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Synthesis of a Novel Composite Imprinted Material Based on Multiwalled Carbon Nanotubes as a Selective Melamine Absorbent

Huabin Zhang,[†] Zhaohui Zhang,*[,†,‡] Yufang Hu,[†] Xiao Yang,[†] and Shouzhuo Yao[‡]

[†]College of Chemistry and Chemical Engineering, Jishou University, Jishou 416000, People's Republic of China

[‡]State Key Laboratory of Chemo/Biosensing and Chemometrics, Hunan University, Changsha 410082, People's Republic of China

ABSTRACT: A novel composite imprinted material, on the basis of a multiwalled carbon nanotube (CNT)-incorporated layer using melamine as a template, methacrylic acid as a functional monomer, and ethylene glycol dimethacrylate as a cross-linker, was synthesized by a surface imprinting technique. The imprinted/CNT sorbent was characterized by a scanning electron microscope (SEM). Adsorption dynamics and a Scatchard adsorption model were employed to evaluate the adsorption process. The results showed that the imprinted/CNT sorbent displayed a rapid dynamic adsorption and a high adsorption capacity of 79.9 μmol g^{-1} toward melamine. Applied as a sorbent, the imprinted/CNT sorbent was used for the determination of melamine in a real sample by online solid-phase extraction−high-performance liquid chromatography (SPE-HPLC). An enrichment ratio of 563-fold, detection limit (S/N = 3) of 0.3 μg L^{-1}, and quantification limit of 4.5 μg L^{-1} were achieved.

KEYWORDS: Molecular imprinting, multiwalled carbon nanotubes, melamine, online solid-phase extraction, high-performance liquid chromatography

INTRODUCTION

Melamine (MEL) is usually used to produce MEL−formaldehyde resin. In plastics manufacturing, MEL−formaldehyde resins are necessary for making surface coatings, laminates, adhesives, and flame retardants.[1] However, unscrupulous traders added MEL to milk or other dairy products to increase the amount of nitrogen, which will falsely show higher determinations of proteins. Infants or young children ingested the problematic dairy products, resulting in an increased incidence of renal failure in infants. Thus, MEL was not allowed as an additive in food or related ingredients.[2] A few previous works were reported on the determination of MEL in real samples by gas chromatography (GC),[3] gas chromatography/mass spectrometry (GC/MS),[4,5] high-performance liquid chromatography/tandem mass spectrometry (HPLC−MS/MS),[6,7] high-performance liquid chromatography with UV detection (HPLC−UV),[8−11] etc. The method based on HPLC−UV is the universal method of the quantitative determination of MEL. However, these determinations of MEL are unsatisfactory because of the lower MEL concentration in the real sample.

Solid-phase extraction (SPE) is an extraction method used to isolate one or one type of analyte from a solution. Owing to enrichment of SPE in sample preparation, it could be used to determine MEL to offset the problem of low content in different matrix samples. However, the sample pretreatment methods based on a conventional SPE technique show a lack of selectivity, resulting in a large amount of matrix interferences being extracted simultaneously,[12] and thus, decreases in the SPE separation and enrichment efficiency. Using molecularly imprinted polymers (MIPs) as SPE sorbent to separate and enrich MEL could solve this problem. MIPs are a kind of artificially synthesized macromolecular material, which have prearrangement of structure and specific molecular recognition abilxity. In the process of molecular imprinting, appropriate functional monomers are introduced to interact with template molecules, and then the functional groups on the monomers are fixed with chemical cross-linkers.[13] Removal of the template molecules leaves predetermined arrangement of ligands and a tailored binding pocket.[14] Therefore, MIPs show higher affinity for the template molecules over other structurally related compounds.[15]

An efficient imprinted adsorbent should consist of a stable and insoluble porous matrix. Multiwalled carbon nanotubes (CNTs) are ideal support materials because of their strong interactions, stability under acidic conditions, lack of swelling, and large surface area.[16] Up to now, CNTs have attracted great attention.[17] CNT-incorporated layer can be prepared in many ways. Immobilization of the vinyl group onto the surface of CNTs reported by Kan et al.[18] was one of the incorporated methods. This effectiveness combination between CNTs and MIPs offers an attractive route for the wide application of MIPs. The effective use of CNTs strongly depends upon the modification of CNTs. This study based on combining CNTs and MIPs is a unique idea to develop the application of the CNT-incorporated layer in the SPE field.

In this work, the poly(acrylic-acid)-functionalized CNTs were synthesized to increase the diameter of CNTs. Then, the vinyl group was introduced to the surface of poly(acrylic-acid)-functionalized CNTs by an amidation. Using MEL as a template molecule, a novel imprinted CNT composite material was fabricated by a thermal polymerization. Applied as a sorbent, the imprinted materials were used for the determination of MEL in the spiked sample by online SPE combined with HPLC.

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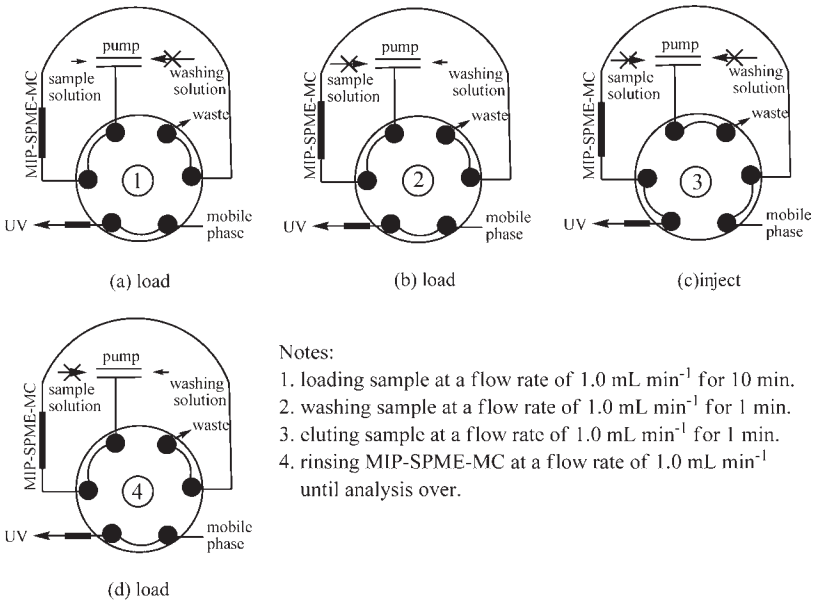


Figure 1. Schematic diagram of the online SPME preconcentration coupled with HPLC.

EXPERIMENTAL SECTION

Materials and Reagents. All of the chemicals used were in analytical grade. Distilled water was used throughout this work. MEL, cyromazine (CYR), and cyanuric acid (CYA) were obtained from Chem Service (West Chester, PA). Multiwalled CNTs (95%, with diameters ranging from 20 to 40 nm and lengths ranging from 500 nm to 50 μm) were obtained from Shenzhen Carbon Nanotechnologies Co., Ltd. (China). Methacrylic acid (MAA), ethyleneglycol dimethacrylate (EGDMA), and acrylamide were purchased from Sigma-Aldrich (St. Louis, MO). Ethoxyethanol, sodium hydroxide, chloroform, 2,2′-azobisisobutyronitrile (AIBN), methanol, ethanol (HPLC grade), acetic acid, ammonium persulfate [$(NH_4)_2S_2O_8$], thionyl chloride ($SOCl_2$), and *N*,*N*′-dimethylformamide (DMF) were obtained from Changsha Chemical Reagent Company (Hunan, China). Ion-pair reagent buffer consisted of 10 mmol L^{-1} 1-octanesulfonic acid sodium salt monohydrate and 10 mmol L^{-1} citric acid (pH 3.0).

Pretreatment of CNTs. A typical free-radical reaction using AIBN as a radical initiator was adopted to obtain carboxylic-acid-functionalized CNTs (CNTs/COOH). Briefly, crude CNTs (0.5 g) and AIBN (0.1 g) were dispersed in 50 mL of toluene under sonication for 10 min. Then, the mixture was stirred continuously at 75 °C for 4 h under the protection of nitrogen. Cooled to room temperature, the mixture was filtered through a 0.22 μm polycarbonate membrane and washed thoroughly with toluene 4 times. The filtered solid was dried under vacuum, obtaining AIBN-modified CNT black solid. The dried black solid (0.5 g) was dispersed in 50 mL of NaOH methanol solution (10 mol L^{-1}) at 60 °C for 48 h under reflux. The resulting solid was collected by filtration and washed repeatedly with 6 mol L^{-1} HCl several times until the pH value of the filtrate was 3.0. Finally, the solid was washed thoroughly with distilled water and dried under vacuum at 70 °C for 8 h to obtain carboxylic-acid-functionalized CNTs (CNTs/COOH).

Synthesis of Vinyl-Group-Functionalized CNTs. The poly(acrylic-acid)-functionalized CNTs were synthesized with a thermal polymerization under $(NH_4)_2S_2O_8$ chain initiation in water, which was described by Shen et al.[19] Vinyl-group-functionalized CNTs were prepared by an amidation reaction of an acid chloride in poly(acrylic-acid)-functionalized CNTs and acrylamide. Briefly, the poly(acrylic-acid)-functionalized CNTs (0.5 g) were suspended in 30 mL of $SOCl_2$ at 60 °C for 24 h under reflux. The mixture was distilled to remove the excess $SOCl_2$. Acrylamide (2.0 g) and DMF (50 mL) were added to the distilled residue. The mixture suspension was stirred at 45 °C for 24 h. Then, the mixture was collected by centrifugation and washed with DMF. Finally, the product was dried overnight in a vacuum desiccator to obtain vinyl-group-functionalized CNTs (CNTs/V).

Preparation of Imprinted/CNT Sorbent. MEL (51.4 mg) and CNTs/V (200 mg) were added to a 100 mL round flask containing 50 mL of mixed solvent ($V_{chloroform}/V_{methanol}$ = 3:2). The mixture was incubated for 1 h at room temperature, resulting in hydrogen bond binding between the amido of the template and the carbonyl group of CNTs/V. Then, 142.5 mg of MAA and 1564.3 mg of EGDMA were added to this mixture, which was incubated 3 h under shaking for prepolymerization. Purged with nitrogen to remove oxygen, the polymerization was initiated by the addition of 30 mg of AIBN. The reaction was allowed to process at 60 °C for 24 h. The resulting products were collected by centrifugation and washed thoroughly with ethanol to discard the reagents. Then, the composites were eluted by the mixing solvent of methanol and acetic acid (9:1, v/v) 3 times to extract the template. The obtained polymers were finally rinsed with distilled water to remove the remaining acetic acid and dried in a vacuum desiccator for 24 h before use.

For comparison, non-imprinted co-polymers (non-imprinted/CNT sorbent) were prepared by the same procedure, only without MEL in the polymerization process.

Adsorption Experiment. In the experiment, 10 mg of sorbent was dispersed in 10 mL of MEL methanol solution. The mixture was agitated in a shaken bed. At different time intervals, the mixture was centrifuged and the supernatant solutions were collected. The concentration of MEL was detected using an ultraviolet−visible (UV−vis) spectrophotometer. The amount of MEL adsorbed by the sorbent (B, μmol L^{-1}) was detected according to the following formula:

$$B = (C_i - C_f)V/m$$

where C_i (mmol L^{-1}) and C_f (mmol L^{-1}) are the initial and final MEL solution concentrations, respectively. V (mL) is the sample volume, and m (g) is the mass of the sorbent. The adsorption isotherm test for the imprinted sorbent was performed in the same method. The test was carried out in triplicate.

Chromatographic Measurement. Various lengths of imprinted solid-phase microextraction (SPME) microcolumns (MIP-SPME-MC, 3.0 mm inner diameter) packed with a certain amount of

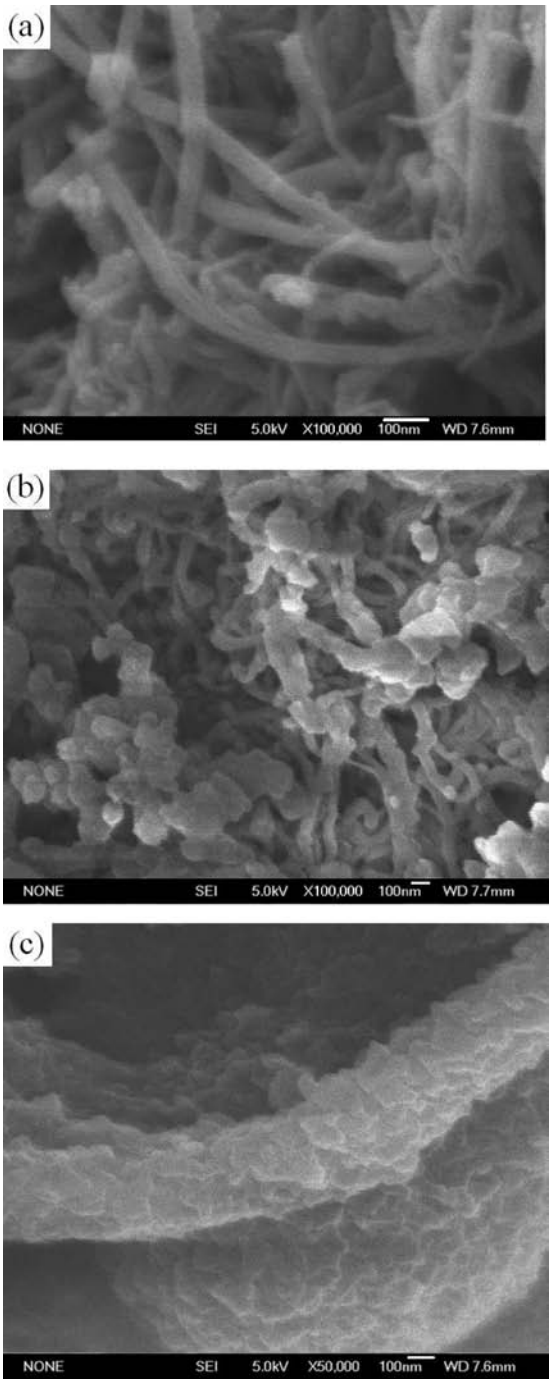


Figure 2. SEM images of the (a) original CNTs, (b) poly(acrylic-acid)-functionalized CNTs, and (c) imprinted/CNT sorbent.

the imprinted/CNT sorbent were prepared. The MIP-SPME-MC coupled with online SPME−HPLC determination of MEL was conducted to evaluate the applicability of the imprinted/CNT sorbent. The configuration of online SPME preconcentration coupled with HPLC for determination of MEL was described in Figure 1. The analytical system was performed on a LC-10AD HPLC system (Shimadzu, Japan) equipped with two LC-10ATvp HPLC pumps, a SPD-10A UV−vis detector, a N2000 chromatographic workstation (Intelligent Information Engineering Institute of Zhejiang University), and a constant flow pump. All separations were conducted on an analytical reversed-phase Shimadzu VP-ODS C_{18} column (4.6 × 150 mm) in a CTO-10A temperature controller. The mobile phase was a mixture of ion-pair reagent buffer/acetonitrile (9:1, v/v), and its flow rate was set at 1.0 mL/min. The UV−vis detector was operated at 240 nm.

The MIP-SPME-MC was pretreated with methanol and acetic acid (9:1, v/v) mixing solution before use. As shown in Figure 1a, the extraction was performed by passing 10 mL of MEL sample solution through the MIP-SPME-MC using the constant flow pump when the six-port injector valve was set to the "load" position. Simultaneously, the mobile phase was directly driven by the HPLC pump through the analytical column to obtain a flat baseline. After sample loading, the MIP-SPME-MC was washed with 5% ethanol aqueous solution at a flow rate of 1.0 mL min^{-1} for 1.0 min (Figure 1b). When the washing step finished, the injector valve switched to the "inject" position rapidly, as shown in Figure 2c. The adsorbed MEL was eluted in the back flush mode by the HPLC mobile phase at a flow rate of 1.0 mL min^{-1} for 1.0 min. Afterward, the injector valve was switched to the "load" position to prepare for the next analysis (Figure 1d). At the same time, the MIP-SPME-MC was rinsed by 5% ethanol aqueous solution uninterruptedly.

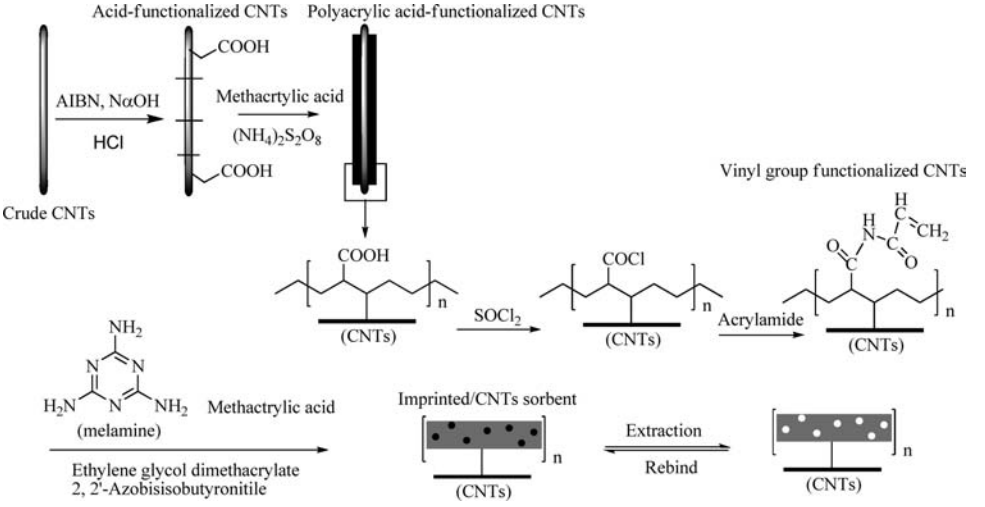
SPME for Spiked Animal Feed Material and Milk Powder. A 5 g portion of blank animal feed material or blank milk (liquid or powder) was added to 100 mL of 5% ethanol aqueous solution. After vortexing for 1.0 min, the resulting suspension was filtered directly through a 0.22 μm filter film. All of the filtered fluid and 1.0 mL of 0.1 mg L^{-1} MEL solution were transferred to a 100 mL volumetric flask, obtaining 1.0 μg L^{-1} MEL sample solution. An amount of 10.0 mL of the prepared spiked sample passed through the MIP-SPME-MC for online SPME−HPLC determination. The recovery and performance of clean-up were evaluated. The samples were analyzed in triplicate.

RESULTS AND DISCUSSION

Preparation of Imprinted/CNT Sorbent. Because of its tensile strength, chemical stability, ultra-small size, and poor solubility, CNTs were selected as attractive structural material for the development of novel analytical devices.[20] However, the ideal π-electron-conjugated structure of original CNTs on the surface had been slightly damaged after the carboxyl-functionalized process.[18] Two common methods have been employed to offset this defect. One is layer-by-layer (LBL) self-assembly, which is a powerful and versatile means for assembling supramolecular structures.[19] Thus, the diameter of CNTs can be obtained from 20 nm to more than 100 nm, which is suitable for an absorbent matrix material. The other is to avoid the destruction of its structure in the carboxyl-functionalized process and the further repair of the conjugate structure after the carboxyl-functionalized process.[21] In this paper, the preparation of the novel imprinted/CNT sorbent was conducted combining with two methods, and the procedures were described in Scheme 1. AIBN was used for the carboxyl function of CNTs because of its strong catalytic action and low oxidizing ability, which prevents it from cutting off CNTs and damaging the structure of the CNT wall. The effect of the mass ratio of AIBN and CNTs was investigated. The results showed, when the mass ratio increased from 0.2 to 1, with the reflux temperature fixed at 75 °C, the percent grafting of CNTs/COOH did not change significantly.

A scanning electron microscope (SEM) was employed to capture the detailed morphology of the original CNTs, poly(acrylic-acid)-functionalized CNTs, and imprinted/CNT sorbent. The results were shown in Figure 2. Random, curled structure and cottonlike features were shown in original CNT images (Figure 2a). The diameter of the wall and length of the original CNTs were about 20 nm and several micrometers, respectively. Functionalized with poly(acrylic acid), the CNTs were coated

Scheme 1. Schematic Representation of the Preparation of the Imprinted/CNT Sorbent



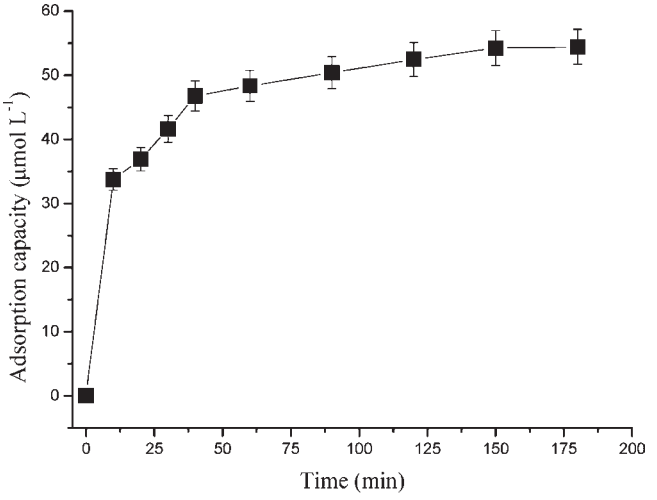


Figure 3. Adsorption dynamics of MEL on the imprinted/CNT sorbent. Experimental conditions: *V*, 10 mL; *C*, 1.0 mmol L^{-1}; and mass of sorbent, 10 mg.

drastically with homogeneous polymer (Figure 2b). The diameter of the poly(acrylic-acid)-functionalized CNTs was 90−100 nm. When the imprinted polymers were introduced to the CNT surface, it exhibited a distinctly thick, rough surface and many micropores distributed on the support (Figure 2c).

Adsorption Performance of the Imprinted Sorbent. The adsorption dynamics of the imprinted/CNT sorbent toward 1.0 mmol L^{-1} MEL was investigated by changing the adsorption time from 0 to 180 min. The fitted curve of the dynamic adsorption was presented in Figure 3. The adsorption capacity increased rapidly in the early 10 min, and then the rate of adsorption increased slowly until obtaining equilibrium adsorption. Because of the large number of imprinted cavities existing on the imprinted sorbent surface, the template molecules (MEL) reach the specific binding sites easily at the early time.[22] When the imprinted cavities were filled up, the rate of adsorption dropped significantly and the adsorption process achieved equilibrium.

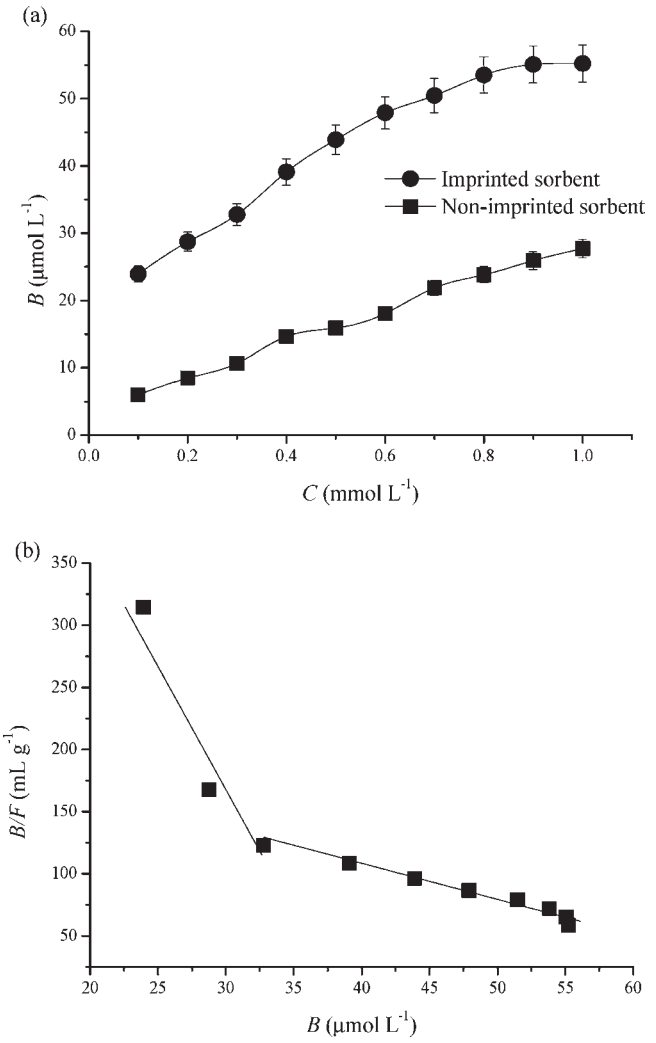


Figure 4. (a) Adsorption isotherms of MEL on the imprinted/CNT sorbent or non-imprinted/CNT sorbent and (b) its Scatchard plots. Experimental conditions: *V*, 10 mL; mass of sorbent, 10 mg; and adsorption time, 12 h.

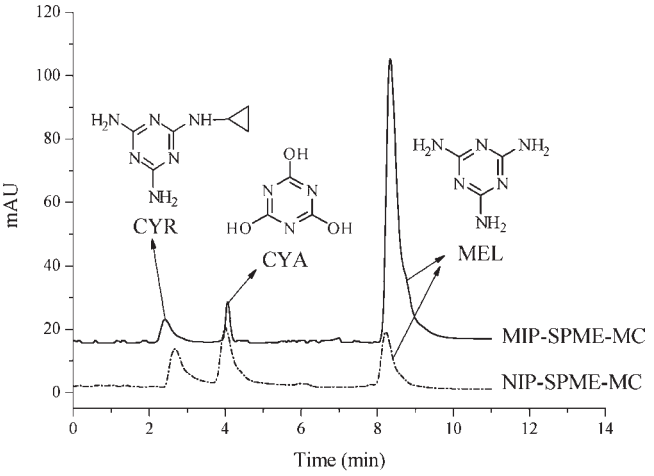


Figure 5. Chromatograms of MEL, CYR, or CYA on MIP-SPME-MC and NIP-SPME-MC. Experimental conditions: sample concentration, 1.0 μg L^{-1}; loading sample volume, 10 mL; loading flow rate, 1.0 mL min^{-1}; analytical reversed-phase Shimadzu VP-ODS C18 column, 150 × 4.6 mm inner diameter; column temperature, 30 °C; detection wavelength, 240 nm; mobile phase ion-pair reagent buffer/acetonitrile, 9:1 (v/v); and flow rate, 1.0 mL min^{-1}.

Table 1. Imprinting Factor (α) and Selectivity Factor (β) of the Sorbents

target	R_{MIP} (%)	R_{NIP} (%)	α	β
MEL	91.3	14.2	6.41	
CYR	13.8	18.2	0.76	8.43
CYA	12.4	19.4	0.64	10.02

As a novel composite imprinted material, the key property of the imprinted/CNT sorbent is special adsorption capacity. The adsorption isotherm experiment for the imprinted/CNT sorbent was carried out in MEL concentration range of 0.1−1.0 mmol L^{-1}. The results were shown in Figure 4a. The imprinted/CNT sorbent exhibited a good imprinting effect for the template (MEL). In the low concentration of MEL (0.1−0.8 mmol L^{-1}), the amount of MEL was not enough to saturate the specific binding cavities and the adsorption capacity of the MEL concentration of 0.8 mmol L^{-1} was about 53.5 μmol g^{-1}. However, with the MEL concentration increasing, almost all of the specific imprinted sites were occupied by MEL and the adsorption capacity of the sorbent with the highest adsorption capacity was about 55.2 μmol g^{-1}.

The statistical data of the adsorption isotherm experiment was further processed with the Scatchard equation[23] to estimate the binding parameters of the imprinted/CNT sorbent. Scatchard plots were constructed by plotting the ratio of the bound amount (B) to free MEL concentration (F) against the bound amount (B), and the equation is as follows:

$$B/F = (B_{max} - B)/K_d$$

where K_d is the equilibrium dissociation constant and B_{max} is the apparent maximum amount. As shown in Figure 4b, two typical straight lines were fitted according to the Scatchard equation. It implied that two kinds of non-equivalent binding sites were formed. The equilibrium dissociation constant K_{d1} and the apparent maximum amount B_{max1} for the higher affinity binding sites can be calculated to be 0.045 μmol L^{-1} and 37.3 μmol g^{-1}, respectively. By the same treatment, K_{d2} and B_{max2} for the lower affinity binding sites were calculated to be 0.38 μmol L^{-1} and 79.9 μmol g^{-1}, respectively.

Selectivity. Selective recognition toward the template molecule, which is based on the imprinted cavities in complement to the size, shape, and functionality of the template molecule, is an important property for a novel imprinted material. An amount of 200 mg of imprinted/CNT sorbent was packed into MIP-SPME-MC (100 mm length and 3 mm inner diameter). The selective extraction of MEL by the MIP-SPME-MC for online SPME−HPLC was tested. According to that described in the Chromatographic Measurement section and Figure 1, 10 mL of standard aqueous solution containing 1.0 μg L^{-1} mixture of MEL, CYR, and CYA for the online SPME−HPLC analysis was carried out. As shown in Figure 5, only MEL appeared obvious in the chromatogram after eluting the MIP-SPME-MC by the mobile phase. However, the peak of MEL after eluting the NIP-SPME-MC by the mobile phase was very low, as well as CYR and CYA. The results indicated that the reservation capacity of the imprinted/CNT sorbent for MEL was much higher than that for CYR and CYA. In the loading process, many specific recognition sites with respect to the template molecule were generated on the imprinted/CNT sorbent surface. Thus, MEL was strongly bound to the MIP-SPME-MC. However, CYR and CYA were taken out by washing solution directly. In the desorption process, MEL bound on MIP-SPME-MC was extracted selectively by the mobile phase. Thus, trace MEL in standard aqueous solution was enriched effectually by the imprinted/CNT sorbent.

Additionally, the imprinting factor (α) and selectivity factor (β) were used to evaluate the specific recognition property of the imprinted sorbent, and the results were listed in Table 1. The imprinting factor is defined as follows:

$$\alpha = R_{MIP}/R_{NIP}$$

where R_{MIP} and R_{NIP} (%) are the recoveries of the target extracted by the imprinted sorbent and non-imprinted sorbent, respectively. The selectivity factor is defined as follows:

$$\beta = \alpha_{tem}/\alpha_{con}$$

where α_{tem} is the imprinting factor toward the template molecule and α_{con} is the imprinting factor toward the contradistinctive molecule. The value of α toward MEL is 6.41, which is greater than that toward CYR (0.76) and CYA (0.64). The β values are 8.43 (CYR) and 10.02 (CYA), which indicated that the imprinted/CNT sorbent reservation for both of the two contradistinctive molecules were equally low.

Optimization of SPME. The applicability of the imprinted/CNT sorbent accumulating on MEL was evaluated by the online SPME−HPLC analysis. The species and volume of the washing solution were optimized to obtain the best selectivity and good retention of MEL on the MIP-SPME-MC. Generally, the species of washing solution was optimized by keeping the total washing solution volume constant. Thus, this part of the optimization addressed two steps: the first step was conducted with the constant washing volume (1.0 mL) at the flow rate of 1.0 mL min^{-1} to ascertain the washing solution species. The second step was carried out to investigate the washing solution volume, when fixing on the first step optimal condition and flow rate of 1.0 mL min^{-1}.

The results obtained from online SPME−HPLC analysis for the spiked animal feed material sample are shown in Figure 6. It

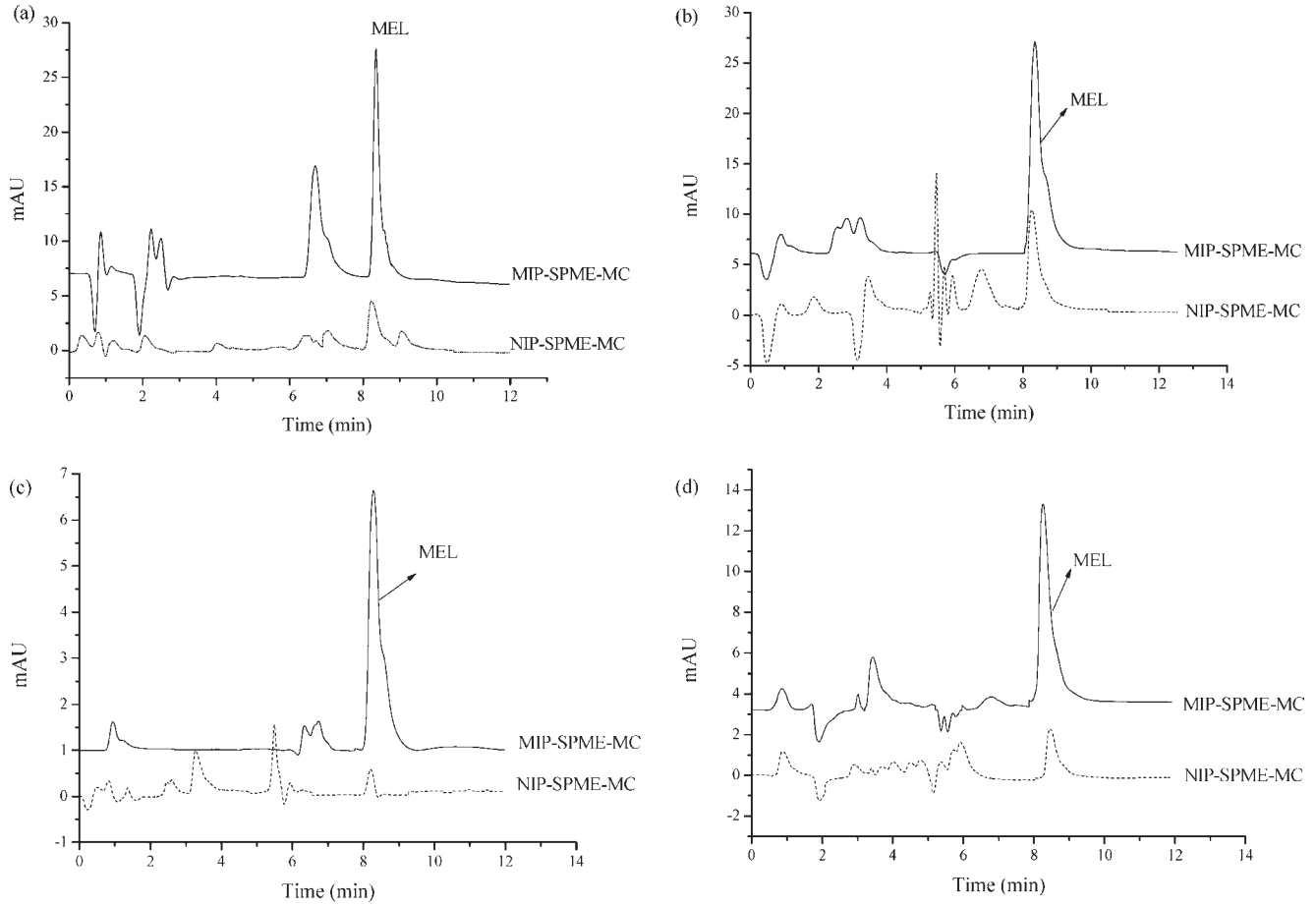


Figure 6. Chromatograms of spiked animal feed material sample on MIP-SPME-MC and NIP-SPME-MC by (a) washing with 1% ethanol at the flow rate of 1.0 mL min^{-1} for 1.0 min, (b) washing with 5% ethanol at the flow rate of 1.0 mL min^{-1} for 1.0 min, (c) washing with 10% ethanol at the flow rate of 1.0 mL min^{-1} for 1 min and washing with 5% ethanol at the flow rate of 1.0 mL min^{-1} for 2.0 min. Experimental conditions: spiked MEL concentration, 1.0 $\mu g\ L^{-1}$; loading sample volume, 10 mL; loading flow rate, 1.0 mL min^{-1}; analytical reversed-phase Shimadzu VP-ODS C_{18} column, 150 × 4.6 mm inner diameter; column temperature, 30 °C; detection wavelength, 240 nm; mobile phase ion-pair reagent buffer/acetonitrile, 9:1 (v/v); and flow rate, 1.0 mL min^{-1}.

was found that 1.0 mL of 5% ethanol aqueous solution was sufficient to remove interfering species presented at the flow rate of 1.0 mL min^{-1} (Figure 6b). However, many impurity peaks appeared in the chromatogram of washing MIP-SPME-MC with 1.0 mL of 1% ethanol aqueous solution (Figure 6a). This indicated that 1.0 mL of 1% ethanol aqueous solution could not remove the impurities effectively. Conversely, after washing with 1.0 mL of 10% ethanol aqueous solution, a large amount of MEL bound on the MIP-SPME-MC was eluted out; thus, the chromatogram peak of MEL was very low (Figure 6c). Therefore, 5% ethanol aqueous solution at the flow rate of 1.0 mL min^{-1} with washing for 1 min was selected to compare to a longer time (2.0 min) in the second step. Figure 6d shows that the chromatogram peak of MEL is also lower. This illuminated the MIP-SPME-MC washed with 5% ethanol aqueous solution at the flow rate of 1.0 mL min^{-1} for 2 min was not suitable to remove the interfering species presented. Thus, the optimization washing solution species, velocity, and washing time (5% ethanol aqueous solution, 1.0 mL min^{-1}, and 1 min, respectively) were selected to disrupt the non-selective interactions for the following experiments.

The ion-pair reagent buffer/acetonitrile had different ratios as the HPLC mobile phase and eluting solution were studied. The results showed that 90% ion-pair reagent buffer acetonitrile solution could separate MEL effectively. Moreover, the quantificational volume (fixed the washing and eluting solvent volume) controlled by the loading and injection time could ensure an exactitude detection. Thus, the effects of the eluting time were studied by eluting the adsorbed MEL with the mobile phase in the back flush at the flow rate of 1.0 mL min^{-1}. It was found that the chromatographic peak height of MEL increased rapidly as the eluting time increased from 0 to 0.8 min, increased slightly as the eluting time increased from 0.8 to 1.0 min, and then leveled off over 1.0 min. Therefore, the injection volume of 1.0 mL at the flow rate of 1.0 mL min^{-1} was controlled in the analysis.

Effect of the MIP-SPME-MC Length on the Backpressure. According to the theory for conventional adsorption, the sorbent quantity largely affects the rebinding of analytes, especially for small molecules. Thus, the SPME of small molecules is usually carried out on a longer SPME column. In this study, the imprinted/CNT sorbent was filled in different length columns proportionately (fixed column diameter of 3 mm), obtaining a series of different MIP-SPME-MC lengths. The effect of the MIP-SPME-MC length on the SPME of MEL for the online SPME−HPLC analysis was investigated. The chromatogram and backpressure were shown in Figure 7. The SPME of MEL

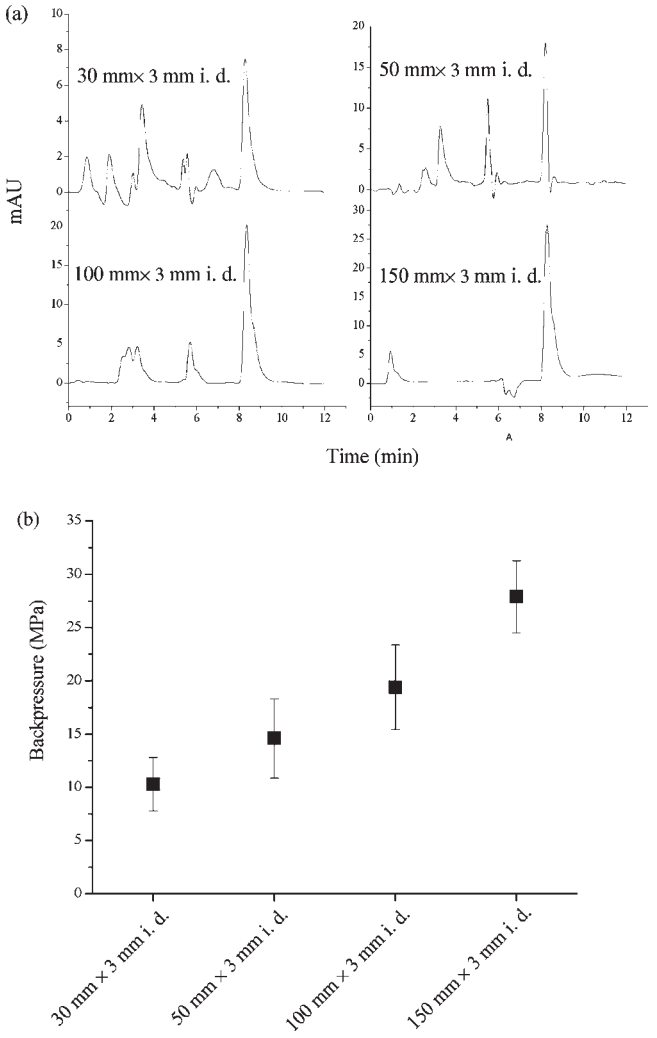


Figure 7. (a) Chromatograms of spiked animal feed material sample on MIP-SPME-MC with different lengths and (b) the relationship of the backpressure. Experimental conditions: spiked MEL concentration, 1.0 μg L^{-1}; loading sample volume, 10 mL; loading flow rate, 1.0 mL min^{-1}; analytical reversed-phase Shimadzu VP-ODS C_{18} column, 150 × 4.6 mm inner diameter; column temperature, 30 °C; detection wavelength, 240 nm; mobile phase ion-pair reagent buffer/acetonitrile, 9:1 (v/v); and flow rate, 1.0 mL min^{-1}.

on the MIP-SPME-MC length of 30 or 50 mm was poor, with lots of impurity peaks appearing in the chromatogram. However, the MIP-SPME-MC length of 150 mm extracting MEL from the spiked animal feed material sample was as successfully the same as the length of 100 mm. This indicated that the extraction could be operated on a certain MIP-SPME-MC length (a certain amount of imprinted/CNT sorbent). Nevertheless, a longer SPME column exhibited a larger resistance to the flow rate than the shorter column. As shown in Figure 7b, backpressure on a shorter SPME column with a length of 30 mm was only 10.3 MPa, whereas it reached 27.9 MPa on a longer SPME column (length of 150 mm) at the same flow rate of 1.0 mL min^{-1}. A reasonable backpressure of 19.4 MPa on the MIP-SPME-MC with a length of 100 mm was achieved in Figure 7b. Therefore, the imprinted column with a length of 100 mm was adopted in the online SPME−HPLC analysis.

Online SPME−HPLC Analysis of MEL. The SPME analysis of the spiked animal feed material sample by the imprinted/CNT sorbent for online SPME−HPLC was executed by passing 10 mL of sample solution containing 1.0 μg L^{-1} MEL at a loading flow rate of 1.0 mL min^{-1}. The data of the online SPME−HPLC for the determination of MEL were calculated and listed in Table 2. With the online SPME−HPLC analysis, the enrichment factor obtained by the slopes of the linear portion in comparison to the direct injection of 10 μL of the same amount of standard sample solution was 563. The relative standard deviation (RSD) of the peak area precision for three replicate extractions was 2.5%. The method detection limit (DL) was determined using progressively lower concentrations of MEL at a peak area when the peak height/noise ratio was 3:1 (S/N = 3) and with an executed volume of 10 mL. The method quantification limit (QL) was calculated at a peak area when the peak height/noise ratio was 10:1. The DL and QL correspond to 0.3 and 4.5 μg L^{-1}. Some methods have been used for the determination of MEL summarized in Table 3. In comparison to these methods, the method developed by this paper was sufficiently accurate and precise to be used for MEL analysis in real food samples and performed better characteristics, such as selectivity, enrichment, robotization, and cleanliness of the extracts.

Table 2. Performance of the Imprinted/CNT Sorbent for the Online SPME−HPLC Method

enrichment factor	563
DL (S/N = 3, μg L^{-1})	0.3
QL (S/N = 10, μg L^{-1})	4.5
RSD (n = 3, %)	2.5

To evaluate the usefulness and reproducibility of the MIP-SPME-MC for online SPME−HPLC, 10 mL of animal feed material or milk powder sample solution spiked with 10 or 100 ng of MEL were detected. At each HPLC analysis, three measurements were repeated. As presented in Table 4, the recoveries of MEL were considered according to the ratio of the detected and added amount. The recovery of the extracted spiking 10 ng of MEL by the imprinted/CNT sorbent was 89.3% with a RSD of 3.3% for the animal feed material sample. The recovery was 91.4% with a RSD of 2.8% for the milk powder sample. The recovery of extracted spiking 100 ng of MEL by the imprinted/CNT sorbent was 91.1% with a RSD of 4.8% for the animal feed material sample, and the recovery of extracted spiking 100 ng of MEL by the imprinted/CNT sorbent was 93.4% with a RSD of 4.1% for the milk powder sample.

Reproducibility. The reproducibility of the imprinted column was investigated. After the repeat of the extraction and elution of MEL 20 times (about 1 week), the chromatograms of SPME did not change remarkably. The RSD of the peak area of the chromatograms for 20 replicate extractions was 4.7%, which indicated that the imprinted column has good reproducibility.

In this study, a novel imprinted SPME material was synthesized in combination with the CNT-incorporated layer. The adsorption experiment showed fast adsorption dynamics and high adsorption capacity of the imprinted material for MEL. Applied as an extracted sorbent, the imprinted/CNTs exhibited excellent extraction characterization for the enrichment of MEL from a real sample by online SPME−HPLC. The results demonstrated that the combination of online SPME−HPLC with the imprinted/CNT sorbent is feasible with an enrichment ratio of 563-fold. The RSD of the peak area precision for three replicate extractions was 2.5%. DL (S/N = 3) and QL were 0.3 and 4.5 μg L^{-1}, respectively.

Table 3. Comparison of the Determination of MEL to Other Methods

pretreatment method	analytical system	selective	enrichment	robotization	detection limit	reference
SPE	HPLC−MS/MS	no	yes	no	4.2 ng mL^{-1}	2
hydrophilic interaction liquid chromatography	HPLC	no	no	no	25.0 ng mL^{-1}	8
extraction under the Food and Drug Administration methods	surface-enhanced Raman	no	no	no	0.033 μg mL^{-1}	9
extraction using 96 well microplate	chemiluminescence detection	yes	yes	no	0.02 μg mL^{-1}	24
MISPE	HPLC	yes	yes	no		25
SPE using Cleanert PCX-SPE cartridges	HPLC	no	yes	no	30 μg L^{-1}	26
MISPE	HPLC	yes	yes	no		27
direct determination	electrochemical	no	no	no	9.6 nmol L^{-1}	28
direct detection	high-performance cation-exchange chromatography	no	no	no		29
direct determination	GC−MS/MS	no	yes	no		30
formed complexation	UV spectra	no	no	no	0.063 mg mL^{-1}	31
SPE	sweeping micellar electrokinetic chromatography	no	yes	yes	10.9 ng mL^{-1}	32
disposable microfluidic device	UV detection	no	yes	no	0.23 μg mL^{-1}	33
extraction using a mixture solution	GC−MS/MS	no	no	no		34
MISPE	HPLC	yes	yes	yes	0.3 μg L^{-1}	this paper

Table 4. Recoveries of 10 or 100 ng of MEL in 10 mL of Spiked Sample Solution

	added (ng)		found (ng)		recoveries (%, mean ± reproducibility, n = 3)	
spiked sample	MIP	NIP	MIP	NIP	MIP	NIP
animal feed material	0	0	0.35	0.02		
	10	10	8.97	1.73	89.7 ± 3.3	17.3 ± 3.2
	100	100	91.1	22.5	91.1 ± 4.8	22.5 ± 3.4
milk powder	0	0	0.63	0.03		
	10	10	9.14	2.35	91.4 ± 2.8	23.5 ± 3.1
	100	100	93.4	25.8	93.4 ± 4.1	25.8 ± 2.9

AUTHOR INFORMATION

Corresponding Author

*College of Chemistry and Chemical Engineering, Jishou University, Jishou 416000, China. Telephone: +86-743-8563911. Fax: +86-743-8563911. E-mail: zhaohuizhang77@163.com.

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