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Biocompatible and bioadhesive hydrogels based on 2-hydroxyethyl methacrylate, monofunctional poly(alkylene glycol)s and itaconic acid

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Summary

Novel poly(Bisomer/HEMA/IA) hydrogels were prepared by radical copolymerization of poly(alkylene glycol) (meth)acrylates, i.e. short chains Bisomers, 2-hydroxyethyl methacrylate (HEMA) and itaconic acid (IA) in a mixture of water/ethanol as solvent. These hydrogels were characterized in terms of swelling in conditions similar to the biological fluids (pH range 2.20-7.40 buffer solutions), compression-strain measurements, thermal properties and morphology. The influence of the type of Bisomer and of the itaconic acid on swelling and mechanical properties, as well as on morphology and thermal behavior of the resulting hydrogels, were investigated.
The *in vitro* study of biocompatibility, carried out with the hydrogels containing different types of Bisomers, showed no evidence of cell toxicity nor any considerable hemolytic activity. All hydrogels showed satisfactory bioadhesive properties, so these materials have potential as drug carriers or biological glue and sealants.

Keywords:

2-Hydroxyethyl methacrylate, itaconic acid, poly(alkylene glycol) (meth)acrylates, hydrogels, physicochemical properties, biological evaluation

Introduction

Hydrogels, cross-linked hydrophilic polymer networks which do not dissolve in water but are able to absorb large amounts of water, have attracted great attention in the past thirty years due to their interesting properties [1–5]. These materials can be classified into: conventional hydrogels and environmentally sensitive hydrogels [6]. Environmentally sensitive hydrogels exhibit marked volume changes with changing external conditions, such as: temperature [7–11], pH [12,13], solvent [14,15], ionic strength [16,17], etc.

The absorption of water by a hydrogel is one of the most important factors which determines its properties and applications. The behavior of swollen hydrogels is, therefore, a function of the network characteristics, such as equilibrium degree of swelling, diffusion parameters, cross-link density, etc., which are intimately related to chemical structure of the hydrogel.

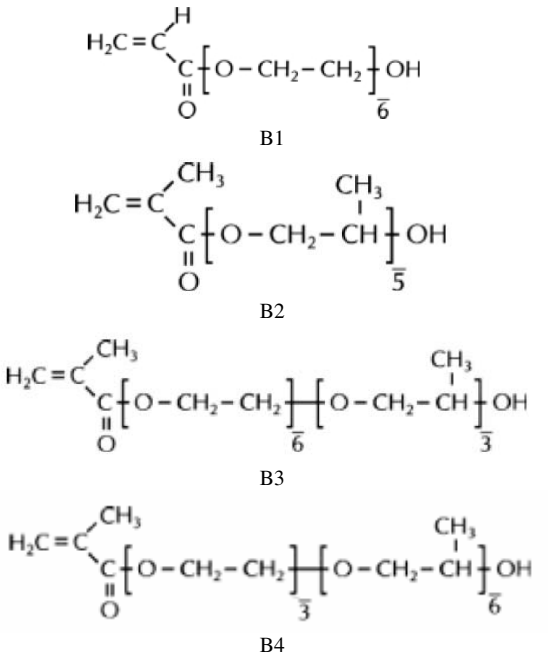
Poly(2-hydroxyethyl methacrylate) (PHEMA) and related hydrogels have been considered for a variety of medical applications [18-21]. Their structure permits a water content similar to that in living tissues. A poly(2-hydroxyethyl methacrylate) hydrogel is inert to normal biological processes, shows resistance to degradation, is permeable to metabolites, is not adsorbed by the body, withstands heat sterilization without damage, and can be prepared in a variety of shapes and forms.

Itaconic acid has two ionisable groups, with different pK_a values, which can form hydrogen bonds. The potential for substitution of acrylic and methacrylic acid in polymers with itaconic acid is high. Itaconic acid is obtained from renewable resources, such as carbohydrate materials as molasses and hydrolyzed starch, by fermentation [22, 23]. Itaconic acid is very hydrophilic and is expected to show high biocompatibility because of its natural source. Sariri et al. [24] found that HEMA/IA copolymers adsorb less protein albumin with increasing IA content, due to the negatively charged surfaces, which was related to its increased biocompatibility. Itaconic acid easily copolymerizes and provides polymer chains with carboxylic side groups, which are highly hydrophilic and are able to form hydrogen bonds with corresponding groups. Small amounts of itaconic acid comonomer in the gel network generally introduce pH sensitivity and increase the degree of swelling [25,26,27]. In addition, incorporation of comonomers which can contribute to H-bonding can increase the mechanical strength of the hydrogel [3].

In this study, hydrogels based on 2-hydroxyethyl methacrylate, itaconic acid and four types of Bisomers, poly(ethylene glycol) acrylate and poly(alkylene glycol) methacrylates, were prepared using ethylene glycol dimethacrylate as the crosslinker. The Bisomer components and itaconic acid were used, respectively, to improve the biocompatibility and to impart pH sensitivity to poly(2-hydroxyethyl methacrylate). The influence of the type of Bisomer on the swelling and mechanical properties, as well as on the morphology and thermal properties was investigated.

Experimental

Preparation of hydrogels. 2-Hydroxyethyl methacrylate (HEMA) (Aldrich, Germany), itaconic acid (IA) (Fluka, Germany), and poly(alkylene glycol) (meth)acrylates, i.e. short chains-Bisomers (B1-PEA6, B2-PPM5S, B3-PEM63P, B4-PPM63E) (Laporte Chemicals, United Kingdom) (Figure 1) were used as components for hydrogel preparation. Ethylene glycol dimethacrylate (EGDMA) (Aldrich, Germany) as crosslinking agent, potassium persulfate (Fluka, Germany) as initiator and tetramethylethylene diamine (TEMED) (Aldrich, Germany) as activator were used in all polymerizations. A mixture of water/ethanol was used as the solvent. Aqueous media with different pH values were prepared using hydrochloric acid (La Chema, Czech Republic), potassium chloride (Alkaloid, Macedonia), potassium dihydrogenphosphate (La Chema, Czech Republic) and sodium hydroxide (Zorka Pharma, Serbia). Distilled water was used for all copolymerizations and the preparation of the buffer solutions.



B1

B2

B3

B4

Figure 1. Chemical structures of Bisomers (B1, B2, B3 and B4).

The copolymer hydrogels of B(1, 2, 3 or 4), HEMA and IA were prepared by radical crosslinking polymerization. The monomers were disolved in 10 ml of water/ethanol mixture (1:1, by volume). The HEMA/B(1, 2, 3 or 4)/IA mole ratio was 70/28/2. 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 number of moles of monomers. The polymerization was carried out at 60 ^{o}C for 24 h. The same procedure was used to prepare the PHEMA hydrogel, with the same monomer to solvent ratio, and the amounts of initiator, activator and crosslinker. The reaction mixture were degassed prior to polymerization and placed between two glass plates sealed with a rubber spacer (2 mm thick). After completion of 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).

Swelling studies. The xerogels discs were immersed in an excess of buffered solutions of different pH values, to obtain equilibrium swelling at 25 ^{o}C. The progress of the swelling process was monitored gravimetrically. The degree of swelling (q) was calculated from the following equation (1):

$$q = \frac{W_t - W_o}{W_o} \qquad (\mathbf{1})$$

where W_o and W_t are the weights of the xerogel at time 0 and of the swollen hydrogel at time t, respectively. The equilibrium degree of swelling (q_e) was calculated as follows (equation (2)):

$$q_e = \frac{W_e - W_o}{W_o} \quad (2)$$

where W_e is the weight of hydrogel at the equilibrium.
Dynamic swelling experiments were also investigated in buffers of the pH range from 2.20 to 7.40, at 25 ^oC, which corresponds to pH values of biological liquids. The swelling measurements were repeated three times.
Dynamic mechanical analysis. Strain-frequency sweeps were performed on hydrogel discs, as synthesized, using a Rheometrics 605 mechanical spectrometer, with parallel plates geometry (25 mm in diameter). The shear moduli were measured as a function of frequency (ω), from 0.1 to 100 rad/s, at 25 ^oC.
Gel morphology. Microstructure characterization was conducted using a JSM 5300 scanning electron microscope. The samples were coated, under vacuum, with thin layer of gold before observation.
Thermal analysis. The thermal properties of all samples were evaluated by TGA. These measurements were carried out on a Perkin Elmer TGA-2 system, in the temperature range of 20–550 ^oC, at a heating rate of 10 ^oC/min, under a dynamic nitrogen atmosphere (flow rate of 26 cm^3/min).
Hemolytic activity. The hemolytic activity of the hydrogels was determined by the direct and indirect contact methods, according to ISO 10 993-4 (1992) [28]. In the direct method, the hydrogel discs were immersed in a physiological solution (PS) (5 ml) to which 0.25 ml of whole rat blood had been added. The PS was used as the negative and distilled water as the positive control. Then the contents of the tubes were gently mixed and incubated in a water bath at 37 ^oC for 1 h. Subsequently, the absorbance of the supernatant liquid in each tube was determined at 545 nm using a Ultrospec III Spectrophotometer (Pharmacia LKB) and the percentage hemolysis was calculated. A mean hemolysis value from two test samples of 5 % or less was considered acceptable. In the indirect contact method were used 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 ^oC 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 bidestilled water. After incubation at 37 ^oC for 24 h, the absorbance of the supernatant was measured at 540 nm and the percentage hemolysis calculated.
Cytotoxicity tests. The cytotoxicity of the hydrogels was tested by the *in vitro* cell viability method according to Ciapetti et al. [29]. The test was carried out by contacting dilutions of the extracts of the hydrogel discs with a mouse subconjuntive tissue cell culture, from NCTC Clone 929 line (ATCC-CCL1). A phenol solution (0.02 mas%) and high density polyethylene (HDPE) extracts were used as the positive and negative control, respectively. The percentage viability was calculated in relation to cell control and graphically plotted to obtain the cytotoxicity index, $IC_{50\%}$, the concentration of extract which cause damage or death of 50 % of the cell population.
Bioadhesion of hydrogels. An adhesionmaster 525 MC (Erichsen GmbH&Co KG, Germany) was used to measure the adhesive force of the hydrogel samples to a polypropylene plate. A polypropylene plate was used instead of intestinal mucosa since there is a relatively good correlation between the adhesive force of the PAA/PEG polymer complexes to pig intestinal mucosa and that of the complexes to a polypropylene plate [30]. The specimens, discs with an area of 3.14 cm^2, were wetted

with a phosphate buffer solution of pH 7.40 at room temperature for 60 s before testing, and then placed between two plastic plates. The plates were then subjected to a pressure of 20.0 N/cm^2 for 60 s before the measurements. The peak force required to detach the disc from the polypropylene plates was measured.

Results and discussion

Swelling studies. Preliminary studies in buffered solution of pH similar to the pH of biological fluids are very important for the application of hydrogels as biomaterials. Swelling curves of PHEMA and copolymeric hydrogels in water are presented in Figure 2.

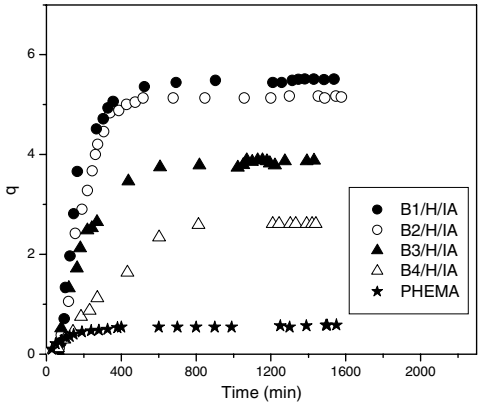


Figure 2. Swelling curves of hydrogels in water at 25 ^{o}C.

Kinetic parameters were calculated from the first part of the swelling curves, using equation (3) [31]:

$$\frac{M_t}{M_e} = kt^n \qquad (\mathbf{3})$$

where M_t and M_e, calculated from equations (1) and (2), are the quantity of fluid absorbed at time t and at equilibrium, respectively, k is a kinetic constant and n is the diffusional exponent. The logarithmic form of equation (3) was used to calculate n and k, from the slope and intercept, respectivelly. This equation is applicable to the initial stages of swelling, where a linear fit of the data was observed.

The values of q_e (in water and buffer solutions at 25 ^{o}C), n and k for all samples are listed in Table 1. It is evident that q_e values for copolymers increase with increasing pH showing a typical pH dependent swelling behavior. The hydrogel containing pure EG acrylate (B1/H/IA), with the acrylate residue in the main chain and EG dangling chains, shows the highest swelling in the whole pH range due to the highest hydrophilicity. As for the copolymers with methacrylic Bisomers, better swelling was obtained for the sample containing pure PG and shorter dangling chains (B2/H/IA)

than for those with mixed EG/PG units in the longer dangling chains (B3/H/IA and B4/H/IA). The highest q_e values for copolymers are in pH 7.40 buffer solution, and are similar to the q_e values in water. Since q_e values for PHEMA are lower than those for copolymers, it can be concluded that introducing Bisomer and itaconic acid components into the HEMA hydrogel leads to higher swelling degrees.

Table 1. q_e, k and n for all hydrogel samples, in water and in a pH 2.20, 4.50 and 7.40 buffer solutions at 25 °C.

	pH	q_e	k	n
B1/H/IA	2.20	4.24	0.31	0.50
	4.50	4.67	0.42	0.54
	7.40	5.66	0.47	0.72
	water	5.50	0.47	0.72
B2/H/IA	2.20	3.67	0.30	0.49
	4.50	4.22	0.39	0.53
	7.40	5.15	0.46	0.68
	water	5.11	0.46	0.70
B3/H/IA	2.20	2.69	0.28	0.48
	4.50	3.30	0.37	0.51
	7.40	3.86	0.42	0.65
	water	3.80	0.42	0.65
B4/H/IA	2.20	1.26	0.27	0.48
	4.50	1.92	0.36	0.52
	7.40	2.63	0.40	0.64
	water	2.60	0.40	0.64
PHEMA	2.20	0.56	0.23	0.47
	4.50	0.57	0.24	0.50
	7.40	0.58	0.25	0.51
	water	0.58	0.25	0.52

It can be seen that PHEMA is practically not pH sensitive, while q_e values of copolymeric hydrogels increase with increasing pH. The maximum extent of swelling was reached in a pH 7.40 for all copolymer samples, due to the very high ionization degree of the IA carboxylic groups. The pK_a values of IA are: pK_{a1} = 3.85, pK_{a2} = 5.44 [32].

Information about the physical mechanism controlling fluid adsorption can be gained from the value of the diffusional exponent. In the case of cylindrical geometry the transport mechanism can be Fickian ($n \leq 0.5$), non-Fickian or anomalous ($0.5<n<1$), Case II transport (n=1) and Super case II transport (n>1). The fluid transport mechanism is Fickian for PHEMA in all employed buffers and for the copolymers between pH 2.20 and 4.50. The mechanism is non-Fickian (anomalous) for all the copolymers in water and at pH 7.40 (Table 1), meaning that both diffusion and polymer relaxation control the fluid transport [33]. The electrostatic repulsions of the equally charged chains, due to the high degree of ionization IA acid groups, forces the

hydrogel to expand, which results in relatively large values of the degree of swelling being attained.

Network parameters. The suitability of a hydrogel as a drug delivery device and other biomedical applications depend to a large extent on its bulk structure. The network structure depend on many factors, such as copolymer composition, amount of crosslinking agent, solvent used, temperature, gel preparation technique, and other factors. The important parameters used to characterize the network structure of hydrogels are the effective crosslinking density (υ_e) and molecular mass of the polymer chain between two neighboring crosslinking points (M_c).
The shear moduli, G, obtained from the dynamic mechanical analysis for the network as formed were used to calculate the network parameters. According to equation (4), it follows [34, 35]:

$$G = \nu_e RT\phi_2^{1/3}(V_u/V_f)^{2/3} \quad (\mathbf{4})$$

where ν_e is the effective cross-link density, ϕ_2 is the volume fraction of polymer, V_u is the volume of dried, unstrained gel, V_f is the volume of the as formed network, R is the gas constant and T the absolute temperature.
The molecular mass between cross-links, M_c, was calculated using equation (5):

$$M_c = \frac{\rho_2}{\nu_e} \quad (\mathbf{5})$$

where ρ_2 is the density of the xerogel. The densities of the xerogels were determined by the picnometric method. The network parameters of hydrogels and the q_e values in water, in order to relate mechanical properties with the swelling results, are presented in Table 2.

Table 2. Network parameters of hydrogels

Sample	G (kPa)	υ_e x 10^3 (mol dm^{-3})	M_c (kg mol^{-1})	q_e (water)
HEMA	3.94	5.31	166.1	1.67
B1/H/IA	2.40	4.60	241.8	6.50
B2/H/IA	2.15	3.51	287.5	6.19
B3/H/IA	1.94	3.05	327.0	4.92
B4/H/IA	1.90	2.92	331.6	3.79

As can be seen from Table 2, PHEMA had the highest G value and the lowest equilibrium degree of swelling, as was to be expected. The G values for the copolymers were lower and varied little with the type of Bisomer. However, it can be noted that copolymers with slightly higher G values swell more. This anomalous behavior can be explained by the fact that the type of Bisomer, which differ in the residue incorporated in the main chain, side chain length and hydrophilicity, has a more pronounced influence on the capability of the copolymer to swell than on its mechanical properties. The side chain length has a predominant influence on the swelling capability, i.e. Bisomers that introduce shorter dangling chains increase the q_e value of the copolymers more, hence the samples B1/H/IA and B2/H/IA swell more than B3/H/IA and B4/H/IA. The hydrophilicity of the Bisomer residue in the main

chain and of the dangling chains is the second factor that influences the swelling of the copolymers. The sample B1/H/IA with acrylate residues in the main chain and short EG dangling chains has a higher value of q_e than B2/H/IA with methacrylate residues in the main chain and short PG dangling chains. The copolymers with longer side chains swelled less than those with short side chains. Samples B3/H/IA and B4/H/IA have longer side chains, but B3/H/IA swells more than B4/H/IA because it has a better ratio of EG to PG units in the side chains (6/3) than B4/H/IA, in which the EG to PG ratio is reversed (3/6).

The lowest value of M_c was obtained for PHEMA and for the copolymer with pure EG acrylate component (B1/H/IA); the gels containing methacrylic Bisomers and longer side chains have higher M_c values (Table 1).

The crosslinking density has a marked influence on the mechanical and swelling properties of hydrogels, varying in our case in the range of 2.92-4.60 x 10^{-3} (for copolymer hydrogels), i.e. the crosslinking efficiencies are low because of the large number of elastically ineffective dangling chains originating from the Bisomers (Table 2). The values of the shear moduli and network parameters are of the same order of magnitude as the values obtained for other similar systems [36].

Gel morphology. Analysis of the SEM micrographs of the air-dried samples reveals that the surfaces of the samples B1/H/IA and B2/H/IA have a buckled structure with microchannels. It can be presumed that the morphology with microchannels enables the molecules of solute to penetrate more easily into the hydrogel interior (Figure 3 (a) and (b)), which is in accordance with the higher qe values of B1/H/IA and B2/H/IA. The compact, smooth surfaces of B3/H/IA and B4/H/IA samples (Figure 3 (c) and (d)) reveal a flat pattern structure with vague spherical or elliptical and cylindrical shapes.

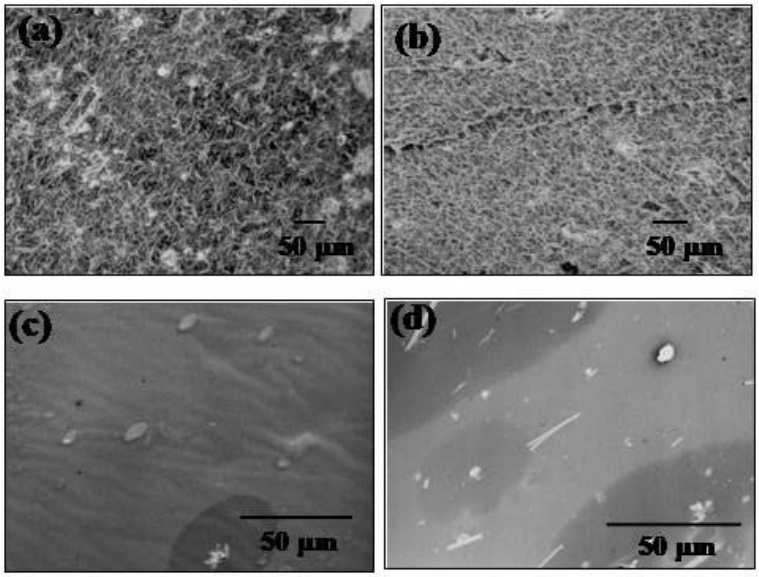


Figure 3. SEM micrographs of hydrogels: (a) B1/H/IA, (b) B2/H/IA (c) B3/H/IA and (d) B4/H/IA.

TGA analysis. The thermal stability of hydrogels was investigated by TGA. Figure 4. shows the weight loss curves of copolymeric hydrogels as well as of poly(2-hydroxyethyl methacrylate) and poly(itaconic acid), for comparison. The copolymeric hydrogel curves show similarity with the PHEMA curve in the temperature range investigated (20-550 ^{o}C). In the temperature range of 300-550 ^{o}C, PHEMA is thermally a little more stable than the copolymers. All the samples show much better thermal stability than the poly(itaconic acid) (Figure 4). The temperatures of the main decomposition are 410, 403, 398 and 388 ^{o}C for B1/H/IA, B2/H/IA, B3/H/IA and B4/H/IA hydrogel samples, respectively. In general, it can be concluded that the presence of itaconic acid and of poly(alkylene glycol)(meth)acrylates slightly reduces the thermal stability of HEMA copolymers in respect to that for pure PHEMA.

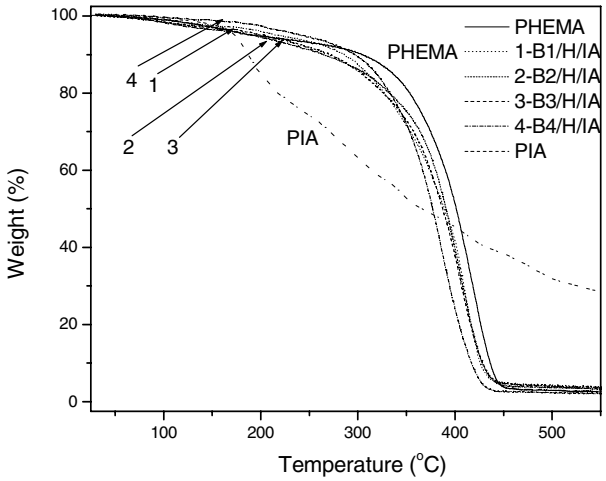


Figure 4. TGA thermograms of PHEMA and copolymeric hydrogels.

Since the proposed hydrogel system is intended to be used for the release of drugs, which normally takes place at the physiological temperature of 37 ^{o}C, the hydrogels are thermally stable in the vicinity of that temperature and up to 150 ^{o}C, which is usual temperature of sterilization.

Hemolytic activity. Hemolysis testing of biomedical materials has been used to measure their hemolytic activity. Under the *in vitro* testing conditions of the B/H/IA hydrogels in contact with blood, they showed a mean hemolysis value less than 1.2% in the direct contact assay, and even less than 0.5% in the indirect contact assay (Table 3). According to the obtained results, these hydrogels are not considered as hemolytic.

Table 3. Hemolytic activity of the hydrogels

Sample	Hemolysis (%)	
	Direct contact	Indirect contact
B3/H/IA	0.2	0.1
B4/H/IA	0.5	0.2
B1/H/IA	0.9	0.3
B2/H/IA	1.2	0.5

Cytotoxicity assay. In the *in vitro* study of the biocompatibility through the cytotoxicity assay, the relative percentage of the number of visible colonies at different concentration of extracts, as a product of the interaction of the cells and the hydrogel, was calculated and is presented in Figure 5.
The concentration of extract which causes the destruction of 50 % of the cell population, $IC_{50(\%)}$ is known as the cytotoxic index. As the cell viability for all the samples was greater then 50 %, none of the hydrogels could be considered cytotoxic. The copolymer samples show better cell viability than the PHEMA sample, indicating that the incorporation of the monofunctionalized poly(alkylene glycols) improves the biocompatibility of the hydrogels, proportionally to the length of the poly(alkylene glycol) chains. Therefore, the best results were obtained in the case of B3/H/IA and B4/H/IA samples with longer dangling chains.

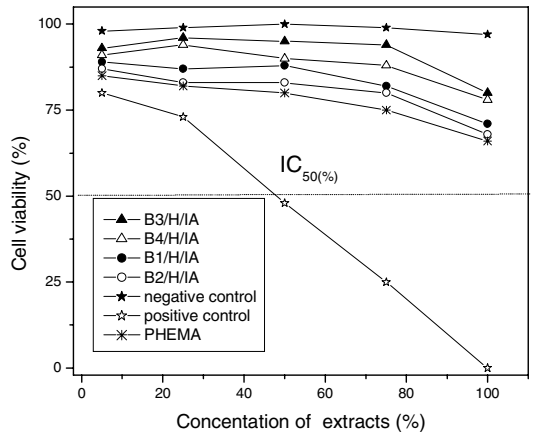


Figure 5. Cell viability curves of the hydrogel samples in the cytotoxicity assay.

Bioadhesion of hydrogels. The adhesive force values for the hydrogel samples and for the commercial Carbopol 971 P adhesive are presented in Table 4. The adhesive force was measured by determining the force required to break the contact between a polypropylene plate and the applied polymer. For the hydrogels containing Bisomers with longer poly(alkylene glycol) chains (B3/H/IA and B4/H/IA), the adhesive force

Table 4. Bioadhesive forces of the hydrogel samples and Carbopol 971 P to a poly(propylene) plate

Sample	Adhesive force (N cm^{-2}) (mean ± S.D.[a])
B3/H/IA	39.4±0.2
B4/H/IA	37.6±0.2
B1/H/IA	36.6±0.1
B2/H/IA	34.3±0.1
Carbopol 971 P	28.4±0.1

[a] Standard deviation.

was slightly greater than in the case of hydrogels with shorter poly(alkylene glycol) chains (B1/H/IA and B2/H/IA). It is supposed that hydrogen bonds are formed between the carboxyl group of IA and the ether group of the poly(alkylene glycol)s which result in better bioadhesion properties of the hydrogels. Our earlier results confirm this interpretation. It was demonstrated that poly(ethylene glycol)/poly(itaconic acid) complexes show good bioadhesion [37]. Generaly, these hydrogels show higher bioadhesivity than Carbopol 971 P.

Conclusion

In this work a new type of hydrogels were synthesized from 2-hydroxyethyl methacrylate, itaconic acid and four different poly(alkylene glycol) (meth)acrylate components (Bisomers). These polymers swell in water at 25 ^{o}C to yield homogeneous transparent hydrogels. All hydrogels show a pH sensitive behavior in buffer solutions of the pH range from 2.20 to 7.40. The presence of these two comonomers, which are added to HEMA, increases the swelling degree of hydrogels and gives gels with better elasticity. The hydrogels are thermally stable in the vicinity of the physiological temperature (37 ^{o}C). The copolymer containing pure poly(ethylene glycol) acrylate units generally has the best properties.
The tests performed with the hydrogels confirmed that they are neither hemolytic nor cytotoxic. The copolymer samples show better cell viability and less hemolytic activity than the PHEMA sample, confirming the assumption that poly(alkylene glycols) improve the biocompatibility of hydrogels.
Due to their swelling and mechanical characteristics, as well as to the very good biocompatibility and bioadhesive properties, poly(Bisomer/HEMA/IA) hydrogels show promise for utilization in the field of biomedicals, especially for the controlled release of drugs.

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