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Intelligent poly(*N*-isopropylacrylamide)-carboxymethyl cellulose full interpenetrating polymeric networks for protein adsorption studies

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Abstract Poly(*N*-isopropyl acrylamide) (PNIPAm)–carboxymethyl cellulose (CMC) full interpenetrating polymeric networks (IPNs), based on PNIPAm and CMC, were prepared and investigated for adsorption of biomolecules utilizing a model protein, bovine serum albumin (BSA). *N*-isopropyl acrylamide monomers were polymerized in the presence of a natural polymer, e.g., carboxymethyl cellulose sodium salt. *N*,*N*′-methylenebisacrylamide (CL) was used to crosslink PNIPAm and CMC chains and IPN formed simultaneously. Spectroscopic and thermal characterization of the hydrogels were done with IR spectroscopy and thermogravimetric analysis. The swelling properties of PNIPAm and PNIPAm–CMC hydrogels were investigated as functions of the medium pH, temperature, ionic strength, and BSA. It was observed that the adsorption of protein molecules onto the hydrogels was mainly dependent on temperature and pH of the environment during the experiments. The maximum adsorption capacity (*X*) was observed at pH 4.7 which is the isoelectric point of BSA and at 40 °C for both hydrogels; and introducing CMC to PNIPAm increased the protein adsorption of the hydrogel. Adsorbed amounts of BSA were 26.70 mg g^{-1} (4 °C) and 38.70 mg g^{-1} (40 °C) for PNIPAm–CMC full IPN hydrogels. Adsorbed BSA (up to 80%) was eluted in the elution medium containing 0.1 mol dm^{-3} NaSCN at pH 8.0. Synthesized cylindrically shaped PNIPAm–CMC full IPN hydrogels can be used for adsorption studies related to the removal of proteins in pH- and temperature-sensitive biotechnological areas.

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Introduction

Interpenetrating polymeric network (IPN) hydrogels, containing intelligent synthetic and/or natural polymers, are capable of undergoing volume changes in response to environmental stimuli including pH, temperature, electric field, light, presence of specific molecules, magnetic field, ionic strength, etc. [1–6]. IPNs are three-dimensional networks which can be formed from homogeneous or heterogeneous polymers crosslinked in the presence of one another. Materials formed from IPNs share properties characteristic of each network. These kinds of polymeric materials are being studied for their application in the fields of biomedicine and biotechnology [7–16]. Dextran, hyaluronic acid, alginate, chitosan, chitin, cellulose derivatives, carrageenan, and gelatin are among the most widely used natural polymers while poly(2-hydroxyethyl methacrylate), polyacrylamide, poly(acrylic acid), poly(*N*-isopropyl acrylamide), poly(3-acrylamidopropyl-trimethylammonium chloride), poly(2-acrylamido-2-methyl-1-propansulfonic acid), poly(*N*-vinyl pyrrolidone), and their derivatives are examples of the most commonly used synthetic polymers to prepare IPNs [2, 8, 17–23].

In this study, pH- and temperature-sensitive poly(*N*-isopropyl acrylamide) (PNIPAm)–carboxymethyl cellulose (CMC) hydrogels were synthesized with full IPN form and tested for adsorption of bovine serum albumin (BSA) from aqueous solutions. Results are given comparatively with that of crosslinked PNIPAm hydrogels. PNIPAm hydrogel is one of the most widely studied gels that displays a temperature-controlled volume phase transition. This gel

swells at temperatures lower than about 34 °C and collapses at temperatures higher than this value. CMC is known to be a derivative of cellulose that is colorless, odorless, nontoxic, and water soluble; it is used in detergents, soaps, food products, and cosmetics, etc. [24]. In addition, CMC is the most abundant, renewable biopolymer and a very promising raw material available at low cost for the preparation of various functional polymers. CMC hydrogels that consist of ionizable carboxylic acid groups can swell or collapse in response to changes of pH [25–27]. Protein adsorption is an important application in protein purification studies. BSA is the most abundant protein in vertebrates and is commercially available at low cost. BSA has also been widely used in biochemical studies as a generic protein. Adsorption of BSA using various materials has been reported by many researchers in the literature [28–34]. These polymer matrixes have certain advantages over other materials such as low cost, ease of protein accessibility, hydrophilic character, and the presence of carboxyl and amino groups on the surface capable of interaction with proteins [35]. PNIPAm–CMC full IPN hydrogels were prepared to combine these characteristics. PNIPAm–CMC full IPN hydrogels were synthesized to take advantage of temperature- and pH-sensitive behaviors of PNIPAm and CMC hydrogels, respectively. These hydrogels were designed in cylindrical form for ease of handling. Hydrogels based on PNIPAm are suitable for developing systems with temperature-sensitive protein adsorption by utilizing their swelling-shrinking behavior [36–41]. No study has been reported in the literature using PNIPAm–CMC hydrogels prepared in IPN form to perform BSA adsorption studies. PNIPAm–CMC full IPN hydrogel maintained its physical unity and mechanical resistance, and adsorbed BSA molecules during the environmentally sensitive adsorption studies.

Materials

Carboxymethylcellulose sodium salt (medium viscosity), *N*,*N*′-methylenebisacrylamide, and Coomassie Brilliant Blue G-250 (CBB) were purchased from Fluka (Steinheim, Switzerland). The model protein, BSA (M_r 67000) was also a product of Fluka (Steinheim, Switzerland). Sodium chloride, disodium hydrogen phosphate, potassium dihydrogen phosphate as buffer components, potassium persulfate (KPS) as a redox initiator and *N*,*N*,*N*′,*N*′-tetramethylethylene diamine (TEMED) as an accelerator were supplied from Merck (Darmstadt, Germany). Acetic acid was a product of Riedel-de-Haën. *N*-isopropylacrylamide (97% w/w) was obtained from Sigma-Aldrich. All the chemicals were of analytical grade and were used as received. Deionized water was used for all the experiments.

Experimental

Preparation of the hydrogels

Poly(*N*-isopropyl acrylamide)–carboxymethyl cellulose hydrogels were simultaneously prepared by redox polymerization and crosslinking *N*-isopropyl acrylamide (NIPAm) with CL monomers and by crosslinking CMC chains with the same crosslinker in water at 18 °C, using KPS and TEMED. CMC and NIPAm were used in 5.0:95.0 weight ratio to prepare PNIPAm–CMC hydrogels. 62.5 μL TEMED and CL (2.5% mol ratio according to NIPAm monomer) were added to an aqueous NIPAm and CMC solution and mixed homogenously. The initiator solution (2% w/w in water, 1.0% mol ratio according to NIPAm monomer) was added under continuous mixing. The solution was injected into a plastic straw and kept at least 10 h to complete the polymerization/crosslinking reaction. The same synthesis procedure was employed for PNIPAm hydrogels without CMC with the ratio of CL and KPS mentioned above. Following cutting to the preferred size and shape (ca. 3 mm in length and 3 mm in radius), hydrogels were washed in deionized water at different temperatures (12 and 40 °C) for 5 days with frequent replenishment of water to remove any impurities and unreacted species.

Fourier transform infrared analysis

Fourier transform infrared (FTIR) spectra of IPN hydrogels were recorded on a Perkin Elmer model (Spectrum BX) FTIR spectrophotometer to determine their structure and intermolecular interactions. Thoroughly ground gel samples were mixed with dried KBr and their disks were prepared by compression under vacuum. Spectra were recorded with a resolution of 1 cm^{-1}, 20 scans.

Thermogravimetric analysis

Thermal stability investigations were conducted with a Shimadzu 50 thermogravimetric analyzer. Thermogravimetric (TG) analyses were performed on ca. 10 mg samples under nitrogen atmosphere with a nominal gas flow rate of 25 mL/min and a heating rate of 10 °C/min to 650 °C.

Swelling studies

The equilibrium swelling behaviors of the hydrogels were studied as a function of pH, temperature, ionic strength, and BSA. To investigate the pH sensitivity of the hydrogels, the pH of the medium was changed between 3.0 and 8.0 by using different buffer systems (CH_3COOH–CH_3COONa for

pH 3.0–5.5, K_2HPO_4–KH_2PO_4 for pH 6.5–8.0). The dried hydrogel was weighed and placed into 25 mL buffer solution (pH 3.0) at 4 °C. Once equilibrium was reached at 4 °C after 24 h, the swollen gel was taken out of the buffer solution and the excess buffer was removed by blotting with filter paper. The weight of the wet gel was measured and it was put into fresh buffer solution at 40 °C. After 24 h, the deswollen hydrogel was removed from the solution and the weight of the hydrogel was measured. The same swelling procedure was employed for all buffer solutions.

To investigate the effect of ionic strength and BSA on swelling behavior of the hydrogels, the same swelling studies were performed in buffer solutions with ionic strength $I = 0.01$ mol dm^{-3} (adjusted by NaCl, pH 4.7) and with BSA in solution (100 mg dm^{-3}, pH 4.7). For temperature-response studies, the hydrogels were equilibrated in deionized water at temperatures ranging from 11 to 60 °C. The hydrogels were allowed to swell in deionized water for at least 24 h at each predetermined temperature, controlled to ±0.1 °C in a constant-temperature water bath. All the swelling experiments were repeated three times and results are reported as average values.

Protein adsorption studies

The effect of temperature, pH, and ionic strength of the solution was investigated for adsorption of BSA onto hydrogels. Gels with dry weight of about 10 mg were added to 10 mL of 100 mg dm^{-3} of BSA solution (pH ranging from 3.0 to 8.0) at 4 °C for 3 and 24 h. Maximum adsorption time was 3 h for all gel samples. The total protein loaded was determined by the mass change of protein in the buffer solutions before and after loading. The protein concentrations of BSA were assayed by the Bradford method, using the CBB. The determinations were provided by a UV–Vis spectrophotometer (Shimadzu UV-1208) at 594 nm. The adsorbed amounts of protein on the adsorbents were calculated from their calibration graphs obtained at each pH. The same adsorption procedures were repeated at 40 °C for 3 h. To investigate the effect of ionic strength of protein solutions, adsorption studies were performed at a constant ionic strength ($I = 0.01$ mol dm^{-3}) at 4 and 40 °C. All the adsorption experiments were repeated three times and results are reported as average values.

Desorption of bovine serum albumin

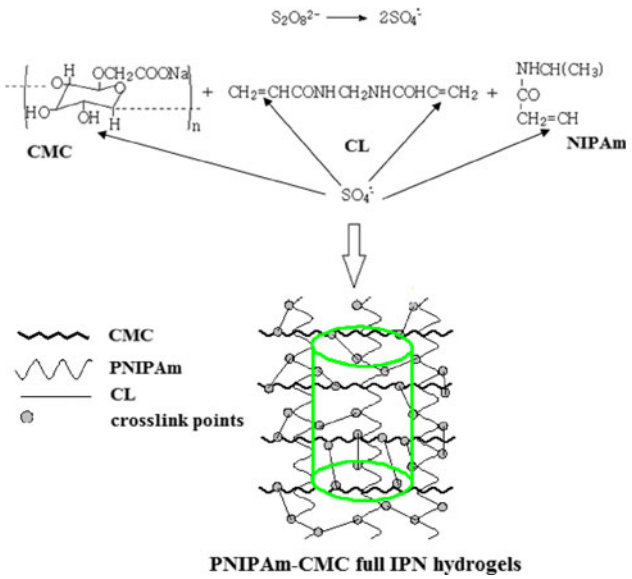
Hydrogels loaded with BSA (pH 4.67, $I = 0.01$ mol dm^{-3}) at 40 °C were used in the desorption studies. Samples were left for 5 h in 0.1 mol dm^{-3} NaSCN solution (pH 8.0) at 18 °C. BSA analysis of this solution was done with Bradford assay.

Results and discussion

Poly(*N*-isopropyl acrylamide)–carboxymethyl cellulose full IPN hydrogels were synthesized by the conventional free radical polymerization and crosslinking of NIPAm monomers, and radical crosslinking of CMC polymer chains using KPS as a free radical initiator and MBA as a crosslinking agent. Scheme 1 depicts schematically the full interpenetrating network hydrogel synthesis. The sulfate anion radical produced from the thermal decomposition of KPS abstracts hydrogen from the hydroxyl groups of the polysaccharide substrate to form corresponding alkoxy radicals on the substrates [25, 42]. These initiator radicals break the double bonds of monomer (NIPAm) and crosslinker (CL) molecules and form radicals on NIPAm and CL. The interpenetrating network hydrogel formation carried out by these free radical centers result in crosslink points. Poly(*N*-isopropylacrylamide) hydrogels crosslinked with CL were synthesized by the same procedure.

Spectroscopic characterization

Fourier transform infrared spectroscopy was used to confirm the structure of PNIPAm and PNIPAm–CMC IPN hydrogels. PNIPAm had a wide band 3000–3700 cm^{-1} for amide groups related to symmetric and anti-symmetric N–H stretching vibrations of NIPAm and MBA repeating units (Fig. 1a). The broadening of this band is due to the existence of intramolecular hydrogen bonding [9, 43]. The



Scheme 1 Schematic presentation of cylindrical shaped PNIPAm–CMC full IPN hydrogel preparation. Hydrogels were prepared by simultaneously radicalic crosslinking of CMC and PNIPAm polymer chains. The CMC/PNIPAm weight ratio was 5.0/95.0 and the hydrogels were prepared at 18 °C. PNIPAm hydrogels were obtained by the same way in absence of CMC too

bands in the range 2700–3000 cm^{-1} (symmetric and antisymmetric stretching vibrations for C–H groups) come from aliphatic $–CH_2$ groups. The bands at 1387 and 1364 cm^{-1} indicate $–CH(CH_3)_2$ groups for PNIPAm. There was a characteristic –C=O stretching vibration band for the amide group at 1658 cm^{-1} (amide I) and a band at 1542 cm^{-1} (amide II) from PNIPAm [17].

In the PNIPAm–CMC spectrum (Fig. 1b), a clear peak at 1457 cm^{-1} and new small peaks occurring at 1660 cm^{-1} are due to –COO groups of the cellulose units. The band intensity related to hydroxyl groups in the range of 3000–3600 cm^{-1} also increased with the introduction of CMC to the hydrogel. This change can be attributed to hydrogen bonding between –C=O groups and –NH groups of NIPAm and –OH groups of cellulose units. Peaks at 1385 and 1366 cm^{-1} indicate –OH bending of the CMC polymer [41]. This data confirmed the existence of PNIPAm–CMC IPN hydrogel formed by polymerization/crosslinking of NIPAm monomers and CMC polymer.

Thermogravimetric characterization

The thermal degradation behavior of the hydrogels was studied in the range 25–650 °C under a nitrogen atmosphere. The thermogravimetry curves obtained for PNIPAm and PNIPAm–CMC polymer are presented in Fig. 2. The results from these curves are listed in Table 1. The first weight loss (25–150 °C) indicates evaporation of water and shows that both polymers are hygroscopic. The TG curves of the gels are wide and show that the weight loss occurs in more than one step. The second step occurs above 250 °C with a maximum decomposition rate at 364 °C for PNIPAm and 371 °C for PNIPAm–CMC. The second degradation temperature of the hydrogels was due to random chain scission. PNIPAm–CMC IPN hydrogel is thermally more stable than PNIPAm crosslinked gels. In the case of full IPN, since the polymer chains are more closely tangled together, the thermal stability of IPN is higher than those of other polymers.

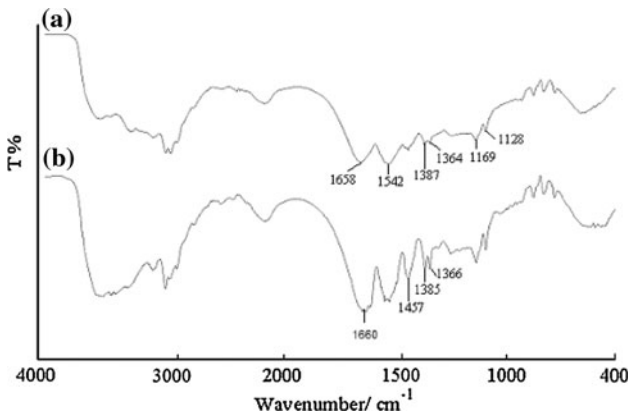


Fig. 1 FTIR spectra of *a* PNIPAm, *b* PNIPAm–CMC full IPN hydrogels. The addition of CMC to PNIPAm hydrogel resulted in an clear absorption band at 1457 cm^{-1} identifying –COO groups of cellulose units in CMC

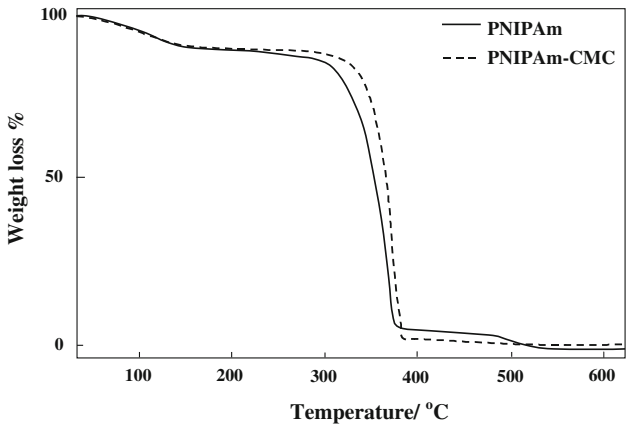


Fig. 2 TG curves of the PNIPAm and PNIPAm–CMC hydrogels in nitrogen atmosphere at heating rate of 10 °C/min

Table 1 Thermal degradation parameters of PNIPAm and PNIPAm–CMC full IPN hydrogels

Hydrogel	T_i/°C	T_h/°C	T_f/°C	T_{max}/°C
PNIPAm	312.0	352.0	374.0	364.0
PNIPAm–CMC	340.0	361.0	380.0	371.0

Thermal analysis parameters such as initial reaction temperature (T_i), final reaction temperature (T_f), half time temperature (T_h), and maximum rate temperature (T_{max}) determined from the thermograms are presented in Table 1. T_i, T_h, T_f, and T_{max} values of PNIPAm–CMC hydrogels were higher than that of PNIPAm hydrogel due to the presence of cellulose units. Properties such as size and polarity of the side groups affected the thermal stability of the polymer. Polysaccharides have been used as additives to synthetic polymers, in amounts of a few percents of the overall compounds, to improve properties of the final gel, i.e., to avoid the formation of bubbles in the bulk material and to enhance mechanical properties [44]. Hence, PNIPAm–CMC can be considered as a threshold toward more thermally stable interpenetrating structures.

pH- and temperature-sensitive swelling studies

Most of the hydrogel systems are pH responsive and, therefore the pH of the immersion medium has direct control over the degree of swelling of the network. The crosslinked PNIPAm–CMC hydrogels contain a number of carboxylate groups in their networks and their group dissociation is dependent on the pH of the medium. Therefore,

in this section, the influence of the pH of the medium (pH 3–8) on the swelling profiles of PNIPAm–CMC IPN hydrogels was studied and the results are shown in Fig. 3 compared to that of PNIPAm hydrogel.

The percentage equilibrium swelling value ($S_e\%$) of the hydrogels was calculated from the following relation;

$$S_e\% = \left(\frac{m_e - m_0}{m_0}\right) \times 100,$$

where m_e is the mass of swollen gel at equilibrium time and m_0 is the initial mass of the dry gel.

It is known that PNIPAm does not exhibit pH dependent swelling behavior because it does not consist of ionizable groups [43]. This feature was also observed during the swelling experiments in this study at both temperatures (Fig. 3). However, it was noticed that the swelling of PNIPAm–CMC IPN hydrogels was strongly dependent on the pH value of the external medium. The pK_a value of CMC is about 4.6 [45]. So the carboxylic groups are ionized at pH $>$ 4.6, while at pH $<$ 4.6 they will be protonated. At higher pHs (above the pK_a of the carboxylic groups), the electrostatic repulsive force between the charged sites (COO^-) causes an increase in swelling. Under acidic pHs (pH $<$ 4.6), most of the carboxylate anions are protonated, so the main anion–anion repulsive forces are eliminated and consequently swelling values are decreased [45–47]. At pH ranges of 4.6–6.5, the swelling ratio of PNIPAm–CMC hydrogels drastically decreased with increasing pH, due to intermolecular complexation via hydrogen bonds (physical crosslinking). As a result, a remarkable decrease in equilibrium swelling is observed (gel collapsing) in this range. It can be said that PNIPAm–CMC IPN hydrogels behaved as polyelectrolyte hydrogels in the pH range 3–9. Polyelectrolyte hydrogels usually exhibit a variation of their equilibrium swelling degree as a function of pH according to the acid–base equilibrium for a weak acid: in an acidic solution, most of the acidic units remain in protonated form while they are ionized under basic conditions [48, 49].

Poly(*N*-isopropyl acrylamide) smart gel is a typical temperature-sensitive gel exhibiting a volume phase transition at approximately its lower critical solution temperature (LCST), i.e., at 34 °C. Below this temperature, the gel swells and it shrinks as the temperature is raised [50]. It has been illustrated in Fig. 3 that both hydrogel samples are temperature-sensitive. At 4 °C, the nonionic PNIPAm gel is below its LCST and it swells, but there was no big change in the equilibrium degree of swelling with pH, as was expected. Hydrogen bonds exist between monomeric units and water molecules in a swollen hydrogel. When the temperature increases above the LCST, these hydrogen bonds disrupt and the hydrogel collapses due to the hydrophobic interaction between the isopropyl groups of the monomers. It was observed that PNIPAm–CMC hydrogels are more temperature-sensitive than PNIPAm hydrogels owing to the effects of hydrophobic groups in CMC. When the hydrophobic interactions between PNIPAm and CMC increased, the hydrogel appeared to be hydrophobic. Similar swelling-temperature dependencies have been reported in the case of other hydrogel systems [36, 51].

Figure 4 illustrates the temperature dependence of the equilibrium swelling ratio of the hydrogels in water when the temperature increased from 11 to 60 °C. As shown in Fig. 4, both hydrogels exhibited phase transitions with increasing temperature. The equilibrium swelling ratio of the hydrogels first decreased drastically up to 11 °C (shrinking case) and remained almost constant in the range of 40–60 °C (collapse case). $S_e\%$ values of PNIPAm–CMC hydrogel with 5.0 wt% CMC were lower with respect to that of PNIPAm hydrogel due to the increase in hydrophobic interactions mentioned above.

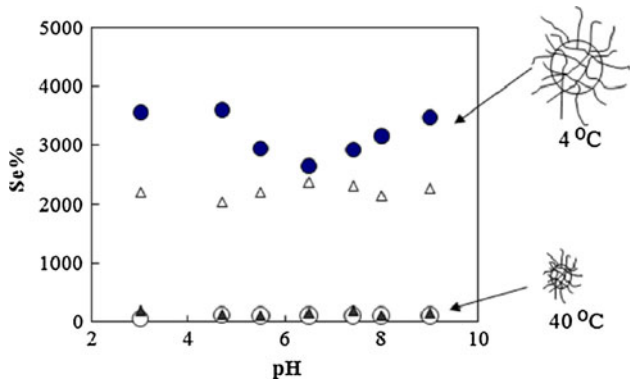


Fig. 3 Equilibrium swelling ratio of temperature-sensitive PNIPAm and PNIPAm–CMC hydrogels as a function of pH, *open triangle* PNIPAm (4 °C), *filled triangle* PNIPAm (40 °C), *filled circle* PNIPAm–CMC (4 °C), *open circle* PNIPAm–CMC (40 °C)

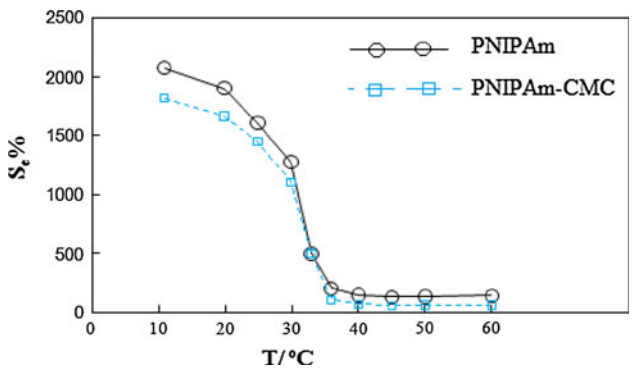


Fig. 4 Variation of equilibrium swelling ratio of PNIPAm and PNIPAm–CMC hydrogels

Ionic strength and bovine serum albumin-sensitive swelling studies

Hydrogels can exhibit phase transitions in response to changes in external conditions such as pH, ionic strength, temperature, electric currents, etc. In this respect, the equilibrium swelling degrees of PNIPAm and PNIPAm–CMC hydrogels were measured in salt (NaCl, $I = 0.01$ mol dm^{-3}, pH 4.7) and BSA (BSA, 100 mg dm^{-3}, pH 4.7) solutions at 4 and 40 °C, and are represented in Fig. 5. In NaCl and BSA solutions, swelling degrees were found to be lower for all hydrogels than in buffer solutions at both temperatures. Solute molecules also influence the swelling behaviors of the hydrogels by hindering penetration of water molecules through the gel [52, 53]. When the ionic strength of the solution is changed, the hydrogel can exchange ions with the solution. By doing so, the hydrogel maintains charge neutrality and the concentration of free counter ions inside the hydrogel increases. An osmotic pressure difference between the hydrogel and the solution arises and causes the gel to swell. When the ionic strength is increased to high levels, the hydrogel will shrink. This is due to the decreasing osmotic pressure difference between the gel and the solution [47].

pH- and temperature-sensitive bovine serum albumin adsorption

Proteins are well-known amphoteric molecules capable of existing in solution as both cations (pH < *pI*), anions (pH > *pI*) and as zwitterions (pH ≈ *pI*), its negative charge increases with an increase of pH and its positive charge increases with a decrease of pH (Scheme 2) [54, 55]. BSA was chosen as a model protein because it is well characterized and commonly used in protein adsorption studies [29, 33, 56–60]. PNIPAm and PNIPAm–CMC hydrogels were used for adsorption of BSA at different pHs (3.0, 4.7, 5.5, 6.5, 7.4, and 8.0) and temperatures (4 and 40 °C). The ionic strength of buffer solutions was constant

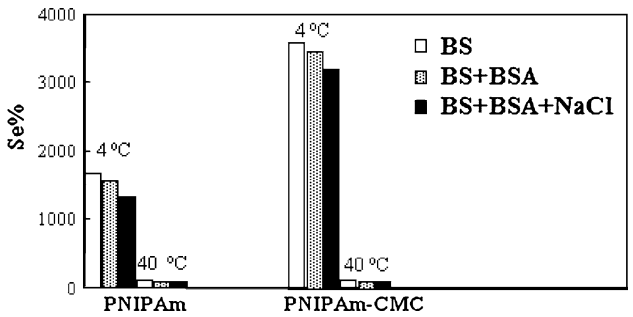
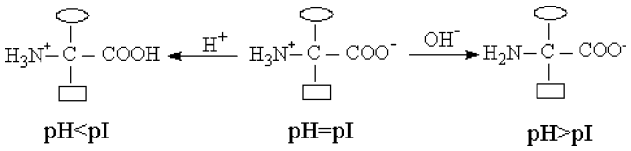


Fig. 5 Effect of ionic strength and BSA on swelling behaviors of PNIPAm and PNIPAm–CMC hydrogels at different temperatures, BS refers acetate buffer solution with pH 4.7



Scheme 2 The change of proteins charge with pH. BSA behaves neutral at pH ≈ *pI*, cationic at pH < *pI*, and anionic at pH > *pI*

($I = 0.01$ mol dm^{-3}) during these adsorption studies. The experiments were conducted at the same initial BSA concentration (100 mg dm^{-3}).In an adsorption system at equilibrium, total solute concentration (C_0, mg dm^{-3}) is

$$C_0 = C_B + C,$$

where, C_B is the equilibrium concentration of the solute on the adsorbent in milligram per liter (bound solute concentration) and C is the equilibrium concentration of the solute in the solutions in mg dm^{-3} (free solute concentration). The binding ratio, X, defined by

$$X = \left(\frac{C_0 - C}{m}\right) \times V,$$

where V is the volume of protein solution and m is the mass of the dry hydrogels, i.e., the adsorbent.

The adsorption curves of BSA at different pH values are shown in Fig. 6. The pH of the medium and chemical structure of the adsorbent can affect protein adsorption. At pH < *pI*, where most of the carboxylate groups (–COO$^-$) on the BSA and CMC converted to the carboxyl group (–COOH) (Scheme 2), BSA carries positive charges and adsorption almost does not occur. The maximum BSA adsorption for both hydrogels was obtained at pH 4.7, which is the isoelectric point of BSA (*pI* 4.7). Proteins do not change at their isoelectric points and so the protein solubility in the aqueous media decreases. However, an acidic or basic medium causes the protein to become

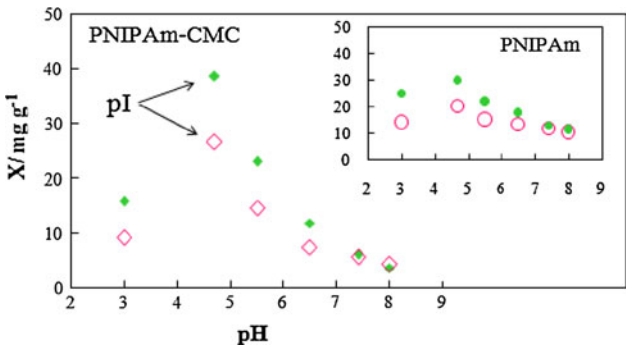
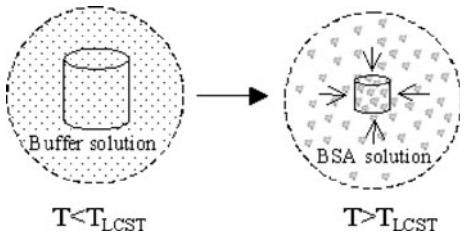


Fig. 6 Effect of CMC, solution pH, and temperature on the protein adsorption of the PNIPAm hydrogel. The *open symbols* represent 4 °C and the *filled symbols* represent 40 °C, the equilibrium concentration of BSA was 100 mg dm^{-3}

positively or negatively charged, increasing the solubility of the protein in the aqueous media. Therefore, lower or higher pH values than isoelectric point resulted in decreased BSA adsorption onto the adsorbent. BSA adsorption can be governed in several ways: hydrophobic binding arises from hydrophobic groups of adsorbent and protein molecules, hydrophilic interactions between adsorbent and protein molecules, or conformational alteration of BSA molecules [38, 61]. At *pI* value any hydrophobic groups such as tryptophan, methionine, alanine, phenylalanine, leucine, and proline on the BSA molecule and the isopropyl and methyl groups on the PNIPAm and PNIPAm–CMC adsorbents were responsible for introducing the hydrophobic binding. In addition, conformational alteration of BSA molecules has some effect on adsorption. Conformational alteration of the protein molecule is not affected at the isoelectric point and the structure of BSA remains in its compact state [53, 62]. It may be said that this position could facilitate the adsorption. Thus, the maximum adsorption of BSA from aqueous solutions was observed at the isoelectric point. Some hydrophilic groups such as amide, carbonyl, and hydroxyl in the hydrogel-BSA system are responsible for hydrophilic interactions. Therefore, some degrees of hydrophobic and/or hydrophilic interactions play a certain role in this case. This implies that binding of BSA to the hydrogel adsorbent was dependent upon the cooperative effects of hydrophobic and hydrophilic interactions. At adsorption pH $>$ 5, the carboxyl groups increasingly dissociate, resulting in an increase of the negative charges on the adsorbent; thus, the negatively charged BSA molecules (pH $>$ *pI*) were retained less on the adsorbent as the adsorption pH increased. It appeared that electrostatic repulsion contributed significantly to the decrease of binding capacity at higher pH values. Similar adsorption results realized at around pH 4.7 have been documented in other BSA adsorption studies in the literature [38, 53, 61, 63]. From Fig. 6 it is seen that BSA adsorption onto hydrogels is temperature-sensitive. Adsorption capacities (*X*, mg BSA g^{-1} gel) of the hydrogels were higher at 40 °C than at 4 °C. This is because PNIPAm becomes more hydrophobic at temperatures above the LCST. Therefore, the hydrophobic interaction leads to a higher binding of BSA. Hydrophobic surfaces tend to have strong interactions between protein and polymer, and result in significant conformational changes in the protein. A schematic diagram showing the effect of temperature on BSA adsorption by hydrogels is given in Scheme 3.

Adsorption capacities of PNIPAm and PNIPAm–CMC hydrogels at both temperatures are also given, respectively, in Fig. 7. Introduction of CMC polymer chains into the PNIPAm increased the adsorption capacity of PNIPAm hydrogel at both temperatures.



Scheme 3 Illustration of BSA adsorption onto temperature-sensitive hydrogels

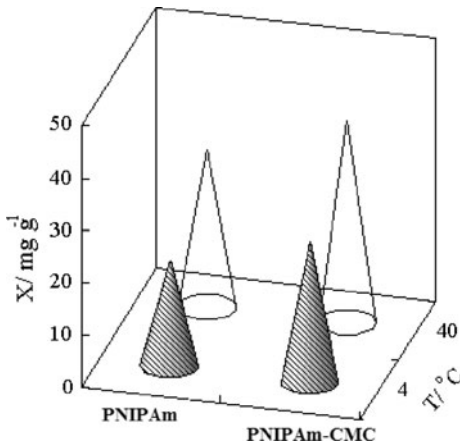


Fig. 7 Presentation of *X* parameters of PNIPAm and PNIPAm–CMC hydrogels comparatively as a function of temperature. Adsorbed amounts of BSA; for PNIPAm–CMC full IPN hydrogels are 26.70 mg g^{-1} (4 °C) and 38.70 mg g^{-1} (40 °C) and for PNIPAm hydrogels are 20.10 mg g^{-1} (4 °C) and 30.0 mg g^{-1} (40 °C)

Desorption studies

Hydrogels loaded with BSA (pH 4.67, $I = 0.01$ mol dm^{-3}) at 40 °C were used in desorption studies. Samples were left for 5 h in 0.1 mol dm^{-3} NaSCN solution (pH 8.0) at 18 °C. The amounts of eluted BSA were found to be 90 and 92% for PNIPAm and PNIPAm–CMC hydrogels, respectively. NaSCN is a suitable desorption agent for protein elution. SCN^-, being a chaotropic ion, disorganized the structure of water thus stimulating the elution of protein [64].

Conclusion

In this article, the aim was to obtain PNIPAm–CMC full IPN hydrogels by combining synthetic and/or natural polymers to provide control of their properties and to expand their applicability. Characterization and protein adsorption studies of PNIPAm–CMC hydrogels prepared in full IPN form were investigated and compared with crosslinked PNIPAm hydrogels. PNIPAm–CMC full IPN

hydrogels exhibited a higher thermal stability than cross-linked PNIPAm hydrogels. The stimuli-responsive swelling behaviors of these hydrogels was investigated at different pHs and temperatures, and it was concluded that synthesized PNIPAm–CMC full IPN hydrogels exhibited sensitivity to both stimuli while PNIPAm hydrogels exhibited only temperature-sensitive behavior. The swelling of the absorbents in saline and protein solutions was appreciably decreased compared to values measured in buffer solutions. Synthesized environmental gels were created to adsorb protein (BSA) due to their dual stimuli response and adsorption experiments onto these hydrogels confirmed that the interaction is temperature- and pH-sensitive. The binding ratios (X, mg BSA g^{-1} gel) of PNIPAm–CMC full IPN hydrogels were 26.70 and 38.70 mg g^{-1} at 4 and 40 °C, respectively, while the X values of PNIPAm were 20.10 mg g^{-1} at 4 °C and 30.0 mg g^{-1} at 40 °C. The study shows that BSA adsorption can be achieved by using suitable PNIPAm–CMC adsorbents in form full IPN and through proper control of the solution pH and temperature values.

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