Swelling Dynamics of IPN Hydrogels Including Acrylamide-Acrylic Acid-Chitosan and Evaluation of their Potential for Controlled Release of Piperacillin-Tazobactam

Mehlika Pulat,[1] Nur Tan,[1] Fatma Kaynak Onurdağ[2]

[1]*Department of Chemistry, Faculty of Arts and Sciences, Gazi University, Teknikokullar, Ankara, Turkey*
[2]*Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Gazi University, Etiler, Ankara, Turkey*

Received 21 December 2009; accepted 9 August 2010
DOI 10.1002/app.33169
Published online 14 October 2010 in Wiley Online Library (wileyonlinelibrary.com).

ABSTRACT: For to be used in controlled releasing of piperacillin-tazobactam, a series of semi and full IPN type hydrogels composed of acrylic acid (AA), acrylamide (AAm) and Chitosan (CS) were prepared via free-radical polymerization. Ethylene glycol dimethacrylate (EGDMA) was used for crosslinking of PAAm and PAA chains to form semi-IPN hydrogels. However, the full-IPN type hydrogels were prepared by using glutaraldehyde (GA) and EGDMA as cocrosslinkers. Characteristics of the hydrogels were investigated by swelling experiments and SEM and FTIR analysis. Generally, full-IPN type hydrogels swell much more than the semi-IPN types. By comparing the full-IPN type hydrogels in between, it is found that the increasing amount of GA causes the decreasing in *S*% values from 4860 to 4300%. Releasing of piperacillin-tazobactam from selected three hydrogels were investigated in phosphate buffer solution at pH = 7.4, 37°C. The kinetic release parameters, n and k were calculated and non-Fickian type diffusion was established for these hydrogels. The behaviors of the piperacillin-tazobactam loaded hydrogels in *Staphylococcus aureus* (*S. aureus*) and Pseudomonas aeruginosa (*P. aeruginosa*) culture suspensions were also studied and the statistically significant differences for the microorganism growth values were determined. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 120: 441–450, 2011

Key words: IPN-hydrogels; piperacillin-tazobactam; swelling; chitosan

INTRODUCTION

Interpenetrating polymer network (IPN) hydrogels are preferred in a number of biotechnological and biomedical applications because of their certain unique biophysical properties such as ease of fabrication to various geometrical forms; minimum mechanical irritation to surrounding tissues; unusual stability to biofluids, etc.[1] IPN structures are also used for the control of overall hydrogel hydrophilicity and drug release kinetic.[2] A wide range of so-called semi-IPN has been prepared dissolving a performed linear polymer in a hydrophilic monomer and crosslinking agent mixture which is subsequently polymerized. In this way a synthetic hydrogel network is formed around a primary polymer chain which modifies the behavior of the hydrogel.[3] The use of semi-IPNs in pH-sensitive or temperature-sensitive drug delivery systems has been well documented.[4] During the last years IPN type hydrogels have often been used for the preparation of controlled-release dosage forms.[5,6] These types of hydrogels are also suitable materials for wound-healing or dressing material. As is known, the wound requires a protective barrier to allow healing to progress. Hydrocolloids, films, foams, alginates, and hydrogels are commercially available preparations for this purpose. The choice of dressing will depend on the location and state of the wound, and also the wound type. Wounds with good granulation tissue would benefit from moisture-providing hydrogels.[7]

Chitosan (CS) stands out by some unique combination of favorable biological properties such as nontoxicity, biocompatibility, biodegradability, along with mucoadhesive, bacteriostatic, and wound-healing properties.[8,9] In addition, this cationic biopolymer has been reported to improve transport across biological barriers.[10] Finally, CS is very abundant and its production is both environmentally safe and of low cost.[11] From a biomedical point of view, CS has demonstrated high activity as wound healing activators or accelerators, and now is used in human and veterinary medications. Several researchers have reported on the mechanism of the activation of wound healing considering the activation of

Correspondence to: M. Pulat (mpulat@gazi.edu.tr).
Contract grant sponsor: Gazi University Research Fund.

polymorphonuclear cells, protective effects against microorganisms, or the promotion of granulocyte tissue formation with angiogenesis.[12] The hydrophilicity of CS, due to the presence of amine and hydroxyl functional groups in its repeat unit, makes the polymer soluble in dilute acidic solutions and yields to a rubbery hydrogel in water. The propensity of CS to absorb water and swell into a soft rubbery material makes it a good matrix material for incorporating hydrophilic drugs.[13] However, this naturally abundant material also exhibits limitations in its reactivity and processability. One strategy to overcome these shortcomings is to incorporate CS in IPN hydrogels.[9]

Because of easy polymerization and biocompatible properties, acrylic acid (AA) and acrylamide (AAm) are widely used to prepare the hydrogels designed for drug release. AAm offer a number of advantages, which include high permeability to both hydrophobic and water-soluble solutes and increased mechanical strength, depending upon copolymer composition and crosslink density.[14] In an anionic polymeric network containing carboxylic acid groups like poly(acrylic acid) (PAA), ionization takes place as the pH of the external swelling medium increases above the pK of the ionizable moiety.[15]

Piperacillin-tazobactam contains two active ingredients; piperacillin and tazobactam. Piperacillin is a penicillin type antibiotic, tazobactam is a medicine to prevent bacteria from inactivating piperacillin. Piperacillin kills many types of bacteria. Although tazobactam also belongs to the penicillin group, it does not have any activity against bacteria but just helps piperacillin to overcome bacteria which might have developed some resistance to piperacillin. Piperacillin-tazobactam has great activity against Gram-positive and Gram-negative organisms such as *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumoniae*, Pseudomonas aeruginosa (*P.aeruginosa*), and the *Enterobacter* species. The piperacillin-tazobactam combination is effective for the treatment of appendicitis, gynecological infections, community-acquired and nosocomial pneumonia, and skin and skin structure infections.[16] These are among the infections most commonly encountered in the community and nosocomial setting. They can range in severity from mild processes such as impetigo and simple cellulitis to more complex infections involving deeper tissue fascia (e.g., necrotizing fasciitis, surgical site infections).[17] These infections are caused by a mixture of aerobic and anaerobic organisms and are responsible for increased morbidity, prolonged hospital stay, and increased health care costs. Frequently isolated gram-negative organism *P. aeruginosa* and gram-negative bacteria *Staphylococcus aureus* (*S. aureus*) are among the commonly implicated anaerobes.[18]

Different from the vast majority of clinical trials about piperacillin-tazobactam in the literature, this presented study is an all original and unique research considering the usage of hydrogels at pointed out compositions for the controlled release of piperacillin-tazobactam. Furthermore, the controlled release of piperacillin-tazobactam through these designed hydrogel-drug systems including hydrophilic and biocompatible components provide some advantages to other systems. While PAA provides high swelling capacity through the anionic structure of the hydrogel, swelling degree could easily be controlled by using the nonionic component PAAm. CS is also added into these designed IPN structures because of its wound-healing and biological properties.

The aim of this presented study is to develop IPN hydrogels based on PAAm, PAA, and CS and investigate the effects of the polymer composition on the swelling behaviors and morphological structure of the hydrogels. Depending on the swelling behaviors, we planned to chose the suitable hydrogel for piperacillin-tazobactam release studies to be carried out at pH = 7.4 and 37°C which is similar to wound media conditions. The kinetic results for piperacillin-tazobactam release were explained on the basis of the swelling values and morphological structures of the hydrogels. As *S. aureus and P. aeruginosa* widely exists in skin wound media, it was also planned to study the effect of the piperacillin-tazobactam loaded hydrogels on *S. aureus and P. aeruginosa* growth at 37°C.

EXPERIMENTAL

Materials

CS (degree of deacetylation is 75%), AA, AAm, ethylene glycol dimethacrylate (EGDMA), acetic acid, and 25% aqueous solution of glutaraldehyde (GA) were purchased from Aldrich, Seelze, Germany. $(NH_4)_2S_2O_8$, $Na_2S_2O_5$, KBr (IR grade) and NaOH were obtained from Merck,Germany. Britton-Robinson buffer (BRB) solutions were prepared as given in the literature.[19] It consists of a mixture of 0.04*M* H_3BO_3 (Merck), 0.04*M* H_3PO_4 (Riedel-de Haën), and 0.04*M* CH_3COOH that has been titrated to the desired pH with 0.2*M* NaOH. Piperacillin-tazobactam was donated by Wheyt. Mueller Hinton Broth, Mueller Hinton Agar and Plate Count Agar used in microbiological experiments were purchased from Merck.

Preparation of the IPN hydrogels

In this study a series of full-IPN and semi-IPN hydrogels based on CS, AA and AAm were

TABLE I
The Amounts of the Components Used to Form the Hydrogels and the Gel Formation Percentages

Hydrogel	CS (g)	AA (mol)	AAm (mol)	GA (amount $\times 10^5$, g)	Gel formation (%)
(PAA/CS)s-1	0.025	0.03	–	–	95.37
(PAA/CS)s-2	0.035	0.03	–	–	95.08
(PAAm/CS)s-1	0.025	–	0.03	–	91.64
(PAAm/CS)s-2	0.035	–	0.03	–	89.75
(PAA/CS)-1	0.025	0.03	–	3,125	92.23
(PAA/CS)-2	0.035	0.03	–	3,125	93.87
(PAA/CS)-3	0.025	0.03	–	15,625	94.19

prepared by radical polymerization procedure.[20] The reactions were initially started by a fixed amount of $(NH_4)_2S_2O_8/Na_2S_2O_5$ redox pair at room temperature. While the semi-IPN structures were composed by using one crosslinking agent, EGDMA, full-IPN hydrogels were constituted by using two crosslinking agents, EGDMA for PAA or PAAm and GA for CS. The detailed procedure is given below:

The different volumes (3.5 mL and 2.5 mL) of CS/acetic acid solution (1.0%) were mixed with the aqueous solutions of AA or AAm into a beaker at the specified amounts given in Table I. The total amount of the mixture was 7.5 mL. The constant amount (0.1 mL) of EGDMA and various amounts of GA were added into the beaker. 0.05/0.05 g/g of $(NH_4)_2S_2O_8/Na_2S_2O_5$ wasinterfused into this mixture as initiator and it was transferred into a glass pan of 5.0×5.0 cm^2 size. The reaction was preceded for 1 h at room temperature. The fresh hydrogel sheets were taken from the pan and 0.5×1.0 cm^2 pieces were cut out. Hydrogel pieces were left overnight at room conditions and washed several times with distilled water to remove unreacted chemicals. The samples were dried first in air and then in a vacuum oven at 37°C and stored for further use.[21] Average thicknesses of dried hydrogels were measured with a micrometer and found in the range of 0.30–0.40 cm according to the gel matrix content.

The gel formation percentages of the samples were gravimetrically determined as follows[20,22]:

Dried hydrogel were weighed and then placed in distilled water for 48 h to extract the unreacted monomers. The hydrogels were then taken out from the extraction medium and dried in a vacuum oven at 40°C to constant weight. The gel formations (%) were determined using the following formula:

$$\text{Gel formation } (\%) = m/m_0 \times 100 \quad (1)$$

where m is the weight of the dried hydrogel after extraction and m_0 is the weight of the dried hydrogel before extraction. All measurements were performed in triplicate.

FTIR measurements

FTIR spectra of (PAA/CS)s-2, (PAAm/CS)s-2 and (PAA/CS)-2 hydrogels were recorded using a Perkin–Elmer 1710 model spectrophotometer. About 1 mg of the samples were pounded and completely mixed with KBr and pellets were prepared using a hydraulic press under a pressure of 600 kg/cm^2. Spectra were scanned between 4000 and 400 cm^{-1}.

Swelling studies

Swelling tests of hydrogel samples were gravimetrically carried out in three steps.[23]

First, dried hydrogel pieces were left to swell in BRB solution (pH = 7.4) at 37°C. Swollen gels were removed from the swelling medium at regular intervals and dried superficially with filter paper, weighed, and placed into the same bath. The measurements were performed until a constant weight was reached for each sample. The percentage swelling (S%) values were calculated from the following equation[21,24,25]:

$$S\% = (m_w - m_d)/m_d \times 100 \quad (2)$$

where, m_w is the wet weight of the sample and m_d is the dry weight of the sample before swelling. The incubation times for all gels were approximately 30 h.

Second, the dried hydrogel pieces were swollen in BRB (pH = 7.4) solutions at different temperatures (4.0°C, 10.0°C, 20.0°C, 30.0°C, 37.0°C, 40.0°C, 50.0°C, and 60.0°C) to investigate the effect of temperature on swelling behaviors. In the third and the last step, same procedure was performed but in different BRB solutions at various pH values (2.0, 4.0, 6.0, 7.4, 8.0, 10.0, and 12.0) for to determine the effect of pH on the swelling behaviors. Temperature was kept constant at 37°C. At the end of the incubation time (30 h), the swollen gels were removed from the swelling medium, dried with filter paper to remove excess water, and weighed. S% values were calculated

using eq. (2). The reproducible results for all swelling studies were obtained with triplicate measurements.

SEM studies

(PAA/CS)s-2, (PAAm/CS)s-2, and (PAA/CS)-2 hydrogel samples swollen to equilibrium in water at room temperature were removed and placed in a deep freezer at −20°C for 24 h and then transferred into a freeze dryer (Christ-Alfa 2-4 Model, Martin Christ GmbH, Germany) at −85°C for one week. The dried and swollen sample straps were coated with 200 A° Au. The surface micrographs of the samples were obtained with a scanning electron microscope (JEOL, JSM 6060A, Japan).

Release of piperacillin-tazobactam from the hydrogels

(PAA/CS)s-2, (PAAm/CS)s-2 and (PAA/CS)-2 hydrogels were chosen for release studies because of their different swelling values at similar compositions. The loading of the model drugs in crosslinked polymer networks can be accomplished by two loading techniques: an equilibrium partitioning and copolymerization/crosslinking in the presence of the drug.[26] In this study, the classic soaking method was chosen to drug load by using 10 mg/mL piperacillin-tazobactam solution.[16] The loaded hydrogel discs were placed into a vessel containing 100 mL of phosphate buffer solution (pH = 7.4). At different time intervals, aliquots of 100 μL were drawn from the medium to follow the drug release; a maximum of 30 aliquots were taken, so the vessel volume can be considered constant. The drug release always maintained at "sink" conditions.[27–29] Piperacillin-tazobactam release was determined spectrophotometrically using a spectrophotometer (Unicam-UV-2100) at λ = 209 nm for 24 h at 37°C. The reproducible results were obtained with triplicate measurements.

Microbiological interaction studies

Microbiological experiments were performed to determine the effect of piperacillin-tazobactam loaded hydrogels on *S. aureus* (ATCC 25923) and *P. aeruginosa* (ATCC 27853) growth.

First, *P. aeruginosa* and *S. aureus* were incubated for 24 h at 37°C on Mueller Hinton Agar and Blood Agar (Mueller Hinton Agar supplemented with 5% blood) respectively, to ensure the purity and viability. Then colonies from each culture were inoculated to Mueller Hinton Broth and after 24-h incubation at 37°C fresh overnight cultures were obtained. The suspensions were diluted 10-fold and 0.1 mL of the diluted suspensions were inoculated to Plate Count Agar and incubated for 24 h at 37°C. The total live *S. aureus* and *P. aeruginosa* numbers in this suspension were determined by the Plate Count Method.[30] In accordance with this method, at the end of the incubation time, the colonies on the plate were counted and the number of microorganisms in 1.0 mL suspension was calculated from eq. (3).

$$N = XD/V \tag{3}$$

where N is the number of microorganisms in 1.0 mL culture (cfu/mL), D is the dilution factor, V is the inoculation volume (0.1 mL), and X is the average number of the colonies.

Piperacillin-tazobactam loaded (PAA/CS)s-2, (PAAm/CS)s-2 and (PAA/CS)-2 hydrogels prepared in disc form at 0.5 cm thickness were sterilized with UV at the wavelength of 254 nm for 3 h and placed into a flask containing 100 mL of the suspension cultures. After incubation at 37°C for 24 h the suspensions were diluted 10-fold and 0.1 mL of the diluted suspensions were inoculated to Plate Count Agar and incubated for 24 h at 37°C. Total number of *S. aureus* and *P. aeruginosa* were determined as explained above. Culture suspensions free from the hydrogels were used as controls. This study was carried out in three parallel studies.[31]

RESULTS AND DISCUSSION

Gel formation

General mechanisms about the formation of semi and full-IPNs are well documented in the literature.[32–34] Similar mechanisms could be suggested for the IPNs produced in this study. Persulfate initiator is reduced to $(SO_4)\_.$ anion-radical. This radical abstracts hydrogen from monomer to form vinyl radicals. Thus, the radically initiated polymerization reactions of AA or AAm monomers were performed.[20,35] It can be thought that PAA or PAAm are the host polymer in this semi-IPN system. It is well known that GA and EGDMA are the selective crosslinkers for CS and PAA/PAAm respectively.[36] While EGDMA provide the crosslinking of vinyl polymer chains (PAA or PAAm), GA conduce the crosslinking of CS chains to compose the full-IPN hydrogel. As PAAm (similar to CS) includes $-NH_2$ groups which could cause the crosslinking with GA, the full-IPN type hydrogel with CS-PAAm was not investigated in this study.

Gel formation percentages of the hydrogels calculated via Equation 1 are given in Table I. In general, high gel formation values were mostly obtained so this procedure is convenient for preparing the IPN type hydrogels with CS and vinylic polymers. By comparing the percentages of the full-IPN hydrogels,

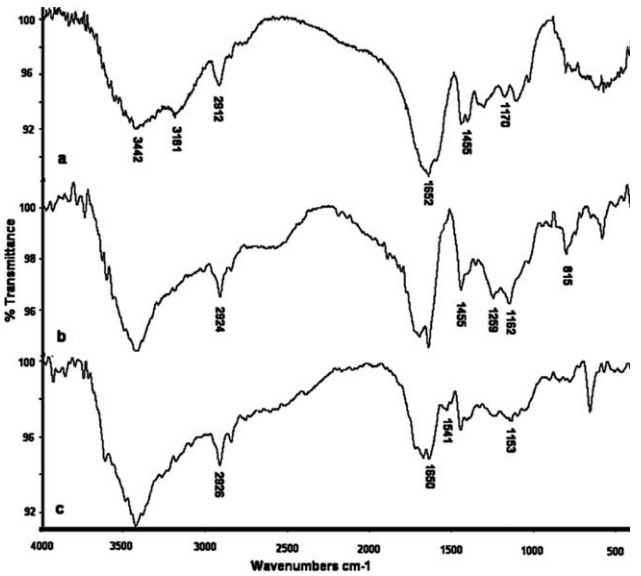

Figure 1 FTIR spectra of (a) (PAAm/CS)s-2 (b) (PAA/CS)s-2 and (c) (PAA/CS)-2 hydrogels.

it can be thought that the increasing amount of GA positively effects gel formation. The highest and the lowest gel formation percentages were obtained for (PAA/CS)s-1 and (PAAm/CS)s-2 hydrogels respectively. This result might be caused from the polymer-additional polymer interactions. As is known CS is a hydro-soluble and positively charged polymer.[37] These properties enable the interaction of CS with negatively charged polymer PAA. Gel formation between CS and PAA chains could be simplified by the anionic structure of PAA, thus gel formation of PAA-CS hydrogels would be favorable than PAAm-CS pairs.

FTIR spectra

The FTIR spectra of (PAAm/CS)s-2, (PAA/CS)s-2 and (PAA/CS)-2 hydrogels are shown in Figure 1. As the components of the hydrogels have some similar groups, the spectrums include identical absorption peaks. In general, all of the curves show the analogous broad band attributed to CS at $\sim$ 3440 cm^{-1} due to *N*—H stretching vibrations and associated —OH stretching vibrations of the hydroxyl group. The absorption bands at $\sim$ 1160 cm^{-1} (antisymmetric stretching of the C—O—C bridge), $\sim$ 1080 cm^{-1} and $\sim$ 1030 cm^{-1} (skeletal vibrations involving the C—O stretching) are characteristic of its saccharide structure. CS also exhibits the distinctive absorption bands at $\sim$ 1650 cm^{-1} (Amide I) and $\sim$ 1300 cm^{-1} (Amide III).[1,13,38]

The band at $\sim$ 1650 cm^{-1} belongs to C=O stretching vibration also confirms the existing of AAm and AA in hydrogels. A relatively high intense peak at $\sim$ 2920 cm^{-1} indicates the aliphatic —CH stretching vibrations. The peak at $\sim$ 3180 cm^{-1} was observed and the related peak at $\sim$ 1650 cm^{-1} corresponds to —NH bending vibrations of the primary amides (see curve a) of AAm.

At curve c, belongs to full-IPN, a new peak appearing at $\sim$ 1540 cm^{-1} due to imine bonds (—C=N) was formed as a result of crosslinking reaction between amino groups in CS and aldehydic groups in GA.[13]

Effect of time to swelling behaviors of the hydrogels

Figure 2 represents the variation of *S*% values with time at pH = 7.4, 37°C. *S*% increased with time initially and then remained constant at close to 30 h. *S*% values were determined to be 4860% for the most swollen hydrogel (PAA/CS)-2, and 1904% for the least swollen hydrogel (PAAm/CS)s-2.

The differences in *S*% values of the semi-IPN type hydrogels can be explained with the hydrophilicty varieties. It is well known that PAA molecules are more hydrophilic than PAAm molecules.[31] AA have —COOH units and the high swelling values of the semi-IPN and full-IPN hydrogels including PAA-CS is due to these ionize-able units. *S*% values of (PAAm/CS)s-1 and (PAAm/CS)s-2 are nearly close to each other. Different swelling values between 80 to 3000% have been reported in the literature for similar hydrogels; depending on the composition or monomer ratio, polymerization route, type of crosslinker, crosslinker density, and so forth. The "ionic charge content" concept was emphasized in those studies.[9] Instead of the swelling value of pure CS hydrogels (nearly 100%), CS hydrogels containing ionic components like PAA were reported to present much more swellable behavior.[39] The results are in agreement with those in the literature.[40,41] Generally, the full-IPN type hydrogels swell much more than

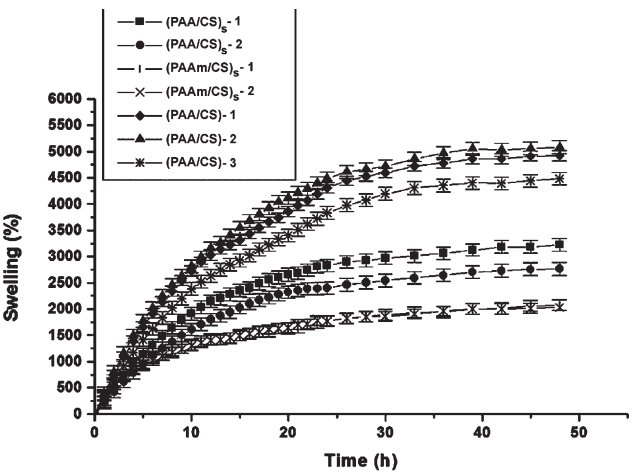

Figure 2 The variation of *S*% values with time at 37°C, pH = 7.4.

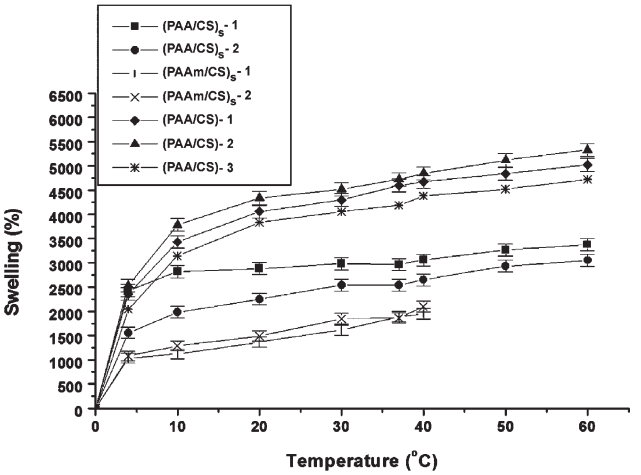

Figure 3 The variation of *S*% values with temperature at pH = 7.4, 30 h.

the semi-IPN types as seen from this figure. By comparing the full-IPN type hydrogels, it is also seen that increasing the amount of GA causes the decreasing of *S*% values from 4860 to 4300%. This situation can be explained by crosslinked density. There are numerous reports in the literature that the more crosslinker gets the intensive crosslinking.

Effect of temperature to swelling behaviors of the hydrogels

Figure 3 presents the variation of *S*% values of hydrogels with temperature at pH = 7.4 and in 30 h time. All hydrogels swelled much more at high temperatures than at low temperatures. The swelling of PAA and PAAm hydrogels is known to be positively dependent on temperature. As the temperature increases, thermal mobility of the polymer chains also increases and hydrogen bonds are broken so hydrogels can easily swell.[42] As the swelling values of all hydrogels exhibit great differences between 4 and 40°C, it is thought that these hydrogels are sensitive to the variations in temperature in this range.

Effect of pH to swelling behaviors of the hydrogels

Figure 4 shows the variation of *S*% values with pH at 37°C and in 30 h time. Low swelling percentages were obtained for all hydrogels based on PAA at pH = 2.0 versus other pH values. In all compositions the maximum extent of swelling was reached at about pH 7.4, this being due to the complete dissociation of acid groups of AA at this pH value. The dissociation constant of AA is pKa = 4.25. The curve of PAA-CS hydrogels start to rise at pH = 4.0 and present a sharp variation near pH = 6.0. As the swelling values of all hydrogels exhibit great differences between pH = 4.0 and 8.0, it is thought that the hydrogels including PAA are sensitive to pH variations at this range.[6,43]

Owing to the nonionic structure, the PAAm hydrogel was not affected by pH variations and was not stable above pH = 8.0.[20,40]

SEM analysis

Microstructures of the network surfaces were investigated by SEM at different magnifications. The micrographs of (PAA/CS)s-2, (PAAm/CS)s-2 and (PAA/CS)-2 hydrogels, chosen for release studies, are presented in Figure 5. The morphological differences between dry and wet states of all hydrogels can be clearly observed in this figure. Dry hydrogels exhibit nonporous surface structures. In contrary, swollen hydrogels possess large number of pores. It can easily be seen that (PAAm/CS)s-2 hydrogel displays a channel like and smaller porous surface which is different in structure than the others. The porosity distribution is relatively more uniform and dense for this hydrogel. As seen in X100 magnified micrographs, PAA-based hydrogels mostly exhibit a sponge type structure. It is well known that charge interactions may occur between two oppositely charged polymers. Thus, besides chemical crosslinking, cationic CS, and anionic PAA molecules might also construct electrostatically crosslinked polymer chains.[44] This morphology and the chemical properties enable easy diffusion and absorption of water into the hydrogel structure. The great variations in swelling values can also be explained with chemical properties of these hydrogels. It can be concluded that, hydrophilic and ionic structure of PAA based hydrogels causes high swelling values.

Piperacillin-tazobactam release kinetics

The baseline pH of most wounds is found to be above 8.5. As the wound condition gets towards

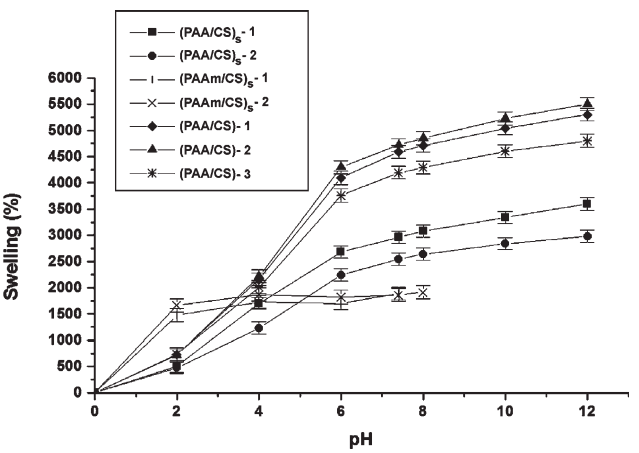

Figure 4 The variation of *S*% values with pH at 37°C, 30 h.

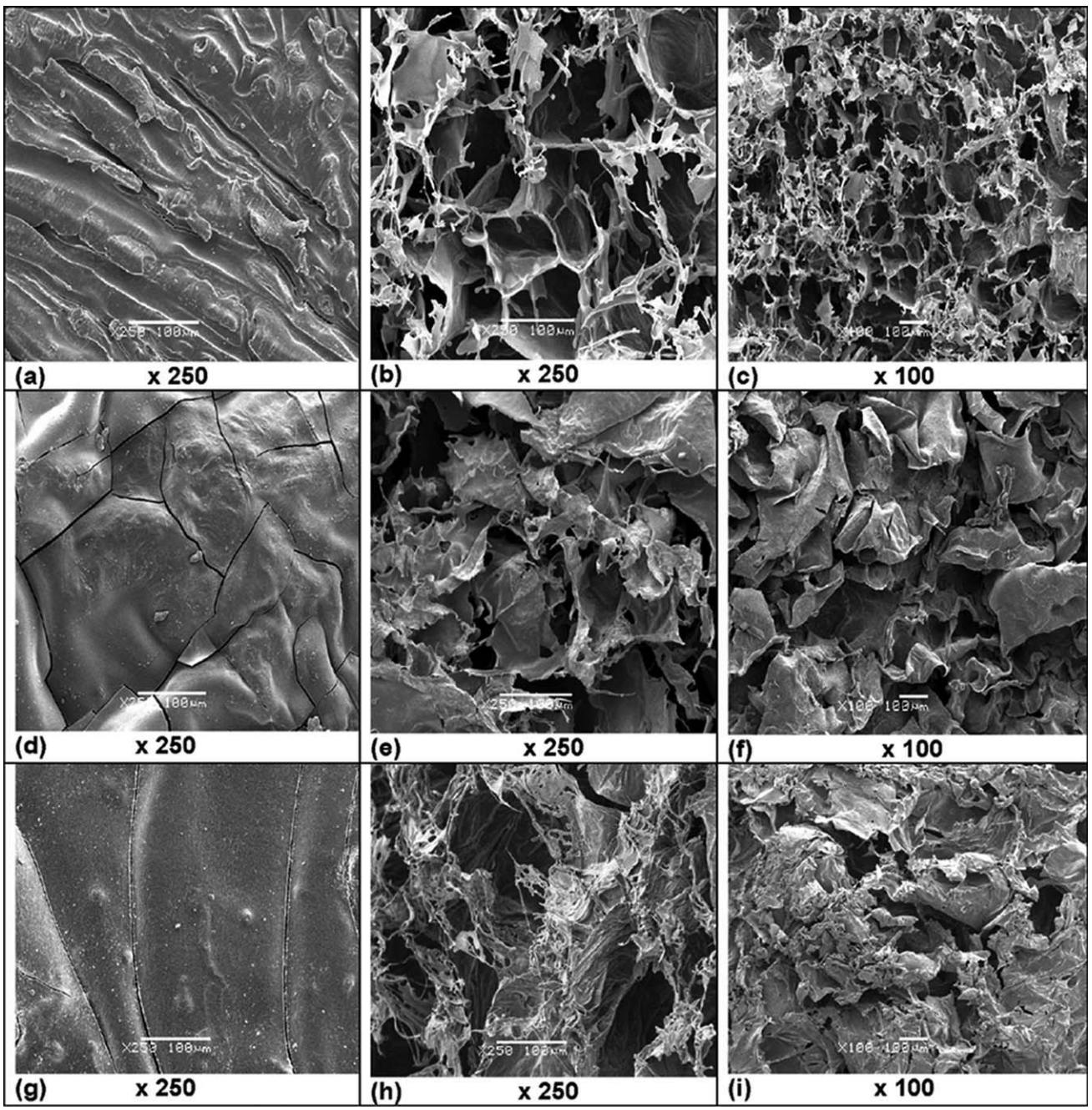

Figure 5 SEM micrographs at different magnifications taken from surfaces of (a) dry (PAAm/CS)s-2, (b,c) swollen (PAAm/CS)s-2 at X250 and X100; (d) dry (PAA/CS)s-2, (e,f) swollen (PAA/CS)s-2 at X250 and X100; (g) dry (PAA/CS)-2, (h,i) swollen (PAA/CS)-2 at X250 and X100.

healing and exudation decreases, pH values also decrease below 8.0. This change in pH can help to predict the likelihood of wound healing.[45] Specific pH values between 5.5 and 8.7 are reported by various researchers. The chosen hydrogels for drug release studies present stable swelling behaviors between pH values of 6.0-8.0. Consequently, release experiments in this study were carried out at pH = 7.4 which could be evaluated as an average pH value of the injured skin.[46,47]

The release profiles of piperacillin-tazobactam from (PAA/CS)s-2, (PAAm/CS)s-2 and (PAA/CS)-2 are presented in Figure 6. As seen from this figure, piperacillin-tazobactam release from (PAA/CS)s-2 and (PAA/CS)-2 hydrogels increases rapidly at first than complies at near 5 h. However, the release from (PAAm/CS)s-2 ends at 10 h. This can be explained by different swelling behaviors of the hydrogels. During the soaking procedure, drug molecules easily diffuse into the gel matrix and settle in

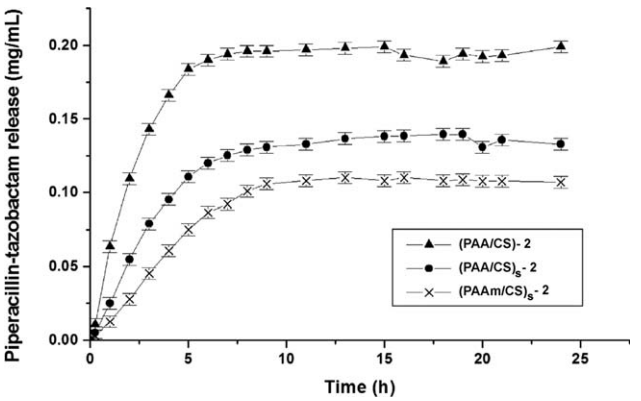

Figure 6 The release of piperacillin-tazobactam from (PAA/CS)s-2, (PAAm/CS)s-2, and (PAA/CS)-2 hydrogels at 37°C.

the interior part of the structure. As emphasized before, (PAA/CS)s-2 and (PAA/CS)-2 hydrogels swell much more than (PAAm/CS)s-2 depending on their chemical properties. While the hydrogel swells, the loaded piperacillin-tazobactam molecules easily escape through the large pores of the including PAA.

A semiempirical equation is introduced to represent the drug release process of swelling polymer.[28,48]

$$F = M_t/M_\infty = kt^n \tag{4}$$

where F is fractional uptake, M_t and M_∞ are the amount of drug released at time t and the maximum amount of drug release respectively. k is the gel characteristic constant; and the swelling exponent n describes the type of diffusion. In general $n = 0.45$–0.50 corresponds to the Fickian type diffusion process while $0.50 < n < 1.0$ indicates the anomalous or non-Fickian type diffusion. $n > 1.0$ implies super Case II transport.[28]

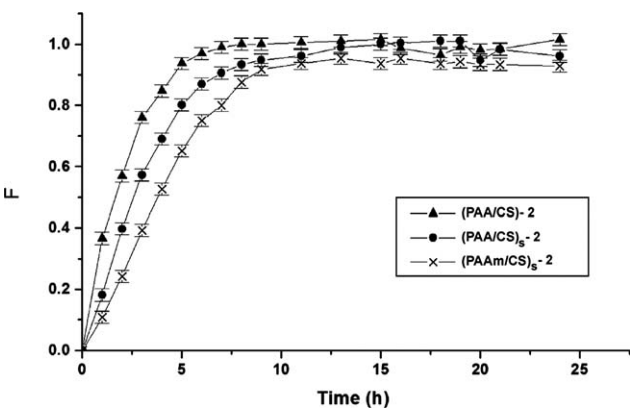

Figure 7 The fractional piperacillin-tazobactam release from (PAA/CS)s-2, (PAAm/CS)s-2, and (PAA/CS)-2 hydrogels at 37°C.

TABLE II
Release Parameters and Diffusion Coefficients of the Hydrogels (r^2: Deterministice Coefficient)

Hydrogel	n	$k \times 10^3$	D $\times 10^6$ (cm^2/s)	r^2
(PAA/CS)-2	1.003	4.14	3.53	0.9736
(PAA/CS)s-2	1.058	2.21	2.77	0.9915
(PAAm/CS)s-2	1.072	1.42	2.03	0.9989

Figure 7 shows the fractional piperacillin-tazobactam release, expressed as M_t/M_∞. The drug release during the first stage could be influenced for the relaxation of polymer chains. Thus, the hydrogels present an initial non-Fickian behavior indicating similar rates of Fickian diffusion and polymer relaxation.

k and n values of (PAA/CS)s-2, (PAAm/CS)s-2, and (PAA/CS)-2 hydrogels were determined from the graphs driven in via Equation 4 and given in Table II. As n values of all hydrogels were calculated near 1.0, it can be mentioned about zero order kinetic for these hydrogels.[1,49]

Diffusion coefficient, D is an important release parameter of some chemical species from polymeric systems. Using n and k, D could be calculated from the following equation[28,31]:

$$k = 4(D/\pi r^2)^n \tag{5}$$

where r is the radius of gel disc.

The diffusion coefficient is a function of the polymer chain mobility, the average pore size and the mobility of the solvent in the gel. The diffusion of piperacillin-tazobactam from the hydrogels mainly occurs through the pores of polymer matrix, according to the results reported in swelling studies.

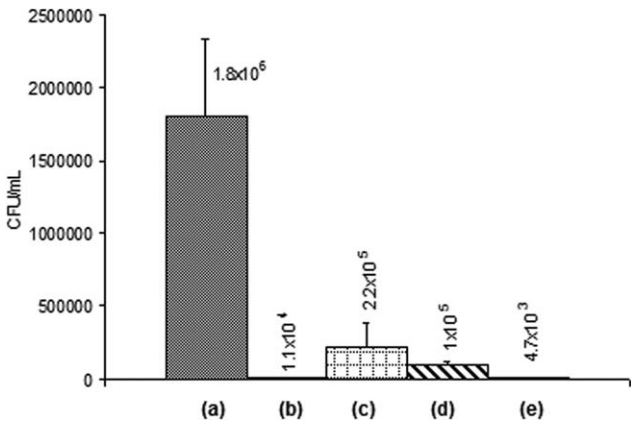

Figure 8 The effect of the piperacillin-tazobactam loaded hydrogel on *S. aureus* growth. (a) *S. aureus*, control; (b) Piperacillin-tazobactam loaded (PAAm/CS)s-2 hydrogel; (c) Piperacillin-tazobactam loaded (PAA/CS)s-2 hydrogel; (d) Piperacillin-tazobactam loaded (PAA/CS)-2 hydrogel; (e) Piperacillin-tazobactam.

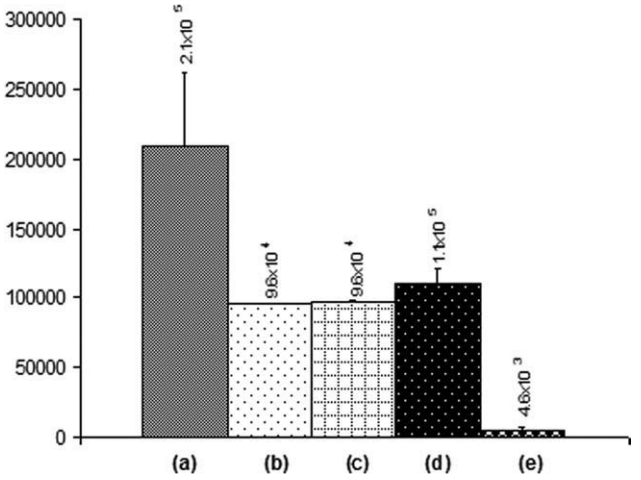

Figure 9 The effect of the piperacillin-tazobactam loaded hydrogel and on *P. aeruginosa* growth. (a) *P. aeruginosa*, control; (b) Piperacillin-tazobactam loaded (PAAm/CS)s-2 hydrogel; (c) Piperacillin-tazobactam loaded (PAA/CS)-2 hydrogel; (d) Piperacillin-tazobactam loaded (PAA/CS) s-2 hydrogel; (e) Piperacillin-tazobactam.

The effects of piperacillin-tazobactam loaded hydrogels on *S. aureus* and *P. aeruginosa* growth

The behavior of piperacillin-tazobactam loaded (PAAm/CS)s-2, (PAA/CS)s-2 and (PAA/CS)-2 hydrogels in *S. aureus* and *P. aeruginosa* culture suspensions were investigated as explained in the experimental part and the results were presented in Figures 8 and 9. The results based on the statistical analysis are concluded as follows:

The growth of *S. aureus* in the control medium was determined as the maximum growth value [Fig. 8(a)]. As seen from the same figure at (e)-column, the growth of *S. aureus* in the medium including piperacillin-tazobactam was nearly found as zero. Piperacilli-tazobactam loaded hydrogels dramatically decreased *S. aureus* growth. The difference in the microbial growth values between the control and the hydrogels containing piperacillin-tazobactam was statistically significant ($P < 0.001$).

The growth of *P. aeruginosa* in the control medium was determined as the maximum growth value [Fig. 9(a)]. Similarly, (e)-column in the same figure shows that the growth of *P. aeruginosa* in the medium including piperacillin-tazobactam is nearly zero. The difference in microbial growth values between the control and the piperacillin-tazobactam loaded hydrogels was also statistically significant ($P < 0.001$).

As statistically significant growth value differences of *P. aeruginosa* and *S. aureus* were found in between the controls and the piperacillin-tazobactam loaded hydrogels, it can be thought that these new formulas performed quite satisfactory. The results clearly showed that our prepared drug-hydrogel systems were highly effective and could be suitable for to be used in local wound healing applications.

CONCLUSIONS

A series of IPN hydrogels based on CS, AA, and AAm for the controlled releasing of piperacillin-tazobactam were prepared via free-radical polymerization using EGDMA and GA as crosslinkers. The variations of swelling percentages with time, temperature and pH were determined for these hydrogels. The full-IPN type hydrogels swell much more than the semi-IPN type hydrogels. It is found that the increasing amount of GA negatively affects *S*% of full type IPN hydrogels. The more swelling percentages were obtained for all hydrogels at neutral and basic mediums rather than acidic. In all compositions the maximum extent of swelling values were reached at about pH = 8.0. Piperacillin-tazobactam release from (PAAm/CS)s-2, (PAA/CS)s-2, and (PAA/CS)-2 hydrogels were studied and non-Fickian type diffusion were established from the kinetic release parameters n and k. The response of piperacillin-tazobactam loaded hydrogel in *S. aureus* and *P. aeruginosa* culture suspensions were also investigated and statistically significant differences in microorganisms' growth values were observed between the controls and piperacillin-tazobactam loaded hydrogels. Drug release and microbial interaction studies in this research showed that our prepared piperacillin-tazobactam loaded hydrogels are potentially suitable to be of use for local wound healing administration.

References

1. Bajpai, A. K.; Bajpai, J.; Shukla, S. React Funct Polym 2002, 50, 9.
2. Bae, Y. H.; Kim, S. W. Adv Drug Deliv Rev 1993, 11, 109.
3. Kayaman, N.; Gürel Hamurcu, E. E.; Uyanık, N.; Baysal, B. M. Macromol Chem Phys 1999, 200, 231.
4. Kim, S. J.; Park, S. J.; Kim, S. I. React Funct Polym 2003, 55, 53.
5. Dziubla, T. D.; Torjman, M. C.; Joseph, J. I.; Tatum, M. M.; Lowman, A. M. Biomaterials 2001, 22, 2893.
6. Pulat, M.; Asıl, D. J Appl Polym Sci 2009, 113, 2613.
7. Menke, M. N.; Menke, N. B.; Boardman, C. H.; Diegelmann, R. F. Gynecologic Oncol 2008, 111, S87.
8. Berger, J.; Reist, M.; Mayer, J. M.; Felt, O.; Gurny, R. Eur J Pharma Biopharma 2004, 57, 35.
9. El-Sherbiny, I. M.; Lins, R. J.; Abdel-Bary, E. M.; Harding, D. R. K. Eur Polym J 2005, 41, 2584.
10. Kotzé, A. F.; Lueßen, H. L.; Boer, A. G.; Verhoef, J. C.; Junginger, H. E. Eur J Pharma Sci 1999, 7, 145.
11. Peter, M. G. J Macromol Sci Part A: Pure Appl Chem 1995, 32, 629.
12. Tanodekaew, S.; Prasitsilp, M.; Swasdison, S.; Thavornyutikarn, B.; Pothsree, T.; Pateepasen, R. Biomaterials 2004, 25, 1453.
13. Krishna Rao, K. S. V.; Vijaya Kumar Naidu, B.; Subha, M. C. S.; Sairam, M.; Aminabhavi, T. M. Carbohydr Polym 2006, 668, 333.

14. Rokhade, A. P.; Patil, S. A.; Aminabhavi, T. M. Carbohydr Polym 2007, 67, 605.
15. Pulat, M.; Ekşi, H. J Appl Polym Sci 2006, 102, 5994.
16. Jang, C. H.; Park, H.; Cho, Y. B.; Choi, C. H. 2008, 72, 1815.
17. Kuti, J. L.; Dowzicky, M.; Nicolau, D. P. Surg Infect 2008, 9, 57.
18. Siami, G.; Christou, N.; Eiseman, I.; Tack, K. J. Antimicrobial Agents Chemotherapy 2001, 45, 525.
19. Britton, H. T. K.; Robinson, R. A. J Chem Soc 1931, 458, 1456.
20. Pulat, M.; Çetin, M. J Bioactive Compatible Polym 2008, 23, 305.
21. Blanco, M. D.; Bernardo, M. V.; Teijon, C.; Sastre, R. L.; Teijon, J. M. Int J Pharmaceutics 2003, 255, 99.
22. Chen, K. S.; Ku, Y. A.; Lin, H. R.; Yan, T. R.; Sheu, D. C.; Chen, T. M.; Lin, F. H. Mater Chem Phys 2005, 91(2/3), 484.
23. Pulat, M.; Akdŏgan, A. J Appl Polym Scis 2002, 85, 193.
24. Pulat, M.; Memiş, E.; Gümüşdereliŏglu, M. J Biomater Appl 2003, 17, 237.
25. Hsiue, G. H.; Guu, J. A.; Cheng, C. C. Biomaterials 2001, 22, 1763.
26. Luo, Y.; Zhang, K.; Wei, Q.; Liu, Z.; Chen, Y. Acta Biomaterialia 2009, 5, 316.
27. Blanco, M. D.; Garcia, O.; Trigo, R. M.; Teijon, J. M.; Katime, I. Biomaterials 1996, 17, 1061.
28. Taşdelen, B.; Kayaman-Apohan, N.; Güven, O.; Baysal, B. M. Radiat Phys Chem 2005, 73, 340.
29. Bajpai, S. K.; Sonkusley, J. J Appl Polym Sci 2002, 83, 1717.
30. USP XVIII, Microbiological Tests, p. 845. Easton, Washington, DC 1970.
31. Pulat, M.; Ekşi, H.; Abbasŏglu, U. J Biomater Sci, Polym Ed 2008, 19, 193.
32. Mahdavinia, G. R.; Pourjavadi, A.; Hosseinzadeh, H.; Zohuriaan, M. J. Eur Polym J 2004, 40, 1399.
33. Solomons, T. W. G. Organic Chemistry; Wiley: New York, 1996.
34. Ravikumar, M. N. V. Reactive Funct Polym 2000, 46, 1.
35. Yin, L.; Fei, L.; Cui, F.; Tang, C.; Yin, C. Biomaterials 2004, 28, 1258.
36. Beppu, M. M.; Vieira, R. S.; Aimoli, C. G.; Santana, C. C. J Membrane Sci 2007, 301, 126.
37. Hamidi, M.; Azadi, A.; Rafiei, P. Adv Drug Deliv Rev 2008, 60, 1638.
38. Peniche, C.; Argelles-Monal, W.; Davidenko, N.; Sastra, R.; Gallardo, A.; San Roman, J. Biomaterials 1999, 20, 1869.
39. Liu, J.; Wang, Q.; Wang, A. Carbohydr Polym 2007, 70, 166.
40. Taşdelen, B.; Kayaman, N.; Güven, O.; Baysal, B. M. Int J Pharma 2004, 278, 343.
41. Şen, M.; Yaka, A. Int J Pharma 2001, 288, 33.
42. Xiao, X. C.; Chu, L. Y.; Chen, W. M.; Zhu, J. H. Polymer 2005, 46, 3199.
43. Şen, M.; Güven, O. Eur Polym J 2002, 38, 751.
44. Hoare, T. R; Kohane, D. S. Polymer 2008, 49, 1993.
45. Shukla, V. K.; Shukla, D.; Tiwary, S. K.; Agrawal, S.; Rastogi, A. J Wound Care 2007, 16, 291.
46. Kang, H. C.; Lee, E. S.; Na, K.; Bae, Y. H. Multifunctional Pharmaceutical Nanocarriers, Stimuli-Sensitive Nanosystems: For Drug and Gene Delivery; Springer: New York, 2008, p 161.
47. Schmaljohann, D. Adv Drug Deliv Rev 2006, 58, 1655.
48. Torre, P. M.; Torrado, S.; Torrado, S. Biomaterials 2003, 24, 1459.
49. Elvira, C.; Mano, J. F.; Roman, J. S.; Reis, R. L. Biomaterials 2002, 23, 1955.