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The characteristics of open-tubular capillary electrochromatography columns with series/mixed stationary phases constructed with magnetic nanoparticle coating

Yaxian Zhu [1], Lingyi Zhang *[,1], Junhong Qian, Weibing Zhang *

Shanghai Key Laboratory of Functional Materials Chemistry, East China University of Science and Technology, Shanghai 200237, PR China

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ABSTRACT

Novel open-tubular capillary electrochromatography (OT-CEC) systems with core/shell magnetic nanoparticles modified by amino or C_{18} groups as stationary phase were constructed by immobilizing nanoparticles in the capillary with permanent magnets. Influence of preparation method of OT-CEC column with series stationary phases (continuous two-dimension) on column performance and effect of dispersant on capability of OT-CEC column prepared by stationary phases with mixed functionalities (mixed stationary phases) were investigated in details to achieve stable preparation. Organic acids were used to evaluate the OT-CEC systems, and the relative column efficiency of salicylic acid was 420,000 plates/m for series stationary phases, while that of benzoic acid reached 480,000 plates/m for mixed stationary phases. The excellent within-column and between-column repeatability ($n=5$) testified with the RSDs of retention time were less than 0.44% and 10.20% for series stationary phases and 1.65% and 4.29% for mixed stationary phases. The two OT-CEC systems were further applied to separation of the aqueous extract of *Rhizoma gastrodiae*. Comparing with normal OT capillary column, the new systems show extra high column efficiency due to large surface areas of nanoparticles and multiple separation mechanisms, and they have great potential in the method development for the analysis of complicated samples.

1. Introduction

Monodisperse silica particles were usually adopted in capillary electrochromatography (CEC) as stationary phase because of easy packing, high column efficiency, good stability and low back pressure [1–3], and have been widely used in different areas [4–6]. According to the Van Deemter equation, the column efficiency increases with the decrease of particle size, which gave birth to ultra-high pressure liquid chromatography (UHPLC) [7–9]. The system using 1.7 μm particles at elevated pressure up to 100 MPa was proposed by Waters (Manchester, UK). The column efficiency was enhanced three times than 5 μm particles under the same flow rate, and the resolution was increased more than 70%. However, high back pressure involved in packing procedure and difficulty of achieving homogeneous packing when using small particles, especially nanoparticles as stationary phase, limit the requirement of higher column efficiency and faster separation speed. The distribution of particle size and the structure of chromatography column, etc. should also be considered. When particle size reduces further to submicron scale (even nano scale), the solvent transport unit which can provide higher pressure will be needed for the homogenous packing of column. Zou and coworkers [10] synthesized 400 nm silica particles and packed them into capillary under 3000 psi for CEC application. The column efficiency of 210,000 plates/m for thiourea was obtained.

Comparing with packed column, OT-CEC has the advantage of none eddy diffusion, which helps to achieve higher column efficiency. In addition, OT-CEC column is suitable for rapid analysis [11–13] because of large EOF. One of the trends in the development of OT-CEC is using nanoparticles as a stationary phase. Bare gold nanoparticles were immobilized in the sol-gel-pretreated fused-silica capillary as stationary phase by Sykora and coworkers [14] for the first time, and it was applied for the separation of hydrophobic polyaromatic hydrocarbons as well as hydrophilic cationic antimicrobial peptides. Yang and coworkers [15] developed enantioselective OT-CEC with thiolated β-CD modified gold nanoparticles as stationary phase. Efficient enantioseparation by this method was demonstrated by the analysis of three drug enantiomers with good run-to-run repeatability. However, the major defect of OT-CEC with

Abbreviations: Id, inside diameter; Od, outside diameter; OT-CEC, open-tubular capillary electrochromatography; OTMS, octadeclytrimethoxysilane; PEDA, N-(3-(trimethoxysilyl)-propyl)-ethylenediamine

* Corresponding authors. Tel.: +86 21 64253068; fax: +86 21 64233161.
E-mail addresses: zhanglingyi@ecust.edu.cn (L. Zhang), weibingzhang@ecust.edu.cn (W. Zhang).

[1] These two authors contributed equally.

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nanoparticles as stationary phase is the tedious coating procedure and unstable coating.

Since 1970s, core/shell magnetic silica coated nanoparticles have been applied as enrichment materials and protein transport carrier due to its good dispersion, fine biocompatibility and easy modified silica surface. Slovakova et al. [16] used strong magnets to immobilize trypsin grafted magnetic beads in a microchip for protein digestion. The device represented an inexpensive way of fabricating an OT-like-column, and high performance and good reproducibility were confirmed by capillary electrophoresis (CE) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS). In our previous work [17], a novel one-dimensional (1D) OT-CEC system using amino modified magnetic nanoparticles coating as stationary phase was successfully constructed by using a series of external magnets to fix magnetic nanoparticles. High relative column efficiency (220,000 plates/m for anthranilic acid) was obtained, and the excellent within-column and between-column repeatability (less than 1.51% and 5.29%, respectively) have been testified.

Multi-dimensional chromatography has become one of the hotspot in analysis of complicated samples, and it shows strong power in investigation of proteomics, environmental science and herbal substance genomics [18,19]. Comparing with conventional 1D separation, multi-dimensional separation can strongly enhance the peak capacity and conveniently adjust separation selectivity. Two-dimensional (2D) system combines two separation modes with different selectivity directly (without interface) or by using interface [20–22]. Continuous 2D system [23–26] (series stationary phases, a separation mechanism followed another separation mechanism) adopts integration mode with no interface and takes the advantages of simple preparation, good repeatability and the property of total analysis. Yates and coworkers [27] described an automated continuous 2D system for multi-dimensional protein identification technology (MudPIT) by packing strong cation-exchange (SCX) resin and reversed-phase (RP) resin into one column, and a dynamic range from 10,000 to 1 between the most abundant and least abundant proteins/peptides in a complicated peptide mixture had been demonstrated. Later, they constructed another triphasic microcapillary column packed with RP material, SCX material and another RP material [28]. The column was useful in discovering co- and post-translational modifications of proteins. According to this strategy, they then assessed the effectiveness for the enrichment of phosphopeptides by using protein-based immobilized metal affinity chromatography (IMAC) as a pre-enrichment step prior to peptide-based IMAC, and 4470 unique phosphopeptides were identified in mammalian cells [29].

Mixed stationary phase, which involves multiple separation mechanisms simultaneously at the same cross section of capillary, has attracted wide interest in the separation of proteins and peptides [30–32]. Lei et al. [33] incorporated Fe_3O_4@SiO_2–NH_2 or SBA-15 nanoparticles into polymethacrylate monolithic column to develop novel stationary phase with mixed mechanism. The relative column efficiency of organic acids reached 290,000 plates/m and the results indicated that the incorporation of nanoparticles enhanced selectivity and column efficiency due to high specific surface area and mixed separation mechanism.

OT-CEC columns prepared with series/mixed magnetic nanoparticles stationary phases will have great potential in a wider research and application area. In this paper, silica surface of core/shell magnetic nanoparticles was modified by N-(3-(trimethoxysilyl)-propyl)-ethylenediamine and octadecyl-trimethoxysilane to obtain ion-exchange (IE) and RP functional groups. Magnetic nanoparticles and external magnetic field were used to construct series/mixed stationary phases OT-CEC columns with nanoparticle coating based on our previous work [17]. Organic acids and the aqueous extract of *Rhizoma gastrodiae* were used to evaluate the systems.

2. Materials and methods

2.1. Reagents and instrumentation

N-(3-(trimethoxysilyl)-propyl)-ethylenediamine (PEDA, 97%), octadecyl-trimethoxysilane (OTMS, 99%), isophtalic acid, salicylic acid, phthalic acid, benzoic acid and anthranilic acid were purchased from ACROS organic (New Jersey, USA); toluene was purchased from Suzhou Qiangsheng Chemical Industry Co., Ltd. (Suzhou, China). All above chemicals are analytical grade. Methanol was purchased from Shandong Yuwang Industrial Co. Ltd. (Shandong, China), and it is chromatographical grade.

Concentric cylindrical permanent magnets of 2.0 mm id × 5.0 mm od × 2.5 mm h (the flux density of each magnet is 0.2 T) were purchased from local store of Dalian, China. LSP01-1A syring pump (Baoding Longer Precision Pump Co. Ltd., China) was used for the preparation of OT-CEC column. The CEC experiments were performed on an EASYSEP GHV001 CEC system (Unimicro Shanghai Technologies Co. Ltd., China). Data collection and instrument control were realized with a Unimicro Trisep-2003 Station. Fused silica capillaries of 75 μm id – 375 μm od were purchased from Xinnuo Optical Chromatography Co. Ltd. (Handan, China). Water used in all experiments was purified by a Sartorius Arium 611 system (SARTORIUS, Germany). JSM-6360LV scanning electron microscope (JEOL, Japan), Magna 550 Fourier infrared spectrometer (NICOLET, USA), EV7 vibrating sample magnetometer (ADE, USA) and NOVA 4200e surface area and pore size analyzer (Quantachrome Instruments, USA) were used for materials characterization.

2.2. Functionalization of core-shell magnetic nanoparticles

Preparation and amination of core-shell magnetic nanoparticles were performed according to reference (see S1 in supplementary materials) [17]. After amination, the surface of Fe_3O_4 @SiO_2 particles was covered by NH_2 groups.

Alkylation of core-shell magnetic nanoparticles was similar to Ref. [10], and the preparation method was performed as follows: 0.68 g of Fe_3O_4@SiO_2 was heated at 100 °C in a water bath for 3 h. After washing by methanol, nanoparticles were dried in oven at 60 °C. Subsequently, 1.36 g of octadeclytrimethoxysilane and 34.11 mL of anhydrous toluene were added into the activated nanoparticles, refluxed for 17 h at 120 °C. After the treatment, the nanoparticles were washed, respectively, by acetone and ethanol for three times, and then filtrated through a 0.45 μm filter membrane by vacuum pump. Fe_3O_4@SiO_2–C_{18} particles were finally produced after drying.

2.3. Preparation of OT-CEC column

2.3.1. Pretreatment of capillary

Briefly, a capillary was orderly washed with HCl (0.1 mol/L), water, NaOH (0.1 mol/L) and water for 30 min, and then purged with nitrogen for 2 h.

2.3.2. Preparation of OT-CEC column with series stationary phases

Thirty pairs of concentric cylindrical permanent magnets were oppositely nipped to the capillary of 30 cm (Fig. 1(a)), and the length of capillary covered by magnets was 13 cm. The first pair of magnets was placed at a distance of 3.5 cm from the inlet of capillary, and the last pair of magnets was placed at a distance of 5.5 cm from the detection window. 2.5 mg/mL of Fe_3O_4@SiO_2–NH_2 suspension (dispersed in H_2O) was pushed into the capillary from the entrance of capillary at a flow rate of 12.50 μL/min