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A novel dianionic amino acid ionic liquid-coated PEG 4000 modified Fe_3O_4 nanocomposite for the magnetic solid-phase extraction of trypsin

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ABSTRACT

A novel magnetic extractant, PEG 4000 modified Fe_3O_4nanomaterial that coated with dianionic amino acid ionic liquid (Fe_3O_4@PEG@DAAAIL), was successfully synthesized and characterized. X-ray diffraction (XRD), transmission electron microscope (TEM), vibrating sample magnetometer (VSM), fourier transform infrared spectrometry (FT-IR), thermal gravimetric analysis (TGA) and zeta potentials were used to confirm that the novel nanocomposite was successfully synthesized. Subsequently, the prepared Fe_3O_4@PEG@DAAAIL nanocomposite was used as the extractant for trypsin coupled with magnetic solid-phase extraction (MSPE). The concentrations of trypsin in the supernatant were detected by UV–vis spectrophotometer at 278 nm. The extraction ability turned out to be better than the other four kinds of extractants prepared in this work. Furthermore, the influence of a series of factors, such as extraction time and temperature, initial trypsin concentration, the value of pH and ionic strength, was systematically investigated. Under the optimal extraction condition, the extraction capacity for trypsin could reach up to 718.73 mg/g, absolutely higher than that of other adsorbents reported. This satisfactory extraction capacity could be maintained unchangeable after at least eight days, and kept over 90% of initial extraction capacity after eight recycles. What's more, the activity of trypsin after extraction retained 92.29% of initial activity, verifying the biocompatibility of the prepared extractant. Finally, the developed Fe_3O_4@PEG@DAAAIL-MSPE method was successfully applied to the real sample analysis with satisfactory results. All of above proves the potential value of Fe_3O_4@PEG@DAAAIL-MSPE in the analysis of biomass.

1. Introduction

Sample preparation plays a role of irreplaceable importance in the analytical process [1,2]. Usually, the sample preparation step consumes the most of time in the whole chemical analysis procedure and may bring some additional errors which could finally influence the result of study. On the basis of the conventional solid-phase extraction (SPE), a novel sample preparation method, magnetic solid-phase extraction (MSPE), has been developed [3–5]. It is qualified with the advantages of SPE, like high recovery, less consumption of volatile organic solvents and so forth. Meanwhile, with the help of an exterior magnetic force, magnetic extractants can be quickly separated from solution, which replaces the filtration or centrifugation procedure, contributing to a significant decrease in the extraction time [6]. As a result of these fascinating superiorities, MSPE method has attracted considerable interest of researchers in recent years [7–9].

For the purpose of fast magnetic separation in the MSPE process, the extractant with magnetism is a must. Most often, magnetic nanoparticles (MNPs) (e.g. Fe_3O_4 and γ-Fe_2O_3) are used as the magnetic part, which can be synthesized by a simple co-precipitation method [10]. In view of the remarkable properties, such as easy to prepare, superparamagnetism and low toxicity [11], magnetic Fe_3O_4 plays an important role. Nevertheless, MNPs are easy to aggregate, so protection on the surface of pure nanoparticles is crucial [12]. Surfactants, polymers [13,14], silica [15], noble metals [16] and carbon materials [17,18] are usually exploited as postsynthesis coatings. These protective materials can not only prevent MNPs from aggregation, but also improve their chemical stability. On account of numerous superiorities, including non-toxicity, flexibility and biocompatibility [19], PEG could be chosen to serve as a robust network to protect MNPs from aggregation. Additionally, the one-step synthesis of PEG modified MNPs is simpler compared with other methods consisting of two or more steps.

In order to achieve various purposes, corresponding modification

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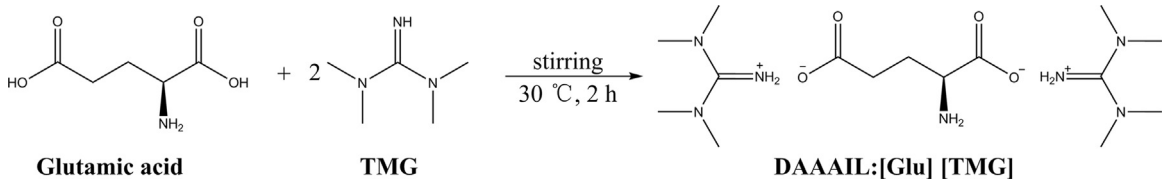

Fig. 1. Synthesis of DAAAIL.

for magnetic materials can be conducted. Ionic liquids (ILs) are generally defined as organic salts with melting points at or below 100 °C [20]. They are regarded as "green solvents" [21], so considerable efforts have been devoted to the use of ILs in chemical analysis [22–24]. So far, a series of studies have been carried out to apply ILs or ILs modified materials in the extraction of analytes [25,26]. Herein, a kind of amino acid ionic liquid with dianionic center could be prepared to modify the magnetic material. Apart from easy to be prepared, it possesses both unique characteristics of ILs (e.g. low volatility, vanishing vapor pressure, high thermal and chemical stability, etc.) and superior properties of amino acids (e.g. biocompatibility and biodegradability), and its dianionic center can improve the electrostatic interaction with analytes.

Proteases can catalyze so many physiological and pathophysiological processes that they play essential roles in metabolism of biosome. Trypsin, a kind of the major digestive enzyme, is a member of the large family of serine endoproteinases [27]. Trypsin possesses excellent substrate specificity that it is able to specifically cleave the carboxyl groups of arginine and lysine residues in proteins and peptide bonds. Because of the potential usage in industries, plenty of studies based on trypsin have been performed so far, such as substrate specificity studies [28,29], immobilization [30,31] and so on. Obviously, it's of great significance to develop an efficient method to accomplish the purification of trypsin to support further analysis.

In this work, PEG 4000 modified Fe_3O_4 nanoparticle that coated with dianionic amino acid ionic liquid (Fe_3O_4@PEG@DAAAIL) was synthesized and applied to the MSPE of trypsin for the first time. The morphologies and properties of the prepared nanomaterials were systematically characterized. The factors that may have impact on the extraction ability were studied and optimized. Subsequently, the extracted trypsin was eluted from the extractant and its activity was examined next. The experiments about regeneration and application in the real sample of the extractant were further conducted.

2. Experimental

2.1. Reagents and instrumentation

Glutamic acid, glycine, $FeCl_3$, $FeCl_2{\cdot}4H_2O$, hydrazine hydrate (88%), dibasic sodium phosphate (Na_2HPO_4), sodium dihydrogen phosphate ($NaH_2PO_4{\cdot}2H_2O$) and PEG 4000 were supplied by Sinopharm Chemical Teagent Co., Ltd. (Shanghai, China). 1,1,3,3-tetramethylguanidine (TMG) was purchased from Adamas Reagent Co., Ltd. (Shanghai, China). Trypsin from bovine pancreas (Try), Bovine serum albumin (BSA), Bovine hemoglobin (BHb) and Ovalbumin (OVA) were supplied by Shanghai yuanye Bio-Technology Co., Ltd. (China). Sodium hydroxide (NaOH), calcium chloride ($CaCl_2$), and hydrochloric acid (HCl) was gained from Shanghai Titianchem Co., Ltd. (China). N_α-benzoyl-L-arginine ethyl ester (BAEE) was obtained from J & K Chemical Technology Co., Ltd. (Beijing, China). All reagents used were of analytical grade without further purification. Ultrapure water was used throughout this work.

The main instruments used in this experiment were listed as following: DZF-6051 vacuum drying oven (Shanghai, China); DF-101S magnetic stirrer (Gongyi yuhua instrument Co., Ltd., China); B-220 thermostat water bath (Shanghai, China); KQ 3200E ultrasonic cleaner (Kunshan ultrasonic instruments Co., Ltd., China); QWC200 incubator shaker (Shanghai, China); INOVA 400NB NMR (Varian, America); UV-2450 UV–vis spectrophotometer (Shimadzu, Japan); FT-IR spectrometer (PerkinElmer, USA); STA 409 thermal gravimetric analyzer (Netzsch, Germany); EV 11 Vibrating Sample Magnetometer (MicroSense, USA); HT-7700 transmission electron microscope (TEM, Hitachi, Japan).

2.2. Preparation of ILs

Four kinds of ILs, bis(1,1,3,3-tetramethylguanidinium) glutamate ([Glu][TMG]), 1,1,3,3-tetramethylguanidinium glycine ([Gly][TMG]), formic acid 1,1,3,3-tetramethylguanidinium ([FA][TMG]) and acetic acid 1,1,3,3-tetramethylguanidinium ([AA][TMG]), were synthesized. Taking the DAAAIL ([Glu][TMG]) as an example (as shown in Fig. 1), 20 mmol of glutamic acid was dissolved in 10 mL of water, and then 40 mmol of TMG was added into the solution dropwise when applying magnetic stirring. The mixture was stirred for 2 h at 30 °C. Finally, the product was dried in a vacuum at 55 °C for 24 h. The structures of the prepared ILs were confirmed by ^{1}H NMR, which were shown in Table 1.

2.3. Preparation of Fe_3O_4@PEG

Fe_3O_4@PEG nanocomposites were prepared by the chemical co-precipitation method referred in the literature with some modification [32–34] (as shown in Fig. 2A). $FeCl_3$ (4.86 g) and $FeCl_2{\cdot}4H_2O$ (3.98 g) were dissolved in 50 mL of ultrapure water under violent mechanical stirring at room temperature, and then 1 mL of 80% hydrazine hydrate and 1 g of PEG-4000 were added in turn. Afterwards, the resulting mixture was placed in a water bath at 60 °C. 50 mL of sodium hydroxide solution (1 M) was poured into the above solution quickly, followed by strong agitation for 30 min. After cooling at the room temperature, the suspension was put on an external magnetic field to collect the product, which was washed by deionized water and ethanol several times, respectively. Finally, the product was dried under vacuum at 55 °C overnight.

2.4. Preparation of Fe_3O_4@PEG@IL

Fe_3O_4@PEG@IL nanomaterials were synthesized as follows: 2 g of IL was dispersed in 8 mL of ethanol, and then 0.5 g of Fe_3O_4@PEG was added into the solution. After sonicated for 20 min, IL coordinated with PEG layer, driven by intermolecular hydrogen bonding interaction force [35,36]. The resulting mixture was treated with magnetic field, washed with ethanol several times and dried in vacuum at 55 °C for 10 h.

2.5. Magnetic solid-phase extraction procedure

In the MSPE procedure, Fe_3O_4@PEG@IL was applied to the extraction of proteins (as shown in Fig. 2B). 5 mg of Fe_3O_4@PEG@IL was added into a 2 mL centrifuge tube, in which there was 1 mL of protein aqueous solution (3 mg/mL). Subsequently, at 25 °C, the mixture was treated in a thermostats cultivating shaker at 200 rpm for 30 min. Afterwards, a magnet was held at the bottom of the centrifuge tube to attract the magnetic extractant. Then UV–vis spectrophotometer was used to detect the absorbance of the super-