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ORIGINAL PAPER

Synthesis and characterization of poly(2-hydroxyethyl methacrylate/itaconic acid) copolymeric hydrogels

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Abstract In the present study poly(2-hydroxyethyl methacrylate-co-itaconic acid) (P(HEMA/IA)) hydrogels were synthesized by free-radical copolymerization of 2-hydroxyethyl methacrylate and itaconic acid in order to evaluate as controlled release drug delivery system. All polymerizations were performed in a mixture of water/ethanol with ethylene glycol dimethacrylate, as crosslinking agent, potassium persulfate, as initiator, and N, N, N′, N′-tetramethylethylene diamine, as activator. The samples were characterized by FTIR and SEM. Swelling kinetics and antibiotic release studies were performed using gravimetry and UV spectrophotometry, respectively. The antibacterial activity studies were performed bearing in mind that infections are the most common cause of biomaterial implant failure and represent a constant menace to the application of medical implants. The hemocompatibility testing was performed as imperative for medical devices intended for direct or indirect blood exposure. In accordance with the results of antibacterial assessment on gels loaded with antibiotics and hemolytic activity testing these gels exerted good bacterial growth inhibition and favorable hemolytic activity. The release profiles of antibiotics, evaluated in vitro conditions, were correspondent for antibacterial therapeutics. Therefore, it was concluded that (P(HEMA/IA)) gels could be propound for the potential application as drug delivery systems for the controlled release of antibiotics.

Keywords Hydrogels · 2-Hydroxyethyl methacrylate · Itaconic acid · Morphology · Hemocompatibility · Controlled drug release · Antibacterial activity

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Introduction

Hydrogels are hydrophilic, crosslinked polymers made of homo- or co-polymers that can absorb significant amounts of water or biological fluids but do not dissolve owing to the presence of chemical or physical cross-links. In chemically crosslinked hydrogels, covalent bonds are present between different polymer chains, while in physically crosslinked hydrogels, dissolution is prevented by ionic interactions, hydrophobic associations, or hydrogen bonds. In general, hydrogels exhibit good biocompatibility. Their hydrophilic surface is characterized by a low interfacial free energy in contact with body fluids, which results in a low tendency for proteins and cells to adhere to these surfaces. Due to their high water content, hydrogels also possess a degree of flexibility similar to natural tissues, which minimizes potential irritation to surrounding membranes and tissues [1, 2]. Thus, hydrogels have found widespread biomedical and pharmaceutical applications. They have been increasingly studied for uses such as matrices for tissue engineering, materials for immobilization of enzymes and cells, contact lenses, and drug delivery devices. The ability of molecules of different sizes to diffuse into and out of hydrogels allows their possible use as drug delivery systems for oral, nasal, ocular, rectal, vaginal, and transdermal routes of administration [3–5].

Recently, there has been an increased interest for preparation and characterization of environmentally sensitive or stimuli-sensitive hydrogels that can control drug release by changing the gel structure in response to some environmental stimuli. These hydrogels are called "intelligent" or "smart" because of their ability to change the swelling behavior, permeability, and mechanical strength in response to changes in the pH, ionic strength, temperature, or composition of the surrounding fluid. Due to the large variations in physiological pH values, as well as the pH variations in pathological conditions, pH-responsive polymeric networks have been studied extensively [2, 3, 5].

The success of the therapy with drugs strongly depends on achieving actual drug concentration on the targeted site for a sufficient period of time. This goal is very difficult to achieve by typical delivery of drugs, accounting for about 90% of all drug formulations, which in some instances lead to serious side effects [6]. Thus, there is a need for new drug delivery systems that increase the residence time of the precisely dosed drug concentration in the targeted site, thereby, reducing wastage and minimizing side effects. To reduce the drug loss and the systemic side effects, and improve the drug efficacy, several approaches have been proposed to obtain more sustained release profiles, such as the addition of thickening, in situ gelling or bioadhesive polymers, or by using of inserts which stimulate tissues, and in particular, its base material often causes allergies [2, 3].

Infections play an important role in various applications of biomaterials. Consequently, in recent years, infections are the most common cause of biomaterial implant failure and represent a significant challenge to the more widespread application of medical implants [7–9]. One method to prevent these biomaterial related infections is to provide the biomaterial with some antimicrobial properties.

The essential idea in this work was to synthesize pH-sensitive poly (2-hydroxyethyl methacrylate-co-itaconic acid) (PHEMA/IA) hydrogels by free-radical

copolymerization and to evaluate them as a multifunctional biomaterial used as controlled release drug delivery system. PHEMA hydrogels are most popularly used multifunctional polymeric biomaterial. It was found that hydrogels based on poly (2-hydroxyethyl methacrylate-co-itaconic acid) copolymers are biocompatible and pH-sensitive [10]. Because pH value of a physiological fluid is different in healthy and sick tissue, one can use pH-sensitive hydrogels to achieve suitable release profile of the drug in the fluid with given pH value. pH of the infected tissue is in the range of 5.5–6.5, which is above both dissociation constants of IA, so the gel swelling increases. During the healing process the pH value raises and therefore, the swelling and drug release is intensified. Gentamicin sulfate and sodium sulfacetamide were used as model drugs in our study.

Experimental

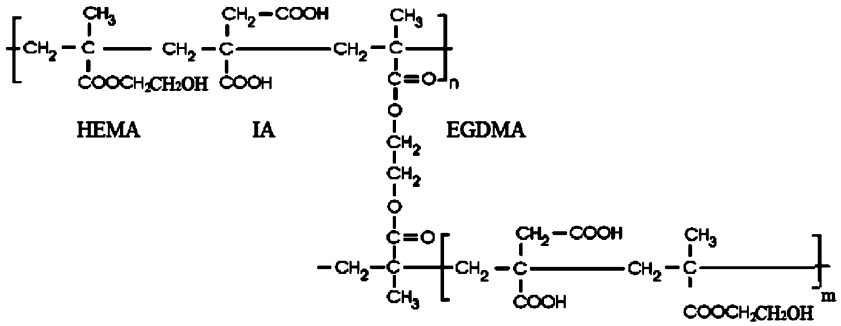
Materials

2-Hydroxyethyl methacrylate (HEMA) (Aldrich), freshly distilled, and itaconic acid (IA) (Fluka), as received, were the monomers used in this study. Ethylene glycol dimethacrylate (EGDMA) (Aldrich), as crosslinking agent, potassium persulfate (Fluka), as initiator, and N,N,N′,N′-tetramethylethylene diamine (TEMED) (Aldrich), as activator, were used in all polymerizations performed in a mixture of water/ethanol as solvent. Gentamicin sulfate (GS) and sodium sulfacetamide (SS) were supplied by Sigma. Potassium hydrogen phosphate (KH_2PO_4 and K_2HPO_4) (Fluka) were used for buffer preparations. Demineralized water was used for all copolymerizations and the preparation of the buffer solution.

Preparation of hydrogels

The P(HEMA/IA) copolymeric hydrogels (Scheme 1) were prepared by radical crosslinking polymerization. The monomers were dissolved in water/ethanol mixture. The IA mole fractions were 2.0, 3.5 and 5.0. According to the monomer mole fraction samples were designated as P(HEMA/2IA), P(HEMA/3.5IA) and P(HEMA/5IA), respectively. The initiator, activator and crosslinker were added to a monomer feed mixture in the amount of 0.25, 0.25 and 0.5 mol%, respectively, with respect to the total moles of monomers. The polymerization was carried out at 60 °C for 24 h. The same conditions, monomer to solvent ratio, amount of initiator, activator and crosslinker were used to prepare the PHEMA hydrogel. The reaction mixture was degassed prior to polymerization and placed between two glass plates sealed with a rubber spacer (2 mm thick). After the reaction, the gels were cut into discs and immersed in water for a week to remove unreacted monomers. The water was changed daily. The discs were dried to xerogels (1 mm thick and 5 mm in diameter).

The amount of uncrosslinked IA was determined by titration of extract against NaOH (0.05 mol l^{-1}) to phenolphthalein end point. On the other hand, the amount of uncrosslinked HEMA was determined using a UV spectroscopy. In both cases,



Scheme 1 P(HEMA/IA) hydrogel network obtained by free-radical copolymerization of HEMA, IA and crosslinking agent (EGDMA)

results indicate that the conversion during cross-linking reaction was nearly complete.

Fourier-transform infrared spectroscopy (FTIR)

Gel samples were crushed in powder and mixed with potassium bromide (Merck IR spectroscopy grade), in 1:100 proportions, and dried at 40 °C. The mixture was compressed to a 12-mm semi-transparent disk by applying a pressure of 65 kN (Pressure gauge, Shimadzu) for 2 min. FTIR spectra over the wavelength range 4,000–700 cm^{-1}, with a resolution of a 4 cm^{-1}, were recorded using FTIR spectrometer (BOMEM Michelfan MB-102 FTIR).

Scanning electron microscopy (SEM)

A scanning electron microscope (SEM), JEOL JSM-5300, was used to observe the specimen morphologies. The samples were prepared by freeze drying using a Modulyo Freeze Dryer System Edwards (England) consisting of a freeze dryer unit and a High Vacuum Pump E2M8 Edwards. All samples were pre-frozen in deep-freezer at −80 °C for 24 h. Subsequently, they were freeze dried. The vacuum during 20 h of freeze drying was around 4 mbar. The gel samples were gold sputter coated under vacuum before observation.

Hemolytic activity of gels

The hemolytic activity of the hydrogels was determined by the direct and indirect contact methods, according to ISO 10 993–4 (1992) [11]. In the direct method, the hydrogel discs were immersed in 5 ml of a physiological solution (PS) to which 0.25 ml of whole rat blood had been added. The PS and distilled water were used as the negative and the positive control, respectively. Then the contents of the tubes were gently mixed and incubated in a water bath at 37 °C for 1 h. Subsequently, the absorbance of the supernatant liquid in each tube was determined at 545 nm using a Pharmacia LKB Ultrospec Plus UV/VIS spectrophotometer and the percentage of

hemolysis was calculated. The mean hemolysis value of 5% or less variation from two tests is considered acceptable. In the indirect contact method 5 ml of an isotonic aqueous extract from a hydrogel disc was used with 0.25 ml of a 10% suspension of rat erythrocytes. To prepare the isotonic aqueous extracts, pieces of each disc were kept for 72 h at 37 °C in 100 ml of sterilized bidistilled water and then 0.9 g NaCl was added. The negative control was 0.9% NaCl solution and 100% hemolysis was obtained in bidistilled water. After incubation at 37 °C for 24 h, the absorbance of the supernatant was measured at 545 nm and the percentage of hemolysis was calculated.

Swelling study

Dynamic swelling experiments were performed in buffer solution of pH 7.40 (simulated physiological fluid) at 37 °C. Swollen gels were removed from the swelling medium at regular intervals, dried superfically with filter paper, weighed and placed in the same bath. The measurements were continued until constant weight was reached for each sample. The amount of solution absorbed was monitored gravimetrically. The degree of equilibrium swelling (q_e) was calculated as follows:

$$q_e = (M_e - M_o)/M_o \quad (1)$$

where M_e is the weight of the swollen hydrogel at equilibrium and M_o is the weight of the xerogel [12, 13]. All swelling experiments were performed in triplicate.

In vitro controlled release of antibiotics

For the investigation of drug release profile of PHEMA and P(HEMA/IA) hydrogels prepared in this study, gentamicin sulfate (GS) and sodium sulfacetamide (SS) were used as model drugs. Dry polymer discs (1 mm thick, 5 mm diameter) were loaded with GS and SS by immersion into aqueous solution of the drug in the absence of light, for 1 week. The amount of the drug loaded into each gel was 5 wt% of dry gel weight. All drug loaded discs were transparent. The swollen drug loaded gels were then dried at ambient temperature for several days to constant mass and used for the release experiments.

In vitro release studies of the antibiotics, gentamicin sulfate (GS) and sodium sulfacetamide (SS), have been carried out in triplicate by placing dried and loaded sample in definite volume of releasing medium (a buffer solutions of pH 7.40 (simulated physiological fluid)) at 37 °C. The amount of antibiotic released was measured spectrophotometrically using a UV spectrophotometer (UV-1700, Shimadzu) by taking the absorbance of the solution in regular time intervals at wavelength 254 nm for gentamicin sulfate and sodium sulfacetamide, respectively.

Mathematical modeling for drug release from polymer matrix

The absorption of fluid from the environment changes the dimensions and physicochemical properties of the hydrogels system and thus, the drug release

kinetics. Although some simple solution may apply to the diffusion, swelling or chemically controlled drug release system, model complexity will increase as other mechanisms, like for example polymer–drug interactions, are involved. Some generalized empirical equations are widely used to describe both the fluid uptake through the swellable glassy polymers and the drug release from these devices [14–20]. An empirical equation developed by Peppas et al. assumes a time-dependent power law function [16, 17]:

$$\frac{M_t}{M_\infty} = k \cdot t^n \tag{2}$$

Here M_t/M_∞ is the fractional release of drug at time t (M_t and M_∞ are the drug released at time t and at equilibrium, respectively), k is the constant characteristic of the drug–polymer system and n is the diffusion exponent characteristic of the release mechanism. Normal Fickian diffusion is characterized by $n = 0.5$ and Case II diffusion by $n = 1.0$. A value of n between 0.5 and 1.0 indicates a mixture of Fickian and Case II diffusion, i.e. non-Fickian, or anomalous diffusion. When the plot ln M_t/M_∞ versus ln t is drawn, for the logarithmic form of Eq. (2), the slope of the plot gives the value of n and the intercept gives the k value. This equation applies until 60% of the total amount of drug is released.

Analysis of the swelling behavior of PHEMA and P(HEMA/IA) hydrogels was carried out using the one-dimensional fluid diffusion equation. The Fickian diffusion equation for one-dimensional fluid transport may be solved under initial and boundary conditions equivalent to the conditions of testing here, to give Eq. (3) [21]:

$$\frac{M_t}{M_\infty} = 1 - \sum_{n=0}^{\infty} \frac{8}{(2n+1)^2\pi^2} \exp\left[\frac{-D(2n+1)^2\pi^2 t}{l^2}\right] \tag{3}$$

Here D is the fluid diffusion coefficient in the polymer.

The initial and boundary conditions are written as:

$$\begin{array}{lll} t = 0 & -l/2 < x < l/2 & c_1 = 0 \\ t > 0 & x = 0 & \dfrac{\partial c_1}{\partial x} = 0 \\ t > 0 & x = \pm l/2 & c_1 = c_{1e} \end{array}$$

The early time approximation of Eq. (3) can be written as:

$$\frac{M_t}{M_\infty} = 4\left(\frac{D_e t}{\pi l^2}\right)^{0.5} \tag{4}$$

Thus, the fluid diffusion coefficient would be easily calculated. Diffusion coefficients were also calculated from the late-time approximation of Eq. (3), written as:

$$\left(\frac{M_t}{M_\infty}\right) = 1 - \left(\frac{8}{\pi^2}\right)\exp\left[\frac{(-\pi^2 D_l t)}{l^2}\right] \tag{5}$$

where D_l is the late-time diffusion coefficient and l is the thickness of the sample. The slope of the plot between ln $(1 - M_t/M_\infty)$ and t was used for the evaluation of D_l.

Antibacterial activity assay

The drug loaded hydrogel samples were inoculated in tubes with saline solution (9 ml). The selected indicator microorganisms were *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923). The number of bacterial cells was c.a. 10^4 ml^{-1} for both strains. The tests were performed in three replicas and the results calculated as a mean value. The tubes were placed into a shaker (120 rpm) and thermostated in the water bath at 37 °C, to assure the better contact of tested hydrogels and bacterial cells. For this purpose, the sterile Petri plates were inoculated with an aliquot (100 μl) of saline solution with tested hydrogels and bacterial cultures and overlaid with melted TSA. After solidification of agar and the incubation at 37 °C for 24 h, the counting of visible colony was performed.

Results and discussion

Spectral analysis of gels (FTIR)

The amount of uncrosslinked IA was determined by titration of extract against NaOH (0.05 mol l^{-1}) to phenolphthalein end point. On the other hand, the amount of uncrosslinked HEMA was determined using a UV spectroscopy. In both cases, results indicate that conversion during cross-linking reactions was almost complete.

FTIR spectroscopic analysis was used to illustrate the composition and nature of bond formation into the hydrogels. The incorporation of ionic monomer in hydrogels was also confirmed using FTIR spectroscopy. The FTIR spectrum of PHEMA (Fig. 1 (black line)) revealed two peaks characteristic of alcoholic (OH) groups, respectively

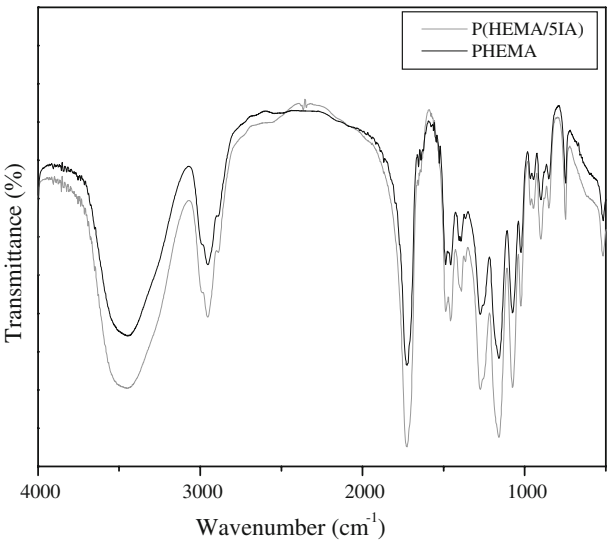


Fig. 1 FTIR spectra of PHEMA and P(HEMA/5IA) gels

at 3,445 cm^{-1} (stretching C–O) and 1,022 cm^{-1} (bending O–H). Ester groups were identified by a peak at 1,728 cm^{-1} (C = O stretching) and at 1,275 cm^{-1} (C–O stretching). The increased peak intensity of the C = O group at 1,730 cm^{-1} in the spectrum of P(HEMA/5IA) gel (Fig. 1 (gray line)) was associates with the presence the additional C = O groups from IA. Spectrum of P(HEMA/5IA) sample shows a broader peak in the range of 3,700–3,100 cm^{-1} which is the evidence of the OH stretching vibrations of carboxylic acid groups of IA. Other characteristic bands represent C–C and C–H vibrations of $–CH_3$ and $–CH_2$ groups [22].

Gel morphology (SEM)

In order to obtain successful delivery devices for bioactive agents or drugs, the adequate hydrogel morphology is required. One of the important factors influencing the drug release profile is the pore size, volume, and structure in hydrogels. The pore size of the polymeric material mainly depends on factors affecting the swelling and micromorphology properties of hydrogels and seriously impact micromorphology. The porosity of PHEMA hydrogels could be improved by incorporations of hydrophylic polyelectrolyte comonomer like itaconic acid. Scanning electron microscopy images of pure homopolymeric PHEMA and P(HEMA/3.5IA) gels are depicted in Fig. 2. In Fig. 2(a) (PHEMA), there is observed a compact, dense and layered structure with small uniformly distributed pores of gel. On the other hand, coral-like structure with larger pores are clearly visible on the surface of the

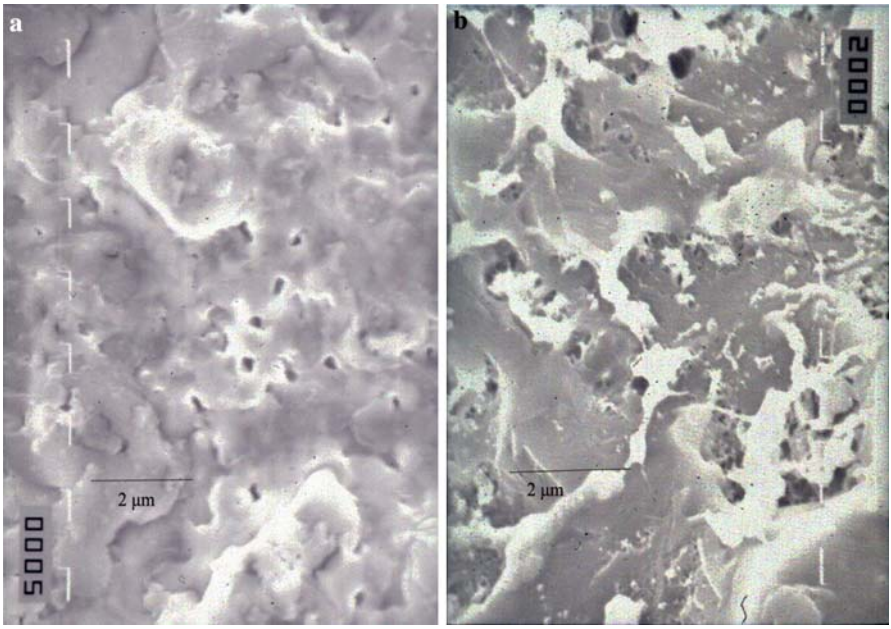


Fig. 2 Scanning electron microscopy (SEM) micrographs of the PHEMA (**a**) and P(HEMA/3.5IA) (**b**) gels (magnification: 750×)

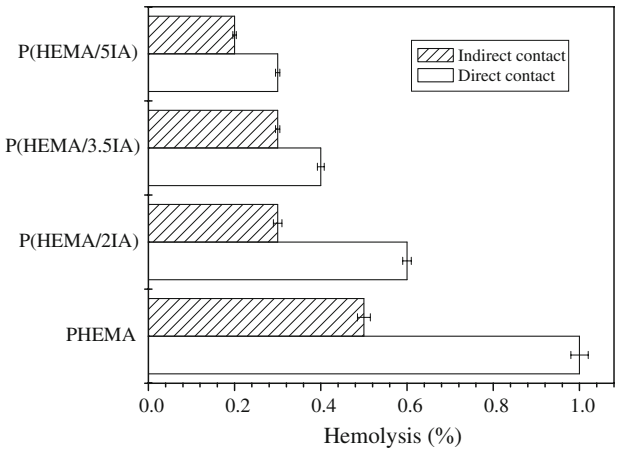


Fig. 3 Hemolytic activity of PHEMA and P(HEMA/IA) hydrogels

P(HEMA/3.5IA) gel (Fig. 2(b)). The reason for the presence of larger pores inside the P(HEMA/3.5IA) gel network can be attributed to the greater incorporations of itaconic acid residues, which contribute to the enlargement of the pore size by increasing the network hydrophilicity and by electrostatic repulsion of the negative charges of itaconic acid carboxylate groups.

Hemolytic activity of hydrogels

The hemocompatibility of PHEMA and P(HEMA/IA) copolymers was evaluated by their testing for hemolysis. For in vitro testing conditions PHEMA and P(HEMA/IA) hydrogels in contact with blood showed a mean hemolysis value less than 1.0% in the direct contact assay, and even less than 0.5% in the indirect contact assay (Fig. 3). According to the results obtained, these hydrogels are not considered as hemolytic. Incorporation of small amounts of itaconic acid, which is obtained from natural renewable sources, in the range of 2–5 mol% in gels improves the hemocompatibility of PHEMA biomaterial. Gels containing higher content of IA showed lower degree of hemolysis namely better hemocompatibility.

Effect of hydrogel composition on swelling

When a hydrogel is in a dehydrated state, the polymer chains are in a collapsed state, allowing little room for molecular diffusion. As the hydrogel swells and reaches an equilibrium swelling value, the swelling pressure on the chains is counteracted by the force holding the chains together, namely, the force of crosslinking. At the swelling equilibrium, the network mesh size is the greatest and molecular diffusion reaches its maximum value [23, 24].

Equilibrium swelling measurements were conducted in a buffer solution of pH 7.40 in order to simulate physiological environment [2]. The gels swollen to equilibrium and degree of equilibrium swelling (q_e) of the PHEMA and P(HEMA/

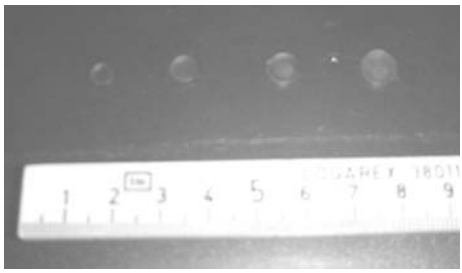


Fig. 4 Photograph of PHEMA and P(HEMA/IA) gels at equilibrium swelling

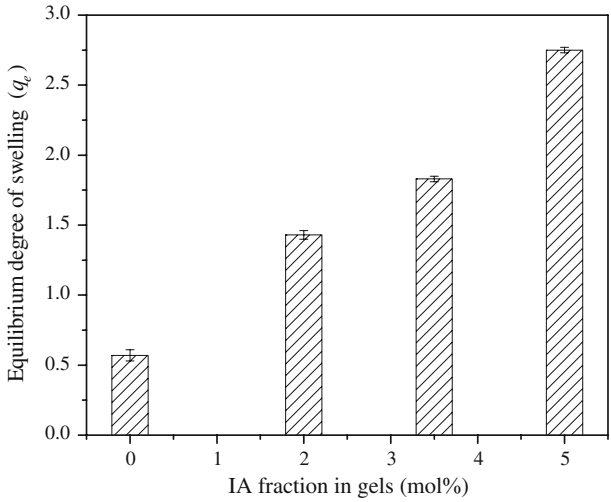


Fig. 5 The equilibrium degree of swelling (q_e) of PHEMA and P(HEMA/IA) hydrogels as a function of IA content

IA) hydrogel series are presented in Figs. 4 and 5, respectively. The amount of incorporated IA in gel composition was 2, 3.5 and 5 mol%. It can be seen that the swelling behaviour is highly influenced by the composition of the hydrogels. The equilibrium degree of swelling increases with the increase of the IA content in the copolymer. The swelling capacities of the homopolymeric PHEMA and P(HEMA/IA) copolymeric gels were between 55 and 298%. These results indicate that the low swelling of PHEMA gel was increased by copolymerization with IA, due to high hydrophilicity and electrostatic repulsion of COO^- groups in IA units.

In vitro controlled release of antibiotics

Among controlled release drug delivery systems, hydrogels are interesting owing to their unique tunable time-dependent swelling behavior. The aim of controlled release systems is to deliver the drug at a specified rate, keeping the drug concentration in the body at the therapeutically effective level, i.e. with convenient drug release profile [25, 26]. Hence, a hydrophilic monomer (IA) was affirmed to improve the swelling capacity, antibacterial activity and biocompatibility of

PHEMA hydrogels in order to evaluate these copolymers as potential delivery systems. Antibiotics typically retain the growth of bacteria and help the body's immune system to fight the bacteria causing the infection. In order to test P(HEMA/5IA) hydrogels as controlled release systems, the antibiotics gentamicin sulfate (GS, $M = 573.6$ g mol^{-1}) and sodium sulfacetamide (SS, $M = 237.2$ g mol^{-1}) were loaded in gel and their release kinetics were studied.

The cumulative release profiles of GS and SS from antibiotic-loaded PHEMA and P(HEMA/5IA) formulations into physiological buffer solution (pH 7.40) at 37 °C are illustrated in Fig. 6. The sulfacetamide release increases rapidly at first and then gradually reaches an equilibrium value in about 49 and 38 h for PHEMA and P(HEMA/IA) gels, respectively. On the other hand, the GS release is slower and the equilibrium value is reached in about 62 and 49 h for PHEMA and P(HEMA/5IA) gels, respectively. No lag-time was observed during the release of both antibiotic-loaded hydrogel formulations. The total amount of GS and SS released from PHEMA gel was 78 and 63%, respectively. The amount of antibiotics released from the drug loaded P(HEMA/5IA) gels within 120 h was 98 and 91% for SS and GS, respectively. Itaconic acid incorporated in gels significantly improves the release profiles of the samples and facilitates higher amounts of released drug. For all the formulations, the sustained release behavior was observed.

The values of the drug release exponent (n) for PHEMA and the P(HEMA/5IA) hydrogels loaded with SS and GS are presented in Table 1. They are near 0.5 in all cases, suggesting that the release process can be described by a Fickian transport mechanism as a diffusion-controlled delivery system, with slab geometry. In the case of SS, the half-times of the release values ($t_{1/2}$) were substantially smaller than those of GS. P(HEMA/5IA) hydrogel had the shortest half-time $t_{1/2}$ for the release of SS (1.42 h) (Table 1).

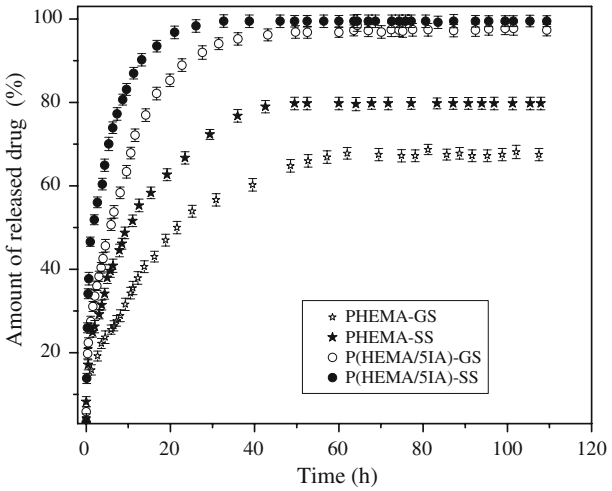


Fig. 6 Release profiles of GS and SS from PHEMA and P(HEMA/5IA) gel formulations in vitro conditions

Table 1 Release characteristics of PHEMA and P(HEMA/5IA) hydrogels

Drug	Hydrogel	k	n	r^2	$t_{1/2}$ (h)	$D_e \times 10^7$ ($cm^2\ s^{-1}$)	$D_l \times 10^7$ ($cm^2\ s^{-1}$)
SS	PHEMA	0.251	0.51	0.99	3.75	0.664	3.759
	P(HEMA/5IA)	0.272	0.49	1.00	1.42	1.303	16.65
GS	PHEMA	0.086	0.48	0.99	16.5	0.135	0.562
	P(HEMA/5IA)	0.131	0.49	0.99	9.82	0.295	1.617

The values of the diffusion coefficients (early and late stages of the drug release process), D_e and D_l, respectively, are also presented in Table 1. Both D_e and D_l values were influenced by IA residues in the copolymer. An introduction of IA in the hydrogel structure leads to an increase of the ionization of the network in the slightly basic buffer, increasing the inherent electrostatic repulsion between like charges. As a consequence, the values for the fluid diffusion coefficients are higher for the samples with IA (Table 1).

For all the studied samples, the late-time diffusion coefficients D_l are higher than the initial diffusion coefficients D_e, due to the larger swelling at equilibrium, following the same trend as in the fluid diffusion process (Table 1). In general, the expansion of the polymer network by the fluid is slower at the earlier stages of the process, since the gel goes from xerogel (glass state) to hydrogel form.

Antibacterial activity of hydrogels

Hydrogels have been widely used for biomedical applications such as wound dressings for burns, contact lenses, artificial skin and in drug release systems [26]. Therefore, the prevention of their contamination with microorganisms is very important. The analysis of antibacterial activity, which is a very valuable property for biomedical applications, has been reported for many polymers [27, 28].

In vitro antibacterial study of antibiotics loaded P(HEMA/IA) hydrogels was performed to examine the efficiency of released antibiotics gentamicin sulfate and sodium sulfacetamide in microbial infections. Because they function by inhibiting bacterial protein synthesis, these antibiotics have a very broad spectrum of activity, they are active against most of Gram-positive and Gram-negative bacteria, so *S. aureus* and *E. coli* were chosen as representative examples of Gram-positive and Gram-negative bacteria, respectively.

In vitro studies carried out by incubation of the antibiotics loaded hydrogel matrices with *S. aureus* and *E. coli* strains demonstrated that the drug was released in the active form. Under the experimental conditions, PHEMA and P(HEMA/5IA) hydrogel matrices displayed range of bacterial growth inhibition during the whole drug release period (Figs. 7, 8). Antibacterial activity lasted for 120 h. Antibiotics loaded PHEMA gels showed lower bacterial growth inhibition against both of tested bacterial strains while both antibiotics incorporated in P(HEMA/5IA) gel indicated much better antibacterial activity, i.e. bacterial inhibition of 75 and 89% for gentamicin sulfate and sodium sulfacetamide, respectively (Fig. 7). The best antibacterial properties are obtained for sodium sulfacetamide loaded P(HEMA/

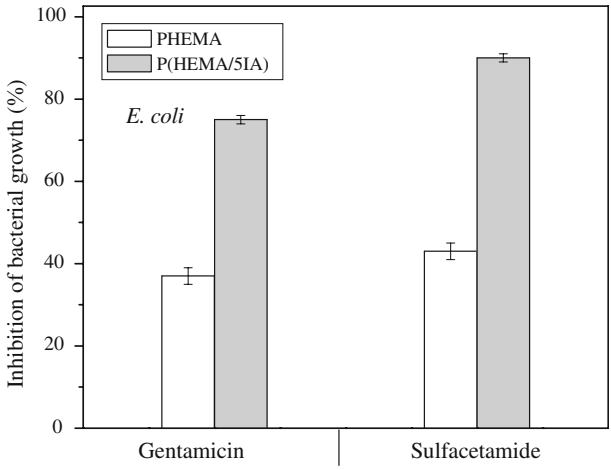


Fig. 7 Inhibition of *E. coli* growth on antibiotics loaded PHEMA and P(HEMA/5IA) hydrogels

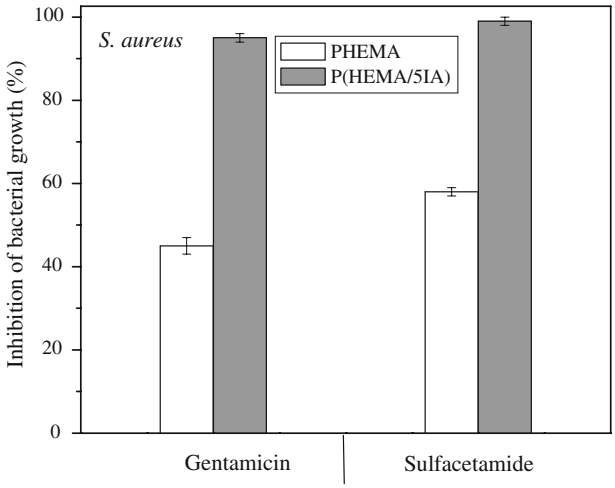


Fig. 8 Inhibition of *S. aureus* growth on antibiotics loaded PHEMA and P(HEMA/5IA) hydrogels

5IA) gel against *S. aureus* (Fig. 8). It is obvious that itaconic acid based hydrogels impart better antibacterial properties of gels. This result can be interpreted by better drug loading and spreading into itaconic acid based gels providing their good antibacterial activity and more released drug.

Conclusion

The aim of the present study was to develop controlled release drug delivery system based on P(HEMA/IA) hydrogels as a polymer matrix for therapeutics. It was

concluded that by introducing highly hydrophilic itaconic acid into PHEMA structure significantly improved the swelling of the gel, its hemocompatibility and drug release characteristics. The release profiles of sodium sulfacetamide and gentamicin sulfate from the P(HEMA/IA) hydrogels were affected by hydrogel composition and antibiotic type. Slower release was observed for gentamicin sulfate. The P(HEMA/IA) hydrogels indicated significant antibacterial activity against *S. aureus* and *E. coli* during the whole period of sulfacetamide release, whilst gentamicin sulfate had lower antibacterial activity against *E. coli* strains. Therefore, P(HEMA/IA) hydrogels prepared in this study can be considered as potential pH-sensitive matrices for the controlled drug release systems suitable for transdermal and ocular route of administration and wound healing dressings.

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