



Synthesis and study on effect of various chemical conditions on the swelling property of collagen-*g*-poly(AA-*co*-IA) superabsorbent hydrogel

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Abstract

In this work, a novel family of salt-responsive polymeric hydrogel based on collagen was prepared via simultaneously graft copolymerization of acrylic monomers, acrylic acid (AA) and itaconic acid (IA) onto collagen backbones by a free radical polymerization technique using ammonium persulfate (APS) as initiator and methylene bisacrylamide (MBA) as a crosslinker. The effect of grafting variables, i.e. AA/IA weight ratio and concentration of MBA and APS, was systematically optimized to achieve a hydrogel with swelling capacity as high as possible. The water absorbency increased with increasing the AA/IA amount in the monomer feed due to formation of polyelectrolyte and then become constant. The swelling of the hydrogel samples in town water and saline solution (0.15 mol/L LiCl, NaCl, KCl, CaCl₂ and AlCl₃) was examined. Results indicated that the swelling capacity decreased with an increase charge of cation. This behavior can be attributed to charge screening effect for monovalent cations, as well as ionic crosslinking for multivalent cations. Finally, a mechanism for hydrogel formation was proposed and the structure of the product was established using Fourier transform infrared (FTIR) and Scanning Electron Microscopy (SEM) spectroscopies. Also, thermogravimetric analysis showed the thermal stabilities of the hydrogels.

Keywords: collagen, hydrogel, acrylic acid, itaconic acid, salt-responsive.

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 hygienics, foods, cosmetics, and agriculture. This accounts for the increase in the worldwide production of superabsorbent polymers (SAPs) from 6000 tons in 1983 to 450000 tons in 1996 (Buchholz & Graham, 1997). Nowadays, the worldwide production of SAPs is more than one million tons in a year. Hence, synthesis and characterization of superabsorbent hydrogels is the main goal of the several research groups in the world (Chu & Yamaguchi, 2002; Chu & Kim, 2007; Crescenzi & Cornelio, 2007; Cheng & Zhu, 2008).

The properties of the swelling medium (e.g. pH, ionic strength and the counter ion and its valency) affect the swelling characteristics (Hoffman, 1996; Eddington & Beebe, 2004). The hydrogels sharply and reproducibly responding to the medium conditions are referred to as "responsive", "smart" or "intelligent". Among these, salt-, pH- and temperature- sensitive hydrogels have been extensively investigated for potential use in site-specific delivery of drugs to specific regions of the gastrointestinal tract and have been prepared for delivery of low molecular weight protein drugs (Kirk & Othmer, 1992; Wu & Wei, 2003).

Although hydrogels made from synthetic polymers, such as polyacrylate, possess excellent water-absorbing properties, their toxicity and non-biodegradability might pose long-time environmental problems and limit their use in drug delivery systems and consumer products. Natural-based SAPs have attracted

much attention in medical and pharmaceutical fields because of their non-toxicity, biocompatibility and biodegradability. Free radical vinyl graft copolymerization onto natural polymers is a well-known method for synthesis of natural-based superabsorbent hydrogels (Barvic & Kliment, 1967; Chirila & Constable, 1993; Pourjavadi & Harzandi, 2004; Lim & Whang, 2001). The first industrial superabsorbent hydrogel, hydrolyzed starch-graft-polyacrylonitrile was synthesized using this method (Hosseinzadeh & Pourjavadi, 2005).

Collagen being a biomaterial its crosslinking is used in various purposes such as collagen swelling, and collagen hydrogels as biodegradable implants to deliver small and macromolecular drugs. Recently, attention has been focused on employing collagen substrate to produce hydrogels with a specific response to a biological environment (Silverstein & Webster, 1998; Mahdavinia & Pourjavadi, 2004). Therefore, following a continuous research on synthesis of natural-based superabsorbent hydrogels this work is an attempt on the synthesis of a novel collagen-based superabsorbent polymer. The swelling behavior in distilled water, town water and various saline solutions was investigated as well.

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. Double distilled water and town-water

were used for the hydrogel preparation and swelling measurements.

Preparation of hydrogel

A general procedure for chemically crosslinking graft copolymerization of AA and IA onto collagen backbones was conducted as follows. Hydrolyzed collagen (1.5 g) was dissolved in water and 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 (65 °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 (0.4-1.6 g and IA 0.4-1.60 g) 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 120 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 cut 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

Collagen-g-poly(acrylic acid-co-itaconic acid) sample (0.10 g) was put into a weighed teabag and immersed in 100 mL distilled water and allowed to soak for 2 h at room temperature. The equilibrated swollen gel was allowed to drain by removing the teabag from water (~20 min). The bag was then weighed to determine the weight of the swollen gel. The absorbency (equilibrium swelling) was calculated using the following equation:

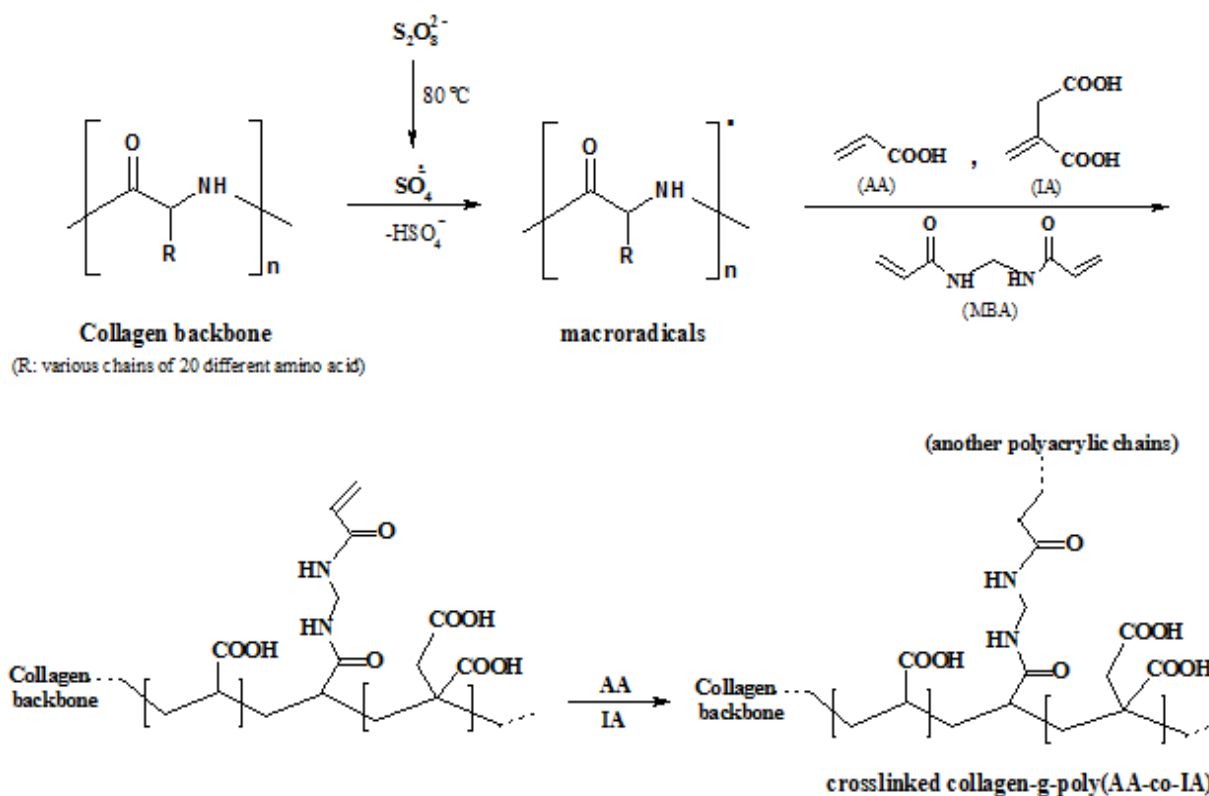
$$\text{Absorbency} = (W_s - W_d)/W_d \quad (1)$$

where W_s and W_d are the weights of the samples swollen in water and in dry state, respectively. So, absorbency was calculated as grams of water per gram of resin (g/g). The accuracy of the measurements was $\pm 3\%$.

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). Thermogravimetric analyses (TGA) were performed on a Universal V4.1D TA Instruments (SDT Q600) with 8-10 mg samples on a platinum pan under nitrogen atmosphere. Experiments were done at a heating rate of 20 °C/min until 600 °C.

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



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 (Mahdavinia & Pourjavadi, 2004).

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 1634 cm⁻¹ can be attributed to C=O stretching in carboxamide functional groups of substrate backbone (Fig.1a). The superabsorbent hydrogel product comprises a collagen backbone with side chains that carry sodium carboxylate functional groups that are evidenced by peak at 1558 cm⁻¹ (Fig.1b). This characteristic band is due to asymmetric stretching in carboxylate anion that is reconfirmed by another peak at 1411 cm⁻¹ which is related to the symmetric stretching mode of the carboxylate anion (Chen & Zhao, 2000).

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 Fig.2. The structure of the hydrogel in compare to pure collagen is very porous, and it could help to form a high-water-content hydrogel, as is generally shown in other hydrogels (Chen & Zhao, 2000).

Thermogravimetric analysis

TGA curves for collagen and collagen-based hydrogel are shown in Fig.3. The grafted collagen has shown improvement in thermal stability as clear from TGA curve. The initial decomposition temperature of the collagen on grafting was increased from 168 to 402 °C with maximum decomposition rate at 523 °C, in comparison to original decomposition temperature of 325 °C of collagen. These observations have clearly indicated that grafting of poly(AA-co-IA) has improved the thermal stability of collagen.

Optimization of effecting parameters onto swelling capacity

Effect of MBA Concentration on Swelling: Crosslinks is necessary to form a superabsorbent in order to prevent

dissolution of the hydrophilic polymer chains in an aqueous environment. The effect of MBA concentration on the water absorbency of the hydrogel was studied by varying the concentration of MBA from 0.006 to 0.035 mol/L. All other parameters in these series of reaction were constant. As the concentration of MBA was increased, the water absorbency of the superabsorbent hydrogel was decreased. This known behavior shown in Fig.4. This is due to a decrease in the space between the copolymer chains as the crosslinker concentration is increased. This decreasing trend is similar to the similar cases found by us (20) and other groups for other superabsorbents. The maximum water absorbency (411g/g) was obtained at 0.006 mol/L of MBA. The known relationship between swelling and concentration of crosslinking agent (C_c) is stated as Eq. (2):

$$\text{Swelling} \cong kC_c^{-n} \quad (2)$$

where k and n are constant values for an individual superabsorbent. Fig. 4 exhibits a power law behavior of absorbency- C_c , with $k = 23.25$ and $n = 0.38$ which is obtained from the fitted curve with Eq. 2.

Effect of monomer ratio on swelling capacity: In this series of experiments, the swelling capacity of the hydrogels prepared with various ratios of monomers is shown in Fig. 5. Since pH of the polymerization mixture was adjusted at 8.0 after the reaction, the superabsorbency of Collagen-g-poly(NaAA-co-NaIA) hydrogel is due to both functional groups of ionic carboxylate from neutralized AA and IA monomers. The presence of the ionic groups in polymer chains results in increasing of swelling because the ions are more strongly solvated in the aqueous medium. Higher swelling capacities are obtained from employing higher initial ratios of NaAA/NaIA. Therefore, the swelling enhancement versus higher NaAA/NaIA ratio can be attributed to the formation of high carboxylate groups in the synthesized samples. The swelling decrease after the maximum may be attributed to (a) preferential homopolymerization over graft copolymerization, (b) increase in viscosity of the medium which hinders the movement of free radicals and monomer molecules, and (c) the enhanced chance of chain transfer to monomer molecules (Lim & Whang, 2001).

Effect of APS Concentration on Swelling: The effect of the initiator (APS) concentration on the water absorbency of the superabsorbent hydrogel was also studied (Fig. 6). The APS concentration was changed from 0.005 to 0.038 mol/L. Water absorbency of the hydrogel was increased initially with increasing the initiator concentration up to 0.02 mol/L, but it was decreased later as shown in Fig 6. The increase of water absorbency with increasing initiator concentration may be ascribed to the increase of the active sites on the backbone of the collagen arising from the attack of sulfate anion-radical. An additional reason, according to Flory (Flory,1953), is imperfection of the polymer networks obtained from high-initiator polymerization systems.

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

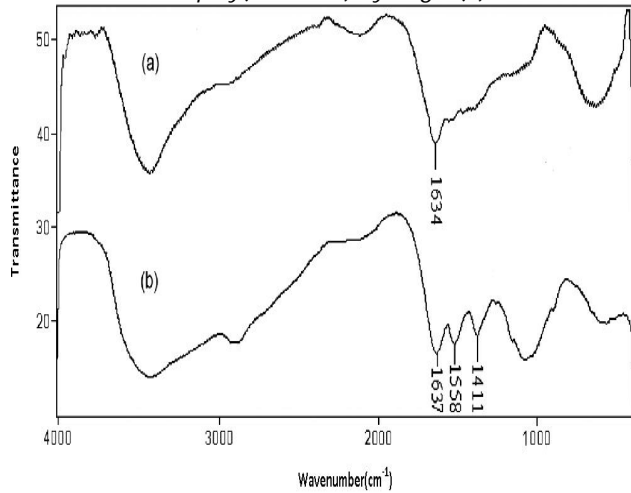


Fig. 2. SEM photograph of pure collagen (a) and the hydrogel (b). Surfaces were taken at a magnification of 1000, and the scale bar is 20 μm

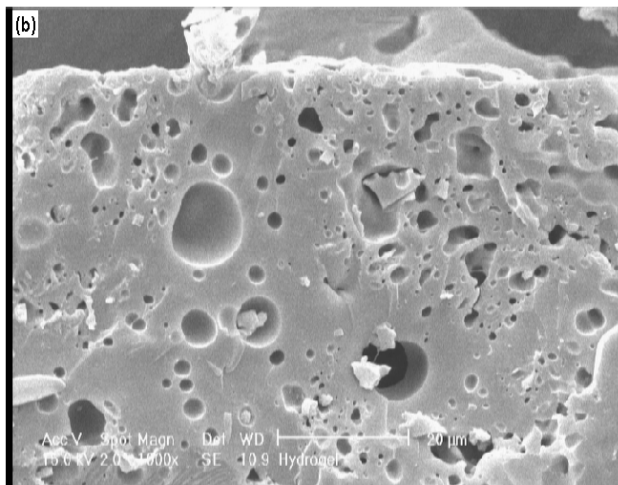
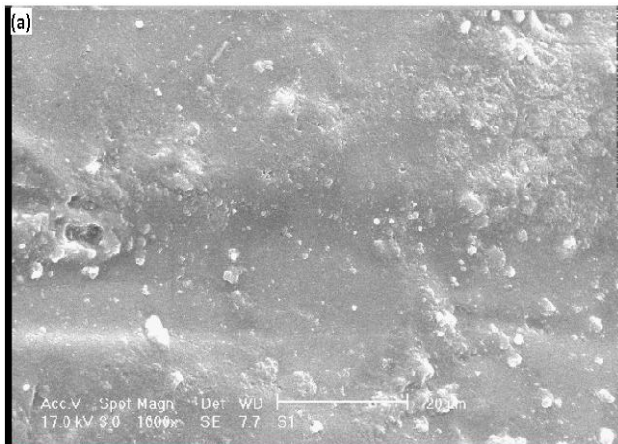


Fig.3. TGA thermograms of (A) collagen and (B) collagen-g-Poly(AA-co-IA) superabsorbent. Heating rate 20 °C/min, under N₂

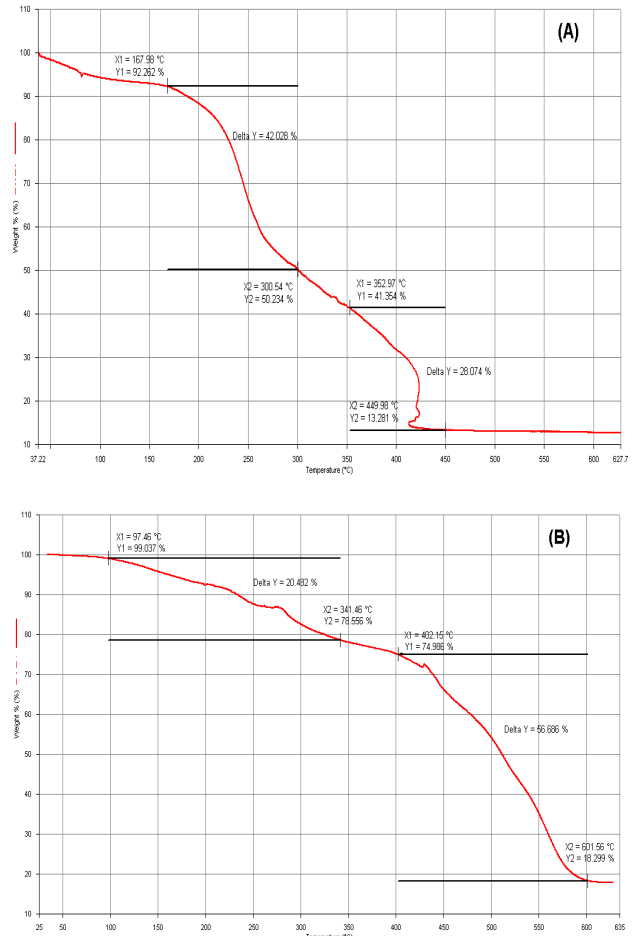


Fig. 4. Effect of MBA concentration on swelling capacity of the collagen-based hydrogels. Reaction conditions: Collagen 1.50 g, AA/IA= 1(w,w), APS 0.015 mol/L, 65 °C, 120 min.

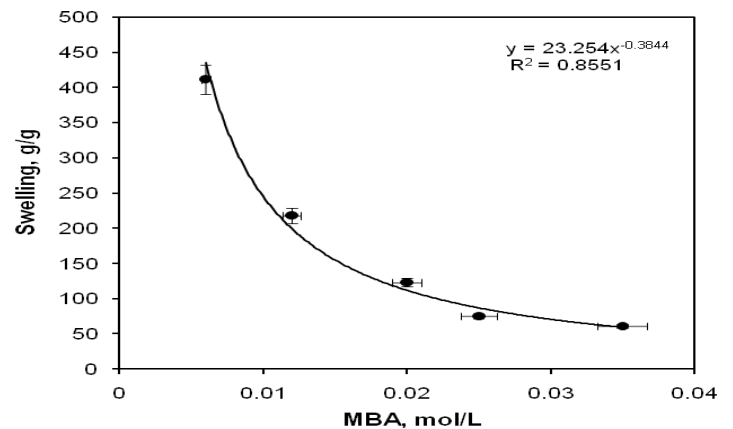


Fig. 5. Effect of monomer ratio on swelling capacity of the collagen-based hydrogels. Reaction conditions: Collagen 1.50 g, MBA 0.006 mol/L, APS 0.015 mol/L, 65 °C, 120 min

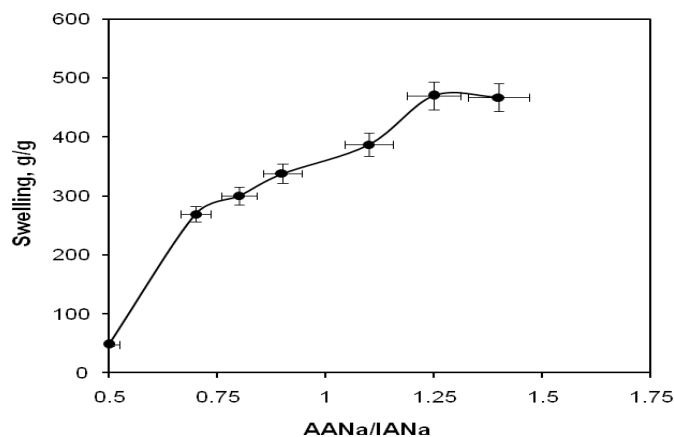


Fig. 6. Effect of MBA concentration on swelling capacity of the collagen-based hydrogels. Reaction conditions: Collagen 1.50 g, MBA 0.006 mol/L, AA/IA= 1.25(w,w), 65 °C, 120 min.

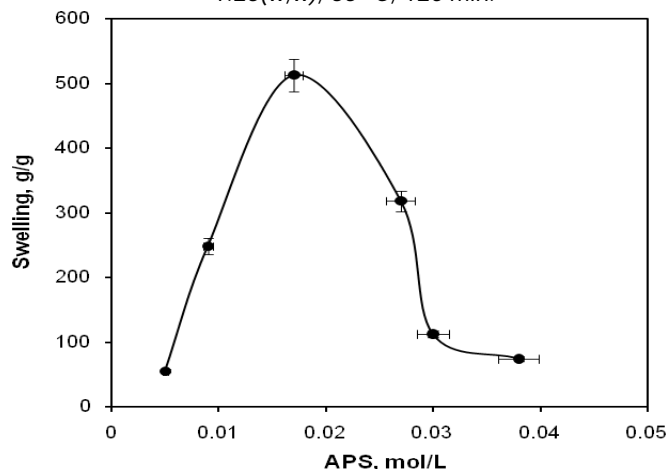
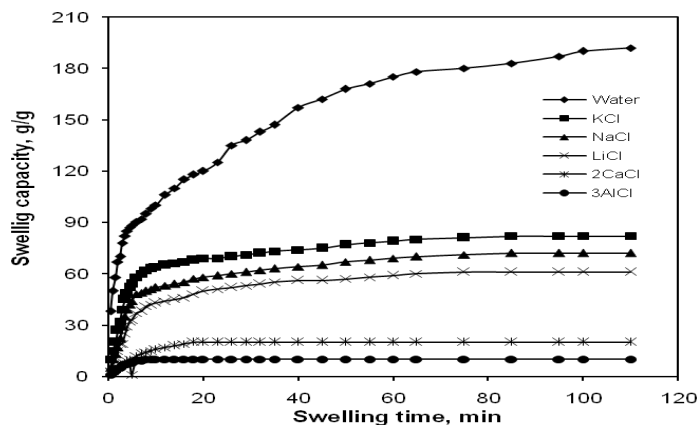


Fig. 7. Swelling capacity variation of the hydrogel in town-water and saline solutions (0.15 mol/L). Reaction conditions: Collagen 1.50 g, MBA 0.006 mol/L, APS 0.02 mol/L, AA/IA= 1.25(w,w), 65 °C, 120 min.



The increase the active sites on the protein led to increase of AANA-IANA grafting onto collagen backbones. The decrease of water absorbency with initiator concentration beyond 0.02 mol/L may be due to: (a) increased number of produced radicals led to terminating step via bimolecular collision, (b) predominance of homopolymerization over grafting, (c) molecular weight loss of the synthetic part of the polymer network (Flory,1953), and (d) free radical degradation of collagen substrate. A proposed mechanism for the latter possibility has been reported in a previous work(Lim & Whang, 2001). Hsu et al. have recently reported a similar observation in the case of degradation of chitosan with potassium persulfate (Hsu & Don, 2002).

Swelling in salt solutions and salt-responsivity

The swelling behavior of hydrogel in town-water and aqueous solutions of 0.15 mol/L NaCl, CaCl₂ and AlCl₃ is compared in Fig.7. It is obvious that swelling decrease strongly depended on the "type" and "concentration" of salt added to the swelling medium. The swelling of the absorbents in saline solutions was appreciably decreased comparing to the values measured in town water. This well-known phenomenon, commonly observed in the swelling of ionic hydrogels is often attributed to a screening effect of the additional cations causing a non-perfect anion-anion electrostatic repulsion, leading to a decreased osmotic pressure (ionic pressure) difference between the hydrogel network and the external solution. The swelling capacity increases with a decrease in charge of the metal cation (Al³⁺ < Ca²⁺ < Na⁺). This may be explained by complexing ability arising from the coordination of the multivalent cations with carboxylate groups present in hydrogel. This ionic crosslinking mainly occurs at the surface of particles and makes them rubbery and very hard when they swell in Ca²⁺ or Al³⁺ solution. Also the effect of cation radius on swelling may also been observed from Figure 6. As reported by Pass et al(24), the carboxylate anion interacts with small cations, e.g.Li⁺, stronger than with large cations, e.g. K⁺. The stronger interactions of carboxylate-small cation have been observed using measurement of activating coefficients of various cations in several salt solutions (Pass & Philips, 1997).

Conclusion

A new salt-responsive copolymer based on collagen hydrogel was synthesized through simultaneous crosslinking and graft copolymerization of acrylic acid itaconic acid (IA) mixtures onto collagen. optimum reaction conditions to obtain maximum water absorbency (512 g/g) were found to be: MBA 0.006 mol/L, AA/IA 1.25(w,w), APS 0.02 mol/L, reaction temperature 65 °C, and reaction time 120 min. The study of FTIR,SEM spectra and thermogravimetric analysis provide the graft copolymerization and crosslinking do takes place. Swelling measurement of

the synthesized hydrogels in different salt solutions showed appreciable swelling capacity, especially in solutions with monovalent cations. However, swelling loss in salt solutions, in comparison with town-water, can be attributed to charge screening effect and ionic crosslinking for mono- and multi-valent cations, respectively.

References

1. Barbucci R, Maganani A and Consumi M (2000) Swelling behavior of carboxymethylcellulose hydrogels in relation to cross-linking, pH, and charge density. *Macromolecules*. 33, 7475- 7478
2. Barvic M, Kliment and Zavadil M (1967) A new biodegradable crosslinked polyethylene oxide sulfide (PEOS) hydrogel for controlled drug release *J. Biomed Mater Res*. 1, 313-323.
3. Buchholz FL and Graham AT Modern (1997) Superabsorbent polymer technology. Wiley, NY.
4. Chen J and Zhao Y (2000) Relationship between water absorbency and reaction conditions in aqueous solution polymerization of polyacrylate superabsorbents. *J. Appl. Polym. Sci.* 75, 808- 812
5. Cheng H, Zhu JL, Sun YX, Cheng SX, Zhang XZ and Zhuo RX (2008) Novel thermo-responsive non-viral gene vector: P(NIPAAm-co-NDAPM)-b-PEI with adjustable gene transfection efficiency. *Bioconjug. Chem.* 19, 1368-1374.
6. Chirila TV, Constable IJ, Crawford GJ, Vijayasekaran S, Thompson DE, Chen YC, Fletcher WA and Griffin BJ (1993) Poly(2-hydroxyethyl methacrylate) sponges as implant materials: In vivo and in vitro evaluation of cellular invasion *Biomaterials*. 14, 26-38.
7. Chu LY, Kim JW, Shah RK and Weitz DA (2007) Monodisperse thermo-responsive microgels with tunable volume-phase transition kinetics. *Adv. Funct. Mater.* 17, 3499-3504.
8. Chu LY, Yamaguchi T and Nakao SA (2002) molecular recognition microcapsule for environmental stimuli responsive controlled release. *Adv. Mater.* 14, 386-389.
9. Crescenzi V, Cornelio L, Di Meo C, Nardocchia S and Lamanna R (2007) Novel hydrogels via click chemistry: synthesis and potential biomedical applications. *Biomacromol.* 8, 1844-1850.
10. Eddington D T and Beebe D J (2004) Flow control with hydrogels. *Adv. Drug Deliv. Rev.* 56, 199-210.
11. Flory PJ (1953) Principles of polymer chemistry. Ithaca, Cornell University Press, NY.
12. Hoffman AS (1996) Polymeric materials encyclopedia. Salamone JC (Ed), CRC Press, Boca Raton, FL. 5. 3282.
13. Hosseinzadeh H, Pourjavadi A, Zohouriaan-Mehr MJ and Mahdavinia GR (2005) *J. Bioact. Compat. Polym.* 20. 475-491.
14. Hsu SC, Don TM and Chiu WY (2002) Free radical degradation of chitosan with potassium persulfate. *Polym. Degrad. Stab.* 75, 73-78.
15. Kirk RE and Othmer DF (1992) Encyclopedia of chemical technology, 4. Kroschwitz JI & Howe-Grant M (eds). John Wiley & Sons, NY. pp:942.
16. Lim DW, Whang HS and Yoon KJ (2001) Synthesis and Absorbency of a superabsorbent from sodium starch sulfate-g-polyacrylonitrile. *J. Appl. Polym. Sci.* 79, 1423-1427.
17. Lim DW, Whang HS, Yoon KJ (2001) Synthesis and Absorbency of a Superabsorbent from Sodium Starch Sulfate-g-Polyacrylonitrile. *J. Appl. Polym. Sci.* 79, 1423-1430.
18. Lim J, Wu J, Yang Z and Pu M (2001) Synthesis and properties of poly(acrylic acid)/montmorillonite superabsorbent composites, *Polymers & Polymer Composites*. 9, 469-471.
19. Mahdavinia GR, Pourjavadi A Hosseinzadeh H and Zohouriaan-Mehr MJ (2004) Superabsorbent Hydrogels from poly(acrylic acid-co-acrylamide) grafted chitosan with salt- and pH-responsiveness properties. *Eur. Polym. J.* 40, 1399-1407.
20. Pass G, Philips GO and Wedlock DJ (1997) Modeling of drug release from delivery systems based on hydroxypropyl methylcellulose (HPMC). *Macromolecules*. 10, 197-201.
21. Pourjavadi A, Harzandi AM and Hosseinzadeh H (2004) Modified carrageenan. 3 Synthesis of a novel polysaccharide-based superabsorbent hydrogel via graft copolymerization of acrylic acid onto kappa-carrageenan in air. *Eur. Polym. J.* 40, 1363-1370.
22. Silverstein RM and Webster FX (1998) Spectrometric identification of organic compounds. 6th Edn., Wiley, NY.
23. Wu J, Lim J, Zhou M and Wei C (2000) Synthesis and properties of starch-g-polyacrylamide/clay superabsorbent composite. *Macromol. Rapid Commun.* 21, 1032-1034.
24. Wu J, Wei Y, Lin J and Lin S (2003) Study on starch-g-acrylamide/mineral powder superabsorbent composite. *Polymer*. 44, 6513-6520.