Analytica Chimica Acta 861 (2015) 36–46

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Preparation of magnetic chitosan and graphene oxide-functional guanidinium ionic liquid composite for the solid-phase extraction of protein

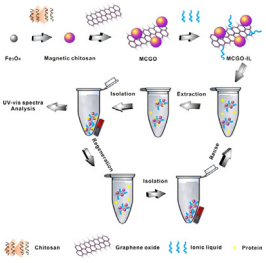
Xueqin Ding, Yuzhi Wang *, Ying Wang, Qi Pan, Jing Chen, Yanhua Huang, Kaijia Xu

State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Hunan University, Changsha 410082, PR China

HIGHLIGHTS

- A strategy for the solid-phase extraction of protein based on magnetic chitosan and graphene oxide-functional guanidinium ionic liquids.
- Trypsin, lysozyme, ovalbumin and bovine serum albumin were used as the analyst.
- The possibility of reusability and regeneration has been evaluated.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 13 October 2014
Received in revised form 1 December 2014
Accepted 4 January 2015
Available online 7 January 2015

Keywords:
Functional guanidinium ionic liquid
Magnetic chitosan and graphene oxide-ionic liquid
Protein
Magnetic solid-phase extraction
Regeneration

ABSTRACT

A series of novel cationic functional hexaalkylguanidinium ionic liquids and anionic functional tetraalkylguanidinium ionic liquids have been synthesized, and then magnetic chitosan graphene oxide (MCGO) composite has been prepared and coated with these functional guanidinium ionic liquids to extract protein by magnetic solid-phase extraction. MCGO-functional guanidinium ionic liquid has been characterized by vibrating sample magnetometer, field emission scanning electron microscopy, X-ray diffraction spectrometer and Fourier transform infrared spectrometer. After extraction, the concentrations of protein were determined by measuring the absorbance at 278 nm using an ultra violet visible spectrophotometer. The advantages of MCGO-functional guanidinium ionic liquid in protein extraction were compared with magnetic chitosan, graphene oxide, MCGO and MCGO-ordinary imidazolium ionic liquid. The proposed method has been applied to extract trypsin, lysozyme, ovalbumin and bovine serum albumin. A comprehensive study of the adsorption conditions such as the concentration of protein, the amount of MCGO-functional guanidinium ionic liquid, the pH, the temperature and the extraction time were also presented. Moreover, the MCGO-functional guanidinium ionic liquid can be easily regenerated, and the extraction capacity was about 94% of the initial one after being used three times.

1. Introduction

Solid-phase extraction (SPE) is the most widely used preconcentration technique mainly because of the variety of different materials employed as sorbents [1,2]. Many problems associated with liquid–liquid extraction, such as the expensive and incomplete phase separations, breakable specialty glassware, less-than-quantitative recoveries, and disposal of large quantities of organic solvents, can be circumvented by using SPE [3]. However, tedious

* Corresponding author at: State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Hunan University, Changsha 410082, PR China. Tel.: +86 731 88821903; fax: +86 731 88821848.
E-mail address: wyzss@hnu.edu.cn (Y. Wang).

http://dx.doi.org/10.1016/j.aca.2015.01.004
0003-2670/

centrifuge steps make traditional SPE very time-consuming. Magnetic solid-phase extraction (MSPE) methods can be seen as novel derivatives of SPE and could overcome this shortcoming [4].

Magnetic adsorbent plays a vital role in the MSPE procedure. The adsorbents with a magnetic core need not be packed into the SPE cartridges, and the centrifuge steps can be substituted by an external magnetic field to achieve solid–liquid separation [5]. MSPE has been proved to be an efficient technique to extract organic and inorganic analytes from complex media. And it is a relatively simple, rapid, and reliable technique in sample preparation [6–8].

Chitosan is an increasingly popular surface modification material used for the preparation of magnetic particles as adsorbents. It is a basic polysaccharide polymer which has bifunctional groups and accordingly possesses unique physiological activity and physico-chemical properties [9]. Chitosan can be obtained from a wealth of sources and is a kind of renewable resource. It also can be easily functionalized, activated and coupled because of a multitude of chemically active amino and hydroxyl groups rich in its molecular structure [10]. In addition, chitosan is nontoxic and has good biodegradability and biocompatibility, so it can be widely used in life sciences. Coupled with Fe_3O_4, magnetic chitosan will have the advantages of both and then has a more wide range of applications [11,12]. So far, there exist many researches on magnetic chitosan which demonstrated chitosan is a kind of good surface modification material [13–16].

Graphene, a crystalline allotrope of carbon with 2-dimensional properties, has attracted a mass of attentions as a potential biomaterial owing to its physico-chemical properties, including the large surface area, high dispersibility and hydrophilicity [17–19]. Graphene oxide (GO) contains a wide range of oxygen functional groups both on the basal planes and at the edges of GO sheets, such as —COOH and —OH [20]. These functional groups are expected to promote interfacial interactions between GO and adsorbate, which are essential for the efficient extraction of proteins. Recently, some researchers have explored the possibility of chitosan–GO composite as bio adsorbents. Where the carboxyl group of GO chemically reacts with the amine group of chitosan and consequently form chemical bond between GO and chitosan [21,22]. However, the chitosan–GO composite still has some disadvantages, such as hard to be separated and easy to aggregate. Therefore, it is very important to develop a kind of novel material to overcome these shortcomings and to efficiently extract biomolecules.

Ionic liquids (ILs) are widely recognized solvents due to their excellent properties. They can be designed to be environmentally benign, with large potential benefits for sustainable chemistry [23]. Nowadays research on ILs is booming, but research on the functional guanidinium ILs is still in the initial stage. Guanidinium IL is a new member of ILs family. Owing to higher thermal stability, better catalytic activity, and stronger biological activity, guanidine compounds have attracted a large number of attentions of pharmaceutical scientists and chemists [24]. Especially in life support system, guanidine group is an emphasis of the life science research because it embraces unique molecular recognition function. Moreover, the high dispersive degree of the cationic parts, the adjustability of the three nitrogen-atoms and other characteristic properties making the synthesis and the application of guanidine salt increasingly popular [25]. Functional guanidinium IL not only has common performances of guanidinium IL but also has its unique potential in extraction field. It is demonstrated that functional guanidinium IL is an environmental friendly solvent which has good designability, and has more potential than traditional imidazolium IL [26].

During the last five years, ILs have been reported several times to be immobilized on GO surface as sorbents in SPE [27–29]. Although the liquid state of ILs is lost when they are immobilized on GO surface, the other unique properties, such as polarity and low volatility associated to non polar and ionic interactions, are maintained [3,30]. Based on the properties of IL as well as the strong electrostatic/chemical interaction between IL and GO, the introduction of IL into functional composites not only could increase the water-solubility of the composite material but also could enhance the extract efficiency of proteins [20,31,32].

In this paper, a series of novel cationic functional hexaalkylguanidinium ILs and anionic functional tetraalkylguanidinium ILs have been synthesized, and then magnetic chitosan graphene oxide (MCGO) composite has been prepared based on Fe_3O_4 and coated with these functional guanidinium ILs (FGILs) to form MCGO-FGILs for the protein extraction by MSPE. After extraction, the concentrations of protein were determined by measuring the absorbance at 278 nm using an ultra violet visible spectrophotometer. The proposed MCGO-FGILs-MSPE method has been applied to extract trypsin, lysozyme, ovalbumin and bovine serum albumin. This paper is only showing the possibility of application of the sorbent for protein extraction, and more realistic evaluation of the protein extraction from a real matrix and analysis of real samples require further investigations.

2. Experimental

2.1. Apparatus

MCGO-FGILs were dried by a 101-0E ventilated drying oven (Beijing, China) and DZF-6051 vacuum drying oven (Shanghai, China). A QYC200 thermostats cultivating shaker (Shanghai, China) was used to provide a certain temperature and rotation speed in the experiment. Ultraviolet detection was carried out on a UV-2450 UV–vis spectrophotometer (SHIMADZU, Japan). A field emission scanning electron microscope (FEI Quanta 200FEG, USA) was used to examine the microstructures of the composite. FT-IR spectra were recorded in the range of 4000–400 cm^{-1} on a Spectrum One FT-IR spectrometer (PerkinElmer, USA). Wide angle X-ray diffraction (XRD) patterns were recorded by a D8 ADVANCE X-ray diffraction spectrometer (Bruker, German).

2.2. Reagents and materials

Chitosan with 80 meshes, 96% degree of deacetylation and average-molecular weight of 6.36×105 was purchased from Aladdin Co. (Shanghai, China). $FeCl_3 \cdot 6H_2O$, $FeCl_2 \cdot 4H_2O$, Bovine Serum Albumin (BSA), H_2O_2 (30%) and graphite were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and N-hydroxyl succinimide (NHS) were obtained from Adams Reagent Co., Ltd. (Shanghai, China). 1,1,3,3-Tetramethylguanidine (TMG, >99%) was purchased from Haohua Chemical Co., Ltd. (Shanghai, China). 4-Chloro-1-butanol (98%) and 6-chloro-1-hexanol (98%) were supplied by Xinyuan Chemical Reagent Co. (Tianjin, China). Formic acid (≥99%), acetic acid (≥99.5%) and propionic acid (≥99%) were obtained from Bodi Chemical Reagent Co. (Tianjin, China). Lactic acid (≥99%) was purchased from Xilong Chemical Reagent Co. (Guangdong, China). Sulfuric acid was procured from Merck, India. All other reagents used in this study were analytical grade, and distilled or double distilled water was used in the preparation of all solutions.

2.3. Preparation of magnetic chitosan and graphene oxide

MCGO was prepared by magnetic chitosan particles and graphene oxide. MC was prepared according to the literature [21] with some modification. A freshly prepared iron solution