Polyacrylamide-Grafted Carboxymethyl Cellulose: Smart pH-Sensitive Hydrogel for Protein Concentration

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Received 26 February 2010; accepted 25 August 2010
DOI 10.1002/app.33283
Published online 27 April 2011 in Wiley Online Library (wileyonlinelibrary.com).

ABSTRACT: A novel application, utilizing polyacrylamide-g-carboxymethyl cellulose (CMC-g-PAM) in concentrating dilute solutions of Bovine serum albumin (BSA), was investigated. The grafting reaction parameters were investigated and the hydrogel smartness was verified. FT-IR proved that the grafting reaction occurred between the hydroxyl group located in anhydroglucose C_2 position of CMC and the π-bond of PAM and SEM confirmed a changed morphology to a fibrillar structure. The pH sensitivity was proved; as the grafted polymer attained its maximum swelling at pH 7.2 while the minimum swelling was observed under acidic conditions (pH 1-3). The rate of water uptake in the grafted polymer hydrogel was higher than that of the homopolymer hydrogel and the swelling behaviors of both hydrogels obeyed second-order kinetics. The tested hydrogel showed a high potency towards concentrating BSA solutions with a concentration factor of 1 to 4.5 times and recovery of 60–90%. The concentration factor increased linearly with increasing both the polymer concentration and the process time and decreased with the increase in the protein concentration. The grafted polymer had stable efficiency in the concentration process for 20 cycles. The obtained results have recommended the employment of the prepared CMC-g-PAM hydrogel in the down stream protein concentration process in the industrial scale. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 122: 469–479, 2011

Key words: carboxymethyl cellulose; graft copolymer; polyacrylamide; hydrogel; protein separation

INTRODUCTION

A great interest in natural-based superabsorbent hydrogel has increased recently, mainly due to high hydrophilicity, biocompatibility, nontoxicity, and biodegradability of biopolymers. These materials are characterized with crosslinked macromolecular networks that can absorb water or physical fluids up to many times of their own weight in a short time without dissolution when directly being in contact with the medium.[1] Removal of the absorbed fluids is hard even with applying pressure. According to their excellent characteristics, superabsorbent hydrogels are widely used in many technological and biotechnological fields, such as disposable diapers, feminine napkins, pharmaceuticals medical applications and agricultural and horticultural.[2–4]

Due to their exceptional properties such as biocompatibility, biodegradability, renewability, and nontoxicity, polysaccharides are used as the core component of the natural-based superabsorbent hydrogels. Carboxymethyl cellulose (CMC), an anionic water-soluble polysaccharide, is the most modified cellulose, which can be used in various fields such as detergent, food, paper, and textile industries. CMC is a reaction product of cellulose with sodium hydroxide and chloroacetic acid. It has a number of sodium carboxymethyl groups (-CH_2COONa), introduced into the cellulose molecule, which promote its water solubility; Scheme 1. Among all the polysaccharides, CMC is easily available, very cheap and has high shear stability. The use of crosslinked (CMC), in gel formulations using grafting technique with selected monomers, mainly polyacrylamide, in addition to other techniques has been reported recently.[5–11]

Most of ionic hydrogels usually undergo volume changes in response to a little change in environmental conditions such as heat, pH, electric field, chemical environments, etc. The hydrogels that sensitive to external stimuli are often referred to as intelligent and smart hydrogels. They have important applications in the field of medicine, pharmacy, and biotechnology. Among these, pH-sensitive hydrogels have been extensively investigated for the potential use in site-specific delivery of drugs to specific regions of the gastrointestinal tract and have been prepared for the delivery of low molecular weight protein drugs.[12,13]

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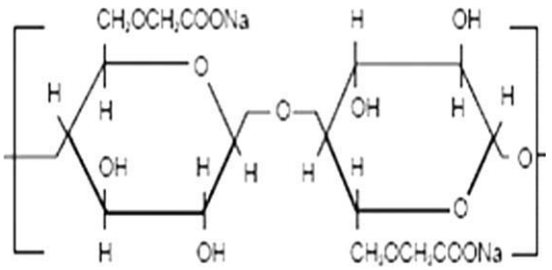

Scheme 1 The structure of carboxymethyl cellulose.

This article investigated the preparation and the swelling of novel smart pH-sensitive of CMC hydrogel by grafting crosslinked polyacrylamide chains onto CMC backbone through free radical polymerization technique. According to the best of our knowledge, the prepared hydrogels have been evaluated, for the first time, in the process of bovine serum albumin (BSA) concentration.

MATERIALS AND EXPERIMENTAL TECHNIQUES

Materials

Carboxymethyl cellulose (CMC), (M.Wt. 90,000, DS = 0.7), was purchased from New Jersey (USA) in the form of sodium salt. Acrylamide, 97% (AM), *N,N'*-methylene-bis-acrylamide, 99% (MBA) as a crosslinker, ammonium persulfate, 98% (APS) as an initiator and bovine serum albumin (BSA) were purchased from SIGMA-ALDRICH CHEMIE GmbH, Germany. All chemicals were of analytical grade and were used without any further purification.

Synthesis of CMC-g-PAM hydrogel

The grafting of CMC was carried out in 100-mL stopper flask. The polymerization was done with 3-8% monomer concentration. The reaction was initiated with 0.06% APS and the polymer was crosslinked with 0.016-0.064% MBA. All the concentrations were based on the weight of CMC. The reaction temperature was 55°C and the CMC: liquor ratio was 1 : 50. At the end of the desired reaction period (about three hours), the product was thoroughly washed with acetone, dried at 60°C and then crushed and sieved to have particles size in the range (500 μm-1 mm). The synthesis of the homopolymer (PAM) was performed using the same conditions of the grafting reaction but without using of CMC.

The percentage of grafting (%G) and grafting efficiency (%*E*)[14] were calculated as follows:

$$\%G = A/B \times 100 \quad (1)$$

$$\%E = C/D \times 100 \quad (2)$$

where A is the mass of AM in the graft polymer, B is mass of original CMC, C is the mass of graft polymer, and D is the mass of (polymer + AM).

The percentage conversion of AM (%*C*) was calculated as follows:

$$\%C = A/M \quad (3)$$

where M is the mass of feeding acrylamide monomer used in the grafting process, and the mass of grafted acrylamide in the graft copolymer was calculated as the mass of graft minus the mass of CMC taken.

Grafting verification

Physicochemical characteristics of synthesized PAM and PAM-g-CMC copolymers were studied using Fourier Transform Infrared Spectrophotometer (Shimadzu FTIR-8400 S, Japan) and Thermogravimetric Analyzer (Shimadzu TGA -50, Japan). Morphological characteristics were followed using analytical Scanning Electron Microscope (SEM) (Joel JSM 6360LA, Japan).

Swelling experiments

Swelling study was conducted on PAM and PAM-g-CMC hydrogels as a function of time.[15,16] Dry sample of hydrogel was immersed in distilled water (pH7.2) at 25°C for different intervals of time up to 8 h. After each time interval, the sample was withdrawn and blotted on filter paper to remove excess water and then weighed. At least three swelling measurement were performed for each sample and the mean value was reported. The swelling of hydrogel can be determined as a function of time as follows:

$$\text{Swelling} = (W_s - W_o)/W_o \quad (4)$$

where W_s is the weight of the swollen hydrogel sample at time t, and W_0 is the weight of the dry hydrogel sample.

For the swelling experiments, buffer solutions were prepared by mixing known amounts of *ortho*-phosphoric acid, disodium hydrogen phosphate, and potassium dihydrogen phosphate to vary the pH, and the ionic strength was kept constant at 0.1*M* by using NaCl. Also, hydrochloric and bicarbonate buffer solutions were used in case of high acid and basic conditions, respectively. The pH values were precisely checked by a pH meter (Metrohm/620, accuracy ± 0.1).

Protein concentration process

The desired amount of polymer (1-5mg/mL) was weighed and placed in a beaker contains an appropriate volume ($V_o = 100$ mL) of an aqueous BSA solution (concentrations; 0.5-3mg/mL, C_o) with the desired pH (3-7.2) at 25°C and left without stirring. After 30 min the final residue volume (V_f) of BSA solution was filtered and measured in a graduate glass. Water between the gel particles was removed using filter paper. After centrifuging the residue BSA solution samples, the final concentration of BSA (C_f) was measured at $\lambda = 280$ nm. Experiments were performed at room temperature (20°C).

The concentration process was analyzed using the following process and performance variables[17]:

$$\text{Concentration factor}: C_F = C_f/C_o \quad (5)$$

$$\text{Recovery}(\%): R = (100V_fC_f)/(V_oC_o) = 100 \times C_F \times (V_f/V_o) \quad (6)$$

The standard deviation in all measurements was close to zero. The total uncertainty for all experiments ranged from 3 to 6%.

RESULTS AND DISCUSSION

Grafting polymerization

The copolymerization mechanism of acrylamide grafting onto polysaccharides, using persulphate as the initiator, has been well studied.[18,19] The persulphate initiator is decomposed under heating to generate sulfate ion radicals, which are well known as free radicals chain carriers. The hydrogen radical is abstracted from the hydroxyl group of the polysaccharide to form alkoxy radicals on the substrate. Therefore, this persulphate- saccharide redox system results in active centers on the substrate, which initiate a radical polymerization of acrylamide and lead to a graft copolymer.[20]

PAM-g-CMC hydrogels, with different grafting percentages and varied crosslinking degrees, have been prepared and characterized for further application in the concentration of BSA solution.

Effect of monomer concentration

Table I shows the effect of monomer (AM) concentration on the percentage of grafting, grafting efficiency, percentage conversion, and swelling. With the increase in the monomer concentration, the %*G* increased continuously, as expected. This behavior may be explained by the fact that an increase in monomer concentration leads to the accumulation of monomer molecules in close proximity to the immobile cellulose macroradicals and a greater availability of grafting sites for the monomer. The greater availability of monomer in the polymerization medium acts in favor of molecular collision, thereby enhancing polymerization in general or homopolymer formation in particular. The decreased in the percentage of graft at more than 7% monomer concentration suggests that (a) homopolymerization prevailed over grafting at a high monomer concentration (℘7%), (b) an increase in the viscosity of the medium, which hindered the movement of free radicals and monomer molecules, and (c) the enhanced chance of chain transfer to monomer molecules.[21] It is worth mentioning that these values of swelling are 100-fold higher than those obtained for *N*-vinylformamide-g-CMC hydrogels,[22] (*N*-vinylformamide is the structural isomer of acrylamide). The enhanced swelling gave our system a priority in improving the efficacy of the protein concentration process as it can be seen in the section Factors affecting protein concentration.

TABLE I
Effect of Monomer (AM) Concentration on the Percentage of Grafting, Grafting Efficiency, Percentage Conversion, and Swelling[a]

AM %	%*G*	%*E*	%*C*	Swelling (g/g)
3	24.28	73.91	75.85	56
4	38.57	84.11	79.61	67
5	48.57	85.78	81.08	80
6	64.28	88.23	84.5	110
7	90.0	95.45	93.85	133
8	87.14	85.91	88.3	130

[a] Grafting conditions: 7% CMC, 7% AM, 0.06% APS at 55°C for 3 h.

Effect of crosslinker concentration

The crosslinking reaction plays an important role in the formation of three dimensional network structures permanently to the hydrogels in the polymerization process. This is also a promising factor directly affecting the swelling ratio of the superabsorbent polymers. The relationship between the swelling ratio and network structure parameters was given by Flory[23] and is usually used as follows:

$$q_m^{5/3} = [(i/2v_\upsilon S^{1/2}) + (1/2 - \chi_1)/v_1]/v_c/V_o \quad (7)$$

where q_m is swelling ratio, i/v_υ is the concentration of the fixed charge of the nonswollen networks, S is the ionic strength of the swollen solution, and v_c/V_o is the crosslinking density that refers to the number of effectively crosslinked density in the unit volume. The expression $([1/2]\text{-}\chi_1)/v_1$ represents the network-medium affinity. The swelling as a function of MBA concentration, for the superabsorbent hydrogels, was

TABLE II
Effect of Methylene-bis-Acrylamide (MBA) Concentration on the Percentage of Grafting, Grafting Efficiency, Percentage Conversion and Swelling[a]

MBA%	%G	%E	%C%	Swelling (g/g)
0.016	82.8	87.69	92.64	119
0.024	85.7	91.32	93.53	126
0.032	90.0	95.45	93.85	140
0.048	78.57	84.09	92.79	111
0.064	75.7	80.91	92.72	92

[a] Grafting conditions: 7% CMC, 7% AM, 0.06% APS at 55°C for 3 h.

investigated as shown in Table II. The results were consistent with Flory's equation, i.e., high values of swelling were obtained at the low concentration of the crosslinker (low crosslinking density). By increasing the MBA concentration more than 0.032%, the swelling decreased which may be attributed to the fact that crosslinking hinders the mobility of the polymer chains and reduces the pore size; thus lowering the swelling.[24] Besides, increasing the crosslinker concentration enhanced the grafting percentage and efficiency to a certain limit, and then both of them had declined due to the formation of homopolymer. The relatively insignificant change in the above-mentioned factors may suggest that the crosslinking reaction rather than the increase in the percentage grafting was the reason of the swelling behavior.

Grafting verification

FTIR spectroscopy

The IR spectra of CMC, PAM, and CMC-g-PAM are shown in Figure 1(a–c), respectively. From the IR spectra of CMC, it showed a broad absorption band at 3444 cm^{-1}, due to the stretching frequency of the —OH group. The band at 2921 cm^{-1} was due to C—H stretching vibration. Appearance of a strong absorption band at 1618 cm^{-1} was due to the presence of COO^- groups. The bands around 1423 and 1326 cm^{-1} were assigned to CH_2 scissoring and —OH bending vibration, respectively. In the case of PAM, a broad absorption band at 3431 cm^{-1} was for the *N*—H stretching frequency of the NH_2 group. Two strong bands around 1689 and 1647 cm^{-1} were due to amide-I (C=O stretching) and amide-II (NH bending), respectively. The bands around 1400 and 2922 cm^{-1} were for the C—N and C—H stretching vibrations, respectively. Other bands at 1458 and 1323 cm^{-1} were attributed to CH_2 scissoring and CH_2 twisting. For IR spectrum of CMC-g-PAM, The presence of a broad absorption band at 3434 cm^{-1} was due to the overlap of —OH stretching band of CMC and —NH stretching band of PAM. A band at 1652 cm^{-1} was due to amide-I (C=O stretching) of the amide group of PAM and the band at 1618 cm^{-1} of CMC and amide-II band of PAM overlapped with each other and led to a broad band at 1628 cm^{-1}. The presence of a band at 1733 cm^{-1} was due to free acid groups. The bands around 1404 and 2922 cm^{-1} were for the C—N and C—H stretching vibrations, respectively. Other bands at 1458 and 1338 cm^{-1} were attributed to CH_2 scissoring and CH_2 twisting. Also, there was an important peak at 1068 cm^{-1} which assigned for the CH—O—CH_2 group resulting from grafting reaction between the hydroxyl group located in anhydroglucose C_2 position and the π-bond of PAM. The primary peaks existed in the CMC-g-PAM characteristic for the groups of AM, and the shift in the band corresponding to OH group, may suggest formation of ether (>CH—O—CH_2) during the grafting copolymerization.[25] Accordingly, it is apparent that FTIR presented a strong evidence of grafting of PAM branches onto the polysaccharide backbone; (since homopolymers were removed by solvent extraction).

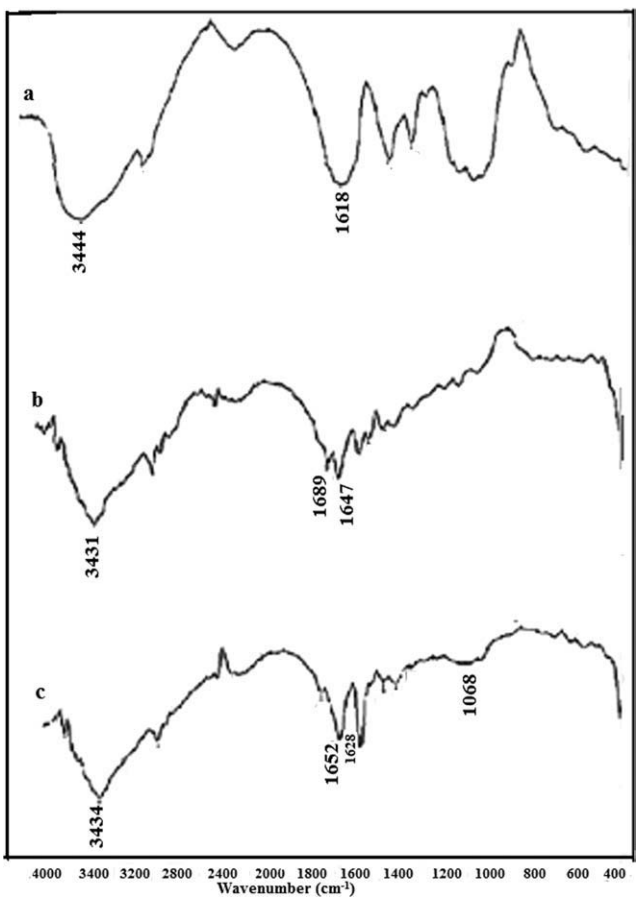

Figure 1 FTIR of (a) CMC, (b) PAM, and (c) CMC-g-PAM.

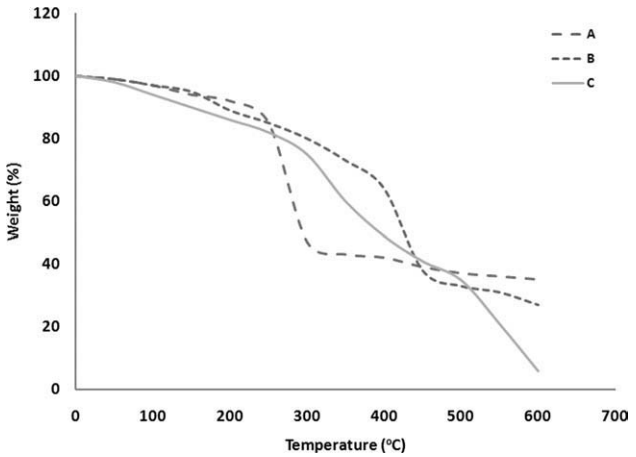

Figure 2 TGA of (a) CMC, (b) PAM, and (c) CMC-g-PAM.

Thermogravimetric analysis

Thermogravimetric curves of CMC, PAM, and CMC-g-PAM in a nitrogen atmosphere are displayed in Figure 2. In the case of PAM, a continuous weight loss starting at the beginning of the heating was observed, and at least four thermal events. Above 240°C the degradation of PAM is due to loss of ammonia with the formation of imide groups via cyclization.[26] Ammonia and water are the only volatile products below 340°C in PAM.[27] Decomposition of the cyclic product was observed starting from 380°C.[26]

In general, the main decomposition of the polysaccharides starts above 200°C. The first stage was attributed to desorption of moisture as hydrogen bound water to the polysaccharide structure. The second and third stages of decomposition took place at 241 and 307°C, respectively, probably due to depolymerization with formation of water, CO, and CH_4.[28]

The pattern of copolymer thermal decomposition, exemplified by CMC-g-PAM, is different from those for the starting materials (CMC and PAM). The graft decomposition was observed in at least four stages.

The presence of PAM chains grafted onto CMC provoked a reduction in residue at 600°C, from 37% (CMC) to 2.2–4.0% (grafts). This behavior has been observed for sodium alginate[29] and cashew gum[28]-grafted polyacrylamide. The grafting of PAM chains onto the polysaccharide did not cause a significant change in the PAM thermal stability, but enhanced the CMC resistance to heat; perhaps due to the crosslinked nature of the homopolymer. This can also be proved by the T_{50} (the temperature at which the half weight loss occurs) which was 297, 417, and 391°C for CMC, PAM, and CMC-g-PAM, respectively.

Scanning Electron Microscope

The scanning electron micrographs of CMC, PAM, and the graft copolymer (CMC-g-PAM) are shown in Figure 3. It is clear that the morphological structure of both PAM and CMC differed from CMC-g-PAM. Surface morphology of CMC before grafting showed a granular structure, which has been changed to fibrillar form after grafting. It became more close to PAM morphology. Thus, the comparison of these figures reveals that grafting has taken place.

Hydrogel swelling parameters

The diffusion process of solutions into the hydrogels can be estimated by studying the swelling behavior of the polymers. The diffusion process generally represents the affinity between the polymer networks and an external solution. The absorption of external solutions can be balanced by three main forces: (1) free energy between chain networks of the polymers and external solvent; (2) electrostatic repulsion (domain effect); (3) elastic response of the networks. Among these three factors the first two forces promote the swelling behavior and the third one suppresses the swelling phenomena of the hydrogels.[30] The absorption of hydrogels depends on the strength of the hydrophilic groups, crosslinking density, polymer network behavior, and elasticity of the polymer networks, type of solvent, and strength of the external solution as well as the characteristics of the external solution, etc. The key properties of superabsorbent polymers are swelling capacity and the elastic modulus of the swollen crosslinked gel.[31] Both of these properties are related to the crosslink density of the networks of the gel. To improve the swelling capacity of the as-prepared grafted copolymer, various reaction parameters were employed. The complete details of the influence of the swelling conditions are given below.

Effect of swelling pH

Superabsorbent hydrogels exhibit swelling changes for a wide range of pH. Therefore, in this series of experiments, equilibrium swelling for the prepared hydrogels, as shown in (Fig. 4), was studied at various pH solutions ranged from 1.0 to 11. No additional ions (pH adjustment was performed via buffer solutions) were added to the medium for setting pH because the absorbency of a superabsorbent is strongly affected by the ionic strength. In addition, it has been reported that the swelling properties of polybasic gels are influenced by the buffer composition and pK_a. A progressive increment of swelling was observed with increase of pH from 3.0 to 7.0 where a maximum swelling of 141 (g/g) was obtained. Such observed sensitivity to pH variation is in agreement with results published by Sunil and Seema[32] in which they studied the effect of pH

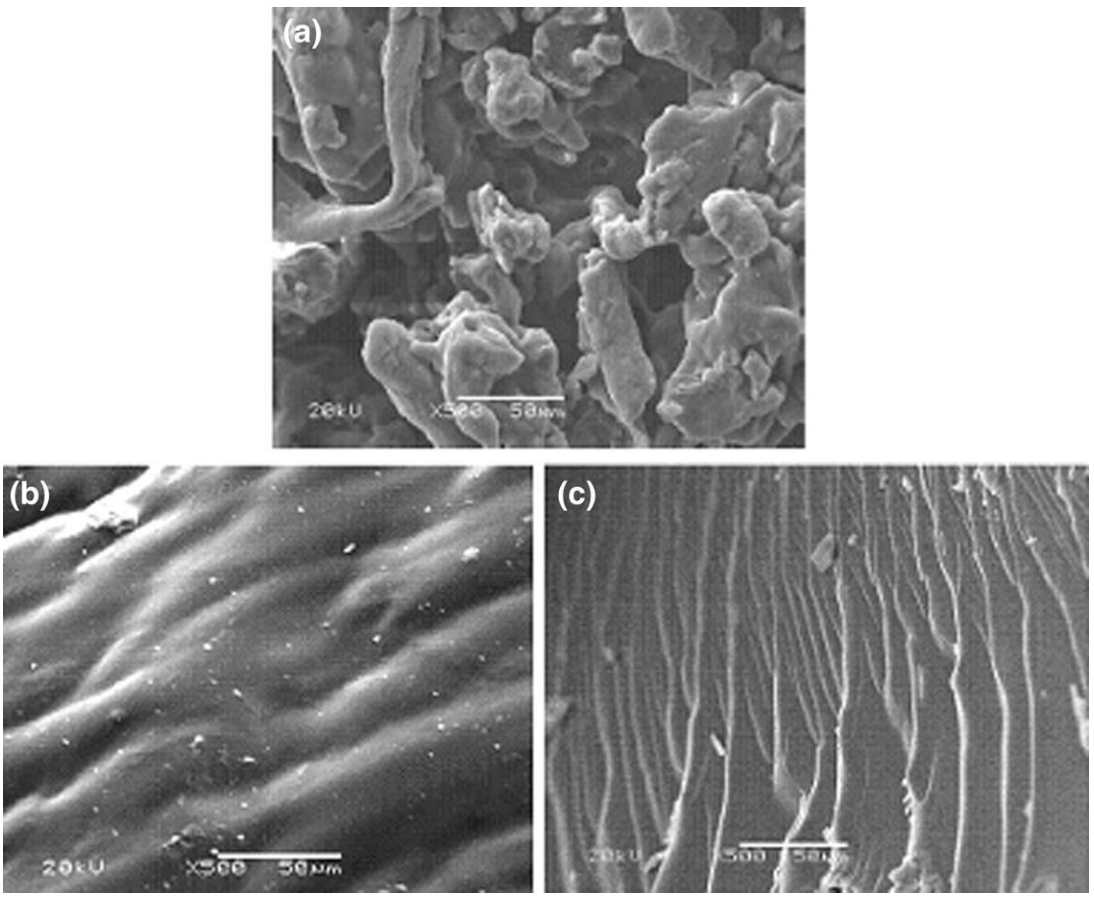

Figure 3 SEM of (a) CMC, (b) PAM, and (c) CMC-g-PAM.

variation on the swelling of pH-sensitive terpolymeric hydrogel system composed of acrylamide, methacrylamide, and acrylic acid. These terpolymeric hydrogels exhibited a fair pH-dependent swelling behavior as the pH of the swelling medium varied from 1.0 to 8.0. The minimum swelling in the acidic pH could be explained on the basis of the formation of complex structures within the gel network due to H-bonding interactions between —COOH and —$CONH_2$ groups. These interactions resulted in the formation of a compact or tight structure which did not permit much movement of polymeric segments within the hydrogel network. Also, —COOH groups within the network remained almost nonionized; thus imparting almost nonpolyelectrolyte behavior to the gel.[33] As the pH of the swelling medium approached neutrality, the degree of swelling increased. In the medium of pH 7.2, the almost complete ionization of —COOH groups resulted in an extensive chain relaxation due to repulsion among similarly charged —COO^- groups present along the macromolecular chains. Moreover, this dissociation also caused an increase in ion osmotic swelling pressure. These two factors were thus responsible for the high degree of swelling in the medium of pH 7.2.

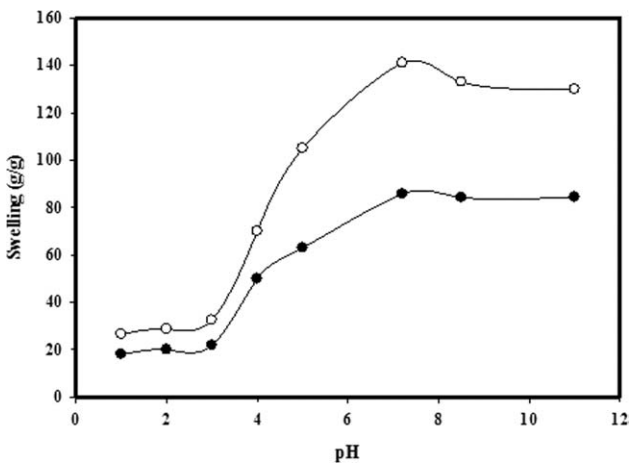

Figure 4 Equilibrium swelling of CMC-g-PAM after (●) 30 min and (O) 3 h at various pH and at 25°C.

Effect of ionic strength

It is important to understand the osmotic and structural changes of hydrogels, induced by addition of salt with respect to many physical and chemical processes in biological systems. Figure 5 presents the effect of the swelling medium ionic strength on the

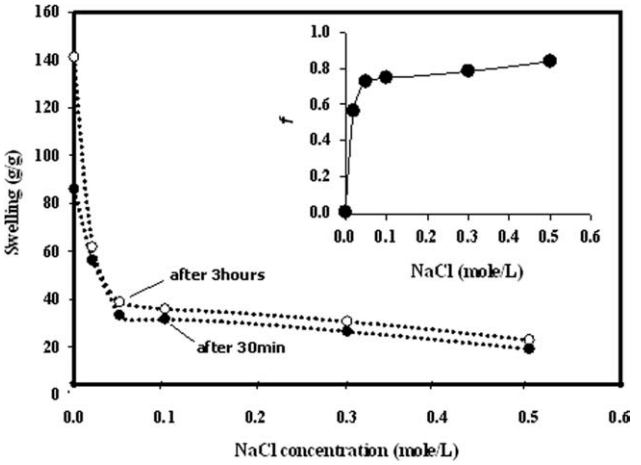

Figure 5 Effect of ionic strength on the CMC-g-PAM swelling after 30 min and 3 h. The inset is the relation between the dimensionless swelling factor, *f*, and the ionic strength.

CMC-g-PAM swelling capacity. The swelling of the CMC-g-PAM hydrogels in saline solutions was appreciably reduced compared with the values measured in distilled water. This well-known phenomenon, commonly observed in the swelling of ionic hydrogels, is often attributed to a screening effect of the additional cations; causing a nonperfect anion-anion electrostatic repulsion, leading to a reduced osmotic pressure (ionic pressure) difference between the hydrogel network and the external solution.

This behavior was found by many authors for different hydrogel systems.[32,34] Moreover, hydrogels, composed of psyllium and polyacrylamide, did not swell appreciably in the presence of electrolyte salts due to ex-osmosis and even the swollen hydrogels shrank dramatically in the presence of salts.[24]

To make a comparative measurement of the sensitivity of the hydrogels to the sort of aqueous fluid, Kabiri et al.[29] have defined a dimensionless swelling factor, *f*, as follows:

$$f = 1 - (\text{Absorption in a given fluid/absorption in deionized water}) \quad (8)$$

Large *f* value means low swelling degree in salt solutions. Therefore, hydrogels with low *f* are usually preferred. Negative values of *f* reveal that the absorbency increases in salt solutions. The hydrogels with betaine structures exhibited such surprising behavior.[35] The *f* values are given in the inset of Figure 5. The effect of increasing cation concentration on the ultimate absorption for the CMC-g-PAM hydrogels can be found from the *f* values, so that the lower the cation concentration, the lower the salt sensitivity is.

Swelling kinetics of PAM and CMC-g-PAM hydrogels

The phenomenon of water sorption by hydrogel depends mechanistically on the diffusion of water molecules into the gel matrix and subsequent relaxation of macromolecular chains of the hydrogel. Water transport mechanism in a swelling hydrogel is greatly contributed by numerous factors such as equilibrium water content, swelling rate, chemical composition of the hydrogel system, etc.[23]

The swelling kinetics of PAM and CMC-g-PAM hydrogel in water (pH 7.2) at 25°C is shown in Figure 6. It can be seen that the swelling greatly increased after a relatively short time (less than an hour) and then tended to reach an equilibrium state after ≈4 h. The grafted CMC hydrogel exhibited higher swelling values than the homopolymer ones. This may be attributed to the hydrophilicity of PAM. Also, it can be seen that the swelling of both hydrogels constantly increased as a function of the swelling time, which is an indicative of homogenous gels. Surprisingly, CMC-g-Poly (acrylic acid) hydrogels,[36] prepared by electron beam, had an equilibrium swelling capacity of 33 (g/g) after 25 h compared with the as-prepared CMC-g-PAM hydrogels whose equilibrium swelling of 156 (g/g) after 5 h.

Simple power law expressions did not satisfactorily fit the experimental data. A sharp transition from the high initial rate to the slow rate towards the end of the swelling process needed to be explained. Accordingly, the swelling kinetics of the superabsorbents can be studied by means of a Voigt-based viscoelastic model (eq. 9)[37]:

$$S_t = S_e(1 - e^{t/\tau}) \quad (9)$$

where S_t is the degree of swelling (g/g) at any moment, S_e, the equilibrium swelling, is swelling at

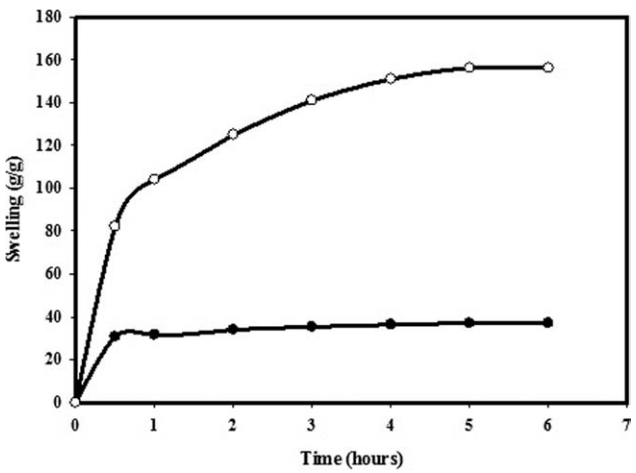

Figure 6 The swelling kinetics of PAM (●) and CMC-g-PAM (O) at pH 7.2 and 25°C.

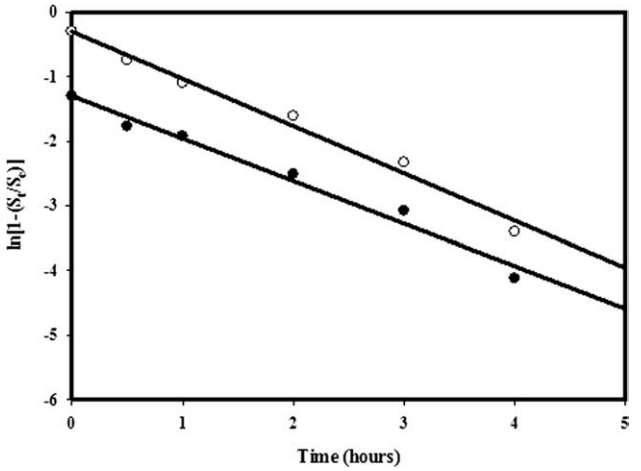

Figure 7 Plot of ln [1-S_t/S_e] versus time of PAM (●) and CMC-g-PAM (O) at pH 7.2 and 25°C.

infinite time or maximum water-holding capacity, t, is the swelling time (h), and τ, the rate parameter (h), is a measure of the resistance to water permeation, the lower the τ value, the higher the rate of water uptake will be.[32]

One can plot ln[1-(S_t/S_e)] versus time (t) (Fig. 7). The slope of the line passing the point zero was determined (slope = $-1/\tau$). Therefore, the rate parameter for the PAM was found to be 1.52 h where that for CMC-g-PAM was 1.37 h in the distilled water. Thus, the rate of water uptake in the grafted polymer hydrogel was higher than that of the homopolymer hydrogel. This difference in the rate parameter may originate from the high hydrophilicity (high affinity of the matrix toward the solvent) in the grafted polymer induced by the presence of both the polysaccharide (CMC) and the hydrophilic monomer (AM). In addition, it may also be attributed to a possible porosity of the matrix due to the grafting process.

We analyzed the swelling kinetics to see whether the swelling follows first-order or second-order kinetics. We adopted the procedure proposed by Quintana et al.[38] For the first-order kinetics, the rate of swelling at any time t is directly proportional to the water content that the hydrogel has to obtain before the equilibrium water content W_∞ is reached. The swelling is then expressed as

$$dW/dt = K(W_\infty - W) \tag{10}$$

where W is the water content of the hydrogel at time "t," and K is the proportionality constant between the swelling rate and the unrealized swelling capacity W_∞-W.

Upon integration of eq. (10) between the limits t = 0 to t and W = 0 to W, the following expression can be obtained:

$$\ln[W_\infty/(W_\infty - W)] = Kt \tag{11}$$

If the swelling process of the hydrogel considered follows the first-order kinetics, the plot of the variation of ln[$W_\infty/(W_\infty$-W)] as a function of time should give a straight line. However, none of the swelling behaviors, studied in distilled water, followed eq. (10), as clear from the inset of Figure 8. Considering second-order kinetics, the swelling rate at any time may be expressed as

$$dW/dt = K(W_\infty - W)^2 \tag{12}$$

Integration of eq. (11) with the limits t = 0 to t and W = 0 to W and after rearrangement, eq. (13) is obtained:

$$t/W = 1/KW_\infty^2 + (1/W_\infty)t \tag{13}$$

According to this equation, the swelling data must fit a straight line with a slope of $1/W_\infty$ and an ordinate intercept of $1/KW_\infty^2$. The variation of t/W against time is plotted in Figure 8 for hydrogel samples of CMC-g-PAM and PAM. It can be observed that the swelling data for both hydrogels gave straight lines. Therefore, the swelling behaviors of both hydrogels obeyed second-order kinetics.

Factors affecting protein concentration

Macromolecules from biological sources are usually present in dilute form at the initial stages of purification processes. In down stream processes, a reduction of volume of such solutions is a necessary step to speed up. Different methods for concentration of protein solutions including precipitation, ion-exchange chromatography, ultra-filtration, freeze drying and dialysis under vacuum have been

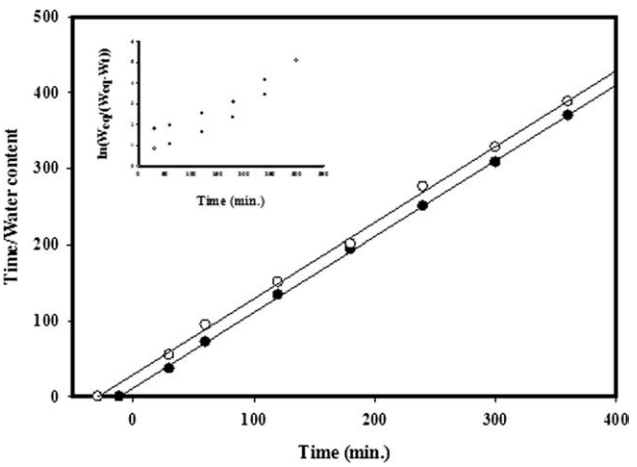

Figure 8 The second order swelling kinetics. The inset is the first order swelling kinetics.

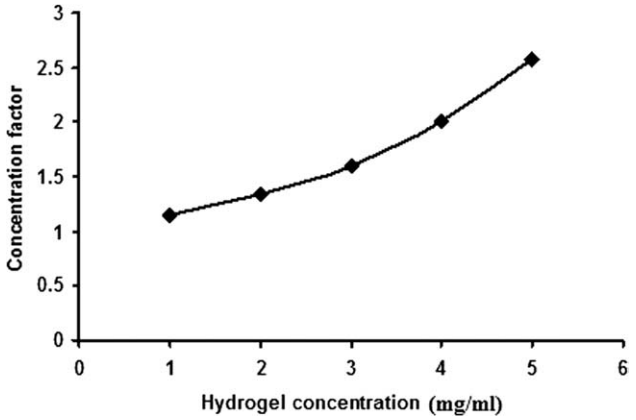

Figure 9 Effect of the CMC-g-PAM hydrogel concentration on the concentration process of 0.5 mg/mL BSA solution, at pH 7.2 and 25°C.

studied. In addition, hydrogels were proposed for the concentration of bio-macromolecules.[39–42] The crosslinked structure of hydrogels enables them from absorbing large quantities of solvents, mainly water, in addition to small solutes, while excluding macromolecules such as proteins and concentrating them as a result. This technique has been used in concentration of proteins, viruses, vitamin B12, polyethylene glycol, and other polymers.[35–38]

This work explores the use of CMC-g-PAM hydrogel for the concentration of dilute solution of BSA. Factors affecting the concentration process such as hydrogel concentration, protein solution concentration and pH, and process time were investigated. Finally, the reuse applicability of the hydrogel was also studied.

Effect of hydrogel concentration

Figure 9 shows the effect of using different hydrogel concentration from 1 to 5 mg/mL in the concentration process of fixed BSA concentration (0.5 mg/mL) in distilled water (pH 7.2) at 25°C. From this figure, it can be seen that there is a direct relationship between the concentration potency of the polymer and its concentration. The concentration factor increased linearly with increasing the polymer concentration to reach its highest value; 2.6. This may be attributed to the direct relationship between the polymer concentration and the amount of water absorbed. Recovery percentage around 95% was obtained at all tested conditions. This indicates that BSA is completely excluded from the gel due to electrostatic repulsion between the protein and the polymer, which could be even totally responsible for this exclusion in addition to the size/shape of the protein.[17]

Prazeres,[17] using 2.5 mg/mL (Hoechst, Sanwet IM-5000-SG; linear sodium polyacrylate), showed a concentration factor up to 3.6 and a recovery percentage around 70% for concentration of 0.2 mg/mL BSA solution. Our results are consistent with those obtained by Cussler et al.[41] who employed polyacrylamide (250 mg/mL) to concentrate BSA solution of 0.82 mg/mL in a batch process. Indeed a proper comparison is not possible here since data concerning the amount of the used polymer, initial BSA concentration, ionic strength and pH are not available.

Effect of protein solution pH

The pH-sensitivity of CMC-g-PAM hydrogel, proved above in distilled water, reflects the importance of studying the performance of the BSA concentration process in different pH. Figure 10 shows the behavior of the concentration factor with pH from 3 to 7 at 25°C. The obtained trend was almost identical with that of swelling behavior in Figure 4. This coincidence may be referred to the variation of absorbed amount of water which consequently affected the concentration factor. On the other hand, the recovery percentage was found unchanged and its values were between 95 and 96%. Such pH-sensitivity reflects a probable use of the prepared CMC-g-PAM hydrogel in the delivery of drug proteins orally through the gastrointestinal system.

Effect of process time

The effect of the process time on the concentration of BSA, at pH 7.2 and 25°C, was investigated (Fig. 11). The results showed that the concentration factor is directly proportional with the process time. It increased to reach its highest value (4.55) after 5 h process time and then leveled off. This behavior coincided with the swelling behavior of the hydrogel. A noticeable decrease in the recovery

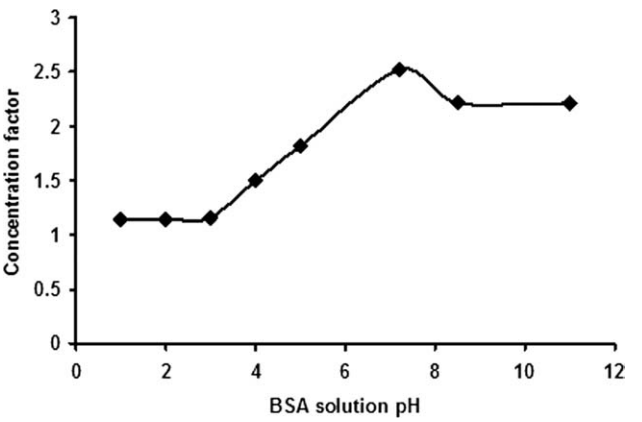

Figure 10 Effect of BSA solution (0.5 mg/mL) pH on the concentration process at 25°C using 5 mg/mL CMC-g-PAM.

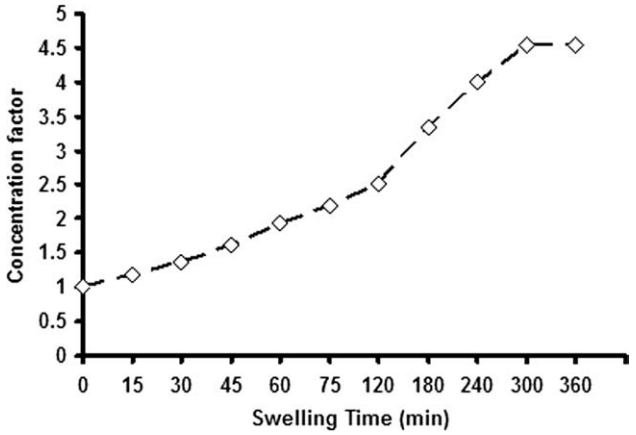

Figure 11 Effect of the time on the concentration process of BSA solution, at pH 7.2 and 25°C, using 5 mg/mL CMC-g-PAM.

percentage, around 60–65%, was observed with samples had a concentration factor higher than 3.0. This finding is consistent with that observed by Prazeres.[17]

Effect BSA concentration

Figure 12 shows the effect of initial BSA concentration (0.5–3.0 mg/mL) on the concentration process using a polymer concentration of 5 mg/mL, at pH 7.2 and 25°C. The results revealed that high concentration factors and recoveries are obtained with high diluted solutions (0.5 mg/mL). This is in agreement with the results described above. Many authors[17,37,39] observed a considerable loss in the efficacy of the concentration process with the increase in the protein concentration. In our case, the loss of protein solution volume was very small and could not be accused for such loss of recovery percentage.

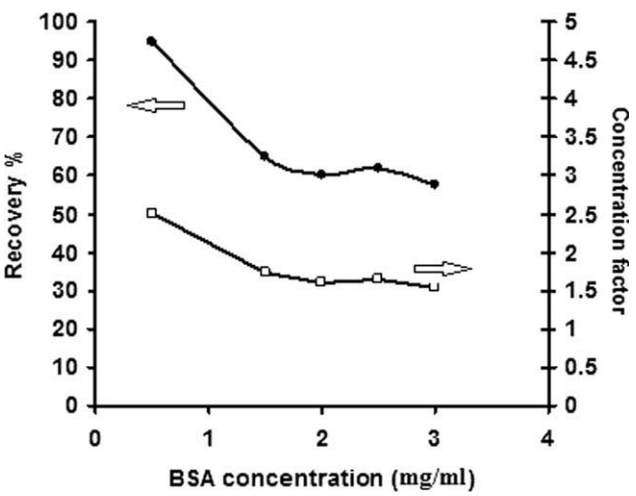

Figure 12 Effect of initial BSA concentration on the concentration process, using 5 mg/mL CMC-g-PAM, at pH 7.2 and 25°C.

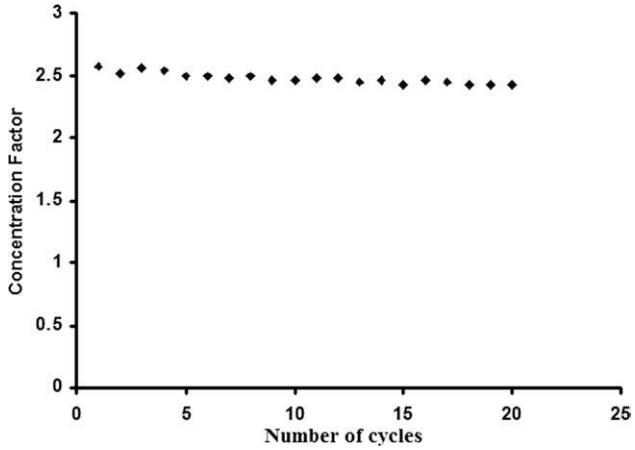

Figure 13 Reusability of CMC-g-PAM in concentrating 0.5 mg/mL BSA solution, at pH 7.2 and 25°C.

The migration of BSA molecules from liquid phase to the hydrogel phase may be the most probable cause since at such high protein concentration; a concentration gradient could be created which might present a driving force "pushing" BSA molecules to the hydrogel polymer phase. This assumption is reinforced by the swelling data of the hydrogel. This leads us to suppose that "opening" of the gel structure, via the swelling process, may facilitate the diffusion process of BSA molecules into the gel phase.

Reusability

One of the main advantages, presented by using hydrogels in the concentration process of bio-macromolecules, is the possibility of reuse them; which has a direct impact on the process costs. The reusability of CMC-g-PAM hydrogel was tested and the obtained results are illustrated in Figure 13. From this figure, it is clear that 20 cycles of employing the same hydrogel sample in concentration of BSA (0.5 mg/mL) solution, at pH 7.2 and 25°C, gave almost the same concentration factor and recovery percentage. Such results recommend the employment of the prepared CMC-g-PAM hydrogel in the down stream protein concentration process in industrial scale. The final slight decrease in concentration factor was explained by the loss of 20% of hydrogel weight. Blocking of the pores with some diffused BSA molecules was probably the main cause. Besides, the dissolution of uncrosslinked chains might most likely occurred after this large number of cycles.

CONCLUSION

Crosslinked polyacrylamide was grafted onto carboxymethyl cellulose, via free radical polymerization in an aqueous system; to be employed in concentrating dilute solutions of BSA. The grafting reaction

parameters were investigated and the hydrogel smartness was verified. Finally, the hydrogel potency towards protein concentration was explored.

The following conclusions are drawn from the present investigation:

1. FTIR proved that the grafting reaction occurred between the hydroxyl groups located in anhydroglucose C_2 position of CMC and the π-bond of PAM.
2. The pH sensitivity was proved as the grafted polymer attained its maximum swelling at pH 7.2 while minimum swelling was observed under acidic conditions.
3. The rate of water uptake in the grafted polymer hydrogel was higher than that of the homopolymer hydrogel and the swelling behaviors of both hydrogels obeyed second-order kinetics.
4. The concentration factor increased linearly with increasing both the polymer concentration and process time and decreased with the increase in the protein concentration.
5. The grafted polymer had stable efficiency in concentrating BSA solution for twenty cycles.

Generally concluding, the technology described here enables the processing of large volumes of dilute protein solutions under very mild conditions, without the drawbacks associated to the more traditional alternative methods of concentration. For instance, there are no problems of contamination and denaturation due to chemical agents (as in precipitation), denaturation due to high shear fields and no extreme temperature, pressure or pH changes occur (as in freeze drying, vacuum dialysis, and precipitation). Also, the process is simple and relatively inexpensive (the polymers are cheaper than ion-exchangers), even if regeneration/reutilization is not considered.

Such results recommend the employment of the prepared CMC-g-PAM hydrogel in the down stream protein concentration process in the industrial scale. Consequently, the utilization of hydrogels in this field may open new doors in various areas of advanced research and technology.

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