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Separation of D, L-amino acids using ligand exchange membranes

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ABSTRACT

Chiral ligand exchange membranes were synthesized for potential use in racemic filtration applicable to the pharmaceutical industry. Regenerated cellulose membranes with 1 kDa molecular weight cutoff (MWCO) were grafted with chiral L-proline-copper complexes through an intermediate epoxy-silane surface functionalization reaction performed for various times (0, 1.5, 3, 6, 12 and 24 h). The resulting membranes were evaluated in single component diffusion experiments with D or L-Phenylalanine (Phe), which showed much higher permeability for D-Phe compared with L-Phe. The membranes were then evaluated under pressure-driven, single enantiomer filtration and all grafted membranes exhibited predicted enantiomeric excess, with the 6 h epoxy-silane modified membrane yielding 100% enantiomeric excess (EE) over the largest throughput. An equimolar racemic mixture of D,L-Phe was subsequently used to challenge the optimum 6 h epoxy-silane modified membrane, and a two-tiered breakthrough curve was obtained. As predicted by the single enantiomer filtration results, the membrane was shown to completely fractionate the enantiomers at Peclet numbers of ~ 400. The high Peclet number during filtration combined with complete enantiomer fractionation is very intriguing as a competitive technology to chiral chromatography. Simply exposing the membrane surface to the epoxy-silane, L-proline, and copper solution presents a facile and scalable method for membrane surface modification.

1. Introduction

An area of industrial separations in which membranes have not been commercially applied is in the fractionation of enantiomers, especially in the pharmaceutical and agricultural industries. There are two categories of methods by which enantiomerically pure chiral compounds can be produced [1]. The first category entails synthetic methods and consists of employing natural chiral compounds or their derivatives directly, synthesizing chiral products via fermentation processes, or through utilization of a selective reaction where the use of special synthetic methods results in the production of only one enantiomer [1,2]. These methods generally involve highly specialized catalysts and synthetic methods, which may limit their use at a large scale industrially. The second category entails the resolution of a racemic mixture (i.e. a solution of enantiomers) into pure enantiomers through the use of a separating agent [1,3]. These methods include enantioselective crystallization, chiral chromatography, and membrane based separations amongst others [1,4]. Although enantioselective crystallization is the prevailing method used industrially to produce enantiomerically pure compounds, it suffers from low versatility, the need to handle excessive amounts of solids, and yields of 50% or less [1]. Chiral chromatography separates enantiomers that possess different binding affinities for chiral stationary phases (i.e. chiral resin) and therefore enantiomers elute at different times. High performing chiral resins contain three-dimensional chiral structures like those found in naturally occurring or synthetically made stereoregular polymers [2,5]. Chiral resins take advantage of a so-called three-point interaction, in which one interaction must be stereochemically dependent [6]. These interactions usually entail combinations of π-π interactions and hydrogen bonding [7,8]. The use of packed-bed chromatography, however, is severely limited by production capacity since the velocity of enantiomers through the column is very low and packed-bed chromatography dilutes rather than concentrates the desired enantiomeric product. A solution to this low production capacity and dilution problem would be to replace packed-bed chiral chromatography with membranes, where the potential for higher flow rates could be greatly increased and the desired enantiomeric product is concentrated. As a rule of thumb, chromatography has higher resolution due to higher binding capacity at low throughput (i.e. larger number of theoretical plates) and dilutes the product, whereas membranes typically have poorer resolution due to decreased surface area for binding albeit at higher throughput (i.e. lower number of theoretical plates) and

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concentrates the product. The challenge is to develop membranes with increased chiral selectivity (i.e. binding capacity) to compete with chromatography while still maintaining its superior process speed.

There have been some attempts at forming membranes for resolution of enantiomers reported in the literature [9]. Depending on the nature of the chiral membrane employed, transport can occur through either facilitated or retarded transport [9]. Facilitated transport is characterized by interactions between one enantiomer and the membrane which shuttle this enantiomer through the membrane more quickly (like a bucket brigade) [10]. Membranes characterized by facilitated transport are also known as diffusion-enantioselective membranes, since on increasing driving forces such as pressure that create convective flow through the membrane, the enantioselectivity decreases abruptly [9]. Retarded transport results when one enantiomer interacts with the membrane in such a way that it becomes bound more strongly or for a longer time and therefore proceeds through the membrane more slowly. Membranes that fall under this category are called adsorption-enantioselective membranes and these generally can maintain selectivity with convective flow. Therefore, chiral membranes exhibiting retarded transport are more attractive commercially.

A rational approach to producing membranes with chiral selectivity can be adapted from chemistry originally developed for chromatography known as ligand exchange chromatography (LEC). Helfferich et al. developed LEC chemistry to separate 1,3-diamino-2-propanol from a dilute aqueous solution containing ammonia [11,12]. The technique combined the use of ion-exchange and coordination chemistry. The method involved the use of a column packed with cation-exchange resin through which a solution of cupric sulfate in aqueous ammonia was passed. This allowed the copper-ammonia complex to bind to the cation-exchange resin. As two different amine-containing molecules were passed through the column, they became differentially adsorbed by the stationary phase, displacing the ammonia ligand originally coordinated to the copper. The amine group forming the weaker complex passed through the column more quickly. This laid the foundation for subsequent use of amine-containing *chiral* ligands for enantioseparation of suitable enantiomers. Two main applications of LEC are the analysis of mixtures of carbohydrates and separation of amino acid enantiomers. Sugar molecules are bound more tightly depending upon the number of hydroxyls that can coordinate with the metal, which directly depends upon the orientation of the hydroxyl groups. An example is separation of fructose from glucose to give high-fructose corn syrup. Fructose binds approximately twice as strongly to the LEC stationary phase as compared with glucose [11]. Similarly, one enantiomer in an amino acid racemate will bind more strongly to the copper complex due to spatial differences in the presentation of ligands in a more favorable manner for binding [13,14]. We propose, for the first time to our knowledge, to apply ligand exchange chemistry to create membranes capable of separating amino acid mixtures and potentially other pharmaceuticals which have functional groups capable of ligating with the metal complex (e.g. propranolol). The method is simple, scalable and inexpensive.

2. Experimental

2.1. Materials

D-phenylalanine (98%), L-phenylalanine (98%), L-proline (99%), copper acetate (98%), (3-glycidyloxypropyl) trimethoxysilane (97%) (epoxy-silane), toluene (99.5%), and tetrahydrofuran (THF) (99%) were purchased from Sigma-Aldrich (St. Louis, MO) and used as received. Milli-Q water with a resistivity of 18.2 MΩ cm was obtained from an in-house water purification system by Millipore/Sigma (Billerica, MA). Costar UV transparent 96-well microplates were purchased from Corning (Corning, NY). Regenerated cellulose (RC) membranes with 1 kDa molecular weight cutoff (MWCO) were provided by Millipore/Sigma. A diffusion cell from PermeGear (Hellertown, PA) was used to conduct enantiomer diffusion experiments. An Amicon 8010 stirred-cell from Millipore/Sigma (Billerica, MA) was used to evaluate membrane performance during pressure-driven filtration experiments.

2.2. Synthesis of ligand exchange membranes

The 1 kDa MWCO RC membranes were pre-soaked in THF for approximately 15 min to remove residual surfactant and preservatives remaining from the manufacturing process. The RC membranes were then transferred to a solution of 10 mM of epoxy-silane in toluene for a predetermined time. Afterwards, the membranes were rinsed with toluene to remove any unreacted epoxy-silane. The functionalized RC membranes were transferred to a methanol solution containing 50 mM L-proline for 24 or 48 h. After this reaction period, the membranes were rinsed with methanol to remove unreacted L-proline. Finally, the RC membranes functionalized with L-proline were placed in an aqueous solution containing 1 mg mL^{-1} copper acetate for 24 h. Membranes were stored in a diluted copper acetate solution until filtration performance was evaluated.

2.3. Attenuated total reflectance – fourier transform infrared spectroscopy (ATR-FTIR)

A Nicolet Magna-IR 550 Series II (Nicolet Instruments, Madison, WI) with an external ATR accessory containing a germanium crystal was used to measure the surface chemistry of the RC membranes and the RC membranes grafted with L-proline. The membrane strips, precisely cut to the dimensions of the germanium crystal (i.e. 7 cm x 0.95 cm), were pressed firmly against the germanium crystal using a spring-loaded assembly which came as part of the accessory. Spectral scans were taken over a given frequency range, typically 700–4000 cm^{-1}. 256 scans were averaged for each spectrum at a 4 cm^{-1} resolution. A fresh background scan was performed before each sample was measured. The absorbances in all the spectra were normalized to the absorbance at ~ 1060 cm^{-1}, which is the highest intensity peak in the RC membrane. It was necessary to normalize ATR-FTIR spectra to account for slight differences in membrane size and differences in rigidity that can occur after chemical reactions are performed on the membrane surface.

2.4. UV absorbance measurements

A Biotek Powerwave XS instrument (Winooski, VT) was used in conjunction with 96-well UV transparent microplates to collect absorbances of 270 μL aliquots of enantiomer solution. Calibration curves for both D and L-Phenylalanine were produced to correlate UV absorbance at 260 nm with amino acid concentration. These calibration curves were used to determine amino acid concentration in the permeate of the filtration experiments.

2.5. Diffusion testing

After the RC membranes were grafted with the L-proline–copper complex, the diffusion of both D and L-Phenylalanine (D-Phe or L-Phe) were independently measured using the diffusion cell. The apparatus contains two chambers capable of holding 10 mL of fluid separated by a membrane. An O-ring on the feed side of the membrane provides a seal separating the feed and permeate compartments such that permeation between the compartments must pass through the membrane. The feed chamber was loaded with 10 mL of 0.05 mg mL^{-1} aqueous solution of the amino acid enantiomer which faces the active (feed) side of the membrane, whereas the permeate side contained only 10 mL of pure Milli-Q water to impose a concentration gradient across the membrane. The permeate was sampled at regular time intervals and the enantiomer concentration determined through UV absorbance at 260 nm. UV readings were performed in triplicate and compared with calibration curves to quantify the concentration of enantiomer. After measuring the