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Carboxyl modified magnetic nanoparticles coated open tubular column for capillary electrochromatographic separation of biomolecules

Wentao Wang, Xing Xiao, Jia Chen, Li Jia *

Ministry of Education Key Laboratory of Laser Life Science & Institute of Laser Life Science, College of Biophotonics, South China Normal University, Guangzhou 510631, China

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ABSTRACT

Carboxyl modified magnetic nanoparticles (Fe_3O_4-COOH MNPs) coated open tubular (OT) columns were prepared for capillary electrochromatography. The Fe_3O_4-COOH MNPs coatings were constructed on the surface of positively charged poly(diallydimethylammonium chloride) (PDDA) modified capillaries through electrostatic self-assembly approach. The as-prepared PDDA@Fe_3O_4-COOH MNPs coated OT columns were characterized by scanning electron microscopy and electroosmotic flow measurement. The electrochromatographic characterization of the OT columns was evaluated by separation of amino acids, dipeptides and proteins. The influences of background solution pH, concentration, and organic modifier content on separation were investigated. The separation of these analytes was primarily based on the electrophoretic mechanism in combination with chromatographic mechanism. The Fe_3O_4-COOH MNPs coatings improved the separation resolution of these analytes due to their large surface area. Three variants of bovine serum albumin, two variants of β-lactoglobulin and nine glycoisoforms of ovalbumin were successfully separated. The relative standard deviations of migration times of analytes representing run-to-run, day-to-day and column-to-column were less than 4.3%. Furthermore, the feasibility of the PDDA@Fe_3O_4-COOH MNPs coated OT column was verified by successful separation of acidic proteins in egg white.

1. Introduction

Developments of proteomics and biotechnology have caused an increasing demand for analytical tools of biomolecules. Capillary electrochromatography (CEC) combining high selectivity of high-performance liquid chromatography and high efficiency of capillary electrophoresis has been widely applied in the analysis of biomolecules including amino acids [1], peptides [2] and proteins [3]. As the core of CEC, various forms of columns including packed, monolithic, and open tubular (OT) columns are commonly used. Among these columns, OT columns have shown a great attraction for the analysis of complex samples owing to no bubble formation, simple column preparation, and simple instrumental handling, etc. However, due to the limited amount of stationary phase coating, low phase ratio and sample capacity of OT columns are the primary drawbacks and restrict their widespread application in electrochromatographic separations. To overcome these shortcomings, a series of innovative approaches such as sol–gel derived phases [4,5], etching [6], porous layers [7,8] and nanoparticles (NPs) phases [9–19] have been developed to increase the inner surface area of columns and enhance the stationary/mobile phase interactions in OT-CEC separations. Among these approaches, NPs modified OT columns show a great promise to enhance the separation efficiencies of complex samples since NPs possess larger surface-to-volume ratio.

For the past two decades, NPs with unique physical and chemical properties have made a significant contribution to the development of stationary phases in CEC, such as carbon NPs [9–12], silica NPs [13,14], metallic and metal-oxide NPs [15–18], and polymer NPs [1]. As one kind of extensively studied NPs, magnetic nanoparticles (MNPs) have received great attention for their superior characteristics such as good dispersion, excellent biocompatibility [20], high field irreversibility, ease of preparation, high ratio of surface-to-volume [19] and remarkable physicochemical stability. They were widely applied in the field of biotechnology, such as selective separation of protein [19,21–24], extraction of nucleic acid [25], capture of bacteria [24,26–28], biomedicine [20] and biosensing [28]. To the best of our knowledge, few MNPs have been explored as the stationary phase in CEC separation. Liang et al.

* Corresponding author.
E-mail address: jiali@scnu.edu.cn (L. Jia)

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[29] reported synthesis of β-cyclodextrin conjugated graphene oxide MNPs and successfully applied them as the stationary phase of chip-based OT-CEC for efficient enantioseparation. Zhu et al. [30] applied core/shell MNPs modified by amino or C_{18} groups as mixed stationary phase for OT-CEC separation of organic acids and the aqueous extract of *Rhizoma gastrodiae* based on ion-exchange and reversed-phase mechanisms. Though these columns mentioned above are effective to be applied for OT-CEC, they greatly depend on an external magnetic field to immobilize the stationary phases on the inner surfaces of the columns and accurate control of magnetic field will be a challenge to experimenters, which would make the analysis process more complicated. In order to simplify the analysis process, in this paper, we used an easy and reliable method named semipermanent coating to fabricate MNPs functionalized OT columns. A semipermanent coating is based on the strong physical adsorption between coating material and the capillary wall, which can effectively avoid the loss of stationary phase [31]. Recently, poly(diallydimethylammonium chloride) (PDDA) has been widely used to fabricate semipermanent coating in OT-CEC via electrostatic attraction [10–12,14,15]. PDDA is a water-soluble cationic polyelectrolyte, which has a wealth of highly hydrophilic and positively charged quaternary ammonium groups. Some NPs have been applied in OT-CEC as novel stationary phases by means of electrostatic self-assembly between PDDA and NPs, such as graphene [10], graphene oxide and reduced graphene oxide [11,12], nano-SiO_2 [14] and cyclodextrin-modified gold NPs [15].

In this work, we reported the utilization of carboxyl modified Fe_3O_4 (Fe_3O_4-COOH) MNPs as a novel stationary phase for OT-CEC separation. A simple, reliable immobilization procedure based on electrostatic self-assembly was utilized. Firstly, the Fe_3O_4-COOH MNPs were synthesized by a solvothermal reduction method. Then, the resulting Fe_3O_4-COOH MNPs with abundant negative charges were immobilized on the inner surface of a capillary which had been pre-modified with positively charged PDDA. The coating condition was optimized by adjusting the amount of Fe_3O_4-COOH MNPs. Furthermore, separation conditions and performance of different kinds of biomolecules were investigated. Efficient separation of amino acids, dipeptides and proteins were obtained in the PDDA@Fe_3O_4-COOH MNPs coated OT column. In addition, the column was successfully applied to separate acidic proteins in egg white samples.

2. Experimental

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 purchased from Hebei Yongnian Ruipu Chromatogram Equipment Company (Handan, China). Ferric chloride hexahydrate ($FeCl_3{\cdot}6H_2O$), sodium acetate (NaOAc), diethylene glycol (DEG), phosphoric acid (H_3PO_4) and *N*,*N*-dimethylformamide (DMF) were purchased from Tianjin Damao chemical reagent factory (Tianjin, China). Sodium acrylate (Na acrylate) was obtained from Beijing Universal Century Technology Company (Beijing, China). PDDA solution, 20% in water, with an average molecular weight (MW) between 400,000 and 500,000 Da, was acquired from Sigma–Aldrich Company (Saint Louis, MO, USA). Acetonitrile (ACN, HPLC grade) was purchased from SK Chemicals (Ulsan, South Korea). Tris(hydroxymethyl)aminomethane (Tris), sodium hydroxide (NaOH), sodium chloride (NaCl), sodium dihydrogen phosphate dihydrate ($NaH_2PO_4{\cdot}2H_2O$), disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4{\cdot}12H_2O$), hydrochloric acid (HCl), triethylamine (TEA) were obtained from Guangzhou Chemical Regent Factory (Guangzhou, China). Ultrapure water used in the experiments was purified by an Elga water purification system (ELGA, London, UK).

Table 1
Physical properties of amino acids, dipeptides and proteins.

Samples	MW	pI	References
Trp	204.23	5.9	[47]
Tyr	181.19	5.7	[47]
Phe	165.19	5.5	[47]
Gly-Trp	261.28	6.0	[47]
Gly-Tyr	238.24	6.2	[47]
Gly-Phe	222.24	6.0	[47]
ConA	78,000	6.0	[48]
α-Lac	14,200	4.8	[48]
BSA	69,000	4.7	[48]
OVA	45,000	4.7	[48]
β-Lg A	36,700	5.1	[48]
β-Lg B	36,600	5.3	[48]

Phenylalanine (Phe), tryptophan (Trp), tyrosine (Tyr), and bovine serum albumin (BSA) were purchased from Shanghai Boao Bio-Science & Technology Company (Shanghai, China). Glycyl-L-phenylalanine (Gly-Phe), glycyl-L-tyrosine hydrate (Gly-Tyr) and glycyl-L-tryptophan hydrate (Gly-Trp) were obtained from Tokyo Chemical Industry Company (Tokyo, Japan). Conalbumin (ConA) and ovalbumin (OVA) from chicken egg white were purchased from GBCBIO Technologies (Guangzhou, China). α-Lactalbumin (α-Lac) and β-lactoglobulin (β-Lg) from bovine milk were purchased from Sigma–Aldrich Company (Saint Louis, MO, USA). Their physical properties are listed in Table 1.

2.2. Instruments

The laboratory-built CEC apparatus was composed of a TriSep-2100 high-voltage power supply and a UV–Vis detector (Unimicro Technologies, Pleasanton, CA, USA). Data acquisition and analysis were performed with the software HW-2000 work station (Qianpu Software, Shanghai, China). A PHD 2000 syringe pump (Harvard Apparatus, Holliston, MA, USA) was used to push reagents or sample solutions through the capillary column. For column preparation, temperature controlling process was carried out using a GC system 7890II (Techcompany, Shanghai, China). The Fourier-transform infrared spectrometry (FT-IR) was recorded on a Bruker FT-IR spectrometer (Bruker, Germany). Scanning electron microscopy (SEM) was carried out on a ZEISS Ultra 55 field emission scanning electron microscope (Carl Zeiss, Oberkochen, Germany). Transmission electron microscopy (TEM) was carried out on a JEM-2100HR transmission electron microscope (JEOL, Tokyo, Japan). A Zetasizer Nano ZS (Malvern, Worcestershire, UK) was employed to measure the zeta potential.

2.3. Synthesis of Fe_3O_4-COOH MNPs

Fe_3O_4-COOH MNPs were prepared by a solvothermal reduction method [32]. The procedure for preparation of Fe_3O_4-COOH MNPs is schematically illustrated in Fig. 1A. Firstly, $FeCl_3{\cdot}6H_2O$ (0.81 g) was dissolved in DEG (30 mL) to form a clear solution with the help of ultrasonication. Secondly, NaOAc (2.25 g) and Na acrylate (2.25 g) were added to the solution, respectively. And the mixture was vigorously stirred at a speed of 500 rpm and 70 °C until a homogeneous dark yellow solution was obtained. Thirdly, the obtained homogeneous solution was transferred into a Teflon-lined stainless steel autoclave with 40 mL capacity and heated at 200 °C for 10 h. After reaction, the autoclave was cooled to room temperature. The acquired products were washed several times with ethanol and water, and dried under nitrogen atmosphere at 50 °C for 12 h.