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Superabsorbent polymer (SAP) hydrogels for protein enrichment

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Abstract:

Superabsorbent polymer (SAP) hydrogels are mainly acrylic based polymer hydrogels which are capable of absorbing water around 100 times of their own weight. Thus these are supposed to be the promising material which can be incorporated as a draw agent in forward osmosis (FO) process. In this paper, superabsorbent polymer hydrogels are used as a draw agent for protein enrichment in FO process so that structure of protein remains intact during dewatering process. Though the free swelling capacity of hydrogels in aqueous medium is high, due to their slow swelling rates water fluxes obtained in FO process are much lower as compared to conventional salts used as a draw solute. Thus in this paper research emphasis was given on increasing swelling rates of hydrogels to improve water fluxes by graft copolymerizing acrylic acid monomer on carboxymethyl cellulose (CMC) polymer. Moreover, for feasible recycling of these draw agents, a thermoresponsive polymer N-isopropyl acrylamide (NIPAM) was copolymerized with superabsorbing acrylic acid monomer and thermal dewatering process was used to regenerate the hydrogels.

The synthesized hydrogels were tested as a draw agent in FO for bovine serum albumin (BSA) protein enrichment experiments. As the enrichment is directly related to the absolute weight of water extracted from the feed solution, the performance was presented in terms of enrichment factors. Thermally regenerated hydrogels were again recycled back to the process, and some reduction in enrichment was observed. Further based on the results obtained with different membrane area, mass of hydrogels, swelling ratios and water recovery rates, parameters for hydrogel assisted FO process are predicted for various applications.

Keywords:- Superabsorbent polymer; Fouling; Hydrogel; Protein enrichment; Swelling ratio

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1. Introduction:

The development and application of membrane technologies is one of the most significant recent advances in chemical, environmental, and biological process engineering for sustainable growth. Forward osmosis (FO) as low energy consuming membrane technology has attracted great attention in last 10 years. FO has already proven to be an efficient process for various applications such as seawater and brackish water desalination (McCutcheon et al., 2005; Tan et al., 2010; Zhao et al., 2012), wastewater concentration and reclamation (Cath et al., 2005; Ge et al., 2012; Chon et al., 2012; Hickenbottom et al., 2013), power generation, (Thorsen et al., 2009) and food concentration (Garcia-Castello et al., 2011; Sant Anna et al., 2012) etc. One of the key challenges in FO processes is the limited choice of efficient and economic draw solutes. While selecting the draw agent, it is desired to meet basic criteria like high osmotic pressure, minimal diffusion through the membranes to avoid possible contamination of the feed, non-toxicity (particularly for potable water production), compatibility with membrane surface and most importantly easy regeneration with low energy consumption. A wide range of inorganic and organic chemicals including salts (Loeb et al., 1997; McGinnis et al., 2002; Iyer and Linda, 2011), fertilizer (Phuntsho et al., 2011), saccharide (Stache et al., 1989; Yaeli, 1992; Su et al., 2012), hydrophilic nano-particle (Ling et al., 2010, Ling and Chung, 2011 a,), polyelectrolytes (Ou et al., 2013, Ge et al., 2012) etc. have been studied to date as draw agents. A brief review on draw agents used in FO process is given in an article considering all its advantages and drawbacks (Ge et al., 2013).

Recently, Li and his group have introduced stimuli responsive polymer hydrogels as a new class of draw agents in FO process (Li et al., 2011 (a)). Polymer hydrogels have been used for the desalination in FO process as these have the ability to swell in water and deswell under the influence of external stimuli such as pH, pressure, temperature, ionic solutions etc. This property of these polymeric hydrogels is believed to solve the problem of regeneration and reuse of draw agent. Li and his group have reported desalination performance using hydrogels synthesized via free radical polymerization of different ionic polymers such as polyacrylamide (PAM), poly(sodium acrylate) (PSA), poly(N-isopropylacrylamide) (PNIPAM), and poly(N-isopropylacrylamide)-co-poly(sodium acrylate) (PNIPAM-co-PSA) using N,N-methylenebisacrylamide (MBA) as a crosslinking agent. Out of which PNIPAM-co-PSA hydrogel was supposed to be the best choice considering the water fluxes in the FO

and dewatering processes (Li et al., 2011 (a)). Razmjou and his group demonstrated the effect of hydrogel particle size on FO performance in detail (Razmjou et al., 2013 (a)).

In more recent studies (Li et al., 2011 (b); Yan et al., 2012; Li et al., 2013; Zeng et al., 2013), hydrophilic carbon particles and graphene oxide were incorporated into a PNIPAM-co-PSA copolymer hydrogel as solar energy absorbents to enhance water releasing property of hydrogels. Though they showed higher FO fluxes and dewatering rates, the water released was mostly in the vapour state and could not meet the main aim of recovering pure liquid water from saline water. Cai's group synthesized semi-IPN hydrogels by polymerizing NIPAM monomers in the presence of linear polyvinyl alcohol (PVA) or PSA. The resulting semi-IPN hydrogels displayed thermoresponsive property at lower temperatures than PNIPAM-co-PSA. In this study, they proposed a quasi-continuous system for desalination with cyclic cooling and heating in the temperature range of 25-40 °C. This semi IPN hydrogels released more than 90% water that was adsorbed (Cai et al., 2013). Still the water fluxes obtained in FO process were very low. In order to recover more liquid water from swollen polymer hydrogels, magnetic field-induced heating was used for dewatering swollen PNIPAM-co-PSA nanocomposite hydrogels with the incorporation of magnetic nanoparticles (γ -Fe₂O₃). Due to presence of magnetic nanoparticles, internal temperature of hydrogels was increased and the liquid water recovery was higher than 50% (Razmjou et al., 2013 (b)). This was explained by lower case hardening phenomenon occurring during the dehydration process.

All these reported studies have been carried out keeping desalination as a main focused application. As the ultimate aim of the desalination process is extracting pure water from seawater, efficient dewatering process for liquid water recovery is required. Moreover, desalination using FO process must involve high fluxes so that it can replace the existing reverse osmosis (RO) desalination systems. Though it is easier to regenerate hydrogels after FO test, their low water fluxes as compared to other inorganic draw agents may limit their use in desalination process.

As most of the pharmaceutical products are labile and heat sensitive, a thermal separation processes are preferred for them. FO separation process is a simpler, lower energy consuming and a higher efficacy process for pharmaceutical enrichments. As membrane is made of polymers which are generally inert to many chemical elements and is the only component coming in contact with the feed solution, the structural degradation in such products can be prevented. Moreover, as the process is carried out at atmospheric conditions, less damage is

caused to those pressure and temperature-sensitive pharmaceutical products. Among such pharmaceutical products protein is an important product. In the production of protein, protein enrichment plays an important role and is accompanied with dewatering processes. Previously two studies have been reported where they used FO process for protein enrichment. One research group demonstrated protein enrichment in FO process with dual-layer polybenzimidazole- polyethersulfone/ polyvinylpyrrolidone (PBI-PES/PVP) hollow fiber nanofiltration (NF) membranes. They obtained average water flux of 12.7 LMH in the FO mode and 17.1 LMH in the PRO mode, using a membrane surface area of around 70 cm². In these experiments an equal volume of the protein solutions were concentrated by a factor of 1.85 using 600 ml 3.125 M MgCl₂ as the draw agent after 3 h. On the other hand, the salt reverse diffusion fluxes were 3.3 gmH (FO mode) and 4.5 gmH (PRO mode) which is sufficient to cause protein denaturing. Moreover, some protein molecules were found to get trapped in the membrane pores and higher fouling caused due to higher water fluxes (Yang et al., 2009). This not only led to loss of protein but also increases cost of membrane as it cannot be reused with the same performance. As it is impossible to reject salt completely during the FO process using salt solution as a draw agent, this cannot prevent the problem of protein denaturing. Thus another study was reported to enrich protein solutions using FO with highly hydrophilic nanoparticles capped with polyacrylic acid (PAA-NPs) as a draw agent to overcome this problem. Protein enrichments were carried out in two stage FO process in which RO retentate was used to regenerate nanoparticle draw agent. The maximum enrichment factors obtained were 1.1 and 1.2 in single and dual stage FO process after 3 hours with 8 cm² membrane areas respectively (Ling et al., 2011 b). Though gel track and circular dichroism spectra showed that protein structure was maintained in the process, lower enrichments in single stage and diffusion of salt in dual stage process were still the problem.

Thus, here we proposed the utilization of fast swelling superabsorbent polymer hydrogels as a draw agent in FO process for protein enrichment. These hydrogels are easy to regenerate and can be recycled back in the FO process. The superabsorbent (SAP) hydrogels were synthesized by grafting acrylic acid (AA) and N-isopropyl acylamide (NIPAM) monomers on polysaccharide chain to improve swelling kinetics so as to obtain high water fluxes in FO and consequently faster enrichments. Moreover, these hydrogels are sizing in the range of microns which is 1000 times larger than pore size of FO membrane used (Ling et al., 2011 b). Thus solves the problem of diffusion of draw agent into feed protein solution.

Many polysaccharides have been reported in literature to improve swelling capacity and swelling rates of acrylic based hydrogels. We used carboxymethylcellulose (CMC) in this study which is anionic carboxymethyl ether of cellulose synthesized by the alkali catalyzed reaction of cellulose with chloroacetic acid. The introduced polar carboxyl groups render the cellulose soluble, chemically reactive and strongly hydrophilic character (Pourjavadi et al., 2007). Due to its wide availability, it is also supposed to reduce cost of hydrogels synthesized from petroleum based superabsorbent polymers.

As regeneration of draw agent is main focused challenge in FO process, to improve deswelling and reswelling behaviour of SAP hydrogels NIPAM is graft copolymerized with AA monomers on CMC chains to render them thermoresponsive properties. Among the various families of thermosensitive polymers, alkyl acrylamide-based polymers, in particular, poly (*N*-isopropylacrylamide) (PNIPAM), are the most investigated for these applications. The ability of NIPAM based networks to change their conformation at temperatures larger than 32°C which is a lower critical solution temperature (LCST) is well-known. Our intention was to associate NIPAM and AA monomer with a polysaccharide to obtain a polymeric product presenting the fast swelling hydrogel properties and at the same time, thermo responsive property (Hamcerencu et al., 2009).

2. Experimental:

2.1 Materials and Methods:

Sodium salt of carboxymethyl cellulose (CMC) was purchased from Sulab reagents, Baroda, India. Ammonium persulfate (APS) was purchased from SDFCL, fine chem. Crosslinking agent epichlorohydrin (ECH) and acrylic acid (AA, 99%) monomer both were purchased from FINAR reagents and were used as received. For neutralization of acrylic monomer NaOH flakes (Finar reagents, 96%) were used. N-isopropylacrylamide (NIPAM, 96%) monomer was purchased from Aldrich Chem. Co., USA. Bovin Albumin fraction V (BSA) supplied (Molecular weight 60kDa) by HPLC, India was used as a model protein enzyme solution to be concentrated and enriched by the FO process. Commercial FO membrane provided by Hydration Technologies Inc. (Albany, OR) is used to test the performance of hydrogel in forward osmosis process. This FO membrane is made of cellulose triacetate with an embedded polyester screen mesh. Deionised (DI) water produced by MilliQ, Millipore, India is used for the experiments.

2.2 Synthesis of hydrogels:

Free radical polymerization of AA monomer was carried out in a 250 ml three-necked flask equipped with a thermometer, a reflux condenser and a nitrogen line. AA was first neutralized with 8 M NaOH solution to obtain sodium acrylate. Then different weight percent of crosslinker ECH with respect to monomer concentration and initiator APS (0.1 g) were added to the reaction flask and stirred using magnetic stirrer. N₂ is purged through the reactor for 30 min. Then temperature was allowed to increase to 80 °C and kept for 5 h to complete polymerization. Nitrogen atmosphere was maintained throughout the reaction period. Thus obtained gel products were then cut into pieces and washed with DI water to remove unreacted components. After that they were dried to a constant mass at 60°C in a vacuum dryer and ground using lab scale mortar and pestle and passed through a mesh sieve (100-150 µm). These as-synthesized hydrogels were named as pure PNaA.

Similar procedure is used to synthesize graft polymer and copolymer. Firstly, CMC is dissolved in 25ml DI water at 60°C. After complete dissolution of CMC, mixture of monomers, initiator and crosslinker is added to the flask and polymerization is continued for 12 hrs at 80°C. In synthesis of graft copolymer, an equimolar ratio (1:1) of NIPAM and AA monomer is used. These obtained hydrogels were kept in 250 ml ethanol solution for 1 week to dewater before drying and then ground to desired size (100-150 µm). These graft polymer and copolymer were named as CMC-g-PNaA and CMC-g-PNaA-co-PNIPAM respectively.

2.3 Characterization:

The morphology of dry hydrogel particles was characterized by scanning electron microscope SEM. A Fourier transform infrared (FTIR) Spectrophotometer- DRS (8400-S-Shimadzu) was used to analyze the chemical structure of the graft polymerized hydrogels. The stability of protein structure in experiments was investigated by SDS-gel electrophoresis (Bio-Rad Mini-Protean Tetra Cell machine) using 7% polyacrylamide gel.

2.3.1 Swelling ratio measurements:

An accurately weighed sample (0.05 g) of the powdered hydrogel with average particle sizes between 100-150 µm was immersed in 500 ml distilled water for 24 hours to ensure

maximum swelling. The equilibrium swelling (ES) capacity was measured at room temperature by 'tea bag' method and using the equation,

$$\text{---} \quad (1)$$

where W_s is the weight of the swollen hydrogel after three days of immersion in water; W_d is the initial weight of the dry hydrogel powder before swelling.

To study the swelling kinetics of the hydrogels, hydrogels were removed from the distilled water at equal interval of time and their weights were measured. Swelling ratios were then calculated using same equation for that particular time.

To recycle the hydrogels, they were dewatered in a convection oven at 60 °C for 1 hour and then again utilized in the FO process.

2.4 Protein enrichment using FO setup:

The drawback of the FO setups used in previous studies is (as shown in figure 1 (a) if the membrane is kept dipped in the feed solution, water passes from feed to draw side due to potential developed by level difference along with the osmotic pressure exerted by the hydrogels. On the other hand, if the membrane is kept just in contact with the water level, membrane loses its contact with feed solution due to lowering of feed level as water flows towards draw side with time. Thus precise performance of hydrogels cannot be checked using this setup. Hence, we propose a FO setup (as shown in figure.1 (b)) in which we use a peristaltic pump to circulate the feed protein solution which can also reduce membrane fouling. The volumetric flow rates at inlet and outlet were maintained same so that level in the feed chamber remains constant.

One run was carried out for 3 hrs and BSA dissolved in buffer solution at pH 4.9 was studied as the model protein solution. Concentration of feed protein solution used was 0.1g/L and 100 ml feed was circulated through the setup. The water fluxes obtained in the process is calculated using the formula,

$$F = \text{---} \quad (2)$$

where ΔV (L) is the volume of water absorbed by the hydrogel, calculated by dividing the mass of the water (i.e., the mass increase of the hydrogel) (g) by its density, Δt (h) is the time and A (m^2) is the effective area of the FO membrane. To calculate the enrichment factor concentration of protein solution was measured after 3 hrs of cycle by using UV-Vis spectrometer (DR 6000) at the wavelength of 280 nm and enrichment factor was calculated as,

$$EF = \frac{C_t}{C_0} \quad (3)$$

Where, C_t is the concentration after 3 hrs and C_0 is the initial concentration of feed solution.

Process optimization was carried out for different weights of hydrogels with different membrane area and based on swelling ratios obtained after FO process enrichment factors were determined (Table 1).

3. Results and discussion:

3.1 FTIR analysis

Figure 2 (a) to fig 2(c) give the comparison between FTIR spectra of pure PNaA, CMC-g-PNaA and CMC-g-PNaA-co-PNIPAM. In FTIR spectrum of CMC, a strong absorption band was observed at 3587 cm^{-1} due to the stretching vibration of -OH group, which gets weakened after PNaA grafting (Mohkami et al., 2011). Other characteristic absorption bands of CMC at 1055 , 1113 and 1176 cm^{-1} (stretching vibration of C-OH groups) were obviously weakened after reaction and shifted to 1056 , 1119 and 1169 cm^{-1} for CMC-g-PNaA. The new bands at 1730 cm^{-1} (C=O stretching of -COOH groups), 1565 cm^{-1} (asymmetrical stretching vibration of -COO- groups), 1453 and 1395 cm^{-1} (symmetrical stretching vibration of -COO- groups) appeared in the spectrum of CMC-g-PNaA. This result indicates that NaA monomers were grafted onto CMC backbone. It can be observed that characteristic peak of CMC (3587 cm^{-1}), not present in spectrum of pure PNaA. Whereas, in the spectrum of CMC-g-PNaA-co-PNIPAM all the absorption peaks 3550 cm^{-1} (-OH stretching) 3244 cm^{-1} (secondary amide N-H stretching), 1726 cm^{-1} (-COO group stretching of NaA), 1650 cm^{-1} (secondary amide C=O stretching, amide I bond), and 1553 cm^{-1} (secondary amide C=O stretching, aka amide II bond) suggesting that grafting of both

the monomers onto the CMC backbone was successfully done (Cui et al., 2006; Ozkahraman et al., 2011)

3.2 SEM analysis:

Water absorbency of hydrogels depends on the porosity and layered surface of the hydrogels. Hence, hydrogel microstructure morphologies are one of the most important properties, which should be considered (Lim et al., 2000). Moreover, it is supposed that these pores are the regions of water permeation and interaction sites of external stimuli with the hydrophilic groups of the graft copolymers (Pourjavadi et al., 2007). Thus, SEM analysis of hydrogels was performed and figure 3 depicts the SEM micrographs of the various superabsorbent hydrogels. The particle size of around 100 microns can be seen in figure 3 (a), which confirms the average particle size used in the experiments. On higher magnification, it can be observed that CMC-g-PNaA exhibits a rough but dense surface (Figure. 3 (b)). This indicates that some CMC chains were crosslinked with ECH retaining their crystallinity in the hydrogels. Also, this surface roughness corresponds to higher surface area which can cause higher water absorption. Figure 3(c) shows the surface morphology of CMC-g-PNaA-co-PNIPAM hydrogel. SEM micrograph of CMC-g-PNaA, (figure. 3(b)) shows dense structure but with some roughness on the surface, while surface of copolymer grafted on CMC shows dense as well as smooth surface. This result indicates all roughness imparted by crystallinity of CMC was lost. It may be due to higher grafting degree achieved in case of PNIPAM incorporated hydrogels than that of PNaA only.

3.3 Swelling kinetics of hydrogels:

Figure 4 (a) represents the swelling kinetics curves of the grafted hydrogels in distilled water. It can be seen that the swelling rate of the hydrogels is faster within 40 minutes and then reached a plateau showing equilibrium stage. On basis of the experimental data, the plots of t/SR_t vs. t give perfect straight lines with good linear correlation coefficient (>0.99 ; Figure 4 (b)), indicating that the swelling of grafted hydrogels observes the Schott's pseudo second order kinetics model which is given by equation (Wang et al., 2011). But, swelling of pure PNaA hydrogels did not observe to obey pseudo second order. They take much higher time to reach equilibrium swelling than that of grafted hydrogels. This result is in accordance with previously reported swelling kinetics of PNaA hydrogels (Liu et al., 2001). This is because PNaA hydrogels are supposed to follow Fickian or non Fickian

diffusion based on particle size of hydrogels (Kostic et al., 2007; Razmjou et al., 2013, (a)). Also, by fitting experimental data using equation 3, the theoretical equilibrium water absorbency (SR_{∞}) and the initial swelling rate (K_{is}) can be calculated by the slope and intercept of lines (Figure 4(b)), and the results are listed in Table 2.

$$\frac{t}{SR_t} = \frac{1}{K_{is}} + \frac{1}{SR_{\infty}} t \quad (4)$$

where, SR_t is the water absorption at a given swelling time t (s); SR_{∞} (g/g) is the power parameter, denoting the theoretical equilibrium water absorption; K_{is} is the initial swelling rate constant (g/g.s).

According to the obtained values of K_{is} and SR_{∞} for each hydrogel, it can be concluded that the initial swelling rate for CMC-g-PNaA is higher than CMC-g-PNaA-co-PNIPAM hydrogels. The R value obtained showed that pseudo second order kinetics does not fit to swelling of pure PNaA hydrogels (Pourjavadi and Hosseinzadeh, 2010). Thus it can be concluded that swelling of grafted hydrogels is controlled by the relaxation of the polymeric chains for long times whereas for pure PNaA swelling is diffusion controlled (Martinez-Vazquez et al., 2007).

3.4 Effect of crosslinking agent on swelling ratios:

Crosslinking density is an extremely important parameter in deciding swelling capacity of superabsorbent hydrogels. Thus we studied the effect of concentration of ECH crosslinker on the swelling ratios of hydrogels shown in figure 5. It is observed that swelling ratio of grafted hydrogels increases upto 8.6 wt % concentration of ECH and reach to a maximum value of 146.3 (g/g of dry hydrogels). It has been reported that concentration of crosslinking agent also affects the grafting percentage of polymer chains on the CMC backbone (Eldin et al., 2013). Thus it can be concluded that upto 8.6 wt % conc. of ECH in the reaction mixture, crosslinking of grafted chains of PNaA and grafting of PNaA chains on CMC backbone i.e. 'grafting from' is favoured, in case of CMC-g-PNaA hydrogels. Moreover, some parts of CMC chains are also get crosslinked contributing to the increase in swelling ratios and consuming some ECH concentration. This is evident from the fact that wet crosslinking of CMC occurs in the presence of ECH crosslinker in the alkaline medium (Young, 2002).

Further increase in the concentration of ECH leads to increase in the crosslinking density of both grafted PNaA chains and PNaA homopolymer chains. Thus the gel network exhibits higher density and thereby restricting penetration of water molecules into the hydrogel networks (Hebeish et al., 2013). Hence, swelling ratios of hydrogels decreased with more than 8.6 wt % concentration of ECH.

3.5 pH responsive behaviour of hydrogels:

In the practical application like FO, water with wide range of contaminants is to be handled. Thus the sensitivity of superabsorbent hydrogels to the external pHs is also a considerable influence factor. In this section, the swelling ratios of prepared hydrogels at solutions with various pHs were evaluated and the results are shown in Figure 6. For porotein application, as required pH to be handled is acidic, it was important to study how hydrogels behave in various pH conditions. It is observed that, all the hydrogels showed similar pH responsive behaviour as it is the intrinsic property of the polymer having -COOH groups at the backbone (Ozkahraman et al., 2011).

It can be seen that the water absorbency of both grafted and nanocomposite hydrogels almost keep constant at a wide pHs range from 4 to 11, but sharply decreases when the external pHs exceeds this region. This behaviour can be explained by the fact that, in acidic medium ($\text{pH} < 4$), the -COO^- groups on the polymer chain get protonated to -COOH groups. Thus the electrostatic repulsion among negatively charged -COO^- groups decreased and the expansion of polymer network was restricted. Moreover, increase of number of -COOH groups strengthened the intramolecular and intermolecular hydrogen bonding interaction and enhanced the physical crosslinking degree resulting in lower swelling ratios at low pH (Wach et al., 2003).

While with increase in pH value of external solutions ($\text{pH} > 4$), the ionized degree of -COOH or -COO(Na) groups increases, hydrogen bonding weakens and the electrostatic repulsion among negative -COO^- groups induces increase in osmotic swelling pressure. (Kiatkamjornwong et al., 2000). Thus all types of hydrogels show higher water absorbency in this region. This behaviour was supposed to cause due to buffer action of -COOH and -COO^- groups (Lee et al., 1996). As pH is increased further, that is at basic condition ($\text{pH} > 11$), the

increased ionic strength of external solution causes the rapid decrease in osmotic pressure and increase in screening effects of cations, which cause the reduction of water-absorbing capability of hydrogels (Wang et al., 2009).

4 Protein enrichment in FO with hydrogels as a draw agent:

4.1 Single stage semi batch FO process:

Hydrogel assisted FO desalination is studied in this report based on swelling reswelling properties of the grafted hydrogels and found to show higher fluxes than the previously reported studies. However, liquid water recovery from the desalinated water is still the challenge in this process. As the ultimate aim of desalination is to extract pure water from saline water, it can be suggested that hydrogels may not be feasible as draw agent for FO desalination. This is because easy water recovery from these hydrogels, specifically in liquid form is difficult and time consuming. Thus here we proposed utilization of hydrogel assisted FO for pharmaceutical processes in which small batches are processed for production of pharma products. Thus we tested protein enrichment by extracting water using FO membrane with hydrogels as a draw agent. In case of protein enrichment, absolute water content of hydrogels after 3 hours of FO cycle is considered instead of water flux. This is because, the water absorbed by the hydrogels is directly related to enrichment obtained in the protein feed solution. The results are reported in terms of swelling ratios. To maximize the enrichment factors, FO process is optimized with area of membrane and mass of hydrogels.

Protein enrichment was carried out with different hydrogels, and the results were obtained as shown in the figure 5. In accordance with swelling ratios of the different synthesized hydrogels, water fluxes were observed. PNaA hydrogels due to their lower swelling rates showed lowest fluxes, while CMC-g-PNaA with their high swelling rates and sorption capacity showed maximum fluxes. This is simply explained by higher driving forces exerted across the membrane by higher swelling hydrogels. From recycled hydrogels, (thermal dewatering at 60 °C for 1 hour) the fluxes obtained are shown in Figure 7 which shows, CMC-g-PNaA-co-PNIPAM hydrogels retained their swelling capacity causing slight decrease in water fluxes due to their thermoresponsive property (Ozkahraman et al., 2011). The water fluxes were decreased by 47% and 59 % in case of pure PNaA and CMC-g-PNaA hydrogels respectively, whereas only 5% loss in flux was observed in case of CMC-g-PNaA-

co-PNIPAM hydrogels. Thus considering these results further study was carried out with CMC-g-PNaA-co-PNIPAM hydrogels.

4.2 Effect of FO enrichment on BSA protein structure:

Gel track of the BSA solutions were performed to assess the integrity of protein before and after the enrichment. If incomplete disruption occurs in the secondary structure of protein, it cannot unfold to full length and may show different migration pattern than expected. Thus it can be concluded that, BSA is maintained after the FO process. As displayed in figure 8 (a) and fig 8 (b), similar patterns were found in case of the original and the concentrated BSA solutions, which imply FO causes no protein precipitations and no conformational changes in structure of protein molecules as reported previously (Sallantin et al., 1990; Cetin et al., 2007).

4.3 Effect of mass of hydrogels and membrane area on FO performance:

Figure 9 depicts the fluxes obtained with different weights of dry hydrogels used as a draw agent. This shows that upto 0.4g of dry hydrogel particles water fluxes goes on decreasing as the time elapsed. While for higher weights initial fluxes are lower in initial 1 hour and then goes on increasing reaching to maximum value after 1.5 hours and again decreases with time. This may be explained as, for higher weights of hydrogels water takes time to travel in core of hydrogels than that from membrane to the immediate layer of hydrogels i.e. particle to particle flow of water is slower than that of membrane to particle transport. After 1.5 hours, as the swelling capacity goes on decreasing driving force was decreased in turn decreasing water fluxes.

From the figure 10 it can be observed that as the area of membrane was increased, water fluxes also increased. Also, absolute water content (AbWC), is higher for higher membrane area. This is obvious, due to higher contact area provided for hydrogels with the feed solution across the membrane. On comparison of three processes with different membrane area, almost similar water fluxes were observed in initial half an hour. This may be due to higher initial swelling pressure exerted by the hydrogels. The fluxes were observed to decrease gradually as the FO process proceeds. Linear decrease in flux was found in case of process with 1.76 cm² membrane area, whereas gradual decrease was seen with 4.9 cm²

area. In case with 8.55cm^2 area fluxes decrease rapidly than that of 4.9cm^2 . This inconsistent effect of membrane area can be related to poor membrane coverage by the hydrogels (Cai et al., 2013). As the quantity of hydrogels used is same in all cases, hydrogels cover the membrane uniformly with 4.9cm^2 area, whereas for 8.55cm^2 area membrane is partly covered by the hydrogels. This in turn reduces the effective contact area between hydrogels and membrane causing rapid reduction in fluxes with time. This can be easily understood as membrane area is inversely proportional to flux (see equation 2) These results suggest that by optimizing the hydrogel thickness and its contact area with the FO membrane, better performance would result and the duration of the FO process to obtain same enrichment factors can be further reduced.

4.4 Optimization of FO process using hydrogels as a draw agent:

Table 1 gives the optimized values of swelling ratios obtained with 1.76, 4.9, 8.55cm^2 of membrane contact area with different hydrogel mass i.e. 0.4 g, 0.8 g and 1 g in 3 hour FO process. The water fluxes are calculated on the basis of water recovery rates. It is clearly seen that, the applications where high water extraction is desired, which means higher swelling ratios at the end of the process is required, larger contact area should be selected. For mass of hydrogels used, 0.4 g of hydrogels is showing maximum swelling ratios with 8.55cm^2 area. Thus it can be suggested that lower mass of hydrogels with larger contact area is beneficial for obtaining higher swelling ratios. This result directly related to the diffusion path for water transport from feed to draw side which is reduced for this case. Although enrichment factors with higher mass of hydrogels are higher, they are dependent on volume of feed solution used and absolute water content of the hydrogel after the process. Thus in the applications such as protein enrichment, algae dewatering or fruit juice concentration using FO, use of higher contact area with moderate mass of hydrogels will be advantageous. While in the applications such as desalination or wastewater treatment, where water recovery is the main aim of the process higher water fluxes will be advantageous. From table 1 it can be seen that as the mass of hydrogels increases for same membrane contact area fluxes goes on increasing. Thus it can be suggested that for such applications, lower contact area with higher mass of hydrogels must be selected to obtain high water recovery in less duration of FO process.

5. Conclusions:

Forward osmosis process with CMC grafted superabsorbent hydrogels as a draw agent is demonstrated for the pharmaceutical products enrichment and concentration from their dilute aqueous solutions. Hydrogels with 8.6 wt.% of epichlorohydrin crosslinker, showed maximum swelling ratios. The grafted hydrogels were found to obey pseudo second order swelling kinetics and thus faster swelling can be achieved as compared to pure PNaA hydrogels. Due to thermoresponsive property of CMC-g-PNaA-co-PNIPAM hydrogels, they were found as most efficient hydrogels to be used in the process as only 5% loss in flux was observed with recycled hydrogels. The proposed semi batch FO setup can effectively determine dewatering performance of hydrogels and circulation may decrease fouling of membrane. Moreover, due to considerably high sizes of hydrogel particles as compared to membrane pore size the problem of reverse draw agent diffusion in the sensitive feed protein solution was completely removed. Thus keeping protein intact and avoiding denaturing of the structure. In addition, the effect of mass of hydrogels and membrane contact area was systematically studied and optimum values to be used in FO process with hydrogel as a draw agent are suggested for particular application. Higher enrichment factors in FO process can be achieved with higher membrane contact area with higher mass of hydrogels or by concentrating small volume of dilute protein solution. Also, by circulating feed solution through number of such small setups connected in series will be efficacious to improve enrichment results.

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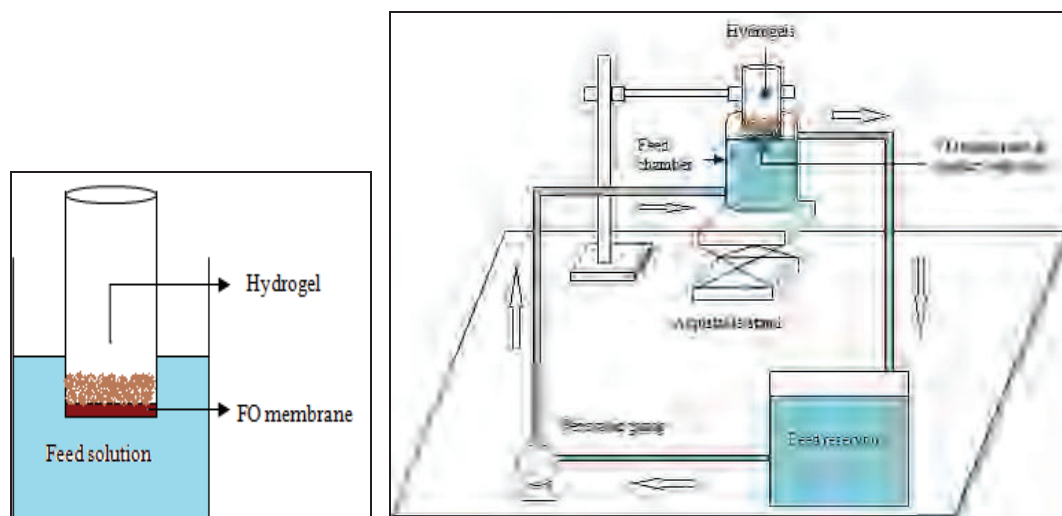


Figure 1: Forward osmosis setup (a) batch process (b) semi batch process.

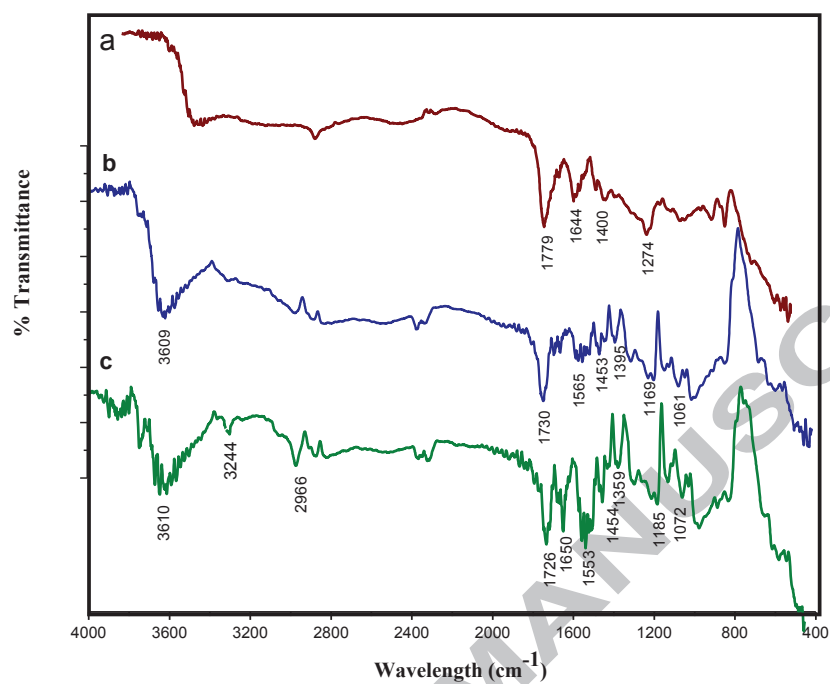


Figure 2: (a) FTIR spectra of (a) PNaA, (b) CMC-g-PNaA, (c) CMC-g-PNaA-co-PNIPAM

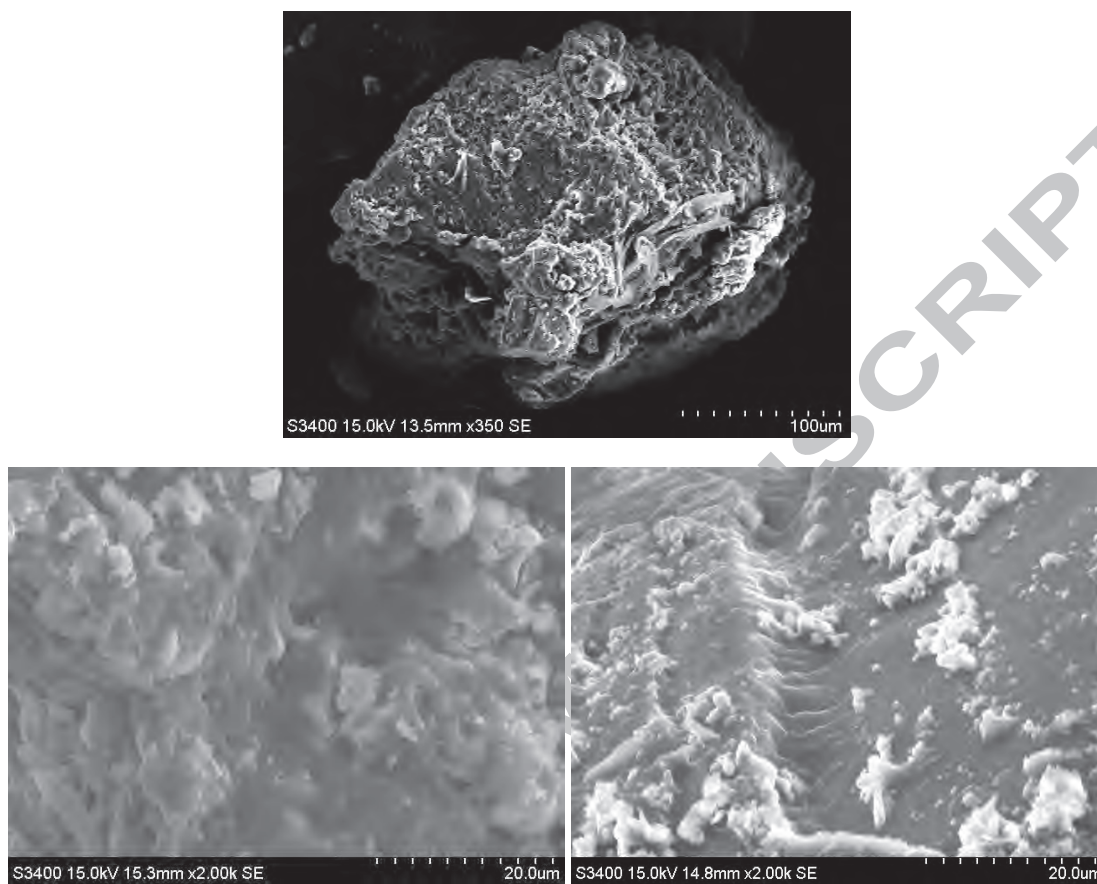


Figure 3: SEM micrographs of hydrogel particles(a) particle size of superabsorbent hydrogel (b) CMC-g-PNaA, (c) CMC-g-PNaA-co-PNIPAM

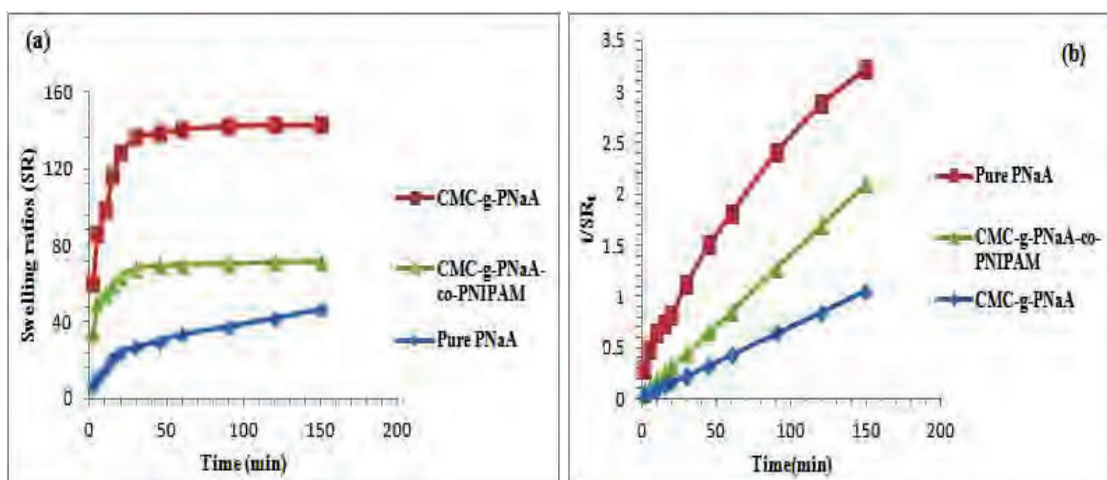


Figure 4: (a) Swelling kinetic curves of various hydrogels and (b) the plots of t/SR_t against t for each superabsorbent.

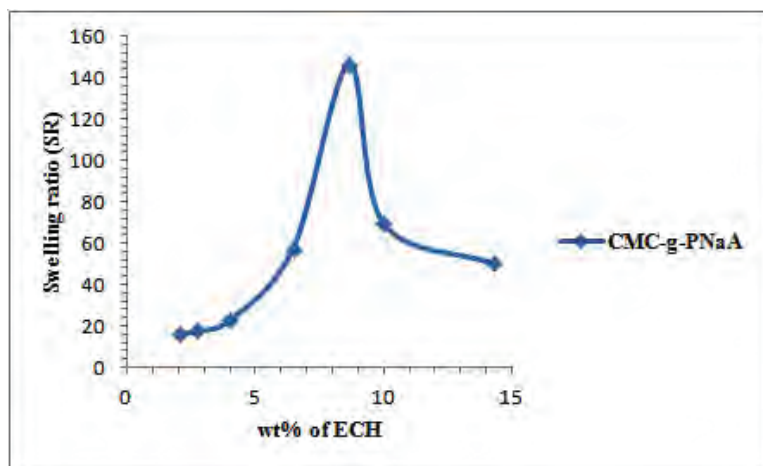


Figure 5: Effect of concentration of crosslinking agent ECH on CMC-g-PNaA hydrogels

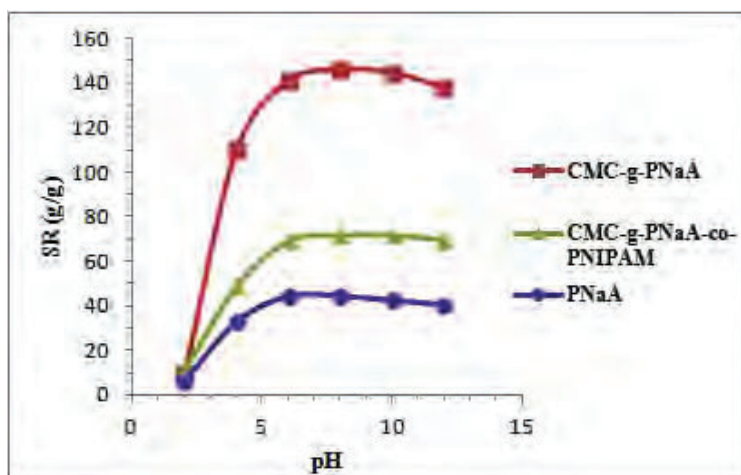


Figure 6: Effect of pH value of external solution on the swelling ratios of different hydrogels.

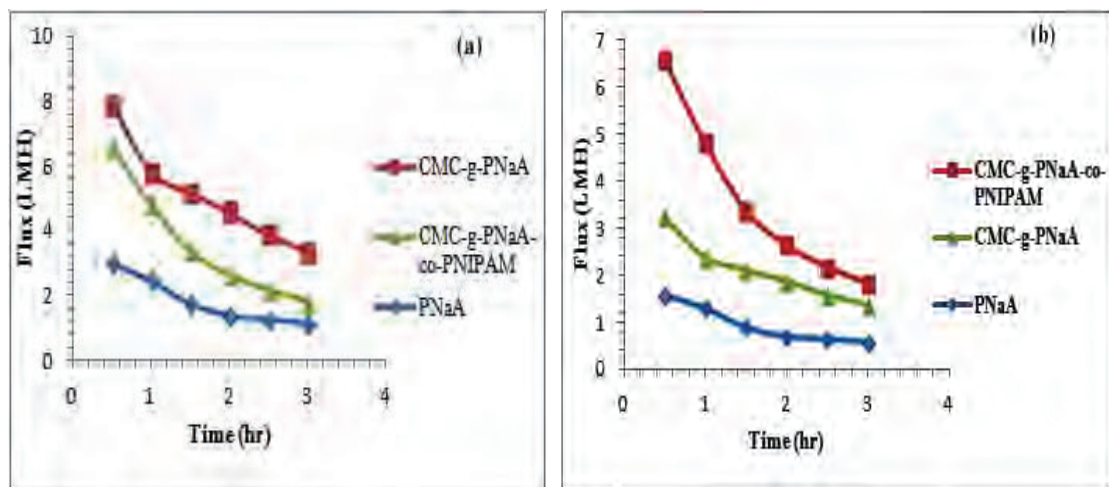


Figure 7: Water fluxes obtained with synthesized hydrogels using 0.4 gm hydrogels as a draw agent with membrane area 8.55 cm² with a) fresh hydrogels, b) recycled hydrogels.

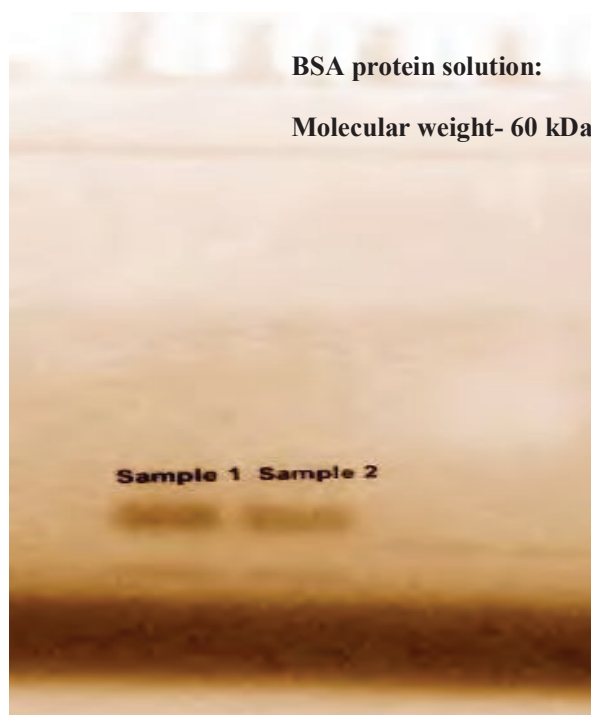


Figure 8: Gel track of BSA of (a) Sample1: original BSA solution before FO test and (b) Sample 2: concentrated BSA solution after FO test.

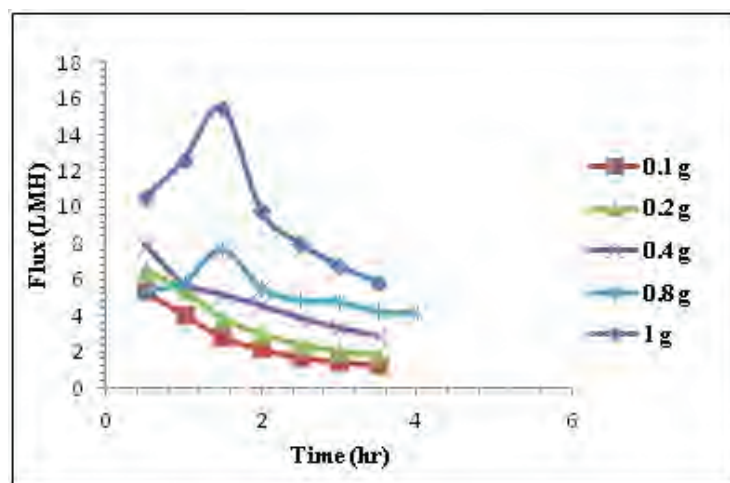


Figure 9: Effect of mass of CMC-g-PNaA-co-PNIPAM hydrogels on water fluxes.

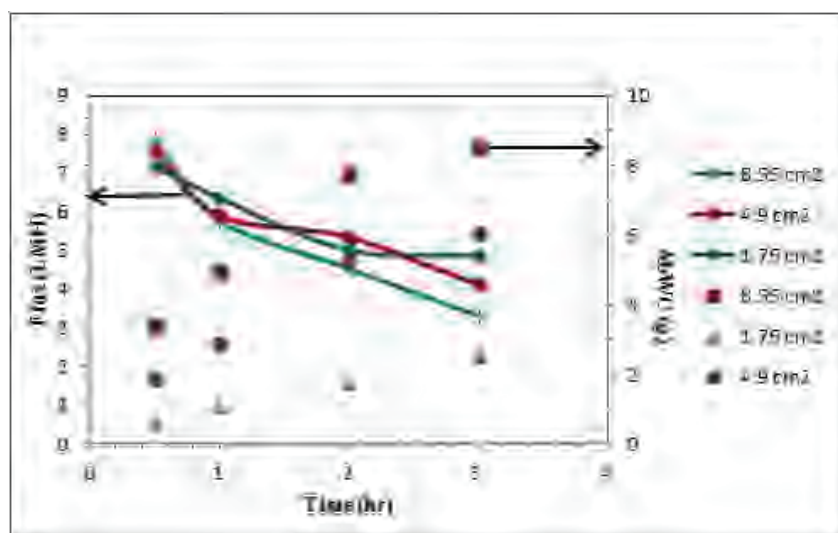


Figure 10: Effect of membrane area on water fluxes and absolute water content (AbWC) of the hydrogels in 3 hour FO test with CMC-g-PNaA-co-PNIPAM hydrogels as a draw agent.

Table 1. Optimization of contact area and mass of hydrogels used as draw agent on FO performance

Area of membrane (cm ²)	Mass of hydrogel (g)	Swelling ratio (g/g)	Water recovery (%)	Water Flux (LMH)	Enrichment factors
1.76	0.4	12.645	70	7.27	1.05
1.76	0.8	8.067	68.5	9.4	1.07
1.76	1	7.258	68	10.63	1.09
4.9	0.4	19.54	68.2	3.8	1.08
4.9	0.8	13.307	53.9	4.2	1.13
4.9	1	11.786	55	4.78	1.14
8.55	0.4	20.323	59	1.96	1.09
8.55	0.8	16.913	46	2.57	1.16
8.55	1	17.468	42	3.1	1.23

Table 2. Swelling kinetic parameters of the hydrogels

Samples	SR_{∞} (g/g)	K_{is} (g/g.min)	R
Pure PNaA	48.31	2.62	0.8715
CMC-g-PNaA	147.06	54.05	0.9995
CMC-g-PNaA-co-PNIPAM	72.46	32.57	0.9997

Highlights:

- CMC-g-PNaA and CMC-g-PNaA-co-PNIPAM superabsorbent polymer hydrogels were prepared.
- Hydrogels were used as a draw agent in FO for bovine serum albumin protein enrichment.
- Maximum swelling ratio was observed at 8.6 wt. % concentration of epichlorohydrin.
- Due to thermoresponsive property, CMC-g-PNaA-co-PNIPAM hydrogel was best hydrogel.