INTRODUCTION

Highly swelling polymers, i.e., superabsorbent hydrogels, are hydrophilic, three dimensional networks that can absorb water in the amount from 10% up to thousands of times their dry weight. They are widely used in various applications such as hygienic, foods, cosmetics, and agriculture. This accounts for increase in the worldwide production of superabsorbent polymers (SAPs) from 6000 tons in 1983 to 450000 tons in 1996. Nowadays, the worldwide production of SAPs is more than one million tons in year. Hence, synthesis and characterization of superabsorbent hydrogels is the main goal of the several research groups in the world.

Hydrogels responding to external stimuli such as heat, pH, electric field, chemical environments, etc, are often referred to as "intelligent" or "smart" hydrogels. These responsive hydrogels have become an important area of

SYNTHESIS OF A NOVEL pH- AND SALT-RESPONSIVE SUPER ABSORBENT HYDROGEL BASED ON COLLAGEN-G-POLY(AA-CO-IA)

MOHAMMAD SADEGHI* and MOJGAN YARAHMADI²

¹Department of Chemistry, Science Faculty, Islamic Azad University, Arak Branch, Arak (Iran).
²Department of English, Humaniti Faculty, Islamic Azad University, Arak Branch, Arak (Iran).
E-mail: m-sadeghi@iau-arak.ac.ir

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ABSTRACT

In this work, a novel family of pH-responsive polymeric hydrogel based on collagen was prepared. Acrylic monomers, acrylic acid (AA) and itaconic acid (IA) were simultaneously graft copolymerized onto collagen backbones by a free radical polymerization technique using ammonium persulfate (APS) as initiator and methylene bisacrylamide (MBA) as a crosslinker. A mechanism for the superabsorbent hydrogel formation was also suggested. Hydrogel formation was confirmed by FTIR spectroscopy. Results from scanning electron microscopy (SEM) observation also showed a porous structure with smooth surface morphology of the hydrogel. The swelling capacity of the hydrogels was also measured in various salt solutions (LiCl, NaCl, KCl, CaCl₂ and AlCl₃). Due to high swelling ability in salt solutions, the hydrogel may be referred as "anti-salt superabsorbent" polymers. Furthermore, the water absorbency of hydrogels was measured in solutions with pH ranged 1 to 13. The collagen-based hydrogel exhibited a pH-responsiveness character so that a swelling-deswelling pulsatile behavior was recorded at pHs 2 and 8.

Key words: Collagen, Hydrogel, pH- and salt-responsive, Vinylic monomers.
research and development in the field of medicine, pharmacy and biotechnology.

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 difficult. Two efficient methods for preparation of aqueous soluble proteins are alkaline and enzymatic hydrolysis. According to the literature survey based on Chemical Abstract Service, a few studies have been reported in the case of protein-based hydrogels. Hence, the target of the current study was to exploit novel pH- and salt-responsive collagen-based hydrogels as a new natural-based polymer with pH, salt-responsiveness properties.

**EXPERIMENTAL**

**Materials**

Hydrolyzed collagen (Parvar Novin-E Tehran Co.) was industrial grade which is available in market and has nearly 25% insoluble phosphate salt. Acrylic acid (AA, Merck) was used after vacuum distillation. Itaconic acid (IA, Merck) and ammonium persulfate (APS, Merck) was used without purification. Methylene bisacrylamide (MBA, Fluka) was used as received. All other chemicals were of analytical grade.

**Preparation of hydrogel**

A general procedure for chemically crosslinking graft copolymerization of AA and IA onto collagen backbones was conducted as follows. Hydrolyzed collagen (1.33 g) was dissolved in 50 mL distilled water and filtered to remove its insoluble phosphate salt. Then the solution was added to a three-neck reactor equipped with a mechanical stirrer (Heidolph RZR 2021, three blade propeller type, 300 rpm). The reactor was immersed in a thermostated water bath preset at a desired temperature (80 °C). Then a definite amount of APS solution (0.1 g in 5 mL H2O) was added to collagen solution and was allowed to stir for 10 min. After adding APS, certain amounts of AA and IA (AA 1.20 g, IA 0.80) were added simultaneously to the collagen solution. MBA solution (0.05 g in 5 mL H2O) was added to the reaction mixture after the addition of monomers and the mixture was continuously stirred. After 60 min, the reaction product was allowed to cool to ambient temperature and neutralized to pH 8 by addition of 1N sodium hydroxide solution. The hydrogel was poured to excess non solvent ethanol (200 mL) and kept for 3 h to dewater. Then ethanol was decanted and the product scissored to small pieces. Again, 100 mL fresh ethanol was added and the hydrogel was kept for 24 h. Finally, the filtered hydrogel is dried in oven at 60°C for 10 h. After grinding using mortar, the powdered superabsorbent was stored away from moisture, heat and light.

**Swelling measurements**

Hydrogel (0.2g) were immersed in 250 mL solution with various pH values (pH 1-13) at 37 °C to reach swelling equilibrium. Swollen samples were then separated from unabsorbed water by filtering through a 100-mesh screen under gravity for 30 min without blotting the samples. The equilibrium swelling (ES) capacity in buffer solution was calculated according to the following equation:

\[
\text{ES (g/g)} = \frac{\text{Weight of swollen gel} - \text{Weight of dried gel}}{\text{Weight of dried gel}} \]

**Instrumental analysis**

Fourier transform infrared (FTIR) spectra of samples were taken in KBr pellets, using an ABB Bomem MB-100 FTIR spectrophotometer (Quebec, Canada), at room temperature. The surface morphology of the gel was examined using scanning electron microscopy (SEM). After Soxhlet extraction with methanol for 24 h and drying in an oven, superabsorbent powder was coated with a thin layer of gold and imaged in a SEM instrument (Leo, 1455 VP).

**RESULTS AND DISCUSSION**

**Synthesis of hydrogels**

The mixture of monomers, acrylic acid and itaconic acid, was simultaneously grafted onto collagen backbones in a homogeneous medium using APS as a radical initiator and MBA as a crosslinking agent. A general reaction mechanism for collagen-based hydrogel formation is shown in Scheme 1. At the first step, the thermally dissociating initiator, i.e. APS, is decomposed under heating 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 NH₂) of the substrate to form corresponding radical. So, these macroradicals initiated monomers grafting onto collagen backbones led to a graft copolymer. In addition, crosslinking reaction was carried out in the presence of a crosslinker, i.e., MBA, so that a three dimensional network was obtained.

Scheme 1: Proposed mechanistic pathway for synthesis of the collagen-based hydrogels.

FTIR spectroscopy
The grafting was confirmed by comparing the FTIR spectra of the collagen substrate with that of the grafted products. The band observed at 1644 cm⁻¹ can be attributed to C=O stretching in carboxamide functional groups of substrate backbone (Figure 1a). The superabsorbent hydrogel product comprises a collagen backbone with side chains that carry sodium carboxylate functional groups that are evidenced by peak at 1561 cm⁻¹ (Figure 1b). This characteristic band is due to asymmetric stretching in carboxylate anion that is reconfirmed by another peak at 1422 cm⁻¹ which is related to the symmetric stretching mode of the carboxylate anion¹³.

Fig. 1: FTIR spectra of collagen (a) and collagen-g-poly(AA-co-IA) hydrogel (b).
Morphology of hydrogel

The morphology of the crosslinked hydrogel was observed by scanning electron microscope (SEM). Although the water inside the hydrogel was sublimed to make cavities, the structure of the hydrogel was preserved. The SEM image is shown in Figure 2. The structure of the hydrogel is very porous, and it could help to form a high-water-content hydrogel, as is generally shown in other hydrogels\textsuperscript{14}.

![SEM photograph of the hydrogel. Surfaces were taken at a magnification of 1000, and the scale bar is 20 µm](image)

Effect of post-neutralization pH

In this series of experiments, after the alkaline hydrolysis, excess NaOH was neutralized by acetic acid solution to a desired pH value (Fig. 3). Without the post-neutralization stage (pH≈13), the decreased absorbency is related to "screening effect" of excess Na\textsuperscript{+} ions in the swelling media (This effect was described in previous sections). In acidic media, a similar behavior is observed due to different phenomena. At pH<8, the carboxylate anions are protonated, i.e. Na\textsuperscript{+} ions are replaced by H\textsuperscript{+} ion. Thus, the main anion-anion repulsive forces are eliminated and, instead, some sort of attractive interactions (H-O and H-N hydrogen bonding) lead to decreased absorbencies. According to Fig. 3, the best pH adjusted after hydrolysis was found to be 8.0.

![Effect of pH of solution on swelling of Collagen-g-poly(AA-co-IA) hydrogel](image)

This behavior has also been observed in the case of commercial acrylic acid-based SAPs\textsuperscript{15} as a standard crosslinked polyelectrolyte.
Indeed, the effective pKa for carboxylic acid groups is ~4.2-4.7. In Figure 4, the dependence of the equilibrium swelling of the Collagen-g-poly(AA-co-IA) hydrogel is characterized by a curve with two maximum at pHs 2 and 8. The remarkable swelling changes are due to the presence of different interacting species depending on pH of the swelling medium. It can be assumed that Collagen-g-poly(AA-co-IA) hydrogel includes collagen, poly(acrylic acid) and poly(itaconic acid) structures. Therefore, based upon pK_a of PAA (~4.7) and pK_a of PIA (4.2), the involving species is COOH (at pHs 1-2), COO^- (at pHs 7-13). At pH 8, the carboxylic acid groups become ionized and the electrostatic repulsive force between the charged sites (COO^-) causes increasing in swelling. Again, a screening effect of the counter ions (Na^+) limits the swelling at pHs 9-13.

The Collagen-g-poly(AA-co-IA) hydrogels were also showed reproducible swelling-deswelling cycles at pH 2.0 and 8.0 as demonstrated in Figure 5. At pH 8.0, the hydrogel swells up to 124.8 g/g due to anion-anion repulsive electrostatic forces, while at pH 2.0, it shrinks within a few minutes due to protonation of carboxylate groups. This sharp swelling-deswelling behavior of the hydrogels makes them as suitable candidate for controlled drug delivery.

**Swelling and Dewelling Kinetics**

The rate of absorbency for Collagen-g-poly(AA-co-IA) superabsorbent hydrogel was measured in town water and in 0.15 molar salt solutions of LiCl, NaCl, KCl, CaCl_2, and AlCl_3 at room temperature. According to Figure 5, the swelling values versus swelling time follow a power low trend. A “Voigt-based” model may be used for fitting the data:

\[
S_t = S_e (1 - e^{-t/\tau})
\]

where \(S_t\) is the swelling at time \(t\), \(S_e\) is the equilibrium swelling (power parameter) and \(\tau\) (min) is the “rate parameter”. The power parameter (equilibrium swelling capacity) according to Figure 5 is 205 g/g for town-water. The \(\tau\) value is a measure of swelling rate (i.e. the lower the \(\tau\) value, the higher the rate of swelling). Therefore, it can be used for comparative evaluating the rate of absorbency of superabsorbents that the particle size of the comparing samples are the same or, at least, in the same range. It is well known that the swelling kinetics for the superabsorbent polymers is significantly influenced by particle size of the absorbents. The size of the dried hydrogel particles used in our experiment was 300-450 µm. For calculate the rate parameter, by using the above formula and a little rearrangement, one can be plot versus time \(t\). The slope of the straight line fitted (slope = -1/\(\tau\)) gives the rate parameter. For example, in the case of town-water the \(\tau\) value is 23 min. It means that the Collagen-g-poly(AA-co-IA) take 23 minutes to absorb 0.65 of its equilibrium capacity of swelling. The rate parameters for the Collagen-g-poly(AA-co-IA) hydrogel in town-water and LiCl, NaCl, KCl, CaCl_2, and AlCl_3 salt solutions are found.
to be 23, 4, 5, 7, 8, and 3 min, respectively. According to the smaller $\tau$ value, the swelling in AlCl$_3$ is faster than in other solutions.

According to Figure 5, the swelling capacities of Collagen-g-poly(AA-co-IA) in salt solutions are decreased comparing with the value measured in town-water (205 g/g). Generally, swelling values for all “anionic” hydrogels in saline media are expectedly decreased$^{17}$. This undesired swelling-loss has been attributed to the “charge screening effect” of the cations led to the reduction of osmotic pressure, the driving force for swelling, between the gel and the aqueous phases. An additional reason is increasing electrostatic attraction between anionic sites of chains and multivalent cations (Ca$^{2+}$ and Al$^{3+}$) leading to increased “ionic crosslinking” degree and consequently loss of swelling. The effect of charge of cation on swelling can be concluded from Figure 5. As shown in the figure, the absorbency of Collagen-g-poly(AA-co-IA) hydrogel in the studied salt solutions is in the order of monovalent $>$ divalent $>$ trivalent cations. With increasing the charge of cation, degree of crosslinking is increased and swelling is consequently decreased.

![Swelling kinetics of hydrogel](image)

**Salt-sensitivity of Collagen-g-poly(AA-co-IA) hydrogel**

Swelling capacity in salt solutions is of prime significance in many practical applications such as personal hygiene products and water release systems in agriculture. The swelling ability of “anionic” hydrogels in various salt solutions is appreciably decreased compared to the swelling values in distilled water. This well-known undesired swelling-loss is often attributed to a “charge screening effect” of the additional cations which causing a non-perfect anion–anion electrostatic repulsion$^{18}$. Also, in salt solution the osmotic pressure resulting from the difference in the mobile ion concentration between gel and the aqueous phases is decreased and consequently the absorbency amounts are diminished. In addition, in the case of salt solutions with multivalent cations, “ionic crosslinking” at surface of hydrogel particles causing an appreciably decrease in swelling capacity.

Since the Collagen-based hydrogels are comprised poly(NaAA-co-AI) chains with carboxylate groups that can interact with cations, they exhibit various swelling capacity in different salt solutions with same concentrations. In the presence of the bivalent calcium ions, the crosslinking density...
increases because of a double interaction of Ca\(^{2+}\) with carboxylate groups leading to “ionic crosslinking”. The swelling–deswelling cycle of the hydrogel in sodium and calcium salts are shown in Figure 6. In sodium solution, swelling of the hydrogel is increased with time. When this hydrogel is immersed in calcium chloride solution, it deswells to a collapsed form. When the shrunk hydrogel is immersed in sodium chloride solution again, the calcium ions are replaced by sodium ions. This ion exchange disrupts the ionic crosslinks leading to swelling enhancement. As a result, when hydrogel is treated alternatively with NaCl and CaCl\(_2\) solutions with equal molarity, the swelling reversibility of hydrogel is observed\(^{19}\).

![Swelling-deswelling cycle of the hydrogel Collagen-g-poly(AA-co-IA) hydrogel in distillated water and sodium chloride salt (a) and in sodium chloride and calcium chloride (b) (Fig. 6)](image)

**CONCLUSION**

The superabsorbent hydrogel, Collagen-g-poly(AA-co-IA), was synthesized by graft copolymerization of methacrylic acid onto collagen, in a homogeneous medium. The maximum water absorbency in distillated water (606 g/g) was achieved. The swelling of the hydrogels exhibited a high sensitivity to pH in 3 and 8. Net effect of H\(^{+}/\)OH\(^{-}\) concentration was examined at various pHs in absence of any buffer solution. It also exhibited ampholytic nature of pH-responsiveness in swelling behavior. Swelling capacity of Collagen-g-poly(AA-co-IA) hydrogel in various salt solutions, especially in LiCl, NaCl, and KCl solutions is appreciable. However, swelling-loss in salt solutions, in comparison with distilled water and town water, can be attributed to charge screening effect and ionic crosslinking for mono- and multi-valent cations, respectively.
REFERENCES


