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Preparation of graphene oxide-modified affinity capillary monoliths based on three types of amino donor for chiral separation and proteolysis

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ABSTRACT

Novel graphene oxide (GO)-modified affinity capillary monoliths were developed employing human serum albumin (HSA) or pepsin as chiral selector. Three types of amino donors for GO immobilization, including ammonium hydroxide (NH_4OH), ethanediamine (EDA) and polyethyleneimine (PEI), were applied to explore the effect of spacer arm on enantioseparation. It was observed that HSA-GO-EDA-based affinity capillary monoliths exhibited better chiral recognition ability in comparison with the other two spacer-based monoliths. Under the optimized conditions, the obtained columns revealed satisfactory repeatability concerning column-to-column, run-to-run and interday repeatability. In addition, the impact of GO concentration on enantiomeric separation was also investigated. HSA-GO-EDA-based affinity capillary monoliths provided higher chiral selectivity for nine pairs of enantiomers compared to the columns without GO. Furthermore, the influence of amino donors and GO on proteolytic activity of pepsin-based immobilized enzymatic reactor (IMER) was discussed. Unfortunately, pepsin-GO-PEI-based affinity capillary monoliths possessed the highest protein digestion capacity, which was different from the effect of amino donors on enantiorecognition. Moreover, GO presented as a favorable choice to improve the enzymatic activity of IMER. These results proved that GO-functionalized affinity capillary monoliths have promising potential for chiral separation and proteolysis.

1. Introduction

New discoveries in material science can have significant impacts on chemical measurements. A good example is the increasing use of nanoparticles (NPs) in modern analytical chemistry [1]. As chromatographic stationary phases, NPs possess large surface-to-volume ratios and multiform morphologies, which are ideal for improving separation performance in terms of efficiency and selectivity. Various NPs, including carbon nanomaterials, gold nanoparticles (GNPs), silica nanoparticles, and metal-oxide nanomaterials have been springing up for electrophoretic analysis with miniaturization becoming a trend in separation systems [2–9].

One interesting application of NPs is to fabricate capillary microsystem for chiral separation. Since the living systems exhibited distinctive physiological responses to different enantiomeric forms of a single drug, chiral discrimination is an important topic in an analytical area [10–14]. To obtain an efficient and environmentally benign separation technique, capillary microsystem has been regarded as a good alternative for chiral separa-

Abbreviations: BSA, bovine serum albumin; BET, Brunauer Emmett-Teller; CDMPC, cellulose tris(3,5-dimethylphenylcarbamate); β-CD, β-cyclodextrin; CSP, chiral stationary phase; EDA, ethanediamine; E-PE, ephedrine-pseudoephedrine; EOF, electroosmotic flow; EDCI, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; FT-IR, Fourier-transform infrared spectroscopy; GO, graphene oxide; GNPs, gold nanoparticles; GO/Fe_3O_4NCs, conjugated GO-magnetic nanocomposites; GPTS, 3-glycidopropyl trimethoxysilane; HSA, human serum albumin; H_3PO_4, phosphoric acid; IMER, immobilized enzymatic reactor; β-Me-PEA, β-methylphenethylamine; NH_4OH, ammonium hydroxide; NPs, nanoparticles; Na_2HPO_4, disodium hydrogen phosphate; NaOH, sodium hydroxide; NEF, (±)-nefopam; OT-CEC, open-tubular capillary electrochromatography; PEI, polyethyleneimine; PS, polystyrene; POSS, polyhedral oligomeric silsesquioxane; PDA/GO/BSA, conjugated polydopamine-GO nanocomposites; PEG, Polyethylene glycol; RSD, relative standard deviation; Rs, resolution; SWNTs, single-walled carbon nanotubes; SEM, scanning electron microscopy; TMOS, tetramethoxysilane; (±)-Trp, (±)-tryptophan.

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tion based on its prominent advantages, including shortened analysis time and reduced reagents consumption [15–18]. In this context, exploring novel chiral stationary phases provides a prospective direction for the development of capillary electrophoresis technique. For purpose of enhancing the phase ratio of open-tubular (OT) capillary column, nanomaterials such as (MCM)-41 mesoporous silica NPs, gold NPs and polystyrene (PS) NPs were used to fabricate enantioselective microsystems [19–21]. In addition to OT capillaries, NPs-based monolithic capillaries also exhibited satisfactory separation ability for racemates. For instance, single-walled carbon nanotubes (SWNTs) were chosen as a new category of chiral selector to prepare polymer capillary monoliths [22,23]. Thiol-functionalized silica and polymer monoliths were modified with chiral molecule-GNP conjugates [24,25]. For developing chiral organic-inorganic hybrid monoliths, octaglycidyldimethylsilyl polyhedral oligomeric silsesquioxane (POSS) incorporated native capillary monoliths were synthesized by ring-opening polymerization for further physically coating with cellulose tris(3,5-dimethylphenylcarbamate) (CDMPC) [26]. The results indicated that NPs-based microsystems presented enhanced performance in relation to separation efficiency and separation stability for enantiorecognition.

Among various NPs, GO is a chemically modified graphene sheet which contained a variety of reactive oxygen functional groups. These organic moieties enable GO to be a satisfactory choice for chemical modification of chiral molecules. Recently, GO-functionalized chiral materials have been utilized for enantioselective crystallization, electrochemical enantiorecognition and chiral separation [27–30]. In the field of enantioseparation, GO was considered as an outstanding nanomaterial to develop novel stationary phases. For instance, Qiu et al. constructed two types of chip-based open-tubular capillary electrochromatography (OT-CEC) by employing β-cyclodextrin (β-CD) conjugated GO-magnetic nanocomposites (GO/Fe_3O_4NCs) or bovine serum albumin (BSA) conjugated polydopamine-GO nanocomposites (PDA/GO/BSA) as chiral stationary phase (CSP) [31,32]. It was found that the large surface and high biocompatibility of GO facilitated the introduction of much more selectors into microfluidic channels. Furthermore, tryptophan enantiomers were successfully separated using these microdevices. In comparison with SWNT-BSA stationary phase, the obtained novel stationary phase presented better chiral separation capacity for (±)-tryptophan. Additionally, a GO-coated capillary was also applied for enantioseparation of ephedrine-pseudoephedrine (E-PE) isomers and β-methylphenethylamine (β-Me-PEA) isomers [33]. Nevertheless, to the best of our knowledge, few studies have specifically focused on developing GO-functionalized capillary monoliths for further chiral selectors immobilization. The monolithic column, with a porous "single particle" structure, has been regarded as a promising alternative to OT and packed column owe to its prominent advantages, including high phase ratio, easy preparation process and rapid chromatographic separation speed [34,35]. Considering the superior properties of GO, novel monolithic microsystem based on GO is expected. Two types of strategy, namely one-step room temperature polymerization and post-modification method have been used to construct GO-based polymer capillary monoliths [36–38]. It was demonstrated that the introduction of GO could significantly enhance separation and enrichment performance.

To take both advantages of nanoparticles and monolithic columns, herein, we developed novel GO-functionalized affinity capillary monoliths by choosing HSA or pepsin as the coated ligand, respectively. In view of our earlier successes in modifying GO onto tentacle-type polymer based OT, we will continue our attempts along this direction to investigate GO-decorated affinity capillary silica monoliths [39]. The obtained monoliths were characterized by scanning electron microscopy (SEM), Fourier-transform infrared spectroscopy (FT-IR), elemental analysis and nitrogen adsorption, which indicated the successful modification of GO on the monolithic matrix. Since the technique for preparing monolithic capillary columns is the "heart" of a microsystem, two important factors, including types of spacer arm and GO concentration, were investigated to discuss the detailed separation mechanisms involved in affinity analysis. It was shown that the effect of spacer arm on chiral separation and protein digestion exhibited distinct trend. Moreover, improved chiral recognition ability and enzymatic activity could be obtained due to the introduction of GO. Affinity chromatography is an important technique for biomedical and pharmaceutical analysis based on its specific selectivity by using biologically related binding agent as the stationary phase. This is the first study which described the use of GO-based affinity capillary monoliths for enantioseparation and proteolysis. The optimized columns exhibited improved chiral selectivity for a variety of enantiomers as well as enhanced enzymatic activity. It opens the way to applying GO-based capillary monoliths for affinity analysis.

2. Experimental

2.1. Chemicals and materials

Polyethylene glycol (PEG), tetramethoxysilane (TMOS), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI), 3-glycidopropyl trimethoxysilane (GPTS) were purchased from Aladdin Co. (Shanghai, China). Commercial graphene oxide dispersion was obtained from Xianfeng Nano-materials Technology Co. Ltd. (Nanjing, China). Acetic acid, ammonium acetate, urea, toluene, sodium hydroxide, hydrochloric acid, phosphoric acid, disodium hydrogen phosphate, NH_4OH, EDA, formic acid were from Nanjing, Chemical Reagent Co. (Nanjing, China). HPLC-grade methanol was from Jiangsu Hanbon Sci. & Tech. Co. Ltd. (Nanjing, China). PEI, pepsin from porcine stomach mucosa, HSA, BSA were from Sigma-Aldrich Trading Co. Ltd. (Shanghai, China). (±)-tryptophan, (±)-phenylalanine, (±)-azelastine, (±)-warfarin, (±)-ibuprofen, (±)-salbutamol, (±)-chlortrimeton, (±)-propranolol, (±)-ritodrine and (±)-nefopam were obtained from Dinghui Chemical industrial Co. Ltd. (Wuhan, China). Triply deionized water was used in all experiments, including synthetic reaction and buffer preparation.

2.2. Sample and mobile phase preparation

The mobile phases of phosphate buffer (pH 6.6, 6.8, 7.0, 7.2, 7.4, 7.6 and 7.8) were prepared by dissolving an exact amount of disodium hydrogen phosphate (Na_2HPO_4) in deionizied water. The pH value of the buffer solutions was adjusted by sodium hydroxide (NaOH) or phosphoric acid (H_3PO_4) solution. Moreover, the pH value of acetic acid-ammonium acetate stock buffer was adjusted by acetic acid. The standard solution (1 mg mL^{-1}) of (±)-phenylalanine, (±)-azelastine, (±)-warfarin, (±)-ibuprofen, (±)-salbutamol, (±)-chlortrimeton, (±)-propranolol, (±)-nefopam were obtained by dissolving analytes in methanol individually. (±)-Trp, (±)-ritodrine and thiourea (1 mg mL^{-1}) used in this study were dissolved in deionizied water. In addition, BSA sample (1 mg mL^{-1}) was prepared in digestion solution 0.5 mol L^{-1} formic acid. All above solutions were stored at 4 °C in a refrigerator and were degassed in an ultrasonic bath (Midmark, Versailles, USA) for 5 min and filtered through 0.22 μm pore size nylon membrane filters from Xinzhou Tech. Co. Ltd. (Tianjin, China) before use.

2.3. Synthesis of GO-modified capillary silica monoliths

Bare fused-silica capillaries (75 μm i.d. × 365 μm o.d.) were supplied by Yongnian Rui-feng Chromatographic Devices Co. Ltd. (Handan, China). Fused-silica capillaries were pretreated with