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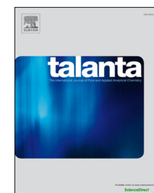

Rapid synthesis of three-dimensional sulfur-doped porous graphene via solid-state microwave irradiation for protein removal in plasma sample pretreatment

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ABSTRACT

In this work, we prepared three-dimensional sulfur-doped porous graphene (3D-SPG) via solid-state microwave method and first introduced it to plasma sample pretreatment as adsorbent for the removal of proteins. The efficient heating effect of solid-state microwave irradiation endowed the as-prepared 3D-SPG with large specific surface area, porous structures and sulfur-doped conjugated π electron surface, thus producing an outstanding adsorbent for proteins adsorption. The adsorption behavior of 3D-SPG towards proteins was explored using bovine serum albumin (BSA) as the model protein and several kinetic models and isotherm models were employed to describe the adsorption process. The results indicated that BSA was adsorbed onto 3D-SPG in a monolayer manner with high adsorption capacity, and chemisorption and intraparticle diffusion was the rate-controlling step in proteins adsorption process. By applying 3D-SPG as adsorbent to remove proteins in real rat plasma, we found that 3D-SPG solid phase extraction (SPE) gained exceedingly high protein removal efficiency compared with other plasma pretreatment methods, suggesting that 3D-SPG SPE could effectively prevent the deterioration of column performance and decrease the interference caused by matrix effect in the follow-up analysis. Furthermore, in comparison with the tandem mass spectra results between 3D-SPG SPE and methanol precipitation, 3D-SPG SPE demonstrated the ability to extract the protein-binding metabolites which usually could not be extracted by methanol precipitation. This ability made 3D-SPG SPE of great value in untargeted metabolomics profiling, because 3D-SPG SPE could be a complementary method to methanol precipitation to improve the coverage of metabolites.

1. Introduction

The metabolites in plasma offer the information concerning metabolism and human health [1,2]. In metabolomics studies, plasma sample preparation is one of the most challenging and error-prone parts of the analytical procedures, because of the deleterious effects of abundant proteins in the plasma sample, in which there are about 10,000 different proteins with the total concentration of 6–8 g dL^{-1} [3]. Methanol protein precipitation is the most commonly used technique for protein removal, because of it is simple to operate, consumes little time and can effectively extract metabolites [4]. However, a few drawbacks limit its applications. For example, methanol protein precipitation could not remove all the proteins from plasma [5], and the remaining proteins can still deteriorate column performance and interfere with the chromatographic procedures by generating matrix effects [5,6]. Additionally, in untargeted metabolomics studies, methanol protein precipitation would result in an incomplete metabolomics profiling because of the loss of some metabolites which were co-precipitating with plasma proteins [4,7]. In order to overcome the disadvantages of methanol protein precipitation and improve the coverage of metabolites in untargeted metabolomics profiling, new protocols which could efficiently remove proteins and extract the metabolites from metabolite-protein complexes need to be developed, because many metabolites prefer to form complexes with plasma proteins, and these metabolites are inclined to co-precipitate with proteins by using methanol protein precipitation [7].

Solid phase extraction (SPE), as an effective sample pretreatment method for eliminating interfering substances and enriching analytes, has been used to remove proteins in plasma [8]. Recently, new SPE adsorbents for plasma sample pretreatment have gained an increasing

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interest. Carbon-based nanomaterials are outstanding adsorbents because of their large specific surface area, high mechanical strength and chemical stability [9]. Many carbon nanomaterials have exhibited strong affinity towards proteins [10,11]. For example, Cuicui Ge et al. [10] reported that carbon nanotubes (CNTs) could adsorb proteins, and proteins adsorbed on the CNTs could maintain their conformational flexibilities. As the structures of proteins remains intact during the adsorption onto CNTs, the interactions between proteins and metabolites are reversible. The reversible interactions ensured the effective contact between elution solvent and protein-binding metabolites, so there would be more possibilities to extract these metabolites from plasma proteins. Therefore, carbon nanomaterials might be a potential adsorbent to remove proteins and extract metabolites which were bound to proteins. Cuicui Ge et al. [10] also pointed out that the main driving force in the adsorption process was the π-π stacking interactions between carbon nanomaterials and the aromatic residues (Trp, Phe, Tyr) of proteins. Additionally, many studies have been conducted on surface modifications of carbon nanomaterials in order to enhance their affinity towards proteins [11]. Noncovalent sulfur interactions have been proved to exist between the electron deficient bivalent sulfur atoms and electron donors, such as oxygen atoms, nitrogen atoms and π-systems [12,13]. Therefore, the addition of sulfur atoms into the carbon layer might further enhance the affinity towards proteins as these could potentially interact with abundant exposed carboxyl, hydroxyl and amino groups on the surface of proteins. Furthermore, the doped sulfur atoms might capture the cysteine residues through sulfur-sulfur bond [14,15], which is much stronger than other noncovalent interactions. Nowadays, three-dimensional (3D) carbon macrostructures have attracted great attention for their great preservation of the large accessible surface area of carbon sheets to ensure high adsorption capacity [16]. Thus, sulfur-doped carbon materials, especially with 3D structures, would have potential to exhibit high adsorption affinity and capacity towards proteins due to their high specific surface area and sulfur-doped conjugated π electron surface.

Chemical doping of sulfur into the carbon framework remains a big challenge, because of the similar electronegativity and different atom size between C and S [17]. Several techniques have been proposed for the synthesis of sulfur-doped carbon materials , mainly including chemical vapor deposition (CVD), solvothermal methods and thermal-annealing graphene oxides (GO) with heteroatom precursors under high temperature [17–19]. However, high vacuum or high temperature conditions during the above synthesis are usually inevitable and these harsh reaction conditions render them unsuitable for the large-scale production of sulfur-doped carbon materials. Furthermore, the direct thermal annealing process generally results in an irreversible stacking of carbon sheets due to the strong π- interactions. As a consequence, the specific surface area of the resulting carbon materials is low, which hampers their adsorption properties [20]. A protocol which can achieve sulfur doping and 3D porous structures simultaneously is needed.

Here, we proposed a simple strategy that has potential for rapid and large-scale preparation 3D sulfur-doped porous graphene (3D-SPG) from graphite via solvent-free solid-state microwave method, which took full use of the strong interactions between microwave and graphite [21]. The preparation of 3D-SPG was carried out by microwave irradiating H2SO4-graphite composite for a short time, which simultaneously achieved sulfur doping and 3D porous structures formation. Scanning electron microscope (SEM), nitrogen adsorption–desorption isotherms and X-ray photoelectron spectroscopy (XPS) were applied to investigate the morphology and modification of 3D-SPG. In this study, the as-prepared 3D-SPG was used as the SPE adsorbent for plasma sample pretreatment for the first time. The adsorbent exhibited high adsorption capacity towards proteins. In the analysis of real plasma sample, 3D-SPG showed the high protein removal efficiency and effective extraction of metabolites which could not be extracted by methanol precipitation method, indicating 3D-SPG would be a potential adsorbent for plasma pretreatment.

2. Experiments

2.1. Reagents and apparatus

Graphite powder (2000 mesh) and Coomassie Brilliant Blue G-250 were purchased from Aladdin Reagents (Shanghai, China). Bovine serum albumin (BSA) was obtained from Ruji Biotech. development Co., Ltd. (Shanghai, China). The chromatographic grade methanol was obtained from TEDIA (OH, USA). The rest reagents such as concentrated sulfuric acid, potassium permanganate ($KMnO_4$), 30% hydrogen peroxide (H_2O_2) solution, hydrochloric acid (HCl) and formic acid were of analytical grade and were purchased from KeLong Chemical Co. Ltd. (Sichuan, China).

The microwave irradiation was conducted by a microwave oven (LWMC-205, LingJiang Technology Development Co. Ltd., Nanjing, China). The characterizations of 3D-SPG were investigated by SEM (JSM-7600F, JEOL Ltd., Tokyo, Japan), XPS (ESCALAB250Xi, Thermo Fisher Scientific, Waltham, USA), automated surface area and pore size analyzer (Quadrasorb 2MP, Quantachrome, Boynton, USA) and zeta potential analyzer (173 Plus, Brookhaven Instruments Corporation, Holtsville, USA). The binding experiments were carried out in a vapor-bathing constant temperature vibrator (SHZ-82, Zhengrong Instrument, Jintan, China). The concentration of proteins was measured by UV–Vis spectrophotometer (Agilent Cary60, Agilent Technologies Inc., Santa Clara, USA). The selective adsorption of plasma proteins on 3D-SPG was evaluated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (all GE Healthcare Bio-Science, Uppsala, Sweden). Plasma sample was analyzed by HPLC-MS/MS system (containing two solvent pumps (LC-30 AD), an autosampler (SIL-30AC), a column oven (CTO-30A) and a triple quadrupole mass spectrometer (LCMS-8060) (all Shimadzu Scientific Instruments, Kyoto, Japan).

2.2. Preparation and characterization of 3D-SPG

The preparation of 3D-SPG involved two steps. Firstly, the preparation of H_2SO_4-graphite composite was conducted by an oxidant-assisted intercalation reaction [22,23]. 1 g graphite powder was put into 23 mL concentrated sulfuric acid, which had been cooled to 0 °C in an ice-bath. While maintaining vigorous stirring, 3 g $KMnO_4$ was added to the suspension. The rate of addition was controlled carefully to prevent the temperature of the suspension from exceeding 20 °C. The mixture was stirred in ice-bath for about 2 h until the mixture became pasty and dark green. Then 138 mL of water was slowly stirred into the mixture to avoid uncontrollable increase in temperature. The diluted suspension was treated with 30% H_2O_2 solution to reduce the residual permanganate and manganese dioxide to colorless soluble manganese sulfate. The suspension was filtered and washed three times with 2 M HCl and water respectively. The H_2SO_4-graphite composite was dried at 50 °C in vacuo for 6 h. Afterwards, the dry composite was placed in an open reactor located in a microwave oven. 3D-SPG was obtained by treating the composite with microwave irradiation at 800 W for 5 s.

The morphology and microstructure of the as-synthesized sample were investigated by SEM. The elemental compositions and sulfur-bonding configurations in the as-prepared 3D-SPG was investigated by XPS. Nitrogen adsorption–desorption isotherms and BET surface area were measured by an automated surface area and pore size analyzer. The zeta potential of 3D-SPG in the PBS buffer solution (used in binding experiments, 0.1 M, pH = 7.4) was measured by a zeta potential analyzer.

2.3. Binding experiments

The adsorption properties of 3D-SPG towards protein were investigated using BSA as the model protein. All the binding experiments were carried out in eppendorf tube, and the samples were shaken with constant agitation (100 rpm) and constant temperature (37 °C). Before

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