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Separation of Naproxen Enantiomers Using Hollow Fiber Molecularly Imprinted Membrane Chromatography

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In this paper, the use of molecularly imprinted membrane (MIM) for chromatographic separation is described. Poly(vinylidene fluoride) hollow-fiber membranes were grafted on an imprinted polymer layer by using 4-vinylpyridine and trimethylolpropane triacrylate as monomer and crosslinker and were applied as the chromatographic media. During separation, naproxen enantiomers were separated efficiently, and the separation factor was 2.36. Molecularly imprinted membrane chromatography (MIMC) showed an apparently opposite transport behavior compared to molecularly imprinted polymer (MIP)-packed chromatography.
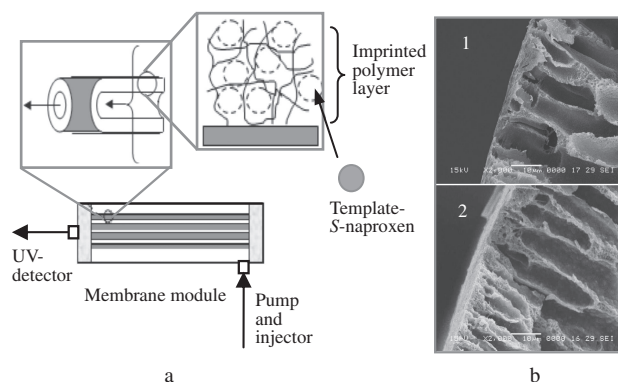
Chromatography has been extensively applied in purification, separation, and analysis owing to good selectivity, efficiency, and high loading capacity. However, separation is limited by using packed beads in this process. For example, high pressure drop, intraparticle diffusion, and channeling lead to increased process time, recovery liquid volume, and complicated scale-up.[1] By using membranes as chromatographic media, some of the major limitations associated with column chromatography can be resolved because of tremendous decrease of mass-transfer resistance.[2] Membranes were first introduced into affinity chromatography by Brandt in 1988.[3] Since then, membrane chromatography has become a fast growing bioseparation technique.[4]

Molecular imprinting is a technique to prepare crosslinked polymers with highly selective recognition sites for a given target or group of target molecules. With the development of imprinted membranes,[5] which are obtained by combination of molecular imprinting technology and membrane separation, molecularly imprinted membrane chromatography (MIMC) as a special affinity chromatography is considered to realize separation and purification of given molecules with higher selectivity and efficiency.

In this paper, hollow fiber MIMC is developed and applied to the separation of naproxen enantiomers. We focus on detailed investigations of the morphology of imprinted membranes, separation efficiency of hollow fiber MIMC, and transport behavior compared to molecularly imprinted polymers (MIPs) as stationary phase in HPLC experiments.

Figure 1a shows a schematic diagram of MIMC. Analytes are pumped into the hollow fiber molecularly imprinted membrane modules (8 mm × 150 mm stainless steel pipe), and permeation solutions are monitored by a UVD-680-1-UV spectrometer detector (Shanghai Kingdom Biochemical Instrument Factory) system. Imprinted membranes (Figure 1 b-2) were obtained by taking *S*-naproxen, 4-vinylpyridine, and

Figure 1. Experimental setup for chromatographic measurement: (a) Schematic illustration of MIMC with hollow-fiber module and (b) SEM images of cross section of membrane (1: initial membrane, 2: imprinted membrane).

trimethylolpropane triacrylate as template, monomer and crosslinker (molar ratio = 1:4:20) copolymerization in the surface of poly(vinylidene fluoride) hollow-fiber membranes (Figure 1 b-1; self-made; porosity: 70%, pure water flux: $12 \, \text{L m}^{-2} \text{h}^{-1} \text{bar}^{-1}$, molecular weight cutoff with BSA67000 as a standard solution: 94.7%). After elution with acetic acid/methanol (1:9, v/v) and methanol, respectively, they were made into hollow-fiber modules.

Chromatographic media is one of the most important factors of separation efficiency. So hollow fiber molecularly imprinted membrane (MIM) plays a key role in MIMC. Seen from Figure 1b, an imprinted polymer layer deposited on the surface of initial membranes and remedied the defects of the membrane surface. At the same time, both a linear path through the membrane and the cylindrical channels are still apparent and interspersed inside the MIM similar to the initial membrane.

The enantioselective separation by MIMC was determined as illustrated in Figure 1. The resulting chromatograms of racemic naproxen and *S*-naproxen are shown in Figure 2. MIMC facilitated the resolution of naproxen enantiomers, and a separation factor (α) of 2.36 was obtained. Here, α is defined as the relationship $\alpha = K_R/K_S$, where K_R and K_S are the capacity factor of the R and S enantiomer, respectively. The capacity factors were determined according to $K_R = t_R/t_0 - 1$ and $K_S = t_S/t_0 - 1$, where t_R and t_S are the retention times of the R and S enantiomer, respectively, and t_0 is the retention time of the dead volume, which was determined by the injection of toluene.

As shown in Figure 2, there was usually extensive peak broadening and asymmetry in the MIMC, which was mainly due
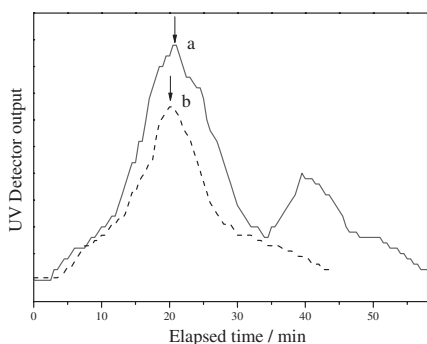
Figure 2. Chromatogram of racemic naproxen (a) and *S*-naproxen (b) by imprinted membrane column (The experiments were carried under the optimized conditions as follows: A flow-rate of $0.2\,\mathrm{mL\,min^{-1}}$ was used over 30 min with 0.1% acetic acid and 99.9% methanol. The concentration of each solutes was $0.02\,\mathrm{mmol\,L^{-1}}$. The detection wavelength was 275 nm. The mobile phase was degassed during measurements).

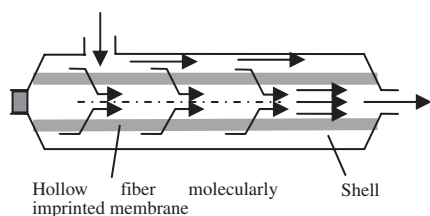Hollow fiber molecularly imprinted membrane   Shell

Figure 3. Solution transport in dead-end hollow fiber membrane module.

to irregular membrane pores, heterogeneous binding sites, and a larger dead volume. Furthermore, *S*-naproxen was eluted first and had a shorter retention time, which can be attributed to the construction of *S*-naproxen imprinted membrane. A strong ionic interaction between 4-vinylpyridine and *S*-naproxen is produced in the polymerization of MIM. After extraction of templates, the matrix of polymers is left with cavities of a complementary size, shape, and arrangement of functional groups to *S*-naproxen. The binding kinetics of template binding to MIP sites in a MIM can be coupled with convection and diffusion transport through the membrane pore thus enabling MIMC separation. In our study, the transport direction includes both radial and axial flow from the use of dead-end hollow fiber membrane filtration (Figure 3). In the axial direction, the liquid initially flows parallel to the membrane surface. In the radial direction, the molecules are gradually directed toward and through the pores due to pressure difference or back to the solvent phase in a longer diffusion time. If the affinity of the binding sites in the membrane is high, the molecule will spend more time in the solvent phase than a molecule with lower affinity, and thus, more of them will diffuse to the other side of membrane. Hence, *S*-naproxen shows a shorter retention in membrane chromatographic separations.

In fact, much research on molecularly imprinted techniques combined with chromatography focused on the transport mechanism. And some discussions on using MIPs as stationary phase draw an apparently opposite conclusion from our study of MIMC. According to them, transport phenomena of MIP bead chromatography lies in convection, diffusion of interbead space, and intraparticle diffusion (Figure 4), and intraparticle diffusion
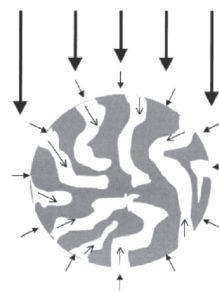

Figure 4. Schematic of chromatography process MIPs taken as stationary phase.[6]

is dominant for the packed chromatography. Owing to complementary recognition sites and configuration with MIPs, analyte molecules that have a higher affinity for the stationary phase will spend more time in complex with the stationary phase and will, therefore, be eluted later.[6]

A new membrane chromatography technique was developed by using hollow fiber molecularly imprinted membrane as separation media in this paper, which provide a new research field for molecular imprinting and set a foundation for the separation of enantiomers. Naproxen enantiomers were efficiently resolved by hollow fiber MIMC. In separation, MIMC showed an apparently opposite transport behavior compared to MIP-packed chromatography. Owing to penetrated membrane pores, membrane chromatography is suitable for large-scale applications. However, there are some limitations and challenges need to be overcome. The separation factor of molecularly imprinted membranes could be enhanced by choosing membranes with suitable pore structure and small pore size distribution, designing and manufacturing membrane modules carefully and efficiently.

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