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Polydopamine assisted fabrication of titanium oxide nanoparticles modified column for proteins separation by capillary electrochromatography

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ABSTRACT

Development of a simple method for preparation of stable open tubular (OT) columns for proteins separation by capillary electrochromatography is still challenging. In this work, the titanium oxide (TiO_2) nanoparticles coated OT column was successfully prepared for separation of proteins by capillary electrochromatography. The polydopamine (PDA) film was first formed in the inner surface of a fused-silica capillary by the self-polymerization of dopamine under alkaline conditions. Then the TiO_2 coating was deposited onto the surface of pre-modified capillary with PDA by a liquid phase deposition process. The plentifully active hydroxyl groups in PDA coating can chelate with Ti^{4+} to boost the nucleation and growth of TiO_2 film. The as-prepared TiO_2 coated OT column was characterized by scanning electron microscopy and measurement of electroosmotic flow. Furthermore, the influence of liquid phase deposition time on the TiO_2 coating was investigated. The TiO_2 coated OT column was used for successful separation of two variants of β-lactoglobulin and eight glycoisoforms of ovalbumin. The column demonstrated good repeatability and stability. The relative standard deviations of migration times of proteins representing run-to-run, day-to-day, and column-to-column were less than 3.7%. Moreover, the application of the column was verified by successful separation of acidic proteins in egg white.

1. Introduction

The developments of proteomics and biotechnology have given rise to an increasing demand for separation tools of proteins. Capillary electrochromatography (CEC) combining high separation efficiency of capillary electrophoresis (CE) and high selectivity of high performance liquid chromatography (HPLC) has been applied in proteins separation [1–3]. As the core of CEC, various forms of columns including packed [4], monolithic [5,6], and open tubular columns are used [7,8]. Among these columns, OT columns have gained a great attraction for proteins separation due to the advantages including simple column preparation, the absence of bubble formation and simple instrumental handling. However, the low phase ratio and sample capacity resulted from the limited amount of stationary phase coating in OT columns impede their wide application in proteins separation. To overcome the limitation, a series of innovative approaches including etching [9,10], porous layers [11–13], sol-gel derived phases [14,15], and nanoparticles (NPs) phases [16–18] have been developed to increase the inner surface area of OT columns and enhance the stationary/mobile phase interactions. Among these approaches, NPs possessing large surface-volume ratio as the coating material in OT columns show a great promise to enhance the separation efficiencies of proteins. NPs are usually modified on the inner surface of capillary through dynamic coating and permanent covalently bonded coating processes [19,20]. Dynamic coating is usually physically adsorbed on the capillary inner surface. Although dynamic coating is simple, the coating suffers from poor stability because of the dynamic equilibrium existed between coating materials in the solution and those deposited on the surface [21]. Chemically modified covalent coating is much more stable than dynamic coating.

Abbreviations: APS, ammonium persulfate; BGE, background electrolyte; CE, capillary electrophoresis; CEC, capillary electrochromatography; ConA, conalbumin; EOF, electroosmotic flow; α-Lac, α-lactalbumin; β-Lg, β-lactoglobulin; LPD, liquid phase deposition; OT-CEC, open tubular column capillary electrochromatography; OVA, ovalbumin; PDA, polydopamine; TiO_2@PDA, TiO_2 coated OT column with the assistance of PDA; Tris, tris(hydroxymethyl)aminomethane.

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However, the preparation procedure is tedious. Therefore, development of a simple method for preparation of stable OT columns for proteins separation is still challenging.

The aim of the current work is to develop a facile and effective modification procedure to prepare an OT column with stable NPs coating for proteins separation by CEC. Our inspiration profits from the self-polymerization of dopamine and the formed polydopamine (PDA) easily attaching onto the surface of many organic and inorganic materials through various types of interactions, including hydrogen bonding, metal-catechol coordination, electrostatic interaction, cation-π interaction, and π-π aromatic interactions [22,23]. Moreover, the PDA coating was found to be an extremely versatile platform for secondary reactions to deposit uniform metal ions, metal, and metal oxide coatings via the metal binding ability of catechol present in the PDA coating [23–29]. Metal oxides have been reported to be used as the stationary phases in metal oxide affinity chromatography (MOAC) for proteins separation [30,31]. In MOAC, titanium oxide (TiO_2) is a popular stationary phase material owing to its chemical stability, hydrolytic stability at extreme pH and amphoteric nature [30]. Herein, TiO_2 NPs film was deposited onto the surface of pre-modified capillary with PDA by a simple liquid phase deposition (LPD) process. The condition for preparation of TiO_2 coated OT column with the assistance of PDA (TiO_2@PDA coated OT column) was optimized by adjusting the time of LPD process. The as-prepared OT columns were characterized by scanning electron microscopy (SEM) and measurement of electroosmotic flow (EOF). The conditions and the performance of the columns for separation of proteins were investigated. The feasibility of the column in the application in real samples was also evaluated by separation of proteins in egg white samples.

2. Materials and methods

2.1. Materials and chemicals

All chemicals and reagents were analytical grade or better. Fused-silica capillaries (50 μm i.d. × 365 μm o.d.) were obtained from Hebei Yongnian Ruipu Chromatogram Equipment Company (Handan, China). Dopamine hydrochloride was purchased from Alfa Aesar (Tianjin, China). Ammonium hexafluorotitanate [$(NH_4)_2TiF_6$] was obtained from Acros (Geel, Belgium). Ammonium persulfate (APS), boric acid (H_3BO_3), disodium tetraborate decahydrate ($Na_2B_4O_7{\cdot}10H_2O$), sodium dihydrogen phosphate dihydrate ($NaH_2PO_4{\cdot}2H_2O$), disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4{\cdot}12H_2O$), tris(hydroxymethyl)aminomethane (Tris), sodium hydroxide (NaOH), and hydrochloric acid (HCl) were supplied by Guangzhou Chemical Regent Factory (Guangzhou, China). Phosphoric acid (H_3PO_4) and *N*,*N*-dimethylformamide (DMF) were purchased from Tianjin Damao chemical reagent factory (Tianjin, China). Ultrapure water used in the experiments was supplied by an Elga water purification system (ELGA, London, UK).

α-Lactalbumin (α-Lac, Mr 14.2 kDa, p*I* 4.8) [32], β-lactoglobulin (β-Lg) having two variants (β-Lg A, Mr 36.7 kDa, p*I* 5.1; β-Lg B, Mr 36.6 kDa, p*I* 5.3) [32] from bovine milk, conalbumin (ConA, Mr 78.0 kDa, p*I* 6.0) [32] from chicken egg white were obtained from Sigma-Aldrich Company (Saint Louis, MO, USA). Ovalbumin (OVA, Mr 45.0 kDa, p*I* 4.7) [32] from chicken egg white was purchased from GBCBIO Technologies (Guangzhou, China). Bovine serum albumin (BSA, Mr 69.0 kDa, p*I* 4.7) [32] was obtained from Shanghai Boao Bio-Science & Technology Company (Shanghai, China).

2.2. Apparatus

The CEC experiments were conducted on a PACE-MDQ Beckman P/ACE™ MDQ CE instrument (Beckman Coulter, Fullerton, CA, USA) equipped with a photodiode array detector. Data collection and analysis were performed with 32 Karat software (Beckman Coulter, Fullerton, CA, USA). For column fabrication, temperature controlling process was carried out using a GC system 7890 II (Techcompany, Shanghai, China). Syringe pump (KD Scientific, Holliston, MA, USA) was used to control the flow rate in the preparation of the columns. SEM was carried out on a ZEISS Ultra 55 field emission scanning electron microscope (Carl Zeiss, Oberkochen, Germany).

2.3. Preparation of TiO_2@PDA coated OT column

To clean and activate the inner surface of a bare fused-silica capillary and expose more hydroxyl groups, a new capillary was first preconditioned by flushing with 1 M HCl (2 h), followed by deionized water (0.5 h), 1 M NaOH (2 h), deionized water (0.5 h), and acetone (0.5 h) at a flow rate of 10 μL/min. Then the capillary was dried with nitrogen stream at 180 °C for 3 h.

The procedure for preparation of TiO_2@PDA coated OT column was schematically illustrated in Fig. 1. The TiO_2@PDA coated OT column was prepared in two steps. Firstly, the PDA coated OT column was prepared using a method previously reported [33]. The formation of PDA coating is schematically illustrated in Fig. 1A. The Tris-HCl buffer (10 mM, pH 8.5) was first prepared by dropwise addition of 12 M HCl to 10 mM Tris solution until the desired pH was obtained. Then the dopamine solution (7.7 mg/mL) was

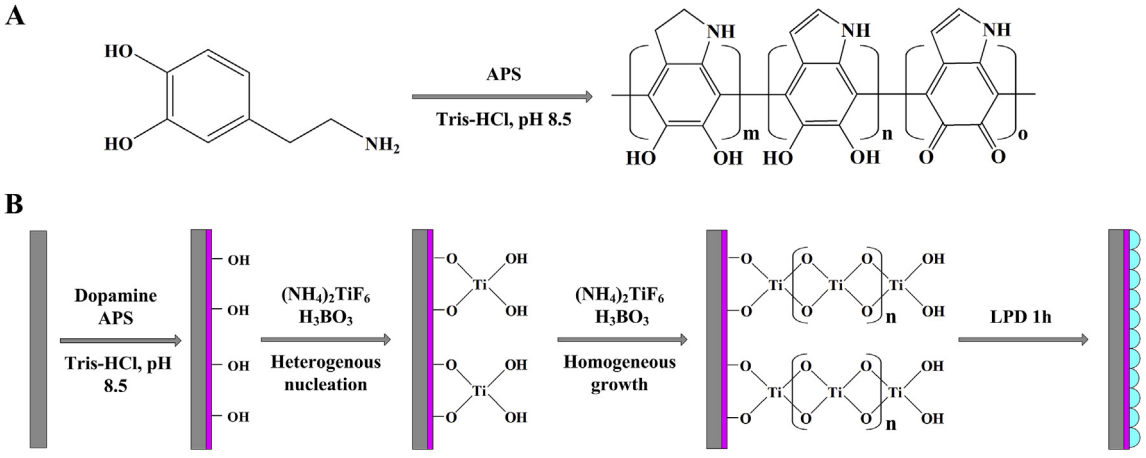

Fig. 1. Schematic illustration of the formation of PDA (A) and TiO_2 NPs deposited on the PDA coated OT column (B).