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# Magnetic solid-phase extraction of protein with deep eutectic solvent immobilized magnetic graphene oxide nanoparticles

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ABSTRACT

As a new type of green solvent, four kinds of choline chloride (ChCl)-based deep eutectic solvents (DESs) have been synthesized, and then a core–shell structure magnetic graphene oxide ($Fe_3O_4$–$NH_2$@GO) nanoparticles have been prepared and coated with the ChCl-based DESs. Magnetic solid-phase extraction (MSPE) based $Fe_3O_4$–$NH_2$@GO@DES was studied for the first time for the extraction of proteins. The characteristic results of vibrating sample magnetometer (VSM), X-ray diffraction (XRD), Fourier transform infrared spectrometry (FT-IR), thermal gravimetric analysis (TGA) and field emission scanning electron microscopy (FESEM) indicated the successful preparation of $Fe_3O_4$–$NH_2$@GO@DES. The concentrations of proteins in studies were determined by a UV–vis spectrophotometer. The advantages of $Fe_3O_4$–$NH_2$@GO@DES in protein extraction were compared with $Fe_3O_4$–$NH_2$@GO and $Fe_3O_4$–$NH_2$, and $Fe_3O_4$–$NH_2$@GO@ChCl–glycerol was selected as the suitable extraction solvent. The influence factors of the extraction process such as the pH value, the temperature, the extraction time, the concentration of protein and the amount of $Fe_3O_4$–$NH_2$@GO@ChCl–glycerol were evaluated. Desorption experimental result showed 98.73% of BSA could be eluted from the solid extractant with 0.1 mol/L $Na_2HPO_4$ solution contained 1 mol/L NaCl. Besides, the conformation of BSA was not changed during the elution by the investigation of circular dichromism (CD) spectra. Furthermore, the analysis of real sample demonstrated that the prepared magnetic nanoparticles did have extraction ability on proteins in bovine whole blood.


## 1. Introduction

Proteins are materials basis of life that play important roles in various forms of life activities. As a kind of biomacromolecules, proteins also have wide applications in fields of research, pharmaceuticals and industrials. So it is significant to prepare pure proteins. However, proteins are sensitive to extreme conditions, which make proteins easily denatured during separation process. Thus, separation and purification of proteins has been a major obstacle of modern biotechnology.

Aqueous two-phase system has been widely applied to protein purification due to its superiority of moderate extraction environment, high extraction efficiency and high biocompatibility in recent years [1–3]. However, proteins which obtained in an aqueous two-phase system are difficult to recycle [4,5]. Under this circumstance, magnetic solid-phase extraction (MSPE), which is based on the application of magnetic or magnetically modified adsorbents, has emerged as an alternative method [6]. In MSPE, magnetic adsorbents are dispersed into sample solution, and the magnetic nanoparticles with captured analytes can be directly separated from sample matrix by using an external magnetic field without additional centrifugation or filtration, which makes separation more simple and effective [7]. Besides, the analytes can be readily eluted from the magnetic adsorbents so that the recovery of analytes and adsorbents are available. Due to these fantastic properties, increased attention has been paid to the development and applications of MSPE [8–10].

The selection of adsorption material is particularly critical for the efficient extraction in MSPE. A variety of solids have already been used as the adsorbents [11]. And graphene oxide (GO) is gaining increasing interest as a promising sorbent material owing to its hydrophilicity, ultrahigh surface area and high dispersion stability [12,13]. GO possess large delocalized π-electron system and much polar oxygen functional groups, such as hydroxy, carboxy and expoxy groups, which enables GO interact with analytes through π–π, hydrogen bonding and electrostatic interactions. For this sake, GO have provided an efficient platform for the separation of biomolecules [14,15], pollutants [16,17] and drugs [18]. Besides, magnetic nanoparticles can be introduced into GO because of the large surface area of GO, which greatly facilitate the separation process. $Fe_3O_4$ is the most frequently used magnetic nanoparticles due to its superparamagnetism, low toxicity and

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ease of preparation [19]. $Fe_3O_4$ deposited GO ($Fe_3O_4$@GO) composites can be easily got by using chemical coprecipitation method and have been widely employed in MSPE in previous works [20,21]. According to the literature [6,22], electrostatic layer-by-layer self-assembly could be applied to the modification of magnetic nanoparticles. Herein we suggested a novel strategy for the fabrication of core–shell structure $Fe_3O_4$–$NH_2$@GO nanoparticles through electrostatic assembly between positively charged 3-aminopropyltriethoxysilane (APTES)-modified $Fe_3O_4$ particles and negatively charged GO, which could keep $Fe_3O_4$ nanoparticles from exposing themselves to external environment.

Recently, ionic liquid (IL)-functionalized magnetic GO composites are drawing increasing attentions as adsorbent materials. Due to the excellent properties of wide solubility and polarity of IL, the introduction of IL into magnetic GO is helpful for the polydisperse and the stability of the complexes in various matrices [23]. Based on this, IL-modified magnetic GO composites have achieved different degrees of success in removal of heavy metal ions from water and extraction of proteins [9,24]. However, the complex synthesis and the toxicity of IL have impeded its large-scale application.

Given the similar physico-chemical properties of deep eutectic solvent (DES) to IL, DES is expected to be a substitute of IL. DES is a eutectic mixture which consists of substituted quaternary ammonium salts and hydrogen bond donors [25]. Choline chloride (ChCl) is one of the most common quaternary ammonium salts used in DES as ChCl is cheap and can be easily extracted from biomass. Compared to traditional ILs, DESs derived from ChCl are non-toxic, biocompatible and biodegradable. Besides, the atom utilization rate in synthesis process is 100% and no further purification is needed. Because of these excellent characteristics, ChCl-based DESs have received considerable interest for its potential applications in fields of organic reaction [26,27], biodiesel preparation [28,29] and nanomaterials construction [30,31].

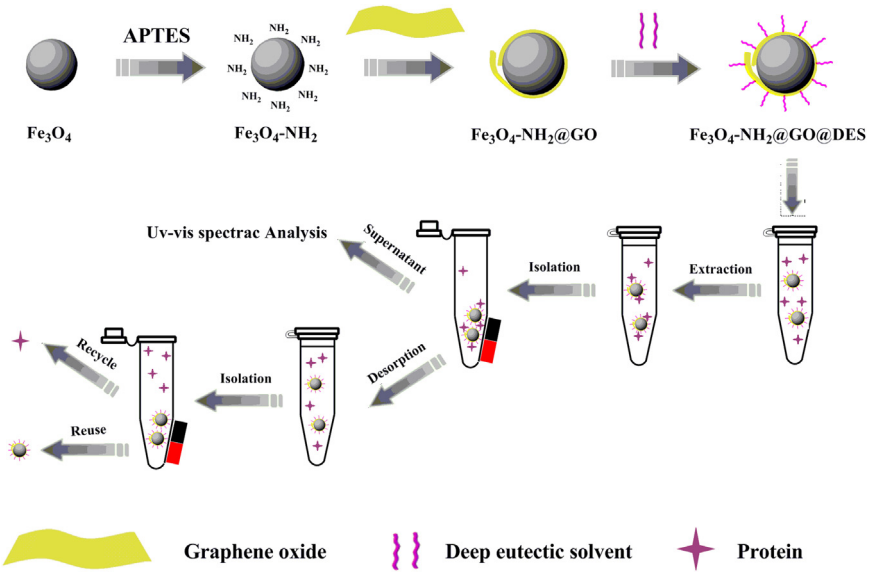
In this paper, four kinds of ChCl/alcohols-based DESs have been synthesized and coated on the surface of core–shell structure $Fe_3O_4$–$NH_2$@GO nanoparticles for the magnetic solid-phase extraction of proteins (as shown in Scheme 1). $Fe_3O_4$–$NH_2$@GO@ChCl–glycerol was chosen to study the affecting factors of the extraction process. VSM, XRD, FT-IR, TGA and FESEM were used to characterize the proposed magnetic nanoparticles. The analysis of real sample and desorption experiment were further investigated.

## 2. Experimental

### 2.1. Materials and apparatus

All materials used in this study were of analytical grade. $FeCl_3 \cdot 6H_2O$, $FeSO_4 \cdot 7H_2O$, ammonium hydroxide, graphite powder, $KMnO_4$, $BaCl_2$, $H_2O_2$ (30%), ethylene glycol, glycerol and Bovine serum albumin (BSA), Ovalbumin (OVA), Lysozyme (Lyz), Bovine hemoglobin (BHb) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Bovine whole blood sample was purchased from Jiaozuo biological technology Co. Ltd. (Jiaozuo, China). Hydrazine hydrate (80%) was obtained from Shanghai Shanpu Chemical Co., Ltd. (Shanghai, China). 3-aminopropyltriethoxysilane (APTES) was purchased from Sun Chemical Technology Co., Ltd. (Shanghai, China). $NaNO_3$ was supplied by Taishan Chemical Co., Ltd. (Guangdong, China). Concentrated sulfuric acid and hydrochloric acid were purchased from Zhuzhou Star Glass Co., Ltd. (Hunan, China). Choline chloride(Shanghai Source Biological Technology Co., Ltd.), D-(+)-glucose (Sinopharm Chemical Reagent Co., Ltd.) and D-sorbitol solution (Kermel Chemical Reagent Co., Ltd.) were dried under vacuum prior to use. Ultrapure water was used throughout the study.

Materials were dried by a DZF-6051 vacuum drying oven (Shanghai,China) and a FD-1C-50 vacuum-freezing drier (Beijing, China). The extraction process was completed in a QYC200 incubator shaker (Shanghai,China). EV11 Vibrating Sample Magnetometer (MicroSense, USA) was used to determine the magnetism of adsorbent. X-ray diffraction pattern was obtained using a D/Max 2500 X-ray diffraction (Rigaku, Japan). Infrared spectrum was recorded using a Spectrum One FT-IR spectrometer (PerkinElmer, USA). Thermogravimetric analyses were performed by a STA 409 thermal gravimetric analyzer (Netzsch, Germany) to analyze thermal behavior of the adsorbent. Microstructure of sample was examined using a MIRA3 LMU field emission scanning electron microscopy (FESEM, TESCAN, Czech). Zeta potentials were determined by a Zetasizer Nano-ZS90 dynamic light scattering (Malvern, Britain). Ultraviolet absorption spectrum was measured by a UV2450 UV–vis spectrophotometer (Shimadzu, Japan). Secondary structure of protein was determined by a Mos-500 circular dichroism (CD) spectrometer.




**Scheme 1.** Fabrication of $Fe_3O_4$–$NH_2$@GO@DES and its application in MSPE of protein.