

Chiral Separation of D,L-Serine Racemate Using a Molecularly Imprinted Polymer Composite Membrane

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ABSTRACT: Molecularly imprinted (MIP) composite membranes were prepared using conventional interfacial polymerization technique that has been often used for the preparation of reverse osmosis (RO) membrane. Target molecules (D-serine) were used together with the piperazine (PIP) and Trimesoyl chloride (TMC) for the interfacial polymerization of the active layer with chiral spaces in it on the surface of the polysulfone ultrafiltration membranes. After formation of the polyamide composite membranes, the target molecules in the active layers were removed and MIP composite membranes were prepared. The MIP membranes prepared so were then characterized

with analytical methods and proved to be effective for the selective permeation of D-serine. When serine racemate was used for optical resolution, the diffusion rate of the D-serine appeared to be faster than that of the L-serine and in permeates, the concentration of the D-serine increased with operation time. When the operating time reached 60 h, enantiomeric excess (% ee) of the serine mixture in permeates became about 80%. © 2007 Wiley Periodicals, Inc. *J Appl Polym Sci* 104: 1866–1872, 2007

Key words: molecular imprinting; enantioseparation; composite membrane; D,L-serine

INTRODUCTION

A chiral compound has optical isomers in mirror images that are called enantiomers, having chirality. The chirality of the chiral compounds is important factor controlling the biosynthesis and metabolism in a human body. Such chiral compounds therefore play an important role in many industries. Since a chiral compound shows its characteristic properties only when it is in a single enantiomer, the important process in the chiral compound production industry is the separation of optical isomers. Though optical isomers have exactly same chemical structure, they cause very different biological properties. So, to use chiral compounds properly for their purpose, they have to be in single enantiomers, not in mixtures.

With knowledge of such properties of chiral compounds, researchers have tried to make single enantiomers by asymmetric synthesis and have succeeded in producing some of the single enantiomers. However, it has not always been possible to synthesize the compounds in a single enantiomer form. In many cases, they have been produced as optical iso-

mer mixtures. So, optical resolution by the separation techniques is necessary for the production of single enantiomers from optical isomer mixtures. So far, many different processes have been employed for optical resolution, but each process showed its limitations. For instance, the most economical process, recrystallization process, has been widely used, but not every optical isomer can be separated by this technique. In the case of HPLC, most often used for the analysis of the purity of the optical isomer, it is not economically feasible because it requires large amounts of solvents for mass production of single enantiomers.

Based on this, membrane process has become an alternative as an economical process for the production of single enantiomers by the optical resolution.^{1,2} Many different types of membranes have been prepared till date, but the performance of the membranes developed is not yet satisfactory. In 1990, Piletsky et al.³ reported the optical resolution by the permselective membranes produced by the molecularly imprinted technology that had been developed by the Wulff et al. Molecular imprinting is a technique that has been used to make a polymeric matrix with a selective affinity to certain molecules, and some researchers have tried to use this technique to make a permselective membrane useful for the optical resolution of chiral compounds. Recently, many different types of environmental friendly molecularly imprinted (MIP) composites in

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membrane shape have been prepared, and they are being used for the molecular recognition in separation systems.⁴⁻¹⁰

In this study, a MIPCM was developed to separate D,L-serine racemate with high efficiency. Because the D,L-serine and their alkyl ester-derivatives are all soluble in water, it is difficult to separate them using a membrane bioreactor with enzyme loaded in it. The MIPCM was prepared by using interfacial polymerization technique using D-serine as a target molecule. The preparation and characterization of the MIPCM for the optical resolution of D,L-serine is elaborated below.

EXPERIMENTAL

Materials

For the formation of microporous support of the MIPCM, polysulfone (Udel P3500, Amoco, USA) was used, and piperazine (PIP), trimesoyl chloride (TMC), and D-serine purchased from Sigma-Aldrich were used for the formation of active layers of the MIPCM with regiospecific chiral space in it. Triethyl amine purchased from Sigma Aldrich (Milwaukee, WI), N-methyl pyrrolidone (NMP) and Hexane from Junsei (Tokyo, Japan) were used as catalyst and solvents. D,L-Serine racemate and phosphate buffer (pH 7) solution were bought from Sigma Aldrich and used for the optical resolution. Perchloric acid used

as a moving phase of the HPLC was obtained from Sigma Aldrich.

Characterization

To ascertain the structure and characteristics of the MIPCM, FTIR-ATR (FT-80, Bio-Rad) and SEM (XL30S FEG Scanning Electron Microscope, Philips) were used. The membrane reactor shown in Figure 1 was designed and made, and used in this study; the chiral column for the analysis of the concentration of serine isomers by the HPLC was the CROWNPAK CR(+) column (4.6 mm i.d. \times 25 cm) purchased from Daicel Chemical (Osaka, Japan). The HPLC system used was obtained from Dionex (Sunnyvale, CA), which was equipped with P580 Pump and UVD170U UV/VIS detector.

Preparation of MIPCM

An NMP solution of polysulfone with a composition of 15/85 (PSf/NMP in wt %) was cast 150 μ m thick on the polyester nonwoven fabric and phase inverted in the water used as a nonsolvent miscible with NMP for the formation of microporous PSf support. To form an active layer containing D-serine as a target molecule, the surface of the PSf support prepared was coated with aqueous solution of PIP well mixed with D-serine in water. The composition of PIP/D-serine/water was 5/1/94 (wt %). It was then immersed into the 1 wt % of hexane solution of

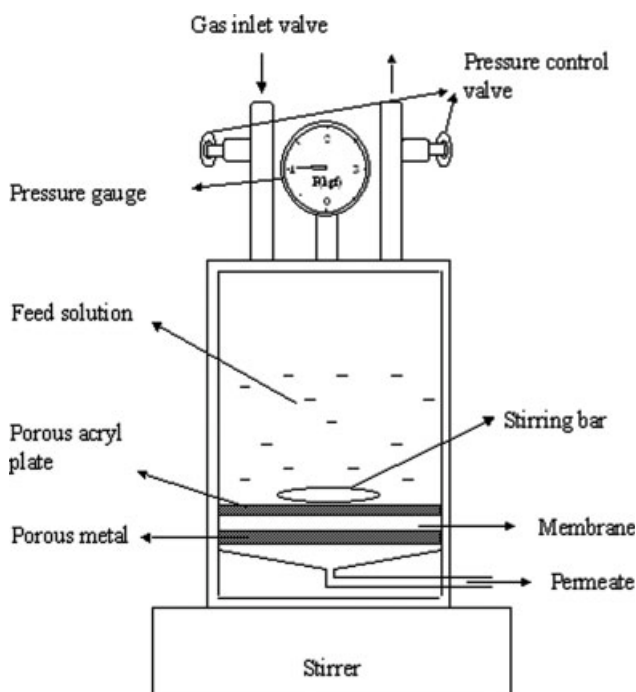


Figure 1 Schematic diagram of the membrane set-up.

TMC for 10 s for the interfacial polymerization of PIP with TMC to form the active layer. The D-serine used as a target molecule was remained in the active layer, forming chirally active sites. Trace amount of TEA was dissolved in the hexane solution of TMC and acted as an HCl absorbing agent, accelerating the interfacial reaction, forming crosslinked polyamide (PA) layers. After which, the PA composite membrane with D-serine in its active layer was kept in 50°C water for 50 h, and D-serine was removed from the crosslinked PA active layer to form the MIPCM.

Optical resolution by the MIPCM

Using the permeation test set-up shown in Figure 1, optical resolution of D,L-serine was carried out in this study. The MIPCM was located on the bottom side of the cell and pressure was applied, using nitrogen gas, through the cell to the D,L-serine aqueous solution, causing selective permeation of D-serine. The gas pressure was controlled by the knob attached to the set-up. During the separating operation, the D,L-serine aqueous solution was stirred by the magnetic bar stirring on the top of the membrane, preventing the membrane from fouling and making the concentration of the solution homogeneous. The D,L-serine aqueous solution was prepared by dissolving 0.2 g of D,L-serine into 150 mL of phosphate buffer solution of pH 7.2. The operating pressure was controlled in the range of 0.5–3.0 bar, and the temperature was 25°C. The permeate collected was analyzed using HPLC equipped with CROWNPAK chiral column and perchloric acid of pH 1.5 as a moving phase. The flow rate of the moving phase through the column was controlled at 0.4 mL/min at 0°C. UV light, 200 nm, was used as the detector of the HPLC.

RESULTS AND DISCUSSION

Preparation of MIPCM

To develop an efficient method for the optical resolution of D,L-serine, in which D-serine has often been used as a starting material for the production of drug useful for the curing of tuberculosis, a MIPCM was prepared in this study as one of the enantioselective membranes.

It has been known that there are two types of membranes useful for the optical resolution: one is enantioselective membrane that has capability for the selective permeation of one of the optical isomers, and the other is enantiospecific membrane that has often been used for membrane bioreactors with enzyme that reacts specifically with one isomer of racemate used. For the efficient operation of a membrane bioreactor using enzyme for example lipase as

a optical resolution tool, the starting materials and the products produced from the reaction with lipase should be dissolved in different immiscible solvents such as water and organic solvents, respectively. If the starting material for example *R,S*-naproxen methyl ester is soluble in organic solvent and the product, *R*-naproxen, is soluble in water, the membrane bio-reactor can effectively separate the *R,S*-naproxen. However, when both the starting material and product are soluble in the same solvent, it is hard to use membrane bioreactor. Unfortunately, D,L-serine was belonged to this case. Both the D,L-serine and D,L-serine alkyl ester were soluble in water. Therefore, for the optical resolution of serine racemate, an enantioselective membrane has to be used.

The MIP membrane has been considered as an effective enantioselective membrane especially useful for the kinetic optical resolution, and some of the MIP membranes were reported to be effective for the separation of optical isomers of α -amino acids and 1,4-butanediol. Based on this, a MIP membrane with a high performance was pursued in this study and ended up with a MIPCM. The MIPCM is a composite membrane, while other MIP membranes reported in the previous papers are homogenous membranes. Generally speaking, the composite membrane consisting of a thin active dense layer and a porous support layer has much higher flux than the homogeneous membrane whose whole structure is dense layer.

As explained in the Experimental section, MIPCM was prepared by coating the surface of the microporous PSf support that was prepared by the phase inversion of PSf solution in NMP with thin polyamide active layer containing regiospecific chiral space in it, using the interfacial polymerization technique. For the formation of crosslinked polyamide active layer with chiral space in it, D-serine, the target material was mixed with PIP in water solution. The PIP-D-serine (5/1, wt %) mixture in aqueous solution coated homogeneously on the surface of the microporous PSf support reacted with TMC in hexane by the interfacial polymerization to form a crosslinked polyamide active layer. The three —COCl groups of TMC reacted with the two amine groups of the PIP, forming a crosslinked polyamide network structure. During the formation of the crosslinked PA network by the PIP and TMC, the D-serine bound to the PIP via the hydrogen bond formation between the amine of PIP and —COOH of D-serine was drawn into the PA network and formed chiral space in it, forming a MIPCM (Fig. 2). Figure 2 shows the schematic diagram of the MIPCM prepared in this study. The D-serine was then removed from the composite membrane by keeping the membrane in 50°C water for 50 h, completing the formation of the MIPCM.

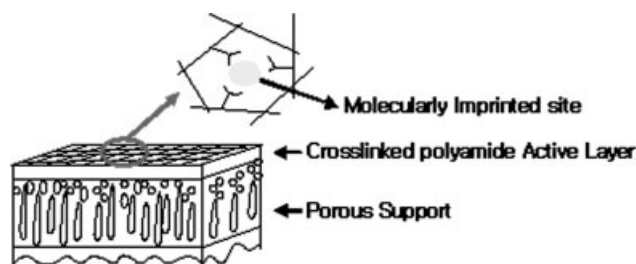


Figure 2 Schematic diagram of the molecularly imprinted composite membrane (MIPCM).

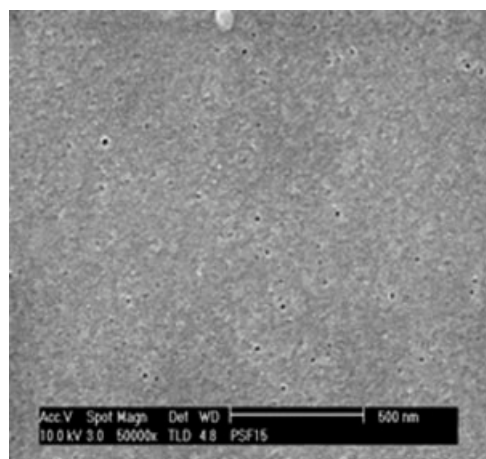
Characterization of the MIPCM

Figure 3 shows the FESEM photographs of the surfaces of the several membranes, PS support, NF PA composite membrane and MIPCM. As one can see, the pores of the microporous PSf support were completely covered by the crosslinked polyamide layers for the formation of both the MIPCM and the just PA composite membrane that was prepared without using D-serine, showing no distinct differences between the MIPCM and PA composite membrane. Especially for the MIPCM, even though D-serine was removed from the active layer at rather high temperature (50°C) to form regiospecific space in the active layer, the PA layer was found to be very smooth and stable.

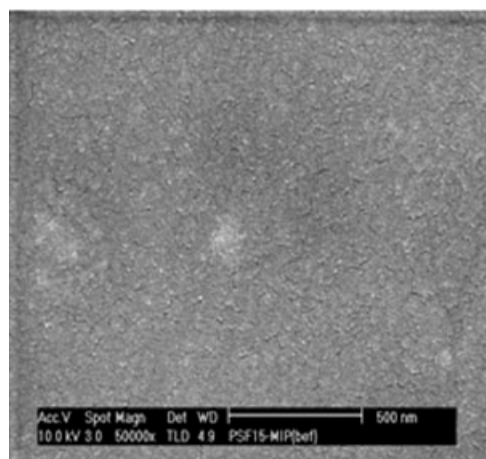
To study the difference in their permeability, the water flux of the MIPCM and the just nanofiltration (NF) PA composite membrane was measured (see Table I), and it was found that the MIPCM have higher water flux than the NF PA composite membrane, suggesting that the free volume of the PA active layer of MIPCM is higher than that of the NF PA composite membrane. This result might be explained by the relatively large chiral spaces formed in the crosslinked PA active layer by removing the D-serine. In other words, water penetrated more easily through the chiral space than through the pores of crosslinked PA layer, because the chiral space is much larger than the pores of the NF PA composite membrane that has usually been known to be 1 nm. The PA composite membrane, which was prepared in this study by the same process of the formation of MIPCM without using D-serine, showed NF performance. Figure 4 shows the flux and rejection of several feed solutions of the NF PA composite membrane being compared with the MIPCM. From the high flux at moderate pressure, 200 psi, and high rejection of Na₂SO₄ and low rejection of NaCl shown in Figure 4, one can confirm that the membrane is a typical NF membrane with pores of about 1 nm.

Optical resolution of D,L-serine using MIPCM

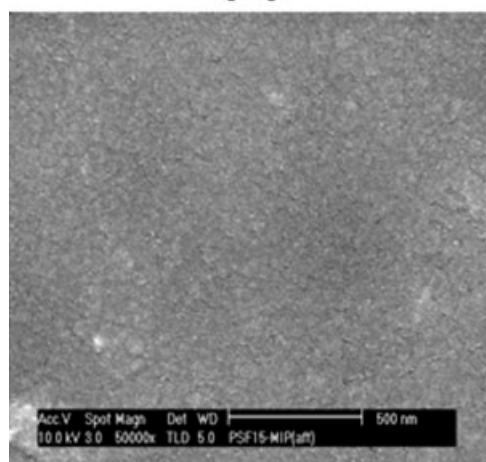
Optical resolution of D-serine was carried out, using the MIPCM. Figure 5 shows the variation of accumu-



(A)



(B)



(C)

Figure 3 FESEM micrographs of the surfaces of the several membranes: (A) PS support, (B) NF PA composite membrane, (C) MIPCM.

TABLE I
Pure Water Flux of the NF PA Composite Membrane
and the MIPCM

Membrane	NF PA composite membrane			MIPCM		
	0.5	1	2	0.5	1	2
Pressure (bar)						
Flux ($\text{m}^3/\text{m}^2\text{day}$)	0.0112	0.0432	0.0592	0.0368	0.0832	0.1376

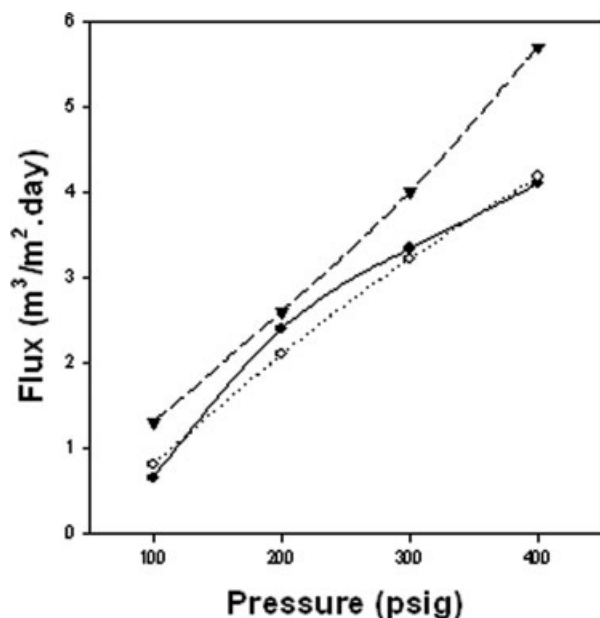
lated D- and L-serine of permeates according to the operating time. For this experiment, 0.2 g of serine racemate was dissolved in 150 mL of phosphate buffer solution (pH 7.2) to prepare a feed solution and the operating pressure and temperature were 2 bar and 25°C, respectively. From Figure 5, it was found that the amount of the D-serine accumulated in permeates increased with operating time almost linearly, while only limited amount of L-serine was accumulated in permeates, indicating that permeation rate through the membrane of D-serine is much faster than that of L-serine.

The optical resolution through the MIPCM of D- and L-serine that have exactly same chemical structure except for their stereochemical structure can be explained by the different degree of resiospecific interaction between the chiral environment in the membrane and the D- and L-serine when they diffuse through the membrane. In other words, the chiral space formed in the MIPCM acted favorably for the diffusion of D-serine rather than L-serine through the regiospecific interaction. Because D-serine was used as the target material for the formation of the MIPCM, it is natural that the chiral space formed by the D-serine in the membrane is easy for D-serine to pass through while L-serine that has different stereochemical structure from D-serine would have some disturbance. This is a kind of key and lock system, like a right key can easily go thorough the lock but a wrong key cannot.

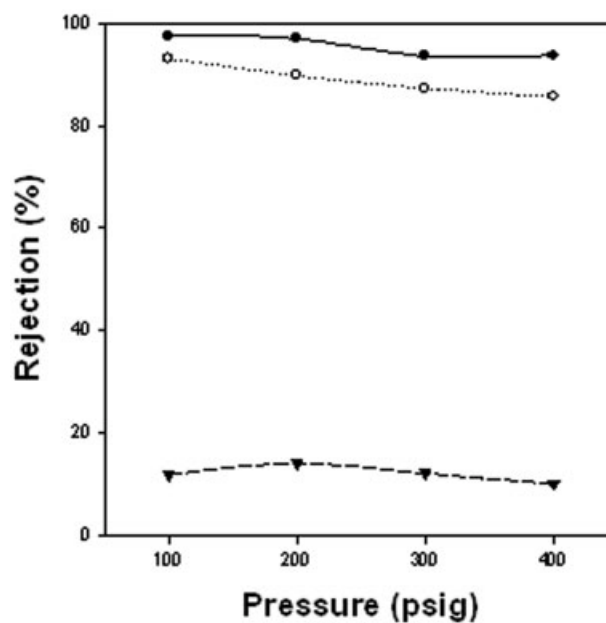
From this result, it can be suggested that the MIPCM prepared in this study is useful for the separation of D,L-serine racemate.

Figure 6 shows the variation of accumulated amounts of D- and L-serine in permeates with operating time when operating pressure was 3 bar. As being compared with the result obtained when the operating pressure was 2 bar, the amount of L-serine that penetrated through the membrane increased substantially, decreasing the enantiomeric excess of permeates. It was found that by increasing operating pressure from 2 to 3 bar, total amount of D- and L-serine that penetrated through the membrane increased, but the composition of the D- and L-serine in permeates became different: it seemed that the diffusion of D-serine was interrupted by the L-serine

competing with D-serine to pass through the chiral space, decreasing the amount of D-serine and increasing the amount of L-serine in permeates. From this result, it can be suggested that the increasing operating pressure is not always positive for an



(A)



(B)

Figure 4 Permselective properties of the NF PA composite membrane prepared using PIP and TMC as monomers as 1000 ppm aqueous solutions of PEG 600, Na_2SO_4 and NaCl used as feed: (A) Flux and (B) solute rejection.

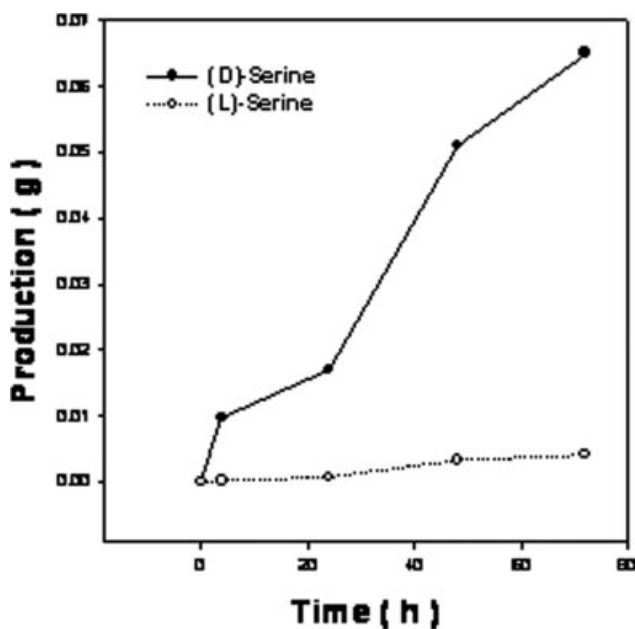


Figure 5 Production of the D- and L-serine by the MIPCM from serine racemate as a function of operating time. Operating pressure and temperature were 25°C and 2 bar, respectively.

efficient operation of the MIPCM membrane system for the optical resolution of D- and L-serine. This suggestion can be confirmed from Figure 7, exhibiting the effect of operating pressure under 2 bar.

Figure 7 shows effect of pressure on the diffusion of D- and L-serine. Up to 2 bar, the amount of L-ser-

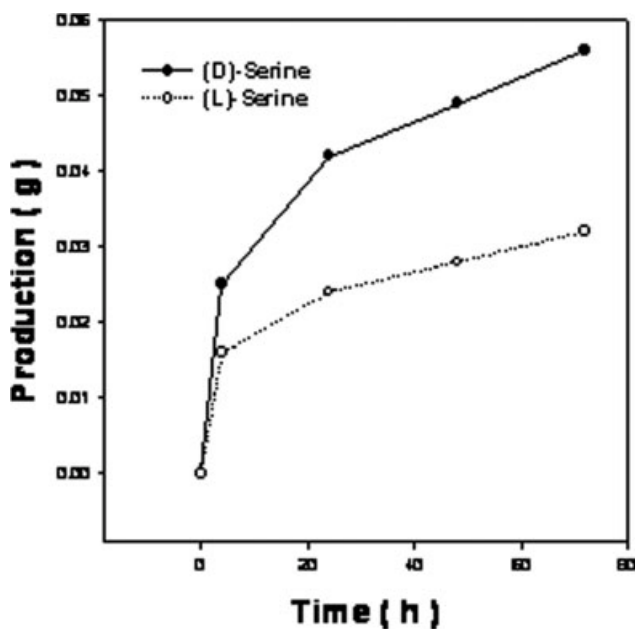


Figure 6 Production of the D- and L-serine by the MIPCM from serine racemate as a function of operating time when the operating pressure was 3 bar. Operating temperature was 25°C.

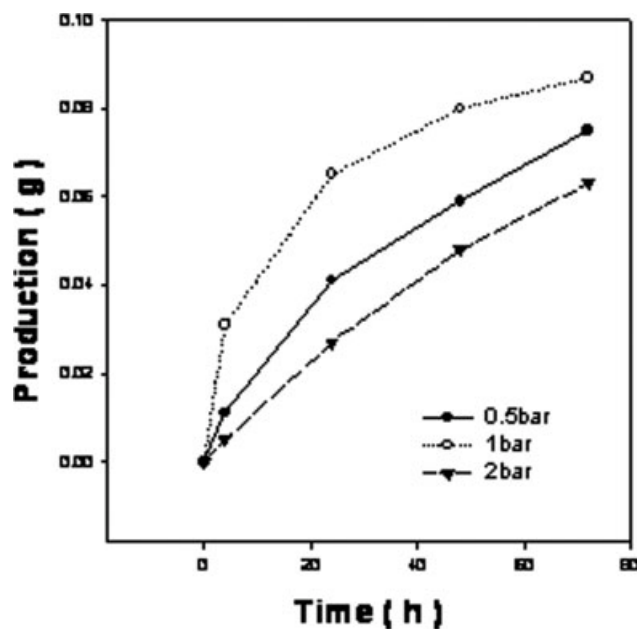


Figure 7 Production of the D- and L-serine by the MIPCM from serine racemate as a function of operating time as the operating pressure was varied from 0.5 to 2 bars. Operating temperature was 25°C.

ine with operating time was almost negligible at least as long as for 75 h. However, the D-serine was affected substantially by the pressure, whose amount was found to be dependent on the pressures employed. By increasing pressure from 0.5 to 2 bar, the amount of the D-serine became maximum at 1 bar, then decreased again. From this result, it would be possible to speculate that the D-serine penetrating through the membrane via the chiral spaces collide each other as the operating pressure is over 1 bar, disturbing the smooth flow of the D-serine, resulting in the decrease in the amount of D-serine in permeates.

CONCLUSIONS

A MIPCM useful for the optical resolution of D- and L-serine was prepared by coating the surface of the microporous PSf support with PA active layer with D-serine, using interfacial polymerization of PIP that strongly interacted with D-serine with TMC, followed by the removal of the D-serine from the PA active layer again. In this case, the D-serine was used as target molecule for the molecular imprinting in the membrane. The MIPCM prepared transpired to be successful for the optical resolution of D- and L-serine. The efficiency for the optical resolution was dependent on the operating conditions, especially on the operating pressure. At 1 bar of operating pressure, best separation results were obtained.

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