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Short communication

Preparation and characterization of lysine-immobilized poly(glycidyl methacrylate) nanoparticle-coated capillary for the separation of amino acids by open tubular capillary electrochromatography

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ABSTRACT

In this study, poly(glycidyl methacrylate) (PGMA) nanoparticles (NPs) were prepared and chemically immobilized for the first time onto a capillary inner wall for open tubular capillary electrochromatography (OTCEC). The immobilization of PGMA NPs onto the capillary was attained by a ring-opening reaction between the NPs and an amino-silylated fused capillary inner surface. Scanning electron micrographs clearly demonstrated that the NPs were bound to the capillary inner surface in a dense monolayer. The PGMA NP-coated column was then functionalized by lysine (Lys). After fuctionalization, the capillary can afford strong anodic electroosmotic flow, especially in acidic running buffers. Separations of three amino acids (including tryptophan, tyrosine and phenylalanine) were performed in NP-modified, monolayer Lys-functionalized and bare uncoated capillaries. Results indicated that the NP-coated column can provide more retention and higher resolution for analytes due to the hydrophobic interaction between analytes and the NP-coating. Run-to-run and column-to-column reproducibilities in the separation of the amino acids using the NP-modified column were also demonstrated.

1. Introduction

Open tubular capillary electrochromatography (OTCEC) is one of the most important methods in CEC. Its advantages include ease of preparation and operation, fritless, minimal effect on on-line detection, *etc.* However, OTCEC consistently yields relatively low phase ratio, which causes low resolution [1,2]. Hence, tailored capillaries, such as etched [3], porous polymer layer-coated [4,5], tentacle-type polymer-modified [6–9] and nanoparticle-coated capillaries [10–18], were employed to increase the surface area available for interactions.

Nanoparticles (NPs), with high surface-to-volume ratio, have been successfully used as coating material for OTCEC. Examples of these NPs include polymer [10–13], gold [14–17], titanium oxide NPs [18], *etc.* Polymer NPs are widely used because they can be easily prepared. For example, Kleindienst et al. prepared a permanently NP-adsorbed capillary using ethylenediamine and 2,3-epoxy-1-propanol functionalized polystyrene(PS)-based NPs for the separation of proteins and peptides [12]. We have recently prepared a bovine serum albumin-immobilized PS-NP capillary coating for chiral separations. The results suggest that NPs-coated capillary can apparently improve resolution, compared with monolayer ligand-modified capillary [13].

Difunctional monomer glycidyl methacrylate (GMA) is widely used for the preparation of CEC stationary phases, including monolithic packing [19], porous capillary coating [5], and tentacle-type polymer coatings [7–9], because it can be easily functionalized by different ligands with a simple ring-opening reaction. This work reports the novel application of poly-GMA (PGMA) NP-coated capillary. The prepared NP-coating can be easily modified by different chromatographic ligands by a simple ring-opening reaction. In this study, lysine (Lys) was used as the functional group that covalently bonded with the immobilized NPs. Three amino acids were used as probes to characterize the novel NP coating stationary phase.

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2. Experimental

2.1. Materials

(3-Aminopropyl)trimethoxysilane (APTS) (97%) was obtained from Jingchun Chemical Reagents Company (Shanghai, China). The amino acids (Lys, tryptophan (Trp), tyrosine (Tyr), and phenylalanine (Phe)) were obtained from Lianxing Chemical Co. (Tianjin, China). GMA was distilled under vacuum before use. Other chemicals were all analytical grade from local suppliers. Untreated fused silica capillaries of 50 μm ID were provided by Yongnian Optic Fiber (Hebei, China).

2.2. Apparatus and analytical procedure

OTCEC separations were performed on a P/CEC system MDQ CE instrument (Beckman Coulter, Fullerton, CA, USA). Scanning electron micrographs of the prepared nanoparticles and their capillary coating were taken using an XL 30 ESEM scanning electron microscope (SEM) (Philips, Amsterdam, Netherlands).

Phosphate buffer (20 mM) of various pHs were prepared for electroosmotic flow (EOF) measurements. For the separation of amino acids, running buffers (100 mM) with different pHs were used which were prepared from citric acid/Na_2HPO_4 (pH 3.0 and 5.0), KH_2PO_4/Na_2HPO_4 (pH 7.0) or boric acid/sodium borate (pH 9.0). All solvents and solutions for CEC were filtered through a 0.22 μm membrane. The amino acids were dissolved in deionized water with a suitable concentration (0.2 mg/mL for Trp and Phe, and 0.1 mg/mL for Tyr) before use. The samples were injected at 0.4 psi for 3 s. 12 kV was used for CEC separations. All experiments were carried out at 25 °C.

2.3. Column preparation

2.3.1. Fabrication of PGMA NPs

The solution (100 mL) containing 20 mM Na_2CO_3, 20 mM $NaHCO_3$, 0.2% (w/w) potassium persulfate, 10% GMA and 0.3% sodium dodecyl sulfate was stirred for 12 h in a 250 mL three-necked round-bottomed flask at 70 °C. Residual monomers were removed by dialysis with water. The prepared PGMA NPs were then diluted into 2.5% (w/w) solution with water.

2.3.2. Immobilization of the PGMA NPs onto the capillary inner wall

A 50 μm ID fused silica capillary was first pretreated with NaOH and HCl following the published method [8]. Then, the capillary was filled with APTS solution to introduce amino groups, as described by Xu et al. [20]. The obtained capillary was flushed with PGMA NP suspension diluted 1:1 with 100 mM borate buffer (pH 8.5) for approximately 30 min at room temperature. The filled capillary was sealed and left to react overnight at room temperature. The capillary was washed with deionized water to remove excess NPs.

2.3.3. Preparation of the monolayer epoxy group-functionalized capillary column

Preparation of the monolayer epoxy group-functionalized capillary coating included pretreatment of the capillary inner wall and silanization (with 3-Glycidoxypropyltrimethoxysilane). The procedure was based on the report of Li et al. [21].

2.4. Lys modification

Both PGMA NP-coated and monolayer epoxy group-functionalized capillary columns were modified by Lys using the procedure described by Ye et al. [19] and Dong et al. [22]. The reaction was performed with Lys (1 M) in the PBS buffer (pH 8.0, 50 mM) at 75 °C. After the reaction, the column was rinsed with water, and then further conditioned with running buffer prior to use.

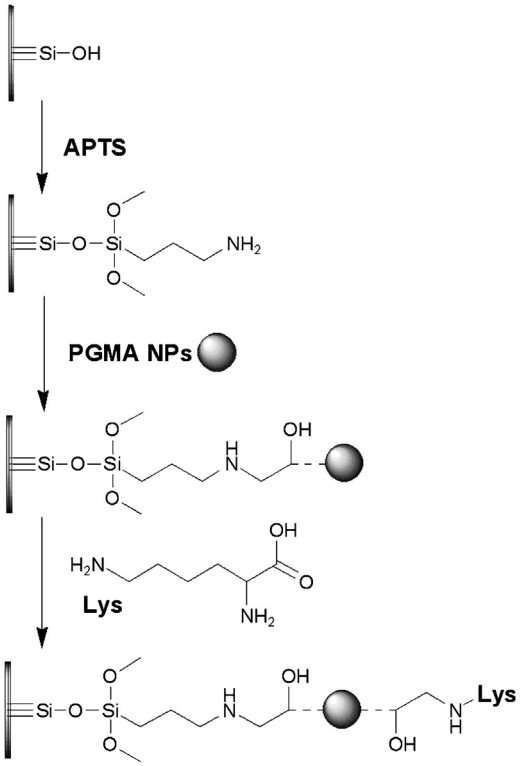

Fig. 1. Reaction scheme of the preparation of the nanoparticle-coated capillary.

3. Results and discussion

3.1. Design and characterization of the NP coating

The objective of this work was to prepare a NP-coated capillary with relatively higher capacity and easier surface functionalization using different chromatographic ligands. This objective was achieved by coating the inner wall with PGMA NPs according to the reaction scheme shown in Fig. 1. As can be seen, a naked capillary was primarily treated with APTS to introduce amino groups. The obtained amino-silylated capillary was then allowed to react with the epoxy groups on the PGMA NPs. Thereafter, the NPs were chemically immobilized. The coating of the pore surface with the NPs was visualized using SEM. The micrograph in Fig. 2 clearly shows that the NPs (Fig. 2a) were bound to the surface in a dense continuous monolayer (Fig. 2b). The prepared NP-coated capillary was finally functionalized with the chromatographic ligand (Lys) by a ring-opening reaction between epoxy and amino (Fig. 1).

The modification of the capillary was also evaluated by testing the electroosmotic mobility (μ_{eo}). Fig. 3 illustrates μ_{eo} values at different pH values in a bare uncoated, APTS-modified, PGMA NP-coated and PGMA/Lys NP-coated capillaries. Positive μ_{eo} was obtained for the naked capillary, and it increased with increasing pH. However, for APTS-modified capillary, negative μ_{eo} values (the direction of EOF was from cathode to anode) were observed at pH 3.0 and 5.0. This result can be attributed to the amino group in ATPS that was immobilized onto the capillary inner wall. When PGMA NPs were immobilized onto the capillary, some of the amino groups reacted with the epoxy groups of the NPs. Therefore, μ_{eo} decreases as the number of charged groups on the capillary inner wall decreases. As shown in Fig. 3, the EOF values of the PGMA