Journal of Applied Polymer Science

In vitro evaluation of biopolymer networks based on crosslinked cellulose with various diamines

Nasim Movagharnezhad, Peyman Najafi Moghadam
Department of Organic Chemistry, Faculty of Chemistry, University of Urmia, Urmia, Iran
Correspondence to: P. N. Moghadam (E-mail: p_najafi27@yahoo.com or p.najafi@urmia.ac.ir)

ABSTRACT: In this study, we investigated some hydrogels based on natural, biodegradable, and biocompatible polysaccharides and cellulose, as sustained release carriers and evaluated their controlled drug release. The crosslinking of bromoacetylated cellulose with various aliphatic and aromatic diamines, such as ethylene diamine, hexamethylene diamine, and paraphenylene diamine, were carried out in *N,N*-dimethylformamide in the presence of triethylamine as a base. On the other hand, the synthesized hydrogels were characterized with Fourier transform infrared spectroscopy, thermogravimetric analysis, scanning electron microscopy, and CHN elemental analysis. Also, the equilibrium swelling behavior of the hydrogels in water was calculated, and this indicated their sustained expansion. The loading of ceftizoxime antibiotic was carried out on the prepared hydrogels, and the *in vitro* and drug-release behaviors were investigated in three different media (HCl solution at pH 3 and bicarbonate buffer solutions at pH 6 and 8). Finally, the release profiles of ceftizoxime from the hydrogels and their loading capacities were determined by ultraviolet–visible absorption measurements at λ_{max} 295 nm. © 2015 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2015**, *132*, 42568.

KEYWORDS: biodegradable; crosslinking; drug-delivery systems

Received 2 February 2015; accepted 28 May 2015
DOI: 10.1002/app.42568

INTRODUCTION

There is continually growing interest in the pharmaceutical industry in drug delivery in sustained or controlled release forms. The release kinetics from various formulations have shown that dissolution depends on several parameters, including the drug solubility, drug loading, polymer characteristics, and presence of additional excipients.[1–5] For this purpose, various polymeric systems have been synthesized for pharmaceutical applications.[6–8] The best way to incorporate a polymer with a drug involves the formation of polymer networks in which drugs are mixed or blended physically, and drug release is achieved by diffusion from the surrounding polymeric network.[9,10] The main functions of polymeric carriers are to sustain drug release, transport drugs to the site of action, or both. By loading the drugs in a polymer network by various techniques in which the drugs are protected from interacting with other molecules, we could change in the chemical structure of the active ingredient that is causing the drug to lose its pharmaceutical action.[11] One of the important polymer networks for pharmaceutical applications is hydrogels.[12–14]

The term *hydrogels* was originally introduced by Wichterle and Lim in the 1960s, and its biological applications were put forward.[15] Hydrogels are crosslinked materials that absorb large quantities of water without dissolving, and with the large amount of water interposed in their three-dimensional polymeric networks, hydrogels are highly needed in biomedical fields.[16,17] These polymer networks have played a vital role in the development of controlled release drug-delivery systems. Three-dimensional network formation occurs by the crosslinking of the polymeric chains. This crosslinking can occur via physical interactions, covalent bonding, hydrogen bonding, and by van der Waal's interactions.[18–20] The water-holding capacity of hydrogels arises mainly from the presence of hydrophilic groups, such as amino, amide, carboxyl, and hydroxyl groups, in the polymer chains. According to Hoffmann,[21] the amount of water presented in a hydrogel may vary from 10% to thousands of times of the weight of the hydrogel.

Hydrogels containing polysaccharides have been widely used in biomedical applications because of their biocompatibility with the human body.[22,23] These natural polymers, such as cellulose, have been found to possess a wide range of properties, and they find applications in biomedical fields, such as in drug-delivery carriers.[24] Cellulose made from monomers consist of glucose units $(C_6H_{10}O_5)_n$ with *n*s ranging from 500 to 5000, and cellulose is the most abundant natural polymer in the world. Moreover, this natural polymer, found in the cell walls of nearly all plants, because of its low production cost, favorable biodegradability, and exceptional biocompatibility, has attracted great attention.[25,26] However, cellulose suffers from a number of disadvantages, including a poor mechanical strength and low

processability. Many efforts have been carried out to overcome these limitations through the chemical modification of cellulose. Various derivatives of cellulose have been prepared, and a large number of medical applications have been reported by several authors, YerriSwamy *et al.*[27] synthesized hydroxypropyl methyl cellulose and poly(vinyl alcohol) blend microspheres by a water-in-oil emulsion method, and ciprofloxacin hydrochloride was loaded into interpenetrating polymer network microspheres crosslinked with glutaraldehyde. Oprea *et al.*[28] prepared mixed hydrogels based on natural, biodegradable, and biocompatible polysaccharides, such as cellulose and chondroitin sulfate, with various mixing ratios, and they evaluated the hydrogels for application in biomedical fields. Najafi Moghaddam *et al.*[29] prepared the chloroacetylated cellulose and bromoacetylated cellulose (BAC). Then, they modified them with the graft copolymerization of acrylamide, hydroxyl ethyl acrylate, and styrene via free-radical polymerization and atom transfer radical polymerization.

On the basis of this background, in this study, we report the preparation of three hydrogels by crosslinking of BAC with three types of aromatic and aliphatic diamines along the chains. The diamines were ethylenediamine (EDA), hexamethylenediamine (HMDA), and paraphenylene diamine (PPDA). The drug-loading and drug-release behaviors of the prepared hydrogels were investigated in three different media (at pH 3, 6, and 8) with ceftizoxime as a model drug. In the last characterization, the swelling and drug-loading and drug-release behaviors of these hydrogels were compared with each other.

EXPERIMENTAL

Materials

Cellulose powder (Merck) were pulverized, dried *in vacuo* for 24 h, and kept in desiccator. Boromoacetyl bromide (>99%), EDA, HMDA, PPDA, *N,N*-dimethylacetamide (DMA), *N,N*-dimethylformamide (DMF), triethylamine, and pyridine (PY) (98%) were purchased from Merck and used without further purifications. LiCl (98%, Merck) was kept *in vacuo* oven overnight to remove any trace of moisture before use. Ceftizoxime was kindly donated by Dena Tabriz Pharmaceutical Co., and it was used as received.

Measurements

IR spectra were measured with a Fourier transform infrared spectrophotometer (Nexus 670, Thermo Nicolet). Thermogravimetric analysis (TGA) of the prepared samples was performed with a LENSESSTAPT-1000 calorimeter (Germany) with scanning from room temperature up to 600°C at a heating rate of 10°C/min. Moreover, the morphology of the hydrogels was observed with scanning electron microscopy (SEM) images, which were obtained with an SEM-3200 scanning electron microscope. The elemental analysis of the hydrogels was performed. In addition, carbon, nitrogen, and hydrogen were analyzed by a PerkinElmer C H N S Analyzer 2400 series 2. Furthermore, an ultraviolet–visible spectrophotometer (T80 – PG Instruments, Ltd., United Kingdom) was used to study the drug release.

Preparations of BAC

N,N-Dimethylacetamide/LiCl (20 mL, 2.5 w/w %) was added to a 50-mL, round-bottomed flask equipped with a magnetic stirring bar. Then, cellulose powder (1 g, 6.17 mmol) was added too. The flask was kept under reflux at 80°C for about 2 h, and then, it was cooled to room temperature. After that, pyridine (1.46 g, 18.51 mmol) and bromoacetyl bromide (4.48 g, 22.22 mmol) were added slowly to the flask with stirring in an ice bath. After the addition was completed, the stirring continued at the room temperature for 18 h. Finally, the mixture was poured into 150 mL of 2*M* HCl, and then, it was filtered and washed several times with distilled water and dried in a vacuum oven at 50°C.

Synthesis of the Hydrogel Networks

First, crosslinking of bromoacetylated cellulose with EDA (c-BAC/EDA) was prepared in a 50-mL, one-necked, round-bottomed flask equipped with a reflux condenser and a magnetic stirrer bar. Second, to achieve this purpose, a mixture of BAC (0.95 mmol, 0.5 g), EDA (0.47 mmol, 0.028 g), triethylamine (4.94 mmol, 0.28 g), and DMF (20 mL) were added and heated with stirring at 100°C for 18 h. After the reaction mixture was cooled, the product was filtered, washed thoroughly with water, and dried *in vacuo* for 24 h (yield = 72%).

To prepare crosslinking of bromoacetylated cellulose with HMDA (c-BAC/HMDA) and crosslinking of bromoacetylated cellulose with PPDA (c-BAC/PPDA) the same procedure was repeated with only differences in the kind of diamine; 0.05 g (0.47 mmol) of HMDA and 0.05 g (0.47 mmol) of PPDA were used, respectively (yield of c-BAC/HMDA = 76% and yield of c-BAC/PPDA = 83%).

Swelling Measurements with the Tea Bag Method

A tea bag with an average particle size between 40 and 60 mesh (250–420 mL) containing an accurately weighed powdered sample (0.1 ± 0.0001 g) was immersed in distilled water (100 mL) and allowed to soak for 30 h at room temperature. To measure the swelling kinetics and the rate of absorption, after a certain time (t), we took the water-absorbed samples from the solution at various time points, and the swelling measurements were done according to the previous procedure. Subsequently, the tea bag was suspended in air for 2 min to remove excess fluid each time. Finally, the swelling measurements were made without extraction by water.[30]

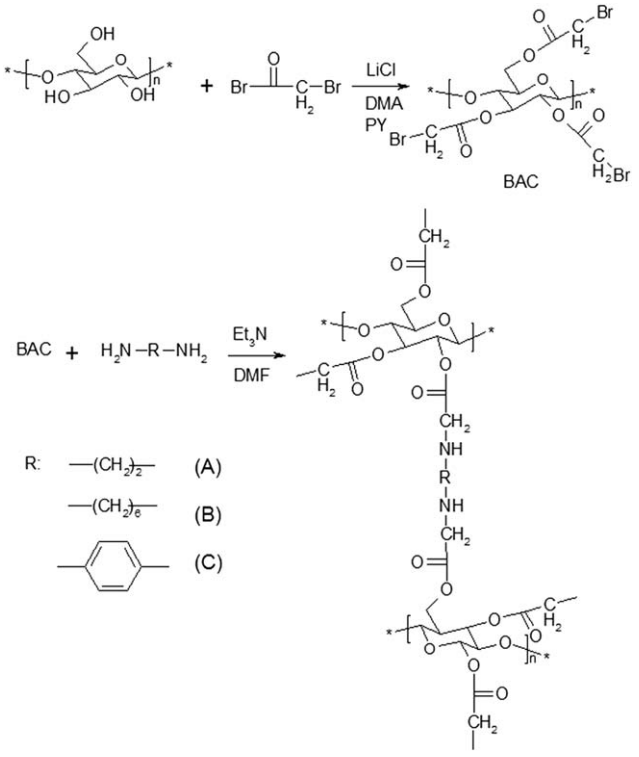
The equilibrated swelling (ES) was calculated twice with the following equation:

$$\mathrm{ES}\left(\frac{\mathrm{g}}{\mathrm{g}}\right) = \frac{(W_s - W_d)}{W_d} \quad (1)$$

where W_s and W_d are the weights of the swollen gel and the dry sample, respectively. Thus, the absorbency was calculated as grams of water per grams of hydrogel (g/g).

Drug-Loading and Drug-Release Behaviors

An accurately powdered weight of prepared sample (0.1 g) was immersed entirely in a dilute solution of 0.5 g of ceftizoxime drug dissolved in 20 mL of distilled water; this was incubated at 37°C for 30 h. Later, the completely swollen hydrogels were loaded with drug and then placed in a vacuum oven and dried *in vacuo* at 37°C.



Scheme 1. Synthetic route for the preparation of (A) c-BAC/EDA, (B) c-BAC/HMDA, and (C) c-BAC/PPDA.

To measure the amount of drug loaded on the hydrogel and the drug released in the release medium, the standard calibration curve of the absorbance as a function of the drug concentration was drawn at 295 nm on the UV spectrophotometer.

The loading capacity (LC) was calculated from the difference between the amount of ceftizoxime that was initially used to prepare the drug-loaded hydrogels and the nonassociated ceftizoxime residues divided by the total mass of the hydrogels:

$$\mathrm{LC}(\%) = \frac{\text{Total amount of ceftizoxime} - \text{Free ceftizoxime in the supernatant}}{\text{Total weight of the hydrogels}} \quad (2)$$

In the *in vitro* release studies, the drug-loaded hydrogels were placed in carbonate-buffered solutions. The samples (0.1 ± 0.0001 g) in a dialysis tube (molecular weight cutoff = 3500) were immersed in 50 mL of bicarbonate buffer (0.1*M*) at different pH values (3, 6, and 8) at 37°C with slow agitation. At a given time interval, 3 mL of the release medium was removed with a syringe. The sample was poured back into the drug-release container. The concentration of the released drug was measured by a UV spectrophotometer at 295 nm.

RESULTS AND DISCUSSION

Synthesis of the Hydrogel Networks

Three types of hydrogels were synthesized according to the synthetic route that is depicted in Scheme 1. The hydrogels were prepared by processing in two steps. In the first step, the hydroxyl groups of activated cellulose reacted with the acyl bromide of bromoacetyl bromide. Slow addition of the bromoacetyl bromide to the cellulose solution was carried out in an ice bath because their reaction was very exothermic, and the released heat might have destroyed the cellulose chains. The crosslinking was carried out in the second step of the reaction. In this step, the reaction was carried out between BAC and various diamines in the presence of triethylamine as the base. The reaction readily proceeded inside a heterogeneous solution in DMF.

Fourier Transform Infrared Spectroscopy

Figure 1(A) shows the IR spectrum of the pure cellulose and indicates the presence of OH groups due to the bands that appeared in the region between 3350 and 3450 cm^{-1}. These were related to the stretching vibrations of the OH ring and side chain (—CH_2—OH). The band at 2900 cm^{-1} was due to C—H stretching of the aliphatic hydrogen groups. The ring-stretching band of glucose units appeared at 1638 cm^{-1}. In addition, the bands in the region 1320–1429 cm^{-1} were due to the symmetrical deformations of the CH_2 and COH groups. The primary alcoholic CH_2OH stretching band and CH_2 twisting vibrations appeared at 1058 and 1034 cm^{-1}, respectively. The weak bands around 664 cm^{-1} were due to the ring stretching and ring deformation of α-D-(1–4) and α-D-(1–6) linkages. By comparing the spectrum that corresponded to the BAC [Figure 1(B)] with that of pure cellulose, we shifted the O—H stretching band at 3402 to 3428 cm^{-1} as a result of the esterification of the O—H and hydrogen bond dissociation. Also, the presence of the band at 787 cm^{-1} corresponding to the carbon–bromine stretching vibrations confirmed the effectiveness of bromoacetylation. The decrease in the intensity of the bands among 1050–1500 cm^{-1}, which confirmed the success of the reaction, was due to the substitution of hydroxyl with acetyl bromide too. The other significant change occurred with the

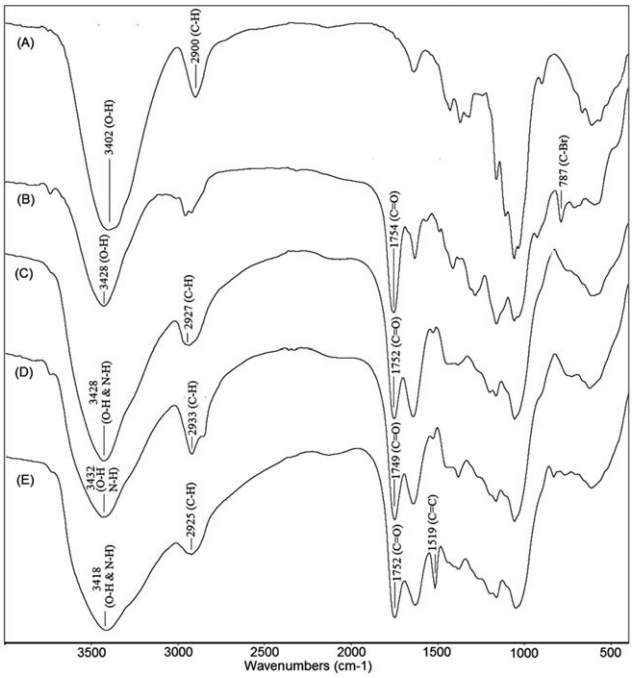


Figure 1. Fourier transform infrared spectra of (A) pure cellulose, (B) BAC, (C) c-BAC/EDA, (D) c-BAC/HMDA, and (E) c-BAC/PPDA,

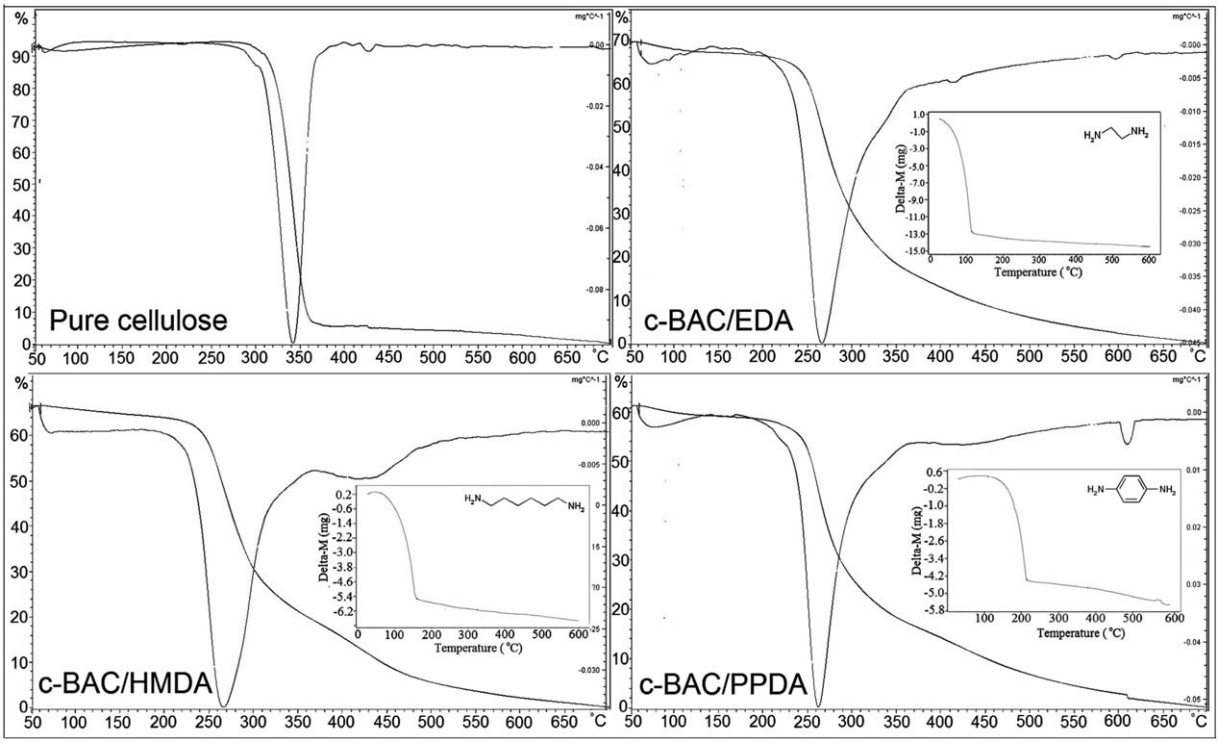


Figure 2. TGA curves for the prepared hydrogels and pure cellulose along with the pure crosslinker.

appearance of a sharp peak at 1754 cm^{-1}, which was related to the formation of ester linkages as a result of the reaction.

The spectrum for the c-BAC/EDA, c-BAC/HMDA, and c-BAC/PPDA hydrogels is shown in Figure 1(C–E). The intensity of the C—H vibration band around 2930 cm^{-1} was slightly stronger in the case of c-BAC/EDA and c-BAC/HMDA because of the introduction of more C—H in the crosslinked hydrogels. Also, the comparison of the spectra for the BAC with different hydrogels showed that the strength band about 3400 cm^{-1} increased because of the N—H band addition in the hydrogels. We also noted that the band present at 787 cm^{-1} in the spectrum of BAC was completely omitted because of the removal of the bromine in the BAC structure after crosslinking with various diamines. Furthermore, in the spectrum of the c-BAC/PPDA, a new peak was appeared at 1519 cm^{-1}, which may have been due to the C=C stretching vibration of the aromatic ring. At last, these results clearly confirmed the successful synthesis.

TGA

The results of the TGA technique were used to characterize the thermal properties of the pure cellulose, the diamines as crosslinkers, and the obtained hydrogels at 10°C/min in a nitrogen atmosphere, as shown in Figure 2, and their thermal analysis data are listed in Table I. These curves highlighted the differences between the pure cellulose, diamines, and hydrogel networks. In general, the degradation of pure cellulose was faster than that of the prepared hydrogels. The pure cellulose showed a one-step characteristic thermogram, which was attributed to cellulose chain degradation. The thermograms of the other hydrogels showed three decomposition stages. The first decomposition stage was attributed to the loss of absorbed water (moisture), which was hydrogen bonded with the appropriate functional groups in the hydrogel structure, and it was not removed in the preceding drying operation. The second stage for the loss of mass, at Temperature (T) = 181–330°C for c-BAC/EDA, T = 172–358°C

Table I. Thermal Analysis Data for the Pure Cellulose, Diamines, and Hydrogels

Sample code	Char yield (%)[a]	Temperature for 5% weight loss	Temperature for 20% weight loss	Temperature for 50% weight loss	Final decomposition temperature
Pure cellulose	8	316	329	341	361
EDA	3.34	49	77	98	112
HMDA	4.25	82	115	143	160
PPDA	4.54	149	179	202	213
c-BAC/EDA	33	235	267	345	580
c-BAC/HMDA	36	256	270	393	600
c-BAC/PPDA	42	238	266	436	620

[a] The char yield is the weight of the sample remaining at 600°C.

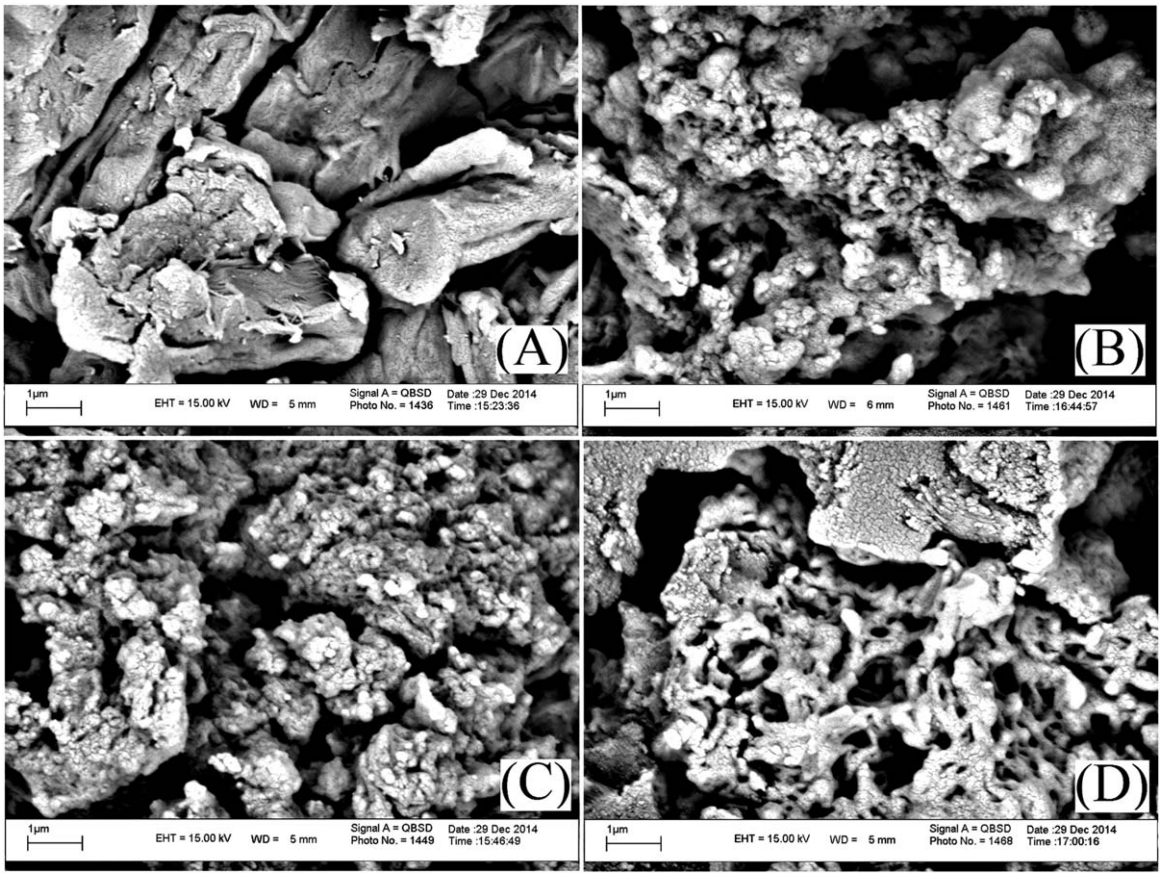


Figure 3. SEM images of (A) pure cellulose, (B) c-BAC/EDA, (C) c-BAC/HMDA, and (D) c-BAC/PPDA.

for c-BAC/HMDA, and $T = 183–357°$ for c-BAC/PPDA, was mainly due to the breaking of ester and amine linkages in the hydrogel. The last stage of weight loss started at almost 332, 360, and 363°C, respectively, and it continued up to 423, 442, and 420°C, respectively. During these stages, there were 60, 57 and 48% weight losses due to the degradation of polysaccharide chains, as we observed. The c-BAC/PPDA containing a rigid benzene group in the diamine segment showed a relatively higher thermal stability in comparison with the other hydrogels, which contained aliphatic diamines. The TGA thermograms of EDA, HMDA, and PPDA, which were added to the main image, showed that the temperatures for 5% weight loss took place at 49, 82, and 149°C, respectively. In addition, the residual weight retentions at 600°C for the diamines were almost 3% for EDA and almost 4% for HMDA and PPDA. These observations showed the low thermal stability of the pure diamines in comparison with the hydrogels; this was another confirmation of the synthesis of the hydrogel network.

SEM

One of the most important properties that we needed to consider was the hydrogel microstructure morphology. The surface morphology of the samples was investigated by SEM. Figure 3 shows the SEM micrographs of the pure cellulose, c-BAC/EDA, c-BAC/HMDA, and c-BAC/PPDA. By comparing the images, we clearly observed morphological changes between the crosslinked samples with pure cellulose. The visible pores that were convenient for the penetration of water into the polymeric network and the interaction sites of the external stimuli with the incorporated drug or hydrophilic groups of the hydrogels were observed at higher magnifications in the hydrogel structure. This indicated that the hydrogels that had a proper crosslinked agent may have been favorable for drug adsorption. On the other hand, it seemed that the c-BAC/PPDA containing a rigid benzene group in the diamine segment had a compact structure that made it better for drug-release studies compared with the other hydrogels.

Elemental Analysis (CHN)

The elemental analyses of all of the synthesized hydrogels are shown in Table II. The CHN data showed that in the prepared hydrogels, there was a considerable amount of N in the prepared hydrogels when compared with the theoretical formula; this confirmed the formation of crosslinked hydrogels in an acceptable percentage.

Swelling Behavior

The swelling behavior of the hydrogel samples in water was studied, and the results are shown in Figure 4. We found that the swelling behaviors of the hydrogels depended on the type of crosslinking agent. The prepared hydrogels, c-BAC/EDA, c-BAC/HMDA, and c-BAC/PPDA, attained swelling ratios of 12, 10, and 15, respectively, according to eq. (1). In c-BAC/HMDA, despite the large pore size of the hydrogel due to the long chain of crosslinker, a decrease in the swelling ratio was observed. On

Table II. CHN Analysis Data for the Prepared Hydrogels

Sample	C (%)	H (%)	N (%)
c-BAC/EDA	50.54	5.89	5.05
c-BAC/HMDA	47.99	5.26	4.97
c-BAC/PDA	47.05	5.66	5.90

the other hand, the relatively rigid structure of the crosslinker in c-BAC/PPDA caused an increase in the swelling values. The pores obtained by the PPDA crosslinker had a more appropriate size for the swelling behavior. Also, the hydrogel network displayed a fast initial swelling followed by a swelling decrease before attaining equilibrium. The fast initial swelling was due to the hydrophilic nature of the hydrogel network. This fast swelling opposed the resistance because of the cohesive forces exerted by the polymer strands. The cohesive forces tended to resist further hydrogel expansion and encourage some of the swelling fluid to exit the hydrogel (swelling decrease stage), and this continued until equilibrium was attained.

LC

LC released kinetics is defined as the amount of drug that can be mixed into the hydrogels. Hydrogels should have a maximum LC so that the drug is released continuously for a longer duration after insertion into the body.[31] The LC percentage of the model drug, ceftizoxime, was calculated in the hydrogel network. We expected these hydrogels to have an exceptionally high drug LC because of their porous structure. The LC of the hydrogels increased from 37.04 to 64.38% through changes in the crosslinker from HMDA to PPDA. An intermolecular hydrogen bond was formed between the electronegative atoms of the hydrogels and the functional groups of ceftizoxime. So, for c-BAC/HMDA, the LC was relatively low (37.04%) because the size of the pores was large, and the possibilities of an appropriate interaction between ceftizoxime and c-BAC/HMDA were subsequently reduced. As a result, this hydrogel was not very suitable for loading the ceftizoxime drug. On the other hand, the interactions increased with decreasing chain length of the crosslinking agent and decreasing pore size; this might have been responsible for the increase in LC when c-BAC/EDA (45.75%) was used.

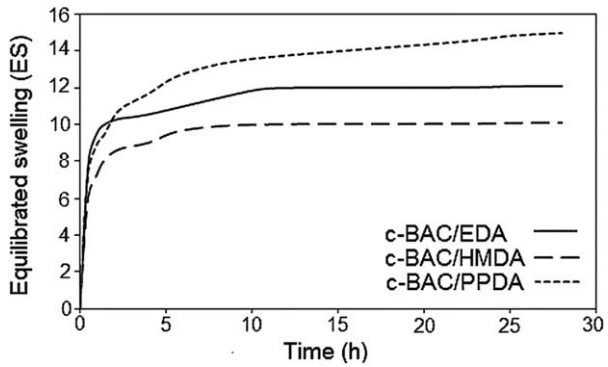


Figure 4. Swelling behavior of the prepared hydrogels.

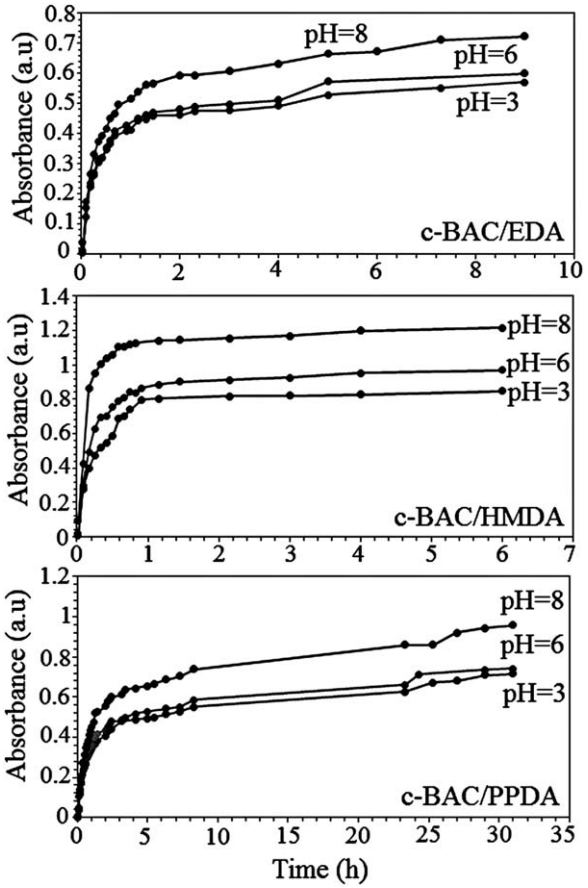


Figure 5. Drug release diagram for (A) c-BAC/EDA, (B) c-BAC/HMDA, and (C) c-BAC/PPDA.

In Vitro Drug-Release Study

The comparative results of the *in vitro* drug-release study of all of the hydrogels are shown in Figure 5. The concentration of released ceftizoxime at selected time intervals was determined by a UV spectrophotometer. In the drug-release profile of c-BAC/EDA and c-BAC/HMDA, we observed that the total amounts of drug were released within 9 and 6 h, respectively, whereas for c-BAC/PPDA, the total amount of drug was released within 30 h. As shown, all of the curves showed a burst release in the first stage; this was attributed to the drug molecules loaded at or near the surface of the particles. This was followed by a slower sustained release. c-BAC/HMDA showed a faster release rate in comparison to c-BAC/EDA and c-BAC/PPDA because of its bigger pores. Also, in the c-BAC/PPDA hydrogel, it seemed that the rigid structure of the crosslinker and the appropriate size of the pores caused to better entrapment of ceftizoxime, so the release profile of this hydrogel was much slower and longer with respect to the other ones. The amount of ceftizoxime released in a specified time from the hydrogels decreased as the pH of the dissolution medium decreased. Indeed, the prepared hydrogels had secondary amine groups, and they become protonated in an acidic environment and helped with better interactions with the loaded drug. However, in alkaline and neutral media, they remain un-ionized and released the loaded drug molecules more quickly into the surrounding medium.

CONCLUSIONS

In summary, a series of hydrogels was synthesized via the crosslinking of BAC with three types of diamines. The structures of the hydrogels were characterized by Fourier transform infrared spectroscopy and TGA. The surface morphology of the hydrogels was studied by SEM and showed a porous structure. The swelling measurement of the prepared hydrogel in water showed appreciable swelling capacity, especially in c-BAC/PPDA with a relatively rigid crosslinking agent. The synthesized hydrogels were investigated for *in vitro* drug release to reveal their potential use in drug-delivery systems. The results of this study indicated that the developed hydrogel networks could be tailored and used for the drug-delivery purposes.

REFERENCES

1. Gorner, T.; Gref, R.; Michenot, D.; Sommer, F.; Tran, M. N.; Dellacherie, E. *J. Controlled Release* **1999**, *57*, 259.
2. Govender, T.; Stolnik, S. M. C.; Illum, L.; Davis, S. S. *J. Controlled Release* **1999**, *57*, 171.
3. Neau, S. H.; Howard, M. A.; Claudius, J. S.; Howard, D. R. *Int. J. Pharm.* **1999**, *179*, 97.
4. Ramkisoon-Ganorkar, C.; Liu, F.; Baudys, M.; Kim, S. W. *J. Controlled Release* **1999**, *59*, 287.
5. Gurski, L. A.; Jha, A. K.; Zhang, C.; Jia, X.; Farach-Carson, M. C. *Biomaterials* **2009**, *30*, 6076.
6. Wang, D.; Tan, J.; Kang, H.; Ma, L.; Jin, X.; Liu, R.; Huang, Y. *Carbohydr. Polym.* **2011**, *84*, 195.
7. Sahu, S. K.; Maiti, S.; Pramanik, A.; Ghosh, S. K.; Pramanik, P. *Carbohydr. Polym.* **2012**, *87*, 2593.
8. Kwag, D. S.; Oh, N. M.; Oh, Y. T.; Oh, K. T.; Youn, Y. S.; Lee, E. S. *Int. J. Pharm.* **2012**, *431*, 204.
9. Duncan, R. *Nat. Rev. Drug Discovery* **2003**, *2*, 347.
10. Duncan, R.; Vicent, M. J.; Greco, F.; Nicholson, R. I. *Endocr. Relat. Cancer* **2005**, *12*, 189.
11. Vilara, G.; Tulla-Puchea, J.; Albericio, F. *Curr. Drug Delivery* **2012**, *9*, 000.
12. El-Sherbiny, I. M.; Smyth, H. D. C. *Carbohydr. Res.* **2010**, *345*, 2004.
13. Pinho, E.; Grootveld, M.; Soares, G.; Henriques, M. *Crit. Rev. Biotechnol.* **2013**, *8551*, 1.
14. El-Sherbiny, I. M.; Abdel-Bary, E. M.; Harding, D. R. K. *J. Appl. Polym. Sci.* **2006**, *102*, 977.
15. Wichterle, O.; Lim, D. *Ceskoslovenska Oftalmol.* **1960**, *16*, 154.
16. Ju, H. K.; Kim, S. Y.; Kim, S. J.; Lee, Y. M. *J. Appl. Polym. Sci.* **2002**, *83*, 1128.
17. Ganji, F.; Vasheghani-Farahani, E. *Iran. Polym. J.* **2009**, *18*, 63.
18. Qiu, Y.; Park, K. *Adv. Drug Delivery Rev.* **2001**, *53*, 321.
19. Shibayama, M.; Tanaka, T. *Adv. Polym. Sci.* **1993**, *109*, 1.
20. Vashist, A.; Ahmad, S. *Orient. J. Chem.* **2013**, *29*, 861.
21. Hoffman, A. S. *Adv. Drug Delivery Rev.* **2002**, *54*, 3.
22. Kim, S. J.; Shin, S. R.; Lee, J. H.; Lee, S. H.; Kim, S. I. *J. Appl. Polym. Sci.* **2003**, *90*, 91.
23. Pourjavadi, A.; Mahdavinia, G. R.; Zohuriaan-Mehr, M. J. *J. Appl. Polym. Sci.* **2003**, *90*, 3115.
24. Amin, M. C. I. M.; Ahmad, N.; Halib, N.; Ahmad, I. *Carbohydr. Polym.* **2012**, *88*, 465.
25. Oprea, A. M.; Ciolacu, D.; Neamtu, A.; Mungiu, O. C.; Stoica, B.; Vasile, C. *Cellul. Chem. Technol.* **2010**, *44*, 369.
26. Kim, J.; Yun, S. *Macromolecules* **2006**, *39*, 4202.
27. YerriSwamy, B.; Venkata Prasad, C.; Reedy, C. L. N.; Mallikarjuna, B.; Chowdoji Rao, K.; Subha, M. C. S. *Cellulose* **2011**, *18*, 349.
28. Oprea, A. M.; Profireb, L.; Lupusorub, C. E.; Ghiciucb, C. M.; Ciolacua, D.; Vasilea, C. *Carbohydr. Polym.* **2012**, *87*, 721.
29. Najafi Moghaddam, P.; Ensafiavval, M.; Fareghi, A. R. *Colloid Polym. Sci.* **2014**, *292*, 77.
30. Gupta, P.; Vermani, K. *Drug Discovery Today* **2002**, *7*, 569.
31. Garg, T.; Singh, O.; Arora, S.; Murthy, R. S. R. *Crit. Rev. Ther. Drug Carrier Syst.* **2012**, *29*, 1.