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Supported Poly(Vinyl Alcohol) Hydrogels for Facilitated Transport of Histidine

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ABSTRACT

A membrane was prepared to facilitate the transport of histidine by impregnating a microfiltration membrane with a chemically activated, polymer hydrogel. The rate of histidine diffusion through the membrane was measured with time; mass transfer coefficients and effective diffusion coefficients were determined for intermediate stages of sequential modification. Both supported and nonsupported hydrogel transport properties were compared and evaluated. Results indicated that the supported membrane was mechanically stable as compared to the unsupported membrane, and more significantly, that the addition of divalent copper facilitated the transport of histidine.

INTRODUCTION

Facilitated transport using metal affinity chemistry as the basis for separation is a novel method for the fractionation of biologicals. Facilitated transport has been applied in a variety of separations using emulsion liquid membranes, liquid membranes, and fixed-site carrier membranes. In order for enhanced mass transport to occur, a reversible, selective chemical interaction between the target solute of interest and facilitating species is required (1, 2). Facilitated transport is therefore a combination of chemical and diffusional processes. Examples of systems which describe/use facilitated transport include: heavy metal ion extraction via phosphate interaction, monosaccharide concentration, fatty acid transport via interaction with fatty-acid-binding proteins, and classic oxygen/nitrogen separation via interaction with metal ions (3–14).

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Germane to this work is the application of fixed-site carrier chemistry which selects for metal affinity in a liquid format (15, 16). This work describes the preparation of a composite membrane for facilitated transport via interaction with a metal ion, and the assessment of membrane characteristics. The interaction between a cation [e.g., Cu(II)] and the R-group of histidine is quite selective under conditions of high salt content, and has been the fundamental cornerstone of immobilized metal affinity chromatography (IMAC), a mature chromatographic technique of approximately 25 years (17, 18). Factors making metal-affinity-based bioseparation attractive include: relative cost of a metal ion compared to another biomolecule such as an antibody, generic application based solely upon histidine topography, and ease of incorporation of genetic modifications to enhance metal affinity in an expression system. A extensive number of proteins have been purified (in some cases to homogeneity) via IMAC or related technique(s) (19–25).

In previous investigations of facilitated transport via metal affinity, unsupported, crosslinked poly(vinyl alcohol) (PVA) was used to provide proof-of-concept data for the enhanced transport of histidine (26). In principal, unsupported membranes could be used for future investigations and/or industrial applications; however, supported materials are desired from the standpoint of mechanical integrity (27). Specifically, we describe the preparation of a supported chelate containing hydrogel of crosslinked PVA. In short, microfiltration membranes were used as supports for metal chelate PVA hydrogels, and the mass transport of histidine was examined using a two cell, stirred batch system.

THEORY

A principal means of thin film characterization is the determination of transport data across the membrane (11, 28, 29). The overall mass transfer coefficient (K) is defined by

$$N = KA(C_f - C_p) = \frac{\partial}{\partial t}(V_p C_p) = -\frac{\partial}{\partial t}(V_f C_f) \tag{1}$$

where N is the rate of solute transfer through a membrane area (A) due to the concentration difference between the feed (C_f) and the permeate (C_p) concentrations of each cell (V_i). Alternately, the overall mass transfer coefficient may be defined by a resistance in series model, i.e.,

$$\frac{1}{K} = \frac{L}{D} + \frac{1}{k_f} + \frac{1}{k_p} \tag{2}$$

where k_f and k_p are the mass transfer coefficients of their respective cells, L represents membrane thickness, and D is an "effective" diffusion coefficient.

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In a system where diffusion via mechanism(s) other than free and random motion is encountered, the diffusion coefficient would differ from that of a free solution; hence an effective D is reported. Due to a consistent and high rate of stirring, k_f and k_p are assumed negligible and constant. Therefore, the overall transfer coefficient will approach the ratio D/L.

To account for changes in the feed and permeate volume during the course of an experiment, Eq. (1) is solved assuming discrete samples are taken at time intervals that cause a volume change for each cell (30). Solving the pseudosteady-state case, KA is determined by

$$\ln \frac{(C_{f0} - C_{p0})}{(C_{fi} - C_{pi})} = KA \sum_{n=1}^{i} \left(\frac{1}{V_{fn}} + \frac{1}{V_{pn}} \right)(t_n - t_{n-1}) \tag{3}$$

where C_{f0} and C_{p0} are the initial feed and permeate solute concentrations, C_{fi} and C_{pi} are the feed and permeate solute concentrations in Sample i, $V_{f,n}$ and $V_{p,n}$ are the liquid volume in the feed and permeate chambers during time period n, t_n is the time at Sample n, and t_{n-1} is the time at Sample $n - 1$. A plot of the left-hand side of Eq. (3) versus the summation term on the right-hand side should be a straight line with a slope equal to KA. Using the overall mass transfer coefficient determined from Eq. (3), the effective diffusion coefficient within a supported membrane can be determined by incorporation of the tortuosity and porosity of the support:

$$D_{eff} = \left(\frac{L\tau}{\varepsilon} \right) K \tag{4}$$

METHODOLOGY

Support membranes (designated "support" herein) were hydrophilic, porous poly(vinylidine fluoride) (PVDF), trade name Durapore, manufactured by Millipore Corp. (Bedford, MA). The nominal pore diameter was 0.22 μm with a porosity of 0.67 as per the manufacturer literature, and equivalent tortuosity factor of 2.3 as determined by Kapur et al. (28). The support thickness (L) was 0.125 mm (28). In order to impregnate the porous membrane, an ultrafiltration (UF) cell model 8050, Fig. 1, from Amicon (Beverly, MA) was used. Prior to chemical modification, stock membranes were reduced from 47 to 43 mm.

PVA Impregnation/Water Flux

PVA gels were prepared by first dissolving high molecular weight PVA powder in water using heat and mechanical agitation. Four milliliters of the resulting 11.5% (by mass) PVA solution were added to 0.227 mL of 2.5% (by mass) glutaraldehyde and 0.149 mL of 50% (by volume) methanol (26).

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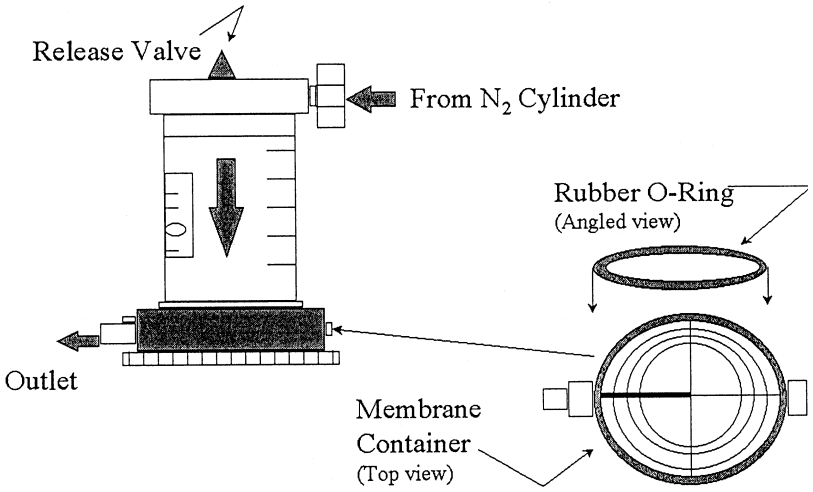

FIG. 1 Amicon UF cell. The support membrane is placed in the unit, and the PVA solution to be crosslinked is added to the cell, warmed, and pressurized.

To prepare the hydrogel, in-situ crosslinking of the PVA solution was accomplished and confirmed using the following procedure. After a support was placed in the UF cell, the PVA solution previously described was slightly heated to decrease its viscosity, added to the cell, and shaken to fully wet the membrane surface. Next, the cell was pressurized with an inert gas, N_2, to 60 psig. Once the solution began to flow out of the permeate tube, the cell pressure was decreased to force bubbles from the membrane. After impregnation, this intermediate was removed from the cell, placed between two pieces of Teflon tape, and gently compressed to remove the excess PVA solution from the surfaces. To initiate crosslinking, a solution of 0.227 mL of 10% (by volume) sulfuric acid, 0.075 mL of 10% (by volume) acetic acid, and 0.075 mL of 50% methanol was used. After four hours, excess crosslinking solution was rinsed by overnight soaking in deionized water with gentle agitation.

At this point, water flux was measured at 5, 10, 15, and 20 psi transmembrane pressure (TMP). If required, the above procedure was repeated until the water flux had been reduced to less than 10^{-4} cm/s at 5 psig TMP. Lastly, the membrane thickness was measured using a caliper. In all cases, thickness did not deviate significantly from the original Durapore membrane thickness; therefore, the assumed thickness (L) of the PVA hydrogel was assumed to be that of the support.

Chemical Activation

The supported PVA hydrogel was placed in a solution of 60 mg of sodium borohydrate in 60 mL of 0.3 M sodium hydroxide (26). During gentle agita-

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tion, 2.5 mL of 1,4-butanediol diglycidyl ether (BDE) was added. After a minimum of 12 hours, membranes were rinsed with deionized water. To prepare the solution of the chelating ligand, 0.625 g of iminodiacetic acid (IDA) was dissolved in 40 mL of 2 M sodium carbonate. The membrane was then placed in the solution and shaken for an additional 12 hours at 60°C, followed by a rinse with deionized water for 2 hours. To charge with Cu(II), the membrane was placed in a 5 mg/mL solution of the sulfate salt for 12 hours. Finally, the membrane was washed with deionized water and stored in a buffer solution (0.05 M phosphate + 0.1 M sodium chloride, 6.5 pH).

Diffusion Experiments

The membrane diffusion experiments were done in accordance with Chai et al. (11, 26) using a two-chamber stirred diffusion cell, as illustrated in Fig. 2. The set-up consisted of two cells (*V* approximately 160–200 mL), 5.5 cm in diameter, and 7.4 cm long. A magnetic stir bar was placed in both cells and rotated in order to achieve constant mixing. The membrane was secured between two glass slides with a known circular area, 11.4 cm^2, across which diffusion can occur. Rubber O-rings were used to prevent leaking.

The feed solution consisted of 2.5 g/L histidine in a buffer at 6.5 pH (0.05 M phosphate + 0.1 M sodium chloride), whereas the permeate solution was buffered at 6.5 pH. Samples were removed from both the feed and permeation cells for 6 to 7 hours. Temperature data were also recorded for each data point. The samples were analyzed with a UV-VIS Spectrophotometer, Model DU-460 (Beckman, USA).

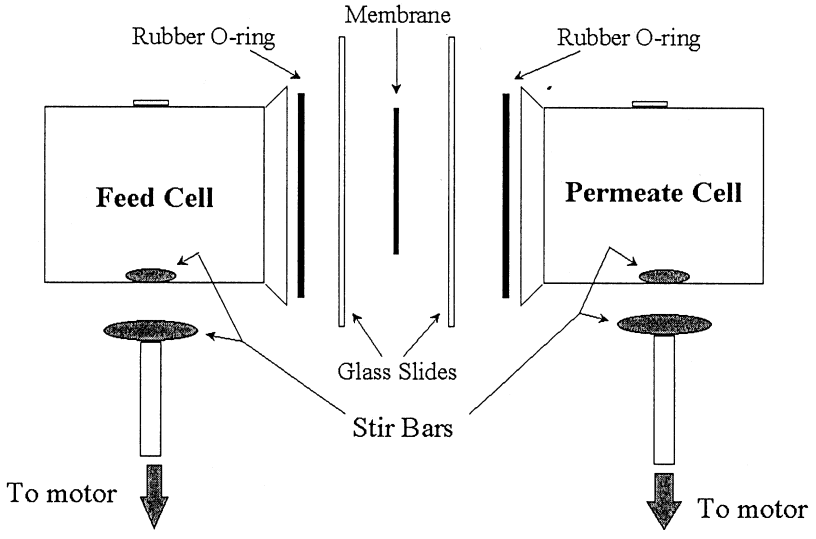

FIG. 2 Stirred diffusion cell. See text for details.

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RESULTS

PVA Impregnation/Water Flux

Water flux helped to determine if crosslinked PVA was maintained within the support. A volumetric flow rate corresponding to approximately 3 mL/day at 5 psi TMP was chosen to define nonhindered passage of fluid. Such a value may seem somewhat arbitrary, but it has previously been used by other investigators in similar settings. Adopting this criterion in order to prepare a hydrogel devoid of pores large enough for free water passage, at maximum three cycles of PVA impregnation and crosslinking were typically required. Corresponding to this flow rate was a water flux below 10^{-4} cm/s at 5 psi TMP.

Diffusion Experiments

Illustrated in Figs. 3 and 4 are data that describe the transport of histidine into the permeate. Three membranes were used in both sets of experiments, and they differ by the presence of Cu(II) within the membranes. From the diffusion experiments, the data of permeate histidine concentration versus time, Figs. 3 and 4, illustrate the linearity and the reproducibility of the experiment. Using Eq. (3), slopes (KA) were determined to be $3.8 \pm 0.2 \times 10^{-4}$ and $8.1 \pm 0.4 \times 10^{-4}$ cm^3/min in the absence and presence of Cu(II), respectively.

Figure 5 summarizes mass transport coefficients for intermediate steps [crosslinking of PVA, addition of BDE, addition of IDA, and finally Cu(II)

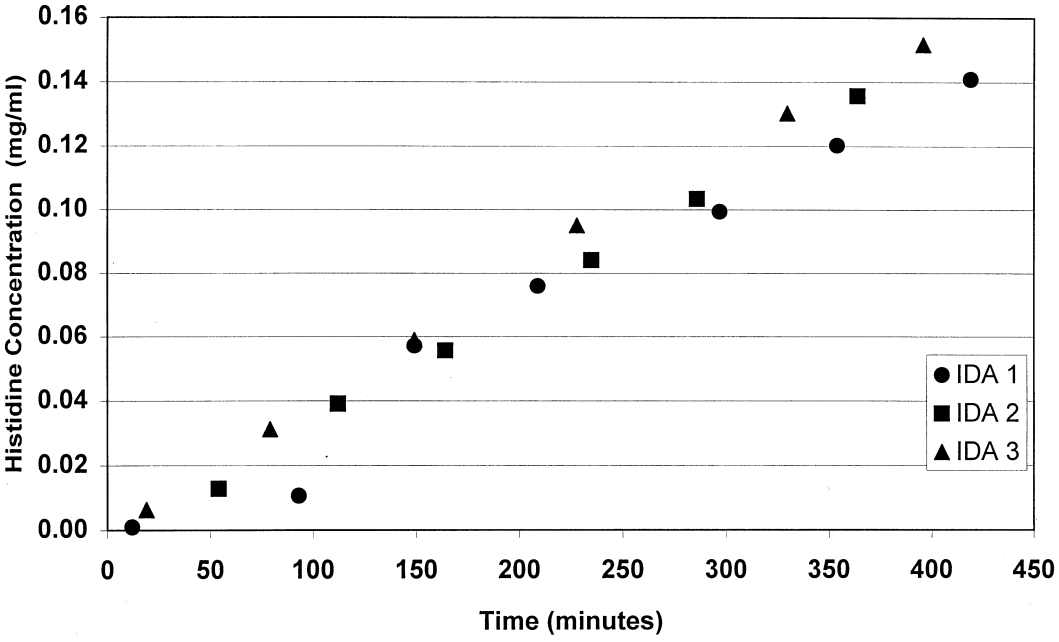

FIG. 3 Permeate histidine concentration vs time for uncharged, chelate-containing membranes. Three membranes [no Cu(II) present] were challenged with a histidine feed.

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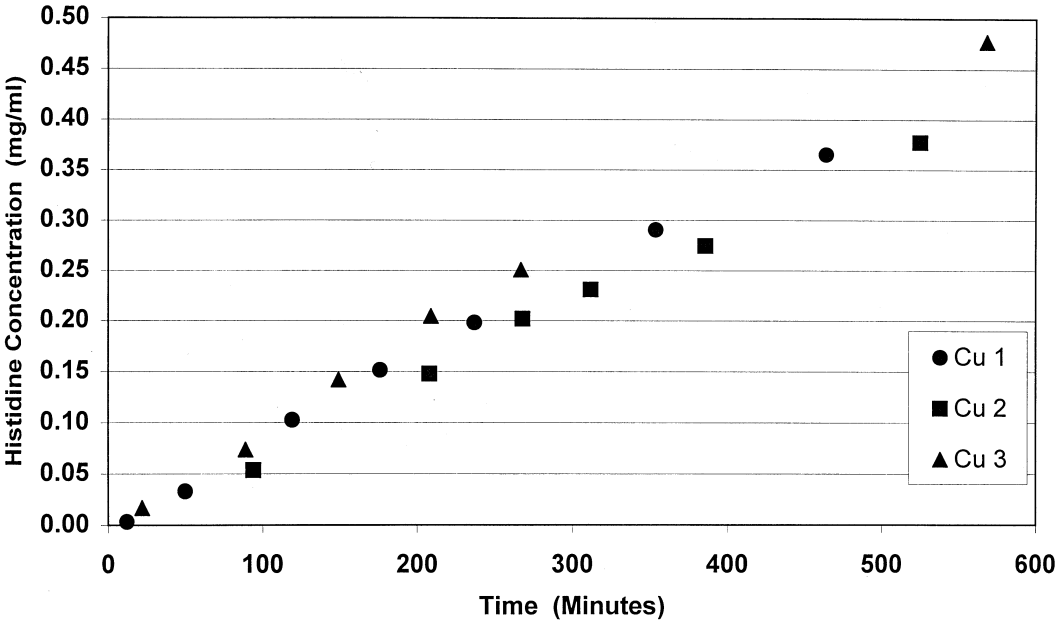

FIG. 4 Permeate histidine concentration vs time for Cu(II) charged, chelate-containing membranes. Three membranes (the same used for the data of Fig. 3) were charged with Cu(II), then challenged with a histidine feed.

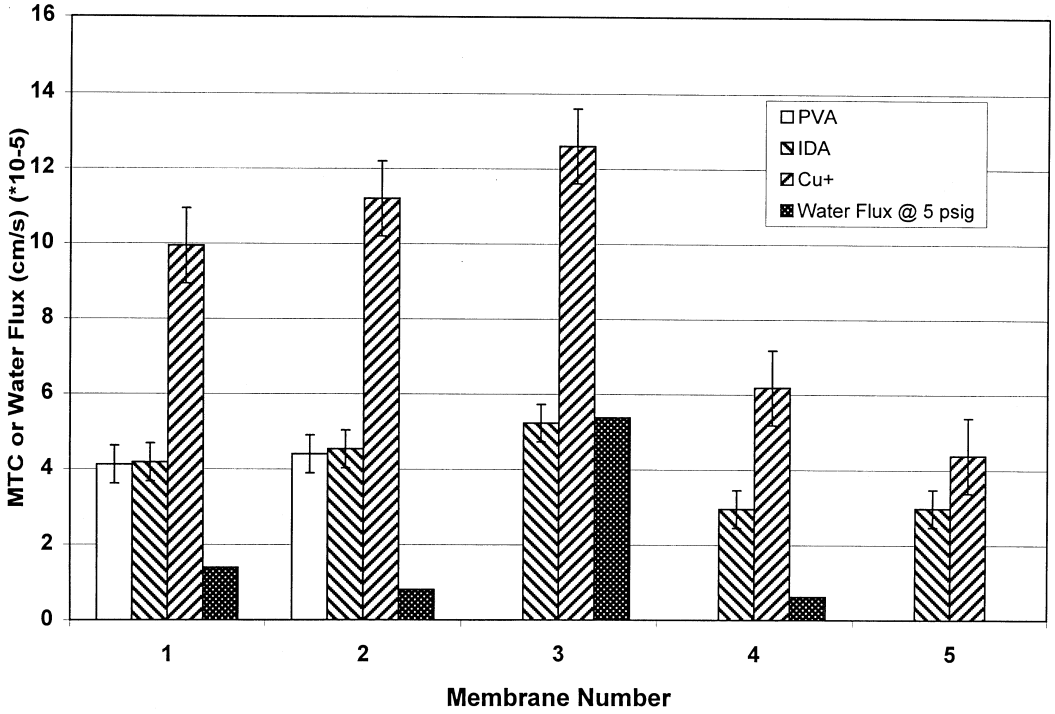

FIG. 5 Summary of mass transfer coefficients and water fluxes. Membranes #1 through #4 are supported, and Membrane #5 is an unsupported membrane.

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charged membranes] and water fluxes. For each parameter, the averages of multiple runs ($n = 3$ or 4) and errors are given. As illustrated in Fig. 5, no significant difference in histidine transport was observed in membranes prior to Cu(II) charging, i.e., the values of the mass transfer coefficients for PVA and IDA steps were nearly identical (approximately 4×10^{-5} cm/s). Histidine transport was attributed in these cases to normal diffusional processes within the hydrogel. Once charged with Cu(II), however, the rate of histidine transport was higher in all cases. For a given membrane (#1 through #4) in all cases the mass transfer coefficient doubled when divalent copper was present. These data support the theory that Cu(II) facilitates the transport by augmenting normal diffusion through a supported membrane. If one considers the first three membranes representative of the whole, a mass transfer coefficient of $K = 11 \pm 1 \times 10^{-5}$ cm/s is obtained. Although both K values drop for Membrane #4, the trend of increasing mass transfer coefficients was still observed when Cu(II) is present.

As a final comparison, the supported membranes were compared to an unsupported PVA chelate-containing membrane charged with Cu(II) (Membrane #5 of Fig. 5). No water flux was measured for this membrane since it would blow out of the membrane holder if moderate pressures were applied. The last data set within Fig. 5 is an unsupported PVA hydrogel of thickness $L = 0.75$ mm. Presumably, the higher mass transfer coefficients were observed with a supported membrane due to a thinner, permselective layer. As before, when Cu(II) was present, a doubling of K was observed.

CONCLUSIONS

Nonselective PVDF microfiltration membranes were made selective by impregnating the pores with a crosslinked polymer, PVA, and functionalizing the polymer with Cu(II). The functional moiety, i.e., chelated Cu(II), was able to facilitate the transport of histidine, an amino acid of commercial significance. Support of the selective hydrogel within the pores of the PVDF increased the membrane's mechanical stability, as repetitive experiments over a 3-month period were possible.

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