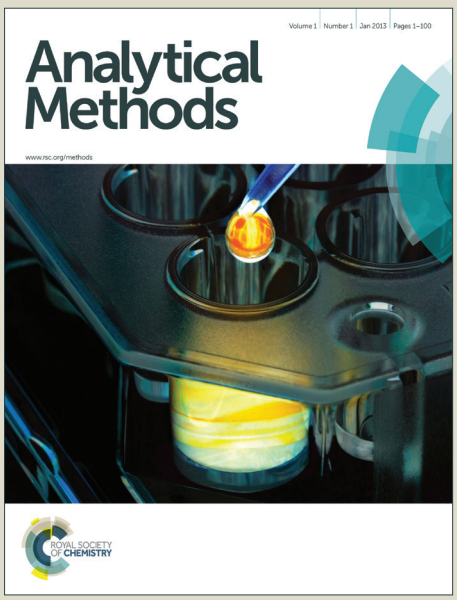
Analytical Methods

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/methods

Magnetic solid-phase extraction of proteins based on hydroxy functional ionic liquids modified magnetic nanoparticles

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

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

Corresponding author: Professor Yuzhi Wang

State Key Laboratory of Chemo/Biosensing and Chemometrics

College of Chemistry and Chemical Engineering

Hunan University

Changsha 410082

P. R. China

Phone: +86-731-88821903

Fax: +86-731-88821848

E-mail: wyzss@hnu.edu .cn

Abstract

Hydroxy functional ionic liquids has been first designed and modified on the surface of silica-coated magnetic Fe_3O_4 nanoparticles (MNPs) for the investigation of the extraction performance of proteins by magnetic solid-phase extraction (MSPE) and characterized by fourier transform infrared spectroscopy (FTIR),electron microscopy (TEM), vibrating sample magnetometer(VSM), X-ray diffraction (XRD) and thermo gravimetric analysis (TGA). BSA was chosen as a model protein to investigate the effect of extraction parameters. Based on the single-factor experiment, an initial serial investigative test was used to identify the optimal conditions of the extraction and the extraction efficiency could reach up to 86.92%. The RSD of extraction efficiencies in precision experiment, repeatability experiment and stability experiment were 1.17% (n=3), 3.79% (n=3) and 4.82% (n=3), respectively. Compared with other four types of ionic liquids, hydroxy functional ionic liquids modified magnetic nanoparticles has the highest extraction rate. Desorption of proteins was up to 94.91% when the concentrations of NaCl greater than 1.1 $mol\ L^{-1}$. Nearly 95% of MNPs could be recovered from each run and extraction rate decreased significantly after five runs.

Keywords: Magnetic nanoparticle, Hydroxy functional ionic liquids，Solid-phase extraction, Protein

1. Introduction

Proteins play a critical role in the basis of life like antibodies in animal immune response, metabolism, gene expression, signal transduction, nutrients, and even as cellular extracellular structures, etc [1]. However, proteins always exist within complex mixtures and poor stability in the conditions of acids, alkali or heating. Therefore, it is necessary to prepare the technology of protein purification and identification.

The classic methods for protein purification include ammonium sulfate precipitation, salting out, electrophoresis [2]. Current techniques for the pre-concentration of protein include ionic or affinity chromatography [3], ionic liquid aqueous two-phase system [4,5], solid phase extraction (SPE) [6], solid-phase microextraction (SPME), pressurized liquid extraction and supercritical fluid extraction.

In recent years, nanoparticles are being paid more and more attention for separation purposes. Nanoparticles have large specific surface areas, thus a large fraction of active sites are available for appropriate chemical interaction [7]. In the numerous nanoparticles, because of the magnetic nanoparticles can be isolated by a magnetic plate from the reaction medium, they have been widely researched. But, the naked magnetic nanoparticles have a low adsorption on account of large solute molecules such as proteins which cannot be specific adsorption. So many organic functional monomers or polymers have been modified on the magnetic nanoparticles

such as polyacrylic acid [8], tetrabenzyl [9], polyacrylamide [10], diphenyl [11], phosphatidylcholine [12] and so on.

Ionic liquids (ILs) are a class of liquids which were first reported by Walden in 1941 [13]. ILs have many fascinating properties including wide liquid ranges, low volatilities (negligible vapor pressure), good thermal stabilities, electrolytic conductivity, wide range of viscosities, adjustable miscibility, reusability, nonflammability and so on [14]. The greatest feature of ionic liquids is designing. By adjusting the combination or introducing an appropriate functional groups, the specific ionic liquids can be obtained. But the ionic liquids which directly used for protein extraction, can not only cause changes in protein conformation, but also loss activity and be difficult to recycle and reuse.

To solve this problem, ionic liquid-modified on the surface of the magnetic nanoparticles (ILs-MNPs) which consist of bulky organic cations combining with inorganic or organic anions, has recently been developed as a new sorbent material. Because the magnetic Fe_3O_4 nanoparticles easy to be oxidized and large aggregated, before the ionic liquid modified on the surface of magnetic nanoparticles, coated with a layer of silicon is very necessary. The silica-shell coated on the surface of Fe_3O_4 nanoparticles can prevent the oxidation of Fe_3O_4 and the surface silanol groups could offer many possibilities for further surface modification, such as the introduction of hydroxyl, carboxyl, amino groups. In addition it also could improve the corrosion resistance, chemically stability and effectively reduce the aggregation of Fe_3O_4 nanoparticles in the liquid.

ILs-MNPs have been applied in magnetic solid-phase extraction (MSPE) of various compounds such as flavonoids [15,16], ergosterol [17], lipase [18], Dye [7,13], DNA [19],metals [20], phthalate esters [21-24], sulfonylurea herbicides [25] and enzymes [26-29]. Some others had studied the applications of ILs-MNPs as recyclable catalyst [30-34]. An alluring prospect is that the protein adsorbed on magnetic nanoparticles modified by ionic liquids can be separated by applying magnetic field, which conducive to recycle and reuse for multiple times.

To the best of our knowledge, adsorption of proteins on magnetic silica nanoparticles modified by hydroxy functional ionic liquids has not been reported. Herein, five environmental-friendly hydrophilic ionic liquids (ILs) were synthesized and modified on the surface of silica-coated magnetic Fe_3O_4 nanoparticles (SiO_2@ Fe_3O_4) for the investigation of the extraction performance of proteins by magnetic solid-phase extraction （as shown in scheme 1）. [Simam][Cl]-MNPs were chosen as a model. The concentrations of proteins in solution were determined by measuring the absorbance at 278 nm for bovine serum albumin (BSA) and ovalbumin (OVA), and at 404 nm for bovine hemoglobin (BHb). BSA was chosen as a model protein to investigate the effect of system parameters. Adsorption isotherms, kinetic of adsorption, recycling and reusing of the sorbent were characterized as well.

2. Experimental

2.1 Instrumentation

The mainly used instruments included: DZF-6051vacuum drying oven (Shanghai, China), D5000 X-ray Diffraction (Siemens, Japan) , Thermostats cultivating shaker

(Shanghai, China), UV-2450 UV-Vis Spectrophotometer (SHIMADZU, Japan), VarianInova-400 NMR spectrometer (Varian, USA), FT-IR spectrometer (PerkinElmer, USA), JEM-1230 transmission electron microscope (JEOL, Japan), STA 409 thermal gravimetric analyzer (Netzsch, Germany) and EV 11 Vibrating Sample Magnetometer (MicroSense, USA).

2.2 Chemicals and reagents

Iron (II) sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$), iron (III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), ammonia solution (27%, w/v), hydrazine hydrate, and 2-propanol were purchased from Fuchen (Tianjin, China). Tetraethyl orthosilicate (TEOS), toluene, N-Methylimidazole, were purchased from Aladdin chemistry Co. Ltd. (Shanghai, China). N, N-dimethylethanolamine (DMEA) and pyrrole were purchased from Tianjin Kermel Fine Chemical Research Institute. N-Ethylmorpholine, 1,1,3,3-tetrame thylguanidine were obtained from Energy Chemical Company (Shanghai, China). BSA, ethyl acetate, and other reagents were supplied by Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All the chemicals from the commercial sources were generally of analytical grade without any further purification.

2.3 Grafting of ILs at the surface of magnetic nanoparticles (IL-SiO_2@ Fe_3O_4)

The nanoparticles of Fe_3O_4 were synthesized by the improved coprecipitation method. In order to get the maximum yield for magnetic nanoparticles, the ideal molar ratio of Fe^{2+}/Fe^{3+} was about 0.5 (as shown in Scheme 2: Step 1). So 5.41 g of $FeCl_3 \cdot 6H_2O$ was dissolved in 30 mL of water, and then 1 mL hydrazine hydrate and 2.78 g $FeSO_4 \cdot 7H_2O$ were sequentially added to the solution. After dissolved

thoroughly, 10 mL ammonium hydroxide (27%, w/v) was quickly added to the solution under violently stirring, then to make sure the pH value of the solution was 9. The reaction was maintained for 30 min at room temperature. Then the mixture was cured for 1 h at 80 ℃. The magnetite precipitates were isolated by a magnetic plate from the reaction medium and washed several times with deionized water until the washing solution was neutral. Finally the magnetic Fe_3O_4 nanoparticles were washed with anhydrous ethanol for three times and dried in vacuum drying oven at 70 ℃ for 24 h.

The method of modified Fe_3O_4 nanoparticles with silicon was reported in our previous work [35]. 600 mg Fe_3O_4 was diluted with 10 mL of water and 50 mL of 2-propanol by ultrasonic vibration for 30 min. 10 mL ammonia solution and 4 mL TEOS were added at room temperature with stirring for 12 h in order to allow the silica shell to grow on the surface of the nanoparticles (as shown in Scheme 2. Step: 1). The suspension was isolated by a magnetic plate and washed with ultra pure water until the pH of washing solution was 7. Finally the particles were dried under vacuum at 70 ℃ for 24 h.

With the moderate modification of the method in the literature [25,31], five kinds of ionic liquids consisting of chloride anions and different cations (as shown in Table 1) were synthesized:1-methyl-3-(triethoxy) silypropyl-imidazolium chloride([Simim][Cl]), (2-hydroxyethyl)-N,N-dimethyl-3-(triethoxy) silypropyl-ammonium chloride([Simam][Cl]), N-ethyl-N-[3-(triethoxy) silypropyl]-morpholinium chloride([Siemp][Cl]), N,N,N',N'-tetramethyl-3-(triethoxy)

silypropyl-guanidinium chloride([Sitmg][Cl]), N-methyl-N-[3-(triethoxy) silypropyl]-pyrrolium chloride([Simpy][Cl]) were directly synthesized in just one step. The synthetic route of the ionic liquids was shown in Scheme 2: Step 2. As an example, a 250 mL round-bottom flask equipped with a magnetic stirring bar and condenser was charged with 20 mmol of 3-Chloropropyltriethoxysilane and 20 mmol of N,N-dimethylaminoethanol. The mixture was refluxed with stirring for 8 h at 120 ℃. After the reaction had cooled to room, the product was purified further by washing three times with 100 mL of ethyl acetate, and then dried in a vacuum for 24h.The five kinds of ionic liquids structures were confirmed by FT-IR, ^{1}H NMR and ^{13}C NMR spectra, which were shown in supplementary data Table S1 and Fig.S1, respectively.

100 mg silica-coated nanoparticles were dissolved in 100 mL toluene by ultrasonication for 15 min. One gram IL was then added to the system and the mixture was stirred at 120 ℃ for two days (as shown in Scheme 2：Step 3). After reaction, the nanoparticles were washed with water for two times and with ethanol for three times. Finally the particles were dried under vacuum at 70 ℃ for 24 h.

2.4 Magnetic Solid-phase extraction procedure

Adsorption of protein from aqueous solutions on the surfaces of biofunctional magnetic nanoparticles was investigated batch-wise. The schematic diagram of the extraction process was shown in Scheme 3. Different amount of the IL-MNPs were added into the protein solutions which were shaken at 200 rpm for a predefined time and temperature. After extraction, the solid phase which contained adsorbed protein

on the surface of ILs-MNPs was magnetically separated. The amounts of protein adsorbed on the magnetic nano-adsorbents were estimated from the concentration change of protein in solution after adsorption by UV–vis spectrometer at absorbance at 278 nm for bovine serum albumin (BSA) and ovalbumin (OVA), and at 404 nm for bovine hemoglobin (BHb) for protein assay. The extraction efficiency (E) was calculated by the following equation:

$$E= (C_o-C_e) \times 100\%/ C_o \quad (1)$$

Where C_o is the initial protein concentrations (mg mL^{-1}), C_e is the equilibrium protein concentrations (mg mL^{-1}).

Desorption of protein from the surface of IL-MNPs (after extracting through optimum extraction conditions) was conductedwith addition of NaCl. The desorption ratios (D) was calculated using the following equation:

$$D=C_r / (C_o-C_e) \times 100\% \quad (2)$$

Where C_r is the concentration of protein in the desorption medium.

2.5 Recycling and reusing of the ILs-MNPs

The recycling and reusing of the ILs-MNPs are highly preferable for a greener process. After protein desorption by NaCl solution with different ionic strength, the magnetic nanoparticles can be isolated by simple magnetic decantation using a magnetic plate. The ILs-MNPs can be reused after washing with ethanol and drying in vacuum at 70 ℃ for 24 h then reused in a subsequent reaction.

3. Results and discussion

3.1 Characterization of the ILs-MNPs

FT-IR spectroscopy was employed to certify the successful modification of magnetic nanoparticles with ILs (as shown in Fig. 1). The strong bond around 569 cm^{-1} corresponds to Fe–O vibrations of the magnetite core which can be observed in every curve. The characteristic bands around 1101.42 cm^{-1}, 925.27 cm^{-1} and 464.06 cm^{-1} which were assigned to the Si–O–Si asymmetric stretching vibration, Si–C stretching vibration and Si–O–Si bending vibration, respectively, can be observed after modified Fe_3O_4 nanoparticles with silicon. The spectrum also displays strong bands around 3370 and 1607 cm^{-1}, which were assigned to O–H stretching, N–H stretching, and H–O–H bending modes of vibration, respectively. The distinguished feature in the FT-IR spectra of ILs bonded to silica-coated nanoparticles was the appearance of the new peak around 2949 and 2882 cm^{-1}, which was assigned to the symmetric and asymmetric methylene (CH_2) and methyl (CH_3) vibrations. It was notable that lots of new peaks (such as 1607.47, 1479.36 cm^{-1}) can be found after modified ionic liquids on the magnetic nanoparticles which proved that the ionic liquids were successfully modified on the surface of SiO_2@Fe_3O_4 MNPs.

TGA was performed to further estimate the relative composition of core and the amount of ionic liquid deposited onto the surface of SiO_2@ Fe_3O_4. Based on the thermograms provided in Fig. 2, it can be seen that there is an initial loss of weight at temperature below 200 ℃ for all samples. This is attributed to the removal of water and solvent residues. The bond energy of Si–O–Fe is great, and the silica coating on the Fe_3O_4 nanoparticles can withstand high temperature, so the weight loss of SiO_2@Fe_3O_4MNPs is very little when the temperature exceeds 200 ℃. When the

temperature continues to rise over 300 ℃, the weight loss of curves c was due to the decomposition of ionic liquid.According to the TGA curves, the ionic liquid content of silica-coated magnetic nanoparticles was evaluated to be in excess of 35% by weight.

The XRD spectra is used for determining the crystallographic identity of the produced material, phase purity and for calculating the mean particle size based on the broadening of the most prominent peak in the XRD profile [36]. The crystal phase of Fe_3O_4 MNPs, SiO_2@ Fe_3O_4 MNPs and IL- SiO_2@ Fe_3O_4 MNPs were investigated by XRD (as shown in Fig. 3). In the 2θ range of 10–80°, the X-ray diffraction peaks for Fe_3O_4 (2θ= 30.1°, 35.5°, 43.1°, 53.4°, 57.0°, and 62.6°) were observed on all the three samples, and the peaks positions were indexed as (220), (311), (400), (422), (511), and (440), respectively (JCPDS Card: 019-0629). The XRD patterns showed a good identity with the standard Fe_3O_4 structure which proved that the particles had got phase stability and the integrity of the structure. The broad peak from 2θ=20° to 30°could be seen obviously after coating the particles with silica. It was consistent with an amorphous silica phase in the shell of the silica-coated Fe_3O_4 nanoparticles [33,37]. No obvious difference was observed between the XRD spectra of SiO_2@Fe_3O_4 MNPs and IL- SiO_2@Fe_3O_4 MNPs except that the intensity of the XRD peaks decreased, which resulted from the preferred orientation of crystalline faces [35]. This result of the XRD spectra indicated that the crystal phase of Fe_3O_4 nanoparticle was not changed during the coating process.

The intensity of magnetism was important for magnetic materials to possess

sufficient magnetic properties in separation from liquid medium.VSM was used to evaluate the magnetization of the MNPs. Fig. 4a shows the VSM magnetization curves of Fe_3O_4 MNPs, $SiO_2@Fe_3O_4$ MNPs and IL- $SiO_2@Fe_3O_4$ MNPs at room temperature. It was found that the saturation magnetization of Fe_3O_4 MNPs and $SiO_2@Fe_3O_4$ MNPs were 71.87 emu g^{-1} and 37.40 emu g^{-1}, respectively.

The saturation magnetization of $SiO_2@Fe_3O_4$ MNPs decreased in comparison with Fe_3O_4 MNPs which was likely resulted from the nonmagnetic silica coating shell. The saturation magnetization has a slight decrease after modified ionic liquids on the magnetic nanoparticles. Nevertheless, the saturation magnetization value of 37.40 emu g^{-1} for the IL-$SiO_2@Fe_3O_4$MNPs was high enough to make them easily and quickly separate from the suspension (as shown in Fig. 4b).

The microscopic structure of the particle was observed by transmission electron microscopy (TEM). Fig. 5a and 5b illustrated that the naked of Fe_3O_4 particles had a mean diameter of about 20 -30nm. Fig. 5c and 5d showed the Fe_3O_4particles coated by silica which had uniform size about 300-400 nm. Moreover, the dark $SiO_2@Fe_3O_4$ MNPs surrounded by a gray liquid could be observed in Fig. 5e after modified with ionic liquids which might be because of the layer of ionic liquids surrounding. It can be observed that all of particles appear to be roughly spherical in shape which were homogeneous, monodisperse, and spherical.

3.2 MSPE of different ionic liquid and different protein

Five kinds of ionic liquids with the common anion of chloride modified on the surface of magnetic nanoparticles have been investigated for the extraction of three

proteins (BSA, BHb, OVA). The extraction efficiencies were shown in supplementary data Table S2. As an example, the values of the extraction efficiencies for BSA change from 47.21% to 18.46%. It is clear that the hydroxyl ammonium-based IL has a higher extraction efficiency, and the poorest effect is shown by the guanidinium-based ionic liquid.

Because the ionic liquids have a wide structural diversity, the extraction efficiency was also differences. It can be seen from Fig. 6, the [Simam][Cl] modified on the surface of magnetic nanoparticles has a higher extraction rate than others, it may be attributed to the hydrogen bond interaction between the hydroxyl of the IL cation and the aliphatic hydrocarbon residue of the protein. Therefore, magnetic solid-phase extraction based on hydroxy functional ionic liquid modified on the surface of silica-coated magnetic Fe_3O_4 ([Simam][Cl]-MNPs) was selected and take BSA as the object for the following study.

3.3 Optimization of MSPE parameters

3.3.1 Effect of the amount of [Simam][Cl]-MNPs

In order to discuss the effect on extraction efficiency of the mass of IL-MNPs, a specific mass of [Simam][Cl]-MNPs and protein solution(0.5 g mL^{-1} and 2.0 mL) systems were adopted, then the suspension was immediately stirred for 30 min at 30 ℃ and the results were illustrated in Fig. 7a. We can see that the extraction efficiency was increased with the addition of IL-MNPs. The reason could be that the more number of [Simam][Cl]-MNPs is, the more adsorption sites would be available for protein molecules adsorbed. The extraction efficiency of BSA has been reached

81.33% when the mass of [Simam][Cl]-MNPs was 60 mg. The extraction efficiency was not obviously increased when the amount of [Simam][Cl]-MNPs attained 70 mg. So 60 mg of [Simam][Cl]-MNPs was chosen in next experiment.

3.3.2 Effect of the mass of protein

To study the effect of sample concentration on the extraction efficiency of [Simam][Cl]-MNPs, a series of concentration of protein solutions (0.50-1.75 mg mL^{-1}) were examined at 30 ℃ and shaken for 30 min. From Fig. 7b, it can be seen that with the increase in concentration of BSA, the extraction yield decreased correspondingly. This observation can be explained by this fact that an extraction system has a limited ability of extraction when the number of adsorbent was the same. So the system is near saturation when the concentration of BSA addition is more than 0.5 mg mL^{-1} the extraction quantity may have a little increase, but the extraction efficiency decreases rapidly. According to the above result, 0.5 mg mL^{-1} BSA solution was selected.

3.3.3 Effect of solution pH

Owing to the proteins as amphoteric molecules, the effect of the initial pH of the solution on the adsorption of proteins onto IL-MNPs surfaces was studied. A series of pH range from 3.0 to 9.0 of protein solutions (0.5 mg mL^{-1}, 2.0 mL) were examined. 60 mg of [Simam][Cl]-MNPs was added and shaken for 30 min at 30 ℃. The results are depicted in Fig. 7c. The extraction yields of BSA was relatively high at pH=6. This may be associated with the following reason. The isoelectric point of BSA is about 4.7–5.2 and consequently a net charge of almost zero is expected for it at about pH 5.0[6]. At pH = 6, BSA has negative charges and the surfaces of IL-MNPA

nanoparticles are positive [7]. The pH value was adjusted to make a strong electrostatic attraction between negatively-charged BSA and positively-charged nanoparticles. Based on these results, a pH value of 6.0 was selected in order to perform next investigations.

3.3.4 Effects of extraction time

The effect of the extraction time in MSPS was investigated by changing the shaking time. From Fig. 7d, it can be seen that the extraction efficiency of BSA increased rapidly in the first 20 min, and over 20 min the extraction efficiency had almost reached the maximum. It can be speculated that when the extraction process is just beginning, there are many adsorption sites on the surface of the [Simam][Cl]-MNPs which would be available for protein molecules adsorbed, so the extraction capacity increases quickly. With the growth of time, more and more adsorption sites were occupied and the extract speed gradually slows down until extraction reached equilibrium.

3.3.5 Effect of solution temperature

To further confirm the temperature range to the influence of extraction efficiency, a series of experiments were performed over a temperature range of 20–70 ℃. Sixty milligram of [Simam][Cl]-MNPs and 2.0 mL of 0.5 mg mL^{-1} protein solution at pH = 6 were used. In light of Fig. 7e, as the temperature increased from 20 to30 ℃, the extraction efficiency of the protein increased with the increasing temperature. But the temperature kept at 30 ℃ or higher, the extraction yield decreased correspondingly. The possible reason for this phenomenon was that the adsorption process of protein onto the [Simam][Cl]-MNPs was endothermic process. But when the temperature continues to rise, the extraction rate was reduced. It means that the temperature was

high to destroy the hydrogen bonding interaction between the ionic liquids modified on the surface of magnetic nanoparticles and the surface water of protein's amino acid residue. Through further study found that when the temperature exceeds 60 ℃, BSA was denaturated. So the extraction was carried out at 30 ℃ because of the relatively high extraction yield. All single-factor experimental data were summarized in supplementary data Table S3.

3.4 Methodological study

Under the optimized extraction conditions, a series of experiments were performed to validate the developed MSPE method for three times by UV detection. Apparatus precision was investigated by the analysis of the solution of BSA for three times. The RSD obtained was 0.55%. The result indicates that the precision of the UV–vis spectra is great. Three copies of the same sample measured respectively under the same conditions. The calculation of RSD was 1.47% (n=3) which indicate that this method has excellent repeatability. Taking a sample detected continuously in three days under the same conditions to verify the stability experiment. The result of the RSD was 1.27% (n=3), which explain that the sample is recoverable within three days （as shown in supplementary data Table S4） .

3.5 Desorption studies

The regeneration of protein is an important factor to be reported for potential applications. Different concentrations of NaCl (2.0 mL) was used and the possible desorption of proteins (0.5 mg mL^{-1} protein already adsorbed on 60 mg [Simam][Cl]-MNPs under optimum extraction conditions) was added. It can be seen

from Fig. 8 that with the increasing of the concentration of NaCl, the percentage of BSA desorbed increased and the desorption ratios reached 95.34% when the concentrations of NaCl greater than 1.1 mol L^{-1}. The results showed that the adsorbed BSA could be easily desorpted from [Simam][Cl]-MNPs.

3.6 Regeneration of the IL-MNPs

To evaluate the sorbent performance, regeneration is a key factor for making the process economic and environmental protection. Nearly 95% of [Simam][Cl]-MNPs could be recovered from each run. Finally, several consecutive adsorption–desorption cycles were performed and the result showed in supplementary data Fig S2. In a test of four cycles, the [Simam][Cl]-MNPs could be reused and the extraction efficiency was about 84.35 -82.64 % which without any significant loss of the extraction efficiency. These results revealed that the sorbent was excellent stable and could endure these reaction conditions. But after five runs a 24.16% decrease in its performance was observed. The reason of the limited reusable times may have the following points. On the one hand, the mass of magnetic nanoparticles will be lost after many times regeneration. Because the adsorbents was be weight for one time and washed many time by ethanol and water in the recycling and reusing test. On the other hand, the mass of ionic liquid modified on the surface of magnetic nanoparticles will become less and it is dissolved in solution after wash many time by ethanol and water. The all will lead to a decline of the extraction rate. Therefore the reuse limit of the proposed sorbent was five cycles.

3.7 Comparison with other methods

Protein can easily be extracted from the mixture through the traditional liquid-liquid extraction process, but it is hard to recycle from the extract. Compared with traditional liquid-liquid extraction method, solid phase extraction has many advantages such as low consumption of organic solvents, high recovery and reuse rate. Magnetic solid phase extraction is an emerging technology for pretreating samples in recent years. It put together liquid-solid extraction and magnetic properties. A novel form of traditional SPE, the magnetic sorbents are easily collected and separated from the solution with an external magnetic field which are convenient, time and effort saving.

We know that C18 has a wide range of applications in the separation of mixture. But the ionic-liquid coated magnetic particles has some advantages which the C18 do not have. For example, the greatest feature of ionic liquids is designing. By adjusting the combination or introducing an appropriate functional groups, the specific ionic liquids can be obtained. In addition, the hydroxy functional ionic liquids modified magnetic nanoparticles have the potential to extract protein from water samples based on hydrogen bond and electrostatic interaction between the ionic liquid and protein which C18 haven't. So this is more conducive to the selective separation of proteins. Considering the results, the sorbent prepared in this study possesses many advantages which is proved to be an efficient, reliable and convenient material for the extraction of protein from water samples.

4. Conclusions

This paper systematically investigated the extraction efficiency of protein based on a novel magnetic nanoparticles which was fabricated by modifying hydroxy functional ionic liquid on the surface of silica-coated magnetic Fe_3O_4 (IL-MNPs). Some parameters such as the mass of IL-MNPs, the protein concentration, pH of solution and temperature were optimized and under the optimal conditions, 86.92% of BSA was extracted. The high adsorption capacity, short contact time, stability, low aggregation, reusability, and selective adsorption ability are the advantages of hydroxy functional ionic liquid modified on the surface of silica-coated magnetic Fe_3O_4 nanoparticles as adsorbent compared with traditional extraction materials. The performances of the method indicate that it have the potential to offer new possibility in the extraction of bio-analysis and proved that [Simam][Cl]-MNPs can be an important tool in bio-separation technology as well as other biotechnological applications.

Acknowledgements

The authors greatly appreciate the financial supports by the National Natural Science Foundation of China (No. 21175040; No. 21375035; No.J1210040) and the Foundation for Innovative Research Groups of NSFC (Grant 21221003).

References

[1] Y. Pei, J. Wang, K. Wu, X. Xuan and X. Lu, Sep. Purif. Technol, 2009, 64, 288-295.

[2] A. R. Piergiovanni, J. Agric. Food Chem, 2007, 55, 3850-3856.

[3] E. Zatloukalova and Z. Kucerova, J. Chromatogr. B, 2004, 808, 99-103.

[4] X. Lin, Y. Z. Wang, Z. Qun, X. Q. Ding and J. Chen, Analyst, 2013, 138, 6445-6453.

[5] Q. Zeng, Y. Z. Wang, N. Li, X. Huang, X. Q. Ding and X. Lin, Talanta, 2013, 116, 409-416.

[6] S. Kamran, M. Asadi and G. Absalan, Microchim Acta, 2013, 180, 41-48.

[7] G.Absalan, M. Asadi, S. Kamran, L. Sheikhian, D. M. Goltz, J. Hazard. Mater, 2011, 192, 476-484.

[8] M. H. Liao and D. H. Chen, Biotechnology Letters, 2002, 24, 1913-1917.

[9] Y. Zou, Y. Z. Chen, Z. H. Yan, C. Y. Chen, J. P. Wang and S. Z.Yao, Analyst, 2013, 138, 5904-5912.

[10] L. Xiong, H. W. Jiang and D. Z. Wang, Acta. Polymerica. Sinica, 2008, 3, 259-265.

[11] F. Bianchi, V. Chiesi, F. Casoli, P. Luches, L. Nasi, M. Careri and A. Mangia, J. Chromatogr. B, 2012, 1231, 8-15.

[12] S. X. Zhang, H. Y. Niu, Y. Y. Zhang, J. S. Liu, Y. L. Shi, X. L. Zhang and Y. Q. Cai, J. Chromatogr. A, 2012, 1238, 38-45.

[13] P. Walden, Bull Acad Imperial Sci, 1941, 1800, 405-422.

[14] P. Sun and D. W. Armstrong, Analytica Chimica Acta, 2010, 661, 1-6.

[15] D. Xiao, D. H. Yuan, H. He, Carbon, 2014, 274-286.

[16] H. He, D. H. Yuan , Z. Q. Gao, J. Chromatography. A, 2014, 1324, 78-85.

[17] Y. Sha, C. Deng and B. Liu, J. Chromatogr. A, 2008, 27, 1198-1199.

[18] Y. Y. Jiang, C. Guo, H. S. Xia, I. Mahmood, C. Z. Liu and H. Z. Liu, J. Molecular Catalysis B:

Enzymatic, 2009, 58, 103-109.

[19] M. Ghaemi and G. Absalan, Microchim Acta, 2013, 158, 521-530.

[20] M. H. Mashhadizadeh and Z. Karami, J. Hazard. Mater, 2011, 190, 1023-1029.

[21] Y. Liu, H. Li and J.M. Lin, Talanta, 2009, 77, 1037-1042.

[22] L. Bai, B. Mei, Q.Z. Guo, Z.G. Shi and Y.Q. Feng, J. Chromatogr. A, 2010, 1217, 7331-7336.

[23] S. Zhang, H. Niu, Y. Cai and Y. Shi, J. Chromatogr. A, 2010, 1217, 4757-4764.

[24] S. Zhang, H. Niu, Y. Cai and Y. Shi, Anal. Chim. Acta, 2010, 665, 167-175.

[25] M. Bouri, M. Gurau, M, Zougagh and Á. Rios, Anal Bioanal Chem , 2012, 404, 1529-1538.

[26] B. Tural, T. Tarhan, S. Tural , Journal of Molecular Catalysis B: Enzymatic, 2014, 102, 188–194.

[27] B. Tural, S. Tural , A.S. Demir, Journal of Moleculer Catalysis. B, Enzymatic, 2013, 95, 41-47.

[28] B. Tural, S. Tural , A.S. Demir, Chirality, 2013, 25, 415-421.

[29] B. Tural, I. Simsek, S. Tural, A. S. Demir, Tetrahedron: Asymmetry 2013, 24, 260–268.

[30] J. Wang, B. L. Xu, H. Y. Sun and G. H. Song, Tetrahedron Letters 2013, 54, 238-241.

[31] R. A. Reziq, D. S. Wang, M, Post and H. Alpera, Adv. Synth. Catal, 2007, 349, 2145-2150.

[32] A. K. Nezhad and S. Mohammadi, American Chemical Society, 2013, 15, 512-518.

[33] Q. Zhang, H. Su, J. Luo and Y. Y. Wei, Green Chemistry, 2012, 14, 201-208.

[34] P. Wang, A. G. Kong, W. J. Wang , H. Y. Zhu and Y. K. Shan, Catal Lett , 2010, 135, 159-164.

[35] X. P. Jia, M. L. Xu, Y. Y. Wang, D. Ran, S. Yang and M. Zhang, Analyst, 2013, 138, 651-659.

[36] M. Faraji, Y. Yamini and M. Rezaee, J. Iran. Chem. Soc., 2010, 7, 1-37.

[37] P. Sun and D. W. Armstrong, Analytica Chimica Acta, 2010, 661, 1-16.

Table 1 The types of ILs

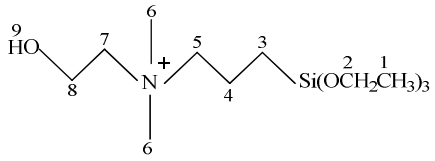
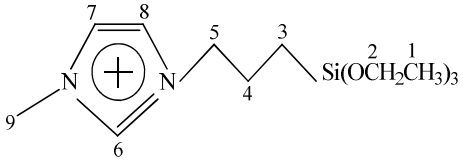
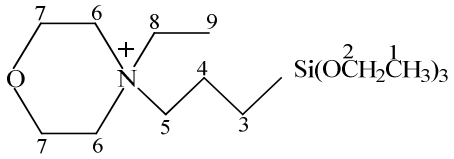
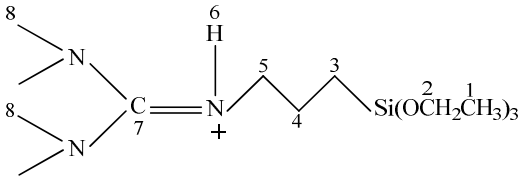
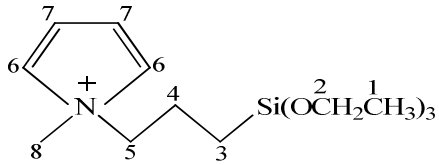
ILs	Cation	Anion
[Simam][Cl]		Cl^-
[Simim][Cl]		Cl^-
[Siemp][Cl]		Cl^-
[Sitmg][Cl]		Cl^-
[Simpy][Cl]		Cl^-

Figure captions

Scheme. 1 Schematic diagram of the synthesis and surface modification of Fe_3O_4 magnetic nanoparticles coated with silica (SiO_2@ Fe_3O_4) for investigation of proteins by magnetic solid phase extraction

Scheme. 2 Schematic diagram of the synthesis five different ionic liquidss at the surface of magnetic nanoparticles (IL-SiO_2@ Fe_3O_4)

Scheme. 3 The schematic charts of extraction proteins by magnetic solid phase extraction

Fig. 1 The FT-IR spectra of the magnetic nanoparticles and the five ionic liquids modified on MNPs.(a) Fe_3O_4 MNPs; (b) SiO_2@ Fe_3O_4 MNPs; (c) [Simim][Cl]-MNPs; (d) [Siemp][Cl]-MNPs; (e) [Simpy][Cl]-MNPs; (f) [Sitmg][Cl]-MNPs; (g) [Simam][Cl]-MNPs

Fig. 2 The TGA analysis of (a) Fe_3O_4 MNPs; (b) SiO_2@ Fe_3O_4 MNPs; (c) [Simam][Cl]-SiO_2@ Fe_3O_4 MNPs

Fig. 3 The XRD patterns of (a) Fe_3O_4 MNPs; (b) SiO_2@ Fe_3O_4 MNPs; (c) [Simam][Cl]-SiO_2@ Fe_3O_4 MNPs

Fig. 4 The VSM analysis of A(a) Fe_3O_4 MNPs; A(b) SiO_2@ Fe_3O_4 MNPs; A(c) [Simam][Cl]- SiO_2@ Fe_3O_4 MNPs, and the magnetic response of [Simam][Cl]-MNPs to external magnetic field (B)

Fig. 5 The micro-pictures of (a), (b) Fe_3O_4 MNPs (TEM); (c), (d) SiO_2@ Fe_3O_4 MNPs(TEM); (e) [Simam][Cl]-SiO_2@ Fe_3O_4 MNPs (TEM)

Fig. 6 Five kinds of ionic liquids modified magnetic nanoparticles were been investigated for the extraction of three proteins (a)BSA, (b)BHb, (c)OVA and

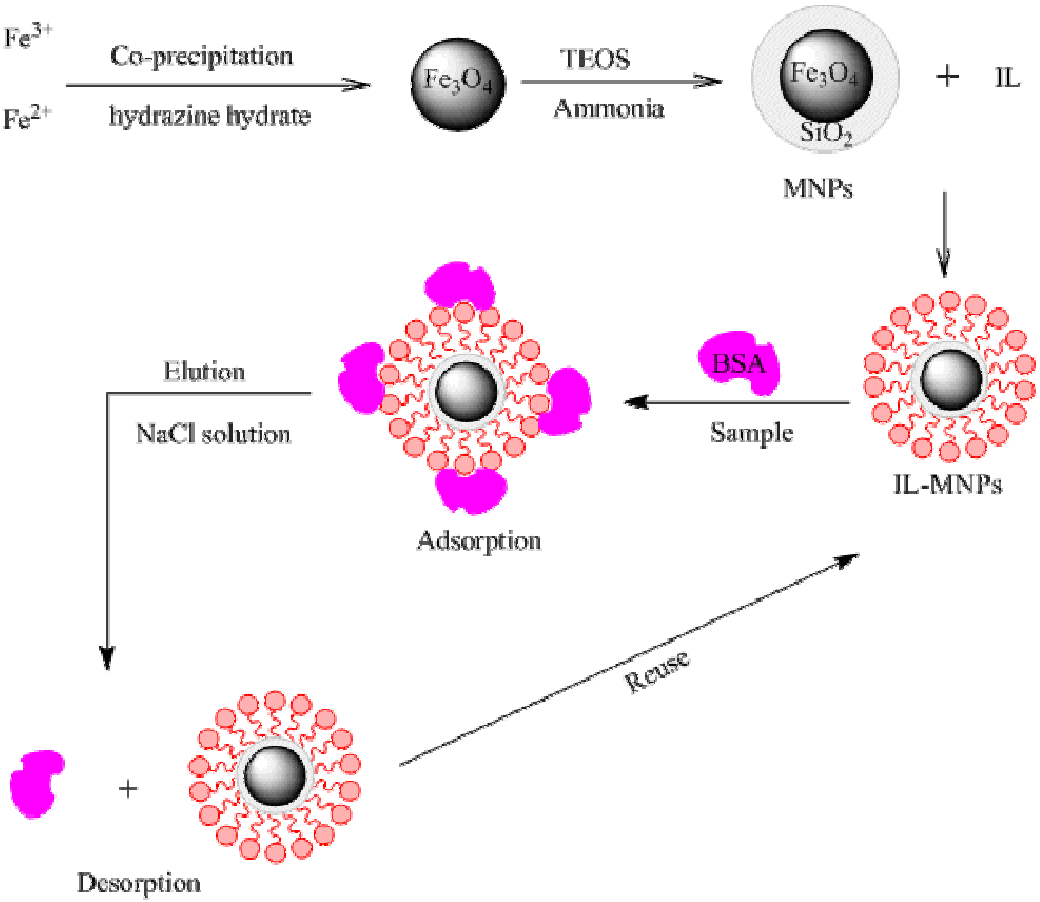
(d) [Simam][Cl]-MNPs extraction of three proteins, respectively.

Fig. 7 Single factor experiments: (a) the mass of [Simam][Cl]-MNPs; (b) protein concentration;(c) the solution pH;(d) the extraction time; (e) temperature

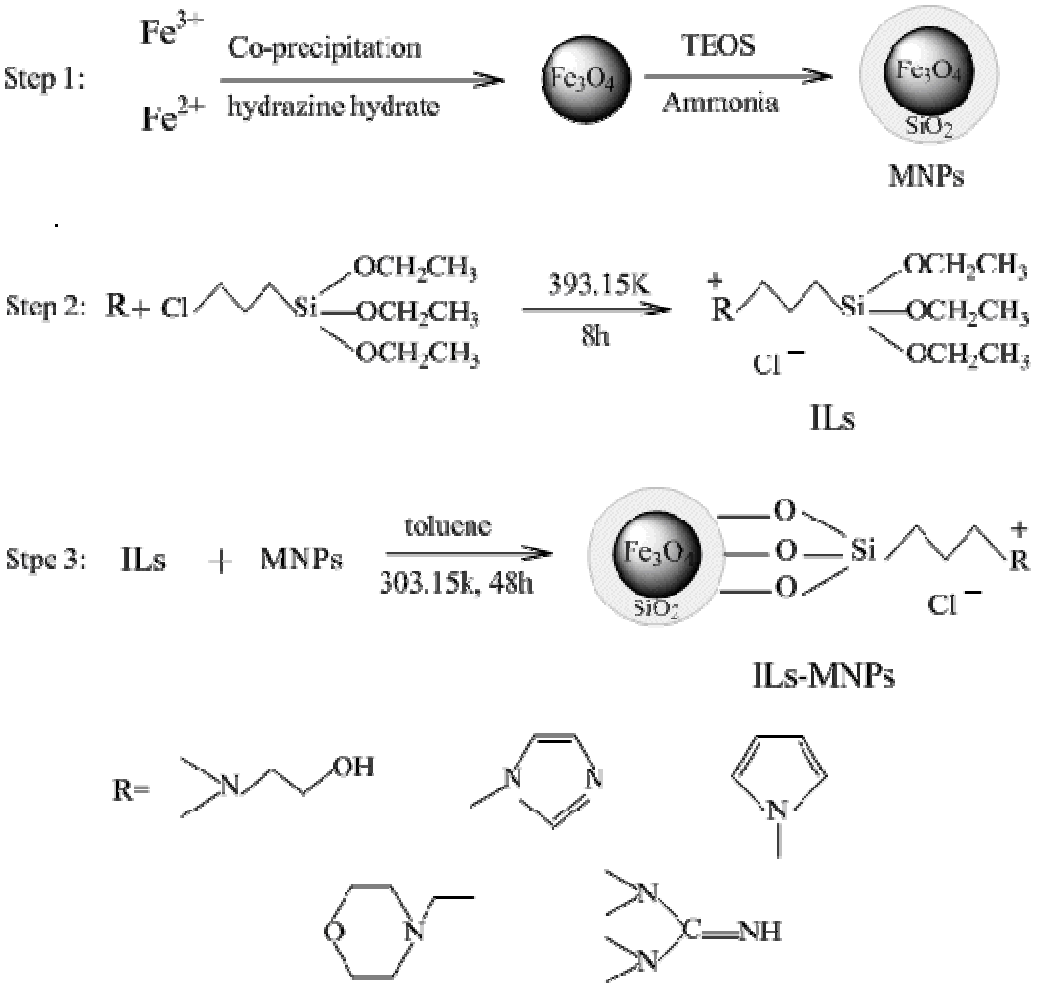
Fig. 8 Desorption BSA from the surfaces of [Simam][Cl]-MNPs experiment.

Fig. S1 Infrared spectroscopy of ILs: (a) [Simam][Cl]; (b) [Simim][Cl]; (c) [Siemp][Cl]; (d) [Sitmg][Cl]; (e) [Simpy][Cl].

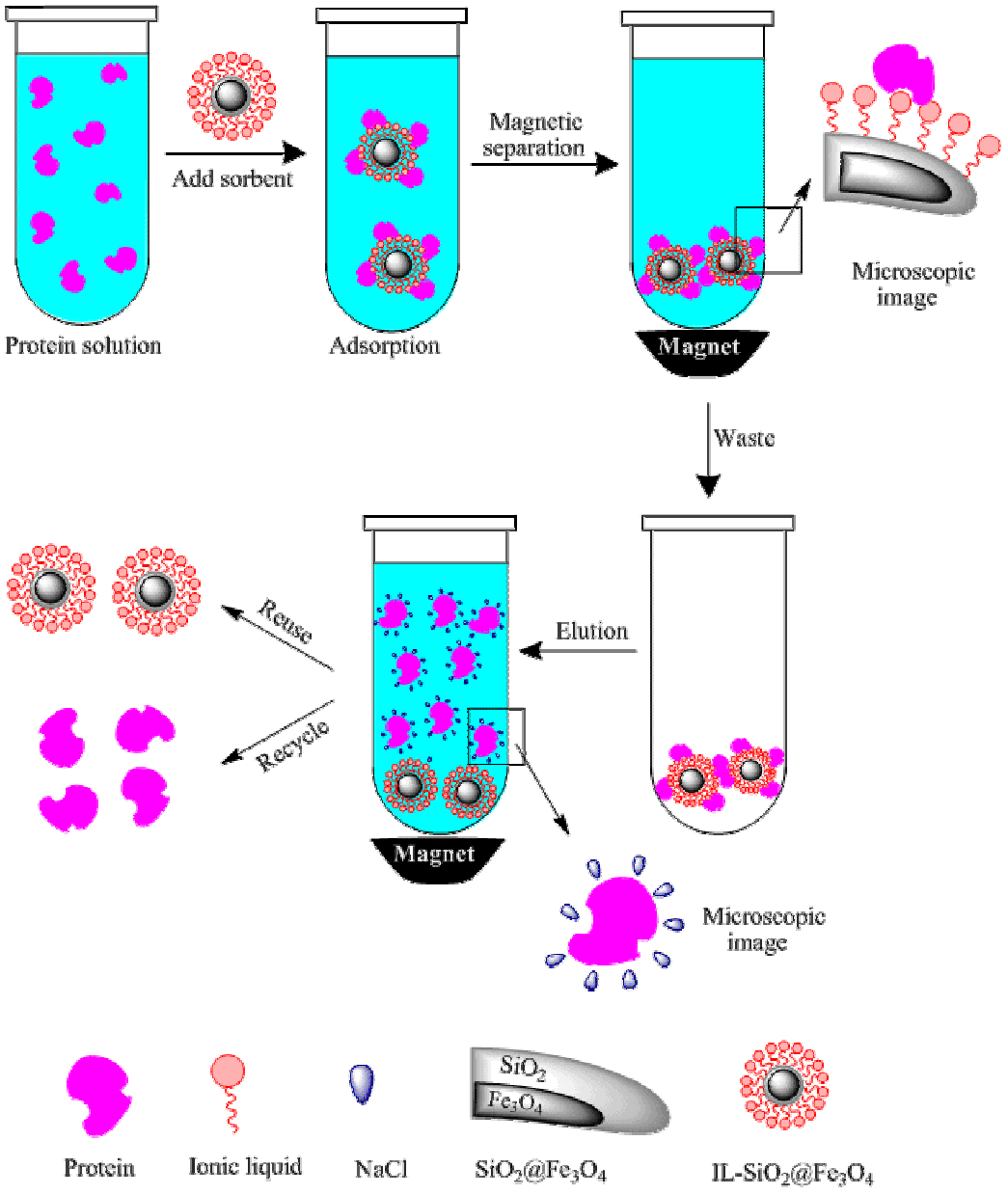
Fig. S2 Reusing experiment of [Simam][Cl]-MNPs.



Scheme. 1 Schematic diagram of the synthesis and surface modification of Fe_3O_4 magnetic nanoparticles coated with silica (SiO_2@ Fe_3O_4) for investigation of proteins by magnetic solid phase extraction.



Scheme. 2 Schematic diagram of the synthesis five different ionic liquidss at the surface of magnetic nanoparticles (IL-SiO_2@ Fe_3O_4)



Scheme. 3 The schematic charts of extraction proteins by magnetic solid phase extraction.

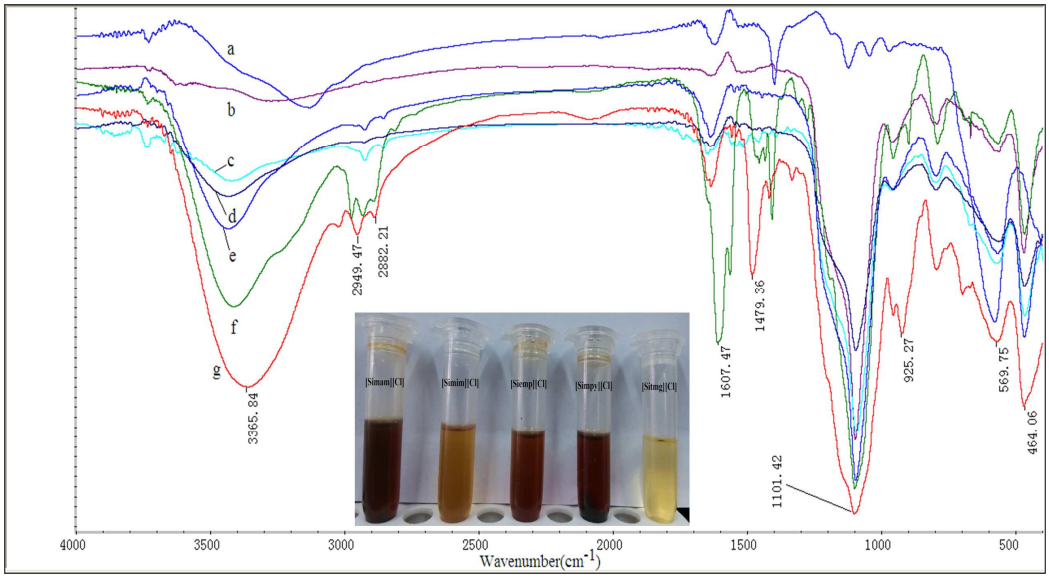


Fig. 1 The FT-IR spectra of the magnetic nanoparticles and the five ionic liquids modified on MNPs.(a) Fe_3O_4 MNPs; (b) SiO_2@ Fe_3O_4 MNPs; (c) [Simim][Cl]-MNPs; (d) [Siemp][Cl]-MNPs; (e) [Simpy][Cl]-MNPs; (f) [Sitmg][Cl]-MNPs; (g) [Simam][Cl]-MNPs

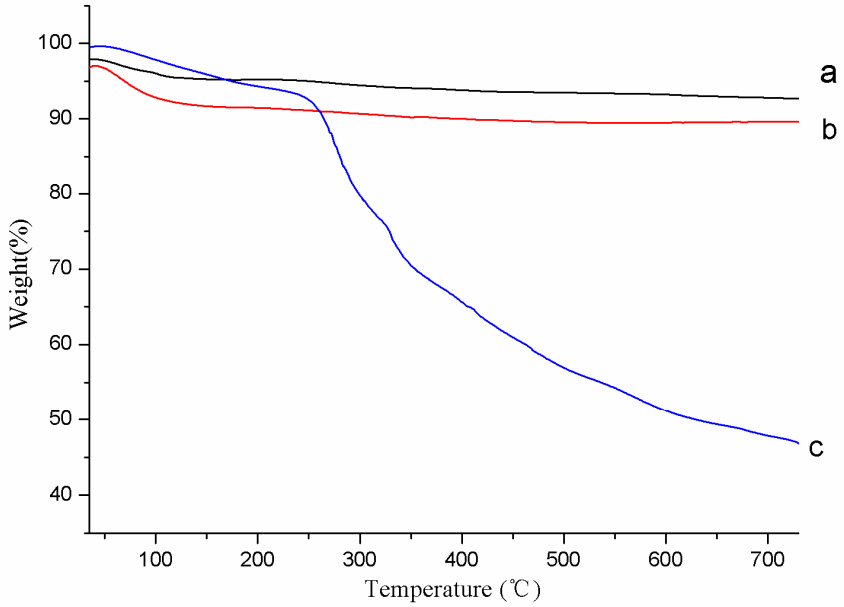


Fig. 2 The TGA analysis of (a) Fe_3O_4 MNPs; (b) SiO_2@ Fe_3O_4 MNPs; (c) [Simam][Cl]-SiO_2@ Fe_3O_4 MNPs

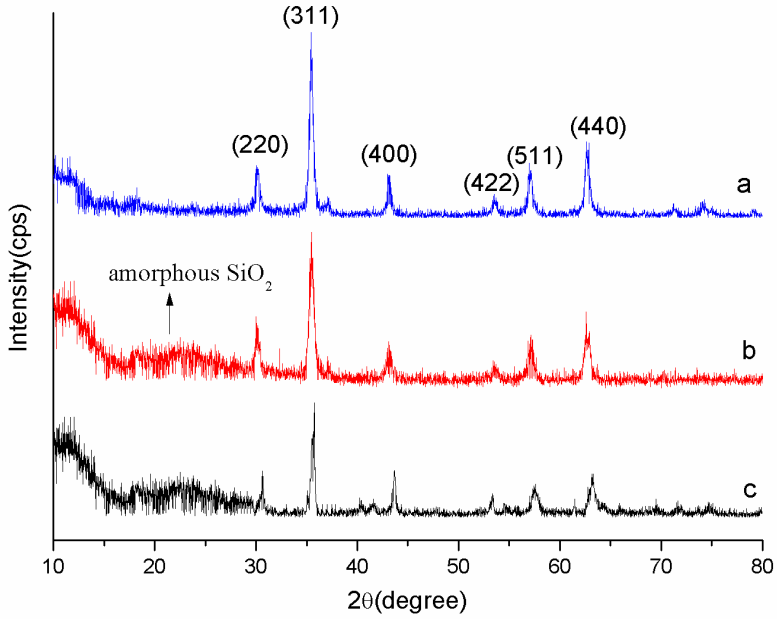


Fig. 3 The XRD patterns of (a) Fe_3O_4 MNPs; (b) SiO_2@ Fe_3O_4 MNPs; (c) [Simam][Cl]-SiO_2@ Fe_3O_4 MNPs

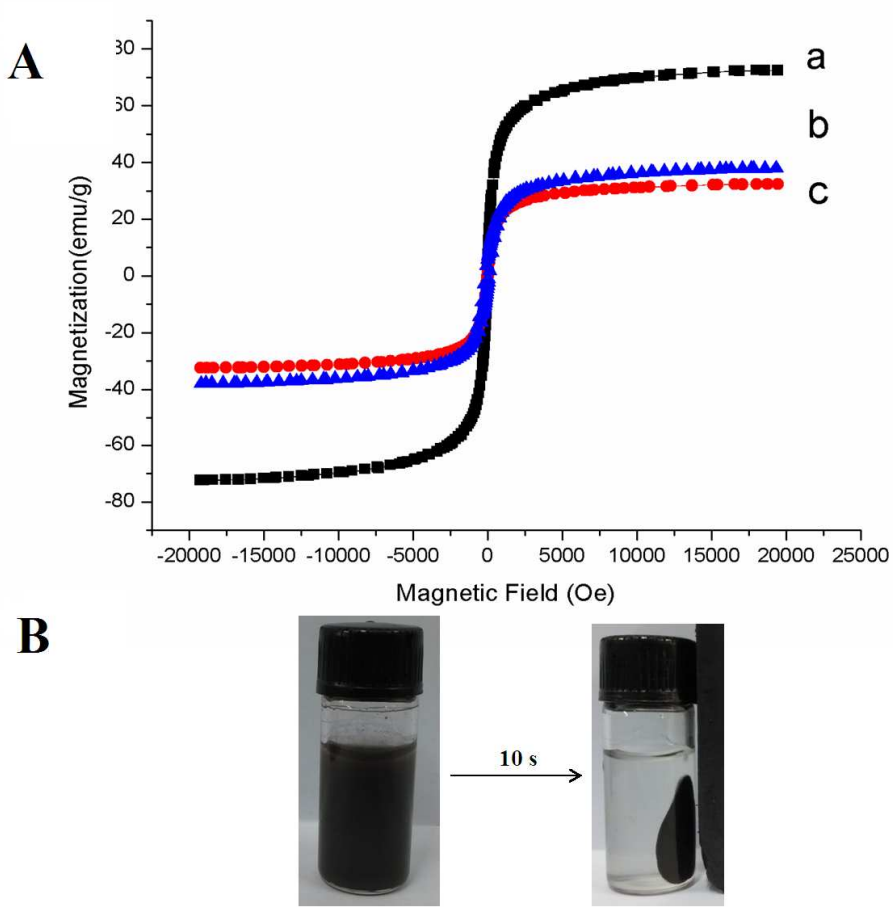


Fig.4 The VSM analysis of A(a) Fe_3O_4 MNPs; A(b) SiO_2@ Fe_3O_4 MNPs; A(c) [Simam][Cl]- SiO_2@ Fe_3O_4 MNPs, and the magnetic response of [Simam][Cl]-MNPs to external magnetic field (B)

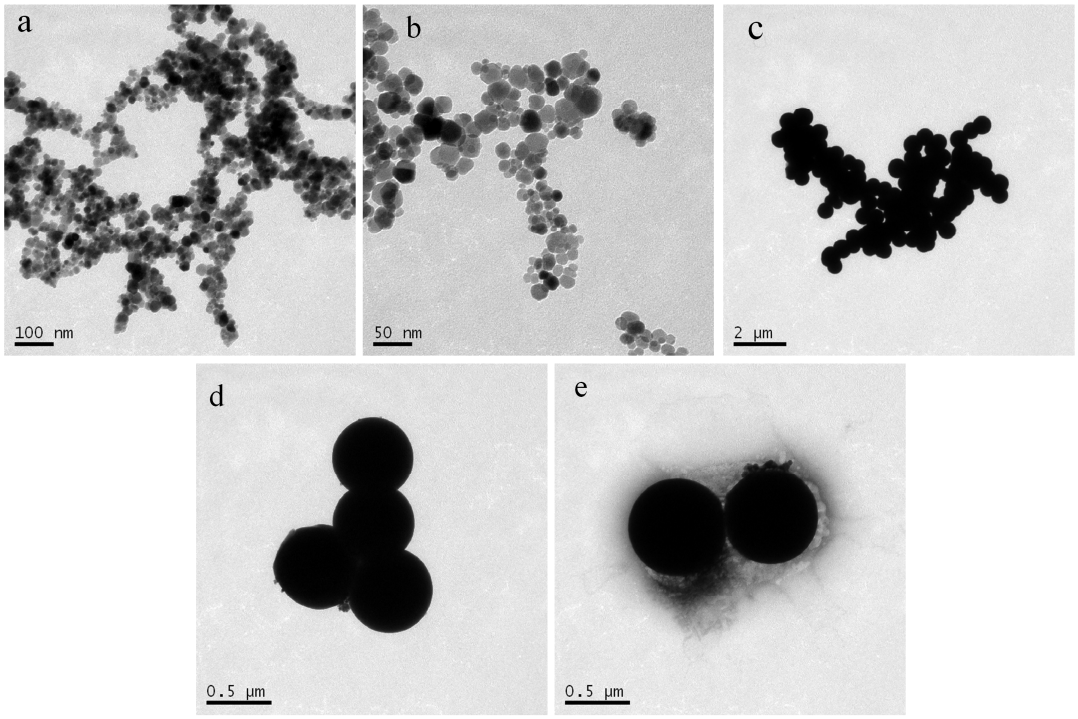


Fig. 5 The micro-pictures of (a), (b) Fe_3O_4 MNPs (TEM); (c), (d) SiO_2@ Fe_3O_4 MNPs(TEM); (e) [Simam][Cl]-SiO_2@ Fe_3O_4 MNPs (TEM)

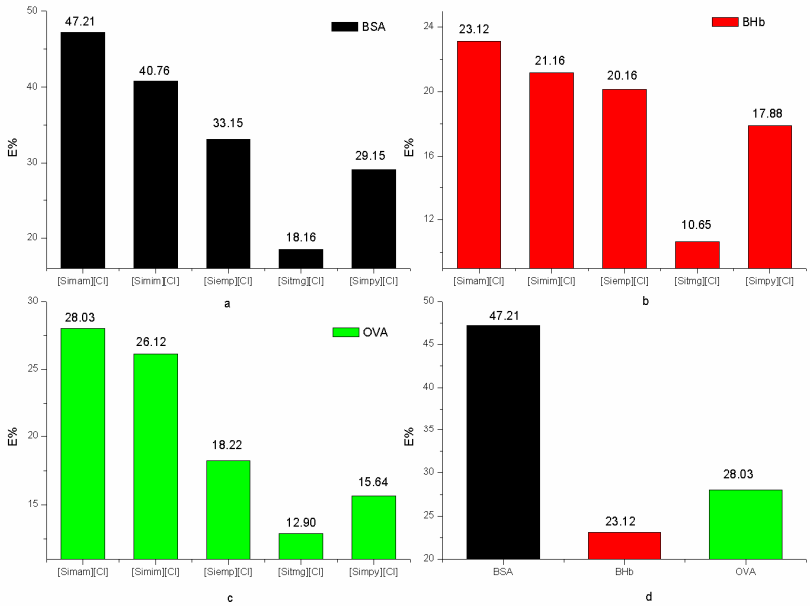


Fig. 6 Five kinds of ionic liquids modified magnetic nanoparticles were been investigated for the extraction of three proteins (a)BSA, (b)BHb, (c)OVA and (d) [Simam][Cl]-MNPs extraction of three proteins, respectively.

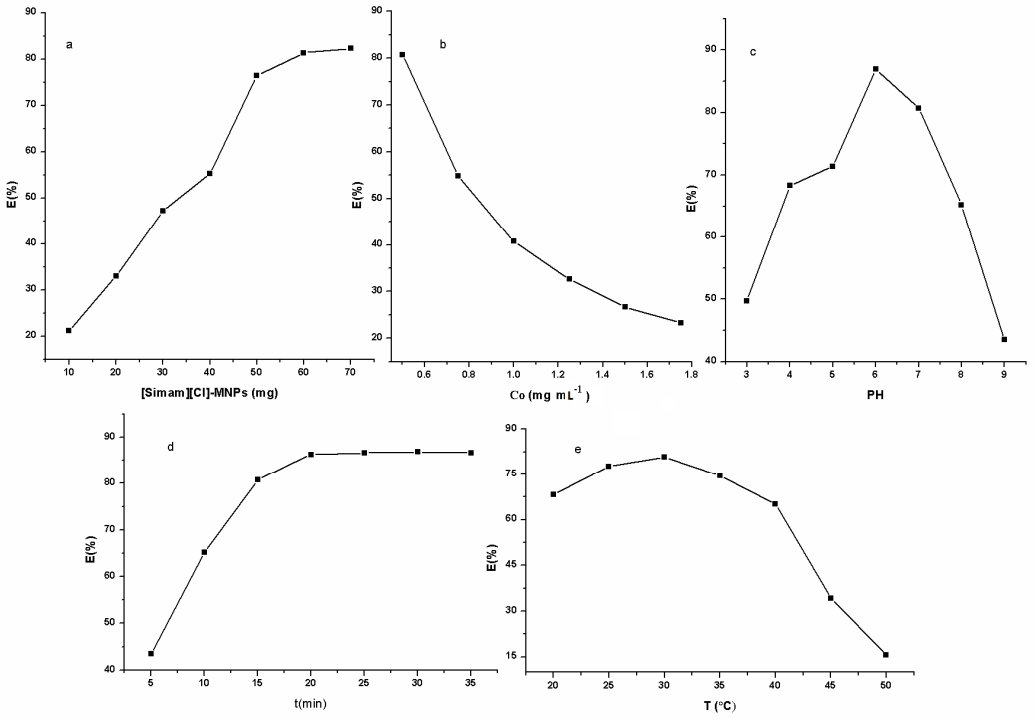
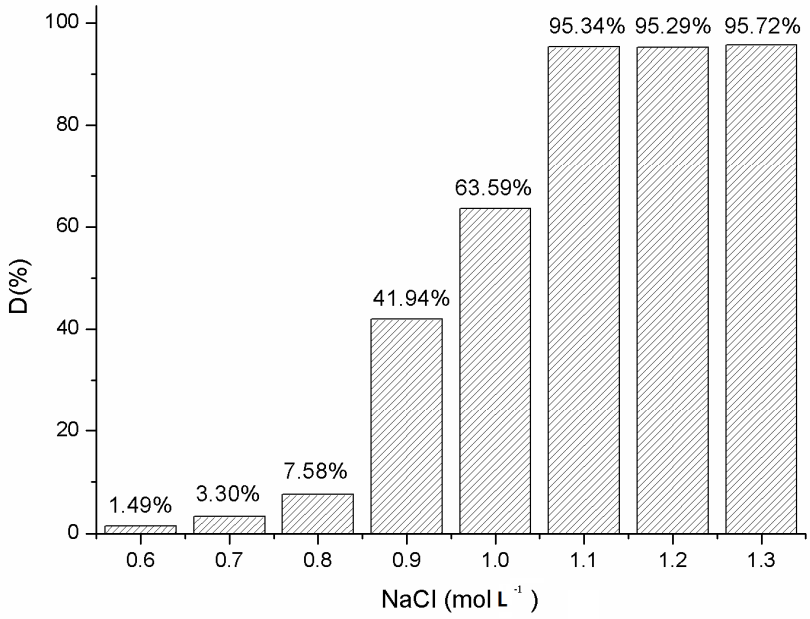


Fig. 7 Single factor experiments: (a) the mass of [Simam][Cl]-MNPs; (b) protein concentration;(c) the solution pH;(d) the extraction time; (e) temperature



692

693 **Fig. 8** Desorption BSA from the surfaces of [Simam][Cl]-MNPs experiment.

694

695