POLY(STYRENE-*ALT*-MALEIC ANHYDRIDE) BASED ON MOLECULARLY IMPRINTED POLYMER MEMBRANE FOR SELECTIVE RECOGNITION OF CHLORAMPHENICOL

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TÓM TẮT

TỔNG HỢP MÀNG POLYME IN DẤU PHÂN TỬ DỰA TRÊN POLY(STYRENE-ALT-MALEIC ANHYDRIDE) ỨNG DỤNG NHẬN BIẾT CHỌN LỌC CHLORAMPHENICOL

Trong nghiên cứu này, một loại màng polyme in dấu phân tử (MIP) dựa trên poly(styrene-alt-maleic anhydride) (PSMA) được chế tạo ứng dụng nhận biết chọn lọc chloramphenicol (CAP). PSMA được tổng hợp đơn giản bằng phản ứng trùng hợp gốc tự do sử dụng chất khơi mào 2,2'-azobisisobutyronitrile trong môi trường khí nitơ ở 60 ^oC. Phản ứng tạo liên kết ngang nối mạng PSMA được thực hiện với sự hiện diện của tác nhân nối mạng và CAP như phân tử mẫu, tiếp theo sau bởi quá trình rửa giải để thu được màng MIP. Màng MIP được đánh giá thông qua các phương pháp phân tích FT-IR, ^1H NMR, và TGA trong khi khả năng bắt giữ CAP được khảo sát bằng phương pháp phân tích sắc ký lỏng hiệu năng cao HPLC. Màng MIP hứa hẹn tiềm năng trong lĩnh vực ứng dụng nhận biết và tách loại CAP.

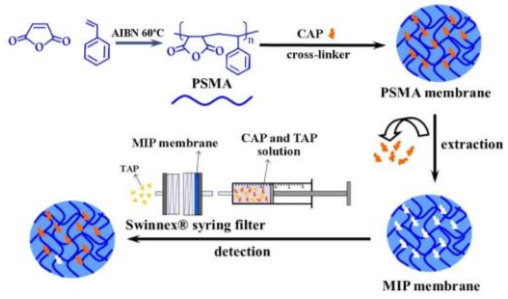
Từ khóa: *polyme in dấu phân tử; poly(styrene-alt-maleic anhydride); chloramphenicol; nhận biết phân tử; HPLC.*

1. INTRODUCTION

Chloramphenicol (CAP), an antibiotic, has been widely used for treatment of typhoid fever and some diseases in both human beings and animals [1]. The antimicrobial effectiveness of CAP is due to its binding capacity to mitochondrial ribosomes which inhibits bacterial protein synthesis [2]. However, it was banned by overuse of CAP for short period in food producing animals because of its toxicity [3]. The persistence of CAP in animals can be transmitted to humans via the food chain [4,5]. The accumulation of CAP in the human body is very dangerous which causes not only harmful side effects such as nausea, vomiting, unpleasant taste, diarrhea, bone marrow suppression, aplastic anemia, and leukemia but also toxic effects such as liver and kidney damage even at low concentrations [6]. Therefore, CAP detection at trace levels plays an important role during analysis procedure in food industry [7]. Obviously, it is quite essential to recognize CAP from biological samples and food products. In this light, CAP residues could be identified by photoelectrochemical [8,9], surface enhanced Raman spectroscopy (SERS) [10], amperometric biosensor [11], fluorescent sensor [12,13], gas chromatography–mass spectrometry (GC-MS) [14], and liquid chromatography–tandem mass spectrometric (LC-MS/MS) method [15]. However, a

significant obstacle limited utilizing of these methods is high cost with low sensitivity and selectivity. On this basis, molecularly imprinted polymer (MIP) has been proposed as a great solution to overcome the problems [16]. The advantages of the MIP are fast and easy preparation, low cost, effectiveness, and simple application. The MIP working principle was based on the selectivity of configuration and stereocenters [17]. The MIP is usually synthesized by the cross-linking polymerization of functional monomer in the presence of specific template which creates a cross-linked polymer matrix with template molecules inside. Then, these templates are removed from polymer matrix under extraction. Consequently, the template leaves complementary imprint which provides a three-dimensional shape and size of specific template [18]. As a result, the recombination of template into the MIP requires very high selectivity.

In this work, MIP membrane was prepared through poly(styrene-*alt*-maleic anhydride) (PMSA) for CAP identification. The PSMA copolymers were used as a polymer matrix and subsequently cross-linked in the presence of CAP templates (Scheme 1). The adsorption capacity and selectivity of MIP membrane were examined by high performance liquid chromatography (HPLC).



Scheme 1. Preparation of MIP membrane and CAP recognition

2. EXPERIMENTAL DETAILS

2.1. Materials

Chloramphenicol (CAP), thiamphenicol (TAP), styrene, and maleic anhydride were purchased from Mecrk. Poly(vinyl alcohol) (500 Da, 89% hydrolyzed), 1-pentanesulfonic acid sodium were purchased from Junsei (Japan). Acetonenitrile was supplied from Fisher. The other chemicals of analytical grade and solvents were used as received.

2.2. Preparation of PSMA copolymer

Poly(styrene-*alt*-maleic anhydride) (PSMA) (M_n 110 kDa, PDI 1.2) was prepared according to our previous work with modification [19]. Briefly, styrene (0.52 g, 50 mmol), maleic anhydride (0.49 g, 50 mmol), and 2,2'-azobisisobutyronitrile (15 mg, 0.09 mmol) were added to a round bottom flask containing 5.0 mL of 1,4-dioxane. The solution was purged with nitrogen for 30 min, the flask was put in a preheated oil bath at 60 ^oC for 3 h. The product was diluted by 8 mL of tetrahydrofuran and precipitated in 250 mL of cool diethyl ether. The final polymer was dried under vacuum at 40 ^oC until constant weight (yield~95%).

2.3. Preparation of MIP membrane

PSMA based MIP membrane was constructed by cross-linking reaction between poly(vinyl alcohol) and maleic anhydride group of PSMA in the presence of triethylamine as a catalyst. Firstly, poly(vinyl alcohol) (0.030 g) was heated and stirred in DMF (2.0 mL) homogeneously. Then, PSMA (0.2 g) was added to this mixture. Meanwhile, CAP (50 mg) and triethylamine (250 µL) were dissoved in 0.5 mL of DMF. This solution was slowly added dropwise to PSMA mixture. The mixture was stirred at 60 ^oC for 2 h. The mixed solution was cast into a petri dish and dried under room temperature for at least 24 h. The obtained membrane with thickness ranging from 150-200 µm was immersed into deionized water under stirring to remove whole CAP templates. The MIP membrane was drained and kept cool before use. Control membrane (non-MIP) was prepared by the same process without using CAP.

2.4. Binding experiment

MIP membrane was cut circular pieces with a diameter of 13 mm and placed into Swinnex® syringe filter holder. The mixture of

CAP and TAP in 1-pentane sulfonic acid sodium buffer solution was passed through the MIP membrane at rate of 0.1 mm/min. Filtrate was collected and acetonitrile was added to analyze by using HPLC spectroscopy. The CAP concentration was calculated from the calibration curve. The CAP bound to MIP was determined by subtracting the concentration of CAP filtrate from the initial CAP feed solution. For recovery test, the MIP membrane was washed with 5 mL of water following by extraction with 2x2.5 mL of pure methanol. The elution aliquots were collected, evaporated, and reconstituted in acetonitrile/buffer solution mixture for HPLC analyses. The same procedure was carried out for the non-MIP membrane. The limit of detection (LOD) was determined according to the 3-fold of the signal to noise ratio (*S/N*=3) [20].

2.5. Characterization

Fourier transform infrared (FT-IR) spectra were examined using a Bruker Tensor 27 (Germany) spectrometer in the 4000–500 cm^{-1} region. ^{1}H NMR spectroscopy were obtained on a JNM-ECP 400 (JEOL) equipment. Thermogravimetric analysis (TGA) was conducted with a Setaram Labsys Evo S60/58988 thermoanalyzer (France) within temperature range of 50-700 °C under nitrogen flow using heating rate of 10 °C/min. HPLC analysis was performed on HPLC Jasco LC-4000 system (Japan) with a UV L-4070 detector at wavelength of 278 nm and Apollo C18 column (250 mm x 4.6 mm, 5 µm) at 30 ^{o}C. The mobile phase was a mixture of acetonitrile:1-pentane sulfonic acid sodium (12 mmol/L) buffer solution (28/72, v/v) with a flow rate of 1.2 mL/min, injection volume of 5 µL.

3. RESULTS AND DISCUSSIONS

The FT-IR spectra confirmed the structure of the MIP membrane as shown in Fig. 1. The absorption peak at 703 cm^{-1} was ascribed to the aromatic ring vibration of polystyrene. After the reaction of maleic anhydride ring-opening, new absorption band of -OH groups at 3430 cm^{-1} and methylene groups at 2924 and 2865 cm^{-1} of poly(vinyl alcohol) were generated in the spectrum of MIP membrane (Fig. 1a). Additionally, the carbonyl stretching bands of maleic anhydride rings were still observed at 1855 and 1780 cm^{-1} indicating that MIP membrane contains unreacted anhydride groups, which is possibly extended to further ring-opening functionalization. The FT-IR spectrum of non-MIP membrane presented a similar absorption peaks comparing to MIP membrane (Fig. 1b) due to the same components. The results illustrated that the formation of MIP membrane was indeed successful by cross-linking reaction.

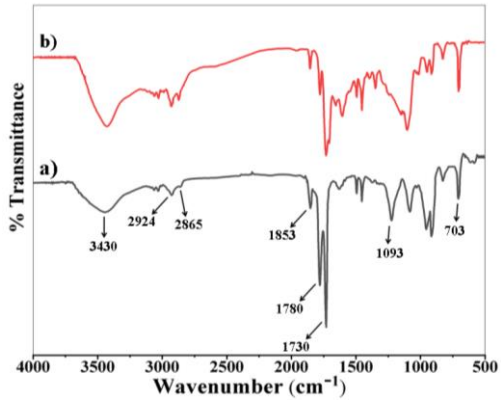


Figure 1. FT-IR spectra of MIP membrane (a) and non-MIP membrane (b)

The ^{1}H NMR spectroscopy was used as a common method to characterize the structures of polymers. In Fig. 2a, a broad peak at 7.23 ppm was associated to the protons of phenyl ring on the PSMA moieties while the peak at 2.1 ppm was due to methine protons of PSMA main chain copolymers. The methylene protons of poly(vinyl alcohol) were visualized at 1.44 ppm while the existence of hydroxyl group was confirmed by a typical peak at 3.98 ppm. It was found that the peak position of non-MIP polymer can be visualized in comparison to the presented MIP polymer (Fig. 2b). In other words, the CAP templates were absolutely removed by extraction from MIP membrane. The ^{1}H NMR results implied an accomplished preparation of MIP membrane.

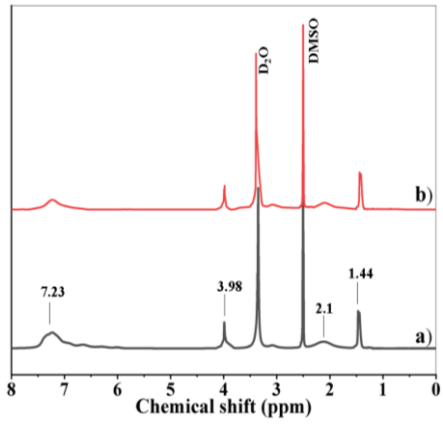


Figure 2. ^{1}H NMR spectra of MIP membrane (a) and non-MIP membrane (b) in DMSO-d_6

In order to evaluate the thermal properties of MIP membrane, TGA was used as shown in Fig. 3. The first derivative of the mass loss (DTG) indicated that the decomposition of the MIP polymer began at about 70 ^oC and it degraded substantially in the range 260–490 ^oC. The thermograms of non-MIP and MIP polymer showed a highly overlapped mass loss fraction demonstrating the same ingredient of them in terms of in the absence of CAP molecules on the MIP membrane.

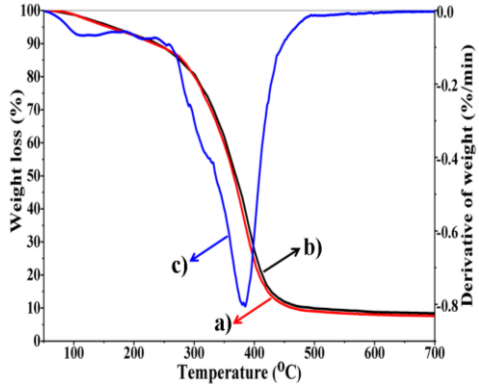


Figure 3. TGA curves of MIP (a), non-MIP membrane (b), and DTG curve of MIP membrane (c)

The recognition capacity and selectivity are important factors for the evaluation of MIP polymer. In this study, TAP, an antibiotic member of the CAP family, is used as a structurally similar molecule to examine the stereoselective adsorption. As shown in Fig. 4a, the MIP membrane presented CAP adsorption more remarkable than TAP. It can be explained by the fact that the MIP membrane contains specific cavities which is occupied only by CAP. On the other hand, CAP can be also absorbed to the nonspecific binding sites of non-MIP membrane with very low level (Fig. 4b). In addition, the LOD was estimated to be 3.59 µg/L (Table 1). The LOD value implied the designed membrane is suitable for recognition of CAP due to its high selective affinity. The recovery of CAP from MIP membrane is found using standard curve with average higher than 90% and relative standard deviation (RSD) of 1.19-1.32% (n=4) (Table 2). The results demonstrated the MIP materials can be utilized to determine whether the presence of CAP in real sample.

Table 1. Linear regression data and the limit of detection (LOD)

Linear range (µg/L)	Regression line			RSD (%)	LOD (µg/L) (*S/N*=3)
	Slope	Intercept	r (n=10)		
0.50 – 40.0	1513.4	2432.4	0.9987	1.17-2.36	3.59

Table 2. Recoveries of CAP samples

CAP (mg/L)	Recoveries (%)				Average recoveries (%)	RSD (%)
10	89.3	91.2	91.7	89.8	90.5	1.25
20	92.1	90.3	90.4	92.7	91.4	1.32
30	92	93.1	91.4	90.5	91.8	1.19

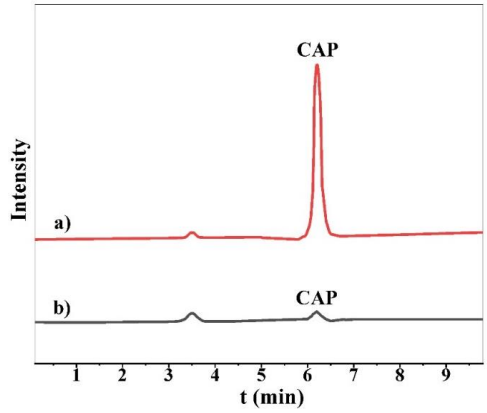


Figure 4. HPLC chromatograms of mixture of CAP and TAP solution extracted with MIP (a) and non-MIP membrane (b)

4. CONCLUSIONS

In conclusion, MIP membrane was constructed by crosslinking process between PSMA copolymer and poly(vinyl alcohol) for CAP determination. The MIP structure was characterized by ^{1}H NMR and FT-IR spectroscopy while component and thermal stability of MIP membrane was confirmed TGA. The adsorption capacity and selectivity tests indicated that the MIP membrane possessed a priority performance towards CAP as evaluated by HPLC analysis. The LOD of MIP membrane was 3.59 μg/L. The CAP recovery was higher than 90% with 1.19-1.32% RSD (n=4). The MIP membrane demonstrates a bright prospect for practical applications.

Acknowledgments

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