

Synthesis and Properties of Biopolymer Based on Gelatin-G-Poly(Sodium Acrylate-Co-Acrylamide) for Cephalexin Controlled Release

[Sefaleksinin Kontrollü Salınımı için Jelatin-G-Poli(Sodyum Akrilat-Co-Akrilamid)'e Dayalı Biyopolimerin Sentezi ve Özellikleri*]

Mohammad Sadeghi¹,
Hossein Hosseinzadeh²

¹Chemistry Department, Science Faculty, Islamic Azad University, Arak Branch, Arak, ²Chemistry Department, Payame Noor University, 19395-4697, Tehran, Iran.

Yazışma Adresi
[Correspondence Address]

Mohammad Sadeghi

Chemistry Department, Science Faculty, Islamic Azad University, Arak Branch, Arak, Iran.
Tel: +98-861-4130037
Fax: +98-861-413227
E-mail: m-sadeghi@iau-arak.ac.ir

*Translated by [Çeviri] Özlem Dalmızrak

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ABSTRACT

Objective: This study investigated the usefulness of a pH-sensitive protein-based hydrogel for in vitro controlled release of cephalexin

Methods: The hydrogels were prepared via graft copolymerization of mixtures of acrylic acid (AA) and acrylamide (AAm) onto gelatin backbones by a free radical polymerization technique using ammonium persulfate (APS) as initiator and methylene bisacrylamide (MBA) as a crosslinker

Findings: The synthesized hydrogels were subjected to equilibrium swelling studies in simulated gastric and intestinal fluids (SGF and SIF). The loading drug yield was found to depend on the incubation time, the amount of encapsulated drug and the crosslinker concentration. In addition, the release of cephalexin from this kind of hydrogel was studied.

Conclusion: In vitro drug release studies in different buffer solutions showed that the most important parameter affecting the drug-release behavior of hydrogels is the pH of the solution. The release rate of cephalexin from hydrogel at pH 7.4 was faster than that at pH 1.2 due to the shrinkage of the hydrogel at pH 1.2.

Key Words: Gelatin, hydrogel, acrylic acid, acrylamide, cephalexin, controlled drug release

ÖZET

Amaç: Çalışmada sefaleksinin in vitro kontrollü salınımında pH'ya duyarlı protein yapısındaki hidrojellerin kullanılabilirliği araştırılmıştır.

Yöntem: Hidrojeller, akrilik asit (AA) ve akrilamid (AAm) karışımının jelatin iskelet üzerine polimerizasyonu ile hazırlanmıştır. Uygulanan serbest radikal polimerizasyon tekniğinde amonyum persülfat (APS) başlatıcı, metilen bisakrilamid (MBA) ise çapraz bağlayıcı olarak kullanılmıştır.

Bulgular: Sentezlenen hidrojeller temsili gastrik ve intestinal sıvı (SGF ve SIF) içerisinde kararlılık şişme çalışmalarına tabi tutulmuştur. Yüklenen ilaç veriminin inkübasyon süresine, etrafı kapsülle çevrili ilaç miktarına ve çapraz bağlayıcı derişimine bağlı olduğu saptanmıştır. Ayrıca hidrojellerden sefaleksinin salınımına da bakılmıştır.

Sonuç: Farklı tampon çözeltileri kullanılarak yapılan in vitro ilaç salınımı çalışmaları, hidrojellerden ilaç salınım davranışını etkileyen en önemli parametrenin çözeltinin pH'sı olduğunu göstermektedir. Hidrojeller pH 1.2'de büzüldüğü için sefaleksinin pH 7.4'de salınım hızı pH 1.2'ye göre daha hızlı olmaktadır.

Anahtar Kelimeler: Jelatin, hidrojel, akrilik asit, akrilamid, sefaleksinin, kontrollü ilaç salınımı

Introduction

Hydrogels have been of interest to biomaterial scientists for many years because of their hydrophilic character and potential to be biocompatible [1-7]. These networks are special soft and flexible polymeric materials that can absorb large quantities of water, saline or physiological solutions while the absorbed solutions are not removable even under pressure [4].

To date, many types of hydrogels as drug carriers have been widely investigated. Some interesting drug delivery systems based on hydrogels have thus been proposed [8-11]. Among hydrogels, however, considerable research attention has been focused on so-called "smart" hydrogels which can transfer their volume in response to environmental stimuli, and these results in environmental stimuli modifying drug release. Among these, pH-sensitive hydrogels have been extensively investigated for potential use in site-specific delivery of drugs to specific regions of the gastrointestinal tract and have been prepared for delivery of low molecular weight drugs. Therefore, these hydrogels have important applications in the field of medicine, pharmacy, and biotechnology.

Controlled or sustained release of drugs provide many advantages in comparison with conventional forms including reduced side effects, drug concentration kept at effective levels in plasma, improved utilization of drug and decrease the dosing times [12].

In the current study, we investigated the synthesis and utility of an anionic hydrogel from graft copolymerization of acrylic acid and acrylamide onto gelatin backbones, for the controlled release of a beta-lactam antibiotic cephalosporin, cephalexin. Drug absorption and release capacities of hydrogel systems and influence of pH of the medium on the release properties were also examined.

Materials and Methods

Materials

The gelatin (Merck) was used as received. Acrylic acid (AA, Merck) was used after vacuum distillation. Acrylamide (AAm) and methylene bisacrylamide (MBA), from Fluka, was employed after crystallization in acetone. Ammonium persulfate (APS, Merck) was used without purification. All other chemicals were of analytical grade. The drug, cephalexin, was obtained from Jaberebne Hayan Pharmaceutical Co. (Tehran, Iran). The chemical structure of cephalexin is shown in Figure 1. Double distilled water was used for the hydrogel preparation and swelling measurements. All the tests including determination of drug content, swelling and *in vitro* drug cumulative release were carried out in triplicate and the averages were reported. Statistical data analysis was performed using the Student's *t*-test with $p < 0.05$ as the minimal level of significance. The reproducibility of the experiments was $n=3$. The average of three independent determinations was considered and the accuracy of the measurements was $\pm 3\%$.

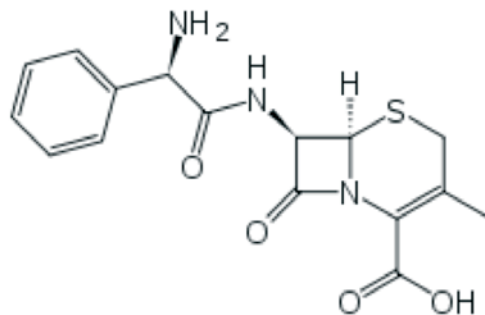


Figure 1. Chemical structure of cephalexin.

Preparation of hydrogel

A general procedure for chemically crosslinking graft copolymerization of AA and AAm onto gelatin backbones was conducted as follows: Gelatin (1.0 g) was added to a three-neck reactor equipped with a mechanical stir (Heidolph RZR 2021, three blade propeller type, 300 rpm), including 35 mL double distilled water. The reactor was immersed in a thermostated water bath preset at 80 °C. Then, a definite amount of APS solution (0.1 g in 5 mL H₂O) was added to gelatin solution and was allowed to stir for 10 min. After adding APS, variable amounts of AA and AAm (AA 0.40–1.60 g, AAm 0.40–1.60 g) were added simultaneously to the gelatin solution. Then, in order to investigate the effect of crosslinker concentration on swelling capacity, methylene bisacrylamide solution (0.03–0.08 g in 5 mL H₂O) 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 1 N sodium hydroxide solution. The hydrogel, gelatin-g-poly(NaAA-co-AAm), was poured to excess non-solvent ethanol (200 mL) and kept for 3 h to remove of absorbed water. Then ethanol was decanted and the product scissored to small pieces. Again, 100 mL fresh ethanol was added and the hydrogel was stored for 24 h. Finally, the filtered hydrogel was dried in oven at 60°C for 10 h. After grinding by mortar, the powdered superabsorbent was stored by protecting from moisture, heat and light.

Swelling measurements

An accurately weighted sample of the superabsorbent (0.2 ± 0.001 g) with average particle size in between 40-60 mesh ($250\text{--}350\mu\text{m}$) was immersed in distilled water (200 mL) and allowed to soak for 3 h at room temperature. The equilibrium swelling (ES) capacity was measured twice at room temperature by using the following formula [13]:

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

Swelling at various pHs

Individual solutions (50 mL) with acidic and basic pHs were prepared by the dilution of NaOH (pH 10.0) and HCl (pH 1.0) solutions (0.1 M) to achieve $\text{pH} \geq 6.0$ and

pH < 6.0, respectively. The pH values were precisely checked by a pH-meter (Metrohm/620, accuracy ± 0.1). Then, 0.5 (± 0.001) g of the dried hydrogel was used for the swelling measurements according to Eq. 1. Sensitivity of the hydrogel to pH was investigated in terms of swelling and deswelling of the final product at two basic (pH 7.0) and acidic (pH 2.0) solutions, respectively. Swelling capacity of the hydrogels at each pH was measured according to Eq. 1 at consecutive time intervals (15 min).

Drug loading on hydrogels

The hydrogels were incubated with drugs using a contact adsorption technique. The swollen hydrogel sample was dried in vacuum overnight until its weight remained unchanged. The vacuum dried powdered samples (1 ± 0.0001 g), with average particle size in between 250–350 μm , were accurately weighted and placed in the aqueous solution of drug (0.6 g dissolved in 50 mL distilled water) at 0°C for 25 h to reach the equilibrium state. The swollen hydrogels loaded with drug were placed in a vacuum oven and dried under vacuum at 37°C.

In vitro drug release

The samples (0.1 ± 0.0001 g) were placed into 50 mL of the release medium (simulated gastric and intestinal fluids, SGF and SIF, respectively) at different pH values (pH 1.2 or 7.4) at 37°C with agitation. At fixed time intervals, 1 mL of the release medium was removed using a syringe attached with a 0.45 μm Millipore filter and after suitable dilution, the concentration of released drug was

measured spectrophotometrically (UV-1201, Shimadzu, Kyoto, Japan) at 276 nm.

Spectroscopic behavior

Fourier transform infrared (FT-IR) spectra of samples were taken in KBr pellets by using an ABB Bomem MB-100 FT-IR spectrophotometer (Quebec, Canada), at room temperature.

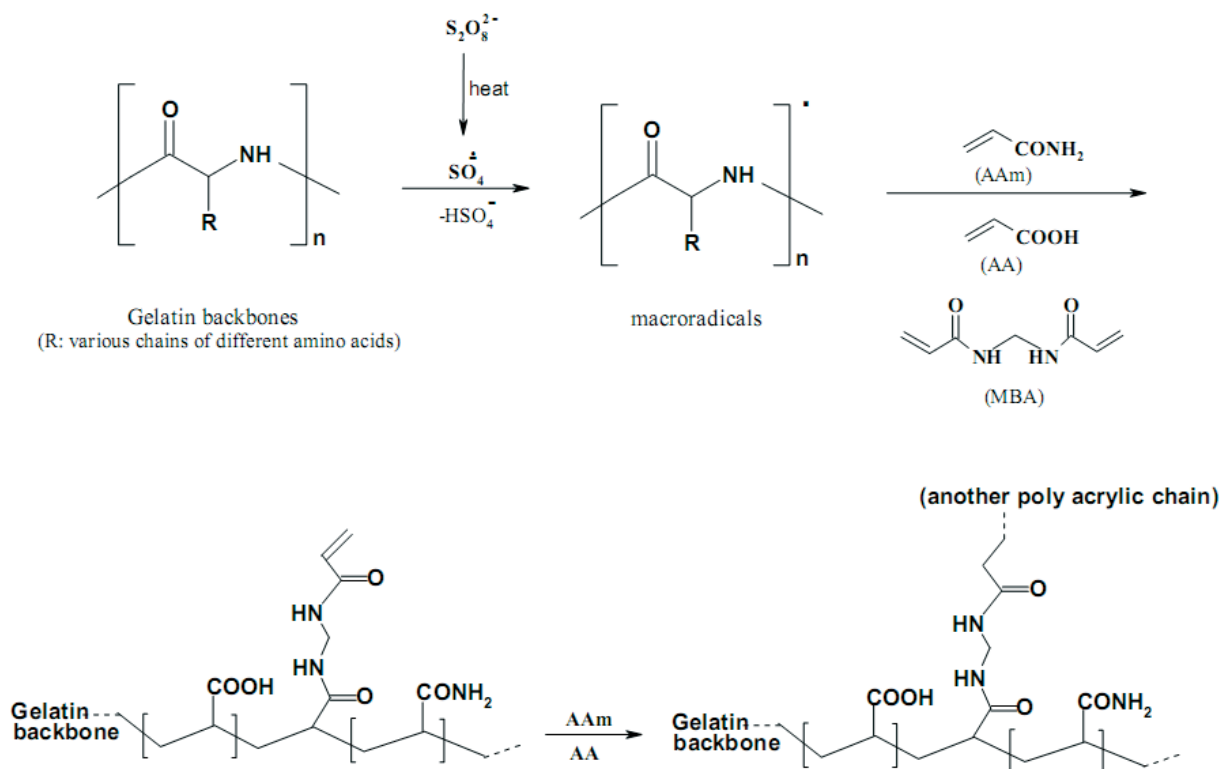
Surface morphology

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 and spectral characterization

The mixture of monomers, AA and AAm, were simultaneously grafted onto gelatin backbones in a homogeneous medium using APS as a radical initiator and MBA as the crosslinking agent. A general reaction mechanism for gelatin-g-poly(NaAA-co-AAm) 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 removed 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 monomer grafting onto gelatin backbones led to



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

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 could be obtained [14].

The grafting was confirmed by comparing the FT-IR spectra of the gelatin substrate with that of the grafted products. The band observed at 1634 cm^{-1} can be attributed to C=O stretching in carboxamide functional groups of substrate backbone (Figure 2a). The superabsorbent hydrogel product comprises a gelatin backbone with side chains that carry sodium carboxylate and carboxamide functional groups that are evidenced by peaks at 1558 and 1637 cm^{-1} , respectively (Figure 2b). The characteristic band at 1558 cm^{-1} is due to asymmetric stretching in carboxylate anion that is reconfirmed by another peak at 1411 cm^{-1} which is related to the symmetric stretching mode of the carboxylate anion. The stretching band of the grafted carboxamide groups overlapped with that of the gelatin portion of the copolymer.

One of the most important properties that must be considered is hydrogel microstructure morphology. The surface morphology of the samples was investigated by SEM. Figure 3 shows the SEM micrograph of the polymeric hydrogels obtained from the fracture surface. The hydrogel has 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.

Effect of crosslinker concentration

Crosslinks have to be present in a hydrogel in order to prevent dissolution of the hydrophilic polymer chains in aqueous environment. The crosslinked nature of hydrogels makes them insoluble in water. Efficiency of the incorporated crosslinker controls the overall crosslink density in the final hydrogel. Figure 4 shows the influence of the crosslinking agent on the swelling capacity of gelatin-g-poly(NaAA-co-AAm) hydrogel. As indicated in Figure 4, higher crosslinker concentration decreases the space between the copolymer chains and, consequently, the resulted in highly crosslinked rigid structure cannot be expanded and hold a large quantity of water.

Effect of monomer ratio on swelling capacity

The swelling capacity of the hydrogels prepared with various ratios of monomers, is shown in Figure 5. In this series of experiments, both monomer concentrations were simultaneously changed from 0.4 to 1.6 g . Since pH of the polymerization mixture was adjusted at 8.0 after the reaction, the superabsorbency of gelatin-g-poly(NaAA-co-AAm) hydrogel is due to both functional groups of ionic carboxylate (from neutralized AA) and non-ionic carboxamide (from AAm). As shown in Figure 5, higher swelling capacities are obtained by employing higher initial ratios of AA/AAm. This behavior can be attributed to the formation of high carboxylate groups in samples. The ionic groups are more strongly solvated than non-ionic groups in the aqueous medium.

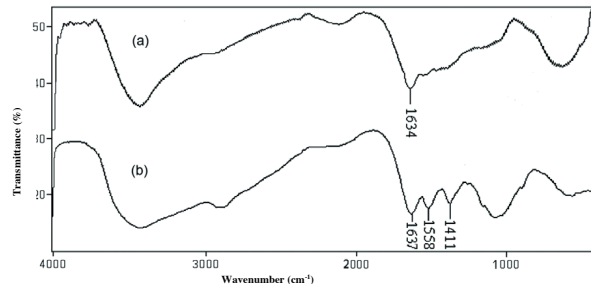


Figure 2. FT-IR spectra of gelatin (a) and gelatin-g-poly(AA-co-AAm) hydrogel (b).

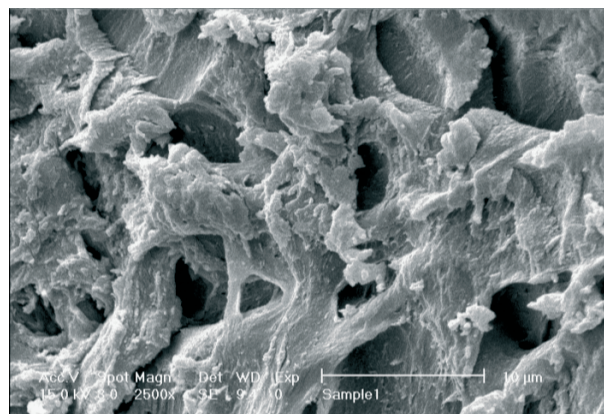


Figure 3. SEM photograph of the hydrogel. Magnification is 2500X and the scale bar is $10\text{ }\mu\text{m}$.

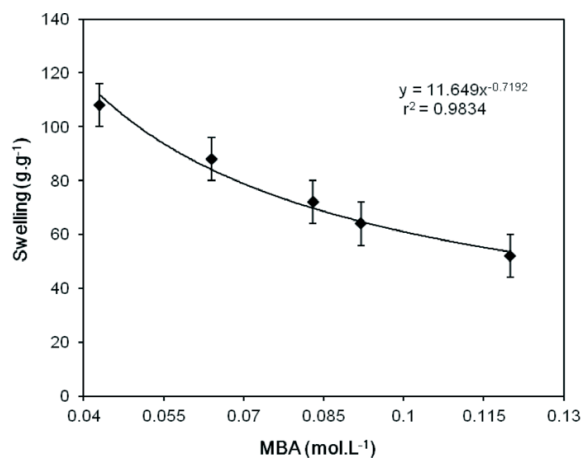


Figure 4. Effect of crosslinker concentration on swelling capacity.

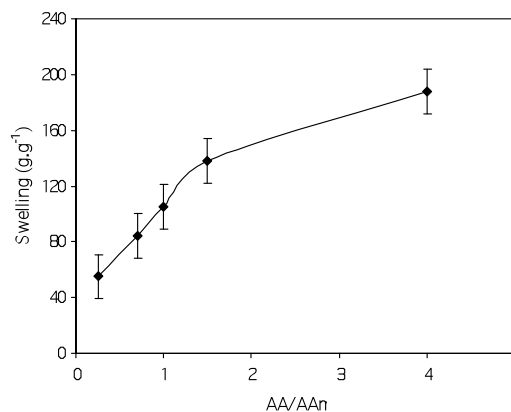


Figure 5. Effect of monomer ratio on swelling capacity of the gelatin-based hydrogel.

Effect of pH on equilibrium swelling

In this series of experiments, swelling ratio for the synthesized hydrogels was measured in different pH solutions ranged from 1.0 to 13.0 (Figure 6). 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 (pH 1.0) solutions were diluted with distilled water to reach desired basic and acidic pHs, respectively. Maximum swelling (51 g.g⁻¹) was obtained at pH 8. In acidic media, most carboxylate groups are protonated, so decreased repulsion of anionic groups leads to a decreased swelling ratio. At higher pHs (3–8), some carboxylate groups are ionized and the electrostatic repulsion between carboxylate groups causes an enhancement of the swelling capacity. The reason of the swelling loss for the highly basic solutions is the charge screening effect of excess Na⁺ in the

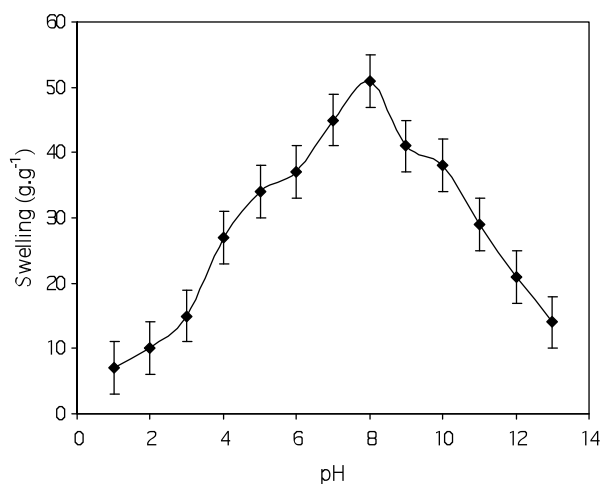


Figure 6. Effect of pH of solution on swelling of gelatin-g-poly(AA-co-AAm) hydrogel.

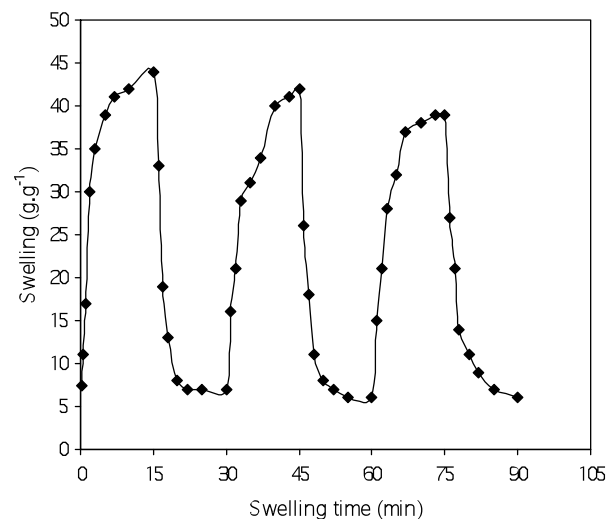


Figure 7. On-off switching behavior as reversible pulsatile swelling (pH 7.0) and deswelling (pH 2.0) of the hydrogel. The time interval between the pH changes was 15 min.

swelling media, which shield the carboxylate anions and prevent effective anion–anion repulsion.

pH-responsiveness behavior of the hydrogel

Since the hydrogels show different swelling behaviors at various pHs, their pH-reversibility was investigated in solutions buffered at pHs 2.0 and 7.0 (Figure 7). The figure shows a stepwise reproducible swelling change of the hydrogel at 25°C with alternating pH between 2.0 and 7.0. At pH 7.0, the hydrogel swells up to 44 g.g⁻¹ due

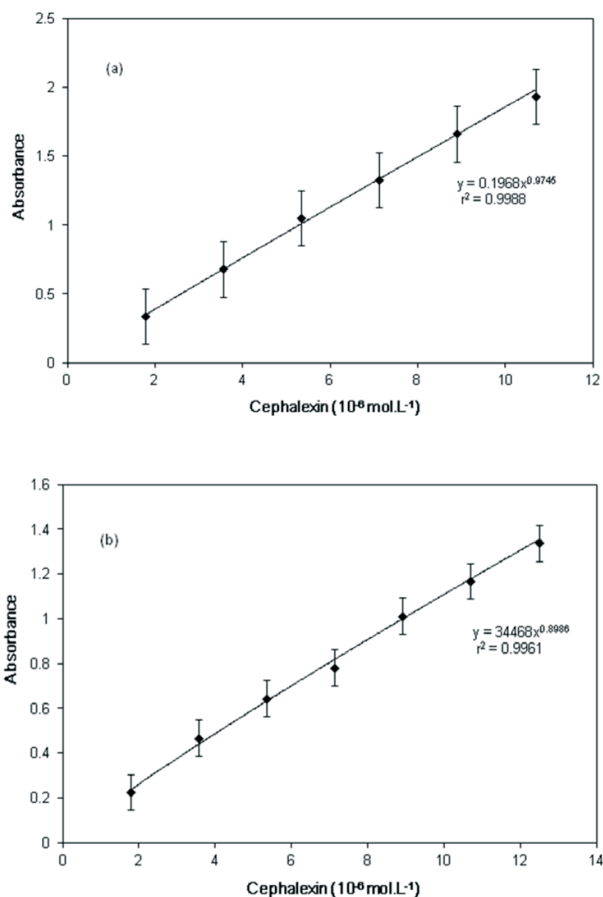


Figure 8. The standard spectrophotometric calibration curves of cephalixin at 266 nm and at pH 1.6 (a) and pH 7.4 (b).

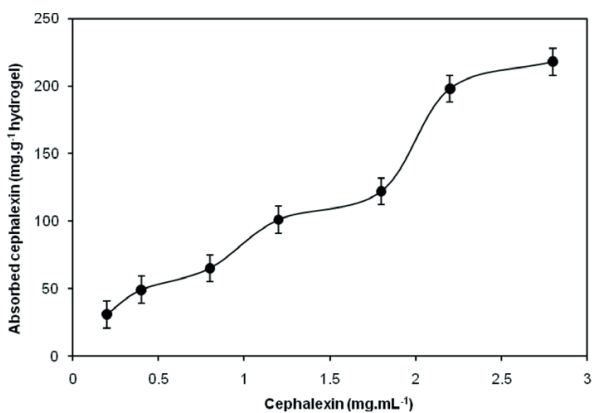


Figure 9. Effect of drug concentration on the adsorption capacities of hydrogel.

to anion–anion repulsive electrostatic forces, while at pH 2.0, it shrinks within few minutes due to protonation of carboxylate groups. This sharp swelling–deswelling behavior of the hydrogels makes them suitable candidates for controlled drug delivery systems.

Calibration curve

The calibration curve of the absorbance as a function of the cephalexin concentration at 256 nm, shown in Figure 8, has a linear relationship with determination coefficients (r^2) of 0.997 and 0.996 at pHs 1.6 and 7.4, respectively.

Cephalexin loading

The amount of drug content entrapped in the hydrogels was determined by an indirect method. After the gel preparation, the washing solutions were collected, filtered with a 0.45 μm Millipore filter and tested at λ_{max} 256 nm using the standard calibration curve of the drug recorded by UV/VIS spectrophotometer.

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

$$\text{Drug entrapment (\%)} = \frac{\text{Amount of drug present in hydrogel}}{\text{Theoretical amount of drug}} \times 100 \quad (2)$$

For the investigation of drug adsorption behavior of hydrogels prepared in this study, hydrogels were firstly swollen in cephalexin solution in concentration range 0.20–2.80 $\text{mg}\cdot\text{mL}^{-1}$. As can be seen from the Figure 9, an increase in drug concentration in the swelling medium increased the amount of adsorbed drug, as observed in many adsorption studies [15–17]. This result can also be obtained from the following equation:

$$q_e = \left(\frac{C_i - C}{m} \right) \times V_t \quad (3)$$

where q_e is in mg adsorbate per gram of dry adsorbent, C_i and C are the initial and equilibrium concentrations of adsorbate solution in $\text{mg}\cdot\text{mL}^{-1}$, V_t is the volume of solution treated in mL , and m is the mass of dry adsorbent, in g . As can be seen from Eq. 3, an increase in concentration of drug in the gel system increased with q_e values.

The amounts of the loaded drug in superabsorbent hydrogels was also significantly affected by the incubation times (Figure 10). It is obvious that with increasing the loading time, the amount of drug loaded increased and then in the beginning decreased. 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 effective time of loading efficiency was 18 h, where the high amount of drug was encapsulated.

In this series of experiments, the drug loading of the hydrogels with different crosslinker content were shown in Figure 11. As can be seen, the amount of drug loaded in the hydrogel beads decrease with increasing the content of crosslinker, MBA. The greater the crosslinking density, the worse the elasticity of the polymer chains, which could restrict the penetration of theophylline into hydrogel, and then leads to the decrease of the loading amount for drug.

In vitro release behavior of hydrogels

To determine the potential application of gelatin-based superabsorbent containing a pharmaceutically active compound, the drug release behavior from this system under physiological conditions was investigated. The percentage of released drug from the polymeric carriers as a function of time is shown in Figure 12. The concentration of cephalexin released at selected time intervals was determined spectrophotometrically. The drug-loaded hydrogels with high drug loading (>85%) were prepared by the swelling–diffusion method. The amount of cephalexin released in a specified time from the gelatin-based hydrogel decreased as the pH of the dissolution medium was lowered (Figure 12). At low pH values, electrostatic repulsion between the carboxylic acid groups of backbone is low, thus decreases gel swelling and minimizes release of cephalexin via diffusion. However, in alkaline media the presence of OH^- increases the electrostatic repulsion between carboxylate groups, thus increases the gels, swelling degree and so the release of cephalexin increased [18,19].

The amount of the released cephalexin from hydrogels was also significantly affected by the drug-loaded content (Figure 13). It was generally noticed that there is an immediate release of drug at the time of immersing the sample in the release medium. This might be due to the surface drug on the hydrogel. This explanation is supported by the fact that this immediate release depends largely on the weight percent of the drug-loaded in the hydrogel. The higher the weight percent of the drug, the higher the percent of the drug immediately released. This immediate drug release is desirable from the practical point of view at the beginning of the applications which must be followed by a period of a controlled release of the drug.

Conclusion

Gelatin-g-poly(NaAA-co-AAm) hydrogel was synthesized through simultaneous crosslinking and graft polymerization of acrylic acid/acrylamide mixtures onto gelatin. Swelling capacity of the hydrogels is affected by the crosslinker (MBA) concentration and monomer ratio, so that the swelling is decreased by increasing the MBA concentration and AAm/AA ratio.

The superabsorbent hydrogels exhibited high sensitivity to pH, so that, several swelling changes of the hydrogel

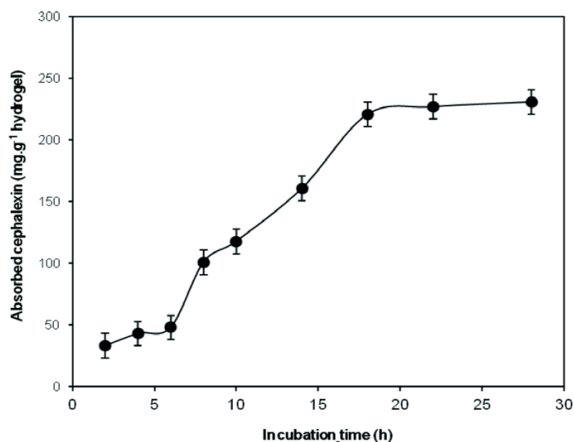


Figure 10. The dependency of the drug loading amount on the incubation time.

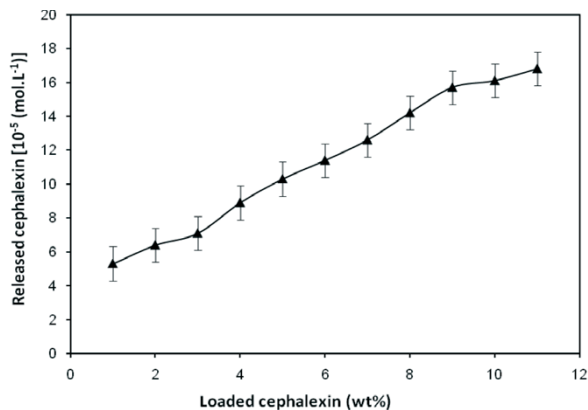


Figure 13. The dependency of the released drug amount to the cephalaxin loaded content.

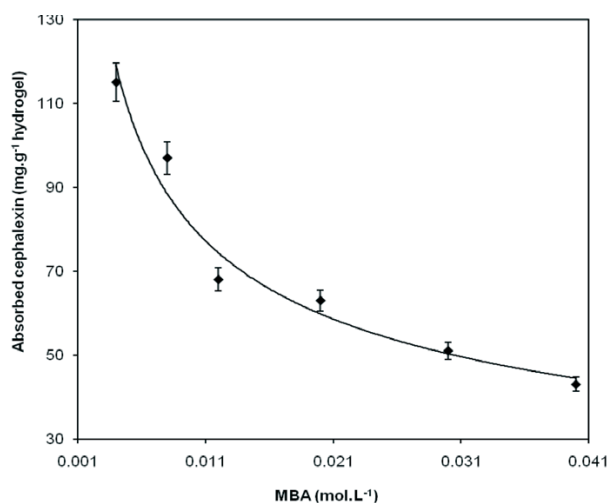


Figure 11. The dependency of the drug loading amount to the crosslinker concentration.

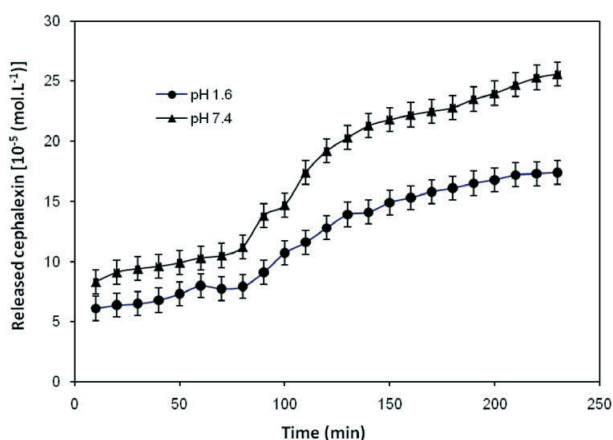


Figure 12. Release of cephalaxin from hydrogel carrier as a function of time and pH at 37°C.

were observed in pH variations of a wide range (1-13). 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. Furthermore, the reversible swelling-deswelling behavior in solutions with acidic and basic pH makes the hydrogels a suitable candidate for controlled drug delivery systems.

The loading and release of cephalaxin from the pH-sensitive hydrogels was effective. The drug loading efficiency was decreased with increasing crosslinker content. The release value of cephalaxin from hydrogels at pH 7.4 was higher than that at pH 1.2 due to the electrostatic repulsion between carboxylate groups. The hydrogels presented in this study may serve as a platform for a wide range of pharmaceutical uses to improve the bioavailability of beta-lactam antibiotic cephalosporin drugs.

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