ISSN: 0970-020 X; CODEN: OJCHEG
Oriental Journal of Chemistry
2011, Vol. 27, No. (2): Pg. 453-460

http://www.orientjchem.org

Synthesis of a Novel pH- and Salt-Responsive Super Absorbent Hydrogel based on Collagen-g-poly(AA-co-IA)

MOHAMMAD SADEGHI[1]* and MOJGAN YARAHMADI[2]

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

(Received: March 10, 2011; Accepted: April 25, 2011)

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 hydrogels was also measured in various salt solutions (LiCl, NaCl, KCl, $CaCl_2$ and $AlCl_3$). 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.

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[1]. This accounts for increase in the worldwide production of superabsorbent polymers (SAPs) from 6000 tons in 1983 to 450000 tons in 1996 [2-4]. 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[3-6].

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

research and development in the field of medicine, pharmacy and biotechnology[8].

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(9-12). Hence, the our 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 avail able 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 H_2O) 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 H_2O) 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:

$$ES(g/g) = \frac{Weight\ of\ swollen\ gel - Weight\ of\ dried\ gel}{Weight\ of\ dried\ gel} \quad ...(1)$$

Instrumental analysis

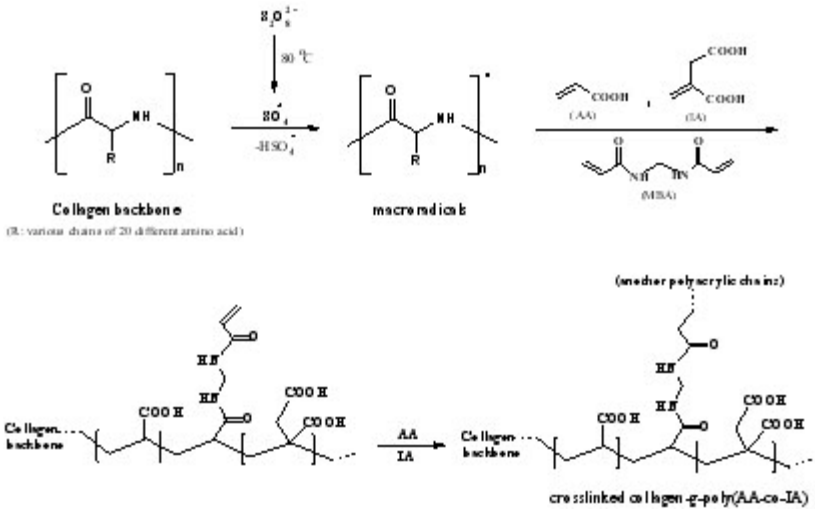
Fourier transform infrared (FTIR) spectra of samples were taken in KBr pellets, using an ABB Bomem MB-100 FTIR spectrophotometer (Quebec, Canada), at room temperature. The surface morphology of the gel was examined using scanning electron microscopy (SEM). After Soxhlet extraction with methanol for 24 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 NH_2) 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 anion[13].

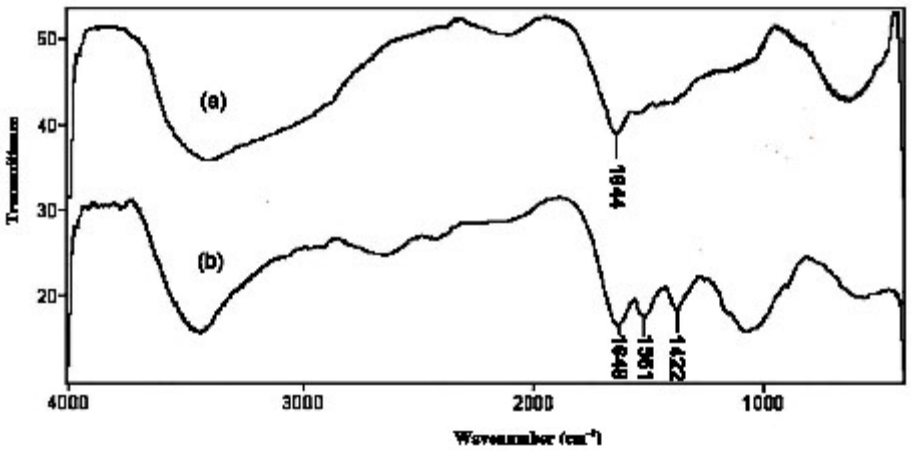


Fig. 1: FTIR spectra of collagen (a) and collagen-*g*-poly(AA-*co*-IA) hydrogel (b).

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[14]

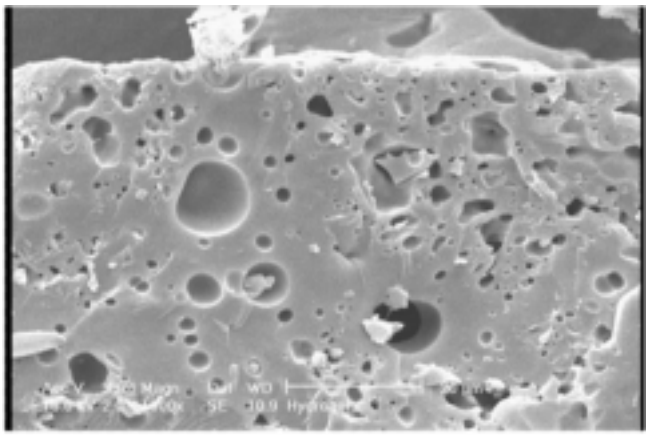


Fig. 2: SEM photograph of the hydrogel. Surfaces were taken at a magnification of 1000, and the scale bar is 20 µm

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^+ 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^+ ions are replaced by H^+ 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.

This behavior has also been observed in the case of commercial acrylic acid-based SAPs(15) as a standard crosslinked polyelectrolyte.

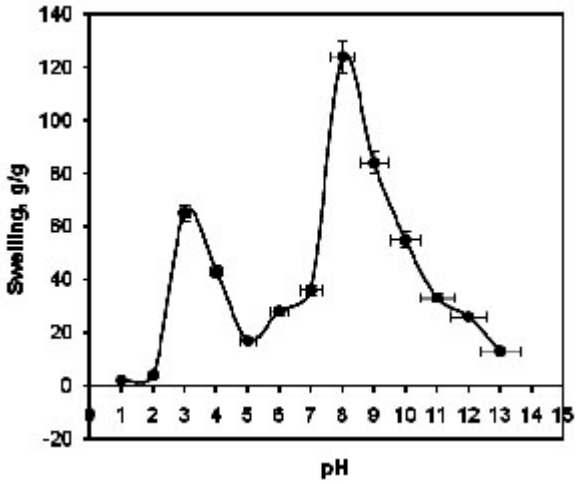


Fig. 3: Effect of pH of solution on swelling of Collagen-g-poly(AA-co-IA) hydrogel

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[16].

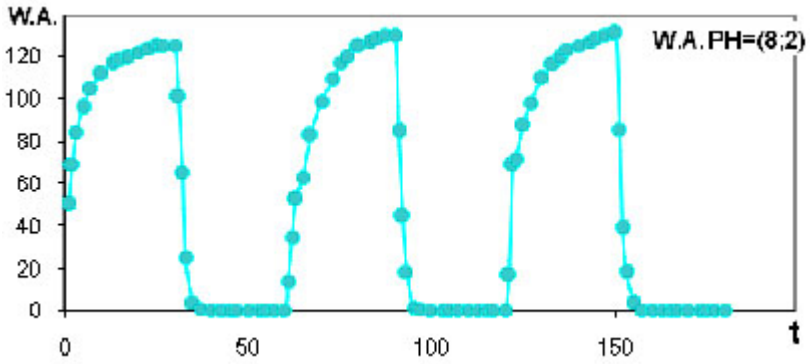


Fig. 4: On-off switching behavior as reversible pulsatile swelling (pH 8.0) and deswelling (pH 2.0) of the hydrogel. The time interval between the pH changes was 50 min

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[17].

$$S_t = S_e(1-e^{-t/\tau}) \qquad ...(2)$$

where S_t is the swelling at time t, S_e is the equilibrium swelling (power parameter) and τ (min) is the “rate parameter”. The power parameter (equilibrium swelling capacity) according to Figure 5 is 205 g/g for town-water. The τ value is a measure of swelling rate (i.e. the lower the τ 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.[33] 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/τ) gives the rate parameter. For example, in the case of town-water the τ 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 τ 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.

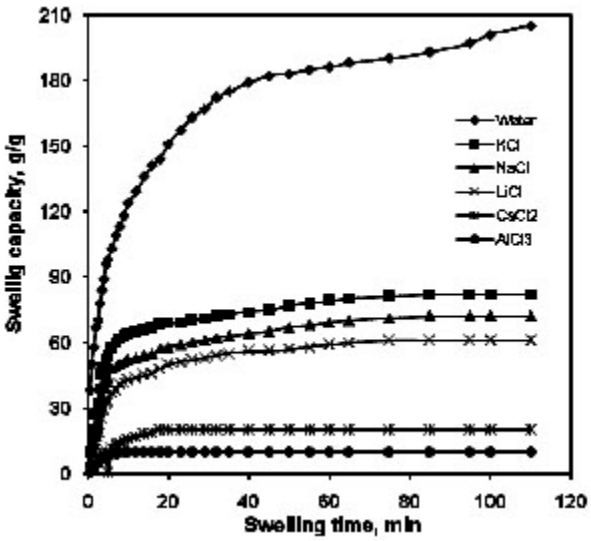


Fig. 5: Representative swelling kinetics of the hydrogel, Collagen-g-poly(AA-co-IA), in town-water and various chloride salt solutions (0.15 M). Particle size of the dried gel was 300-450 µm

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+}þ 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 shrinked 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].

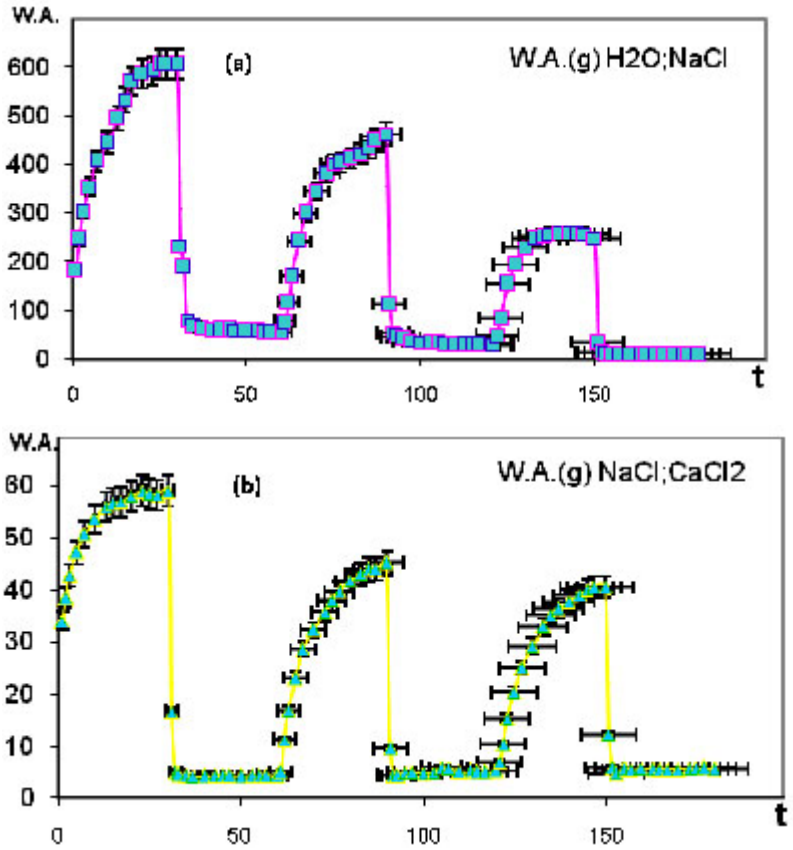


Fig. 6: swelling-deswelling cycle of the hydrogel Collagen-g-poly(AA-co-IA) hydrogel in distilated water and sodium choloride salt (a) and in sodium choloride and calcium choloride (b)

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

1. Buchholz, F.L., Graham, A.T. Modern Superabsorbent Polymer Technology, New York: Wiley(1997).
2. Cheng, H., Zhu, J.L., Sun, Y.X., Cheng, S.X., Zhang, X.Z., Zhuo, R.X. Novel thermo-responsive non-viral gene vector: P(NIPAAm-co-NDAPM)-b-PEI with adjustable gene transfection efficiency. *Bioconjug. Chem*. **19**: 1368-1374 (2008).
3. Chu, L.Y., Kim, J.W., Shah, R.K., Weitz, D.A. Monodisperse thermo-responsive microgels with tunable volume-phase transition kinetics. *Adv. Funct. Mater*. **17**: 3499-3504 (2007).
4. Chu, L.Y., Yamaguchi, T., Nakao, S. A molecular recognition microcapsule for environmental stimuli responsive controlled release. *Adv. Mater*. **14**: 386-389 (2002).
5. Crescenzi, V., Cornelio, L., Di Meo, C., Na rdecchia, S., Lamanna, R. Novel hydrogels via click chemistry: synthesis and potential biomedical applications. *Biomacromolecules* **8**: 1844-1850 (2007).
6. Eddington, D.T., Beebe, D.J. Flow control with hydrogels. *Adv. Drug Deliv. Rev*. **56**: 199-210 (2004).
7. Hamidi, M., Azadi, A., Raûei, P. Hydrogel nanoparticles in drug delivery. *Adv. Drug Deliv. Rev*. **60**: 1638-1649 (2008)
8. Koo, H., Jin, G., Kang, H., Lee, Y., Nam, H.Y., Jang, H., Park, G.S. A new biodegradable crosslinked polyethylene oxide sulûde (PEOS) hydrogel for controlled drug release. *International Journal of Pharmaceutics*. **374**: 58-65 (2009).
9. Kakinoki, S., Taguchi, T., Saito, H., Tanaka, J., Tateishi, T. Injectable in situ forming drug delivery system for cancer chemotherapy using a novel tissue adhesive: characterization and in vitro evaluation. *Eur. J. Pharm. Bio*. **66**: 383-90 (2007).
10. Kim, S.J., Spinks, G.M., Prosser, S., Whitten, P.G., Wallace, G.G., Kim, S.I., Surprising shrinkage of expanding gels under an external load. *Nat. Mater.* **5**: 48-51(2006).
11. Kranz, H., Bodmeier, R.Structure formation and characterization of injectable drug loaded biodegradable devices: in situ implants versus in situ microparticles. *Eur. J. Pharm. Sci*. **34**: 164-72 (2008).
12. Kwon, I.C., Bae, Y.H., Kim, S.W. Electrically erodible polymer gel for controlled release of drugs. *Nature*, **354**: 291-293 (1991).
13. Oh, J.K., Drumright, R., Siegwart, D.J., Matyjaszewski, K.he development of microgels/nanogels for drug delivery applications. *Prog. Polym. Sci*. **33**: 448-77 (2008)
14. Siepmann, J., Peppas, N.A.Modeling of drug release from delivery systems based on hydroxypropyl methylcellulose (HPMC). *Adv. Drug Deliv. Rev*. **48**: 139-57 (2001).
15. Soppimath, K.S., Aminabhavi, T.M., Dave, A.M., Kumbar, S.G., Rudzinski, W.E.Stimulus- responsive “smart” hydrogels as novel drug delivery systems. *Drug Dev. Ind. Pharm*. **28**: 957-974 (2002).
16. Tatsuma, T., Takada, K., Miyazaki, T.UV-light-induced swelling and visible-light-induced shrinking of a TiO2-containing redox gel. *Adv. Mater*. **19**: 1249-1251 (2007).
17. Thornton, P.D., Mart, R.J., Ulijn, R.V. Enzyme-responsive polymer hydrogel particles for controlled Release. *Adv. Mater*. **19**: 1252-1256 (2007).
18. Wang, W., Liu, L., Ju, X.J., Zerrouki, D., Xie, R., Yang, L., Chu, L.Y. A novel thermo-induced self-bursting microcapsule with magnetic-targeting property. *Chem. Phys. Chem*. **10**: 2405-2409 (2009).
19. Yang, M., Chu, L.Y., Wang, H.D., Xie, R., Song, H., Niu, C.H. A thermoresponsive membrane for chiral resolution. *Adv. Funct. Mater*. **18**: 652-663 (2008).