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Graphene oxide decorated monolithic column as stationary phase for capillary electrochromatography

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ABSTRACT

In this work, GO bonded monolith (pAS-GO@PS-DVB) as the stationary phase for capillary electrochromatography was fabricated, which was achieved by a simple one-step *in-situ* copolymerization of styrene and vinylized GO in the presence of divinylbenzene as a cross-linker. GO functionalization was primarily completed using *p*-aminostyrene based on condensation reaction between amino and carboxyl groups. The characterization by infrared spectroscopy, X-ray photoelectron spectroscopy, X-ray diffraction and scanning electron microscopy proved the covalent bonding of GO on the monolith. The average pore diameter via Barrett–Joyner–Halenda, specific surface area and pore volume via Brunauer-Emmett-Teller equation by nitrogen adsorption/desorption were determined to be 112.4 nm, 485.8 $m^2\,g^{-1}$ and 1.4 $cm^3\,g^{-1}$, respectively. The pAS-GO@PS-DVB monolithic column gave effective separation for a wide range of aromatic compounds, which was based on hydrogen bonding and π-π interactions of GO with polar and/or non-polar organic compounds. The reproducibility in terms of the precisions of migration time, peak height and peak area was estimated below 6% using thiourea and other aromatic compounds. Furthermore, the differences of migration time, peak height and peak area between the first-week analysis and the forth-week analysis were less than 19%, indicating good stability of the proposed monolithic column in one month. The applicability of the pAS-GO@PS-DVB monolith was also demonstrated by baseline separation of three phenols and three anilines.

1. Introduction

Graphene (G) is an allotrope of carbon in the form of a two-dimensional sp_2, single-layer scale (0.34 nm thick), hexagonal lattice. The networking material offers extraordinary properties including excellent thermal and mechanical properties and an ultra-high specific surface area (2630 $m^2\,g^{-1}$ [1]), and thus it has recently gained much research interest from a variety of scientific fields. For instance, potential applications of G as field-effect electrochemical detector [2], field effect transistor [3,4], sensor [5,6], storage battery [7], polymer nanocomposite [8], *etc*, have already been reported. However, graphene also suffers from aggregation (induced by the high surface energy and strong interlayer van der Waals interactions) and poor water solubility (resulted from strong lipophilic structure), which to some extent limits the applicability of graphene. As a result, chemical modification of graphene is usually carried out and graphene oxide (GO) is one of the most commonly functionalized graphene-based materials. Compared with G, GO sheets maintain the two-dimensional, atomic scale and hexagonal lattice of G but contain a range of new oxygen functional groups such as epoxy, hydroxyl and carboxyl. Therefore, GO exhibits significantly superior dispersion and water solubility over G besides comparable thermal, mechanical and specific surface properties to G.

Owing to the ultra-high specific surface area and strong π-π interactions, GO has been extensively used as a solid-phase adsorbent in microextraction and preconcentration of aromatic compounds [9] such as polycyclic aromatic hydrocarbons (PAHs) [10–12], and phloxine B [13]. Recently, Liu et al. [14] synthesized a novel solid-phase extraction adsorbent by covalently bonding GO sheets with aminosilica (GO@SiO_2). The GO@SiO_2 adsorbent offered strong adsorption capacities and high efficiencies for various analytes from small pollutants to biomolecules such as proteins and peptides. The adsorption capacity and high efficiency of GO-based materials may be further improved by chemical functionalization. Therefore the extensive applications of GO in wastewater processing [15], pollutants elimination [16], environmental monitoring and protection [17] have also been demonstrated.

Besides sample pretreatment, GO also exhibits potentials as a stationary phase in chromatographic separation. Zhang et al.

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[18] synthesized G and GO bonded aminosilica microspheres (G@ SiO_2) according to the method by Liu et al. [14], and then fabricated G@ SiO_2 and GO@ SiO_2 packed columns for the separation of both neutral and polar aromatic compounds by high performance liquid chromatography (HPLC). The separation was achieved by mixed actions of π-π stacking and electron-donor–acceptor interaction, hydrophobic effect and hydrogen bonding. Based on the potential of GO as stationary phases, much attention has been paid to the fabrication of GO-based open-tubular columns (OTC) and subsequent chromatographic applications. Qu et al. [19] firstly bonded GO onto the inner surface of capillary column (GO@OTC) using 3-aminopropyldiethoxymethyl silane as coupling agent. The GO@OTC was then transformed into graphene-bonded open-tubular column (G@OTC) through hydrazine reduction of GO. Both columns were applied for the separations of proteins, alcohols, polycyclic aromatic hydrocarbons and other aromatic substances by capillary electrochromatography (CEC) [19] and gas chromatography [20]. Gao et al. described another fabrication method for GO@OTC [21]. The inner wall of the silanized capillary was primarily grafted with tentacle-type polymer (glycidyl methacrylate (GMA)), then flushed with ammonium hydroxide, and finally bonded with GO through the reaction between amino groups of derivatized capillary and abundant reactive oxygen functional groups of GO. Zhang et al. presented a novel layer-by-layer strategy for graphene-based OT-CEC [22]. The column was prepared by first introduction of polyaniline (PANI) on the capillary wall as a sub-layer and next grafting with poly dopamine (PDA) following with GO to generate a multilayer GO-PDA-PANI@capillary. Thereafter, the preparation of similar GO-modified open-tubular capillary column for the CEC analysis of phenyl homologs and enantiomers was also reported by Xu et al. [23], and Ye et al. [24]. Liu et al. [25] reported another preparation method for the fabrication of GO-functionalized OTC columns. Positively charged poly(diallyldimethylammonium chloride) (PDDA) was firstly adsorbed on the inner surface of a bare capillary due to electrostatic attraction toward negatively charged silanolate. The negatively charged GO (owing to $-COO^-$) was then electrostatically deposited on the PDDA coating layer. The physical modified GO-OTC was also capable of distinguishing three acidic nitro-phenol isomers, three basic nitro-aniline isomers, and four neutral PAHs from each other.

In comparison with packed and silica-based monolithic capillary columns, polymer monolithic columns offer significant merits such as, large specific surface area, good separation efficiency and easy preparation. Wang et al. [26] firstly introduced GO into the monolith by one-step room temperature polymerization. GO sheets were primarily dissolved into cyclohexanol, and then mixed with ethyleneglycol dimethacrylate (EDMA), methacrylic acid, and HNO_3. The polymerization was initiated by nitric acid at room temperature and completed after 24 h. Although GO was immobilized in the monolith via physical adsorption, the GO-incorporated monolithic column still gave baseline separation of phenyl homolog and anilines. Tong et al. [27] reported GO decorated monolith via a three-step *in-situ* copolymerization for the analysis of sarcosine. *In-situ* copolymerization of glycidyl methacrylate and ethyleneglycol dimethacrylate in the capillary was first performed for preparing polymer GMA-EDMA monoliths. The poly GMA-EDMA monoliths were grafted with ethylenediamine based on condensation reaction between amino and carboxyl, followed by covalent attaching of GO to amine groups of poly(GMA-EDMA) monolith to obtain GO@poly(GMA-EDMA) monolithic column. The monolithic column afforded satisfactory enhancement factor (32-fold) and good recoveries. However, to the best of our knowledge, GO modified polymer monolith based on chemical bonding for electrochromatography has not been reported.

Polystyrene-based polymers have attracted much attention in HPLC because of high chemical stability and excellent mechanical property under a wide pH range, and their applications were extended from small molecules [28], to biomolecules such as peptide [29,30], protein [31], ribonucleic acid [32] and so on. If ionizable monomers such as ethenyl-benzenesulfonic acid, diallyldimethylammonium chloride, *etc*, were used in the polymerization mixture, negatively or positively charged functionalities may be decorated to neutral polystyrene monoliths, hence generating adequate electroosmotic flow (EOF). Thereby polystyrene-based monolith could be applied as the stationary phase for CEC [33–37]. Jin et al. [37] successfully prepared negatively charged polystyrene-based monolith by a single polymerization step using methyl methacrylate (MAA). However, MAA can only generate a stable EOF at pHs higher than 7.0, and thus this monolithic column is preferable for the analyses of basic compounds. Huang et al. [35] utilized vinylbenzenesulfonic acid as the EOF modifier and achieved complete separation of acidic analytes since the modifier provided enough EOF even at low pHs, providing the chance of polystyrene-based monolithic column to be applied for the analyses of acidic analytes.

In this work, we fabricated GO bonded monolithic column for CEC based on one-step copolymerization. GO was firstly grafted with vinyl groups based on condensation reaction between amino of 4-vinylaniline and carboxyl of GO. The vinylized GO (pAS-GO) was then copolymerized with styrene (St) and divinyl-benzene (DVB) in the mixture of cyclohexanol and toluene to produce GO covalent bonding monolith (pAS-GO@PS-DVB). The polymerization conditions were optimized and effects of CEC parameters on EOF were investigated. Analytical performance of the pAS-GO@PS-DVB column in CEC analysis was estimated by using neutral aromatic compounds. The feasibility of pAS-GO@PS-DVB in CEC was finally demonstrated by the separation of acidic phenolic compounds and basic anilines.

2. Experimental

2.1. Chemicals and reagents

All of the organic reagents were of analytical grade and used without further purification unless otherwise indicated. Ultrapure water with a resistivity of 18.2 MΩ cm prepared from a Milli-Q Plus water purification system (Millipore, Bedford, MA, USA) was used throughout. Thiourea, 3-(trimethoxysilyl)propyl methacrylate (γ-MPS), 2-acrylamido-2-methyl propane sulfonic acid (AMPS) and 2,2-azobisisobutyronitrile (AIBN) were obtained from Aladdin Chemistry (Shanghai, China). Disodium hydrogen phosphate ($Na_2HPO_4 \cdot 12H_2O$), sodium hydroxide, phosphoric acid and hydrochloric acid were also purchased from Aladdin Chemistry. Styrene, divinylbenzene, *p*-aminostyrene (pAS), *N*,*N*′-dicyclohexylcarbodiimide (DCC), benzene, naphthalene, fluorene, phenanthrene were all purchased from J & K Chemical Technology (Beijing, China). Toluene, cyclohexanol, *p*-nitroaniline, *o*-nitroaniline, *m*-nitroaniline, acetone, thiourea were provided by Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Acetonitrile (ACN), *N*,*N*′-dimethyl formamide (DMF), *o*-diphenol, *p*-diphenol, phenol and methanol from Amethyst Chemicals (Beijing, China) were also used. Fused silica capillaries of 100 μm i.d. × 365 μm o.d. were purchased from Yongnian Ruifeng Optical Fabric Factory (Handan, China).

The standard stock solutions of 1.0 mg mL^{-1} of all the analytes were prepared individually in ACN and stored at 4 °C in the fridge. The daily working solutions of standards were appropriately diluted from the above stock standard solutions with ACN. The electrolyte solutions (10 mM phosphate buffer solutions (PBS) at pH 5.0–9.0) were prepared by dissolving exact amounts of disodium hydrogen phosphate in ultrapure water with pH adjustment by using 1.0 M NaOH and 1.0 M phosphoric acid. Before use they were