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Impact of Mobile Phase Parameters on Transport Properties of Metal Affinity Hydrogel Membranes

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Abstract: Hydrogels have been the focus of considerable interest for use in a range of applications including bioseparations, controlled release devices, and artificial organs. While they have attractive properties, hydrogels typically provide limited selectivity and have low protein loading. Metal affinity ligands were grafted to poly (vinyl alcohol) hydrogels to provide a selective means to enhance protein loading and improve protein separation characteristics. The impact of solution conditions on the solubility and transport properties of chicken egg white lysozyme (CEWL) and Ribonuclease A (RnaseA) were determined. The metal affinity ligands provided a selective means to increase CEWL and RnaseA loading. In addition, the solubility of CEWL and RnaseA were moderated by changes in either temperature or solution pH. The impact of metal affinity ligands and solution pH on the mass transfer coefficient of CEWL and RnaseA through the hydrogel membranes will be discussed.

Keywords: Bioseparations, chicken egg white lysozyme, immobilized metal affinity chromatography, poly vinyl alcohol, ribonuclease A

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INTRODUCTION AND BACKGROUND

Separation systems are critical to biochemical processes for concentrating or purifying bioactive compounds including controlled release systems, membrane chromatography, artificial organs, membrane separations, and sensors (1–4). The development of robust materials that provide high selectivity separations is key to improvements of biochemical processes. Hydrogels are attractive materials for these applications because of their open mesh structure, biocompatibility, and mild operating conditions (4–15). These materials consist of a crosslinked hydrophilic polymer network that can swell considerably in the presence of aqueous solutions while maintaining a solid state (1–3). Solute transport occurs through a solution-diffusion mechanism in which the solute molecules dissolve and diffuse within the solution-filled region of the polymeric matrix. The diffusivity is governed by the size and shape of the solute combined with the mesh size of the hydrogel matrix. The open mesh of the hydrogels results in advantageously high diffusivity that can be controlled by variations in the degree of crosslinking and/or hydrophobicity of the polymer matrix. Since separations in hydrogels are based upon size exclusion, these materials typically have poor selectivity for molecules of similar size and shape (4–6). Additionally, hydrogels often exhibit low solubility or loading for proteins and lower molecular weight drugs and have release characteristics that are governed by the mesh size, which can limit their commercial applicability (7–10).

Several approaches have been used to improve control of transport properties of hydrogels including development of materials that exhibit dramatic swelling or collapse in response to small changes in environmental conditions (10–15). The ability to control swelling with environmental factors allows the design of materials with adjustable transport properties. For example, poly(isopropylacrylamide) exhibits a significant decrease in swelling in response to subtle changes in temperature which makes this an attractive material for use in sequential extraction schemes (10). For a simple example separations process, the hydrogel is placed in a solution, swells, and solutes smaller than the mesh size are absorbed into the hydrogel matrix. The hydrogel is placed into a second solution where a small temperature change is used to collapse the gel resulting in desorption of these species. Note that such a process will allow for nonselective concentration of protein solutions and would require a great deal of hydrogel. Barbari et al. synthesized poly(vinyl alcohol) hydrogels with mesh size asymmetry to decrease the mesh size of one surface of the hydrogel to provide a more selective surface layer without significant loss in flux (6). While this improved hydrogel selectivity, diffusion based

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separations are limited to proteins with large size variations and have little impact on protein loading.

Affinity chromatography (i.e. Ion Exchange Chromatography (IEC), Size Exclusion Chromatography, Immobilized Metal Affinity Chromatography (IMAC)) are popular methods for purification of recombinant proteins (16). Because of better mass-transfer performance, affinity chromatography using membrane materials has emerged as a viable alternative to the conventional packed bed chromatography methods (16–21). Affinity membrane separations exhibit reduce mass transfer, pressure drop, and flow limitations that are associated with packed columns. Proper design of the membrane systems requires a fundamental understanding of the underlying transport phenomena and sorption equilibrium behavior. Factors that influence the interaction of the biomolecule and the affinity membrane system include: membrane material, ligand type, solution parameters, etc. (22) For example, the surface properties of an affinity membrane may affect the adsorption capacity and behavior of targeted molecule (22). In recent studies, a number of novel membranes that incorporate affinity chromatography to improve separation have been investigated (19–22).

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Bayramoglu et al. synthesized 2-hydroxyethyl methacrylate and chitosan via UV-initiated photo-polymerization to form an interpenetrating network (IPN) membrane and subsequently grafted immobilized metal affinity (IMA) groups to study the binding characteristics and selectivity for a model protein lysozyme (20). These novel IPN metal-chelate affinity membranes were reusable, had high protein capacities and were effective in processing large volumes of biological fluid containing a target protein. The advantage of using IPN compared to hydrogels is that IPN possess a high mechanical strength (20). In another study, Bayramoglu et al. synthesized acrylic hydrogel co-polymer membranes with a dye-ligand (Cibacron Blue F3GA) as the affinity ligand to investigate the separation properties of bilirubin from human serum. These affinity hydrogel exhibited a high adsorption capacity, reusability, biocompatibility, and could provide economic advantages for large-scale bioseparation (21).

Affinity hydrogels have considerable potential for use as controlled release or chromatography media in small devices, as well as, components of membrane based separations media. Incorporation of highly specific affinity groups within hydrogels can simultaneously enhance the flux and selectivity in facilitated transport membrane schemes as well as improving protein loading and controlling release characteristics. Facilitated transport membranes use a rapid, reversible chemical reaction to augment the solute diffusion through a semi-permeable membrane (23,24). Poly(vinyl alcohol) (PVA) and poly(isopropyl acrylamide) were functionalized with the metal affinity ligand, iminodiacetic acid (IDA),

via a spacer 1,4 butane diol diglycidyl ether (BDE) and were shown to bind divalent metals (17,18). Histidine, which exhibits strong affinity for chelated metals, was used to probe the impact of affinity groups on transport characteristics of the PVA. Phenylalanine, which does not exhibit metal affinity, was used as the control penetrant to compare the transport environment in the control and affinity membranes. While there was approximately a two-fold increase in the rate of transport for histidine in the affinity membrane relative to the control membrane, the presence of chelated Cu^{+2} had no impact upon the mass transfer coefficient of the phenylalanine. The increase in the transport rates was attributed to the presence of the metal affinity carrier bound to the PVA backbone. While these results demonstrate the potential of affinity membrane systems for selective recovery of small analytes, the focus of this paper will be the characterization of affinity hydrogels for protein systems which are of greater commercial interest.

The transport properties of fixed carrier membranes are affected by a number of factors including:

1. strength of binding,
2. mobile phase characteristics, and
3. concentration of carrier sites (23,24).

For optimum operation of fixed carrier membranes, the binding strength should allow for a balance between binding of solutes of interests, as well as, ease of desorption and transfer of solute to adjacent affinity sites. For affinity hydrogel membranes, two distinct sorption modes exist:

1. absorption in the liquid region, and
2. binding or adsorption to the affinity sites.

Application of affinity hydrogels as either an adsorbent media or for carrier mediated membrane transport requires analysis of transport characteristics including affinity binding and mass transport studies.

This paper presents a study of the transport properties of model proteins within affinity hydrogel membranes based upon poly(vinyl alcohol) with grafted metal affinity ligands. Immobilized metal chelating ligands

1. are quite stable and easily synthesized,
2. operate over a diverse range of conditions,
3. can be readily grafted to hydrogel backbone,
4. have easily controlled binding affinities.

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Chelate groups are strong Lewis acids that form several coordinate bonds with the metal ion through the sharing of three or more pairs of electrons (17). The amino acid histidine, in particular, is of central focus to metal affinity based recovery systems, as the association constant for Me^{+2}-His can be quite high (2,14,25–28). Under controlled conditions of pH and salt content, the divalent metal ion-histidyl interaction may be tuned toward the recovery or release of a target species that contains solvent exposed histidyl groups (i.e. Chicken Egg White Lysozyme, (CEWL)).

Iminodiacetic acid (IDA) provides a balance between binding of the metal ion to the chelate and strong protein affinity which makes this an ideal model chelating ligand for this study. Transition metal ions were employed as the affinity ligand, as the ability to choose between divalent copper, cobalt, zinc, and nickel provides a high degree of flexibility in designing recovery schemes. Divalent copper, which was used for this study, exhibits the strongest binding affinity followed by Ni^{+2}, Zn^{+2} and Co^{+2} (25–28).

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Modifications to the aqueous phase composition (e.g. inclusion of salt, pH, and/or detergents) provides a measure of freedom, since one or more of these components can attenuate adsorption differences between competing species (28,29). Selectivity and strength of binding will depend on the type of salt employed, as well as its concentration. The inclusion of a chaotropic salt (e.g. NaCl) performs several important functions including: suppression of ionic effects, and promotion of adsorption. In addition, there is a sharp variation in Me^{+2}-histidine binding affinity with reductions in solution pH, which is primarily due to protonation of the imidazole ring of histidine. The impact of solution pH on the sorption and transport properties of CEWL and RnaseA in affinity hydrogels will be discussed in this paper.

EXPERIMENTAL

Materials

The hydrogels used for this study were prepared from poly(vinyl alcohol) (PVA) of molecular weight 70,000–100,000, crosslinked with glutaraldehyde using standard techniques (30,31). The metal chelating group (IDA) was grafted to the PVA backbone via a spacer arm (BDE) as shown in Fig. 1. All reagents were purchased from J.T. Baker or Aldrich Chemical Company and were used without further purification. CEWL, which has one surface exposed histidyl residue, was used as the model protein for the sorption studies. Ribonuclease A (RnaseA), which has

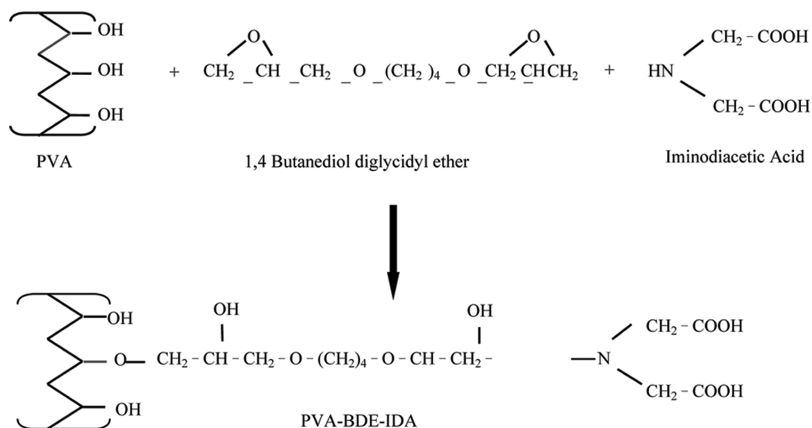

Figure 1. Grafting reaction for metal affinity ligand (IMA-Cu^{+2}) on PVA backbone via 1,4 BDE spacer.

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two surface exposed histidyl residue was used as the comparative protein for sorption and permeation studies. All protein solutions were buffered over a pH range from 5.0 to 8.0 using 0.05 M sodium phosphate in a solution of 0.50 M NaCl to minimize nonspecific binding of the positively charged lysozyme with the IDA groups.

Hydrogel Formation and Functionalization

Prior to membrane formation, a casting solution was prepared by adding the following reactants in aqueous solution to 16 ml of 0.125 g/ml PVA solution: 1.82 ml 10% (v/v) sulfuric acid, 0.596 ml 10% (v/v) acetic acid, 0.596 ml 50% (v/v) methanol solution, and 1.82 ml 2.5% (v/v) glutaraldehyde (27,28). Hydrogels were cast onto a glass plate using a doctor's blade set at 0.5 mm thickness, and allowed to crosslink for 2 hours at room temperature. Following crosslinking, the membranes were washed in deionized water to remove any residual reactants and stored in deionized water.

The metal affinity groups were grafted to PVA backbone of hydrogel using a well-defined reaction scheme that has been widely used for immobilized metal affinity chromatography (IMAC) systems (16). The functionalization procedure consisted of reaction of one epoxide group of the spacer arm (BDE) with a hydroxyl group on the PVA backbone followed by the reaction of the metal chelating group (IDA) with the available epoxide group of the bound BDE (Fig. 1). For the BDE reaction step,

a PVA membrane was incubated at room temperature in a solution containing 60 ml 0.3 M sodium hydroxide, 60 mg sodium borohydride and 7 ml BDE for 12 hours. Twelve hours was determined to be the optimal reaction time for the BDE functionalization step. An excess of BDE was used to minimize crosslinking reaction of the two epoxide groups on BDE with hydroxyl groups on the PVA backbone. The PVA-BDE hydrogel was removed from the BDE solution and washed in deionized water to remove excess reactants.

For the IDA reaction step, the PVA-BDE gel was placed in a solution of 70 ml of 2 M sodium bicarbonate and 5 g of dissolved IDA at 60°C for 24 hours. The metal chelating group was formed through reaction of the IDA with available epoxide groups on the bound BDE group. These hydrogels were used as the controls for subsequent studies as they exhibit no affinity for the target protein and are designated PVA-BDE-IDA. To produce the affinity membrane (PVA-BDE-IDA-Cu^{+2}), a PVA-BDE-IDA membrane was incubated in a $CuSO_4$ solution. The pH of the $CuSO_4$ solution was not determined for this study.

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Prior to hydrogel characterization, an incubation procedure was developed to ensure that all the affinity sites were saturated with copper and all unbound copper had been washed from the hydrogels. For this analysis, a set of hydrogels was functionalized with BDE for 12 hours followed by IDA reaction at 60°C for 24 hours. The hydrogels were placed in a 0.5 M $CuSO_4$, washed multiple times with deionized water, and placed in EDTA solution to strip the bound copper. Additionally, a virgin PVA gel was incubated in 0.5 M $CuSO_4$ solution and washed twice in deionized water and once in EDTA solution to ensure that the number of deionized water washes was sufficient to remove any unbound copper from the polymer matrix.

While the majority of unbound copper was stripped from each hydrogel following the first wash step, there was a significant amount of copper desorbed from the gels in the second water wash. There was little copper removed in the EDTA wash for the virgin PVA membrane. Similar results were seen for the PVA-BDE-IDA membranes following the first two wash steps. For gels incubated in high molarity $CuSO_4$, it is possible that there would be residual unbound copper within the membrane following two water wash steps. Therefore, incubation in a 0.05 M $CuSO_4$ solution followed by two water washes was used for preparation of affinity membranes (PVA-BDE-IDA-Cu^{+2}). These conditions were chosen to ensure that the affinity sites were saturated and that the copper content measured in the EDTA stripping solution was strictly from copper bound to metal affinity sites.

Note that the bound metal can easily be stripped from the affinity membranes using a stronger soluble metal chelate such as ethylene

diamine tetraacetic acid (EDTA) to reproduce the control membrane. To eliminate variations due to subtle difference in hydrogel formation and functionalization and allow direct comparison of material properties, the hydrogels were used as both control and affinity membranes for all characterization studies.

Membrane Characterization

Degree of Functionalization

The concentration of bound copper within the membrane was used as an indirect measure of the degree of functionalization with metal chelating ligands. An assumption of one-to-one binding of divalent copper ion with a chelating ligand is consistent with results from metal affinity chromatography systems (32). To determine the bound copper concentration, PVA-BDE-IDA-Cu^{+} membranes were washed twice in deionized water to remove any unbound copper and placed in a known volume of EDTA solution. The concentration of copper in the EDTA solution was measured using an Inductively Coupled Plasma Spectrometer (ICP) at the Instrumentation Center at the University of Toledo. A simple mass balance was used to determine the concentration of bound metal within each membrane.

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Equilibrium Solution Content

The equilibrium solution content (ESC) was used as a relative measure of swelling and to provide data to estimate mesh size of the hydrogel for the control and affinity membranes. The ESC was determined in water and phosphate buffer over the entire pH range used for the protein sorption studies. The ESC for membranes that were equilibrated in either phosphate buffer or deionized water was calculated using a simple mass balance.

$$ESC = \left(\frac{W_{wet} - W_{dry}}{W_{wet}}\right) \times 100\% \qquad (1)$$

For the wet weight (W_{wet}), the membranes were placed in aqueous solution at room temperature for more than 12 hours to ensure equilibrium hydration, removed, patted dry, and weighed. The dry weight (W_{dry}) was measured for membranes that had been dried in a vacuum oven for at least 12 hours at 100°C.

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Mesh Size Calculations

The mesh size describes the space available for solute transport in the region between the polymer chains of the hydrogel matrix. When the size of the network is larger than that of the solute, the solute can diffuse through the network. The method described by Matsuyama et al. was applied to calculating the mesh size of the hydrogels (33). These equations are valid for hydrophilic tetrafunctinoal crosslinked membrane systems where Gaussian distribution is assumed. The average mesh size, (ξ), of the swollen network was obtained by the following equation:

$$\xi_s = l_c \left(\frac{2M_c}{M_r} \right)^{1/2} C_n^{1/2} v_2^{1/3} \tag{2}$$

where ξ is the average mesh size, l_c is the C–C bond length (1.54 Å), c_n is the characteristic ratio (8.9 for PVA (32)), M_c is the average molecular weight between crosslinks (4,123 g/g_{mol}), M_r is the molar mass of the repeat unit (44 g/g_{mol}), and v_2 is the polymer volume fraction at swelling. The polymer volume fraction was obtained from the following equation:

$$v_2 = (1 - H) \tag{3}$$

where H is the water volume fraction of each membrane from equilibrium swelling experiments.

Protein Sorption Isotherms

The overall solubility of two proteins, CEWL and RnaseA, in control and affinity membranes over a pH range of 5.0 to 8.0 for CEWL and 5.0 to 7.2 for RnaseA was measured using standard experimental techniques (34,35). Sorption isotherms were measured for CEWL at room temperature and 37°C and for RnaseA at 37°C. The hydrogels were equilibrated in a phosphate buffer of appropriate pH, weighed, and placed in a known volume of protein solution. The protein concentration as a function of time was monitored using a Shimadzu 2401 PC UV-Vis spectrophotometer at 281.0 nm until steady state solution concentration was reached. The solubility of protein in the membrane (Q_t) was determined using a simple mass balance

$$Q_t = \frac{(C_0 - C_t) \times V_{sol}}{m_{membrane}} \tag{4}$$

where C_0 is the initial solution concentration of protein, C_t is the solution concentration of protein at time t, V_{sol} is the solution volume, and $m_{membrane}$ is the weight of the wet membrane equilibrated in buffer at the appropriate pH.

The sorption results can also be reported in terms of a solubility coefficient, K_{equ}, which is the ratio of equilibrium concentration of protein in the membrane (Q_{equ}) to the equilibrium solution concentration (C_{equ}). The K_{equ} will be reported in units of (mM protein/g wet membrane)/(mM protein/ml solution). Note that for the affinity membranes Q_{equ} includes both unbound protein that is absorbed within the membrane and protein that is adsorbed to affinity groups. The K_{equ} in the control membranes provides a measure of the relative concentration of bound and unbound protein in the affinity membranes. The bound copper content (BCC) of the affinity membranes were stable following exposure to both proteins during sorption studies which indicates that there was no detectable loss of affinity ligands.

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Permeation Experiments

Permeation studies were performed at 37°C using a side-by-side temperature controlled super diffusion cell (Crown Glass, Somerville, NJ). These experiments were used to measure the flux of the proteins through control and affinity membranes. The apparatus consisted of two half-cells with a volume of 50 ml and sampling ports at the top. A magnetic stir bar was placed in the well of each half-cell for continuous agitation at a constant rotation speed of 380 rpm to minimize boundary layer resistance. The cells were treated with Sigmacote® prior to the diffusion experiments to inhibit protein adsorption onto the glass walls. A pre-equilibrated membrane of approximately 0.060 cm thickness was sandwiched between the two half-cells and the sample ports were capped to prevent solution evaporation. The upstream (feed) side was filled with 50 ml of protein solution at the desired concentration and pH. The initial concentration used was 2 mg/ml for CEWL and 1 mg/ml for RnaseA. The downstream (permeate) was filled with 50 ml of buffer solution at the desired pH. At regular intervals, 1.5 ml solution was removed from the permeate cell, analyzed using a UV-Vis Spectrophotometer, and subsequently returned to the cell. CEWL was evaluated at a wavelength of 280 nm and 277 nm for RnaseA. These wavelengths are consistent with the values reported in the literature and are valid at the operating conditions examined in this study (25,27). Due to the relatively low extinction coefficient for RnaseA, it took much longer to reach a detectable permeate concentration for RnaseA than CEWL. Therefore, permeation

experiments for the control were conducted from 4 to 6 days RnaseA relative to 24 hours for CEWL.

The pH gradients used in this study examined solution conditions where the binding strength between the protein and copper ion were varied. The feed-permeate pH gradients used were selected to cover the protonated and deprotonated state of the histidyl residue on the protein's surface. Thus, the pH was used as a mechanism to modulate the relative binding strength of affinity groups at the feed and permeate membrane faces.

RESULTS AND DISCUSSION

Impact of pH on Affinity Hydrogel Properties

Bound Copper Concentration and Equilibrium Solution Content

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The BCC was used to quantify the degree of functionalization of the hydrogels with metal affinity ligands. The BCC for the membranes used for the studies at 37°C was 0.6 ± 0.10 mg Cu^{2+}/g membrane and 1.00 ± 0.10 mg/g for room temperature studies. Note that the hydrogels used for room temperature sorption studies had a greater extent of functionalization and will not be compared directly with the data at 37°C. The degree of functionalization was consistent with each of the different hydrogels used at a given temperature, so that any difference in protein sorption can be attributed to pH differences. In addition, these samples were used for both the control and affinity sorption studies over the entire pH range.

Prior to sorption studies, the impact of buffer pH on the mesh size of the control and affinity gels was monitored using the equilibrium solution content (ESC). The ESC in PVA, control and affinity membranes over the entire pH range is listed in Table 1 for hydrogels used for the experiments conducted at 37°C. The results are consistent for both systems and are similar at room temperature. The control membranes had a higher hydration level in water than in phosphate buffer solution at each pH. The charged sites of IDA in control membranes would be more hydrated than the IDA-Cu^{+2} sites in affinity membranes where the charge was dispersed over the metal complex. Salt ions in the buffer would replace the hydrating water molecules in the control membranes, thereby decreasing the difference in the ESC between an IDA group and an IDA-Cu^{+2} group. The control membranes had very similar ESC levels in buffer solution at all pH values studied.

Table 1. Equilibrium solution content of control and affinity membranes in aqueous and buffer solutions at room temperature

	Equilibrium solution content			
Membrane	Water	pH 5.0	pH 6.5	pH 8.0
PVA	0.900 ± 0.009	0.870 ± 0.005	0.870 ± 0.015	0.850 ± 0.010
PVA-BDE-IDA	0.933 ± 0.003	0.876 ± 0.005	0.878 ± 0.004	0.876 ± 0.004
PVA-BDE-IDA-Cu^{+2}	0.886 ± 0.004	0.869 ± 0.003	0.875 ± 0.005	0.873 ± 0.005

The water content of the affinity membrane and PVA are similar and lower than that of the control membrane. In the affinity membrane, Cu^{2+} replaces water molecules to interact with the electron donors on IDA, thereby resulting in a lower water content in affinity membranes. Unlike the control membranes, the swelling of the affinity membranes does not show significant difference in the water and buffer solution. The electron donor sites of IDA are already combined with copper ions in the affinity membranes so that the water molecules cannot interact with groups on the affinity membrane. The affinity and control membranes had a very similar ESC in buffer over the entire pH range. The stability in membrane swelling for both control and affinity membranes will allow for direct comparison to determine the impact of metal affinity groups on the transport properties of proteins within these membranes (i.e. CEWL solubility).

An ESC of 0.870 for the PVA in buffer solution was used to estimate the average mesh size of the base hydrogel at 161 Å (33). The ESC was similar for the affinity and control membranes in buffer solutions and the method used to estimate mesh size does not readily account for impact of large pendant groups. Therefore, the mesh size determined for the PVA in buffer was used for the affinity and control membranes. However, for these hydrogels solute diffusion would be affected by the mesh size of the PVA backbone along steric effects from covalently bound affinity groups. The length for the BDE-IDA group was estimated to be 13 Å using Spartan program in which the chemical structure was drawn, the energy minimized, and the length determined. Therefore, the randomly bound affinity ligands may further limit the space available for the solutes to diffuse through the hydrogel mesh.

Chicken Egg White Lysozyme Sorption at Room Temperature

The overall solubility at 25°C and 37°C of chicken egg white lysozyme (CEWL) in PVA-BDE-IDA-Cu^{+2} and PVA-BDE-IDA membranes was

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studied over a wide range of pH values. CEWL has one surface exposed histidine group and is commonly used as a model protein in metal affinity studies. Note that the pHs used for these experiments ranged from 5.0 to 8.0, encompassing the pKa of the histidyl residue on the lysozyme surface. The sorption isotherms in the control and affinity membranes are shown in Fig. 2 for CEWL at 25°C. To simplify the figure and allow for direct comparison, the sorption isotherms for the control membranes at all pH's are collapsed to one isotherm. The sorption isotherm is presented in terms of millimoles of protein adsorbed per gram wet membrane at equilibrium (Q) as a function of equilibrium solution concentration (C_e). There was no noticeable pH effect on lysozyme sorption in the control membranes.

Over the concentration range studied at 25°C, the sorption isotherms in the control membranes were linear and could be modeled using Henry's law isotherm. Solubility in affinity membranes results from contributions from absorption in the solution region and adsorption to affinity sites. While the solubility in the affinity membranes was linear in the low concentration range, there was a deviation from linearity at higher concentrations, which is consistent with the onset of saturation of affinity sites. This negative deviation from linearity is particularly distinctive for

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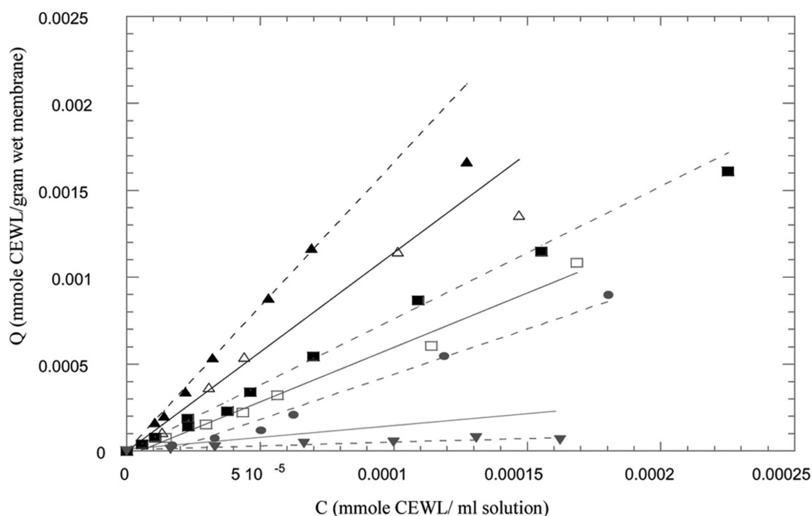

Figure 2. Sorption isotherms for CEWL at room temperature in control and affinity membranes as a function of solution pH: Control (▼), Affinity pH 5.0 (○), Affinity pH 5.56 (●), Affinity pH 6.0 (□), Affinity pH 6.5 (■), Affinity pH 7 (△) and Affinity pH 8 (▲).

the isotherm at pH 8.0 in the affinity membrane where the binding affinities were highest between CEWL and IMA groups. The maximum concentration tested was limited by the solubility level of CEWL of 5 mg/ml in the 0.5 M NaCl buffer that was used for this study (17,18). Since the solution concentration range was not sufficient for saturation of adsorption sites, it is not possible to distinguish between absorbed and adsorbed CEWL in these membranes and results will be discussed in terms of an overall solubility.

For each pH tested, the affinity membranes exhibited much larger solubilities than the control membranes. While the CEWL solubility exhibited no pH dependence in the control membranes, and there was a sharp increase in CEWL solubility with pH in the affinity membranes. The solubility coefficients for the control and affinity membranes were calculated by fitting a line through the sorption isotherm at each pH and are given in Table 2. At 25°C, the solubility coefficient in the control membrane remained relatively consistent at 0.43 with the increasing pH from 5.0 to 8.0. And, for the affinity membrane the solubility coefficient increased from 1.3 to 13.6 with increasing pH from 5.0 to 8.0.

The increase of the solubility coefficient in the affinity membrane with increasing pH is due to the strong coordinate complex formed between the immobilized copper ions and the histidyl residue on the surface of lysozyme molecule. Since the binding affinity between the imidazole group and copper ion is provided primarily by the electron-donating capability of the imidazole group, the binding affinity will be promoted as the extent of the deprotonation of the imidazole group on the surface of lysozyme is increased. A higher pH environment would result in a greater extent of deprotonation of the imidazole group on the surface of the lysozyme and therefore would cause higher lysozyme solubility in the affinity membrane. This pH dependence of affinity binding can be used to easily attenuate protein recovery in a hydrogel extraction process or mass transfer coefficient in a facilitated transport process.

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Table 2. Solubility coefficients at room temperature as a function of solution pH for CEWL in control and affinity membranes in (mmole CEWL/g hydrogel)/(mmole CEWL/ml solution)

	Solubility Coefficient (K)					
Membrane Type	pH 5.0	pH 5.5	pH 6.0	pH 6.5	pH 7.0	pH 8.0
Control	0.43	NA	NA	0.43	NA	0.47
Affinity	1.4	5.2	6.3	7.6	11.4	16.6

Chicken Egg White Lysozyme Sorption at 37°C

Sorption isotherms were measured for CEWL in PVA-BDE-IDA-Cu^{+2} and PVA-BDE-IDA membranes over a wide range of pH values. The experiments were conducted for solution concentrations that ranged from 0.5 to 7.0 mg CEWL/ml solution for the affinity membranes and 0.5 to 3.0 mg CEWL/ml solution for the control membranes. At higher temperatures, the solubility of the CEWL in the NaCl buffer was higher so a wider range of solution concentrations was examined. The sorption isotherms for CEWL in control and affinity membranes are shown in Fig. 3 and estimated solubility coefficients are shown in Table 3. The solubility, Q isotherm was linear with solution concentration for both control and affinity membranes. The affinity membranes exhibited higher CEWL solubility than the control membranes particularly at higher pHs which is consistent with results at lower temperature.

At pH 5.0, the solubility coefficient of CEWL in the affinity membrane was very similar to the values observed for CEWL sorption in control membranes. However, as the solution pH increased, a sharp increase in solubility was observed. The differences in the CEWL adsorption isotherms were more apparent when the pH value increased from 5.0 to 6.5

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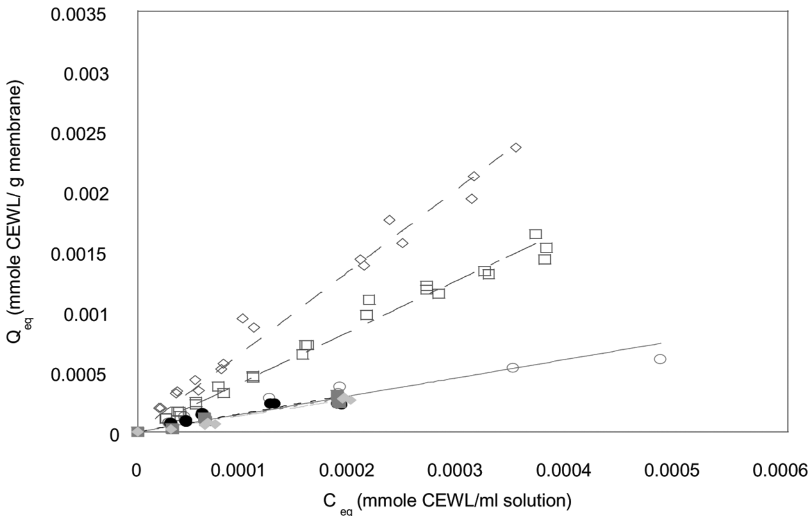

Figure 3. Sorption isotherms for CEWL at 37°C in control and affinity Membranes as a function of solution pH: Control pH 5.0 (◆), Control pH 6.5 (■), Control pH 8.0 (•) Affinity pH 5.0 (○), Affinity pH 6.5 (□) and Affinity pH 8.0 (◇).

Table 3. Solubility coefficients at 37°C as a function of solution pH for CEWL in control and affinity membranes in (mmole CEWL/g hydrogel)/(mmole CEWL/ml solution)

Membrane Type	pH 5.0	pH 6.5	pH 8.0
Control	1.38	1.43	1.57
Affinity	1.52	4.27	6.85

than 6.5 to 8.0. As the pH increases from 5.0 to 6.5, the solubility coefficient triples, whereas, there was a 50% increase in the solubility coefficient as the pH increased from 6.5 to 8.0. The solubility of CEWL in the affinity membranes was much higher than in the control membrane at pH values 6.5 and 8.0. The solubility coefficient in the control membrane ranged from 1.38 to 1.57 with the increasing pH from 5.0 to 8.0. The solubility coefficient increased gradually from 1.52 to 6.85 with increasing pH from 5.0 to 8.0. Note that the solubility coefficient decreased for 6.5 and 8.0 at 37°C compared to 25°C approximately by half. This observation is consistent with temperature affects on the solubility coefficient noted in IMAC literature combined with the lower concentration of IMA ligands in these samples used for sorption studies at 37°C (27).

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Ribonuclease A Sorption

The adsorption isotherms were measured for RnaseA at 37°C. RnaseA is similar in molecular weight to CEWL but has twice the number of surface exposed histidyl groups. A pH of 7.2 was maximum used for these studies because RnaseA tends to denature. Since no significant pH dependence was observed in the control membranes for CEWL, the control experiments for RnaseA were performed at pH 6.5 only.

For the control membrane, the adsorption isotherm at pH 6.5 was linear over the concentration range studied, as shown in Fig. 4. As expected, the solubility coefficient estimated using Henry's law was low and similar in value to the CEWL in control membrane. For the affinity membranes, as the pH was increased from 5.0 to 7.2 a significant increase in the solubility of RnaseA was observed. The sorption isotherms generated were linear over the concentration range over the lower concentration regions with some deviation from linearity at higher concentration for pH 7.2, The solubility coefficients was calculated for the linear region and are reported in Table 4. The increase in solubility was more defined as the pH was increased from 5.0 to 6.5 and than 6.5 to 7.2. The solubility

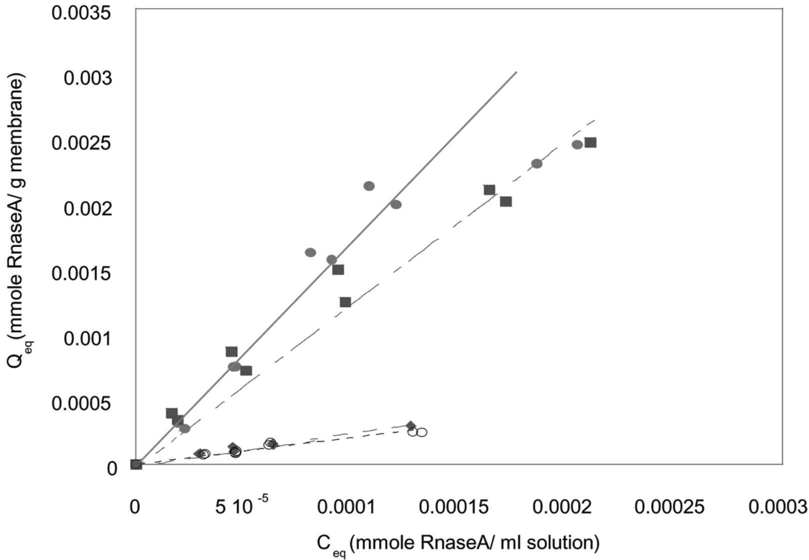

Figure 4. Sorption isotherms for RnaseA at 37°C in control and affinity membranes as a function of pH: Control pH 6.5 (○), Affinity pH 5.0 (◆), Affinity pH 6.5 (●) and Affinity pH 7.2 (■).

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coefficient increased approximately by 85% as the pH increased from 5.0 to 6.5, whereas, the solubility coefficient showed little variation as the solution pH increased from 6.5 to 7.2. These results follow a similar trend as those for CEWL. At 37°C, the solubility coefficient in the control membrane was 1.56 for pH 6.5. The solubility coefficient increased gradually from 1.52 to 11.2 with increasing pH from 5.0 to 7.2 for the affinity membrane.

As shown in Tables 3 and 4, there was an increase in K_{equ} for the affinity membranes with increasing pH for both proteins studied. The sorption coefficients for the two proteins in the control membranes were

Table 4. Solubility coefficients at 37°C as a function of solution pH for RnaseA in control and affinity membranes in (mmole RnaseA/g hydrogel)/(mmoleRnaseA/ml solution)

Membrane Type	pH 5.0	pH 6.5	pH 7.2
Control	Not determined	1.56	Not determined
Affinity	1.52	10.2	11.2

similar. At pH 5.0, K_{equ} for both proteins was low and similar to the values reported for their respective control membranes. This suggests that at low pH, the interaction between the protein and copper ions is weak due to the electron donating groups on the surface of the protein being in a protonated state. Lowering the solution pH decreases the dissociation degree of the proton from the nitrogen reaction between the copper ion and protein or peptide and the solubility that occured was primarily due to the absorption of the species into the solution region of the membrane which is similar to the control membranes.

At pH values greater than 5.0, there was a significant increase in K_{equ} for both proteins. As the solution pH shifts across the pK_a of the histidine group(s) on the protein surface, which ranges from 5.5 to 8.5 for the proteins observed, a significant increase in protein binding was achieved. Incremental increases occurred as the solution pH nears the individual isoelectric point (pI) of each molecule, where maximum adsorption was expected.

In general, the trend of sorption RnaseA and CEWL are similar; however, as the solution pH changed from 6.5 to 7.2 no significant increase in K_{equ} value was observed. RnaseA with an isoelectric point (pI) of 9.2 is closer to neutrality at higher pHs than the CEWL with a pI of 11.2. Thus, the pH differences from 6.5 to 7.2 for RnaseA were not large enough to observe a significant impact on K_{equ}–value.

PERMEATION STUDIES AT 37°C

CEWL in Cu^{+2} Affinity Hydrogel

The mass transfer coefficient (MTC) of CEWL was determined for the PVA and control membranes to provide a basis for comparison with affinity membranes. Since, swelling of PVA and protein sorption in control membranes were not affected by changes in pH, the solution pH should not impact the MTC in these membranes. The permeation experiments for the PVA and control were conducted using a feed of pH 8.0 and a permeate pH of 5.0. Permeation studies were run for sufficient time to ensure steady state and to observe a substantial increase in permeate concentration. The MTC at these conditions for CEWL in PVA, control membranes and affinity membranes are summarized in Table 5.

The overall MTCs of CEWL in the PVA and control membranes were the same. Transport in the control and PVA membrane occurs by diffusion in the solution region of the membrane. In simple diffusion, the rate of transport is affected by both protein and membrane

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Table 5. Mass transfer coefficient of CEWL in PVA, control, and affinity membranes for varying pH feed-permeate gradients 37°C

Hydrogel	Feed pH	Permeate pH	Overall MTC (cm/s)
PVA	8.0	5.0	$3.17 \times 10^{-6} \pm 6.2 \times 10^{-8}$
Control	8.0	5.0	$3.15 \times 10^{-6} \pm 8.5 \times 10^{-8}$
Affinity-Cu^{+2}	8.0	5.0	$1.60 \times 10^{-6} \pm 2.8 \times 10^{-8}$
Affinity-Cu^{+2}	6.5	5.0	$1.84 \times 10^{-6} \pm 5.0 \times 10^{-8}$
Affinity-Cu^{+2}	6.5	6.5	$2.09 \times 10^{-6} \pm 3.5 \times 10^{-8}$
Affinity-Cu^{+2}	5.0	6.5	$2.06 \times 10^{-6} \pm 3.0 \times 10^{-8}$

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characteristics. For example, protein size and shape and hydrogel mesh size will determine the effective protein diffusivity. The degrees of swelling as determined by ESC of these membranes were very similar, so that there was little difference in the mesh size. These results suggests that grafting of the large BDE-IDA group to the PVA backbone did not result in significant steric hindrance or affect the diffusion environment in the control membranes.

The overall MTCs for CEWL in affinity membranes using multiple pH gradients are listed in Table 5. The pH values were selected to cover the pK_a range of histidyl residues and to provide a wide range of protein binding affinity on both membrane faces. For the affinity membranes, there was a decrease in MTC for CEWL relative to the control membranes for all pH gradients used. Additionally, the decrease was largest for affinity membranes where high feed pHs were utilized. While there was a 50% decrease in MTC for affinity membranes at pH 8.0 in feed relative to the control, the decrease was 42% for pH of 6.5 feed. Therefore, an increase in binding affinity with feed side pH resulted in decreases in the MTC. When a constant pH of 6.5 was applied across the membrane, the MTC in affinity membranes were slightly higher than with the 6.5 feed-5.0 permeate gradient. Finally, a reversed pH gradient of 5.0 in the feed and 6.5 in the permeate was investigated to modify the driving force for permeation. There was little change in the MTC relative to the system with constant pH of 6.5.

The results for pH studies suggest that the complexation-decomplexation reaction with affinity groups has a negative effect on the rate of CEWL transport in immobilized metal affinity hydrogel membranes. This may be due to strong binding of the protein to the affinity site and/or steric hindrance resulting from large bound proteins (MW = 14200) that could effectively reduce the diffusion

pathway. A weaker binding divalent metal can easily be substituted for Cu^{+2} to moderate the equilibrium binding kinetics of CEWL in the membrane and reduce the concentration of bound protein within affinity membrane. For example, the binding capacity for Cu^{+2} is typically more than an order of magnitude greater than that of Ni^{+2} in IMA chromatography systems (25). Additionally, a lower crosslink density can be used in producing the PVA backbone which would provide a large mesh size for protein diffusion and minimize the steric effect of bound species in solution.

RnaseA in Cu^{+2} Affinity Hydrogel

The MTCs for RnaseA which exhibits much higher solubility than CEWL were measured at similar conditions to monitor the impact of concentration of bound protein on diffusion in the hydrogel. While RnaseA has two surface exposed histidyl residues compared to one for CEWL, these molecules are similar in size and have similar diffusivity in water. The PVA and control experiments were conducted using a feed pH of 8.0 and a permeate pH of 5.0. The affinity membrane experiments were conducted using feed-permeate gradient of 6.5 and 5.0. The results for RnaseA transport in PVA and control membranes are summarized in Table 6. As was seen for the CEWL, grafting BDE-IDA groups to the PVA backbone had no effect on the rate of mass transport of the RnaseA. The MTC of RnaseA in control membranes was similar to CEWL which is consistent for proteins with similar solubility coefficients and protein size.

Since the protein concentration in the permeate side did not reach the lower detection limit within 4 to 6 days, there was no detectable mass transport for RnaseA in the affinity membranes. The solubility coefficient of RnaseA is more than twice that of the lysozyme, therefore, any space filling effect of bound protein would be further magnified.

Table 6. Mass transfer coefficient of RnaseA in controal and affinity membranes at 37°C

Hydrogel	Feed pH	Permeate pH	Overall MTC (cm/s)
PVA	8.0	5.0	$3.18 \times 10^{-6} \pm 6.95 \times 10^{-8}$
Control	8.0	5.0	$3.16 \times 10^{-6} \pm 3.54 \times 10^{-8}$
Affinity-Cu^{+2}	6.5	5.0	Not detectable

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CONCLUSIONS

A series of PVA hydrogels were functionalized with the metal affinity ligand, IDA via a spacer arm, 1,4 BDE. The effect of mobile phase parameters (i.e. protein size and affinity and solution pH) on the protein sorption and mass transfer was investigated in the affinity and control hydrogels. Since the swelling was independent of solution pH in both the affinity and control membranes, the diffusion environment was stable for the entire pH range studied. The affinity hydrogels exhibited considerably higher solubilities than the corresponding control membranes at each pH studied with an increase in solubility with increasing solution pH. The solubility in the control membranes was independent of solution pH. The ease of controlling the binding affinity using pH with no change in the diffusion environment makes this an ideal system for study of affinity membranes. The incorporation of affinity ligands within these hydrogels resulted in very high loading for both CEWL and RnaseA. The RnaseA with two surface exposed histidyl residues had higher adsorption to binding sites within the affinity hydrogel which resulted in a higher overall solubility.

The MTCs for CEWL and RnaseA were similar in the control and PVA membranes which indicates that the presence of the grafted metal affinity ligands did not affect the diffusion environment. A general decrease in the MTCs for the affinity membranes relative to the control membranes was observed for both proteins irrespective of the feed-permeate pH gradient employed. The best results were achieved for systems that operated at low pH (pH 5.0) where adsorbed concentrations were lower. The reduction in MTC in the affinity membranes may be attributed to strong binding of the protein to affinity groups, which reduces the mobility of bound proteins and/or reduction in mesh size effective transport area due to the space filling effect of bound proteins within the hydrogel matrix. There was no detectable transport of RnaseA within the affinity membranes, which is consistent with hypothesis that bound proteins provided significant steric hindrance in the affinity hydrogels.

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REFERENCES

1. Ramirez-Vick, J.E.; Garcia, A.A. (1996–97) Separation and Purification Methods. In: *Recent Developments in the Use of Group-Specific Ligands for Affinity Bioseparations*, Waskat, P.; Van Oss, C., eds.; 25, 85–129.
2. Wong, J.W. (1991) Separation and Purification Methods. *Immobilized Metal Ion Affinity Chromatography (IMAC) Chemistry and Bioseparation Applications*, Wankat, P.; Van Oss, C., eds.; 20, 49–106.
3. Nova, C.J.M.; Paolucci-Jeanjean, D.; Belleville, M.-P.; Barboiu, M.; Rios, G. (2008) Elaboration, characterization and study of a new hybrid chitosan/ceramic membrane for affinity membrane chromatography. *Journal of Membrane Science*, *321*: 81–89.
4. Takamura, A. (1992) Drug release from poly(vinyl alcohol)gel prepared by freeze-thaw procedure. *Journal of Controlled Release*, *20*: 21–28.
5. Gerald-Gerlach, M.; Sorber, J.G.; Suchanecle, G.; Arndt, K.-F.; Richter, A. (2005) Chemical and pH sensors based on swelling behavior of hydrogels. *Sensors and Actuators*, *B111–112*: 555–561.
6. Li, R.H.; Barbari, T.A. (1994) Protein transport through membranes based on toluene diisocyanate surface-modified poly(vinyl alcohol) gels. *Journal Membrane Science*, *88*: 115–125.
7. Kim, J.J.; Park, K. (1999) Smart hydrogels for bioseparations. *Bioseparation*, *7*: 177–184.
8. Dinarvand, R.; D'Emanuele, A. (1995) The use of thermoresponsive hydrogels for on-off release of molecules. *J. of Controlled Release*, *36*: 221–227.
9. Cussler, E.L.; Stokar, M.R.; Varberg, J.E. (1984) Gels as size selective extraction solvents. *AICHE J.*, *30*: 578–582.
10. Kwon, I.C.; Bae, Y.H.; Kim, S.W. (1991) Electrically erodibl polymer gel for controlled release of drugs. *Nature*, *354*: 291–293.
11. Sawahata, K.; Hara, M.; Yasunaga, H.; Osada, Y. (1990) Electically controlled drug delivery using polyelectyte gels. *J. Controlled Release*, *14*: 253–266.
12. Tang, M.; Zhang, R.; Bowyer, A.; Eisenthal, R.; Hubble, J. (2004) NAD-sensitive hydrogel for the release of macromolecules. *Biotechnology and Bioengineering*, *87* (6): 791–796.
13. Tang, M.; Zhang, r.; Bowyer, A.; Eisentahl, R.; Hubble, J. (2003) A reversible hydrogel membrane for controlling the delivery of macromolecules. *Biotechnology and Bioengineering*, *82* (1): 47–53.
14. Kabra, B.G.; Gehrke, S.H.; Spontak, R.J. (1998) Microporous, responsive hydroxypropyl cellulose gels. 1. Synthesis and microstructure. *Macromolecules*, *31*: 2166–2173.

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15. Gehrke, S.H.; Uhden, L.H.; McBride, J.F. (1998) Enhanced loading and activity retention of bioactive proteins in hydrogel delivery systems. *J. Controlled Release*, *55*: 21–33.
16. Sulkowski, E. (1989) The Saga of IMAC and MIT. *BioEssays*, *10* (5): 170–175.
17. Chai, S.A.; Beitle, R.R.; Coleman, M.R. (1996) Facilitated transport metal affinity membranes. *International Journal of Biochromatography*, *2*: 125–131.
18. Iyer, G.; Iyer, P.; Tillekeratne, L.M.V.; Coleman, M.R.; Nadarajah, A. (2007) Controlling phase transition behavior of thermally responsive metal affinity hydrogels: A molecular design approach. *Macromolecules*, *40*: 5850.
19. Changa, G.S. (2001) Twenty-five years of immobilized metal ion affinity chromatography: Past, present, future. *Journal of Biochemistry Biophysics Methods*, *49*: 313–334.
20. Bayramoglu, G.; Kaya, B.; Arica, M. (2002) Procion Brown MS-5BR attached and lewis metals ion-immobilized poly(hydroxyethyl methacrylate)/chitosan IPNs membranes: Their lysozyme adsorption equilibria and kinetics characterization. *Chemical Engineering Science*, *57*: 2323–2334.
21. Bayramoglu, G.; Yalcin, E.; Arica, M. (2005) Characterization of polyethylenimine grafted and Cibacron Blue F3GA immobilized poly(hydroxyethylmethacrylate-co-glycydylmethacrylate) membranes and application to bilirubin removal from human serum. *Colloids and Surfaces A: Physiocochem. Eng. Aspects*, *264*: 195–202.
22. Klein, E. (2000) Affinity membranes: A 10-year review. *Journal of Membrane Science*, *179*: 1–27.
23. Goyette, M.; Longin, T.; Noble, R.; Koval, C. (2003) Selective photofacilitated transport of sodium ions through liquid membranes: Key factors in experimental design, transport results and comparison with a mathematical model. *Journal of Membrane Science*, *212*: 151–160.
24. Kim, J.D.; Woo, J.; Kang, Y.; Jang, H. (2006) The structural transitions of π-complexes of poly(styrene-b-butadiene-b-styrene) block copolymers with silver salts and their relation to facilitated olefin transport. *Journal of Membrane Science*, *281*: 369–376.
25. Hearn, M. (1996) Protein interaction with immobilized methal ion affinity ligands under high ionic strength conditions. *Analytical Biochemistry*, *242* (0426): 45–54.
26. Ching-Fa Wu, W.-Y.C.; Jiunn-Fwu, Lee. (1996) Microcalorimetric studies of the interactions of imidazole with immobilized Cu(II): Effects of pH value and salt concentration. *Journal of colloid and Interface Science*, *183* (538): 236–242.
27. Ataai, M.M. (1991) Immobilized metal affinity chromatography and related techniques. *AIChE Symposium Series*, *88* (290): 34–42.
28. Porath, J. (1987) Metal ion-hydrophobic, thiophillic, and II-electron governed interactions and their application to salt-promoted protein adsorption chromatography. *Biotechnology Progress*, *3* (1): 14–21.
29. Porath, J.; Olin, B. (1983) Immobilized metal ion affinity adsorption and immobilized metal affinity chromatography of biomaterials. Serum

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protein affinities for gel-immobilized iron and nickel ions. *Biochemistry*, *22*: 1621–1630.
30. Chai, S.-A. *Facilitated Transport Using Immobilized Metal Affinity Functionalized Poly(vinyl alcohol) Gel Membranes*, in Chemical Engineering. 1996, University of Arkansas: Fayetteville.
31. Crookston, D. *The effect of Spacer Arm Length on the Separation of Amino Acids and Proteins with Facilitated Transport Membranes*, in Chemical Engineering. 1998, University of Arkansas: Fayetteville.
32. Fanou-Ayi, L. Metal-chelate affinity chromatography as a tool. *Biochemical Engineering III, Annals of the New York Academy of Sciences*, *413*: Published by NY Academy of Sciences.
33. Matsuyama, M.T.; Urano, H. (1997) Analysis of solute diffusion in poly (vinyl alcohol) hydrogel membranes. *Journal of Membrane Science*, *126*: 151–160.
34. Beitle, R.R.; Ataai, M.M. (1992) New Developments in Bioseparations. In *Immobilized Metal Affinity Chromatography and Related Techniques*, Ataai, M.; Sikdar, S., eds.; *AICHE*, NY, 34–44.
35. Agarwal, S. (2001) Interactions of proteins with immobilized metal ions: A comparative analysis using various isotherm models. *Journal of Colloid and Interface Science*, *288*: 126–140.

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