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Polymer-modified fibrous mesoporous silica nanoparticles as coating material for open-tubular capillary electrochromatography☆

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ABSTRACT

A novel fibrous mesoporous silica nanoparticles (fSiO$_2$) stationary phase grafted with polymer (Poly (2-(dimethylamino) ethyl methacrylate) (PDMAEMA) was developed for open tubular capillary electrochromatography (OT-CEC). The preparation procedure included synthesizing fSiO$_2$ through biphase stratification approach, removing the surfactants, silanization and in situ graft polymerization with monomers via atom transfer radical polymerization (ATRP). Subsequently, PDMAEMA-modified mesoporous silica nanoparticles (P-fSiO$_2$)/ethanol solution was immobilized onto the inner surface of the pretreated capillary and functionalized with octadecylsilane to fabricate the open-tubular column. Separation of polycyclic aromatic hydrocarbons (PAHs) and proteins were carried out to evaluate the performance of the column in CEC. The run-to-run, day-to-day and column-to-column reproducibility in terms retention time of naphthalene was 1.9%, 2.2%, and 3.7%, respectively. The effects of solvent concentration and pH on the separation were evaluated. The method was also used for the separation of real bio-sample, egg white proteins.

1. Introduction

Open-tubular (OT) capillary column has been proved promising for separation because of its easy preparation and simple instrumental handling [1]; and it does not require the fabrication of frits and particles packing as in the case of packed column. However, OT capillary column suffers from problems of low sample capacity and phase ratio. To date, several options, such as etching [2], coating with porous layers [3–5] and depositing nanoparticles [6–9] on the inner surface of the capillary have been used to overcome the above problems.

Nanoparticles (NPs), which can create efficient surface area (the smaller nanoparticles used, the higher the phase ratio and the ligand capacity, thus the higher resolution [10]) for capillary after their non-covalent or covalent bonding onto the columns, causes widespread interest of researchers [11]. Silica nanoparticles (SiO$_2$ NPs) are the subject of intense research [12,13] and have been widely used as stationary phases for chromatographic separation since they exhibit low cytotoxicity, excellent chemical stability, and ease of chemical modification. Their large surface-to-volume ratio can not only facilitate mass transfer but also provide a platform for the attachment of the large number of functionalities needed for separation. To increase the surface area and exposure of binding groups to the sample, liquid phase deposition was used to coat the inner surface of the capillary with SiO$_2$ NPs [6]. Na et al. described the use of SiO$_2$ NPs to develop a capillary column with a new type of ionic liquid-dispersed silica nanoparticles (IL-SNs) for gas chromatography [14]. Liang et al. prepared a kind of octadecylsilane functionalized silica/graphene oxide stationary phase for high performance liquid chromatography of polycyclic aromatic hydrocarbon (PAHs) [15]. Qu and coworkers fabricated an octadecyltrichlorosilane derivatized silica nanoparticle-based coating using a multilayer by multilayer deposition approach for separation of PAHs [16]. Dong et al. immobilized crystalline MCM-41 mesoporous SiO$_2$ NPs (MSNs) onto the inner wall of an open-tubular capillary with a coating of cellulose tris (3,5-dimethylphenyl-carbamate) for enantio-separation in capillary electrochromatography (CEC), so that eight pairs of acidic, neutral and basic enantiomers were resolved [17]. Also, SiO$_2$ NPs could be used as a pseudo-stationary phase for capillary

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electrophoresis (CE) for separation of proteins with good reproducibility throughout the analysis [18].

A number of reviews have published on the development in the synthesis of MSNs and its applications in biotechnology and biomedicine [19–21]. MSNs are slica materials, which contain hundreds of empty channels arranged in a 2D network of honeycomb-like porous structure [22]. Moreover, they offer several unique and advantageous structural properties, such as high surface area (>700 $m^2 g^{-1}$), pore volume (>1 $cm^3 g^{-1}$), stable mesopore structure, tunable pore diameter (2–10 nm), two functional surfaces (exterior particle and interior pore faces), and modifiable morphology (controllable particle shape and size) [23]. However, to the best of our knowledge, they have not been used as coating materials for chromatography. Our research was focused on the immobilization of the fibrous mesoporous SiO_2 NPs onto the inner wall of a fused silica capillary to meet the need of increasing the phase ratio of OT capillary column. Poly [(2-(dimethylamino)ethyl methacrylate)] (PDMAEMA), a well-studied positively charged polymer brushes, whose charges change in response to solution pH [24], can easily adsorbed to inner wall of a silica capillary through strong electrostatic interaction. In addition, this type polymer stationary phases, functionalized with phenylalanine [25] or polyethyleneimine (PEI) [26,27], have been wildly used for OTCEC to increase phase ratio. Thus, P-*f*SiO_2 was firstly synthesized by modifying the surface of *f*SiO_2 with PDMAEMA. The coating of P-*f*SiO_2 on OT capillary column was convenient and fast to complete by comparing with the other silica-based OT columns. To demonstrate the chromatographic properties of such P-*f*SiO_2-coated OT capillary column, the uses of this column for the separation of three neutral samples, three basic proteins, and a real proteins sample are presented. This is the first report on the use of fibrous mesoporous SiO_2 NPs for open-tubular CEC (OTCEC), which would expand the applicability of mesoporous silica nanoparticles in separation field.

2. Experimental

2.1. Materials

Octadecyltrichlorosilane was purchased from Aladdin Chemistry (Shanghai, China). Analytical grade thiourea, toluene, naphthalene, biphenyl and HPLC-grade methanol (MeOH) were all purchased from Shanghai Chemical Reagent of Chinese Medicine Group (Shanghai, China). Lysozyme, ovotransferrin, ovalbumin, ovomucoid, cytochrome C and α-chymotrypsinogen A were obtained from Aladdin Chemistry (Shanghai, China). All chemicals were used without any further purification. Fused silica capillary (50 μm i.d. × 363 μm o.d.) was purchased from Yongnian Rui-feng Fiber Plant (Handan, China).

2.2. Synthesis and PDMAEMA-modification of silica nanoparticles (P-fSiO_2) and P-fSiO_2@C18

Fibrous mesoporous silica nanoparticles (*f*SiO_2) were synthesized through biphase stratification approach reported in the literature with slight modification [28–30]. 0.5 g (1.3 mmol) of hexadecyl trimethyl ammonium bromide (CTAB) and 0.3 g (5.0 mmol) of urea were dissolved in 15 mL of water. Subsequently, 15 mL of cyclohexane and 0.46 mL (6 mmol) of isopropanol were added to the solution. With vigorous stirring, 1.25 g (6 mmol) of tetraethyl orthosilicate (TEOS) was added dropwise to the mixed solution. After vigorous stirring for 30 min at room temperature, the reaction mixture was heated up to 72 °C, and maintained for 10 h. Then, the mixture was allowed to cool to room temperature and the silica was isolated by centrifugation, washed with distilled water and acetone, and air dried for 24 h. Finally, as-synthesized materials were transferred to 50 mL 0.6% (w/v) NH_4NO_3 ethanol solution and kept at 60 °C for 4 h to remove the surfactants CTAB from the *f*SiO_2.

2-(Dimethylamino)-ethyl methacrylate (DMAEMA) brushes were grown onto 300 nm initiator modified *f*SiO_2 via atom transfer radical polymerization (ATRP). The synthesis of initiator-modified *f*SiO_2 was achieved in two steps. First, the *f*SiO_2 (1 g) were suspended in 50 mL of 0.06 M (3-aminopropyl)-triethoxysilane (APTES) dry acetonitrile solution for 24 h at room temperature. The amine-modified *f*SiO_2 were collected and rinsed with acetonitrile via repeated centrifugation. The amine-functionalized particles (1 g) were then suspended in 50 mL of dry dichloromethane solution of 0.15 M dry triethylamine, 0.13 M 2-bromoisobutyryl bromide, and a catalytic amount of 4-dimethylaminopyridine (DMAP) for 12 h at room temperature. The initiator-modified particles were isolated and rinsed with dichloromethane by centrifugation.

The grafting of PDMAEMA brushes onto the initiator-modified *f*SiO_2 (1 g) was carried out in 10 mL of a 2:1 mixture of degassed methanol and water, containing DMAEMA (0.5 M), 2,2′-dipyridyl (0.08 M), $CuCl_2$ (0.006 M), and CuCl (0.02 M) at room temperature. After polymerization, the sample were quenched and rinsed with MeOH and water. After drying in an oven (60 °C) for 2 h, P-*f*SiO_2 was obtained. Then, P-*f*SiO_2 nanoparticles was modified with 10% octadecyltrichlorosilane in toluene at 110 °C for 24 h and obtained P-*f*SiO_2@C18.

2.3. Preparation of capillary column

Firstly, a new fused silica capillary was rinsed and filled with 1 M NaOH for 30 min, 1 M HCl for 30 min, acetone for 30 min, respectively. A total of 2 mg of PDMAEMA-modified mesoporous silica nanoparticles (P-*f*SiO_2) were added to 1 mL ethanol under agitation and the resulting suspension was transported into the pretreated capillary by using a homemade apparatus. After evaporation of solvent, the obtained column was rinsed with methanol and water and dried with N_2 (denoted as P-*f*SiO_2 column). P-fSiO_2 column was flushed with 10% octadecyltrichlorosilane in toluene, sealed by two GC septa at both ends and heated at 110 °C for 24 h, then rinsed with methanol and water, respectively and dried with N_2 (denoted as P-*f*SiO_2@C18 column). The C18 modified column based on bare capillary was denoted as C18 column. Total length of the open-tubular column was 65 cm with an effective length of 45 cm. Scheme for the preparation of OT column are shown in Fig. 1.

2.4. Chromatographic separation

Stock solutions of 1.0 mg mL^{-1} of neutral sample and 2.0 mg mL^{-1} of protein sample were respectively prepared in MeOH and deionized water. Buffer solutions (sodium phosphate and sodium borate) were prepared in distilled water and kept in a refrigerator. The pH value of buffer solution was adjusted to the range of 4–9 with 0.1 M NaOH or 1.0 M phosphoric acid solutions. The mobile phase was prepared by mixing the phosphate solution with water and MeOH. Chicken eggs were obtained from the local market. The egg white was separated from egg yolk, it was then diluted with borate buffer solution (30 mM sodium borate, pH 8.6) in 1: 6 ratios and filtered through a 0.22 μm membrane prior to use. All solutions were filtered using 0.22 μm membrane filter and sonicated for 15 min before used.

2.5. Instruments

A CE-1000 system (Unimicro (Shanghai) Technologies, Shanghai, China) with an on-column UV detector was used for all experiments. The data acquisition software, version 2.27 allowed instrument control and data analysis. The scanning electron