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High-throughput and selective solid-phase extraction of urinary catecholamines by crown ether-modified resin composite fiber

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ABSTRACT

In the present study, we developed a simple and high-throughput solid phase extraction (SPE) procedure for selective extraction of catecholamines (CAs) in urine samples. The SPE adsorbents were electrospun composite fibers functionalized with 4-carboxybenzo-18-crown-6 ether modified XAD resin and polystyrene, which were packed into 96-well columns and used for high-throughput selective extraction of CAs in healthy human urine samples. Moreover, the extraction efficiency of packed-fiber SPE (PFSPE) was examined by high performance liquid chromatography coupled with fluorescence detector. The parameters affecting the extraction efficiency and impurity removal efficiency were optimized, and good linearity ranging from 0.5 to 400 ng/mL was obtained with a low limit of detection (LOD, 0.2–0.5 ng/mL) and a good repeatability (2.7%–3.7%, n = 6). The extraction recoveries of three CAs ranged from 70.5% to 119.5%. Furthermore, stable and reliable results obtained by the fluorescence detector were superior to those obtained by the electrochemical detector. Collectively, PFSPE coupled with 96-well columns was a simple, rapid, selective, high-throughput and cost-efficient method, and the proposed method could be applied in clinical chemistry.

1. Introduction

Crown ether is a host molecule with binding sites for guest molecules, which has been mainly investigated in supramolecular chemistry. A great deal of attention has been paid to crown ether due to its ability to selectively interact with cations since it has been discovered by Pedersen [1]. It is known that discrete crown ether compounds can form complexes with metal ions. Meanwhile, discrete crown ether compounds are used to separate metal ions and act as chromogenic reagents for photometric analysis of metal ions [2,3]. However, discrete crown compounds are chemically unstable and can be dissolved in most organic and inorganic solvents, leading to their complicated separation and recovery. Nevertheless, crown ether moieties chemically anchored to a polymer backbone can solve these problems [4,5]. After incorporating crown ether units into the polymer backbones, the mobility of the polymer structure is restricted, and the charge density along the polymer chain is increased, resulting in enhanced complexing properties. Moreover, immobilization of crown ethers onto polymers allows easy handling, regeneration, easy purification, low toxicity and adaption to continuous processes for this important set of complexants. High polymer crown ether (PCE) compounds are easy to spin fiber and make into film, which are suitable for various applications.

In the late 1970s, Cram and co-workers have reported the first example of chiral crown ethers attached to a polymer resin [6]. This work has led to numerous examples of crown ethers incorporated as a stationary phase in chromatography. Hyun et al. have demonstrated that enantiomeric compounds possessing a primary amino group can be separated by crown ether-based separation [7]. Lee and co-workers have reported that the crown ether-modified polymer resin can be used as solid phase extraction (SPE) adsorbents for the purification of catecholamines (CAs) in urine. Since then, SPE by crown ether resin has been applied in pretreatment process [5]. Moreover, more attention has been paid to the supramolecular interaction between PCE and RNH_3^+ [8,9].

Currently, crown ether-grafted polymers are obtained by immobilizing crown ethers on organic supports (such as polystyrene beads) [10] or inorganic supports (such as magnetic-multiwalled carbon nanotubes) [4]. Lee and co-workers have prepared PCE via crown ether monomers grafted with macroporous resin promising in SPE of CAs [5]. It is an improved idea to selectively extract CAs using PCE material. However, it is still a traditional method of SPE. Moreover, condensation of the target remains difficult, since Lee's method provides a limit of quantity at 40 ng/mL and it is

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unable to realize the actual analysis of CAs in urine (actual urine E: 8.7–18.3 ng/mL, NE: 23.9–56.6 ng/mL, DA: 70–120 ng/mL [11]). Chen et al. have reported two types of PCE composite nanofibers in enrichment and purification of urinary CAs and plasma CAs [12–14]. However, the adsorption of CAs varies with different PCE structures and biological matrices. Therefore, the studies on PCE composite fibers with different functional groups are necessary to find out the proper PCE composite fibers for pretreatment of different biological matrices and optimize the extraction and purification conditions.

Compared with the conventional particle-packed SPE cartridge, the fibers prepared by electrospinning can be good adsorbents of SPE, offering a reduced volume of both the solid phase and desorption solvent [15,16]. Moreover, fiber-packed SPE can exert sufficient binding sites of the functional groups and reduce the production cost at the same time, conferring an efficient and inexpensive adsorbent. In the present study, 4-carboxybenzo-18-crown-6 ether modified XAD resin (4CB18C6-XAD)/polystyrene (PS) composite fibers were prepared by electrospinning. We assessed the adsorption properties of 4CB18C6-XAD/PS composite fibers in selective separation of urinary CAs. Furthermore, a comparative study was performed to characterize selective adsorption properties of 4CB18C6-XAD/PS composite fibers and 4CB18C6-XAD resin material. In combination with 96-well columns packing composite fibers, a high-throughput and efficient kit was developed for extraction of urinary CAs, which could solve the bottleneck problem of sample pretreatment [17]. In addition, we confirmed that packed-fiber SPE (PFSPE) was superior to conventional particle-packed SPE cartridge. Therefore, the detectable amount of CAs could be directly adsorbed from urine samples by using PFSPE, presenting a broad application prospect. Moreover, the high performance liquid chromatography coupled with fluorescence detector (HPLC-FD) was successfully applied to determine urinary CAs, and the results seemed to be satisfactory. HPLC coupled with electrochemical detector (HPLC-ECD) is regarded as golden standard for determination of urinary CAs [18,19]. However, we found that HPLC-FD was more stable and applicable in this study. Therefore, the method established in this study might be useful in clinical laboratories.

2. Experimental

2.1. Reagents

Epinephrine hydrochloride (E), norepinephrine bitartrate (NE), dopamine hydrochloride (DA), 3,4-dihydroxybenzylamine hydrobromide (DHBA) and diphenylborinic acid 2-aminoethyl ester (DPBA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). HPLC-grade methanol and acetonitrile were obtained from Tianjin Chemical Reagent Company (Tianjin, China). All other reagents were of analytical grade unless otherwise indicated. PS (Mw = 185,000) was provided by Shanghai Chemical Agents Institute (Shanghai, China). 4CB18C6-XAD was synthesized in the laboratory of Tianjin Medical University.

Solutions of CAs (1 mg/mL) and internal standard (DHBA) were prepared by dissolving appropriate amounts of respective chemicals in water and stored in the dark at 4 °C. DPBA solution (0.2 mg/mL) was prepared as above-mentioned and used as a complexing reagent [the reason for reducing the DPBA dosage was shown in Fig. S1 in Electronic Supplementary information (ESI)].

2.2. Apparatus and chromatographic conditions

HPLC-FD was carried out on a Waters-1225 HPLC connected to a Waters 2487 fluorescence detector (Waters, USA). The wavelengths of excitation and emission were 286 and 318 nm, respectively. HPLC-ECD was performed on a Waters 1225 HPLC connected to a Waters 2465 electrochemical detector with a glassy carbon working electrode (set at 0.7 V for determination). A 50-μL volume was injected into a YMC-Pack ODS-A column (100 × 4.0 mm, 3-μm particle size) via a Waters 2707 autosampler. A HPLC software package (Waters, USA) was used for the data analysis. The mobile phase consisted of 5.5% acetronitrile, 45 mM sodium dihydrogen phosphate, 35 mM citric acid, 2 mM sodium heptanesulfonate and 0.25 mM EDTA, the pH was adjusted to 4.2 by 2 M NaOH, and the flow rate was set at 0.5 mL/min. The temperature of the column oven was set to 30 °C.

2.3. Preparation of crown ether-modified XAD resin

4CB18C6 was synthesized according to a previously reported method [20]. 4CB18C6-XAD was synthesized via immobilizing 4CB18C6 on macroporous adsorption resin support based on a method reported by Lee and co-workers [5]. 1H and 13C NMR spectra (Figs. S2–S3 in ESI) were recorded with a Bruker Avance III spectrometer (Bruker, German) using DMSO-*d*6 as the solvent. EI-LCMS spectra (Fig. S4 in ESI) were obtained at 70 eV on an Agilent 6890N mass spectrometer (Agilent, America). Fourier transform infrared (FTIR) spectra (Fig. S5 in ESI) were recorded with a Bruker Tensor 27 spectrometer (Bruker, German).

2.4. Electrospinning

4CB18C6-XAD/PS composite fibers were fabricated by electrospinning. Briefly, 5% 4CB18C6-XAD solution (w/v) was prepared by dissolving an appropriate amount of 4CB18C6-XAD in dimethylsulfoxide. Next, 10% (w/v) PS solution was prepared by dissolving an appropriate amount of PS in a mixture of dimethylformamide and tetrahydrofuran (4:6, v/v). The 4CB18C6-XAD solution was slowly dropped into the PS solution (2:5, v/v), and the mixture was blended until it became homogeneous.

4CB18C6-XAD/PS solution was loaded into a 10-mL glass syringe through a stainless steel needle with a diameter of 0.5 mm and a flat tip, which was connected to the anode. The aluminum foil collecting equipment was connected to the cathode. The distance between the needle tip and the collector was 15 cm. A voltage of 20 kV was supplied by a Dongwen high-voltage generator (model DW-P403-1AC, Tianjin, China). The feed rate of the precursor solution was fixed at 2.0 mL/h. The fibers were examined using a Nanosem 430 scanning electron microscopy (FEI, America) and an infrared (IR) spectroscopy (Fig. S5 in ESI).

2.5. Preparation of column for SPE

In this experiment, 1-mL microcolumn adapted for the 96-well microplate device was used as SPE column. The PFSPE columns were prepared as described in a previous paper [12]. Filter support was not necessary for packing fiber (3 mg) into 1-mL microcolumn. However, two cylindrical filter supports were inserted at the bottom and upper side of the microcolumn for particle-packed SPE, and then 4CB18C6-XAD material (4 mg) was packed in the middle. Fig. S6B in ESI illustrates that 1-mL microcolumn adapted for the 96-well microplate device was packed with the 4CB18C6-XAD/PS composite fiber, and Fig. S6C in ESI shows the 96-well microplate device loaded with 1-mL microcolumns.

2.6. Extraction of CAs

The SPE was performed on the MULTI-SPE M96 (positive pressure solid phase extraction device). Briefly, the microcolumn was firstly installed onto the 96-well microplate device (Fig. S6C in ESI) and placed in a fixed position of the MULTI-SPE M96 (Fig. S6A in