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Capillary electrochromatography using knitted aromatic polymer as the stationary phase for the separation of small biomolecules and drugs

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ABSTRACT

Hypercrosslinked polymers (HCPs) are currently receiving great attention due to their unique characteristics and potential uses in diverse areas. However, the field of HCPs for open-tubular capillary electrochromatography (OT-CEC) separations has not been explored. Here, a knitted aromatic polymer (KAP) was in-situ grown on the inner wall of the capillary column for OT-CEC for the first time. The silylating reagent containing phenyl was served as monomer, immobilized on the inner wall of the capillary column, and then KAPs-modified capillary column was prepared through in-situ hypercrosslinking reaction. The surface structure and morphology of KAPs-modified capillary column was characterized by Fourier transform infrared spectra (FT-IR) and scanning electron microscopy (SEM). The prepared capillary columns showed good separation performance for neutral compounds, small biomolecules, such as nucleosides, amino acids, small peptides, and non-steroidal anti-inflammatory drugs, sulfa drugs. In addition, the KAPs-modified capillaries showed good reproducibility, with relative standard deviations for intra-day, inter-day and column-to-column runs less than 1.59%, 2.55%, and 5.19% respectively. The strategy of in-situ immobilization of KAPs provides a new approach for the application of the material in the analytical fields.

1. Introduction

In recent years, the research on microporous materials, especially with pores smaller than 2 nm has been at the forefront of materials research due to their outstanding properties and potential applications in gas storage and separation, conductivity and catalysis [1]. Among these materials, the most popular are zeolites, activated carbons, metal organic frameworks (MOFs) and microporous organic polymers (MOPs) [2–4], etc. Particularly, MOPs, which are constructed from various light, non-metallic elements such as C, H, O, N, and B, have received an increasing interest and are becoming an important kind of microporous materials [2,4]. Generally, MOPs could be synthesized through various chemical reactions and a series of building blocks, making it possible to obtain the desirable characteristic by material design [5,6]. Besides, the robust covalent bonds interlinked by building blocks can avoid the instability observed in MOFs [7].

Owing to the numerous synthetic methods, MOPs have been classified into several types, including covalent organic frameworks (COFs) [8,9], hypercrosslinked polymers (HCPs) [10,11], porous aromatic frameworks(PAFs) [12], conjugated microporous polymers (CMPs) [13], and polymers of intrinsic microporosity (PIM) [14,15], etc. Among the different types of MOPs, HCPs are composed by Friedel-Crafts alkylation reaction. Compared to other MOPs (e.g. PAFs, CMPs), the synthesis of HCPs does not need the transition-metal catalysts or noble metal catalysts, which make it easy for mass production and practical application [16,17]. However, despite the numerous advantages of HCPs, the applications of the material in the fields of chromatographic separation and analysis are limited. Although there are very few reports about the application of HCPs in gas chromatography [18], solid-phase microextraction [19–22] and high performance liquid chromatography (HPLC) [23]. the application of these promising polymers as stationary phase in open-tubular capillary electrochromatography (OT-CEC) remains underdeveloped.

CEC has received much attention as it has advantages of high selectivity in HPLC and high separation efficiency in capillary electrophoresis (CE) [24]. OT-CEC does not need frits-making and particles-packing and has no bubble formation in packed capillary [25]. However, the OT-CEC suffers from the drawback of relatively low column capacity and phase ratio due to the limited coating surface area [26]. To date, the key to solve the above problem is to apply diverse functional

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material with large surface area as stationary phase to avoid the shortage of OT-CEC, and meanwhile enhance the separation selectivity [27–33]. Combined with their lightweight properties, high thermal stability and high surface areas, HCPs can be considered as promising chromatographic stationary phase materials for CEC.

Herein, we report the first example of in-situ growth of HCPs on the inner wall of the capillary columns for OT-CEC. The selected material named knitted aromatic polymer (KAP), which is prepared by the "knitting" method [34]. In the strategy, simple aromatic compounds like benzene or biphenyl was connected with rigid methylene bridges in the presence of external crosslinker formaldehyde dimethyl acetal (FDA) via the anhydrous $FeCl_3$ catalyzed Friedel–Crafts reaction. The "knitting" method avoids the requirement for monomers with specific functionalities and thus variety of aromatic molecules can be directly crosslinked to form highly porous structure. Furthermore, the large surface area of KAPs networks can be achieved. In this study, we take advantages of silylating reagent containing phenyl as monomer, immobilized on the inner wall of the capillary columns [35], and then obtain the KAPs-modified capillary column through in-situ hypercrosslinking reaction. The strategy of in-situ immobilization of KAPs provides a new approach for the application of the material. Besides, owing to the highly hydrophobic character of KAPs, the currently reported analytical targets of KAPs mainly focused on aromatic compounds (including benzene, toluene, ethylbenzene and m-xylene), PAHs and highly hydrophobic long-chain n-alkanes. In this context, we hope to broaden the choice of analytical targets for the better application of the material in the analytical fields. The results showed that the prepared capillary columns could be applied to the separation of neutral compounds, small biomolecules, such as nucleosides, amino acids, small peptides, and non-steroidal anti-inflammatory drugs, sulfa drugs.

2. Experimental

2.1. Chemicals

Methanol and acetonitrile of HPLC grade, ketoprofen, ibuprofen, flurbiprofen, Phe-Gly, Phe-Gly-Gly, Gly-Phe were purchased from Sigma-Aldrich (MO, U.S.A.). Toluene, 1,2-dichloroethane, FDA, sulfadiazine, L-phenylalanine, L-tryptophan, L-tyrosine, methylbenzene, ethylbenzene, n-propylbenzene, phenyltrimethoxysilane, guanosine, uridine, adenosine, sulfamethazine, sulfamethoxazole chlorobenzene, 1,2-dichlorobenzene, 1,2,4-trichlorobenzene were from Aladdin (Shanghai, China). Ferric chloride, sodium tetraborate decahydrate ($Na_2B_4O_7{\cdot}10H_2O$), disodium phosphate dodecahydrate ($Na_2HPO_4{\cdot}12H_2O$), sodium hydroxide (NaOH), hydrochloric acid (HCl), thiocarbamide were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Fused silica capillaries with a dimension of 50 μm i.d. × 365 μm o.d. were obtained from Ruifeng Chromatographic Devices (Yongnian, Hebei, China). Deionized water was purified by a Milli-Q system (MA, USA). Other reagents used in the experiments were of analytical grade and from commercial sources.

2.2. Instrumentation

The surface morphology of the KAPs-modified OT-CEC column was characterized by a Carl Zeiss Ultra Plus Field Emission scanning electron microscope (FESEM, Carl Zeiss, Germany) at an accelerating voltage of 20 kV. Fourier-transform infrared spectra was determined on a Thermo Nexus 470 FT-IR system (MA, UAS). All CEC separation were carried out on an Agilent 7100 CE system (Waldbronn, Germany), which equipped with an auto-sampler, a diode array detector and a temperature controlled column compartment. The data acquisition and treatment was performed on the chromatographic workstation. A precise syringe pump (Longer Pump Company, Baoding, China) was used to push the solutions through capillaries.

2.3. Background electrolyte and sample solution preparation

The background electrolyte solution includes phosphate electrolyte solution (10 mM $Na_2HPO_4{\cdot}12H_2O$) and borate electrolyte solution (5 mM $Na_2B_4O_7{\cdot}10H_2O$). The pH of phosphate solution was adjusted to 5–9 using phosphoric acid, and that of borate solution was adjusted to 9.5 by NaOH and 6.5 by HCl. Prior to analysis, the above solutions were mixed with appropriate amount of methanol or acetonitrile. All solutions were stored in a refrigerator at 4 °C and filtered by syringe-driven filter. The standard solutions of methylbenzene, ethylbenzene, n-propylbenzene chlorobenzene, 1,2-dichlorobenzene, 1,2,4-trichlorobenzene were prepared in methanol at a concentration of 3 mg mL^{-1}individually, and ketoprofen, ibuprofen, flurbiprofen, sulfadiazine, sulfamethazine, sulfamethoxazole were also prepared by the same solvent at a concentration of 1 mg mL^{-1}. The standard solutions of 1 mg mL^{-1} L-tryptophan, L-tyrosine and L-phenylalanine were prepared in 0.1 M NaOH individually. The standard solutions of Phe-Gly, Phe-Gly-Gly, Gly-Phe, guanosine, uridine, adenosine were respectively dissolved in deionized water at a concentration of 1 mg mL^{-1}. All standard solutions were refrigerated at 4 °C.

2.4. Fabrication of KAPs-modified open-tubular capillary column

The preparation process of KAPs-modified capillary is illustrated in Fig. 1. Firstly, the capillary was treated by rinsing with 1 M NaOH for 2 h, ultrapure water for 10 min and 0.1 M HCl for 10 min, ultrapure water 30 min, sequentially, dried under nitrogen stream and kept at 100 °C in an oven for 1 h. Secondly, the treated capillary was filled with a solution of phenyltrimethoxysilane in toluene (1/1, v/v), and kept in the oil bath at 110 °C for 12 h with both ends sealed, and then rinsed with methanol to remove the residual phenyltrimethoxysilane and dried with a flow of nitrogen. Thirdly, repeat the second step of modifying process to ensure sufficient phenyl group was coated, and rinse the capillary for 30 min by using 1,2-dichloroethane. Finally, 100 mg mL^{-1} ferric chloride was dissolved in the 1, 2-dichloroethane by ultrasonic, and a clear solution was gained by centrifugation, afterwards, FDA was mixed with the ferric chloride solution at a concentration of 46.8 mg mL^{-1} [34]. The resultant solution was filled into the capillary, then the capillary was kept in water bath at 80 °C for 24 h with both ends sealed. Before applied for OT-CEC separation, the prepared column was flushed with methanol for 2 h to remove the residual reactant. The flow rate kept unchanged at 0.05 mL h^{-1} throughout the process.

The procedure to prepare the KAPs-modified microscope cover glass was the same as above process.

2.5. OT-CEC separation

The modified capillary column (total length, 33.5 cm; effective length, 25.0 cm) was conditioned by rinsing with background electrolyte solution for 5 min for the first use, and just for 2 min between two

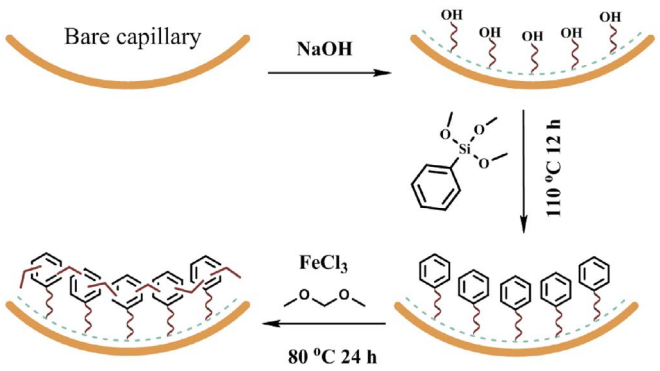

Fig. 1. Schematic illustration for the fabrication of the KAPs-modified capillary column.