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Synthesis, characterization and controlled release of cephalexin drug from smart poly(2-hydroxyethyl methacrylate/poly(alkylene glycol)(meth)acrylates hydrogels

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ABSTRACT

In this work, novel hydrogels based on 2-hydroxyethyl methacrylate (HEMA) and different poly(alkylene glycol)(meth)acrylates (BIS) were prepared by radiation-induced copolymerization. The influence of different BIS types with variation in chain length, based on ethylene glycol (EG) and/or propylene glycol (PG) pendant units, on the nature and inherent properties of P(HEMA/BIS) copolymeric hydrogels was the main idea of this paper. Swelling studies were conducted for all types of P(HEMA/BIS) copolymeric hydrogels in a wide pH and temperature range. Additional characterization of structure, morphology and thermal behaviour of the obtained hydrogels was conducted by Fourier transform infrared (FTIR) spectroscopy, scanning electron microscopy (SEM), and thermogravimetric (TG) analysis. The modelling of drug release and diffusion characteristics was tested using cephalexin (CEX). The results indicate that P(HEMA/BIS) hydrogels' properties are significantly dependent on the type of BIS. The hydrogels with ethylene glycol (EG) pendant chains show a noticeable pH and/or temperature sensitivity and can be considered smart hydrogels. The introduction of propylene glycol (PG) units, pure and mixed with ethylene glycol (EG) pendant chains, can additionally tune the characteristics of such gels. Furthermore, drug release studies indicate that these types of P(HEMA/BIS) copolymeric hydrogels are suitable candidates for controlled drug release systems.

1. Introduction

The development of new polymeric biomaterials for medical and pharmaceutical purposes is of great interest to life-care science and engineering. Hydrogels, as polymeric materials which do not dissolve but swell considerably in contact with aqueous and physiological media, are positioned on high level of significance. They provide a swollen three-dimensional matrix with a predetermined amount of water or physiological fluids, resembling to some degree the environment of the native tissue [1–6]. Special types of hydrogels known as stimuli-responsive have been investigated for the development of "smart" materials in various fields. The term "stimuli-responsive" implies that marked changes of key properties can be induced by an external stimulus. In aqueous media, stimuli-sensitive systems are generally aimed at changing the hydrophilic character of functional groups into a hydrophobic one, or vice versa [1,7]. Both chemical and physical stimuli can be employed for that purpose. Chemical stimuli include, for instance, acid–base reactions, complexation, bond breaking or making redox and electrochemical reactions, or photochemical reactions. Physical stimuli comprise, for example, changes of the pH-value, of ionic strength, of temperature or pressure, light, or electrical and magnetic fields [7]. The simplest stimuli-responsive polymers are based on acid–base reactions or pH changes. Smart hydrogels are of special interest in controlled drug release applications because of their soft tissue biocompatibility, simple loading and dispersing of the drugs in the matrix, and a high degree of control achieved by the selection of the physical and chemical properties of the polymer network and the response by volume variation to some external stimuli [8,9].

Poly(2-hydroxyethyl methacrylate) (PHEMA) is favourable because of its excellent biocompatibility and physicochemical properties similar to those of living tissues [10,11]. It also exhibits good chemical and hydrolytic stability and good tolerance to entrapped cells. It has also been widely used as the backbone for synthesizing stimuli-responsive hydrogels [12]. Numerous studies have been conducted to modify PHEMA with the aim of improving its mechanical properties [12–14], its electro-responsive properties [15], and of eliciting better physiological responses [16]. Copolymers of HEMA with methacrylic [17,18], acrylic [14,19,20], and

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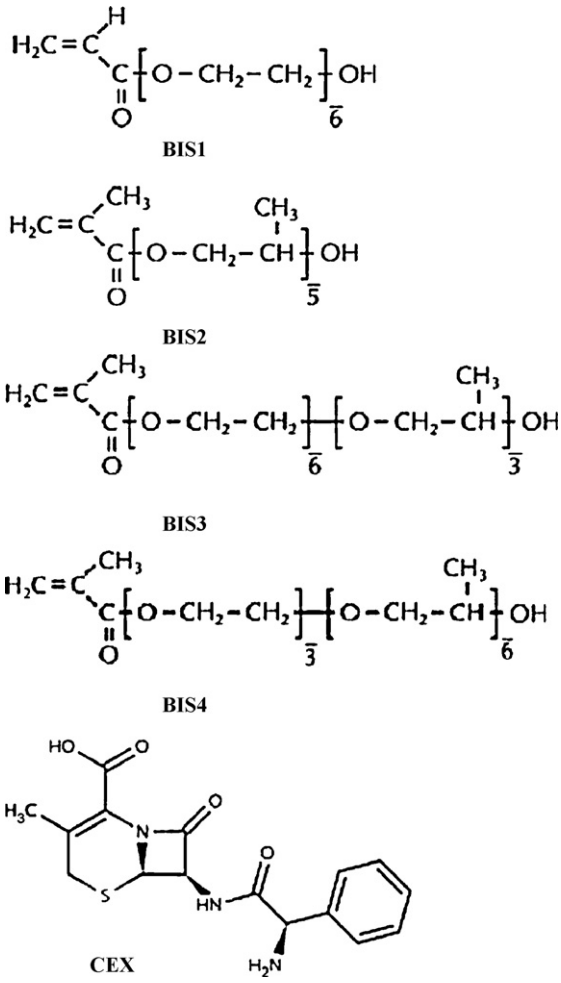


Scheme 1. Chemical structures of bisomers (BIS1, BIS2, BIS3 and BIS4) and antibiotic drug cephalexin (CEX).

itaconic acid [21–23] as pH sensitive components, and some itaconic acid mono alkyl esters [24] have been reported previously; in general, all these copolymers are made by addition of ionic component to non-ionic HEMA. On the other hand, poly(ethylene glycol) (PEG) is a water-soluble, nontoxic, and nonimmunogenic polymer [25]. PEG-based oligomers, mono- and difunctionalized polymeric derivatives are interesting biomaterials because they exhibit low degrees of protein adsorption and cell adhesion [26,27]. Due to good biocompatibility and safe toxicity profile, they are applied in various biomedical areas such as drug delivery, wound healing, and tissue repair systems [28–31]. According to the aforementioned, it is presumed that new class of P(HEMA/BIS) hydrogels, based on HEMA and different poly(alkylene glycol)(meth)acrylates (BIS), can be a beneficial synergetic non-ionic combination for biomedical applications. Thus, in the preparation of new stimuli-responsive hydrogels addition of ionic component was intentionally avoided, since this component could change the cell metabolism, in terms of binding ions, and reduce biocompatibility of hydrogels [32,33].

Radiation polymerization/crosslinking has many advantages over conventional chemical and photochemical methods. It is a simple environmentally friendly additive-free process that occurs at room temperature and the degree of crosslinking can easily be controlled by altering the irradiation conditions. The hydrogels prepared by the radiation method have the potential for use in biomedical applications due to the absence of extraneous toxic additives (chemical initiators, crosslinkers, etc.) [34,35]. Another advantage of this method is that the resultant product is simultaneously sterilized during the irradiation process.

In recent years, there has been an increased interest in the controlled release of drugs, which is another efficient technique for the use of medicines. The ideal drug delivery system should be inert, biocompatible, mechanically strong, comfortable for the patient, capable of achieving a high drug loading for the required blood levels, immune to accidental release, simple to apply, and easy to fabricate [1,5].

In order to evaluate P(HEMA/BIS) hydrogels as potential controlled release systems, the antibiotic cephalexin (CEX) was loaded in gels and the release kinetics was investigated. CEX is a first-generation cephalosporin antibiotic which retains the growth of bacteria and helps the body's immune system to fight the bacteria causing the infection. It is used to treat urinary tract infections, respiratory tract infections, and skin and soft tissue infections. It is also sometimes used to treat acne. In addition to being a rational first-line treatment for cellulitis, it is a useful alternative to penicillins in patients with penicillin hypersensitivity [36]. The interest of formulation of antimicrobial agents in the hydrogels is prevention of infection and protection of the medical implants and devices made of hydrogels from infection during their use in medicine.

In this study, four novel P(HEMA/BIS) copolymeric hydrogels consisting of 2-hydroxyethyl methacrylate (HEMA) and different poly(alkylene glycol)(meth)acrylates (BIS) were prepared by high-energy radiation-induced radical copolymerization. The obtained hydrogels were characterized by swelling studies in the wide pH (2.2–8.0) and temperature range (25–70 °C). Fourier transform infrared (FTIR) spectroscopic analysis was used to determine hydrogel structure, while scanning electron microscopy (SEM) and thermogravimetric (TG) analysis were used to investigate microstructure and thermal stability of the obtained hydrogels. The studies of drug release from hydrogels were conducted *in vitro* with cephalexin (CEX). Modelling of antibiotic release from P(HEMA/BIS) hydrogels was used to determine drug diffusion characteristics in the early and late release phase.

2. Experimental

2.1. Materials

2-Hydroxyethyl methacrylate (HEMA) (Aldrich), and poly (alkylene glycol) (meth)acrylates, i.e. short chains-bisomers (BIS1-PEA6, BIS2-PPM5S, BIS3-PEM63P, BIS4-PPM63E) (Laporte Chemicals) (Scheme 1), freshly distilled, were used as components for hydrogel preparation. Bulk cephalexin powder (CEX) was supplied by Sigma (Scheme 1). Buffer solutions with different pH values were prepared using hydrochloric acid (La Chema), potassium chloride (Fluka), potassium mono and dihydrogenphosphate (Fluka) and sodium hydroxide (Fluka). Demineralized water was used for all polymerizations and the preparation of the buffer solutions.

2.2. Preparation of hydrogels

The P(HEMA/BIS) hydrogels were prepared by gamma-irradiated radical copolymerization. The reactants were dissolved in a water/ethanol mixture. The BIS mole fraction was 30. The feed compositions for the hydrogels are listed in Table 1. According to the type of BIS, the samples were designated P(HEMA/BIS1), P(HEMA/BIS2), P(HEMA/BIS3), and P(HEMA/BIS4). The same conditions were used to prepare the PHEMA hydrogel. The reaction mixture was degassed prior to polymerization and placed between two glass plates sealed with a PVC spacer (2 mm thick). The reaction solutions were irradiated in a ^{60}Co radiation source, under ambient conditions, at a dose rate of 0.5 kGy/h, to an absorbed dose of

Table 1
Feed compositions for the P(HEMA/BIS) hydrogels prepared by gamma irradiation.

Component	P(HEMA/BIS1)	P(HEMA/BIS2)	P(HEMA/BIS3)	P(HEMA/BIS4)
HEMA (mol%)	70	70	70	70
BIS (mol%)	30	30	30	30
HEMA + BIS (wt%)	10	10	10	10
Demineralized water (wt%)	45	45	45	45
Ethyl alcohol (wt%)	45	45	45	45

25 kGy. After the reaction, the gels were cut into discs and immersed in water for a week, to remove unreacted components. The water was changed daily. The discs were dried to xerogels. The amount of unreacted components HEMA and BIS were determined using a UV spectroscopy. In both cases, results indicate that the conversion during the crosslinking reaction was nearly complete.

2.3. Fourier-transform infrared spectroscopy (FTIR)

Xerogels were crushed into a powder and mixed with potassium bromide (Merck IR spectroscopy grade) in the proportion 1:100, 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 of 4000–700 cm^{-1}, with a resolution of 4 cm^{-1}, were recorded using a FTIR spectrometer (BOMEM Michelfan MB-102 FTIR).

2.4. Scanning electron microscopy (SEM)

A scanning electron microscope (JEOL JSM-6460 LV) was used to observe specimen morphologies of xerogels. The xerogels were prepared by two different procedures. The oven dried xerogels were prepared by drying in a vacuum-oven at 40 °C for 48 h. The lyophilized (freeze dried) samples were prepared by the following procedure. The hydrogels swollen to equilibrium were pre-frozen in deep-freezer at −80 °C for 24 h. Subsequently, they were freeze dried, using a Modulyo Freeze Dryer System Edwards, consisting of a freeze dryer unit and a High Vacuum Pump E2M8 Edwards. The vacuum during 20 h of freeze drying was around 4 mbar. The xerogels were gold sputter coated under vacuum before observation.

2.5. Thermal analysis (TGA)

Thermal properties of xerogels were evaluated by thermogravimetric analysis (TGA). These measurements were carried out on a PerkinElmer TGA-2 system, in the temperature range of 20–550 °C, at a heating rate of 10 °C/min, under a nitrogen atmosphere (flow rate of 26 cm^3/min).

2.6. Swelling studies

Dynamic swelling measurements were performed in a wide range of pH buffer solutions, important for biomedical studies (simulated physiological fluids), and in the temperature range from 25 to 70 °C. Swollen gels were removed from the swelling medium at regular intervals, dried superficially 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 equilibrium degree of swelling (q_e) was calculated as follows:

$$q_e = \frac{M_e - M_o}{M_o} \quad (1)$$

where M_e is the weight of the swollen hydrogel at equilibrium and M_o is the weight of the xerogel [37,38]. All the swelling experiments were performed in triplicate.

2.7. In vitro controlled release of antibiotic from polymer matrices

For the investigation of the drug release behaviour of P(HEMA/BIS) hydrogels prepared in this study, the cephalexin (CEX) drug was used. The drug powder (about 5 wt% of the xerogel weight) was dissolved in water; the obtained drug concentration in the initial loading solution was $c = 4 \times 10^{-3}$ mol/dm^3. All xerogel discs (approximately of the same weight) were immersed in this solution and swollen to equilibrium. The swollen drug-loaded samples were then dried at ambient temperature for several days to constant mass and used for the release experiments. Release studies of the antibiotic have been carried out *in vitro* by placing the dried and loaded sample in a definite volume of the release medium (a buffer solutions of pH 7.40 (simulated physiological fluid)) at 37 °C, repeated in triplicate. The amount of antibiotic released was measured spectrophotometrically, using a UV spectrophotometer (Shimadzu UV-Vis Spectrophotometer UV-1800), by taking the absorbance of the solution at regular time intervals, at a wavelength of 262 nm for CEX. The data were analysed using the commercial Origin Microcal 8.0 software.

Extensive research has been conducted to elucidate the mechanisms and models of drug release in hydrogels [39,40]. Modelling of a diffusion process in hydrogels can be complicated by non-constant diffusion coefficients due to large solute loading and solvent/polymer interactions, multi-dimensional diffusion resulting in complicated solutions, changes in free volume due to solvent transport (i.e. polymer swelling/deswelling) and multi-component transport instead of single solute diffusion. These complications partially explain why no universal model has been developed that accurately describes the solute-hydrogel diffusion mechanism. The models used to describe the diffusion process in this work were based on the solutions to Fick's law. This assumes diffusion in one dimension, with constant boundary conditions [41]. The solution to this equation is an infinite series

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

where t is the time, l is the total thickness of the hydrogel sheet, and D is the diffusion coefficient, M_t is the concentration of the drug in puffer media at $t = t$ and M_∞ is the concentration at long times, when release has reached equilibrium.

To enable reasonable modelling of the diffusion process, two well-accepted approximations of Eq. (2) were used: (a) the 'early-time' approximation (Eq. (3)) and (b) the 'late-time' approximation (Eq. (4)) [42,43]. The early-time approximation is valid for the first 60% of the release and the late-time is valid for the remaining 40%. These models were used to determine diffusion coefficients for the drug diffusion. An example of the model fits is shown and is the representative of the data reported in this work.

$$\frac{M_t}{M_\infty} = 4\left(\frac{D_e t}{\pi l^2}\right)^{0.5} \quad (3)$$

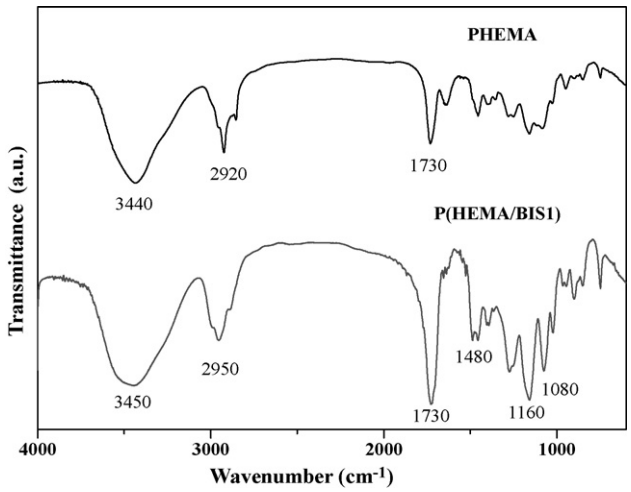


Fig. 1. FTIR spectra of PHEMA and P(HEMA/BIS1) xerogels.

$$\left(\frac{M_t}{M_\infty}\right) = 1 - \left(\frac{8}{\pi^2}\right) \exp\left[\frac{(-\pi^2 D_l t)}{l^2}\right] \quad (4)$$

A complementary modelling method for the early-time approximation is a power law approach (Eq. (5)) [42,43], where the relative release of solute (M_t/M_∞) is proportional (k) to time raised to a power (n).

$$\frac{M_t}{M_\infty} = k\ t^n \quad (5)$$

The proportionality constant can be used to calculate the diffusion coefficient, and n denotes the type of transport mechanism. When n is equal to 0.5, the transport rate is Fickian (as was assumed for the early-time approximation equation) and the drug release rate is time dependent. When n is between 0.5 and 1.0, the drug release rate is time dependent, but other factors such as polymer relaxation and swelling control solute transport. Case-II type diffusion occurs when n is equal to 1.0, which indicates that the release rate is time-independent. Super-case II transport occurs when n is greater than 1.0; in that case, the release rate is time dependent.

3. Results and discussion

3.1. Spectral analysis

FTIR spectroscopic analysis was used to illustrate the composition and nature of bond formation within hydrogels. FTIR spectra of all copolymeric P(HEMA/BIS) hydrogels are recorded and they show large similarity. There are no qualitative differences between observed spectra of P(HEMA/BIS) copolymeric hydrogels, but only hardly noticeable quantitative differences (i.e. in peaks magnitude) in alkylene glycol region (1600–1000 cm^{-1}). Since, it was important to confirm incorporation of BIS in PHEMA based hydrogel, only PHEMA and P(HEMA/BIS1) spectra are presented as representative. Both the PHEMA and P(HEMA/BIS1) xerogels had the characteristic stretching vibration band of hydrogen-bonded alcohol (O–H) around 3450 cm^{-1}, the C=O stretching vibration of the ester group also appeared at 1730 cm^{-1}, and an absorption band with a weak shoulder around 2950 cm^{-1}, which correspond to the stretching of aliphatic $-CH_2-$, C–H and $-CH_3$ groups, respectively (Fig. 1). On the other hand, several bands appeared in the fingerprint region for alkylene glycol units between 1600 and 1000 cm^{-1} on the P(HEMA/BIS1) structure. These peaks were assigned to the $-CH_2$ scissoring band of alkylene glycol units at 1480 cm^{-1} and the antisymmetric and symmetric stretching bands (–O–R) of alkylene glycol units at 1160 and 1080 cm^{-1}, respectively. Other characteristic bands represent C–C and C–H vibrations of $-CH_3$ and $-CH_2$ groups [44].

3.2. Morphology of P(HEMA/BIS)

Fig. 2 represents the scanning electron microscopy (SEM) micrographs of the homo-PHEMA and copolymeric P(HEMA/BIS1) xerogels under various magnifications. The left column of Fig. 2 shows micrographs of xerogels which were not lyophilized; surfaces are compact, smooth and dense. On the right hand side of Fig. 2, lyophilized samples are presented. Despite lyophilization, porous microstructure of homo-PHEMA xerogel is not observed. On the other hand, P(HEMA/BIS1) copolymeric xerogel has a pronounced porous structure which is also noticeable to a lesser extent in the microstructure of P(HEMA/BIS3). The microstructures of the other two P(HEMA/BIS) (e.g., P(HEMA/BIS2) and P(HEMA/BIS4)) copolymeric xerogels are more similar to the homo-PHEMA xerogel microstructure.

3.3. Thermal behaviour of P(HEMA/BIS)

The thermal stability of hydrogels was investigated by TGA. Fig. 3 depicts the thermograms of P(HEMA/BIS) xerogels; the obtained parameters concerning thermal stability are presented in Table 2. It is obvious that thermograms of different P(HEMA/BIS) show a similar trend as homo-PHEMA. Thermal properties of PHEMA homopolymer have been studied extensively and degradation mechanism was proposed [45]. The thermal degradation of poly(n-hydroxylalkyl methacrylate)s typically produces monomer as a result of depolymerization, and/or cyclic anhydride-type structures are formed by intramolecular cyclization. The side products arising from ester decomposition were a six-membered glutaric anhydride type of ring, an oxolane, 2-isopropenyl ethyl methacrylate, methacrylic acid and CO_2 [45]. The TG curves of homo-PHEMA and P(HEMA/BIS) copolymers indicate one reaction stage which is reflected as a single pick on the DTG curves. This behaviour shows that the thermal degradation reaction mechanism of P(HEMA/BIS) networks is almost the same as the PHEMA homopolymer. However, from Fig. 3 and Table 2 it is obvious that PHEMA shows higher thermal stability than copolymers; additionally, it is also evident that a variation of the BIS type has minor influence on the thermal stability of copolymeric networks. Lower thermal stability can be ascribed to homolysis of C–O bond which arises from the presence of polyethers (alkylne glycols) structure in copolymeric networks [46]. As a main conclusion concerning the thermal behaviour of P(HEMA/BIS) copolymers, it can be stated that the introduction of BIS components in PHEMA hydrogels has a negligible influence on the thermal stability of P(HEMA/BIS) copolymers in the temperature range of interest for biomedical applications.

3.4. Swelling studies and network parameters

Hydrogel networks obtained using HEMA and poly(alkylene glycol) (meth)acrylates components (BIS) are presented in Scheme 2. Short pendant poly(alkylene glycol) chains inside the network impart specific (environmental sensitive, i.e. smart) hydrogel behaviour in such a way to regulate diffusion of fluid into/from hydrogel network.

Swelling studies were performed to investigate the influence of the hydrogel composition and external conditions (change of pH and temperature) on the dynamic and equilibrium swelling properties of P(HEMA/BIS) hydrogels. The pH and temperature sensitivities of P(HEMA/BIS) hydrogels are presented in Fig. 4a and b as (q_e) versus pH and temperature dependences in a wide range of physiological pH values and in the temperature range from 25 to

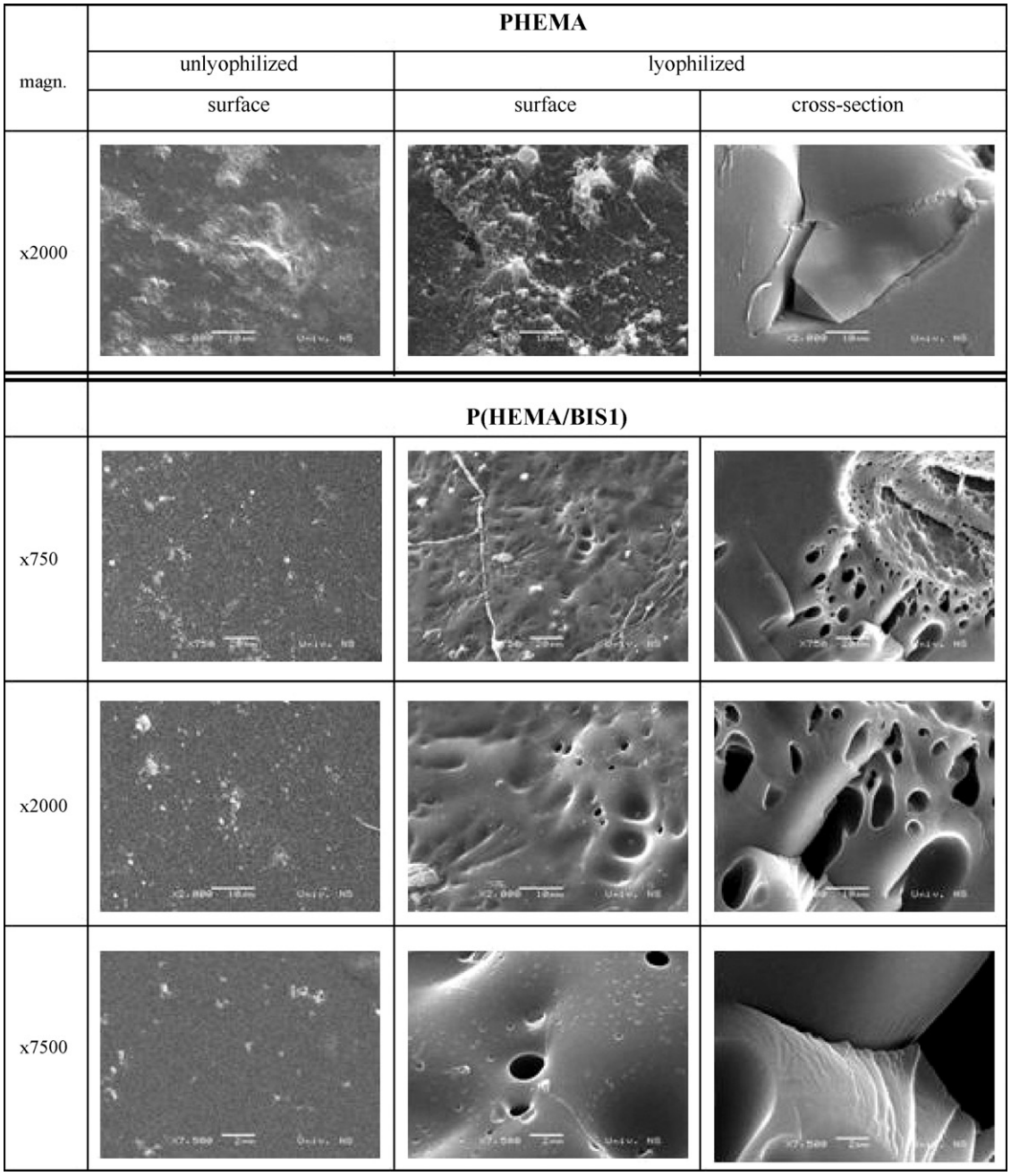


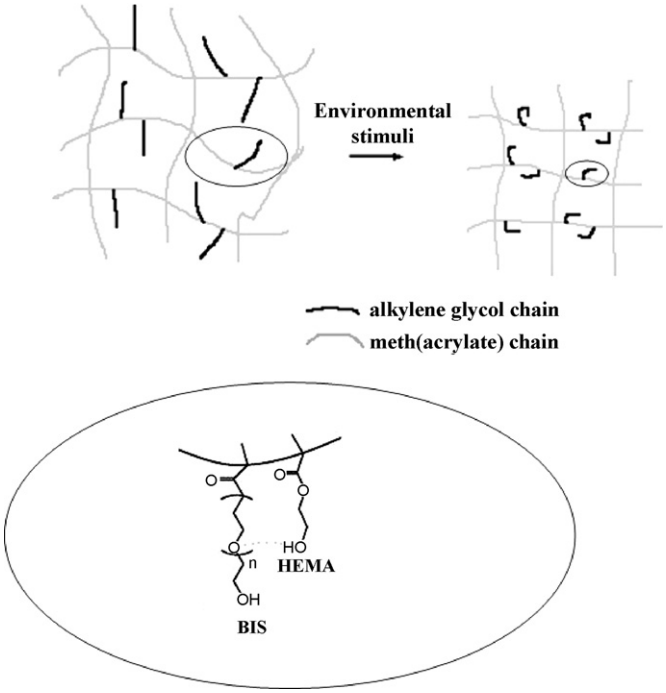
Fig. 2. SEM micrographs of the PHEMA and P(HEMA/BIS1) xerogels under various magnifications.

70 °C. In general, stimuli-responsive hydrogels with these swelling degrees are beneficial for drug delivery systems; they can absorb sufficient quantities of drug for different delivery applications and at the same time, due to their sustained release profile, can provide continuous drug release for a longer time (burst effect is lower than in the case of hydrogels with higher swelling degrees) [17–23].

pH-sensitive hydrogels play a significant role in controlled drug delivery systems. These kinds of delivery systems show different values of swelling degree in various physiological media. Thus,

Table 2
Temperature of 10% of mass loss and the main degradation, as well as corresponding mass loss of P(HEMA/BIS) and PHEMA xerogels in the main decomposition stage.

Sample	Temperature of 10% of mass loss (°C)	Peak temperature in DTG (°C)	Mass loss in the main decomposition stage (%)	Mass loss up to 550 °C (%)
PHEMA	297.0	428.0	79.23	98.39
P(HEMA/BIS3)	285.0	410.7	72.20	96.97
P(HEMA/BIS1)	265.0	405.8	71.55	96.61
P(HEMA/BIS2)	263.0	407.9	71.36	96.36
P(HEMA/BIS4)	254.0	405.0	70.18	96.39



Scheme 2. The influence of environmental stimuli on the swelling of P(HEMA/BIS) hydrogel network.

a pH-sensitive drug delivery system can protect the stability of drug and releases the drug in the targeted site, depending upon the composition of the hydrogel. In this study, the pH-sensitivity of the P(HEMA/BIS) hydrogels was investigated by varying the pH of the swelling medium in the range of 2.2–8.0. Equilibrium swelling results were depicted in Fig. 4a, which reveals that equilibrium degree of swelling (q_e) shows complex dependence on pH for P(HEMA/BIS1) and P(HEMA/BIS3) samples. The P(HEMA/BIS2), P(HEMA/BIS4) and homo-PHEMA samples are almost non-pH-sensitive. On the other hand, with increasing temperature some of the samples display the exponential fall in the degree of swelling (Fig. 4b). Such dependence on temperature is most pronounced for the P(HEMA/BIS1) sample with pure EG pendant chains, while for the P(HEMA/BIS3) sample with more EG units in the pendant chains it is less marked and practically very small for the P(HEMA/BIS2) and P(HEMA/BIS4) samples with more PG units in the pendant

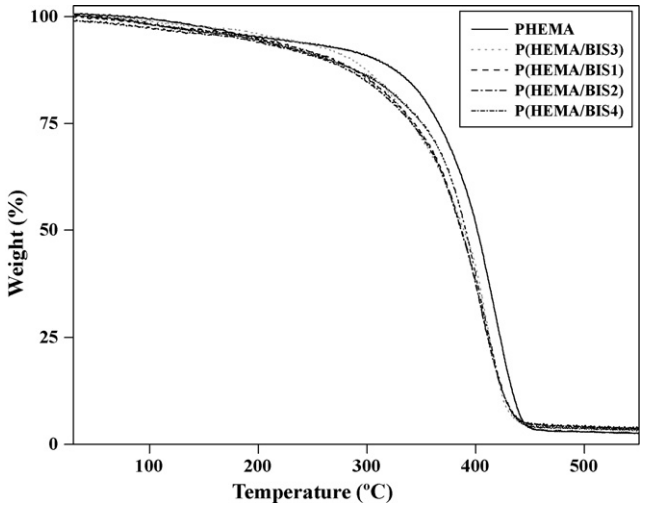


Fig. 3. TGA thermograms of PHEMA and copolymeric P(HEMA/BIS) xerogels.

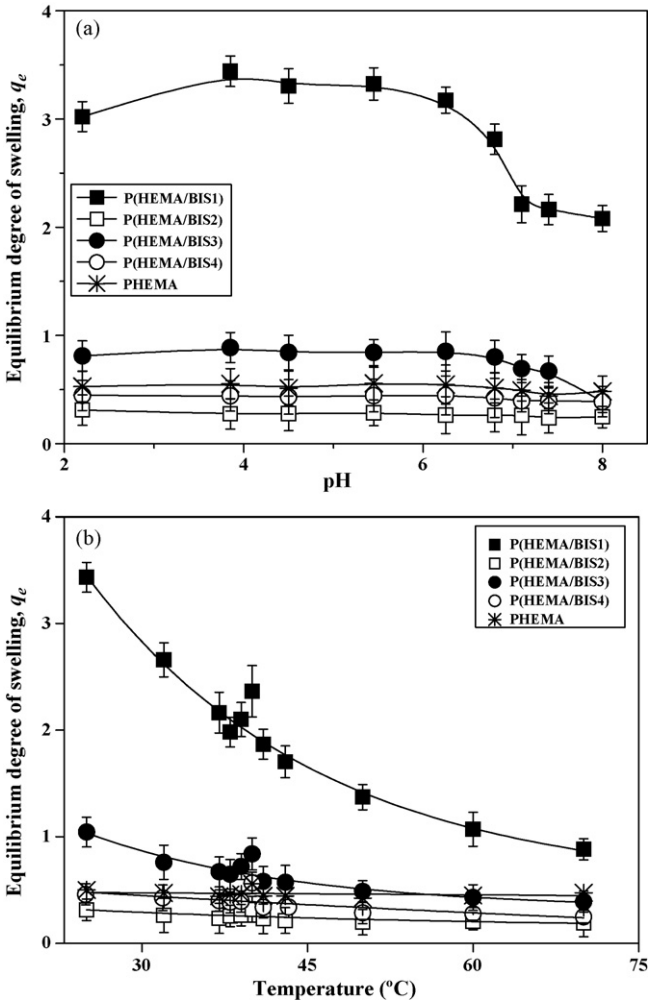


Fig. 4. pH (a) and temperature (b) sensitive swelling behaviour of PHEMA and P(HEMA/BIS) hydrogels.

chains, whereas this dependence for PHEMA is represented as a straight horizontal line. Therefore, dual (pH and temperature) sensitivity is observed for the new type of non-ionic P(HEMA/BIS) hydrogels and can be ascribed to the HEMA and BIS capability to participate in the formation of hydrogen bonds in various media. Hydrogen bonds, between ether oxygen from BIS and hydroxyl group of HEMA (Scheme 2), are responsible for dual sensitivity of non-ionic P(HEMA/BIS) hydrogels. At lower pH, the presence of acidic buffer component inhibits formation of hydrogen bonds and, therefore, the hydrogel swells more. At higher pH, there are favourable conditions for the hydrogen bonds formation and, as a consequence, the hydrogel swells less and the obtained swelling degree is lower. From Fig. 4a, for novel non-ionic P(HEMA/BIS) hydrogels it is evident that this transition is abrupt and occurs at pH of 6.2–7.1. Alternation from acidic to basic conditions leads to a decrease in the swelling degree, due to the transition of BIS pendant chains from extended to globular conformation.

The temperature sensitivity of hydrogels is associated with hydrogen bonding of hydroxyl groups from HEMA and ether oxygen from BIS with water hydroxyl groups and hydrophobic interactions [47,48], so that more hydrophilic samples exhibit a temperature-sensitive behaviour. At low temperatures, since the hydrogen bonds in hydrogel with water interactions considerably lower the free energy of mixing, hydrogels swell more in aqueous media. However, at higher temperatures, the hydrogen bonds weaken; at the same time, the system tends to minimize the contact

between the water and hydrophobic groups, so that the interactions between the hydrophobic groups increase and water is expelled from the hydrogels [49,50]. Dual sensitivity is specially pronounced for P(HEMA/BIS) hydrogels containing EG units; pH and temperature variation in equilibrium degree of swelling are largest for P(HEMA/BIS1) and also observable for P(HEMA/BIS3). The sensitivity of PG units to gamma radiation results in P(HEMA/BIS) copolymeric hydrogels with high crosslinking density. Owing to high crosslinking degree, the motions of pendant BIS chains are restricted to a greater extent in P(HEMA/BIS2) and P(HEMA/BIS4) networks. This gives explanation for barely perceptible sensitivity of P(HEMA/BIS) copolymeric hydrogels with PG units.

From all aforementioned results it follows that the swelling behaviour is significantly influenced by the composition of the BIS component in hydrogels. The hydrogel containing pure EG units (P(HEMA/BIS1)) shows the highest swelling due to the highest hydrophilicity. As for the copolymers with mixed (EG/PG) pendant chains (P(HEMA/BIS3) and P(HEMA/BIS4)), higher swelling degree was obtained for the sample containing more EG units (P(HEMA/BIS3)). The lowest value of swelling is obtained for the P(HEMA/BIS2) gel, which has only PG pendant chains. This phenomenon could be explained by crosslinking reactions during the gamma radiation process. The propylene glycol units (PG) are more susceptible to a crosslinking reaction than the ethylene glycol units in poly(alkylene glycol) pendant chains, leading to higher degrees of crosslinking, which, in turn, additionally reduces free space between polymeric chais for fluid uptake and then gives lower swelling degree. Therefore, the q_e values of P(HEMA/BIS4) and P(HEMA/BIS2) are lower than those for the samples containing more EG units, P(HEMA/BIS1) and P(HEMA/BIS3).

The most important parameters characterizing a hydrogel network structure are the molar mass of the polymer chain between two neighbouring crosslinking points (M_c), and the effective crosslinking density (ν_e). Peppas and Merill [51] described the molar mass of the polymer chain between two neighbouring crosslinks for neutral polymer networks:

$$\bar{M}_c = -\frac{V_1(\varphi_{2,s}^{1/3} - \varphi_{2,s}/2)}{\bar{\nu}\left[\ln(1-\varphi_{2,s}) + \varphi_{2,s} + \chi\varphi_{2,s}^2\right]} \quad (6)$$

where $\varphi_{2,s}$ is the volume fraction of polymer in the swollen hydrogel, $\varphi_{2,r}$ is the polymer volume fraction in the relaxed state (after crosslinking, but before swelling), V_1 is the molar volume of water, $\bar{\nu}$ is the specific volume of the polymer and χ is the Flory polymer–solvent interaction parameter [52]:

$$\chi = \frac{\ln(1-\varphi_{2,s}) + \varphi_{2,s}}{\varphi_{2,s}^2} \quad (7)$$

The effective crosslinking density (ν_e) was calculated as $\nu_e = \rho/\bar{M}_c$, where ρ is the polymer density. The relevant experimental parameters to be used with Eqs. (6) and (7) are as follows: molar volume of the water, $V_1 = 18\ \mathrm{cm^3\ mol^{-1}}$, the experimentally measured polymer volume fraction of the gels in their equilibrium-swollen state, $\phi_{2,s} = 0.316$, 0.599, 0.714, 0.810 and 0.694 for P(HEMA/BIS1), P(HEMA/BIS3), P(HEMA/BIS4), P(HEMA/BIS2), and PHEMA, respectively. The obtained χ values for P(HEMA/BIS) hydrogels are between 0.61 and 0.75. The χ parameter of the crosslinked pure PHEMA has a value of 0.73. Xerogels' densities were determined by picnometric method [21].

According to the swelling performances of P(HEMA/BIS) hydrogels and the potential application as drug delivery systems for transdermal and topical route, the calculations were done for the results obtained at pH 7.40 and 37 °C (Fig. 5). Network parameters for P(HEMA/BIS) hydrogels depend on the type of BIS, i.e. aklylene glycol pendant chains in the polymeric network (Fig. 5). The values of the effective crosslinking density (ν_e) are in the range of

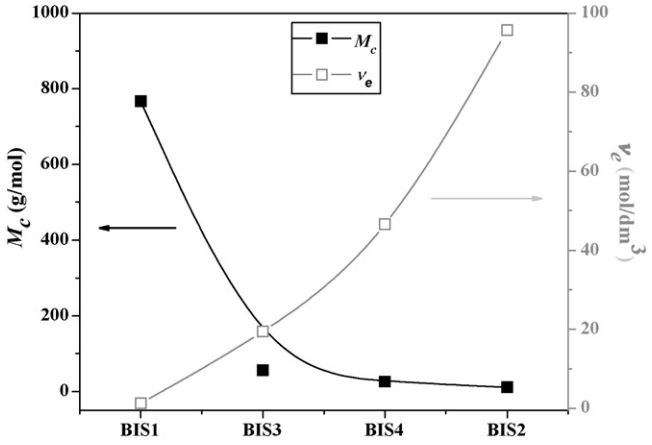


Fig. 5. The effect of BIS component on the molar mass of the polymer chain between two neighbouring crosslinking points (M_c) and the effective crosslinking density (ν_e) of P(HEMA/BIS) hydrogels.

1.27–95.7 mol/dm^3. The M_c and ν_e values follow the same trend as in the case of equilibrium swelling values: the sample with the highest degree of swelling (P(HEMA/BIS1)) has the highest M_c value and the lowest ν_e value, and they change in accordance with the hydrophilic character of the BIS component and the crosslinking degree of the sample.

3.5. Modelling of drug diffusion process

The aim of controlled release systems is to deliver the drug at a specified rate, keeping the drug concentration in the body at a therapeutically effective level, i.e. to provide a convenient drug release profile [1,50,53]. Among controlled release drug delivery systems, hydrogels are interesting owing to their unique tunable time-dependent swelling behaviour. In general, the drug release behaviour depends on the nature of the hydrogel and drug, their mutual interactions, as well as the release conditions. Since poly(alkylene glycol)(meth)acrylates components (BIS) significantly influence the swelling capacity of P(HEMA/BIS) hydrogels, cephalexin was used to evaluate these copolymers as potential delivery systems.

The release profiles from CEX-loaded PHEMA and P(HEMA/BIS) hydrogels into a physiological buffer solution (pH 7.40) at 37 °C are illustrated in Fig. 6. For all the hydrogel formulations, the sustained release behaviour was observed. The fastest release of CEX with a marked "burst" effect is from P(HEMA/BIS1) sample, where CEX releases rapidly at first and then gradually reaches an equilibrium value. This is the consequence of the lowest degree of crosslinking and the highest degree of swelling and porosity. Samples with mixed EG/PG and PG units in the pendant chain showed slower release rates and practically no burst effect (P(HEMA/BIS2), P(HEMA/BIS3) and P(HEMA/BIS4)). Since the amount of released CEX is lowest for P(HEMA/BIS2), the obtained relative ratio of the total amount of released drug among investigated hydrogels is: P(HEMA/BIS2)/PHEMA/P(HEMA/BIS4)/P(HEMA/BIS3)/P(HEMA/BIS1) = 1/1.10/1.14/1.95/2.80. No lag-time was observed during the release of antibiotic loaded hydrogel formulations. It is obvious that the type of BIS component incorporated in hydrogels has a significant impact on the release profiles of the hydrogel samples. Transport of drug and fluid inside hydrogels during drug release could be observed as a dynamic and complex process. Hydrophilic character of pendant chains of BIS has a dominant influence on drug release and therefore hydrogels containing EG units (P(HEMA/BIS1) and P(HEMA/BIS3) hydrogels) showed faster drug release than the gels containing

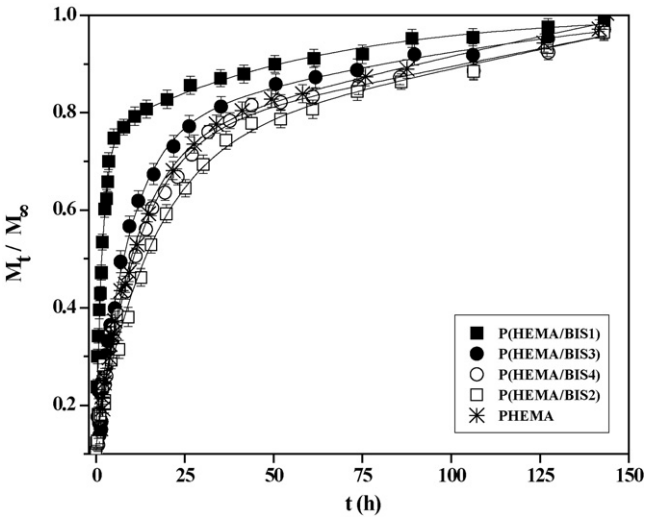


Fig. 6. Release profiles of cephalexin (CEX) from PHEMA and P(HEMA/BIS) hydrogels in *in vitro* conditions.

mixed EG/PG units (P(HEMA/BIS4) and P(HEMA/BIS2)). Drug release from homo-PHEMA is similar to that for P(HEMA/BIS4 and P(HEMA/BIS2).

The values of kinetic constant (k), diffusion exponent (n) and diffusion coefficients for early (D_e) and late (D_l) stages of the drug release process are presented in Table 3. The values of n vary from 0.290 to 0.376, suggesting that the release process can be described by a Fickian transport mechanism as a diffusion-controlled delivery system, with slab geometry. In Fickian diffusion the rate of diffusion of drug from the polymer matrix is slower compared with the rate of polymer chain relaxation. The P(HEMA/BIS1) sample shows the highest value of n. Further, n decreases for P(HEMA/BIS3), PHEMA, P(HEMA/BIS4), and P(HEMA/BIS2), respectively. The decrease of n values indicates that the mobility of CEX is slower.

The values of the diffusion coefficients for early and late stages of the drug release process, D_e and D_l, were also influenced by BIS residues in the copolymer. It is obvious that the swelling performances of P(HEMA/BIS) hydrogels have a dominant influence on the release process in such a way that higher swelling improves and accelerate the drug release. As swelling decreases, drug release slows down. The highest values of D_e and D_l are for P(HEMA/BIS1), as a consequence of the fastest release rate. The diffusion coefficients for the other three gel samples are lower and decrease from P(HEMA/BIS3), P(HEMA/BIS4), then PHEMA, and finally from P(HEMA/BIS2) (Table 3). For all samples, the early-time diffusion coefficients D_e are higher than the late diffusion coefficients D_l. It is obvious that the drug release is faster in the early than in the late phase, due to intensive motions of pendant chains through polymeric network, which has a significant influence on the release in the initial phase. In the late phase constant release is achieved and drug diffusion is slower.

Table 3
Drug release characteristics of P(HEMA/BIS) and PHEMA hydrogels.

Gel	D_e ($\times 10^7$ cm^2/s)	D_l ($\times 10^7$ cm^2/s)	k (h^{-n})	n
P(HEMA/BIS1)	24.43	8.264	0.410	0.376
P(HEMA/BIS3)	21.53	6.887	0.240	0.353
P(HEMA/BIS4)	13.94	6.657	0.195	0.305
PHEMA	12.94	6.647	0.194	0.303
P(HEMA/BIS2)	4.05	6.405	0.151	0.290

4. Conclusions

Novel copolymeric hydrogels based on 2-hydroxyethyl methacrylate (HEMA) and different poly(alkylene glycol)(meth)acrylates (BIS) were prepared by radiation-induced copolymerization; different BIS types were used, with variations of chain length, based on ethylene glycol (EG) and/or propylene glycol (PG) pendant units. Incorporation of BIS components into the polymeric network was confirmed by Fourier-transform infrared spectroscopy. Porous microstructure of lyophilized P(HEMA/BIS) samples was observed by scanning electron microscopy. Swelling studies indicate that P(HEMA/BIS) hydrogel properties are significantly dependent on the BIS type. The hydrogels with ethylene glycol (EG) pendant chains show a significant pH and/or temperature sensitivity and can be considered smart hydrogels. The release behaviour of the P(HEMA/BIS) hydrogels was affected by the hydrogel composition, too. The release rate and the amount of released cephalexin (CEX) drug were highest in the P(HEMA/BIS1) hydrogel with pure ethylene glycol (EG) pendant chains. Incorporation of propylene glycol (PG) units, pure and mixed with ethylene glycol (EG) pendant chains, attenuates the drug release characteristics compared with the P(HEMA/BIS1) hydrogel.

As a general conclusion, it may be stated that new class of P(HEMA/BIS) hydrogels, based on HEMA and different poly(alkylene glycol)(meth)acrylates (BIS), is a beneficial synergetic non-ionic combination for biomedical applications. P(HEMA/BIS) hydrogels based on ethylene glycol (EG) pendant units act as smart systems, showing pH and temperature sensitivity, despite their non-ionic nature. Such stimuli-responsive behaviour could be explained in terms of intermolecular and intramolecular hydrogen bonds between ether oxygen of the BIS component and hydroxyl group of HEMA. The ability of these novel copolymers to deliver a drug at a controlled rate, which can be adjusted by the choice of the BIS type, suggests that all types of P(HEMA/BIS) copolymeric hydrogels are suitable candidates for controlled drug release systems.

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