of hydrogel.

Absorbency under load

The AUL is an important factor to determine the swollen gel strength in the practical application of superabsorbents, which is usually given in the patent literature and technical data sheets offered by industrial hydrogel manufacturers. The AUL of samples is determined by using an AUL tester according to a procedure reported earlier.¹⁷ The results are shown in Figure 11. As shown, the minimum time needed for the highest AUL in the case of each load was determined to be 60 min. After this time, the AUL values were unchanged. In addition, AUL slightly decreases with increasing the amount of loading which confirms low sensitivity of the hydrogel to different loads.

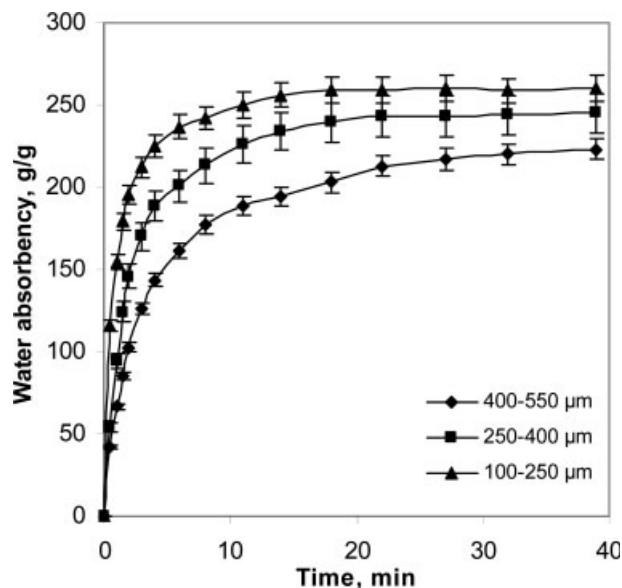


Figure 10 Representative swelling kinetics of the hydrolyzed collagen-g-poly(AAm-co-AMPS) hydrogel with various particle sizes.

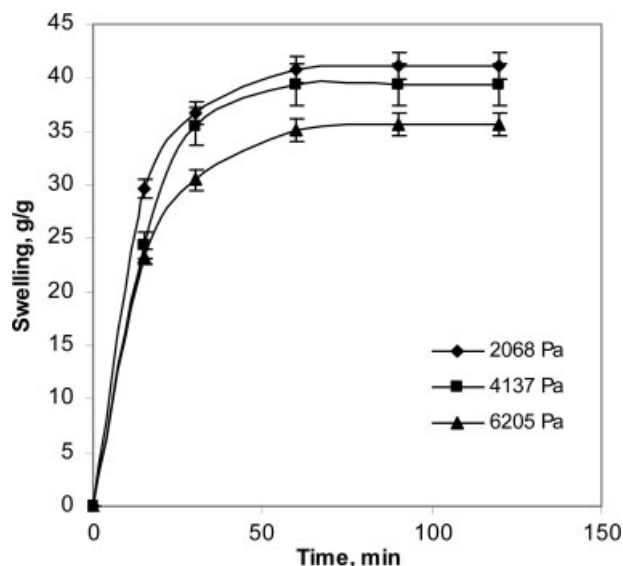


Figure 11 Time dependence of the AUL values for optimized sample of hydrolyzed collagen-g-poly(AAm-co-AMPS) hydrogel swollen in saline solution.

CONCLUSION

In the present study, we prepared a novel superabsorbent hydrogel by crosslinking graft copolymerization of hydrolyzed collagen. The optimum reaction conditions to obtain improved water absorbency (342 g/g) and gel content (67%) were found to be: MBA 5.6 mmol/L, AMPS/AAM 0.33, and APS 3.8 mmol/L. Swelling measurement of the optimized hydrogel in different salt solutions showed appreciable swelling capacity, without considerably swelling loss in small cation solutions, in comparison with our previous works. Also, the superabsorbent hydrogel exhibited high sensitivity to pH, so that, several swelling changes of the hydrogel were observed as a function of pH variations in a wide range (1–13). Furthermore, the reversible swelling–deswelling behavior in solutions with acidic and basic pH makes the hydrogels as a suitable candidate for various probable applications. In addition, because of the use of protein as a natural backbone, it is expected that the resulted gels show more compatibility with human body. The AUL of optimized hydrogel was also investigated at various applied pressures. Finally, dynamic swelling kinetics of the hydrogels shows that the rate of absorbency is increased with decreasing the particle size of superabsorbing samples.

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