



Magnetic solid-phase extraction of protein by ionic liquid-coated Fe@graphene oxide

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ABSTRACT

Amino functional dicationic ionic liquid (AFDCIL) was prepared and then coated on the surface of magnetic graphene oxide (GO) as a new magnetic adsorbent (Fe@GO@AFDCIL) for the magnetic solid-phase extraction (MSPE) of protein. The Fe@GO@AFDCIL composite was characterized by vibrating sample magnetometer (VSM), X-ray diffraction (XRD), Fourier transform infrared spectrometry (FT-IR), thermal gravimetric analysis (TGA), field emission scanning electron microscopy (FESEM) and zeta-potential nanoparticles. The bovine hemoglobin (Bhb) was used as the analyte, and the extraction performance of Fe@GO@AFDCIL was investigated in the MSPE procedure. The concentration of Bhb in samples was determined by a UV–vis spectrophotometer. A comparative investigation of Fe@GO@AFDCIL composite and traditional IL-coated Fe@GO composites (Fe@GO@IL) exhibited the benefits of Fe@GO@AFDCIL. The adsorbed Bhb could be eluted from the Fe@GO@AFDCIL by 4% sodium dodecyl sulfate (SDS) solution. The Fe@GO@AFDCIL exhibited favorable stability which could be reused at least 15 times. Under the optimized condition, the real samples were investigated, which demonstrated that the Fe@GO@AFDCIL was able to be applied in extracting bovine hemoglobin (Bhb) from real biological samples.

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1. Introduction

Proteins are primary components of human body. Many of the researches have devoted to separating and purifying various kinds of protein samples (for example hormone, antibody and plasma proteins). Proteins are of large amphiphilic and intrinsically surface active [1]. They can be extracted by intermolecular forces including strong hydrophobic, π - π and electrostatic interaction. Furthermore, the protein-surface interactions were governed by the nature of protein and the surface characteristics [2].

Several separation methods have been applied for extraction of protein, such as solid-phase extraction, liquid-phase extraction [3], gel electrophoresis [4], reverses micelles extraction [5], molecular imprinting [6] and two aqueous phase extraction [7]. In addition, a novel magnetic solid-phase extraction (MSPE) method, which uses magnetic materials as the adsorbents, has great potential for extraction of proteins from mixed samples [8].

MSPE is an improved solid-phase extraction method, in which the adsorbent has a directly impact on the performance of extraction process. The magnetic adsorbent can be modified with

special functionalities, thus improving the selectivity, affinity and capacity of the extraction procedures [9]. Functional materials, which consisted of ionic liquids (ILs) and magnetic composite, presented excellent properties for extraction [10].

The common magnetic particles are Fe, Fe₃O₄ and γ -Fe₂O₃. Fe particles have high specific surface area and large number of metal sites on its surface [11,12]. Based on these advantages, the Fe particles show a great prospect in MSPE. However, Fe particles are likely to aggregate and oxidize that limit its application. Modifying the surface of magnetic particles is a classical way to conquer these limitations. Generally, the modified materials are GO [13], silica [14], carbon nanotubes [15] and nanoporous carbons [16].

It is generally known that GO has excellent electrical properties, thermostability, favorable biocompatibility and superior high surface areas [17,18]. GO possesses plentiful reactive functional groups at its basal plane or sheet edge. These functional groups are inclined to establish π - π , Van der Waals interactions and hydrogen bond with proteins, which show great promise for extraction application. Modifying Fe particles with GO is not only capable of improving the specific surface area and the extraction capacity of adsorbent, but also preventing Fe particles from being oxidized and agglomerating [19]. However, previous studies have emphasized that only modified the magnetic particles with GO was deficient due to its poor dispersity in solutions [20,21].

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ILs are organic salts with a melting point of 100 °C or less, which are widely regarded as promising solvents and functional materials [22–24]. ILs possess many beneficial properties, such as high thermal and chemical stability, negligible vapor pressure, nonflammability and electroconductivity [25–27]. Besides, ILs have attracted much attention as tunable and green functional materials for MSPE [28,29]. The dicationic ILs are formed of two positively charged moieties anchored to a central core and corresponding anions. Compared with traditional monocationic ILs, dicationic ILs have many beneficial features for extraction. In addition, magnetic GO composite can be modified with ILs. It would be possible to improve the selectivity and the solubility of materials [30].

In this study, the Fe particles were covered by GO to form magnetic GO (Fe@GO), and then the Fe@GO composite was modified with AFDCIL (Fe@GO@AFDCIL). The Fe@GO@AFDCIL composite was applied as the magnetic adsorbent for extraction of protein. Bovine hemoglobin (Bhb) was to serve as the model protein. The extraction performance of the Fe@GO@AFDCIL in the MSPE procedure was investigated. Subsequently, the Bhb could be eluted from the adsorbent with 4% SDS solution and the concentration was probed by UV–vis spectrophotometer at 406 nm.

2. Experimental

2.1. Apparatus

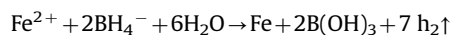
All products were dried by a DZF-6051 vacuum drying oven (Shanghai, China) and a FD-1C-50 vacuum-freezing drier (Beijing, China). The MSPE procedure was conducted in a QYC200 incubator shaker (Shanghai, China). New synthesis of ILs were investigated by INOVA 400NB NMR (Varian, America) and Vario EL III (ELEMENTAR, Germany). The magnetism of sorbents was studied by EV11 Vibrating Sample Magnetometer (MicroSense, USA). The morphology of GO and magnetic sorbents were obtained using a MIRA3 LMU field emission scanning electron microscopy (FESEM, TESCAN, Czech). Infrared spectrum was recorded using a Spectrum One FT-IR spectrometer (PerkinElmer, USA). Thermal gravimetric analysis was studied by a STA 449C thermal gravimetric analyzer (Netzsch, Germany). X-ray diffraction pattern was achieved using a D/Max 2500 X-ray diffraction (Rigaku, Japan). Zeta potentials were studied by a Zetasizer Nano-ZS90 dynamic light scattering (Malvern, Britain). Ultraviolet absorption spectrum was studied by a UV2450 UV–vis spectrophotometer (Shimadzu, Japan). Secondary structure of protein was proved by a Mos-500 circular dichroism (CD) spectrometer (Biologic, France).

2.2. Chemicals and reagents

All chemical reagents in this work were of analytical grade. $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, polyethylene glycol (PEG-4000), ethanol, graphite powder, H_2SO_4 , KMnO_4 , H_2O_2 (30%), HCl, N,N,N',N'-tetramethylethylenediamine, sodium dodecyl sulfate (SDS), n-butyl bromide and bovine hemoglobin (Bhb) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). KBH_4 , NaNO_3 and NaOH were supplied by Taishan Chemical Co., Ltd. (Guangdong, China). Bovine blood sample and porcine blood sample was obtained from Jiaozuo biological technology Co., Ltd. (Jiaozuo, China). Poly (diallyldimethylammonium chloride, PDDA) solution (20%) was purchased from Aladdin (Shanghai, China). 2-Chloroethylamine hydrochloride, 3-chloropropylamine hydrochloride, 1,1,3,3-tetramethylguanidine and N,N-dimethylethylamine were achieved from Adamas Reagent Co., Ltd. (Shanghai, China).

2.3. Synthesis of Fe particles and GO

The preparation of Fe particles was referring to the literature [31], which was generated on the basis of following equation.



Firstly, 2.78 g of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ and 0.5 g of PEG-4000 were completely dissolved in 30 mL alcohol-water system (ethanol: ultrapurewater, 4:1), then bubbled with high-purity nitrogen and mixed with a stirring speed of 2000 rpm for 20 min. Secondly, 21 mL of KBH_4 mixed solution (20 mL 0.02 mol/L KBH_4 and 1 mL 0.5 mol/L NaOH) was added into the above mixture at the rate of 2 drops per second. Finally, after reaction for 30 min the production was washed with water and ethanol three times each respectively, and then vacuum drying.

GO were synthesized by improved hummers method [32]. 0.5 g of graphite, 0.5 g of NaNO_3 and 23 mL of H_2SO_4 were mechanically stirred at ice water bath for 30 min 3 g of KMnO_4 was added slowly then continue to stir 2 h. Raised temperature to 35 °C and mixed for further 30 min, then added 100 mL ultrapure water gradually. The reaction was kept at 95 °C for 30 min. At last, terminated the reaction by adding 3 mL of H_2O_2 (30%) to the solution which finally turned to yellow. After cooling, the yellow suspension was centrifuged. Washed with 1 mol/L HCl until the SO_4^{2-} ions were removed thoroughly. And further washed with ultrapure water to make product neutral. In the end, freeze-dried loose brown power was obtained.

2.4. Synthesis of Fe@GO composite

The Fe@GO composite was synthesized by electrostatic interaction. 0.06 g of ready-prepared Fe particles were ultrasonically dissolved into PDDA solution (2 mg/mL) for 10 min and then stood for additional 15 min. Next, the magnetic composite were ultrasonically washed by ultrapure water for 3 times. Eventually positive charged PDDA-modified Fe particles were prepared. There are lots of oxygen-containing functional groups on the surface of the ready-prepared GO, so GO was able to cover PDDA-modified Fe particles. 0.06 g of ready-prepared GO was dispersed in 120 mL ultrapure water via ultrasonication for 2 h, and then centrifuged to remove impurities. 30 mL of PDDA-modified Fe particles dispersion (2 mg/mL) were gradually added into above GO suspension with a mild stirring for 1 h. The Fe@GO composite was obtained by magnetic isolation, washed with ultrapure water for 3 times and vacuum drying.

2.5. Synthesis of ILs

In this paper, we have synthesized five kinds of ionic liquids as following: AFDCIL (1,2-Ethanediaminium, N,N'-bis(2-aminoethyl)-N,N,N',N'-tetramethyl-, chloride (1:2)); IL₂ (Guanidine, N''-(2-aminoethyl)-N,N,N',N'-tetramethyl-, chloride (1:1)); IL₃ (Guanidine, N''-(3-aminopropyl)-N,N,N',N'-tetramethyl-, chloride (1:1)); IL₄ (Guanidine, N''-butyl-N,N,N',N'-tetramethyl-, chloride (1:1)); IL₅ (1-Butanaminium,N-ethyl-N,N-dimethyl-, chloride (1:1)). Three of the amino-functional ionic liquids were prepared according to the literature [33]. Taking synthesis of AFDCIL for example (Fig. 1), 14.9 mL of tetramethylethylenediamine (0.1 mol) and 23.2 g of

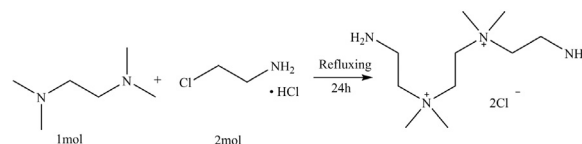


Fig. 1. Synthesis of ionic liquids (AFDCIL for example).

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