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PREPARATION AND PROPERTIES OF CAFFEINE MOLECULAR COMPOSITE IMPRINTED MEMBRANES

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Caffeine complex imprinted polymeric membranes are prepared using polypropylene as a support membrane, acrylamide as a functional monomer and ethylene glycol dimethacrylate as a crosslinker. The imprinted membranes are analyzed by FT-IR, thermogravimetric analysis(TGA) and scanning electron microscope (SEM) to determine the optimal reaction conditions, the structure of imprinted membranes is different from the blank membrane, the surface of the support membrane is covered with an imprinted polymer layer after polymerization. Scatchard analysis indicates that there are two binding sites in the molecularly imprinted membranes. The optimum polymerization condition is 0.2 mmol caffeine, 0.8 mmol acrylamide and 2 mmol ethylene glycol dimethacrylate in acetonitrile at 60 °C for 48 h.

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1. Introduction

Since Vlatakis [1] and Wulff respectively introduced the non-covalent method[2]and the covalent method, molecularly imprinted technology has been broadly used in various research fields, including sensors[3,4] drug separation[5-7], chromatographic separation[8,9] and biological receptors[10]. This technique requires functional monomers surrounding a template molecule in solvent, and the mixed liquor can be polymerized by thermal initiation or irradiation with UV light. As a result of the reaction, target molecule is surrounded by functional monomers, and a selected interaction is secured. Adding a crosslinker in the polymerization process provides the imprinted polymers with mechanical strength, stability and rigidity. The final extraction of the target molecule enables the imprinted sites to selectively bind the template. Molecularly Imprinted Polymer (MIP) has emerged as a promising application in many fields since it can be prepared with ease and stability.

Membrane separation can sensitively separate out a special target molecule from other molecules, and it also could be operated easily and continuously with less energy consumption. Thus, membrane separation has been rapidly developed in green chemistry during recently years. Integrating molecular imprinting technique (MIT) with membrane separation, molecularly imprinted membrane (MIM) could be easily prepared. And now MIM has already attracted the attention of many scientists because of the effective way in molecular separations[11-16] Sergeyeva[17] prepared Atrazine-imprinted membranes by UV-initiated polymerization. The

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water flux could reach 3045L/(m^2•h) due to an earlier onset of phase separation which facilitated the generation of large pores.

Caffeine is used in treating symptoms and apneas in premature infants and as a stimulant for brain to respond energetically and relieve fatigue. It is very important to ensure the quality of caffeine of the drink, thus, the separation of caffeine has attracted attention. A number of caffeine extracting methods have been developed, such as extraction microwave assisted extraction and ultrasonic assisted extraction. The disadvantages of these methods are low selectivity, inconvenience and inefficiency. MIM can be characterized by selective transport of caffeine and rejection of other molecules, which is easier and more efficient.

The aim of this study is to prepare a molecularly imprinted membrane for selective caffeine recognition. The membrane was prepared by using a polypropylene microporous membrane as the support, caffeine as the template, acrylamide (AM) as the functional monomer and ethylene glycol dimethacrylate (EGDMA) as the cross-linker. In this study, the adsorption properties of the imprinted membrane were investigated by UV-Vis spectrophotometry, the chemical structures were analyzed by FTIR spectrometry, morphologies were captured by SEM, and the thermal stability was characterized by TGA

2. Experimental

2.1 Materials

AM and caffeine were purchased from Sinopharm Chemical Reagent Co., Ltd (China), Polypropylene microporous membranes with a nominal pore size dn = 0.45 μm and a diameter of 25 mm were purchased from Haining Taoyuan medical Chemical Co., Ltd (China), EGDMA (98%) was purchased from Sigma–Aldrich (China) and was purified by distillation under vacuum. 2,2-Azobiisobutyronitrile (AIBN) was purchased from Shanghai No.4 Reagent & H.V Chemical Co., Ltd (China). Acetonitrile, methanol and acetic acid were purchased from Xilong Chemical Co., Ltd (China).

2.2 Instruments and apparatus

To measure the UV spectrum of caffeine in aqueous solution, a UV-5600 spectrometer (Shanghai Yuanxi Analysis Instrument Factory, China) was used. The features of surface of membranes were analyzed by an S-3400N SEM (Hitachi, Tokyo, Japan). FTIR spectra (4000–400 cm^{-1}) were recorded using an IS10 FTIR spectrometer (Nicolet, USA). TGA of the samples was performed on a STA 449 F3 analyzer (Netzsch, Germany) over a temperature range of 25–800 °C at 15 °C min^{-1} under N_2. X-ray diffraction (XRD) patterns were collected on a Rigaku D/max-2400 powder X-ray diffractometer (0.08° step/s).

2.3 Synthesis of the imprinted polymer

Place a circular polypropylene microporous membrane on a slide with a solution of AIBN (10 mg) in acetonitrile. And the membrane was dried under vacuum for further use. To a stirred solution of caffeine, the monomer (AM) and the crosslinker (EGDMA) in acetonitrile was degassed for 3 min with nitrogen. The pre-treated polypropylene microporous membrane was coated by the premix solution by the dropper and dried with nitrogen. After that the treated membrane was cover with a cover slide, sealed and heated at 60 °C for 48 h. The membrane was then treated with a methanol/acetate acid (1/9, v/v) solution at 90 °C for 48 h to remove caffeine and AIBN, and dried. A control membrane and a nonimprinted membrane (NIM) were also

prepared under the same conditions, except caffeine. The formulations prepared are described in Table 1.

Table 1 Formulation of the composite imprinted membranes

NO.	1	2	3	4	5
Caffeine (mmol)	0.08	0.14	0.20	0.26	0.32
AM(mmol)	0.8	0.8	0.8	0.8	0.8
AIBN(mg)	10	10	10	10	10
EGDMA(mmol)	4.0	4.0	4.0	4.0	4.0

NO.	6	7	8	9	10
Caffeine (mmol)	0.2	0.2	0.2	0.2	0.2
AM(mmol)	0.8	0.8	0.8	0.8	0.8
AIBN(mg)	5.0	8.0	1.0	12.0	15.0
EGDMA(mmol)	4.0	4.0	4.0	4.0	4.0

NO.	11	12	13	14	15
Caffeine (mmol)	0.2	0.2	0.2	0.2	0.2
AM(mmol)	0.8	0.8	0.8	0.8	0.8
AIBN(mg)	10	10	10	10	10
EGDMA(mmol)	1.5	3.0	4.0	5.0	6.5

2.4 Binding tests

Static binding experiments were conducted to evaluate the recognition property of the membranes toward the target molecule. Each binding experiment was a 50ml solution of caffeine in water for different initial concentrations at room temperature. The amount of caffeine retained by the membrane Q is calculated from Eq. (1):

$$Q = \frac{(C_1 - C_2)}{m} \times V \tag{1}$$

Where, C_1 is the concentration of caffeine in the feed solution, C_2 is the final concentration in the collected permeates, V is the volume and m is the mass of the dried membrane. Blank control membranes were exposed to the same procedure.

2.5 Swelling ratio of the membrane

The wet membrane was prepared by immersing the dry membrane in deionized water for 24 h at room temperature, centrifugation at 3000 rpm for 2 min and weighing. The swelling ratio (δ) of the membrane was calculated by Eq. (2) [18]:

$$\delta = \frac{V_1 - V_2}{V_1} \times 100\% = \frac{h_2 - h_1}{h_1} \times 100\% \tag{2}$$

Where V_1 and V_2 are the volumes of the dry and the wet membrane, respectively, h_1 and h_2 are the thickness of the dry and wet membrane. Due to area altered little, thickness of the membranes,

which was measured with a ruler or micrometer, could be used instead of the volumes of the dry and wet membrane to calculate δ.

3. Results and discussion

3.1. Swelling ratio

From all the data given in the Table 2, the average of swelling ratio was 5.24%, which showed that the membrane can be used in distilled water for a long duration. The structure of membrane was not changed, which indicates that the separation ability of the membrane is stable[19].

Table 2 Thickness and swelling of the imprinted membrane around immersion

NO.	1	2	3	4	5	6	7	8
h_1/mm	0.170	0.186	0.175	0.171	0.187	0.186	0.181	0.178
h_2/mm	0.179	0.195	0.185	0.180	0.198	0.195	0.191	0.187
δ(%)	5.29	4.84	5.71	5.26	5.88	4.84	5.52	5.06

NO.	9	10	11	12	13	14	15
h_1/mm	0.174	0.197	0.170	0.225	0.193	0.171	0.182
h_2/mm	0.183	0.208	0.178	0.238	0.186	0.180	0.192
δ(%)	5.17	5.58	4.71	5.78	4.49	5.18	5.26

3.2. Optimization of reaction conditions

To investigate the effect of reaction conditions on the binding capability of the molecularly imprinted membranes (MIMs) and obtain an optimum reaction condition, different amounts of caffeine, AIBN and EGDMA were used, and the specific binding capacities were measured. Table 3 shows that the binding capacity of the MIM increased to 0.2533μmol/g when the concentration of caffeine reaches 0.2 mmol. Increases in the caffeine concentration increased the binding capacity, while the amounts of AIBN, AM and EGDMA were unchanged. The reason is there is less content of caffeine, only a few caffeine could react with AM, the imprinted membranes had low binding capacity. And the amount of caffeine increased, the excess caffeine could not form complexes with AM, thus, the binding capacity declined. There was less content of AIBN, initiating less free radical, small part of caffeine could react with functional monomer, the imprinted membranes had low adsorption. And while increasing the content of AIBN, the binding capacity increased. That because there have excess AIBN and the functional monomer have already completed used up, the binding capacity declined. Table 4 shows that the binding capacity of the MIM increased to 0.2496 μmol/g as the amount of AIBN was increased to 0.0100 g. The binding capacity decreased as higher concentrations of AIBN were used, while the amounts of caffeine, AM and EGDMA were unchanged. Table 5 shows that the binding capacity of the MIM increased to 0.2589 μmol/g as the amount of EGDMA increasing 4.0 mmol, and the binding capacity decreased as the concentration of EGDMA was increased, while keeping the amounts of caffeine, AIBN and AM the same. As the amount of crosslinking increased, the degree of crosslinking of the imprinted membranes is enhanced which could maintain the spatial structure of cavities of imprinted polymers, and the polymers had specific recognition of the template. The degree of crosslinking is too high to remove the template. Thus, the maximum binding capacity of the MIM was obtained when the reaction condition was optimized to 0.2 mmol caffeine, 0.0100 g AIBN, 4.0 mmol EGDMA and 0.8mmol AM at 60 °C for 48 h.

Table 3 Effects of the proportion between Caffeine and AM on adsorption performance of the imprinted membranes

NO.	1	2	3	4	5
Caffeine(mmol)	0.08	0.14	0.2	0.26	0.32
Caffeine:AM	1:10	1:5.7	1:4	1:3.1	1:2.5
Quantity(μmol/g)	0.1327	0.1868	0.2533	0.2761	0.2803

Table 4 Effects of dosage of initiator reagent on adsorption performance of the imprinted membranes

NO.	6	7	8	9	10
AIBN(g)	0.0050	0.0080	0.0100	0.0120	0.0150
Quantity(μmol/g)	0.1483	0.1975	0.2496	0.1654	0.0853

Table 5 Effects of the proportion between AM and EGDMA on adsorption performance of the imprinted membranes

NO.	11	12	13	14	15
EGDMA(mmol)	1.5	3.0	4.0	5.0	6.5
AM:EGDMA	1:1.9	1:3.8	1:5.0	1:6.3	1:8.1
Quantity (μmol/g)	0.1135	0.2148	0.2589	0.2561	0.1948

3.3. Polymer preparation and characterization

To confirm the presence of the co-monomers in the synthesized copolymers, the FTIR spectra of the polypropylene microporous membrane and imprinted membrane were performed. As shown in Fig. 1(a), the absorption peaks at 2949, 2917, 1452, and 1375 cm^{-1} indicate polypropylene. Due to the –C=O stretching vibrations, a peak at 1715 cm^{-1} of the imprinted membrane does not present in the polypropylene's spectrum (Fig. 1b). The features around 1246 cm^{-1} indicate the stretching vibration of C–N, and the absorption peak of 1140cm^{-1} is assigned to the asymmetric stretching vibration of C–C of AM.

It is inferred that the surface of the Polypropylene microporous membrane has been covered by the imprinted polymer layer. It leads to the typical peaks of PP disappeared and the typical peaks of imprinted polymer appeared [20-21].

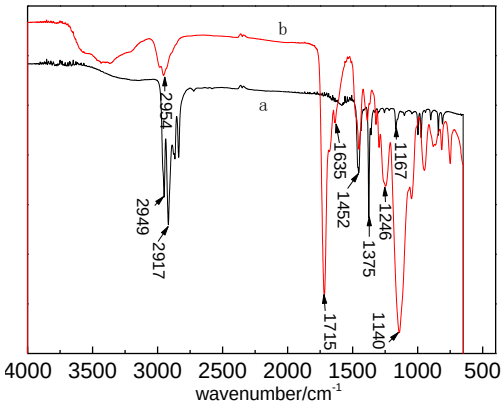

Fig. 1 FT-IR spectra of Polypropylene microporous membrane (a) and imprinted membrane(b)

Fig. 2(a) shows the asymmetric structure of the blank polypropylene microporous membrane. The structure of the MIM underwent a clear change following the polymerization reaction. Fig. 2(b) indicates that there is no pore of the surface of the NIM. Fig. 2(c) show that the number of pores in the support membrane decreased as the polymer layer was formed. These pores are different from those of the blank membrane[22].

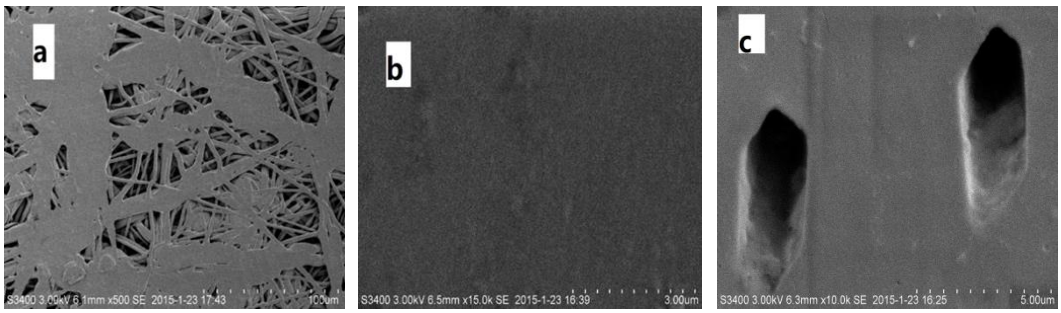

Fig. 2. SEM images of the membranes (a) Polypropylene microporous membrane 0.5k×, (b)the nonimprinted membrane 15k×, (c)the imprinted membrane 10k×

TGA[23] plots of MIM and NIM are shown in Fig. 3. There was a slight weight loss of the two samples at temperatures between 50 and 300 °C, due to the loss of water, acetonitrile, methanol and other volatile residuals. In the case of MIM, the large weight loss region between 300 and 400 °C indicates a faster weight loss rate of MIM compared with that of NIM, which is because of the number of pores on the surface of MIM and the open structure of MIM. Thus, the thermal stability of MIM is poorer than that of NIM. After 500 °C, all of the membranes are completely decomposed.

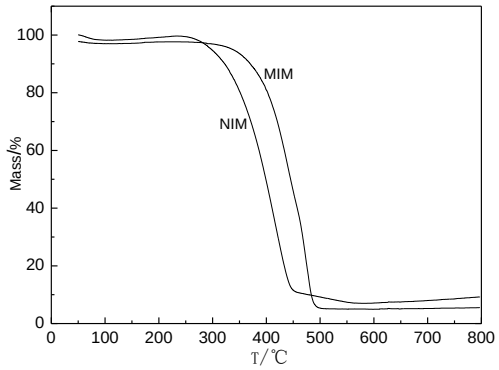

Fig. 3 Thermogravimetric curves of MIM and NIM

3.4 Binding isotherms

The binding performance of the imprinted membrane was studied using static binding tests and the Scatchard analysis.

$$\frac{Q}{C} = \frac{Q_{max} - Q}{K_d} \quad (3)$$

Where K_d is the equilibrium dissociation constant, Q_{max} is the maximum adsorption capacity, C is the final equilibrium concentration of caffeine in solution, Q is the adsorption capacity of caffeine adsorbed on the membrane at equilibrium.

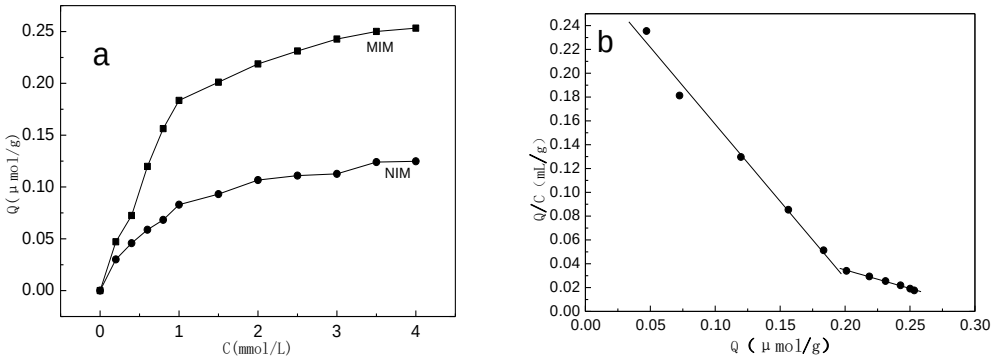

Fig. 4 Adsorption isotherms of MIM and NIM (a) and scatchard plots of MIM (b)

The binding isotherms plots of MIM and NIM in Fig. 4(a) show that MIM can adsorb much more caffeine than NIM. The adsorption data were plotted using the Scatchard equation and shown in Fig. 4(b), indicating that the binding sites in the MIM were heterogeneous. The data points are nonlinear between Q/C and Q. However, a straight line could be obtained for $0 \leq Q \leq 0.19$ μmol/g and $0.20 \leq Q \leq 0.26$ μmol/g. The linearized plots of the two straight lines were obtained. For $0 \leq Q \leq 0.19$ μmol/g, the equation of linear regression was $y = -1.2936x + 0.2865$, and the related coefficient was 0.9890. For $0.20 \leq Q \leq 0.26$ μmol/g, the equation of linear regression was $y=-0.3129x + 0.0975$, and the related coefficient was 0.9953. The first dissociation constant Kd1 of the high-affinity sites was 0.7730 mmol/L and the corresponding Qmax1 was 0.22 μmol/g. The dissociation constant, Kd2, of the low-affinity sites was 3.1959 mmol/L, and Qmax2 was 0.32 μmol/g. These results indicate that there are two binding sites in the MIM, and they showed different affinities for caffeine when the concentration was between 0 and 4.0 mmol/L. The reason because the imprinted caffeine has two binding sites[18].

4. Conclusions

Polypropylene microporous membrane substrate covered with a caffeine imprinted layer through polymerization of AM as a functional monomer and EGDMA as a crosslinker was successfully prepared. The optimum polymerization condition for the preparation is 0.2 mmol caffeine, 0.8 mmol AM and 2 mmol EGDMA in acetonitrile at 60 °C for 48 h. These show that the caffeine imprinted polymer membranes were simple to prepare.

The Scatchard model described the adsorption behavior of the imprinted complex membrane suitably, and suggests that two binding sites exist in the membrane, K_{d1}=0.7730 mmol/L, Q_{max1}= 0.22 μmol/g and K_{d2} =3.1959 mmol/L, Q_{max2} =0.32 μmol/g. All in all, the complex imprinted polymeric membranes can be easily synthetized with the method and effectively applied in separating caffeine, which opens a way to extract caffeine from caffeine methylating mother liquid and natural products.

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