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Salt and pH Sensitive Semi-Interpenetrating Polyelectrolyte Hydrogels Poly(HEMA-*co*-METAC)/PEG and Its BSA Adsorption Behavior

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ABSTRACT: A novel semi-interpenetrating poly(2-hydroxyethyl methacrylate) (pHEMA) based polyelectrolyte hydrogel [p(HEMA-*co*-METAC)/PEG] was prepared by copolymerizing HEMA with the cationic monomer 2-methacryloyloxyethyltrimethyl ammonium chloride (METAC) in the presence of polyethylene glycol (PEG) with different content and molecular weight (MW 4000 and 400). The chemical structure of the gels was confirmed by FT-IR spectroscopy, morphology study was performed by scanning electron microscope (SEM), thermal stability was revealed by thermogravimetric analysis (TGA), and the mechanical properties were determined by electronic universal testing machine. Swelling studies showed introduction of cationic monomer METAC led to high water content, and the obvious salt and pH sensitive properties were observed which proved the smart behavior of the semi-interpenetrating polymer networks (IPNs) gels. In addition, the effect of temperature and some important biological solution on swelling behavior were reported. Cytotoxicity test demonstrated that synthesized gels owned satisfactory cytocompatibility and were convenient for the application as biomaterials. Finally, the weak bovine serum albumin (BSA) adsorption on semi-IPNs by introducing METAC and controlling the content of PEG in gels demonstrated that they were of good protein resistance effect in biomedical applications. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2015**, *132*, 41537.

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INTRODUCTION

Hydrogels are cross-linked polymer with three-dimensional network structures and hydrophilic property, which can absorb large amount of water or biological fluids without dissolving.[1] The excellent biocompatibility has made them be applied in the medical and pharmaceutical fields.[2–6] One of the most widely studied hydrogels in biomedical is the hydrophilic neutral polymer—poly(2-hydroxyethyl methacrylate) (pHEMA), with highly biocompatibility, viscoelastic behavior, permeability, thermal and dielectric properties, which has been used in biomedical field, such as contact lenses, drug-delivery system, wound dressings, and artificial scaffolds etc.[7–10] However, the application of pHEMA hydrogels is always restricted owing to the limited water intake and poor biological stimulus response.[11,12] Therefore, in order to improve the poor performance of pHMEA materials, different kinds of pHEMA based gels have been synthesized by copolymerizing HEMA with other monomers. For instance, polyelectrolyte hydrogels by introducing ionic groups into neutral hydrogels could regulate their water sorption capacity well and improve the biological stimulus response.[13–16] In addition, studies have shown that the performance of cell adhesion and spreading on gels with cationic groups tended to much better than gels with anion groups.[14,17] The pHMEA based hydrogels by copolymerizing HEMA with cationic monomer 2-methacryloyloxyethyltrimethyl ammonium chloride (METAC) have showed excellent biocompatibility, good cell adhesion and spreading, and highly swelling extent. Goel et al.[13] developed p(HEMA-*co*-METAC) hydrogels by high-energy ^{60}Co gamma radiation, and found that except for the

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Table I. Feed Compositions for the p(HEMA-co-METAC)/PEG Semi-IPNs with PEG Molecular Weight 4000 and 400

Sample name	HEMA (based value)	METAC (n/n)	PEG4000 (w/w)	PEG400 (w/w)
p	1	0	0	0
pc	1	0.1	0	0
ppL	1	0	0.1	0
pcpL5%	1	0.1	0.05	0
pcpL10%	1	0.1	0.1	0
pcpL15%	1	0.1	0.15	0
ppS	1	0	0	0.6
pcpS5%	1	0.1	0	0.05
pcpS10%	1	0.1	0	0.1
pcpS15%	1	0.1	0	0.15
pcpS40%	1	0.1	0	0.4
pcpS60%	1	0.1	0	0.6

obviously increasing swelling ratio (SR) with the METAC content, the gels also showed an excellent responsive to the ionic medium, which had an important effect on gaining the optimum SR of gels for various applications.

In addition, pHEMA based interpenetrating polymer networks (IPNs) hydrogels[18] could also optimize the physicochemical properties of gels. Polyethylene glycol (PEG), as a hydrophilic polymer material, has been employed in synthesizing the IPNs[19,20] gels for its good biocompatibility, water solubility, non-toxicity, and especially non-immunogenicity.[21,22] Bajpai et al.[23] prepared a binary IPNs gels comprising of PEG and pHEMA, and studied the bovine serum albumin (BSA) adsorption on the IPNs. The results showed that the IPNs gels with higher PEG and lower HEMA displayed the least weight of blood-clot formed on its surface, which meant a good degree of antithrombogenicity. Jung et al.[24] studied the properties of pHEMA based hydrogels modified by a sulfonated PEG (SPEG) graft, and found that the SPEG-modified gels had a lower level adsorption of fibrinogen than pHEMA gel.

Here we prepared a novel semi-interpenetrating pHEMA based polyelectrolyte hydrogel—p(HEMA-*co*-METAC)/PEG by copolymerizing HEMA with cationic monomer METAC in the presence of PEG with different content and molecular weight and then investigated its physicochemical properties and BSA adsorption behavior. The purpose of our study was to obtain a novel environmental sensitive semi-IPNs hydrogel known as "smart" or "intelligent" polymer,[25] and to achieve optimum level of swelling by employing METAC as main regulator and PEG as auxiliary regulator in different environmental stimuli. In addition, the weak BSA adsorption on the semi-IPNs gels by introducing METAC and controlling the content of PEG is another aim.

EXPERIMENTAL

Materials

2-Hydroxyethyl methacrylate (HEMA) and METAC were purchased from Aladdin. HEMA and METAC are known to contain 250 and 600 ppm MEHQ as stabilizer, respectively. And both the monomers were used without further purification. 2,2′-Azobis(2-methylpropionitrile) (AIBN) was purchased from Sinopharm Chemical Reagent and was recrystallized before polyreaction as the initiator. PEG (MW 4000) was purchased from Shanghai Lingfeng Chemical Reagent, and PEG (MW 400) was from Aladdin. Dulbecco's Modified Eagle Medium (DMEM) and bovine serum (BSA) were purchased from Invitrogen (USA). 3-(4,5-Dimethylthiazol-2-yl)−2,5-diphenyltetrazolium bromide (MTT) and dimethylsulfoxide (DMSO) were purchased from Sangon Biotech (Shanghai, China).

Hydrogels Synthesis

The semi-IPNs p(HEMA-*co*-METAC)/PEG were prepared by radical copolymerizing monomers HEMA and METAC in the presence of PEG with different molecular weight (MW 4000 and 400) in appropriate molar ratio. AIBN was used as thermal initiator at a concentration of 0.1% w/w respect to monomer mixtures (HEMA + METAC weight). We obtained different hydrogels with constant mole ratio of HEMA/METAC (10 : 1) and volume ratio of HEMA/H_2O (1 : 1), and different the weight fraction of PEG/HEMA as shown in Table I.

The synthetic method was performed based on the previously literature with some modification.[12] All the reactants were dissolved in deionized water and mixed together under magnetic stirring in a three-necked flask before polymerization. The mixture was degassed for 15 min under dry pure nitrogen flow, placed in a sealed glass bottle, and then cured in a water bath according to the following heating temperature: 60°C for 2 h, 70°C for 4 h, and 85°C for 1 h. After reaction, the products were washed three times with deionized water to remove the residual unreacted monomers, cut into pieces for the following characterization, and then dried at 40°C for 48 h. The obtained dried IPNs were stored in an airtight container for further use.

Characterization

Fourier transformed infrared spectroscopy (FT-IR) measurements were performed on a Nicolet Nexus 670 FT-IR spectrophotometer. Samples were crushed into powder, mixed with KBr, and then compressed into a transparent disc for FT-IR

analysis with the wavenumber from 500 to 4000 cm^{-1}. The thermal stability was determined on a Mettler Toledo thermogravimetric and differential thermal combined analyzer (TGA/SDTA851e). The gels were tested in aluminium pans under nitrogen atmosphere at a heating rate of 10°C/min from 25°C to 500°C. The morphology was studied on a scanning electron microscope (SEM) (Hitachi S-4800). The samples swollen to equilibrium in water were pre-frozen at −80°C for 24 h, and then freeze dried in a vacuum freeze-drier (Labconco FreeZone6) at −40°C for 3 days. The freeze dried gels were directly sticked onto electronic conductive and then photos were taken by SEM. The mechanical properties were determined by using an electronic universal testing machine (Model UTM2203) with a crosshead speed of 2 mm/min. The samples were swollen to equilibrium in PBS (pH 7.4) and cut into dumbbell shapes according to GB 1039. Young Modulus (E), maximum strength (σ_{max}), and maximum strain (ε_{max}) values were obtained and experiments were run in triplicate.

Swelling Studies

The swelling behavior was studied in different solutions by using an analytical balance (Sartorius Beijing). In particular, dried pre-weighed gels (0.01 g) were immersed into a definite volume of swelling medium, taken out over the desired time intervals, wiped gently by using filter papers to remove excess water from gels surface and finally weighed in balance. Moreover, the swelling temperature was controlled by water bath. The equilibrium degree of swelling (EDS) and SR of the gels were determined as follows:

$$\mathrm{EDS}(\%) = (W_s - W_d)/W_d \times 100\% \quad (1)$$

$$\mathrm{SR} = W_s/W_d \quad (2)$$

where w_s means swollen sample weight, and w_d represents dried initial sample weight. All the swelling experiments were performed in triplicate.

The effect of medium on swelling was carried out at 37°C for 24 h in different NaCl solution (0.01, 0.02, 0.05, 0.10, 0.20M), different buffer solution [citrate buffer 0.01M/pH 3–6, phosphate buffer (PBS) 0.01M /pH 5.7–8], glucose and urea solution (0.01, 0.05, 0.1, 0.2M). In addition, the influence of temperature on swelling was also investigated by changing the swelling temperature from 25 to 70°C in PBS of pH 7.4 for 12 h.

Cytotoxicity Test

The cytotoxicity test was performed by means of the elution test method (ISO 10993-5:2009). Briefly, 3T3-L1 cells were maintained in DMEM supplemented with 10% bovine serum, 100 units/mL penicillin and 100 μg/mL streptomycin at 37°C in humidified air containing 5% carbon dioxide (CO_2). Fluid extracts were obtained by incubating the test materials with cell culture medium for 24 h at 37°C. For MTT assay, 3T3-L1 cells were seeded onto 96-well plates and incubated in cell culture medium. After 24 h incubation, the cell medium was replaced by fluid extracts (100 vol %) or fluid extracts diluted with culture medium (50 vol %). The cells were exposed for 24 h incubation time. The MTT reagent was added to each well and cells were incubated for an additional 4 h. Then all the mediums were removed and 100 μL of DMSO was added and the plates were placed on a shaker to allow mixing at 37°C for 10 min. Thereafter, the absorbance of each well was quantified in an ELISA microplate reader at 570 nm with the reference at 630 nm (Bio-tek, Synergy). All experiments were performed three times and in triplicates. The relative cell viability was calculated. Data are expressed as means ± standard deviations of a representative of three similar experiments carried out in triplicate. In all statistical evaluations, $P < 0.05$ was considered as statistically significant.

Protein Adsorption Test

The BSA adsorption experiments were conducted in 0.1M phosphate buffer solution (PBS) at physiological pH 7.4. Before the adsorption experiments, samples (0.1 g) with the size of about 1.5 × 0.5 cm^2 were equilibrated in 0.1M PBS (pH 7.4) at 37°C for 12 h. And then the swollen samples were wiped gently by using filter papers to remove excess buffer from gels surface, followed by immersing in 10 mL fresh BSA solution (2 mg/mL). The adsorption was carried out by shaking BSA solutions containing the swollen gels at 37°C for 24 h (the time to reach equilibrium adsorption being about 2 h). The concentration of protein solutions were determined by using a UV–Vis spectrophotometer (Unico, UV–Vis 2801) at 280 nm, which is the wavelength of protein UV–Vis absorption. The amount of adsorbed BSA on gels was obtained by using the following equation:

$$\text{Adsorbed BSA}(\mathrm{mg}\ /\ \mathrm{g}) = (C_o - C_e)V/m \quad (3)$$

Where C_o and C_e (mg/mL) are the initial and equilibrium concentrations of BSA solutions, respectively, V (mL) is the volume of BSA solution and m (g) is the mass of dried gels. All the experiments were performed in duplicate.

RESULTS AND DISCUSSION

FT-IR Studies

The FT-IR studies were used to illustrate the chemical structure of the hydrogels. Figure 1 shows the FT-IR spectra of samples PEG, p, pc, pcpL10%, and pcpS10%. And the groups from monomers (HEMA and METAC) and PEG in gels were proved by characteristic bands of FT-IR spectra.[19,20,26] The FT-IR spectra of p, pc, pcpL10% and pcpS10% revealed the characteristic peaks of HEMA: the O-H stretching vibration from 3436 to 3412 cm^{-1}, C═O and C—O—C stretching vibration of the ester group appeared at 1726 cm^{-1}, and an absorption band with a weak shoulder around 2946 cm^{-1}, which corresponds to the stretching of aliphatic —CH_2—, C—H and —CH_3 groups. The main chemical structures of MAETC and HEMA are the same, except the terminal groups, i.e., —OH in HEMA and —$N(CH_3)_3Cl$ in MAETC. Thus, the FT-IR spectra of p, pc, pcpL10%, pcpS10% were almost similar, except for the extra peak of the group —$N^+(CH_3)_3$ at 1363 cm^{-1} in gels pc, pcpL10% and pcpS10% as shown with the black arrows, whereas there was no absorption peak at 1363 cm^{-1} in gels p. So the presence of METAC in the semi-IPNs gels was fully proved by the group —$N^+(CH_3)_3$. Moreover, it was found that the absorption peak of O—H was at 3436 cm^{-1} in gels p and pc while that was at about 3412 cm^{-1} in PEG. With the introduction of PEG into gels, the O—H absorption peak was shifted

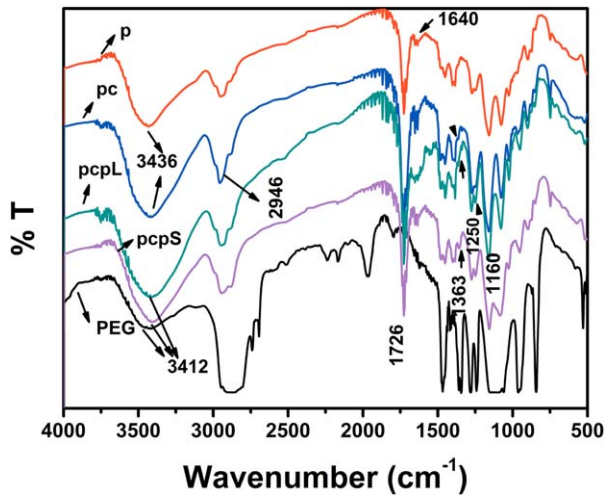

Figure 1. FT-IR spectra of PEG, p, pc, pcpL10%, and pcpS10%. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

from 3436 cm^{-1} to 3412 cm^{-1} in gels pcpL and pcpS, which should be attributed to hydrogen bond between PEG and p(HEMA-*co*-METAC).[27] And the IR spectra also confirm the presence of PEG in the gels as evident from the C—O stretching vibrations at 1250 cm^{-1}, asymmetric C—O—C stretching at 1160 cm^{-1}, and —CH_2— stretching at 2946 cm^{-1}.[19,20]

In addition, the polyreaction is via the opening C=C bond in monomers HEMA and METAC to form free-radicals, and then the gels are obtained by the free-radical addition reaction. So it could be proved that the gels were prepared through the disappearance or reduction of C=C stretching vibration in the FT-IR spectrum. It is generally known that the C=C stretching vibration at 1640 cm^{-1} in monomers HEMA and METAC is quite remarkable according to previous studies, whereas we found that the absorption peak at 1640 cm^{-1} was very weak in gels p, pc, pcpL10% and pcpS10% as shown in the figure. In detail, before polymerization, there was much C=C in the reaction medium which led to the strong absorption peak at 1640 cm^{-1}.[13,28–30] And after reaction, the gels was obtained and there was little C=C in the gels which resulted in the weak absorption peak. Therefore the disappearance of C=C absorption peak at 1640 cm^{-1} in gels p, pc, pcpL10%, and pcpS10% provided potent proof for the prepared gels. In brief, the FT-IR studies indicated the semi-IPNs p(HEMA-*co*-METAC) /PEG gels were successfully fabricated.

Thermal Stability Analysis

For biomaterials, the thermal stability is quite important in biomedical applications. Figure 2 shows the TGA results of different gels. The curves are all very similar in their shape except for gel p, which could be caused by the introduction of monomer. The gel p had one obvious weight loss starting from 300°C to 450°C, while gels pcpL5%, pcpL15%, pcpS5%, and pcpS15% all had two different weight loss zones, which were about 280–395°C and 395–460°C respectively. Compared with the initial degradation temperature of gel p (~300°C), the semi-IPNs gels pcpL and pcpS ranged from 260 to 285°C and showed a slightly reduced thermal stability. Moreover, although the thermal stability of gel pcpL5% seemed a little higher than other semi-IPNs gels, there was no obvious difference for all semi-IPNs gels with different content and molecular weight PEG, which meant that the thermal stability of semi-IPNs gels was nearly independent of content and molecular weight of PEG.

Morphology of the Gels

The morphology of different freeze-dried gels was observed by SEM, and the results were showed in Figure 3. All the gels have a honeycomb-like porous structure, whereas the average pore size of different gels varies widely. For gels p and ppL, the pores appear to be rather homogeneous with about 0.5-μm diameter, and the increase of pore size is not apparent with introduction of PEG. However, the pore size of gels pcpL5%, pcpL15%, pcpS5%, and pcpS15% is all approximately 100 μm, which is about 200 times larger than gels p and ppL. In fact, the marked increase of pore size is mainly due to the introduction of cationic monomer METAC, which is consistent with the following swelling behavior studies.

Mechanical Analysis

Results of mechanical tests [Young Modulus (E), maximum strength (σ_{max}), and maximum strain (ε_{max}) values] were reported in Table II. As shown, the maximum E was gel p (121.3 ± 20.6 KPa), then was followed by ppL (94.4 ± 0.9 KPa), and finally were pcpL5%, pcpL15%, and pcpS5% with all about 33 KPa. The σ_{max} and ε_{max} also mainly followed the change rule. From these data, it was found that the semi-IPNs gels pcpL5%, pcpL15%, and pcpS5% with lower values of elastic modulus than gels p and ppL, which led to the more extensible gels. The change rule was consistent with the previous SEM studies and the following swelling behavior studies. In short, the mechanical strength of the semi-IPNs gels are slightly lower than gels p and ppL.

Swelling Studies

Equilibrium Swelling Theory. When dry gels are dropped into solution to swell, water molecules diffuse into the networks and gels would swell until reaching finally swelling equilibrium. At

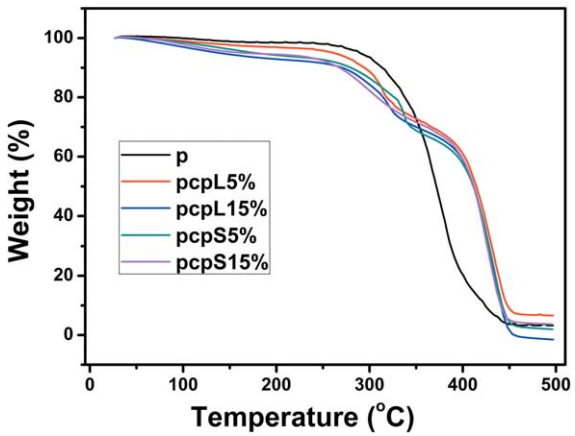

Figure 2. Thermogravimetric analysis (TGA) of pcpL and pcpS gels containing different proportion PEG. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

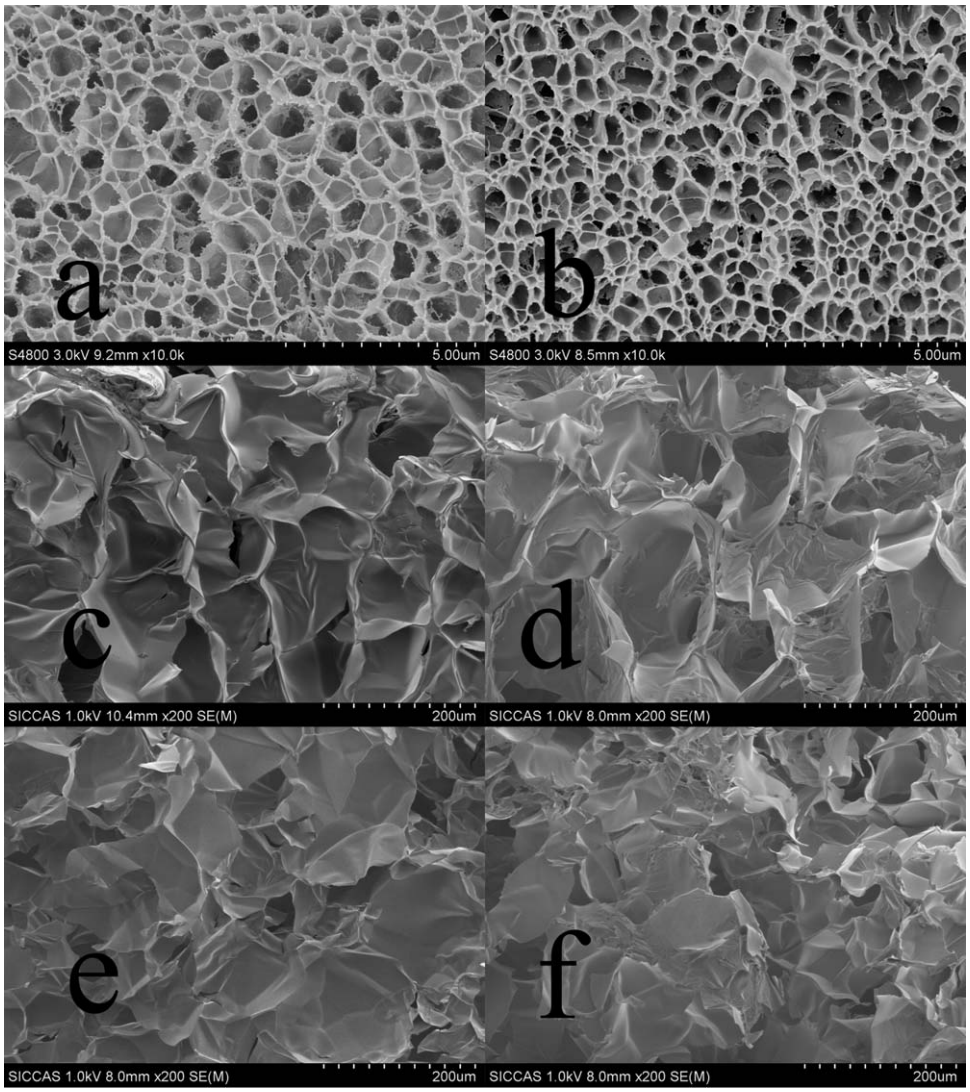

Figure 3. SEM images of freeze-dried samples of (a) p; (b) ppL; (c) pcpL5%; (d)pcpL15%; (e) pcpS5%; (f) pcpS15%.

equilibrium, there are different kinds of pressure which are responsible for balance and determine the degree of swelling of hydrogels.[31,32] For non-ionic gels like p or ppL in swelling equilibrium, the equation of total osmotic swelling pressure for gels is shown as follows:

$$\pi_{tot} = \pi_{mix} + \pi_{elast} \quad (4)$$

where π_{mix} is the contribution due to mixing of solvent molecules with polymer chains, π_{elast} is the result of elastic response to changes in the configuration of the polymer networks. Therefore, parameters π_{mix} and π_{elast} which are related to cross-link density and the Flory–Huggins parameter (χ) determine the swelling degree of non-ionic gels in solvent.

However, for polyelectrolyte gels containing ionic groups, there would be another two kinds of pressure—π_{ion} and π_{elect} which represent the pressure of mixing of ions from solution and the polymer network and changes in the electrostatic interactions of ionized groups upon swelling, respectively, contributing to the total osmotic swelling pressure, and then the equation would be obtained:

$$\pi_{tot} = \pi_{mix} + \pi_{elast} + \pi_{ion} + \pi_{elect} \quad (5)$$

For polyelectrolyte hydrogels, the π_{ion} and π_{elect} contribute significantly to the swelling degree of gels in solvent and the Donnan equilibrium theory evaluates the osmotic pressure π_{ion} of the hydrogel system by following equation:[19,20]

Table II. Mechanical Properties of Various Gels under Wet State

Sample name	E (KPa)	σ_{max} (KPa)	ε_{max} (%)
p	121.3 ± 20.6	329.6 ± 14.4	388 ± 8
ppL	94.4 ± 0.9	313.3 ± 31.0	392 ± 21
pcpL5%	33.7 ± 1.1	41.3 ± 1.5	134 ± 4
pcpL15%	32.9 ± 1.7	39.8 ± 1.1	119 ± 6
pcpS5%	32.9 ± 1.3	40.5 ± 1.9	121 ± 8

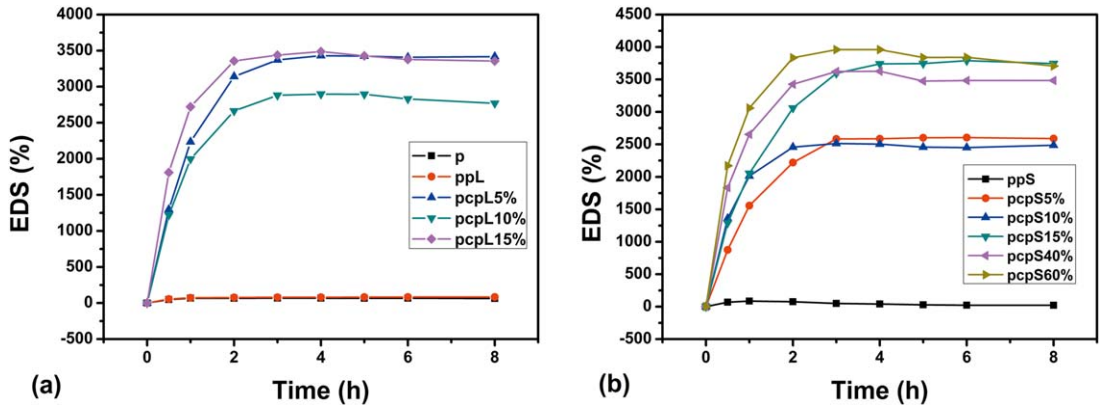

Figure 4. Swelling kinetics of p(HEMA-*co*-METAC)/PEG4000 (a) and p(HEMA-*co*-METAC)/PEG400 (b) at 37°C. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

$$\pi_{ion} = RT\sum_i \left(C_i^g - C_i^s\right) \quad (6)$$

where C_i is the mobile ion concentration of species i and superscripts g and s represent gels and solution phases, respectively. The equation indicates that the greater ionic concentration difference between the interior and external of gels, the greater osmotic pressure π_{ion} would be and the larger swelling degree of the gels would be. The ions inside the gels mainly derived from dissociation of ionic groups of polyelectrolyte and the diffusion from external solution into the gels. As a result, π_{ion} is high and water molecule would diffuse into the gels to maintain the π_{ion} balance, causing the gels to swell. Due to the great influence on π_{ion} of polyelectrolyte, the swelling degree of gels could change sharply in the different pH or ionic strength medium.

Kinetics of Swelling. The swelling kinetics was studied in deionized water at 37°C (Figure 4). Some valuable messages could be obtained from the Figure 4: (1) All the samples were proved to reach rapidly swelling equilibrium within 3 h; (2) Compared with other gels, the neutral gel p had the lowest EDS (∼60) in the same conditions. With the introduction of PEG, the EDS of gels ppL or ppS (∼85) would be slightly improved due to the increasing of hydrophilic groups, while the EDS of gels pcpL or pcpS (above 2500) would be significantly enhanced due to the contribution of ionic group $-N^+(CH_3)_3$. As the fixed charges are generated on the polymer chains, this would result in electrostatic repulsion between the chains of semi-IPNs, which tend to stretch polymer chains from a closed coiled state to an extended state.[1,13] In addition, ionic group $-N^+(CH_3)_3$ has a net structure which could cause effect on the water binding sites because of the high electric fields. This not only could polarize, immobilize and attract the nearest neighbor molecules, but also induce additional order beyond the first layer of water molecules.[13,32] Hence, the EDS of p(HEMA-*co*-METAC)/PEG was the greatest among all the gels; (3) The study found that there would be some slight influence on EDS by changing the content or molecular weight of PEG. For Figure 4(a), the EDS of pcpL5% and pcpL15% were both around 3400, while pcpL10% was around 2700. And for Figure 4(b), the EDS of pcpS5% and pcpS10% were both approximately 2500, while pcpS15%, pcpS40%, and pcpS60% were above 3500. Based on these data, there was an anomalous increase of EDS to pcpL5% but not to pcpS5% with the possible reasons that: (i) The PEG4000 are highly elastic and more flexible than PEG400, so it will induce higher swelling[26]; (ii) When small amounts of PEG4000 was introduced in gels, the METAC content was relatively high and played a leading role on EDS in gels; (4) Finally, the EDS of pcpS were found to

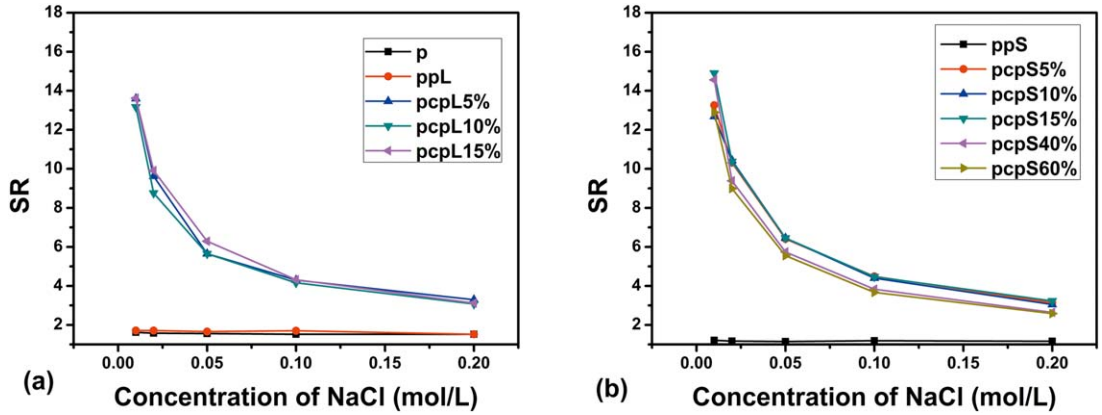

Figure 5. Swelling ratio of p(HEMA-*co*-METAC)/PEG4000 (a) and p(HEMA-*co*-METAC)/PEG400 (b) in different NaCl solutions. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

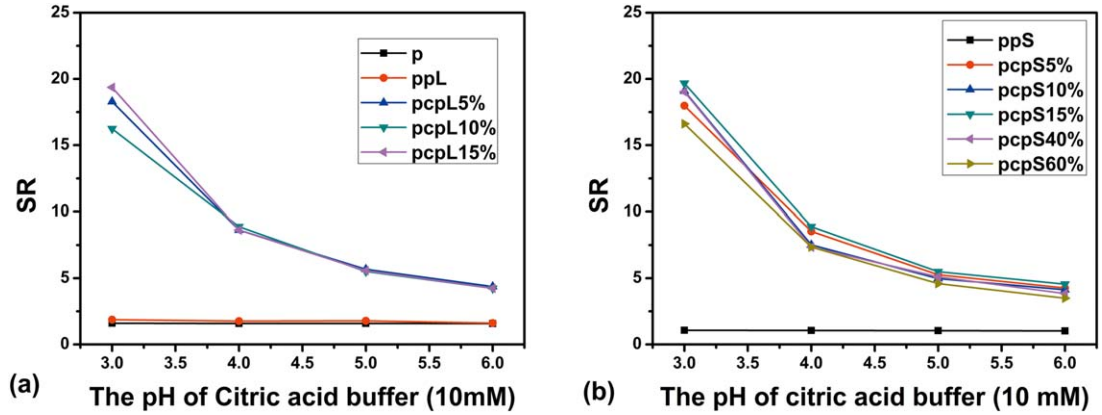

Figure 6. Swelling ratio of p(HEMA-*co*-METAC)/PEG4000 (a) and p(HEMA-*co*-METAC)/PEG400 (b) in different pH citrate buffer system. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

have the tendency of firstly sharply increasing, then slightly decreasing, and finally keeping balance, while the EDS of pcpL first increased and then kept balance. The stability of pcpS was a little lower than pcpL owing to the shorter molecule chains of PEG400 that lead to a weaker physical entanglements than PEG4000, which is also consistent with the results of thermal stability analysis.

Effect of Ionic Strength on Swelling. As a matter of fact, the ionic strength in the swelling medium has a remarkable influence on SR of the semi-IPNs, which is quite important for the applications in biomedicine. Figure 5 shows the SR in different NaCl solutions (0.01–0.2*M*). As expected, with the increasing of ionic strength an obvious reduction of SR (from 14 to 3) was observed for all gels except for p, ppL, and ppS, due to their neutral nature, which has demonstrated that the novel semi-IPNs are responsible for ionic strength sensitive behavior. And according to the data, the semi-IPNs gels have the greatest SR (about 14) in 0.01*M* NaCl solution, which is far less than in deionized water with SR above 26 obtained by transforming previous EDS (above 2500) into SR. In addition, the difference of SR for gels with different PEG content or type is negligible, which is owe to the presence of METAC in the semi-IPNs. In brief, it can be explained that π_{ion} is much higher in the absence of NaCl solution, due to the large difference in the ion concentration inside the gel and external solution. When NaCl is added to the swelling medium, the ionic concentration C_i^s increases and the values of $(C_i^g - C_i^s)$ and π_{ion} accordingly decrease. Therefore, a lower SR is obtained. In addition, the salt ions in the swelling medium have a screening effect of the electrostatic repulsion between ionized groups on the polymer chains,[13] which reduces the π_{elect}, resulting in shrinking of polymer chains and decreasing of SR. In brief, we obtained the semi-IPNs gels with obvious salt sensitive property.

Effect of pH on Swelling. The pH sensitive hygrogels always play a considerable role in controlling drug-delivery systems, due to the significant SR change of gels.[26] The effect of pH on swelling were studied in citrate buffer and phosphate buffer (PBS) respectively with the same ionic concentration (0.01*M*) at 37°C. And the results are depicted in Figures 6 and 7, which both reveal that the SR of semi-IPNs p(HEMA-*co*-METAC)/PEG decreases with increasing pH in the range 3.0–6.0 and 5.7–8 while the SR change of neutral gels p, ppL, ppS is almost omitted. It can be explained that when the ion concentration is the same, the degree of ionization of the semi-IPNs gels reduces with increasing pH. It is mainly caused by the cationic group $-N^+(CH_3)_3$, which results in the number of fixed charges

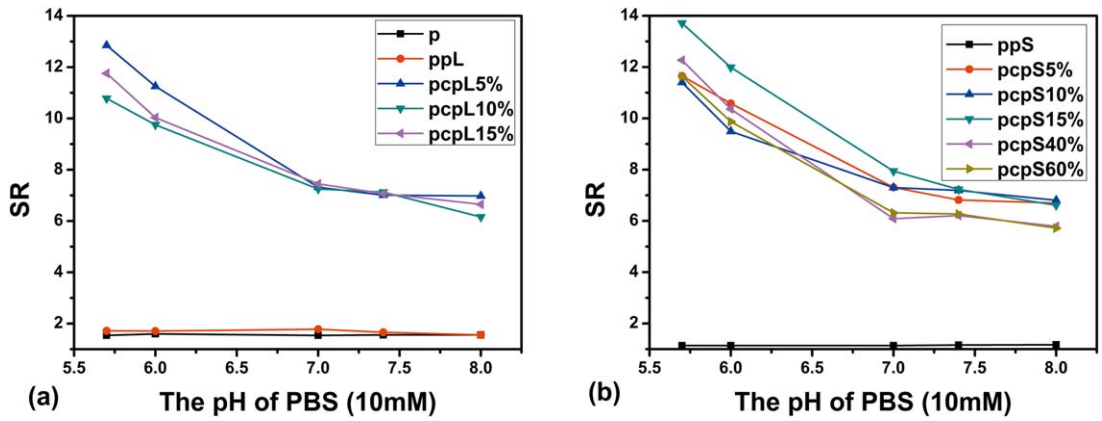

Figure 7. Swelling ratio of p(HEMA-*co*-METAC)/PEG4000 (a) and p(HEMA-*co*-METAC)/PEG400 (b) in different pH PBS system. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

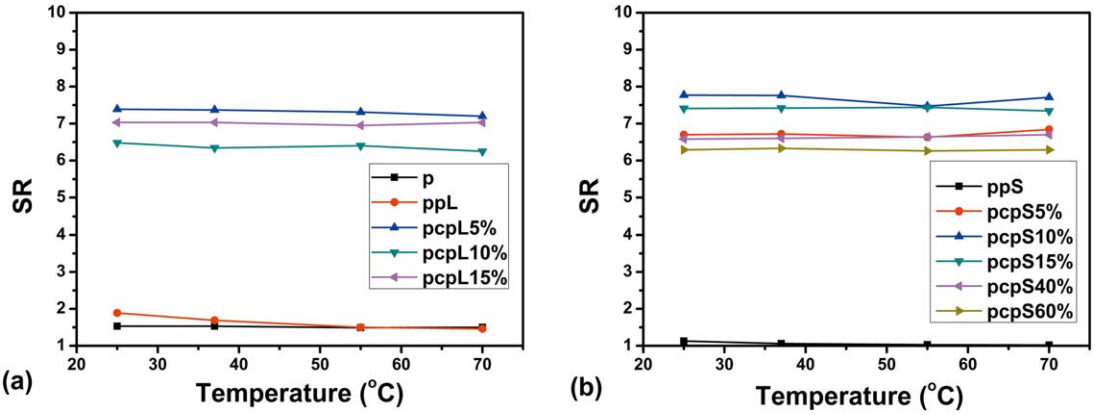

Figure 8. Swelling ratio of p(HEMA-*co*-METAC)/PEG4000 (a) and p(HEMA-*co*-METAC)/PEG400 (b) at different temperature. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

lessening and diminished electrostatic repulsions between polymer chains. Therefore, a lower SR is obtained.

From Figure 6, the SR of gels pcp is somewhat different in pH 3.0 citrate buffer with the value of 19.36 for pcpL15%, 18.29 for pcpL5%, 16.74 for pcpL5% [Figure 6(a)], and 19.67 for pcpS15%, 19.08 for pcpS10%, 19.03 for pcpS40%, 17.98 for pcpS5%, 16.61 for pcpS60% [Figure 6(b)], while it is similar in the range pH 4.0–6.0. Whereas, the difference is more obvious in PBS than in citrate buffer with the same ion concentration 0.01*M* by comparing Figures 6 and 7. Based on these data, the distinction of swelling behavior between semi-IPNs p(HEMA-*co*-METAC)/PEG is basically in accordance with the result of in deionized water, due to the factors of METAC relative content, elasticity of PEG molecule chains and stability of polymers as previously described.

In addition, the SR varies widely when swelling medium is citrate buffer and PBS with the same pH 6, which is quite different with previous studies that swelling degree was consecutive in different buffer with the same pH value.[14] Based on the data, the SR is around 4 and 10 in pH 6 citrate buffer and PBS respectively, which maybe relate with the nature property of medium. As expected, the pH sensitive semi-IPNs gels are obtained which is quite significant in biomedical application.

Effect of Temperature on Swelling. The influence of temperature on the swelling has been investigated by varying the temperature in the range 25–70°C. The results were showed in Figure 8 which indicated that the SR did not change significantly with temperature which showed temperature-independence property of the gels.

Effect of Other Medium on Swelling. In addition, the SR of gels is known to be affected by the presence of solutes of the biologically important additives, such as urea and glucose.[13,20,32] Therefore, the SR of different gels was investigated in urea and glucose solution with different concentration, and the results were shown in Figures 9 and 10. Compared with the decreasing SR with the increasing NaCl concentration, the change of SR was not obvious with the increasing urea and glucose concentration. It could be explained that the ionic strength was invariant with the increasing urea or glucose concentration, that is, the value of $(C_i^g - C_i^s)$ is constant and the π_{ion} is changeless. This could also explain that all the SR is similar in water, urea and glucose solution. In addition, the SR in urea and glucose

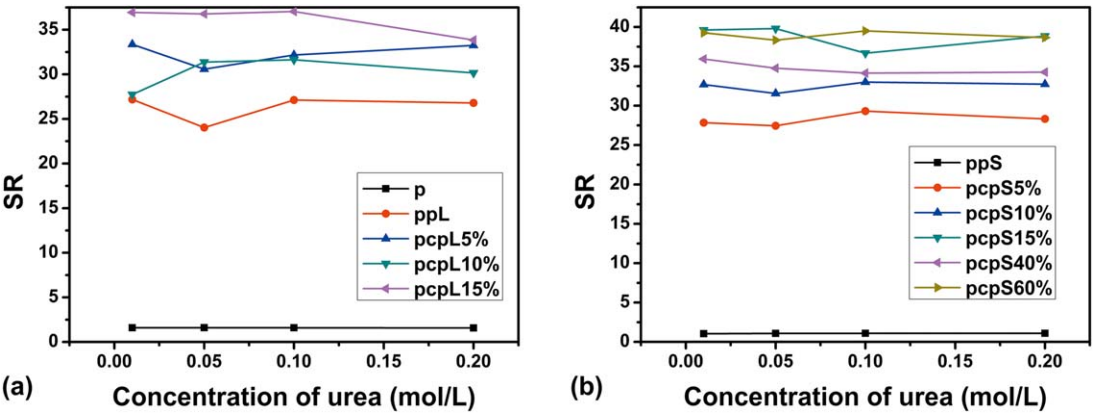

Figure 9. Swelling ratio of p(HEMA-*co*-METAC)/PEG4000 (a) and p(HEMA-*co*-METAC)/PEG400 (b) in urea solution. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

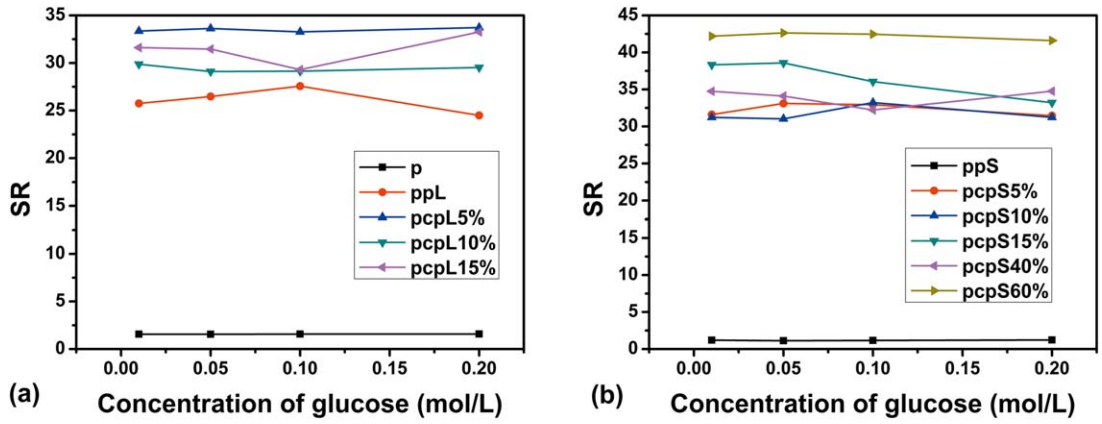

Figure 10. Swelling ratio of p(HEMA-*co*-METAC)/PEG4000 (a) and p(HEMA-*co*-METAC)/PEG400 (b) in glucose solution. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

solution was much higher than in NaCl solution under any concentration.

Cytotoxicity Test

It is known that the cytocompatibility of biomaterials is extremely important for their biomedical applications. Therefore, the cytotoxicity of the semi-IPNs gels was evaluated using mouse embryonic fibroblast cell 3T3-L1 by MTT assay, and the results of different gels are presented in Figure 11. According to ISO 10993-5, samples with cell viability larger than 75% can be considered as noncytotoxic. The results show that though the cell viability of pcpL5% and pcpS15% was 81 and 80% in 100 vol % hydrogel extract, respectively, the cell viabilities of the rest samples were all higher than 100% in 100 vol % and 50 vol % hydrogel extract. This result confirms that the semi-IPNs gels p(HEMA-*co*-METAC)/PEG are cytocompatible and convenient for the applications as biomaterials.

Protein Adsorption Test

For the application as biomaterials, the good protein resistance effect is so important that we studied the BSA adsorption on the semi-IPNs by introducing METAC and controlling the content of PEG in gels. The BSA adsorption on the gels reached their maximum values after 2 h and remained constant since then (Supporting Information Figure S1.). The maximum amount of adsorbed BSA on the gels samples are presented in Figure 12. For gels ppL and ppS without cationic monomer METAC, the adsorption values of BSA are 91 mg/g and 50 mg/g respectively, which are obviously higher than the values of gels pcpL and pcpS containing cationic monomer METAC.

Generally, protein adsorption is influenced by external (such as pH, protein type and concentration) and internal (such as hydrogen bond, hydrophobic interaction, hydrophilic interaction, and electrostatic effect) factors.[33–35] In our work, an interesting result is obtained that the swelling higher gels pcpL and pcpS have lower BSA adsorption than the swelling lower gels ppL and ppS. The isoelectric point of BSA is 4.7, so the protein carries a negative charge at a physiological pH of 7.4.[36] In a general way, gels pcpL and pcpS containing cationic monomer METAC should adsorb more negative BSA because of the electrostatic effect between gels and BSA. It can be explained that the electrostatic effect is ignored and the reason of the result may be attributed to the hydrophobic interaction. That is, since water interacts weakly with hydrophobic surfaces, the entropy would increase with the adsorption of hydrophilic protein, which results in more protein adsorption on surfaces of higher hydrophobicity compared with hydrophilic surfaces. In general, protein adsorption is less effective when hydrophilic monomers are introduced and lower water content materials have shown

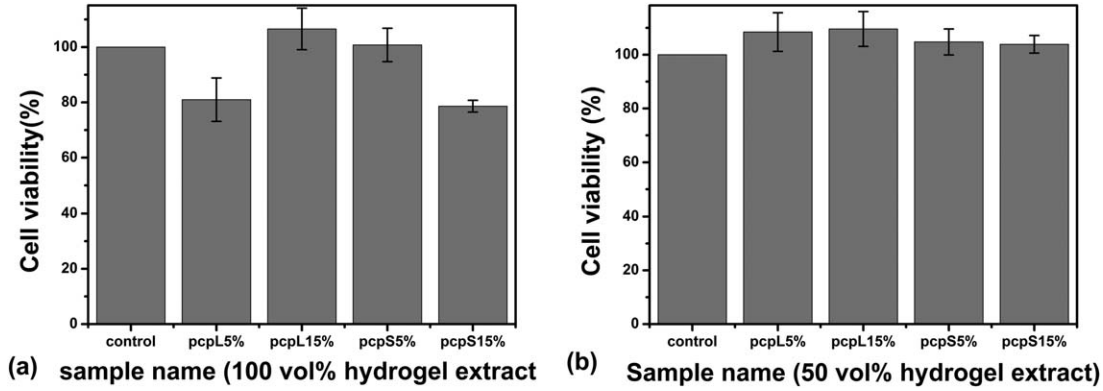

Figure 11. Cell viability of pcpL and pcpS hydrogels in 100 vol % (a) and 50 vol % (b) hydrogel extract.

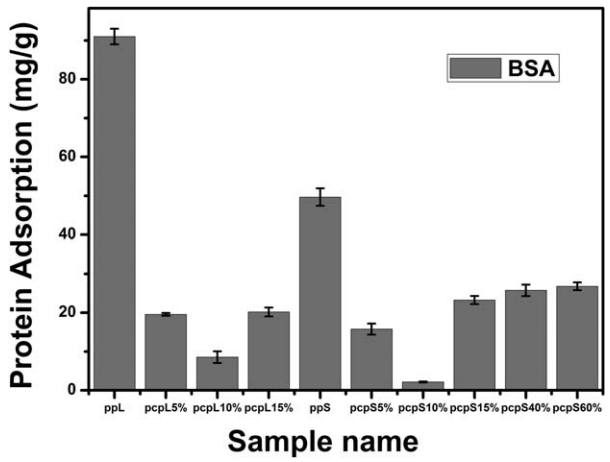

Figure 12. BSA adsorption on pcpL and pcpS gels containing different proportion PEG.

better protein adsorption than high water content materials.[2,37] Therefore, the swelling higher gels pcpL and pcpS have lower BSA adsorption than the swelling lower gels ppL and ppS.

In addition, the gels pcpL and pcpS containing PEG 10% both have the minimum adsorption value 8 mg/g and 2 mg/g, respectively, while the adsorption value of other proportion gels were approximately 20–25 mg/g. Though the protein resistance effect of PEG in the study is less obvious than the cationic monomer METAC, the role as auxiliary regulator is still very significant.

CONCLUSION

The novel semi-IPNs hydrogels p(HEMA-*co*-METAC) /PEG were prepared by copolymerizing HEMA with METAC in the presence of PEG solution. The FT-IR analysis showed that the hydrogels had the expected chemical structure and SEM indicated that the pore size of the semi-IPNs gels pcpL and pcpS are much larger than gels p and ppL. The TGA revealed that the thermal stability of semi-IPNs gels was slight lower than gel p, and nearly independent of content and molecular weight of PEG. The mechanical strength of the semi-IPNs gels pcpL and pcpS are slightly lower than gels p and ppL. The swelling studies showed the SR of the semi-IPNs gels is much higher than gel p by introducing cationic monomer METAC, and PEG as the auxiliary regulators control the swelling behavior of the semi-IPNs gels. In addition, the SR of the semi-IPNs gels in neutral solution is much higher than in salt solution, which mainly owe to the present of osmotic pressure π_{ion} in polyelectrolyte gels. Therefore, the semi-IPNs gels performing salt or pH sensitive swelling behavior can be considered as smart hydrogels. The swelling behavior also revealed the temperature-independence property of gels. The results of cytotoxicity study in vitro showed good cytocompatible and being convenient for the application as biomaterials. Finally, the BSA adsorption results showed that the gels pcpL and pcpS containing PEG 10% both have the minimum adsorption value 8 and 2 mg/g, respectively, which proved good protein resistance effect in biomedical applications.

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