Study on Preparation and Properties of PA6/PSMA-OA Molecularly Imprinted Composite Membranes

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Oleanolic acid (OA) as template molecules, polyamide-6 (PA6) as basement membrane and poly(styrene-co-maleic acid) (PSMA) were used to prepare PA6/PSMA-OA molecularly imprinted composite membranes (PA6/PSMA-OA MICMs) by phase inversion method. The template molecules (OA), polymers [poly(styrene-co-maleic anhydride) (PSMAH), PSMA], MICMs imprinting OA and MICMs after elution were all characterized by Fourier Transform Infrared Spectroscopy (FTIR). And the optimum condition was obtained as follows, polymer concentration 14%, temperature of distilled water 15°C and dipping time 20 h, respectively. After template molecules being removed, the MICMs had effective selectivity hydrogen bonding to separately bind in the binary components to the template molecules–OA.

Keywords: Oleanolic acid; Phase inversion; Molecularly imprinted composite membrane; Binding experiments

Molecularly imprinted membrane (MIM) first studied by Piletsky et al. [1] was an emerging technology, which has the advantages of both molecular imprinting technique and membrane separation technology. In recent years, MIM has aroused general interest in the research filed of molecular imprinting technology. Selective separation of individual substance could not be achieved by using the current commercial membranes, such as ultrafiltration (UF), microfiltration (MF) and reverse osmosis (RO) membranes. However, MIM offered us a new approach to selectively recognize the molecules in complicated systems. The main methods to prepare MIMs include phase inversion in the presence of template molecules [2-9], surface imprinting [10-12] and in situ polymerization by bulk polymerization [13-15].

Presently, in the field of membrane science and technology, “functional complex” has become a developing trend of membrane materials. In this membrane, a thin dense layer is overlaid at the porous support layer (basement membrane), and the cortex and the support layer are made from different materials. The advantages of composite membranes are to select the appropriate cortex and support layer to accomplish optimal performance in the following areas, such as selectivity, permeability, chemical and thermal stability. Composite membranes are generally consisted of selective separation layer (0.02-0.1 μm) and porous support layer (about 100μm). Porous support membranes are commonly used as basement membrane for composite membranes. The main support membranes mainly include polysulfones (PSF), polyethersulfone (PESF), polyimide (PI), polyamide (PA), polyvinylidene fluoride (PVDF), polycarbonate (PC), polyetherimide (PEI), polypropylene (PP), cellulose ester and inorganic microporous membranes. Composite membrane technique is one of the most effective methods to prepare NF and RO membranes. And they have a broad range of applications, such as gas separation, pervaporation, and membrane reactors [16-22].

In the present study, polyamide (Nylon-6) was used for basement membrane. Nylon 6 fibres are tough, possessing high tensile strength, as well as elasticity and lustre. They are wrinkle-proof and highly resistant to abrasion and chemicals such as acids and alkalis. Polyamide/ poly(styrene-co-maleic acid)-oleanolic acid molecularly imprinted composite membrane (PA6/PSMA-OA MICM) was prepared in aqueous solution, and methanol was used in the process of eluting template molecules. Therefore, Nylon membrane of hydrophilic and resistant to organic solvents immersion was selected as a support film.

Experimental part

Materials and reagents
Poly(styrene-co-maleic anhydride) (PSMAH, Mw 224,000) was obtained from Sigma-Aldrich. Polyamide film (Nylon-6) was purchased from (Zhejiang, China); non-woven fabrics (Polyester fiber, TA3631) from Shanghai Tianlue Industrial Textile Co. Ltd (Shanghai, China); oleanolic acid and ursolic acid from (Hunan, China). High purity (99.9%) carbon dioxide (CO2) used for production of compressed liquid CO2 was supplied by (Anhui, China). Before use, all the chemicals were further purified.

Preparation of molecularly imprinted composite membranes
To obtain interactive sites of oleanolic acid template molecules in PSMA, hydrolysis of PSMA was carried out as follows. 5 g of PSMA was added into 60 mL of tetrahydrofuran (THF) in a three-neck flask. The solution was stirred until all PSMA dissolved. Then, 3 mol/L of NaOH solution was added slowly into the flask and refluxed at 80°C for 2 h. The solution was precipitated in 3 mol/L of HCl solution, then the resultant white polymer was washed with a large amount of distilled water for 3 times. The polymer was dried in vacuum to obtain PSMA. The hydrolysis process of PSMA to PSMA containing change of chemical structure is shown in figure 1.

Porous imprinted membranes were prepared by phase separation in distilled water as coagulation medium. Figure
by controlling the flow rate of valve b. Then the device of water bath (8 in fig. 3) was opened to make the extraction container up to the desired temperature (40°C); (iv) the whole system was in a dynamic process and maintained for 1-2 h. It is important to control the flow rate of flowmeter (9 in fig. 3); (v) then, the system was slowly depressurized and CO₂ could be recycled into an empty bottle; (vi) after being taken out, the molecularly imprinted membranes were eluted with the eluant. Then the eluant was evaporated completely to attain required solid substances. The substances were weighed and prepared to be a certain concentration solution. The purity of OA was detected by HPLC; (vii) a series of OA standard solutions were prepared for the standard curve by HPLC; (viii) the solutions in (vi) were detected by HPLC and the purities of OA were calculated by the standard curve. Adsorption rate and purity of OA after adsorption are calculated as follows:

\[
\text{Adsorption rate (Ar) = } \frac{(W_2 - W_1)}{W} \times 100\% \\
\text{Purity of OA after adsorption (Pa) = } \frac{W_3}{(W_2 - W_1)} \times 100\% 
\]

where:
- \(W_1 (g)\) was the weight of MICMs before adsorption;
- \(W_2 (g)\) - the weight of MICMs after adsorption;
- \(W_3 (g)\) - the weight of pure OA in adsorbed substances.

**Binding experiments of substrates by PA6/PSMA-OA MICMs Analysis of oleanolic acid**

0.2 mg/mL OA methanol solution was prepared and scanned in the wavelength range of 200-400 nm. The result showed that the maximum absorption wavelength of OA was 210 nm. A series of OA standard solutions were prepared for the standard curve by HPLC, shown in figure 4. Agilent 1100 series HPLC system (Agilent, USA) was operated at 210 nm. HPLC analysis was performed on Waters Symmetry Shield C₁₈ column (3.9 mm × 300 mm, 4 μm, Waters, USA)
at temperature of 25°C and a flow rate of 1.0 mL/min. The mobile phase was composed of acetonitrile and water (90:10, v/v). A very good linear relationship between sample concentration and peak area was obtained, with a correlation coefficient (R) of 0.99948 in the range of 0.03-0.6 mg/mL.

**Binding experiments**

In order to study the adsorption kinetics of MICMs, 100 mg PA6/PSMA-OA MICMs were added into 8 mL substrate methanol solution. And the mixture was oscillated at room temperature for 8 h. Appropriate combined solution was diluted to a certain volume with methanol. The concentration of free substrate in the equilibrium adsorption solution was measured by HPLC-UV at 210 nm. The amounts of substrate binding to the MICMs (Q, μmol/g) were calculated by the substrate concentration change between before and after binding in the solution. Three parallel experiments were carried out, and then averaged amount of substrate binding to the MICMs was obtained. Amounts of substrate bound to the MICMs were calculated by the following equation:

\[ Q = (C_0 - C_t) V / W \]

where, \( C_0 \) and \( C_t \) were the substrate concentration (μmol/mL) measured at initial and after interval time (hour) for equilibrium, \( V \) and \( W \) were the volume of the substrate solution (8 mL) and the weight of dry MICMs (100 mg) used for the binding experiment, respectively.

Binding partition coefficient (K_D) of substrate between the membrane and solution was calculated by the following equation:

\[ K_D = C_p / C_s \]

where, \( C_p \) was the substrate concentration (μmol/g) bound to the MICMs, and \( C_s \) was the substrate concentration (μmol/mL) in the equilibrium adsorption solution.

Selective factor (α) of the MICMs was the relative value of ursolic acid (UA) substrate bound to the MICMs compared with that of OA template and was calculated by the following formula:

\[ \alpha = K_D(OA) / K_D(UA) \]

where, \( K_D(OA) \) (mL/g) was partition coefficient of OA in MICMs after binding, and \( K_D(UA) \) for UA.

**Results and discussions**

**Characteristic of imprinted membranes**

The hydrolysis reaction of PSMAH to PSMA was monitored by infra-red analysis. As shown in figure 5 (a) and (b), the absorptions at 3082, 3059, 3026, 3001 cm\(^{-1}\) were the stretching vibration of C-H of the phenyl groups. The asymmetrical and symmetrical stretching vibration absorptions of C-H were 2925 and 2851 cm\(^{-1}\), respectively. The IR bands at 1601, 1583, 1493, 1452 cm\(^{-1}\) were the stretching vibration of C=C of phenyl groups on the backbone. The absorptions at 758 and 699 cm\(^{-1}\) were the bending vibration of C=C of the phenyl groups. The characteristic absorptions at 1856 and 1778 cm\(^{-1}\) (a) were the asymmetrical and symmetrical carbonyl adsorption of anhydride groups, respectively. The peak intensity of the high wave number at 1856 cm\(^{-1}\) was much weaker than that of the lower frequency at 1778 cm\(^{-1}\), which is one of the remarkable features of five-membered cycloanhydride. The 1256 cm\(^{-1}\) band was attributed to the C-O-C stretching adsorption of maleic anhydride unit. After hydrolysis completed, it was clearly shown on (b) spectrum that the carbonyl stretching of anhydride band was changed into carboxylic acid band at 1719 cm\(^{-1}\), and the peaks due to the unreacted anhydride groups were clearly disappeared at 1778 and 1856 cm\(^{-1}\). The IR band at 3386 cm\(^{-1}\) was for O-H stretching vibration of carboxylic acid groups [3].

The FT-IR spectra of OA, MICM imprinted with OA and MICM after extraction were shown in figure 6. In (a), the bands at 1686 and 3421 cm\(^{-1}\) were characteristic of OA, which showed the stretching vibration adsorption of O-H and C=O groups, respectively. Compared with the OA spectrum (a), the absorption of C=O group (b) shifted.
toward a higher wave number at 1718 cm⁻¹ and the peak intensity was strengthened notably, indicating that the O-H and COOH groups of OA interacted with the COOH segment of PSMA. In addition, the absorption at 1718 cm⁻¹ in (c) disappeared after completed extraction of OA. In (b), the peak intensity was lower than that observed in the spectrum (c) at 3386 cm⁻¹ of O-H stretching vibration of COOH group in PSMA. This implied that the inter- or intra-molecular hydrogen bonding between COOH segments of the PSMA was disrupted by the added OA template molecules.

Influencing factors on preparation of PA6/PSMA-OA MICMs

Three influencing factors such as polymer concentration, temperature of distilled water and dipping time, on preparation of polyamide 6/poly(styrene-co-maleic acid)-oleanolic acid molecularly imprinted composite membranes (PA6/PSMA-OA MICMs) were investigated in this study. Then the resultant membranes were studied in the supercritical equipment for the adsorption of oleanolic acid (purity of 50%). The adsorption rate of MICMs and the purity of OA after adsorption were the basis to judge the performance of membranes.

Influence of polymer concentration on preparation of PA6/PSMA-OA MICMs

Polymer concentration is an important factor on structure morphology and properties of membrane. The polymer concentration in casting solution is generally in the range of 10%-20%. The strength of membranes is too bad when the concentration is too low; while the concentration is too high, polymer does not dissolve very well, and membrane defect is easy to appear and membrane reproducibility is poor. In figure 7, at first, with polymer concentration increasing, the adsorption rate of MICMs and the purity of OA after adsorption increased. It indicated that with the increase of polymer concentration, the surface thickness of the resultant membranes increased, the porosity and the degree of interconnection between the pores declined, and the pore size decreased. At the same time, the increase of polymer concentration eliminated the formation of macropore and decreased permeability. However, when polymer concentration was more than 14%, the adsorption rate of MICMs and the purity of OA after adsorption were getting lower with polymer concentration increasing. It indicated that polymer didn’t dissolve very well in the casting solution, resulting in the resultant membranes having some defects.

Influence of temperature of distilled water on preparation of PA6/PSMA-OA MICMs

In the phase inversion method, the resultant membrane properties strongly depend on the preparation conditions, such as coagulation temperature. It is known that the phase inversion process involves two stages: one is solvent exchange in polymer environment and another is polymer coagulation in non-solvent [23]. The condition of the coagulation medium influences mainly the rate of solvent exchange between casting solvent and non-solvent. The penetration level of non-solvent into the polymer-casting solution medium becomes lower at low temperature, because of high viscosity and low solubility. Consequently, polymer coagulates slowly at low temperature. In the present study, the temperature of the water medium was changed in order to examine the dependence of coagulation temperature on substrate binding into the OA-imprinted membranes. Figure 8 showed that the uptake of the OA substrate strongly depended on the coagulation temperature. The decrease of the coagulation temperature caused an increase of OA taken into the copolymer membrane. For the membranes prepared at 15 and 40 °C, Ar and Pa were 43.4, 88.7 and 38.7, 79.8%, respectively. The values of the former were higher than those of the latter. The increase of the OA binding maybe due to the resultant membranes coagulated at low temperature having a number of OA imprinted sites only for OA substrate.

Influence of dipping time on preparation of PA6/PSMA-OA MICMs

In figure 9, in the range of 10-20 h, with the dipping time increasing, both of adsorption rate and purity of OA after adsorption increased. It maybe due to the rate of solvent exchange between casting solvent and non-solvent, and polymer deposition also increased with the dipping time increasing. Membrane thickness increased, the amounts of binding increased significantly, and selectivity increased correspondingly. However, when dipping time was more than 20 h, both solvent exchange and polymer deposition reached the dynamic equilibrium. With the dipping time increasing, the interaction between polyamide films and molecularly imprinted polymers was getting worse, becoming the main influencing factor. Therefore, imprinted sites in MICMs were getting less and less, and selectivity declined correspondingly.
Similar binding experiments with OA were carried out by using the PA6/PSMA – OA MICMs for ursolic acid (UA, fig. 10) substrate. Recognition experiments of OA and UA were performed under the same conditions. In table 1, it was apparent that the value of Q for OA (21.3 μmol/g) was higher than that of UA (7.9 μmol/g). The results indicated that the OA imprinted membranes showed selective recognition of OA, which was used as a template. However, the values of Q were not significantly different for PA6/PSMA-OA NMICMs. This indicated that the PSMA imprinted membranes recognized the OA molecules because of efficient formation of the OA imprinted sites, while NMICMs did not.

According to the equation (4) and (5), the binding partition coefficients ($K_D$) of substrate between the membrane and solution, and selective factor ($\alpha$) of the MICMs were obtained respectively (table 2). The values of $K_D$ for OA and $\alpha$ by PA6/PSMA-OA MICMs reached 149.5 mL/g and 5.8, respectively. The results indicated that PA6/PSMA-OA MICMs had the unique recognition on OA template molecules, and they had good capacity to separate substrates. However, the value of $\alpha$ by PA6/PSMA-OA NMICMs was only 1.1, implying that PA6/PSMA-OA NMICMs did not have good ability to separate substrates. It could be speculated that molecularly imprinted polymers (MIPs) prepared by phase inversion had specific recognition sites on the template molecules and bound to the OA well. Otherwise, there were no specific recognition sites on UA, so MIPs showed non-specific binding to UA with low binding capacity. PA6/PSMA-OA NMICMs did not have OA imprinted sites, so the interaction between OA and NMICMs was obstructed. Therefore, the binding amounts of OA by PA6/PSMA-OA NMICMs were also low. It indicated the difference between the binding amounts of OA and those of UA was not significant, and there existed a kind of non-specific binding, and a low selectivity factor ($\alpha$), which were listed in table 2.

**Conclusions**

In the present work, PA6/PSMA-OA molecularly imprinted composite membranes were successfully

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**Table 1**

<table>
<thead>
<tr>
<th>Membrane</th>
<th>Q (μmol/g)*</th>
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<tbody>
<tr>
<td>PA6/PSMA-OA MICMs</td>
<td>21.3</td>
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<tr>
<td>PA6/PSMA-OA NMICMs</td>
<td>10.2</td>
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* Initial concentration of substrate $C_0$: 0.4 mmol/L; $V$=8 mL; $t$=25 °C; Binding time: 8 h; Weight of membranes: 100 mg.

**Table 2**

| Membrane             | $K_D$ (mL/g) | $\alpha$* |
|----------------------|--------------|
| PA6/PSMA-OA MICMs    | 149.5        | 5.8        |
| PA6/PSMA-OA NMICMs   | 38.7         | 1.1        |

* $\alpha = K_{D(OA)}/K_{D(UA)}$; Other conditions were the same as Table 1.
prepared in distilled water by phase inversion method. The distilled water was efficient to fix the shape of OA template molecules into PSMA membrane through hydrogen bonding. Under the optimum conditions that polymer concentration 14%, temperature of distilled water 15°C and dipping time 20 h, the largest adsorption rate and purity of OA after adsorption of the resultant MICMs were obtained. From the binding experiments, it was found that the PA6/PSMA-OA MICMs had effective and selective recognition for OA. Therefore, the preparation of OA imprinted membranes provided a feasible method to separate OA from compounds selectively.

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References


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