Asian Journal of Chemistry; Vol. 24, No. 3 (2012), 1175-1178

ASIAN JOURNAL OF CHEMISTRY

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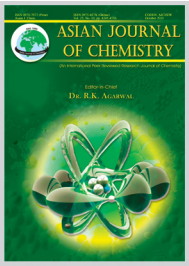

Selective Solid Phase Extraction of Aromatic Amines in Mainstream Tobacco Smoke Solution Using Molecularly Imprinted Polymer

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(*Received*: 28 February 2011; *Accepted*: 11 November 2011) AJC-10618

A new molecularly imprinted polymer (MIP) has been synthesized using 1-naphthylamine as the template molecule. Equilibrium adsorption and selectivity adsorption experiments showed that the molecularly imprinted polymer has better binding ability and higher selectivity for 1-naphthylamine, comparing to non-imprinted polymer (NIP). The molecularly imprinted solid phase extraction (MI-SPE) was prepared by using the molecularly imprinted polymer as sorbent and used to extract and pre-concentrate 1-naphthylamine, 2-naphthylamine, 3-aminobiphenyl and 4-aminobiphenyl from the mainstream tobacco smoke. Compared to non-imprinted solid phase extraction (NI-SPE), MI-SPE can effective to extract selectively the four Hoffmann aromatic amines from the mainstream tobacco smoke under the optimized conditions.

Key Words: Molecularly imprinted polymer, Solid phase extraction, Aromatic amines, Mainstream tobacco smoke.

INTRODUCTION

Aromatic amines (AAs) are toxic compounds, environmental pollutants and suspected human carcinogens. Recently, the four aromatic amines *i.e.*, 1-naphthylamine (1-NA), 2-naphthylamine (2-NA), 3-aminobiphenyl (3-ABP) and 4-aminobiphenyl (4-ABP), are included in Hoffmann lists as representative harmful ingredients of amines in cigarette smoke[1]. Therefore, cigarette smoke analysis for aromatic amines is continuing research focus. Several analytical techniques including GC, CE or HPLC are available for quantification of aromatic amines in various sample matrixes[2]. However, smoke is a very complex mixture in which Hoffmann aromatic amines are at trace levels. Consequently, a selective sample pre-treatment step to reduce the matrix interference and enrich the aromatic amines is highly desirable[3].

Solid phase extraction (SPE) is used to extract and pre-concentrate analytes of interest present at low levels of concentration[4]. In recent years, many new functionalized polymeric sorbents and highly cross-linked polymers have appeared as alternatives to conventional SPE materials in order to facilitate the trace enrichment of polar analytes[5]. Molecularly imprinted polymers (MIPs) are synthetic materials which can selectively recognize a target molecule or its related analogous compounds[6]. As molecularly imprinted polymers show good binding affinity and high selectivity for the template, they are being extensively investigated as highly selective SPE sorbents for the clean-up and the pre-concentration of samples[7,8].

In this study, the 1-naphthylamine molecularly imprinted polymer was synthesized and the binding affinity and selectivity of the molecularly imprinted polymer were studied using equilibrium adsorption experiments and Scatchard analysis. Then, the molecularly imprinted polymer was used as the sorbent of solid-phase extraction (MI-SPE) for the selective extraction and pre-concentration of four Hoffmann aromatic amines (2-naphthylamine, 1-naphthylamine, 4-aminobiphenyl and 3-aminobiphenyl) in mainstream tobacco smoke prior to HPLC. The results were compared to those obtained with non-imprinted polymer SPE cartridge (NI-SPE). To the best of our knowledge, this is the first time that 1-naphthylamine molecularly imprinted polymer has been used as the sorbent in SPE to extract selectively the four Hoffmann aromatic amines from mainstream tobacco smoke effectively.

EXPERIMENTAL

1-Naphthylamine (1NA, 99.5 %), 2-naphthylamine (2-NA, 99.0 %), 3-aminobiphenyl (3-ABP, 97.8 %), 4-aminobiphenyl (4-ABP, 98.5 %), 2,6-dimethylaniline (2,6-DMA, 99.0 %), aniline (AL, 99.5 %), 1-methylnaphthalene (1-MNP, 97.0 %) and 1,8-diaminonaphthalene (1, 8-DAN, 97.0 %) were purchased from Dr. Ehrenstorfer GmbH (Germany). Methacrylic acid (MAA) and ethylene glycoldimethacrylate (EGDMA) were

from Aldrich and were cleaned to remove the inhibitor prior to polymerization. Azobisisobutyronitrile (AIBN) was from Factory of Special Reagent of Nankai University (Tianjin, China). All other chemicals were of analytical grade and solvents were of HPLC quality. Ultrapure water used for sample preparation was obtained from a MILLI-R04 purification system, (Millipore, Germany).

Chromatographic evaluation was performed on an Agilent 1100 series high performance liquid chromatography (HPLC) with UV detection. Chromatographic separation was carried out with a ZORBAX Eclipse XDB-C_{18} column (150 mm × 4.6 mm i.d., particle size 5 μm). The mobile phase was MeOH/acetic amide solution (5 mmol/L, pH = 3) (30:70, v/v) and the detection was carried out at 210 nm. The column was thermostated at 30 ºC.

Synthesis, binding affinity and selectivity evaluations of 1-naphthylamine molecularly imprinted polymer: 1-Naphthylamine molecularly imprinted polymer and non-imprinted polymer were synthesized on non-covalent interaction according to our previous work[9]. Equilibrium adsorption experiments was used to evaluate the binding affinity of the molecularly imprinted polymer. The molecularly imprinted polymer particles (20.0 mg) were mixed with 2.0 mL MeOH solution of 1-naphthylamine at different concentrations ranged from 0.02-3.0 mmol/L. The mixture was incubated for 20 h at 20 ºC accompanied by continuous shaking with a horizontal shaker. Following centrifugation, the concentrations of free 1-naphthylamine were analyzed by HPLC. Meanwhile, Scatchard analysis was also used for evaluation of binding affinity[10].

The selectivity factor (a) was used to evaluate the selectivity of the molecularly imprinted polymer 11.20 mg molecularly imprinted polymer particles were mixed with 10 mL 1.5 mmol/L 1-naphthylamine and four structural analogs (Fig. 1). Then adsorption experiments were carried similarly. The value of a was calculated as follow: $\alpha = K_{Di}/K_{Dj}$ (i = j, $\alpha = 1$), where K_{Di} and K_{Dj} are the retention factors[11] of the 1-naphthylamine and structural analogs.

Sample preparation of mainstream tobacco smoke: Mainstream smoke was generated on a smoking machine under internationally agreed standard conditions. Total particulate matter of 20 cigarettes was collected on four Cambridge filter pads. The filter pads were extracted with 100 mL 15 % HCl in an ultrasonic bath for 0.5 h and the pH was change to neuter with 50 % NaOH. For recovery studies, smoking extraction was spiked with 20 μg/mL standard solutions of four Hoffmann aromatic amines (2-naphthylamine, 1-naphthylamine, 4-aminobiphenyl and 3-aminobiphenyl).

Extraction of target aromatic amines in tobacco smoke solution: SPE columns were prepared by packing 200 mg suspensions of the molecularly imprinted polymer and non-imprinted polymer polymers in MeOH into an empty PTFE SPE cartridge (3 mL cartridge from Supelco, Shanghai, China), respectively. PTFE frits (porosity 10 μm, Supelco) were placed above and below the sorbent bet.

Prior to sample application, the cartridge was conditioned with 1.0 mL of MeOH and 2.0 mL of water. A total of 2.0 mL of each sample was forced to pass through the MI-SPE cartridge at a flow rate of 1.0 mL/min by negative pressure. Then the cartridge was washed with 2 mL methanol-water 3:2 (v/v). The target analytes retained in the cartridge were eluted with 2 mL acetonitrile. All of the loading, washing and elution fractions were analyzed by HPLC. For comparison, NI-SPE was also tested in the same conditions.

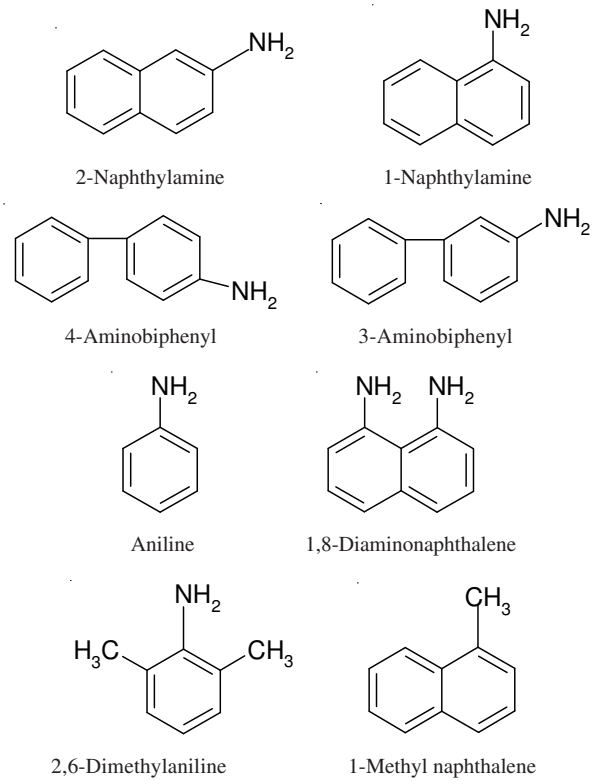

Fig. 1. Chemical structures of aromatic amines

RESULTS AND DISCUSSION

Affinity and selectivity of the 1-naphthylamine molecularly imprinted polymer: Equilibrium adsorption experiments and subsequent Scatchard analysis showed the 1-naphthylamine molecularly imprinted polymer had better imprinting capacity and one distinct group with specific binding property. The calculated dissociation constant (K_d) was 7.85×10^{-4} mol/g and the apparent maximum number of binding sites (B_{max}) was 93.4 μmol/g.

Selectivity adsorption experiments among structural analogs were used for the evaluation of selectivity of the molecularly imprinted polymer. The results (Table-1) showed that, for the molecularly imprinted polymer, the K_D of 1-naphthylamine is 2.71, which is significantly higher than the four structural analogs. The α value for aniline, 1,8-diaminonaphthalene, 2,6-dimethylaniline and 1-methylnaphthalene are 2.36, 2.46, 2.63 and 2.71, respectively. However, for the non-imprinted polymer, the K_D of the four structural analogs are very similar and the α-value is nearly 1.0. The absorption amounts of 1-naphthylamine are significantly higher than that of other structural analogs while the adsorption capacities of different substrates on the non-imprinted polymer are very close. It is because that the molecularly imprinted polymer has a specific adsorption to 1-naphthylamine as it possesses molecular recognition sites designed for 1-naphthylamine which are absent in non-imprinted polymer. Thus, it can be obtained that

TABLE-1
SELECTIVITY OF 1-NAPHTHYLAMINE IMPRINTED POLYMER

Sorbents	1-Naphthylamine		Aniline		1,8-Diaminonaphthylaniline		2,6-Dimethylaniline		1-Methylnaphthalene	
	K_D	α	K_D	α	K_D	α	K_D	α	K_D	α
MIP	2.71	1.00	1.15	2.36	1.01	2.46	1.03	2.63	1.00	2.71
NIP	1.03	1.00	1.33	0.91	1.04	0.99	1.00	1.03	1.08	0.95

the molecularly imprinted polymer is a kind of potential SPE separation material which can be taken forward and applied to selectively extract the target aromatic amines including 1-naphthylamine from mainstream tobacco smoke.

Conditions optimization of the SPE process: The different concentrations of MeOH aqueous solutions were tested as washing solvent when a standard solution of seven aromatic amines was applied to the MI-SPE cartridge. The outflows were collected and analyzed by HPLC. The results showed that when using 2 mL of 60 % MeOH aqueous solution as the washing solvent, the analytes non-specifically loaded on the MI-SPE cartridge were completely removed, while most of the specific binding of analytes were still retained. And the recoveries of the four specific binding of aromatic amines were higher than 86 %, so 2 mL of 60 % MeOH in water was selected as the washing solvent for all further experiments.

For optimizing the conditions of the elution step, the cartridge was eluted with 2 mL of either water, chloroform, dichloromethane, acetonitrile or MeOH. It can be seen that almost all of the seven aromatic amines were removed only when using acetonitrile or MeOH. Because the recoveries of the seven aromatic amines using acetonitrile were higher than that using MeOH. The acetonitrile was choosed as the elute solvent.

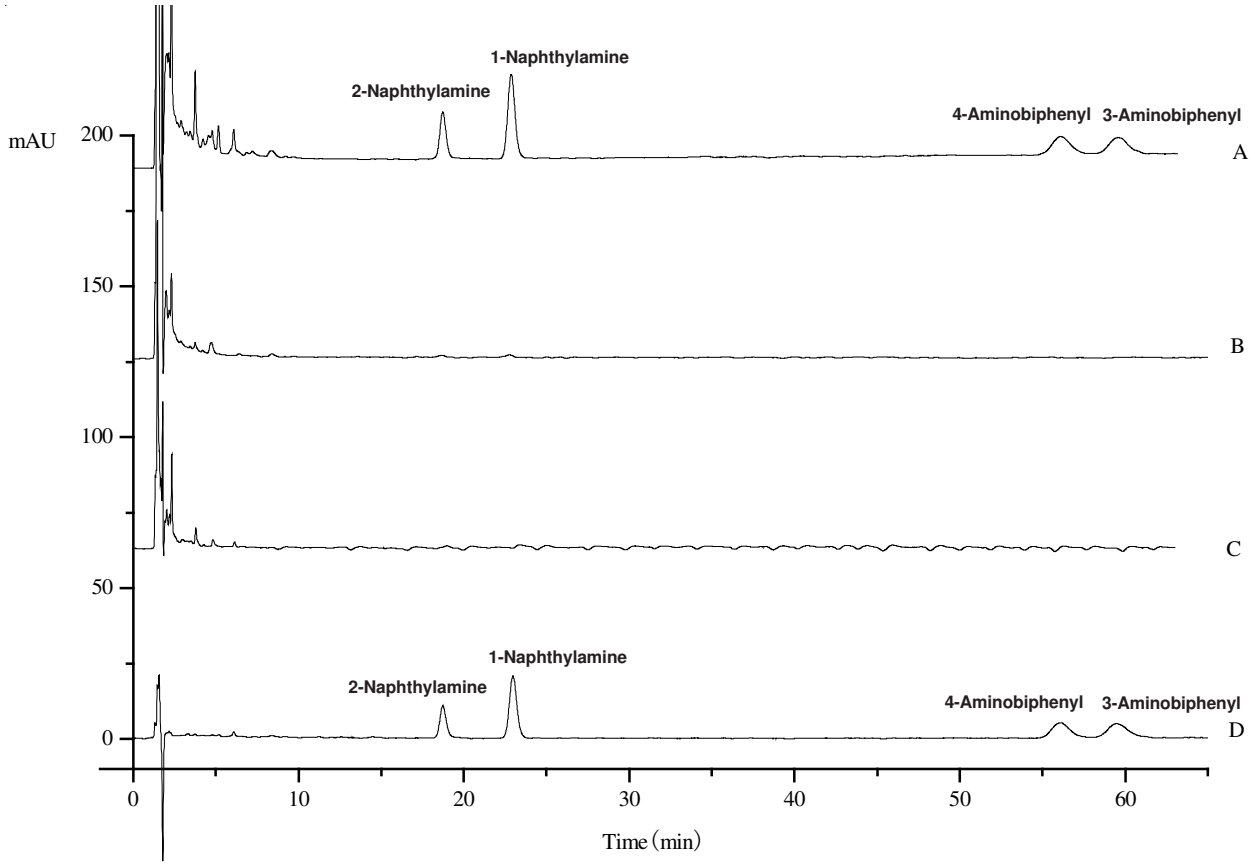

Fig. 2. HPLC chromatograms of the extraction from mainstream smoke spiked with 20 μg/mL 4-aromaticamines standard solution (A) and the outflows at loading step (B), washing step (C) and elution step (D) of MI-SPE process

TABLE-2
RECOVERIES OF THE FOUR AROMATIC AMINES ON MI-SPE AND NI-SPE AT LOADING, WASHING AND ELUTION STEPS

Compounds	Recovery[a] (%)					
	MI-SPE			NI-SPE		
	Loading	Washing	Elution	Loading	Washing	Elution
2-Naphthylamine	n.d.[b]	13.9 ± 0.6	85.2 ± 1.3	n.d.	82.6 ± 1.1	5.5 ± 0.4
1- Naphthylamine	n.d.	n.d.	89.6 ± 2.3	n.d.	85.2 ± 1.9	4.1 ± 0.3
4-Aminobiphenyl	n.d.	n.d.	91.6 ± 4.1	n.d.	87.0 ± 2.2	n.d.
3- Aminobiphenyl	n.d.	n.d.	94.1 ± 4.7	n.d.	90.2 ± 3.6	n.d.

[a]Average of three measurements. [b]Not detectable.

Effection evaluation of MI-SPE for aromatic amines: Fig. 2 showed the HPLC chromatograms of the extraction from mainstream smoke spiked with 20 μg/mL four target aromatic amines standard solution (Fig. 2A) and the outflows at different steps of SPE process. It was clearly demonstrated that there was no breakthrough in loading step (Fig. 2B). Then after the washing step (Fig. 2C), almost all of the sample matrixes were removed, while the target aromatic amines were still totally retained on the MI-SPE cartridge. Finally after the elution step (Fig. 2D), all of the four aromatic amines were recovered effectively. It indicates that MI-SPE is effective to extract selectively the four Hoffmann aromatic amines from mainstream tobacco smoke.

The intuitionistic results (Table-2) showed that the target aromatic amines were totally retained on the MI-SPE cartridge after washing and could be quantitatively eluted using acetonitrile. In contrast, it was clear that unknown compounds can not be separated from aromatic amines by NI-SPE without an additional cleanup step while the MI-SPE cartridge proved to be effective to separate and enrich selectively the four aromatic amines from mainstream tobacco smoke extract. It can be explained that the mechanism of NI-SPE sobents is based on non-specific adsorption[12] but the MI-SPE sobents is based on specific adsorption.

Conclusion

In this work, a new molecularly imprinted polymer using 1-naphthylamine as the template molecule was prepared. The molecularly imprinted polymer showed excellent affinity and high selectivity to 1-naphthylamine and was therefore suitable for the application in SPE. The developed MI-SPE proved to be a powerful tool for the selective extraction of the four Hoffmann aromatic amines (2-naphthylamine, 1-naphthylamine, 4-aminobiphenyl and 3-aminobiphenyl) from mainstream tobacco smoke sample. Its low cost of preparation showed the protocol as a new methodology for enriching the trace aromatic amines from mainstream tobacco smoke sample selectively and efficiently.

ACKNOWLEDGEMENTS

The authors gratefully acknowledged the financial support from Ministry of Education of Anhui Province, P.R. China (Project No. KJ2010A350).

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