African Journal of Biotechnology Vol. 10(56), pp. 12085-12093, 26 September, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.567
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Synthesis and properties of carboxymethylcellulose (CMC) graft copolymer with on-off switching properties for controlled release of drug

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.

Accepted 9 June, 2011

Novel biopolymer-based superabsorbent hydrogels were prepared by grafting crosslinked poly (N-vinyl pyrollidin and 2-Acrylamido-2-methyl propan sulfonic acid (PNVP-*co*-PAMPS) chains onto CMC backbones through a free radical polymerization method. A proposed mechanism for superabsorbent hydrogel formation was suggested and the hydrogel structure was confirmed using FTIR spectroscopy. The morphology of the samples was examined by scanning electron microscopy (SEM). The concentration of released metronidazole loaded was monitored at 245 nm on the UV spectrophotometer. Water absorption of the hydrogel could be switched on and off swiftly by control of pH of the surrounding environment. Therefore, the synthesized hydrogels in this study can be used as a drug delivery system and that the drug release can be controlled by the pH of solution. The release rate of metronidazole from hydrogel at pH 7.4 was higher than that at pH 1.6, due to the increased swelling capacity of the hydrogel.

Key words: CMC, hydrogels, release drug, N-vinyl pyrollidin, 2-acrylamido-2-methyl propan sulfonic acid.

INTRODUCTION

Hydrogels have been of interest to biomaterial scientists for many years because of their hydrophilic character and potential to be biocompatible (Yoshida, 1995). These networks are special soft and pliable polymeric materials that can absorb large quantities of water, saline or physiological solutions, while the absorbed solutions are not removable even under pressure (Buchholz and Graham, 1997).

Indeed, superabsorbent hydrogels have great advantages over traditional water-absorbing materials such as cotton, pulp and sponge. They are widely used in sanitary goods like disposable diapers and hygienic napkins.

Hydrogels responding to external stimuli such as heat, pH, electric field, chemical environments, etc, are often referred to as "intelligent" or "smart" hydrogels. These hydrogels have important applications in the field of medicine, pharmacy and biotechnology (Hoffman, 2002).

*Corresponding author. E-mail: m-sadeghi@iau-arak.ac.ir. Tel: +98-916-1613256. Fax: +98-861-3670017.

Following a continuous research on modification of carboxymethylcellulose (CMC) sodium salt (Peppas and Harland, 1990), in this study, we attempted to investigate the pH-reversibility properties and influence of pH of the medium on the drug release of hydrogel systems.

MATERIALS AND METHODS

The polysaccharide used in this study was carboxymethylcellulose (CMC) with D.S. 0.52. Ceric ammonium nitrate (CAN) was purchased from Merck and was used without purification. It was as freshly prepared 0.1 M solution in 1 M HNO_3. N-vinyl pyrollidin, (NVP, Merck) and 2-Acrylamido-2-methyl propan sulfonic acid (Fluka, Buchs, Switzerland) were used after distillation for removing inhibitor. The antibiotic metronidazole was purchased from Sigma Aldrich. The chemical structure of metronidazole loaded is shown in Figure 1. All other chemicals were of analytical grade.

Preparation of hydrogel

A pre-weighed amount of carboxymethylcelloluse (20 g/l) was dissolved in 50 ml distilled water. Then, the solution was added to a1 L reactor equipped with a mechanical stirrer (RZR 2021, a three-

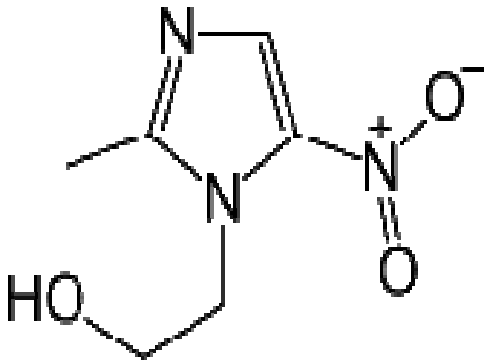


Figure 1. Chemical structure of metronidazole.

blade propeller type, Heidolph, Schwabach, Germany) and stirred (300 rpm) for 10 min. The reactor was placed in a thermostated water bath to control the reaction temperature at 65°C. Then, N-vinyl pyrollidin, (0.05 to 0.2 mol/l) and 2-azcrylamido-2-methyl propan sulfonic acid, (0.02 to 0.7 mol/l), ceric ammonium nitrate (0.1 mol/l) and methylene bisacrylamide (0.004 to 0.008 mol/l, dissolved in 5 ml water) were added simultaneously to the reactor. The temperature was maintained at 65°C and the reaction mixture was stirred continuously (300 rpm) for 90 min. At the end of the propagation reaction, the gel product was poured into ethanol (300 ml) and was dewatered for 24 h. Then, the product was cut into small pieces, washed with 500 ml ethanol and filtered. The particles were dried in an oven at 50°C for 12 h. After grinding, the powdered superabsorbent composite was stored in the absence of moisture, heat and light.

Swelling and deswelling measurements

An accurately weighed sample (0.20 g) of the powdered superabsorbent with average particle sizes between 40 and 60 mesh (250 to 350 µm) was immersed in distilled water (200 ml) or desired acid and base solution (100 ml) and allowed to soak for 3 h at room temperature (Po, 1994). The equilibrium swelling (ES) capacity was measured twice at room temperature according to a conventional tea bag (that is, a 100 mesh nylon screen) method (Peppas and Mikes, 1986), using the following formula:

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

The deswelling water ratio of each sample was evaluated from the following equation:

$$\text{Deswelling water ratio}\ (\%) = \frac{W_t}{W_{t0}} \times 100 \quad (2)$$

Where, W_{t0} and W_t are the initial weight of the fully swollen sample and the weight of sample at the deswelling time, t, respectively.

Drug loading on hydrogels

Loading model drug into a hydrogel was performed using a contact adsorption technique. The vacuum dried powdered samples (1±0.0001 g), with average particle sizes between 40 and 60 mesh (250 to 350 µm), were accurately weighted and immersed in the aqueous solution of drug (0.6 g dissolved in 50 ml distilled water) at 0°C for 25 h to reach the equilibrated state. The swollen hydrogels loaded with drug were placed in a vacuum oven and dried under vacuum at 37°C (Kost, 1995). The hydrogels was washed with distilled cold water.

Standard absorbance curve

The standard calibration curve of the absorbance as a function of drug concentration was studied at 245 nm on the UV spectrophotometer.

Determination of loading efficiency

The amount of drug content entrapped in the hydrogels was determined by an indirect method. After the gel preparation, the washings were collected, filtered with a 0.45 µm Millipore filter and tested at λ_{max} 256 nm using UV/VIS spectrophotometer (UV-1201, Shimadzu, Kyoto, Japan).

The drug entrapped exhibited the same λ_{max} as the free drug. This clearly indicates that the drugs entrapped have not undergone any possible chemical reaction during the matrix formation. The difference between the amount of drug initially employed and the drug content in the washings is taken as an indication of the amount of drug entrapped:

$$Drug\ entrapment\ (\%) = \frac{Mass\ of\ drug\ present\ in\ hydrogel}{Theoretical\ mass\ of\ drug} \times 100 \quad (3)$$

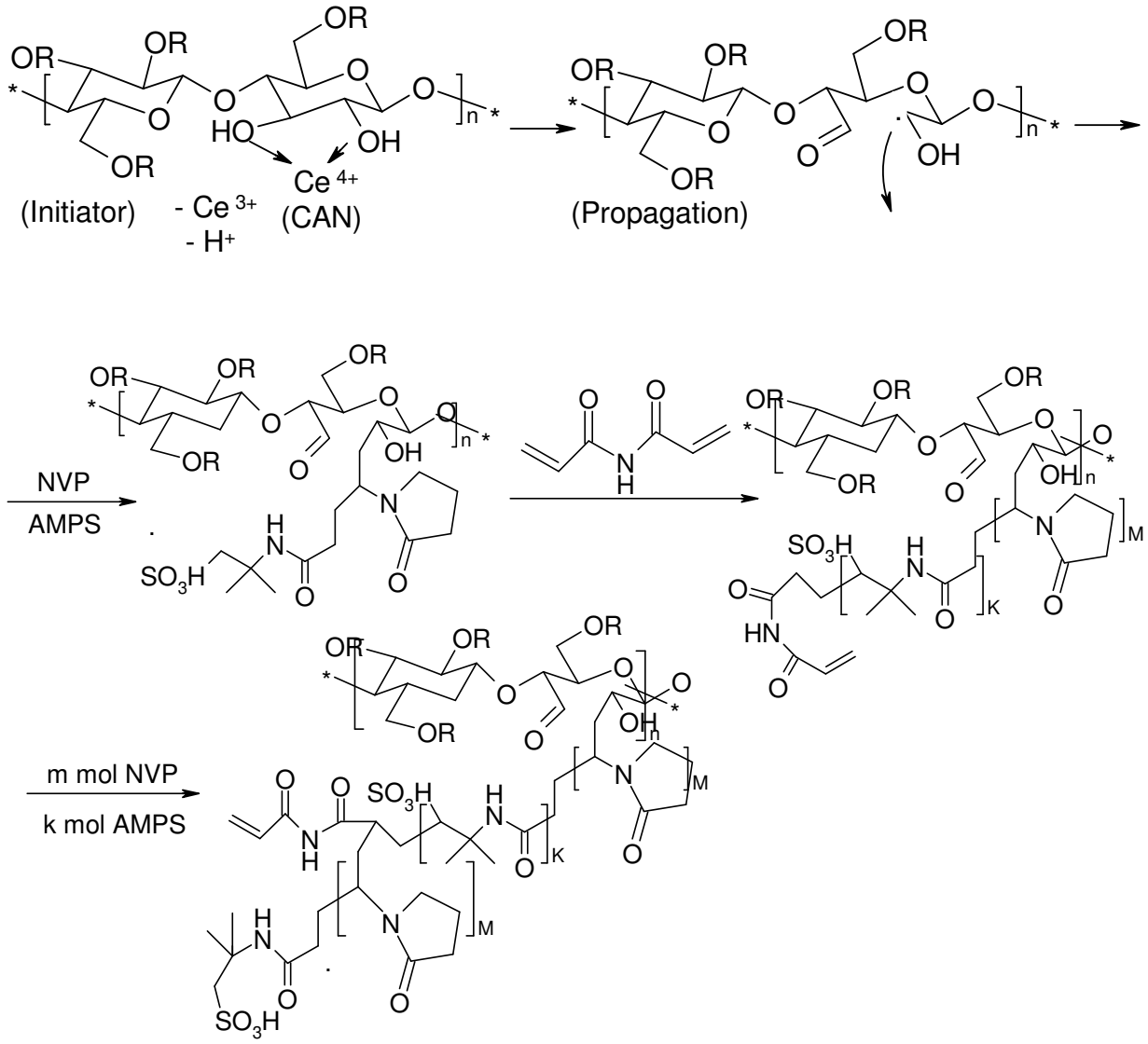
In vitro drug release of hydrogels

The release of metronidazole was followed as a function of time by measuring the light-absorbance of the outer aqueous phase at 245 nm using an UV/VIS spectrometer. The samples (0.1±0.0001 g) were immersed into 50 ml of the release medium (simulated gastric and intestinal fluids, SGF and SIF) with different pH values (pH 1.2 or 7.4) at 37°C with agitation using a magnetic stirrer. The same volume of fresh release medium was used to replace what was removed (Ju, 2001).

RESULTS AND DISCUSSION

Synthesis and spectral characterization

Scheme 1 shows a simple structural proposal of the graft copolymerization of N-vinyl pyrollidin and 2-acrylamido-2-methyl propan sulfonic acid monomers on the CMC backbones and crosslinking of the graft copolymer. At the first step, a complex between the Ce^{4+} ion with the oxygen atom at the C-3 position and the hydroxyl group at the C-2 position was formed. This ceric-CMC complex is the abstracts hydrogen atom from polysaccharide to produce CMC macroradicals. The monomer molecules, which are in vicinity of the macroradical sites, become acceptor of CMC radicals resulting in chain initiation and thereafter themselves become free radical donor to the neighboring molecules leading to propagation. These



Scheme 1. A brief proposed mechanism for ceric-induced grafting of N-vinyl pyrollidin and 2-acrylamido-2-methyl propan sulfonic acid monomers onto CMC.

grafted chains are terminated by coupling to give the graft copolymer. Mino and Kaizerman (1958) for the first time utilized ceric ammonium nitrate (CAN) as a very effective redox initiator. CAN is an efficient oxidizing agent that can create free radicals capable of initiating graft copolymerization of vinyl monomers onto polysaccharides. Crosslinking reaction also occurred in the presence of the crosslinker, that is, MBA (Zhang and Peppas, 2000). It should be pointed out the graft product was soluble in reaction medium. Hence, we used the crosslinker agent during the grafting of vinyl monomers onto CMC.

FTIR spectroscopy was used for the identification of the hydrogel. Figure 2 shows the IR spectra of the CMC and the resulted hydrogel. The band observed at 1623 and can 1430 cm^{-1} could be attributed to C=O stretching and bending in carboxylate (COO^-) functional groups of substrate backbone (Figure 2a). The broad band at 2500 to 3500 cm^{-1} is due to the stretching of -OH groups of the CMC. In the spectra of the hydrogel, the characteristic bands at 1553, 1230 and 1670 cm^{-1} were attributed to N-H, S=O (AMPS monomer) and C=O (vinyl pyrollidin) stretching, respectively.

To obtain additional evidence of grafting, a similar polymerization was conducted in the absence of the crosslinker. After extracting the homopolymers, PNVP or PAMPS and unreacted monomers using a cellophane membrane dialysis bag (D9402, Sigma-Aldrich), an appreciable amount of grafted CMC (87%) was observed. The graft copolymer spectrum was very similar to Figure 2b. Also, according to preliminary measurements, the sol (soluble) content of the hydrogel networks was as little as 1.6%. This fact practically proves that all NVP and AMPS

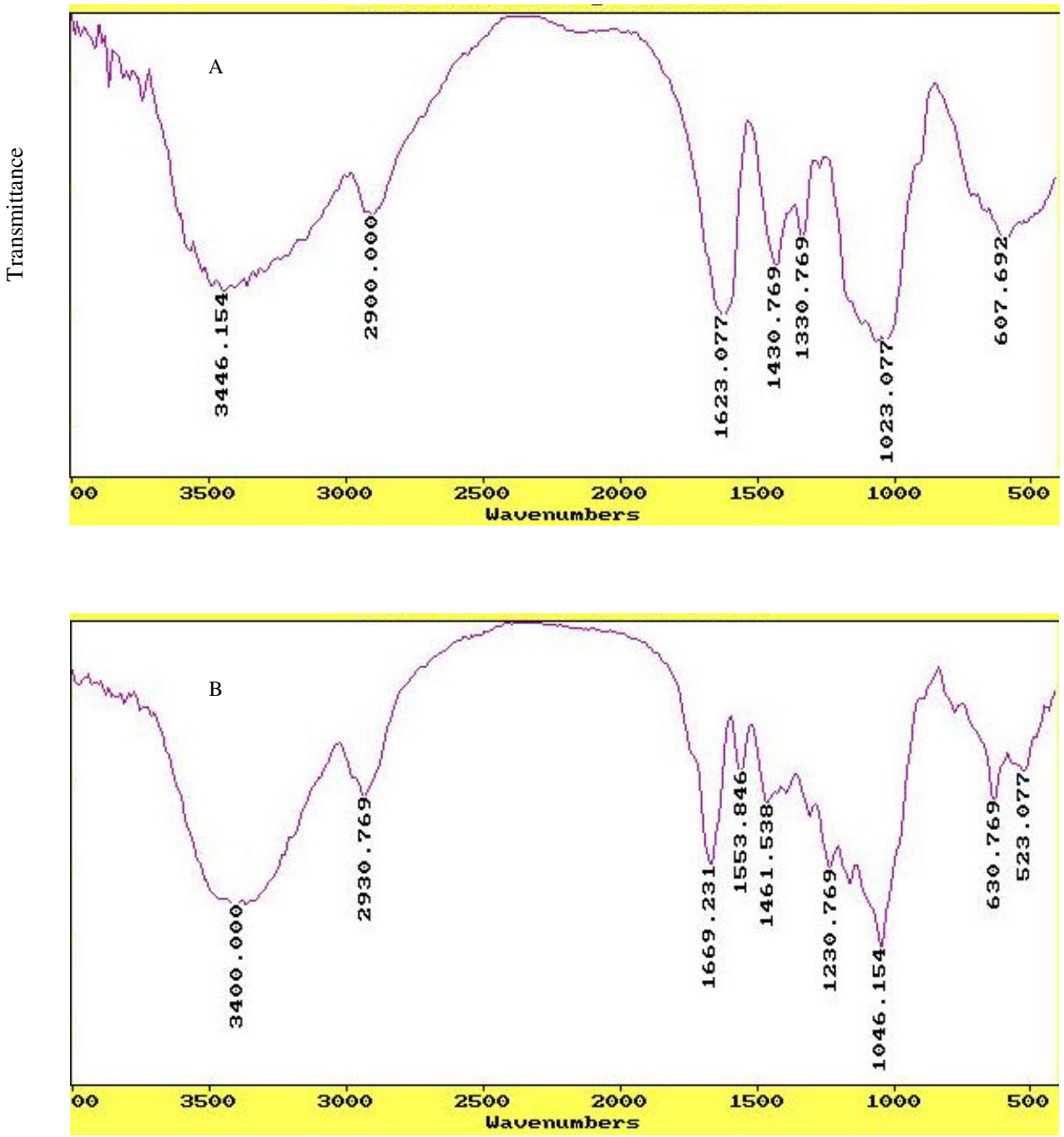


Figure 2. FTIR spectra of CMC (a) and crosslinked CMC-*g*-(PNVP-*co*-PAMPS) hydrogel (b).

are involved in the polymer network. So, the monomers percent in the network will be very similar to that of the initial feed of reaction.

One of the most important properties that must be considered is hydrogel microstructure morphologies. Figure 3 shows the scanning electron microscope images of the hydrogel. This picture verifies that the synthesized polymer in this study have a porous structure. It is supposed that these pores are the regions of water permeation and interaction sites of external stimuli with the hydrophilic groups of the graft copolymers.

pH-sensitivity and pulsatile behavior

Equilibrium swelling studies indicated that the ionic hydrogels were sensitive to environmental pH. Therefore, in this series of experiments, swelling ratio for the

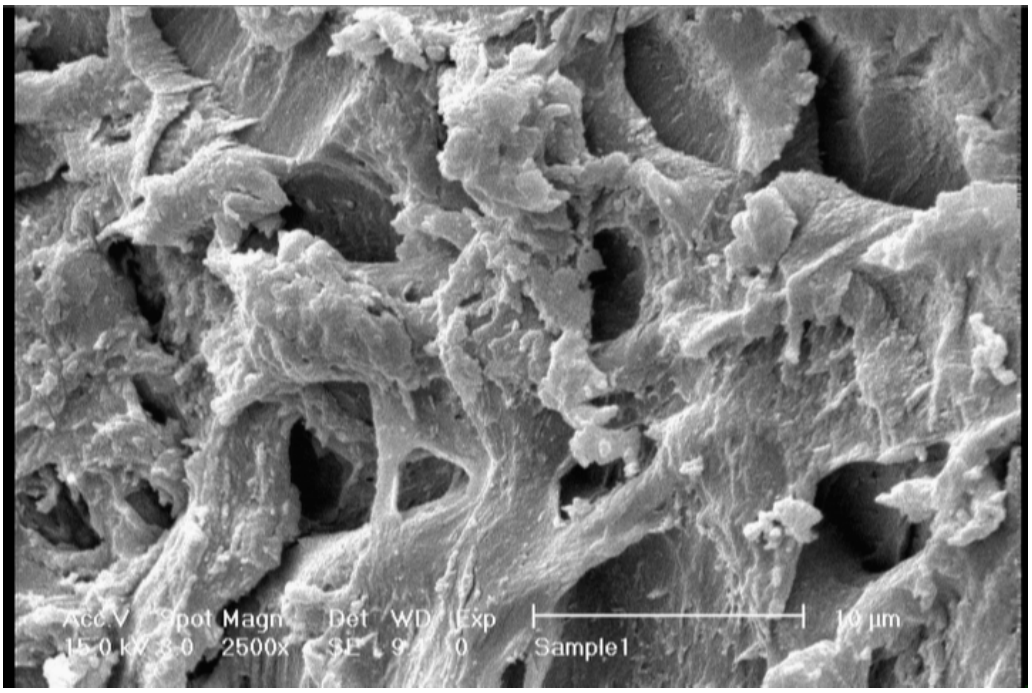


Figure 3. SEM photograph of the hydrogel. Surfaces were taken at a magnification of 2500 and the scale bar is 10 µm.

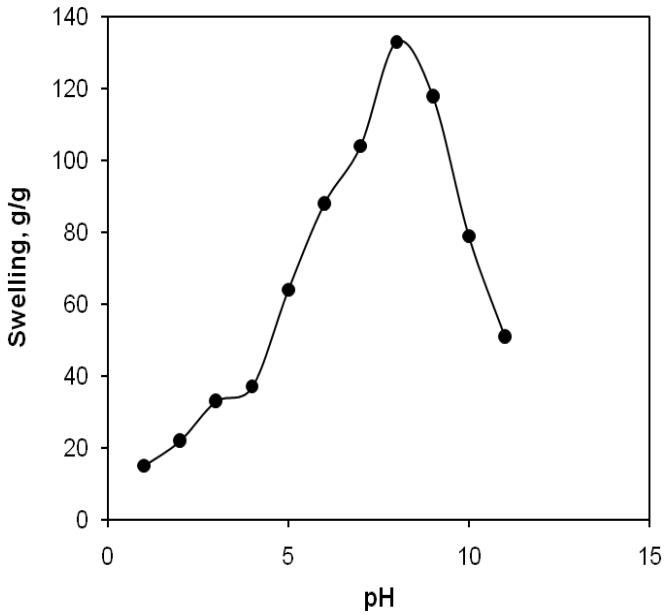


Figure 4. Effect of pH on the swelling capacity of CMC-*g*-(PNVP-*co*-PAMPS) hydrogel.

synthesized hydrogels was measured in different pH solutions ranging from 1.0 to 13.0 (Figure 4). Since the swelling capacity of all "anionic" hydrogels is appreciably decreased by the addition of counter ions (cations) to the swelling medium, no buffer solutions were used. Therefore, stock NaOH (pH 10.0) and HCl (1.0) solutions were diluted with distilled water to reach desired basic and acidic pHs, respectively. Maximum swelling (133 g/g) was obtained at pH 8. In acidic media, most of the solfonic groups are protonated, so decreased repulsion of anionic

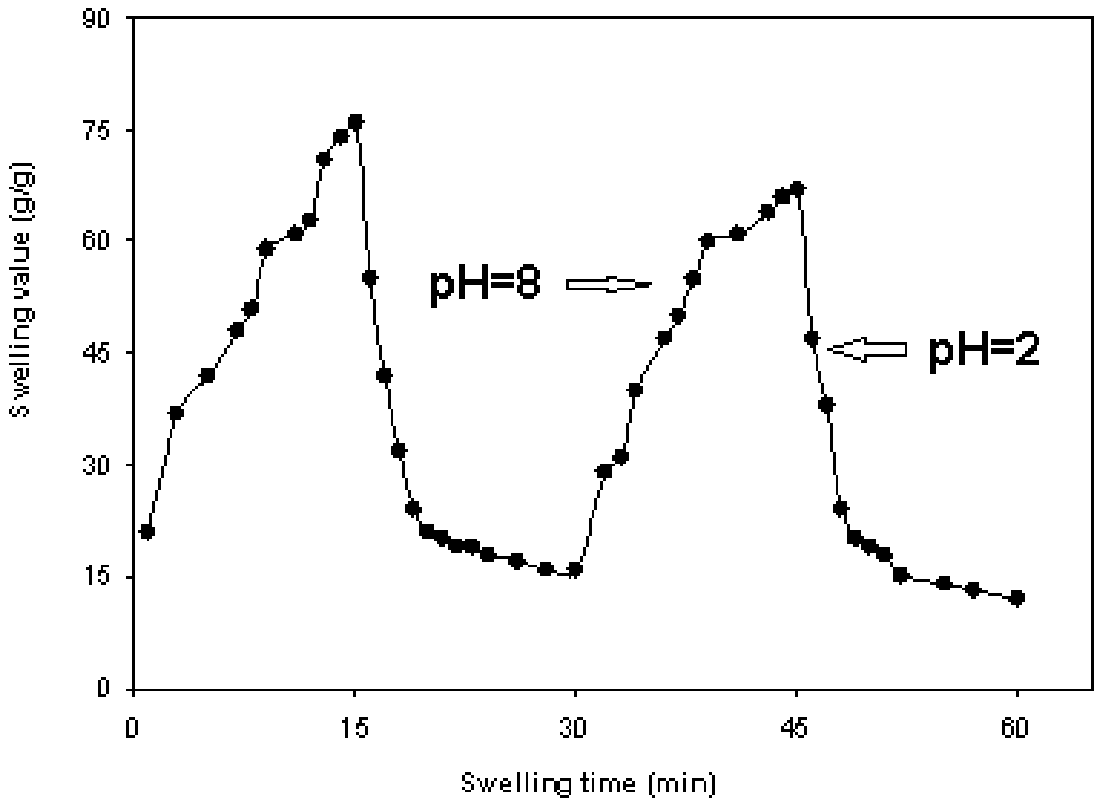


Figure 5. The pH-responsiveness behavior of CMC-*g*-(PNVP-*co*-PAMPS) superabsorbing hydrogel in solutions with pH= 2.0 and pH= 8.0.

group leads to a decreased swelling ratio. At higher pHs (5 to 8), some of solfonic groups are ionized and the electrostatic repulsion between SO_3^- groups causes an enhancement of the swelling capacity. The reason of the swelling-loss for the highly basic solutions is "charge screening effect" of excess Na^+ in the swelling media which shield the solfonic anions and prevent effective anion-anion repulsion (Zhang, 2004).

The CMC-*g*-(PNVP-*co*-PAMPS) hydrogels 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 133 g/g due to anion-anion repulsive electrostatic forces, while at pH 2.0, it shrinks within a few minutes due to protonation of solfonic groups. This sharp swelling-deswelling behavior of the hydrogels makes them as suitable candidate for controlled drug delivery systems. Such on-off switching behavior as reversible swelling and deswelling has been reported for other ionichydrogels (Liu et al., 2004).

Standard calibration curve

The calibration curve of the absorbance as a function of the metronidazole concentration at 245 nm, shown in Figure 6, has a linear relationship with a correlation coefficient (r) of 0.979 and 0.995 at pHs 1.6 and 7.4, respectively.

Drug loading

The amounts of the loaded drug in superabsorbent hydrogels was also significantly affected by the impregnation times (Figure 7). It is obvious that with increase in the loading time, the amount of drug loaded is initially increased and then begins to level off (in both pH= 1.6 and 7.4). The initial increment in the amounts of the loaded drug with increasing loading time can be ascribed to the increased drug diffusion into the swollen matrix. The most efficient time of loading efficiency in pH= 7.4 was 99 h, where a major amount of drug was encapsulated. Although, the swelling equilibrium attained faster in pH= 1.6 than in pH=7.4, the amount of loaded drug pH= 7.4 is higher than that in pH= 1.6 because of the higher swelling capacity of the hydrogel in pH= 7.4.

Controlled metronidazole release

To determine the potential application of CMC-based superabsorbent containing a pharmaceutically active

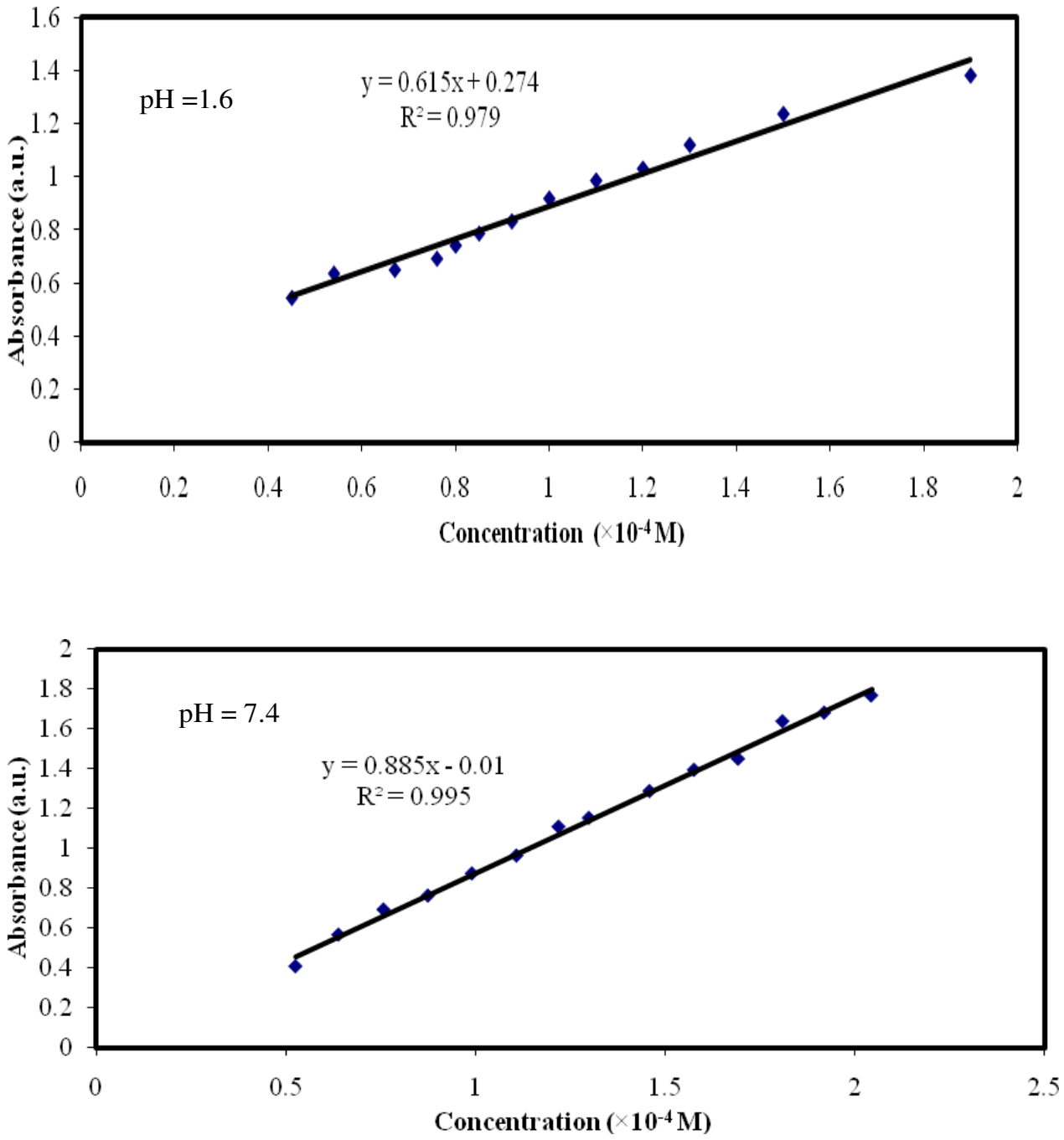


Figure 6. The standard calibration curve of the absorbance as a function of metronidazole concentration at 245 nm on the UV spectrophotometer at pH 1.6 (a) and pH 7.4 (b).

compound, we have investigated that the drug release behavior forms this system under physiological conditions. The concentration of released drug from the polymeric carriers as a function of time is shown in Figure8. The concentration of metronidazole released at selected time intervals was determined by UV spectro-photometer. The amount of metronidazole released in a specified time from the CMC-based hydrogel decreased as the pH of the dissolution medium was lowered (Figure 8). At low pH values, electrostatic repulsion between the solfonic acid groups of backbone is low, thus, decreases gel swelling and minimizes release of metronidazole via diffusion. However, in alkaline media, the presence of OH^- increases the electrostatic repulsion between solfonic groups, thus, increases the gels swelling degree and so the release of metronidazole increased.

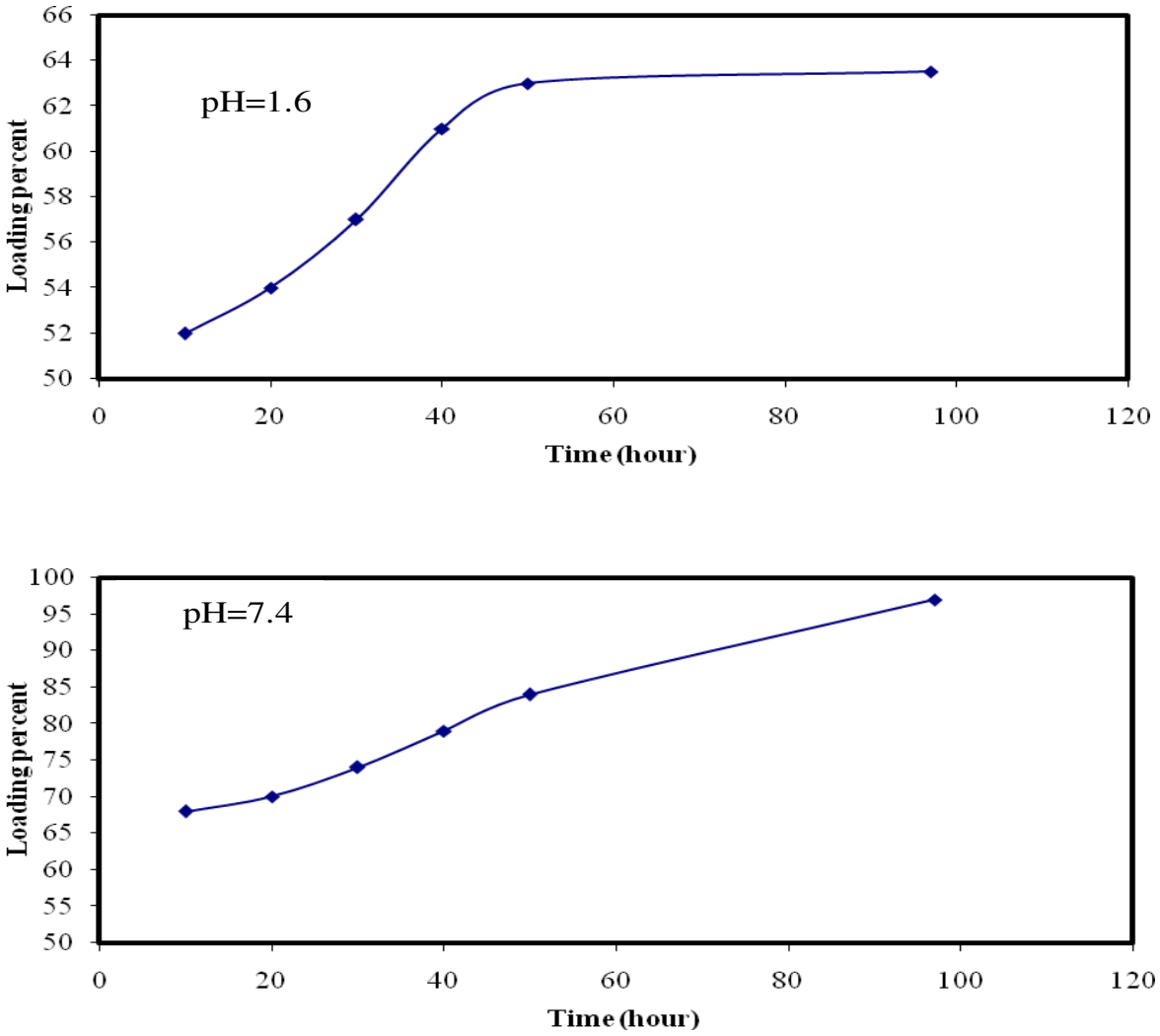


Figure 7. The dependency of the drug loading amount to the loading time in pH=1.6 and 7.4.

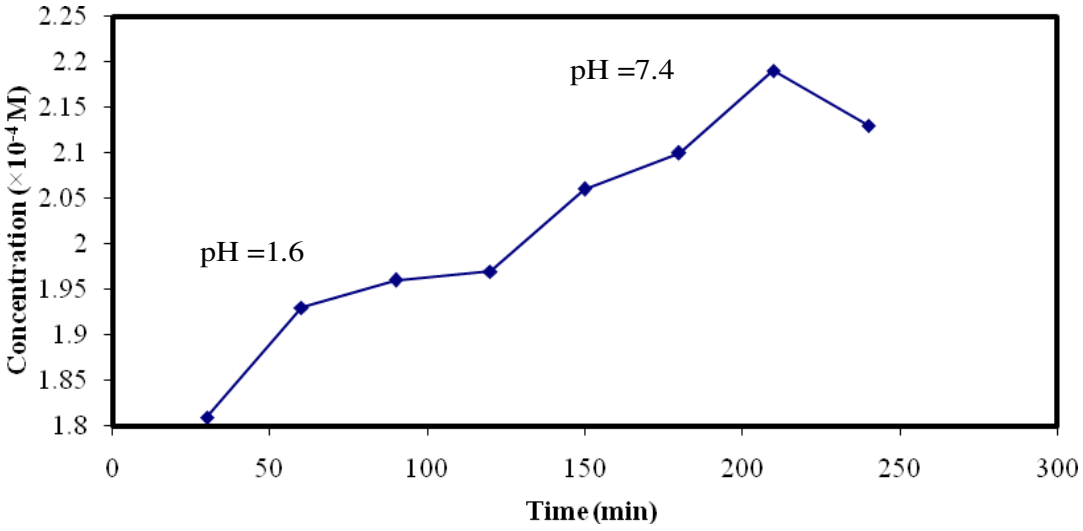


Figure 8. Release of metronidazole from hydrogel carrier as a function of time and pH (pH=1.6 and pH=7.4) at 37°C.

Conclusion

In this study, CMC-*g*-(PNVP-*co*-PAMPS) superabsorbent hydrogel was synthesized in an aqueous solution using a ceric ammonium nitrate initiator and a hydrophilic crosslinker. The swelling of hydrogel exhibited high sensitivity to pH. The study of the effect of H^+/OH^- concentration carried out at various pHs shows that the swelling of hydrogel causes several large volume changes. So, we investigated the pH-sensitivity of the hydrogel. Ionic repulsion between charged groups incorporated in the gel matrix by an external pH modulation could be assumed as the main driving force responsible for such abrupt swelling changes, because this superabsorbent network intelligently responding to pH can be considered as an excellent candidate to design novel drug delivery systems. Therefore, we investigated the release behavior of metronidazole from this kind of responsive hydrogel. The release value of drug from hydrogels at pH 7.4 was higher than that at pH 1.6 due to the electrostatic repulsion between solfonate groups and the higher swelling capacity of the hydrogel.

REFERENCES

Buchholz FL, Graham AT (1997). Modern Superabsorbent Polymer Technology, New York, Wiley.

Hoffman AS (2002). Hydrogel for biomedical applications. Adv. Drug Deliver. Rev. 43: 3-12.

Ju HK, Kim SY, Lee YM (2001). pH/temperature-responsive behaviors of semi-IPN and comb-type graft hydrogels composed of alginate and poly(N-isopropylacrylamide). Polymer, 42: 6851-6857.

Kost J (1995). Encyclopedia of Controlled Drug Delivery, New York, Wiley.

Liu YY, Fan XD, Kang T, Sun L (2004). A cyclodextrin microgel for controlled release driven by inclusion effects. Macromol. Rapid Commun. 25: 1912-1916.

Mino G, Kaizerman S (1958). A new method for preparation of graft copolymers: Polymerization initiated by ceric ion redox systems. J. Polym. Sci. 31: 212-217.

Peppas NA, Mikes AG (1986). Hydrogels in Medicine and Pharmacy. Florida, CRC Press, Boca Raton.

Peppas LB, Harland RS (1990). Absorbent Polymer Technology, Amsterdam, Elsevier.

Po R (1994). Water-absorbent polymers: a patent survey. J. Macromol. Sci. Rev. Macromol. Chem. Phys. C34: 607-662.

Yoshida R, Uchida K, Kaneko Y, Sakai K, Kikuchi A, Sakural Y, Okano T (1995). Comb-type grafted hydrogels with rapid deswelling response to temperature changes. Nature, 374: 240-242.

Zhang J, Peppas NA (2000). Synthesis and characterization of pH- and temperature-sensitive poly(methacrylic acid)/poly(N-isopropylacylamide) interpenetrating polymeric networks. Macromolecules, 33: 102-107.

Zhang XZ, Wu DQ, Chu CC (2004). Synthesis, characterization and controlled drug release of thermosensitive IPN-PNIPAAM hydrogels. Biomaterials, 25: 3793-3805.