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CHIRAL SEPARATIONS USING ULTRAFILTRATION WITH A STEREOSELECTIVE BINDING AGENT

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ABSTRACT

Recent studies have demonstrated the feasibility of using ultrafiltration with a stereospecific binding agent in free solution to separate chiral molecules. The objective of this study was to develop an improved understanding of the key factors that determine the effectiveness of this type of chiral separation. Experiments were performed using a model system of bovine serum albumin as a stereospecific binding agent for the amino acid tryptophan. Batch ultrafiltration data showed strong stereospecific binding of L-tryptophan with a selectivity of 11 at low amino acid concentrations. Actual separations were performed using a constant volume diafiltration system to wash the less strongly bound isomer through the membrane. The diafiltration process avoids problems associated with the accumulation of retained species during filtration and makes it possible to achieve higher purification factors. The L-tryptophan was recovered in the retentate with final purity greater than 90% at 60% yield. Model calculations performed us-

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ing the equilibrium binding constants for the two stereoisomers were in good agreement with the data. Simulations were used to examine the performance of this membrane system for chiral separations.

Key Words: Membrane filtration; Chiral separations; Stereoselective binding; Diafiltration; Ultrafiltration.

INTRODUCTION

Many pharmaceuticals, nutraceuticals, and agricultural chemicals exist as stereoisomers, with each enantiomer having very different biological activity. In some cases, only one of the isomers has the desired activity, while the other chiral form may produce undesirable (toxic) side effects (1). For example, it has been reported that the horrendous teratogenic side effects of the drug thalidomide may reside exclusively in the s-enantiomer (2). The FDA and the Committee for Proprietary Medicinal Products (CPMP) now require pharmaceutical companies to produce only a single enantiomer as the therapeutic agent or to clearly demonstrate the appropriateness of using the racemic mixture (2,3). This has generated a considerable demand for separation techniques appropriate for the large-scale resolution (purification) of chiral molecules.

The most widely used methods for the separation of racemic mixtures are column chromatography (4–8), diastereomeric salt crystallization (9,10), and stereoselective enzyme catalysis (10–12). Liquid membranes and solid membranes with immobilized chiral ligands have also been used for chiral separations (13–15), although these techniques can be difficult to apply for large-scale commercial systems. An alternative approach is to use an affinity ultrafiltration system in which a large stereoselective ligand is added to the bulk solution to selectively bind, and thus retain, one of the stereoisomers.

The idea of using affinity binding in ultrafiltration dates back to the early work by Mattiason and Ramstorp (16) on the purification of concanavalin A by exploiting its binding to the sugar residues on the membrane of heat-killed *Saccharomyces cerevisae*. The first application of this technique for optical resolution using a stereoselective ligand wasn't reported until 1993 (17). Higuchi et al. (17) examined the use of bovine serum albumin (BSA) as a stereoselective binding agent for optical resolution of the amino acid tryptophan. Limited experimental studies demonstrated the general feasibility of this approach, with the maximum selectivity for the permeation of D-tryptophan (relative to the L-isomer) of approximately 7. Subsequent work using the BSA system for the separation of phenylalanine showed a monotonic increase in selectivity with increasing filtration time over periods of at least 6 hours (18). The authors hypothesized that this

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transient increase might have been due to the formation of a protein gel layer on the membrane surface, although no independent evidence for this effect was provided. The selectivity also increased with decreasing feed concentration, with a maximum selectivity of about 20 at a phenylalanine concentration of 0.015 mM (18,19). In contrast, data obtained with racemic leucine showed a maximum in the separation factor at an intermediate feed concentration (18). The authors indicated that this maximum could have been due to the presence of multiple binding sites on BSA with different affinity for the two enantiomers, although no detailed analysis of this concentration-dependence was provided. In addition, Overdevest et al. (20) have used affinity ultrafiltration to separate phenylalanine enantiomers by enantioselective micelles. In this case, the enantioselective micelles preferentially form a complex with one of the enantiomers, allowing the other (uncomplexed) enantiomer to pass through the membrane (20).

Poncet et al. (21) and Garnier et al. (22) also examined the use of bovine serum albumin as a chiral separating agent for racemic tryptophan. Ultrafiltration experiments at pH 9 yielded 91% purity and 89% recovery of D-tryptophan in a single stage, although no details were provided on the process actually used to achieve this separation. Poncet et al. (20) also performed numerical simulations showing the effects of initial tryptophan and BSA concentrations on the recovery and purity. However, no details were provided on the basis for these calculations making it difficult to assess the applicability of the results. Very high purity was predicted at small concentration ratios of tryptophan to BSA, although the tryptophan recovery in the filtrate was quite poor under these conditions. No calculations were provided for the more retained product, even though many commercial processes would require recovery of both enantiomers. Although both Garnier et al. and Poncet et al. describe the tradeoff between purity and recovery that occurs as the solution pH is decreased from 11 to 6, they implicitly assumed that a given ultrafiltration process yielded only a single recovery and purity. This completely neglects the inherent trade-off between recovery and purification that naturally occurs during the course of the ultrafiltration process.

The studies by Higuchi et al. (17–19), Poncet et al. (21), and Garnier et al. (22) have clearly demonstrated the feasibility of using ultrafiltration with a stereoselective-binding agent for the separation of chiral molecules. However, these studies have not provided a clear understanding of the factors that determine the effectiveness of this type of chiral separation. The data show conflicting effects of feed concentration and filtration time, and there is still considerable uncertainty over how to properly evaluate the yield and recovery for an actual separation process. The objective of the current study is to present an appropriate framework for describing the performance of ultrafiltration systems for chiral separations, using data obtained in the BSA-tryptophan system to illustrate the key phenomena governing the behavior of these systems.

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THEORY

BSA is known to have several binding sites for hydrophobic amino acids like tryptophan (23–25). The L-stereoisomer is bound predominantly to a single stereospecific site, with additional binding occurring at one or more non-stereospecific sites (23,24). D-tryptophan binds in a competitive fashion to both types of sites, although with a much lower affinity to the stereospecific site (23,24). Previous studies have demonstrated that the binding constants are strong functions of solution pH, with maximum binding of tryptophan occurring around pH 9 (21–23).

Since BSA is fully retained by the ultrafiltration membrane used in this system, only the free (unbound) tryptophan is able to pass through the membrane. The filtrate concentration (C_{fi}) can thus be expressed in terms of the total feed concentration (C_{Ti}):

$$\frac{C_{fi}}{C_{Ti}} = (1 - f_i)S_i \tag{1}$$

where f_i is the fraction of the stereoisomer that is bound by BSA and the subscript *"i"* denotes the optical isomer, D- or L-tryptophan. S_i is the observed sieving coefficient for the free tryptophan, which is equal to the ratio of free tryptophan in the filtrate solution to that in the bulk feed. S_i should be approximately one for the transport of small tryptophan molecules (MW = 240 g/mol) through the 50,000 g/mol nominal molecular weight cut-off membranes used in these experiments. The binding characteristics of the L- and D-tryptophan are discussed in more detail subsequently. The intrinsic selectivity or separation factor for the affinity ultrafiltration process is defined as the ratio of the concentration of the product and impurity in the filtrate to that in the feed:

$$\alpha = \frac{C_{fD}/C_{fL}}{C_{TD}/C_{TL}} = \frac{1 - f_D}{1 - f_L} \tag{2}$$

where we have assumed that the sieving coefficients for the free stereoisomers are identical and that there is no leakage of the binding agent into the filtrate solution. High selectivities thus require very strong binding of the L-tryptophan ($f_L \approx 1$) with only moderate (or minimal) binding of the D-tryptophan.

In order to effectively separate the L- and D-isomers using a membrane system, it is desirable to use a diafiltration process in which fresh buffer is added to the feed solution at the same rate at which filtrate is removed. Diafiltration maintains a constant feed volume throughout the process, thereby eliminating the problems that can arise from the build-up of a very high concentration of retained BSA in the feed reservoir. In addition, the diafiltration process allows the D-tryptophan to be effectively washed through the membrane, and away from the L-tryptophan, even if the D-isomer is partially bound by the BSA. More detailed information on

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the design and operation of diafiltration processes is available in the literature (26–28).

The tryptophan mass balance during a constant volume diafiltration is simply:

$$V\frac{dC_{Ti}}{dt} = -AJ_vC_{fi} = -AJ_vS_i(1 - f_i)C_{Ti} \tag{3}$$

where C_{Ti} is the total tryptophan concentration in the feed reservoir at time t, V is the (constant) feed volume, A is the membrane area, and J_v is the volumetric filtrate flux. Although Eq. (3) looks similar to the mass balances seen during conventional diafiltration processes, the actual integration can be very complex since f_i will generally be a function of the concentration of both the L- and D-isomers due to competitive binding at multiple sites. This is discussed in more detail subsequently. Under conditions where f_i remains approximately constant during the diafiltration, Eq. (3) can be integrated directly to give:

$$\frac{C_{Ti}}{C_{Tio}} = \exp[-NS_i(1 - f_i)] \tag{4}$$

where C_{Tio} is the initial feed concentration and N is the number of diavolumes, which is equal to the total collected filtrate volume ($\int J_vAdt$) divided by the constant feed volume (V). The recovery (or yield) of L-tryptophan (the more retained species) in the retentate solution at the end of the constant volume diafiltration is defined as:

$$Y_L = C_{TL}/C_{TLo} \tag{5}$$

with the purification factor defined as:

$$P_L = \frac{C_{TL}/C_{TLo}}{C_{TD}/C_{TDo}} \tag{6}$$

Although Eq. (6) looks nearly identical to the definition of the selectivity in Eq. (2), it must be recognized that the purification factor is defined over an entire diafiltration process, while the selectivity provides an instantaneous measure of the relative transmission of the D- and L- isomers into the filtrate solution. Under conditions where f_i remains approximately constant during the diafiltration, both the D- and L-tryptophan concentrations can be evaluated in terms of the number of diavolumes using Eq. (4), giving the following analytical expressions for the L-tryptophan yield and purification factor:

$$Y_L = \exp[-NS(1 - f_L)] \tag{7}$$

$$P_L = \exp[NS(f_L - f_D)] \tag{8}$$

assuming that $S = S_D = S_L$. The yield of L-tryptophan starts at 100% and decreases throughout the diafiltration due to passage of the unbound isomer

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through the membrane. The purification factor for the L-tryptophan increases continuously during the process due to the greater removal of the D-isomer into the filtrate. This trade-off between recovery and purification has been discussed in some detail by van Reis and Saksena (28) in the context of protein separations.

The corresponding equations for the yield and purification factor for the D-tryptophan collected in the filtrate solution are:

$$Y_D = \frac{V_f \overline{C}_{fD}}{V C_{TDo}} \tag{9}$$

$$P_D = \frac{\overline{C}_{fD}/C_{TDo}}{\overline{C}_{fL}/C_{TLo}} \tag{10}$$

where $\overline{C}_{fD}$ and $\overline{C}_{fL}$ are the average concentrations of the D and L-isomers collected in the filtrate over the full diafiltration process and V_f is the total collected filtrate volume. Under conditions where f_i remains approximately constant during the diafiltration, the D-tryptophan yield and purification factor can be expressed analytically in terms of N, S, f_D, and f_L:

$$Y_D = 1 - \exp[-NS(1 - f_D)] \tag{11}$$

$$\mathrm{P}_D = \frac{1 - \exp[-NS(1 - f_D)]}{1 - \exp[-NS(1 - f_L)]} \tag{12}$$

The yield of the D-tryptophan begins at zero and increases throughout the diafiltration, while the purification factor starts at its maximum value (with the first drop of filtrate solution) and decreases during the process.

MATERIAL AND METHODS

Racemic mixtures of D- and L-tryptophan were obtained from Sigma Chemical (Catalog #T-3300, St. Louis, MO). The tryptophan was dissolved in 10 mM borate buffer (pH 9.1) prepared from sodium tetraborate (Fisher Scientific) in deionized distilled water (resistivity $>$ 18 Mohm-cm) obtained from a Barnstead water purification system (Dubuque, IA). All buffer solutions were prefiltered through a 0.2 μm Supor®-200 microfiltration membrane (Gelman Sciences, Ann Arbor, MI) to remove particulates prior to use. Bovine serum albumin (Sigma A-8022) was used as the stereoselective-binding agent. BSA was added to the tryptophan solution to give a final protein concentration of 0.60 mM (41 g/L). The resulting solution was gently stirred for approximately 3 hrs to ensure equilibrium binding of the tryptophan. The pH of the final solution was measured using an Acumet 915 pH meter (Fisher Scientific) as pH 8.5.

Tryptophan concentrations were evaluated by capillary zone electrophoresis using α-cyclodextrine (Sigma C-4642) as a chiral resolving agent. A 50 mM solution of α-cyclodextrine in 50 mM phosphate buffer at pH 2.2 was used as the background electrolyte. Electrophoresis was performed using an ISCO model 3850 capillary electropherograph (ISCO Inc., Lincoln, NE) equipped with a dual-polarity variable high-voltage dc power supply and a variable wavelength UV-visible absorbance detector. Data were obtained using a 50 cm long fused silica capillary (CElect–FS75 CE column; 362 μm OD, 75 μm ID) with an applied voltage of 13kV. Detection was at 278 nm, with the tryptophan concentration determined from the area under the electropherograms. Tryptophan concentrations in the presence of BSA were evaluated by first adding 13 μL of a 1 M HCl/0.2 M KCl solution to a 1 mL sample. Tryptophan binding to the positively charged BSA at this pH was negligible. The resulting solution was then filtered through a 50,000 molecular weight cutoff membrane to remove BSA, with the concentration of D- and L-tryptophan in the collected filtrate measured by capillary zone electrophoresis.

All filtration experiments were performed with Biomax polyethersulfone membranes (Millipore Corp., Bedford, MA) having nominal molecular weight cutoff of 50,000 g/mol. The membranes were thoroughly flushed with deionized distilled water to remove any glycerin, which was used as a wetting and storage agent. The membrane hydraulic permeability (L_p) was then evaluated from the slope of data for the filtration velocity (J_v) for the borate buffer as a function of the transmembrane pressure drop (ΔP):

$$L_P = \frac{J_v \mu}{\Delta P} \tag{13}$$

where μ is the solution viscosity. The permeability was also evaluated at the completion of each filtration experiment to provide a measure of the extent of fouling.

Binding Experiments

The binding constants for the L- and D-tryptophan were evaluated using a 25 mm diameter stirred UF cell (Model 8010, Amicon Corporation, Beverly, MA). The device was filled with a BSA-tryptophan solution of known protein and tryptophan concentration. The stirrer was then set to 550 rpm using a Strobotac Type 1531-AB strobe light (General Radio Co., Concord, MA). The device was air pressurized to $\Delta P = 69$ kPa, and the solution flowrate was measured by timed collection using a Type 1518 electronic balance (Sartorious, Westbury, NY) with an accuracy of 1 mg. Filtrate and bulk samples were obtained periodically for off-line evaluation of the tryptophan concentrations.

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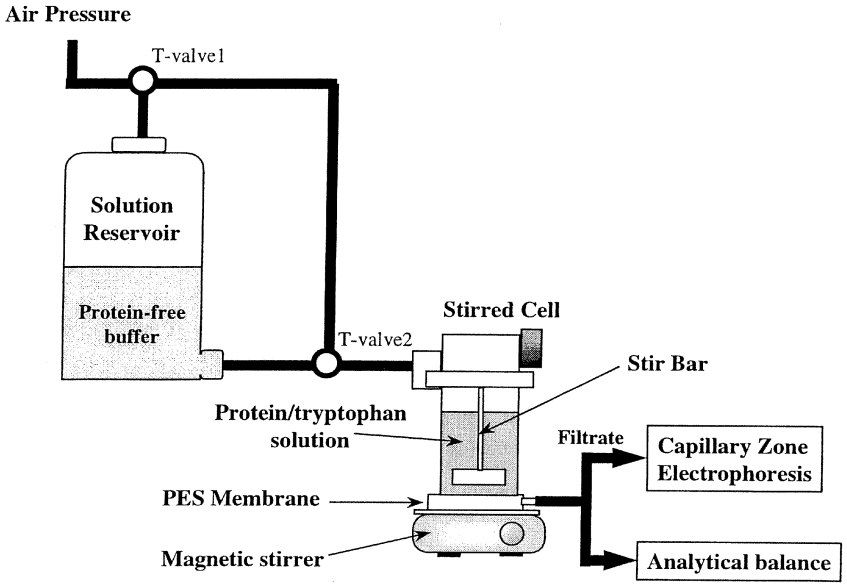

Figure 1. Schematic diagram of the diafiltration apparatus.

Diafiltration Experiments

The optical resolution of a racemic tryptophan solution was performed using the same Amicon stirred cell but in a diafiltration mode as shown schematically in Fig. 1. The solution reservoir was filled with pure borate buffer (1 mM sodium tetraborate at pH 8.5), while the stirred cell contained the BSA-tryptophan mixture. Two T-valves were used to control the reservoir and stirred cell. The stirred cell was filled to the desired level, T-valve2 was tightly closed, and then the solution reservoir was attached. A single air source was used to simultaneously pressurize both the reservoir and stirred cell. T-valve2 was then opened so that the filtration flux caused fresh buffer to be drawn into the stirred cell from the reservoir at a rate equal to the volumetric filtration rate. Filtrate solution was collected continuously, with small samples taken for subsequent analysis of the unbound L- and D-tryptophan concentrations. Samples were also taken periodically from the stirred cell over the course of the diafiltration to measure the total L- and D-tryptophan concentrations in the retentate.

RESULTS AND ANALYSIS

The extent of tryptophan binding by BSA was determined using the stirred cell filtration device. Typical capillary electropherograms of the feed and filtrate

solutions are shown in Fig. 2 for a system with 0.6 mM BSA, 0.25 mM of L- and D-tryptophan, and a pH of 8.5. The electropherograms for the feed solution were obtained prior to addition of BSA. The filtrate sample was obtained near the start of a constant pressure filtration ($\Delta P = 69$ kPa), which yielded a filtration velocity of 1×10^{-5} m/s. The tryptophan concentrations were calculated directly from the peak areas by numerical integration. Results for the feed gave $C_{TL}/C_{TD} = 0.98 \pm 0.05$, consistent with the racemic mixture of the two stereoisomers. The filtrate sample showed considerably more D-tryptophan, with $C_{fL}/C_{fD} = 0.11 \pm 0.05$. Since the filtrate sample was taken near the start of the filtration (i.e., with $V_f/V < 0.05$), the tryptophan concentrations in the stirred cell were essentially equal to

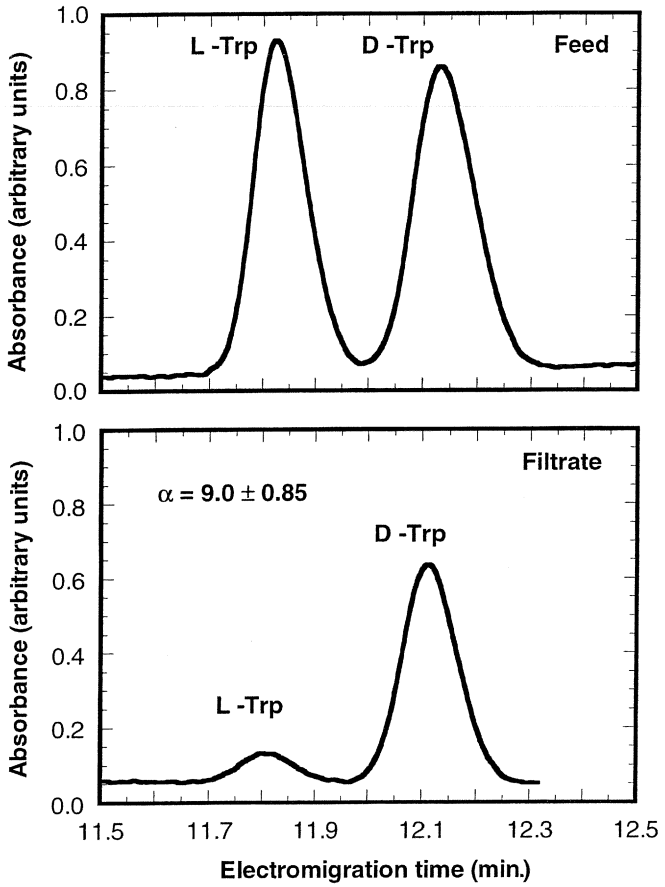

Figure 2. Electropherograms for initial feed (top panel) and filtrate (bottom panel) solutions at pH 8.5 and 22°C.

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their initial values. Thus, the selectivity or separation factor can be evaluated directly from Eq. (2) as $\alpha = 9.0 \pm 0.8$, which is in relatively good agreement with the literature data for the BSA-tryptophan system under similar conditions (17,18). Data obtained over a range of filtration velocity from 10^{-6} to 10^{-4} m/s showed essentially identical values of the selectivity. This indicates that the selectivity was independent of the extent of protein concentration polarization over the range of conditions examined in this study. The selectivity was also independent of the filtration time (for times up to 3 h), which was consistent with the relatively small amount of fouling seen in these experiments (changes in hydraulic permeability before and after the filtration were generally less than 10%).

Experimental data for the selectivity as a function of the total tryptophan concentration (C_{Ti}) are shown in Fig. 3. These data were obtained with a constant BSA concentration of 0.6 mM after a 3-h agitation of the bulk solution. The solid curve is a model calculation, which is described in more detail subsequently. The selectivity increased with decreasing tryptophan concentration, going from a value of less than 2 at $C_{Ti} > 2$ mM to as high as $\alpha = 11$ at $C_{Ti} = 0.3$ mM.

Binding Isotherms

Experimental binding isotherms for L- and D-tryptophan at pH 8.5 and a total BSA concentration of 0.6 mM are shown in Fig. 4. The data were obtained with

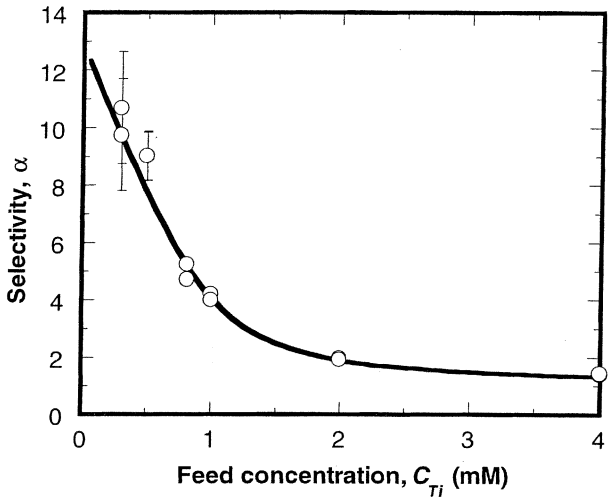

Figure 3. Separation factor as function of the total tryptophan concentration at a BSA concentration of 0.6 mM.

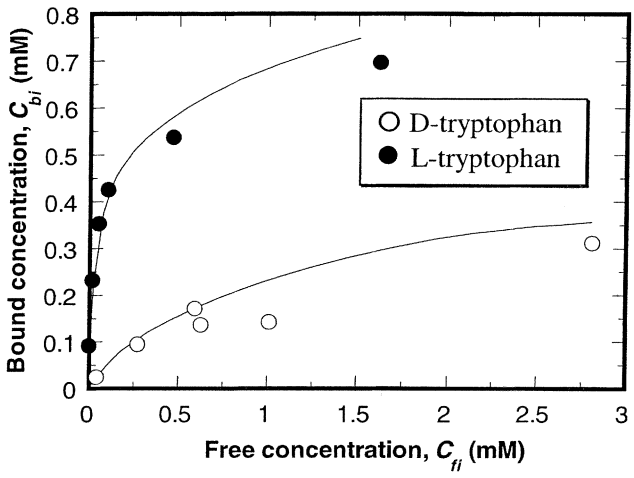

Figure 4. Binding isotherms for L- and D-tryptophan as a function of the free tryptophan concentration at a BSA concentration of 0.6 mM. Solid curves represent model calculations using equilibrium binding constants; $K_L = 39{,}000\ M^{-1}$, $K_D = 510\ M^{-1}$, $K_2 = 300\ M^{-1}$, and binding sites $n_1 = 0.83$ and $n_2 = 2$.

racemic mixtures of the two stereoisomers, with the results plotted as a function of the free tryptophan concentration. The y-axis represents the concentrations of D- and L-tryptophan bound to BSA, which were calculated directly from the measured values of the filtrate concentration using simple overall mass balances. The concentration of L-tryptophan bound to BSA was significantly greater than that for the D-tryptophan due to the strong stereospecific binding of the L-isomer.

The amount of bound tryptophan (L- plus D-) at high feed concentrations was significantly larger than the BSA concentration (0.6 mM), which is consistent with the presence of multiple tryptophan binding sites. L-tryptophan is known to bind predominantly to one site on BSA in a highly stereospecific manner and to several secondary sites with a much lower affinity (21–25). Previous competitive binding studies with racemic tryptophan methyl ester (an analog of tryptophan) suggest that weakly bound D-isomer can displace the L-isomer at the stereospecific site (24). This study also described data at high L-tryptophan concentration that suggested a secondary binding site on human serum albumin, although these results were never fully reported (25). Since the secondary site(s) are non-stereospecific, it is likely that both enantiomers would bind competitively with the same binding constant. This suggests the use of a 2-site competitive binding model for the tryptophan stereoisomers:

$$C_{bi} = \frac{n_1 K_i C_{fi} C_{BSA}}{1 + K_L C_{fL} + K_D C_{fD}} + \frac{n_2 K_2 C_{fi} C_{BSA}}{1 + K_2 C_{fL} + K_2 C_{fD}} \tag{14}$$

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where n_1 and K_i are the number and binding constant for the stereospecific binding site, n_2 and K_2 are the number and binding constant for the secondary sites, and C_{BSA} is the total BSA concentration. The presence of two binding sites was also consistent with Scatchard plots of the binding data that showed two distinct linear regimes. It is possible that the non-stereospecific binding might occur with multiple values of the binding constant, but the scatter in the data made it impossible to confirm or invalidate this.

The model curves in Figs. 3 and 4 were constructed using the best fit values of the model parameters $n_1 = 0.83$, $n_2 = 2$, $K_L = 39{,}000\ \mathrm{M}^{-1}$, $K_D = 510\ \mathrm{M}^{-1}$, and $K_2 = 300\ \mathrm{M}^{-1}$ as determined by minimizing the sum of the squared residuals between the model and data. The best fit value for K_L is in good agreement with literature data for L-tryptophan binding to serum albumin, e.g., $K_L = 4.4 \times 10^4 \mathrm{M}^{-1}$ at pH 7.4 (25) and $K_L = 5.5 \times 10^4\ \mathrm{M}^{-1}$ at pH 8.0 (23). The model calculations for the selectivity are in good agreement with the experimental data (Fig. 3) properly capturing the large increase in α at low tryptophan concentrations caused by f_L approaching a value of one (Eq. 2).

Diafiltration Experiments

To avoid problems associated with the accumulation of retained BSA in the stirred cell, the tryptophan separation was performed with a diafiltration process. During this process, the total volume of solution in the stirred cell was kept constant by continuous addition of protein-free buffer using the apparatus shown in Fig. 1. The diafiltrations were performed at relatively low pressure (20–55 kPa) and high stirring speed (600 rpm) to minimize fouling. Data for the hydraulic permeability after the diafiltration experiments were generally within 8% of the initial value.

Experimental data for the overall purification factor and yield of D-tryptophan in the collected filtrate solution are shown in Fig. 5 for diafiltrations performed for two tryptophan mixtures having 0.3 mM and 0.9 mM total tryptophan concentration and a BSA concentration of 0.6 mM. The data are plotted as a function of the number of diavolumes (N), which is equal to the total volume of collected filtrate divided by the initial solution volume ($V = 7.2$ and 7.5 mL for the runs with 0.3 and 0.9 mM tryptophan, respectively). The D-tryptophan yield increased throughout the course of the diafiltration, although the rate of increase decreased with increasing N due to the dilution of the D-tryptophan remaining in the stirred cell. In contrast, the purification factor for D-tryptophan decreased as the diafiltration proceeded. This decrease in purification factor was a direct result of the "leakage" of unbound L-tryptophan through the membrane and into the filtrate. The D-tryptophan purification factor was much greater for the run with the lower initial tryptophan concentration, while the D-tryptophan yields were nearly

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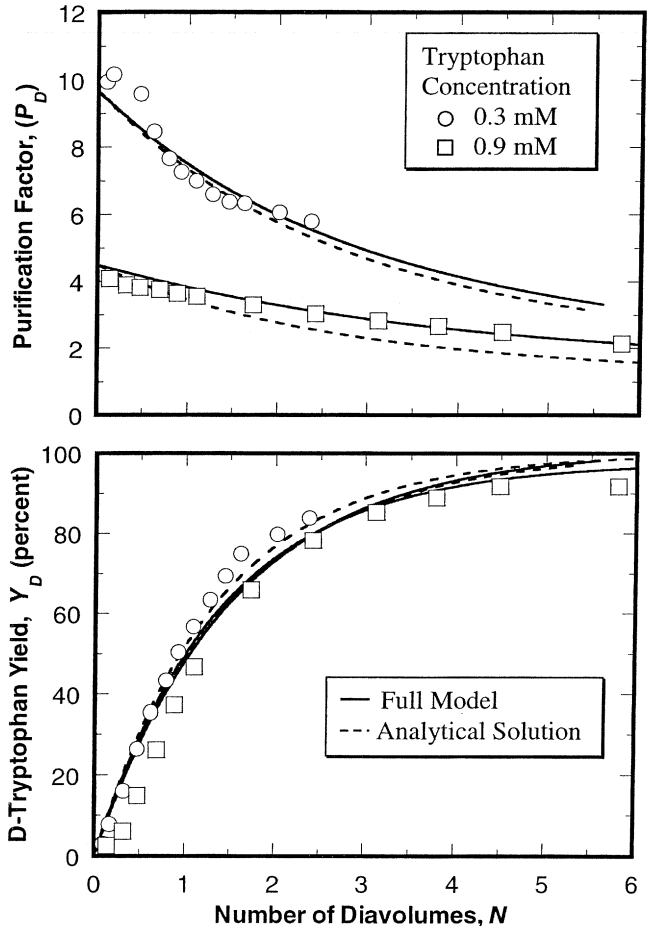

Figure 5. D-tryptophan purification factor (top panel) and yield (bottom panel) in the filtrate solution for the diafiltration of 0.9 mM and 0.3 mM tryptophan solutions at pH 8.5 and C_{BSA} = 0.6 mM. Solid curves are model calculations as described in the text.

identical for the two runs. The increase in purity at the lower concentration was due to the greater extent of L-tryptophan binding, for example, at the start of the diafiltration $f_L = 0.93$ for the run with 0.3 mM tryptophan, compared with $f_L = 0.83$ for the run with 0.9 mM tryptophan. The D-tryptophan yield was largely unaffected by the total tryptophan concentration since there was relatively little D-tryptophan binding under these conditions ($f_D = 0.34$ and 0.25) for the runs with 0.3 and 0.9 mM tryptophan, respectively.

The solid curves in Fig. 5 are the model calculations developed by numerical integration of Eq. (3), with f_i evaluated from the 2-site binding model

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using the best fit values of the binding constants. All calculations were performed assuming that the observed sieving coefficient equals one for both D- and L-tryptophan. The numerical integration was performed in MATLAB (version 5.3) using a forward finite difference scheme. The model is in excellent agreement with the data over the entire diafiltration at both the low and high tryptophan concentrations. Calculations performed using a single site binding model (i.e., $n_2 = 0$) showed considerably larger discrepancies between the model and data. The dashed curves in Fig. 5 represent the model calculations based on the analytical solution (Eqs. 11 and 12) developed assuming that the f_i remained constant throughout the diafiltration at their initial values. The analytical solution properly captures the qualitative behavior, but tends to underpredict the purification factor and overpredict the yield. These discrepancies are a direct result of the increase in both f_L and f_D that occurred during the diafiltration due to the reduction in the retentate concentration of both the L-and D-isomers. This effect was greatest at the higher initial feed tryptophan concentration since there was a much greater relative increase in tryptophan binding. For example, f_L was predicted to increase from 0.83 to 0.93 over the 6-diavolume diafiltration for the run with 0.9 mM tryptophan feed.

The corresponding values for the purification factor and yield of the L-tryptophan in the retentate solution are shown in Fig. 6. The open symbols represent the values determined from the filtrate concentration data using an overall mass balance. The filled symbols represent the results determined from direct samples taken from the stirred cell at several points during the diafiltration. The two results are in good agreement, with the overall mass balance closure for these experiments being better than 15%. The L-tryptophan yield began at 100% and steadily decreased during the diafiltration due to the loss of unbound L-tryptophan through the membrane. The purification factor for L-tryptophan increased throughout the experiment due to the much greater rate of removal of D-tryptophan from the stirred cell. In contrast to the results for the D-tryptophan, the L-tryptophan purification factor was nearly independent of the initial feed concentration, while the L-tryptophan yield was considerably larger for the run with 0.3 mM concentration. This behavior was again a direct result of the differences in binding characteristics for the two stereoisomers. The L-tryptophan purification factor was determined primarily by the rate at which the D-tryptophan was removed from the feed. This rate was largely unaffected by the initial feed concentration since D-tryptophan binding was relatively small. The L-tryptophan yield was determined primarily by the rate at which L-tryptophan leaked through the membrane. The increase in feed concentration caused a significant increase in the concentration of free L-tryptophan, with the initial value of $1 - f_L = 0.068$ for the run with 0.3 mM tryptophan compared to $1 - f_L = 0.162$ for the run with 0.9 mM tryptophan. The model calculations and data are again in very good agreement, with a final L-tryptophan purification factor $P_L = 38$ after 6.4 diavolumes. This

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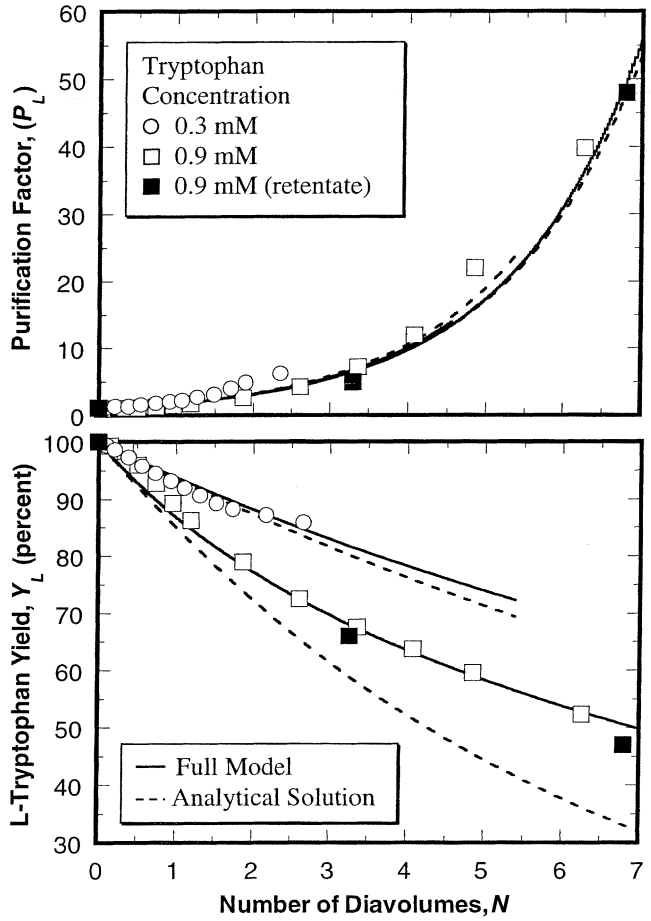

Figure 6. L-tryptophan purification factor (top panel) and yield (bottom panel) in the retentate solution for the diafiltration of 0.9 mM and 0.3 mM tryptophan solutions at pH 8.5 and $C_{BSA} = 0.6$ mM. Solid and dashed curves are model calculations as described in the text.

purification factor corresponds to a final product that has an enantiomeric excess, $ee = (C_{TL} - C_{TD}) / (C_{TL} + C_{TD})$, of L-tryptophan of 95%. Figure 7 shows the electropherogram of this final retentate solution used to calculate both the L-tryptophan purity and recovery after the 6.8 diavolume filtration. The sample was nearly pure L-tryptophan, with the concentration of D-tryptophan being less than 0.004 mM, compared to the initial value of 0.15 mM.

The dashed curves in Fig. 6 represent the model calculations based on the analytical solution (Eqs. 7 and 8) developed assuming that the f_i remained con-

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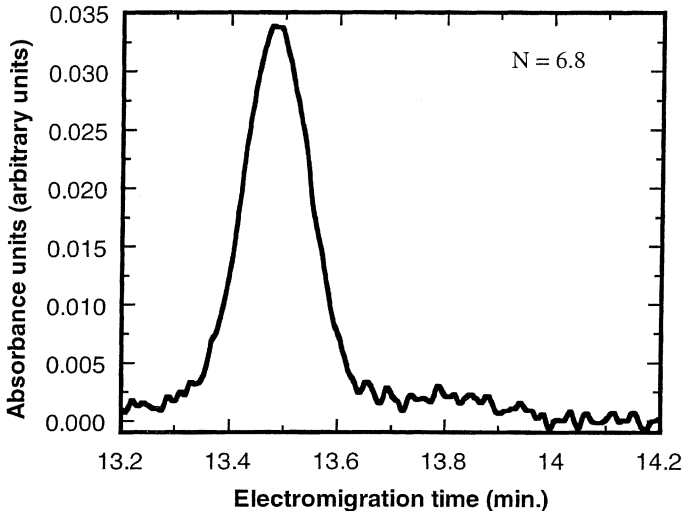

Figure 7. Electropherogram for final retentate product collected after a 6.8 diavolume process at pH 8.5.

stant throughout the diafiltration at their initial values. The analytical solution does a fairly good job of describing the purification factor, since the difference between f_L and f_D remained nearly constant as the tryptophan concentration decreased. The analytical solution significantly underpredicted the L-tryptophan yield, particularly for the run with 0.9 mM initial feed concentration. The L-tryptophan yield (Eq. 7) was determined entirely by the extent of binding of the L-isomer. As discussed previously, f_L increased significantly over the course of the diafiltration due to the reduction in concentration of the L- and D-tryptophan in the retentate solution, an effect that was not considered in the simple analytical solution.

CONCLUSIONS

Although previous studies (17,18) have demonstrated the feasibility of performing chiral separations using membrane systems with a stereoselective binding agent, there has been considerable uncertainty about how to translate these results for "differential separations" into predictions for the performance of an actual separation process. For example, almost all of the previous studies have assumed that the membrane process yields only a single recovery and purity, which completely neglects the inherent trade-off between recovery and purification that naturally occurs during the course of the ultrafiltration process. The data and analysis presented in this manuscript provide the first detailed description of

a diafiltration process for chiral separations, accounting for both the competitive binding characteristics and the effects of diafiltration on yield and purification.

Our work with the tryptophan-BSA system clearly shows that it is possible to obtain high degrees of purification using a diafiltration process. For example, the purification factor for L-tryptophan in the retentate solution after 6 diavolumes was greater than 35, corresponding to an enantiomeric purity of 97% (or an enantiomeric excess of 94%) of L-tryptophan. Even higher purities would be obtained with more diafiltration due to the continuous removal of the D-tryptophan during the constant volume diafiltration.

The effects of the feed tryptophan concentration on the separation process were quite interesting. The initial feed concentration had relatively little effect on the yield of D-tryptophan in the filtrate solution and on the purification factor of the L-tryptophan collected in the retentate, since D-tryptophan binding was relatively small over the concentration range examined in this study. However, the purity of the D-tryptophan in the filtrate and the yield of L-tryptophan in the retentate were both significantly improved at low feed concentrations due to the greater fractional binding of the L-tryptophan under these conditions. Model calculations performed using the best-fit values of the binding parameters suggest that the use of even lower feed concentrations (e.g., 0.05 mM tryptophan) would give a final retentate product with greater than 35-fold purification and 75 % recovery of the L-stereoisomer. Similar improvements in performance could be achieved using higher BSA concentrations, although this would be at the cost of having to operate at lower filtrate flux due to concentration polarization effects.

The optimal trade-off between yield and purification for this type of diafiltration process would be determined by the final purity requirements for the two stereoisomers, the detailed binding characteristics of the stereoselective binding agent, the overall economics of the membrane process, and the availability/effectiveness of other separation techniques that might be used in combination with (or instead of) the membrane process. Note that it might also be desirable to stage the membrane units, so that any L-tryptophan that leaks through the membrane could be recycled back to the feed tank. The effective application of this type of staged process really requires the use of a tangential flow filtration system having a much higher ratio of membrane area to volume than was possible in the stirred cell used in this study. The tangential flow filtration device would also make it possible to obtain the required number of diavolumes in a much shorter filtration time, which would significantly increase the overall attractiveness of this membrane process.

NOTATION

A	membrane area (cm^2)
C_{bi}	bound concentration of D- or L-tryptophan in the feed solution (M)

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C_{BSA}	total concentration of BSA in the feed solution (M)
C_{fi}	free concentration of D- or L-tryptophan (M)
$\overline{C}_{fi}$	average free concentration collected in the filtrate over the diafiltration (M)
C_{Ti}	total concentration (free + bound) of D- or L-tryptophan (M)
C_{Tio}	initial total concentration of D- or L-tryptophan in the stirred cell (M)
f_i	fraction bound of D- or L-tryptophan (C_{bi}/C_{Ti})
J_v	filtrate flux (m/s)
K_i	intrinsic stereospecific binding constant for D- or L-tryptophan (M^{-1})
K_2	intrinsic nonstereospecific (secondary) binding constant (M^{-1})
L_p	membrane hydraulic permeability (m)
n_1	number of stereospecific binding sites on BSA
n_2	number of nonstereospecific (secondary) binding sites on BSA
N	number of diavolumes
P_i	purification factor of D- or L-tryptophan
ΔP	transmembrane pressure drop (kPa)
S_i	sieving coefficient for D- or L-tryptophan
V	volume of solution in the stirred cell (cm^3)
V_f	total collected filtrate volume (cm^3)
Y_i	yield of D- or L-tryptophan

Greek Letters

α	intrinsic selectivity or separation factor for the membrane process
μ	buffer solution viscosity (kg/m/sec)

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