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Magnetic graphene oxide modified with choline chloride-based deep eutectic solvent for the solid-phase extraction of protein

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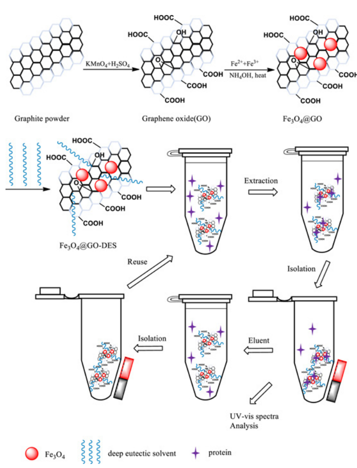
HIGHLIGHTS

- A strategy for extraction of protein based on DES-coated magnetic graphene oxide.
- The deep eutectic solvents were based on choline chloride.
- Bovine serum albumin was used as the analyte.
- The material prepared works for the acidic but not the basic or the neutral proteins.

GRAPHICAL ABSTRACT



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ABSTRACT

Four kinds of green deep eutectic solvents (DESs) based on choline chloride (ChCl) have been synthesized and coated on the surface of magnetic graphene oxide (Fe_3O_4@GO) to form Fe_3O_4@GO-DES for the magnetic solid-phase extraction of protein. X-ray diffraction (XRD), vibrating sample magnetometer (VSM), Fourier transform infrared spectrometry (FTIR), field emission scanning electron microscopy (FESEM) and thermal gravimetric analysis (TGA) were employed to characterize Fe_3O_4@GO-DES, and the results indicated the successful preparation of Fe_3O_4@GO-DES. The UV–vis spectrophotometer was used to measure the concentration of protein after extraction. Single factor experiments proved that the extraction amount was influenced by the types of DESs, solution temperature, solution ionic strength, extraction time, protein concentration and the amount of Fe_3O_4@GO-DES. Comparison of Fe_3O_4@GO and Fe_3O_4@GO-DES was carried out by extracting bovine serum albumin, ovalbumin, bovine hemoglobin and lysozyme. The experimental results showed that the proposed Fe_3O_4@GO-DES performs better than Fe_3O_4@GO in the extraction of acidic protein. Desorption of protein was carried out by eluting the solid extractant with 0.005 mol L^{-1} Na_2HPO_4 contained 1 mol L^{-1} NaCl. The obtained elution efficiency was about 90.9%. Attributed to the convenient magnetic separation, the solid extractant could be easily recycled.

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1. Introduction

Protein is an essential material of organisms. It plays an important role in life activities [1]. It is extremely necessary to obtain the protein that contains no harmful levels of contaminants. Whereas, the target protein always mix with other impurities, along with the self-owned particularity and complexity of protein, which make the degeneration of protein being extremely easy to occur during the separation and purification process. Consequently, the separation and purification of proteins become the bottleneck of biotechnology industry development.

Separation methods based on magnetic materials have attracted widespread attention in recent years [2,3]. The magnetic materials used in solid phase extraction (SPE) do not need to pack into the cartridge, and the problems of high pressure in SPE column can be prevented. Furthermore, the centrifugation or filtration step, which is time-consuming and requires additional apparatuses, is not needed [4]. The use of external magnetic field in magnetic SPE (MSPE) can save processing time [5]. Obviously, MSPE can improve the extraction efficiency and simplify the process of preprocessing [6,7]. Additionally, the interfacial area between sorbents and analytes increases due to the use of dispersive extraction mode, and the vast majority of magnetic sorbents can be easily recycled and reused subsequently [8,9]. MSPE shows a comprehensive advantage of simplicity and saving time and labor, which makes it a technique with bright application prospect for the sample preparation [10,11].

In MSPE, it is particularly important to select appropriate sorbent in order to obtain high recovery. In recent years, intense interest has grown in graphene oxide (GO) as novel sorbents in analytical chemistry [12]. GO possesses a large number of oxygenated groups both on the basal plane and at the edges of GO sheets, such as carboxyl and hydroxyl [13]. The existence of these functional groups makes GO more hydrophilic. Moreover, these functional groups can form hydrogen bonding or electrostatic interaction with adsorbates containing oxygen- and nitrogen-functional groups [12]. Furthermore, GO possesses huge surface area. As a consequence, GO seems to be ideal sorbents in SPE and it is potential for the efficient extraction of proteins. However, GO is hard to be separated from aqueous solution. To overcome this drawback, the combination of magnetic materials with GO to form composites have become a research hotspot [14]. Due to the large surface area of GO, there is a platform available for loading magnetic nanoparticles (MNPs). Among the widely used MNPs, Fe_3O_4 nanoparticles are well received because of their low cost and low toxicity [15]. Combined with Fe_3O_4, magnetic GO (Fe_3O_4@GO) will get new and/or enhanced functionalities which cannot be achieved by either component alone [14] and show great promise for MSPE applications. Recently, magnetic GO has been reported to be used as adsorbent toward heavy metal ions (such as cobalt [16], copper [17], arsenic [18] and mercury [19]) and dye [20,21]. In addition, there is research focused on magnetic GO as adsorbent toward simultaneous removal of heavy metal ions and dyes [22].

Ionic liquids (ILs) as novel green solvents have become one of the hotspots of green chemistry. That is because ionic liquids have unique properties and special characters, which are different from the traditional solvent. A new kind of IL analogues, which is defined as deep eutectic solvent (DES), has emerged to address the shortcomings of ILs that include high cost and toxicity. It has been found that DESs have similar characteristics and properties to ILs [23]. In general, two or three cheap and safe components, which can be associated with each other through hydrogen bond interactions, are capable of forming a DES [24]. One of the most common components used in DES is a quaternary ammonium salt, which is called choline chloride (ChCl). That is because of the fact that ChCl is very cheap, biodegradable and non-toxic. In addition, ChCl can be obtained either by means of extracting from biomass or by synthesizing from fossil reserves through a very high atom economy process [25]. Combined with another component that includes hydrogen bond containing functional groups (called hydrogen bond donors), such as urea, acid amides, carboxylic acids and polyols, ChCl is easy to form a DES. DESs based on ChCl present many advantages as compared with traditional ILs [26], such as it is relatively simple to synthesis, further purification is not needed, the majority of them are biodegradable [27], biocompatible [28] and non-toxic [29]. What is more, the atom utilization rate in the synthesis process can reach 100%, absolutely conforming to the requirements of the atom economy of green chemistry. Therefore, it is worthwhile to study DESs, making it serve various fields better.

Materials functionalized with ionic liquids (ILs) are receiving considerable attention. During the last five years, a few studies have been focused on ionic liquid-coated magnetic GO as adsorbents for extraction of heavy metal ions [30], nitrobenzene compounds [31] and proteins [32]. Although ILs lose liquid state when immobilized on the surface of GO, the other unique properties such as polarity and low volatility are maintained [33]. Moreover, the introduction of ionic liquid moieties could improve the performance of the resulting materials and attract a broad range of applications [34]. For this reason, the introduction of DES into magnetic GO is expected to increase the water-solubility of magnetic GO and improve the extraction efficiency of proteins.

In this paper, four kinds of deep eutectic solvents, which are based on ChCl, have been prepared and coated on the surface of magnetic GO to form Fe_3O_4@GO-DES. To our knowledge, it is the first time that DESs derived from ChCl are applied to the modification of magnetic GO. The resulting DES-coated Fe_3O_4@GO has been used for magnetic solid-phase extraction of proteins. Bovine serum albumin (BSA) was chosen as the model protein due to its relatively high structural stability [35]. After extraction, the concentration of the protein in the supernatant was measured by the UV–vis spectrophotometer at 278 nm. The presented Fe_3O_4@GO-DES-MSPE technique has also been used to extract ovalbumin (OVA), lysozyme (Lys) and bovine hemoglobin (BHb).

2. Experimental

2.1. Apparatus

The main instruments: UV-2450 UV–vis spectrophotometer (Shimadzu, Japan); FT-IR spectrometer (PerkinElmer, USA); JSM-6700F field emission scanning electron microscopy (JEOL, Japan); STA 409 thermal gravimetric analyzer (Netzsch, Germany); EV11 Vibrating Sample Magnetometer (MicroSense, USA); D/Max 2500 X-ray diffraction (Rigaku, Japan); Mos-500 circular dichroism (CD) spectrometer (Biologic, France); incubator shaker (QYC 200; FuMa Experimental Equipment Co., Ltd., Shanghai, China); ultrapure water instrument (RM 220; LiDe Experimental Equipment Co., Ltd., Shanghai, China).

2.2. Chemicals and reagents

All reagents used were of at least analytical grade and needed no further purification. Graphite powder, $KMnO_4$, $BaCl_2$, NaCl, Na_2HPO_4, H_2O_2 (30%), $FeCl_3{\cdot}6H_2O$, $FeSO_4{\cdot}7H_2O$, ammonium hydroxide, methanol, D-(+)-glucose, urea, glycerol and bovine serum albumin (BSA) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). $NaNO_3$ was supplied by Taishan Chemical Co., Ltd. (Guangdong, China). Concentrated sulfuric acid and hydrochloric acid were obtained from Zhuzhou Star Glass Co., Ltd. (Hunan, China). Hydrazine hydrate (80%) was purchased from