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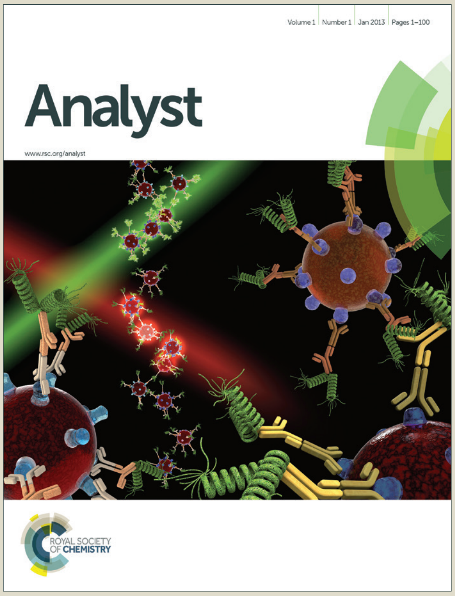

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Magnetic Multiwall Carbon Nanotube Modified with Dual Hydroxy Functional Ionic Liquid for the Solid-phase Extraction of Protein

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Abstract

A novel adsorbent based on silica-coated magnetic multiwall carbon nanotubes (MWCNTs) surface modified by dual hydroxy functional ionic liquid (FIL) ([OH]-FIL-m-MWCNTs@SiO_2) have been first designed and used to the purification of lysozyme (Lys) by magnetic solid-phase extraction (MSPE). Fourier transform infrared spectroscopy (FTIR), vibrating sample magnetometer (VSM), transmission electron microscopy (TEM), X-ray diffraction (XRD) and thermogravimetric analysis (TGA) were employed to characterize [OH]-FIL-m-MWCNTs@SiO_2. After extraction, the concentration of Lys was determined by UV-Vis spectrophotometer at 278nm. A series of single-factor experiments were test to identify the optimal conditions of the extraction and the extraction amount could reach up to 94.6 mg g^{-1}. The RSD of the precision, the repeatability and the stability experiments were 0.37% (n = 3), 0.47% (n = 3) and 0.52% (n = 3), respectively. Comparison of [OH]-FIL-m-MWCNTs@SiO_2 with silica-coated magnetic Fe_3O_4 (Fe_3O_4@SiO_2), silica-coated magnetic multiwall carbon nanotubes (m-MWCNTs@SiO_2) and alkyl quaternary ammonium ionic liquid-modified on m-MWCNTs@SiO_2 were carried out by extracting Lys. The extraction of bovine serum albumin (BSA), trypsin (Try) and ovalbumin (OVA) had been done by the proposed method. Desorption of Lys was carried out by 0.005 mol $L^{-1}$$Na_2HPO_4$-1 mol L^{-1}NaCl as the eluen solution and the desorption ratio reached to 91.6%. Nearly 97.8% of [OH]-FIL-m-MWCNTs@SiO_2 could be recovered from each run, and the extraction amount decreased less after five runs. The circular dichroism spectral experiment analysis indicated that the secondary structure of Lys was unchanged after extract.

Keywords: dual hydroxy functional ionic liquid; magnetic multiwall ; lysozyme; solid-phase extraction

1 Introduction

Lysozyme (Lys) is a basic protein which plays a critical role in life activities. It is a glycoside hydrolase and can damage bacterial cell walls by catalyzing the hydrolysis of 1, 4-β-linkages between the muramic acid and N-acetylglucosamine of the mucopolysaccharides. The traditional purification methods of Lys include crystallization, precipitation and affinity chromatography method. With the emergence of various new biological separation techniques, the purification method is also more and more, such as ionexchange chromatography [1, 2], membrane chromatography [3], affinity based bioseperation technique [4, 5, 6], reversed micellar extraction [7] and aqueous two-phase extraction [8] .

Separation methods based on magnetic materials have attracted widespread attention in recent years[9, 10]. 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 is not needed. The use of external magnetic field in magnetic SPE (MSPE) can save processing time. Obviously, MSPE can improve the extraction efficiency and simplify the process of preprocessing.

In MSPE, it is particularly important to select appropriate sorbent in order to obtain high recovery. Carbon nanotubes (CNTs) have been the focus of intense research since they were firstly discovered by Iigyma in 1991 [11]. Multiwall carbon nanotubes (MWCNTs) are kinds of CNT-based materials which has many unique advantages, such as ultra-high surface area, high mechanical strength, excellent chemical and thermal stability and rich electronic polyaromatic structure [12]. Magnetic multiwall carbon nanotubes (m-MWCNTs) can combine the advantages of MWCNTs and magnetic particles. MWCNTs can be functionalized with magnetic particles (such as Fe_3O_4) by chemical modifications and then the magnetic absorbents are easily collected and separated from the solution with an external magnetic field. Therefore, a novel form of magnetic solid-phase extraction (MSPE) based on magnetic multiwall carbon nanotubes (m-MWCNTs) have been widely researched [13,14]. However, there are still some drawbacks about the naked magnetic multiwall carbon nanotubes like low adsorption on large molecules and less specifically adsorbed. Thus, many organic functional monomers or polymers have been modified on m-MWCNTs, such as polymerized ionic liquid [15-17], organic acid [18,19] and poly ethylene glycol [20].

Ionic liquids (ILs) are a class of liquids and become one of the hotspots of green solvent due to its unique properties and special characters, including wide liquid ranges, good thermal stabilities, low volatilities, wide range of viscosities, electrolytic conductivity, adjustable miscibility, nonflammability and reusability [21]. By adjusting the combination or introducing appropriate functional groups, functional ionic liquids can be obtained. One of the most common functional ionic liquids is hydroxy functional ionic liquids, which not only has the unique propertie of ionic liquid but also has stronger hydrogen bond and biological activity.

Magnetic materials functionalized with ionic liquids (ILs) are receiving considerable attention. Although ILs lose liquid state when modified on the surface of the materials, the other unique properties such as polarity and low volatility are maintained. Moreover, the introduction of ionic liquid moieties could improve the performance of the resulting materials and attract a broad range of applications.

In order to combine the above advantages, a novel form of MSPE has been developed based on magnetic multiwall carbon nanotubes surface modified by the ionic liquid (IL- m-MWCNTs). These methods have been applied in magnetic solid-phase extraction of various compounds such as fluoroquinolones [22], flavonoids [23], dye [24,25], drug [26,27], estrogens [28,29], acid [30,31], pesticides [32].

In this paper, two kinds of dual hydroxy functional ionic liquids were synthesized and modified on the surface of silica-coated magnetic multiwall carbon nanotubes for the investigation of the extraction performance of Lys by magnetic solid-phase extraction (as shown in scheme 1). After extraction, the concentration of Lys was determined by UV-Vis Spectrophotometer at 278nm. The presented [OH]-FIL-m-MWCNTs@SiO_2-MSPE technique has also been used to extract bovine serum albumin (BSA), trypsin (Try) and ovalbumin (OVA).

Compared with our previous work, by choosing hydroxy functional ionic liquids and magnetic multiwall carbon nanotubes as functional solid phase extraction agent, it provides more hydrophilic and selective adsorption sites and can effectively save the time as well as improve the extraction rate in the process of the solid-phase extraction of protein.

Because the ionic liquids can be designed, the performances of the method also have the potential to offer new possibilities in the extraction of protein and DNA or other bio-analysis.

2 Experimental

2.1 Materials and reagents

Multi-walled Carbon Nanotubes (MWCNTs) were obtained from Nanjing XFNANO Materials Tech Co., Ltd (Nanjing, China). Iron (III) chloride hexahydrate ($FeCl_3·6H_2O$), iron (II) sulfate heptahydrate ($FeSO_4·7H_2O$) and ammonia solution (27%, w/v) were purchased from Fuchen (Tianjin, China). Tetraethyl orthosilicate (TEOS) and ethanol were purchased from Aladdin chemistry Co. Ltd. (Shanghai, China). N, N-dimethylethanolamine and N, N-dimethylethylamine were purchased from Tianjin Kermel Fine Chemical Research Institute. 1-Chlorohexane and 6-Chloro-1-hexanol were obtained from Energy chemical Company (Shanghai, China). Bovine serum albumin (BSA), lysozyme (Lys), trypsin (Try) and ovalbumin (OVA) were supplied by Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). The ultra pure water (18.25 MΩ cm^{-1}) used throughout the experiment was purified using the laboratory purification system. All the chemicals from the commercial sources were generally of analytical grade without any further purification.

2.2 Apparatus

Ionic liquids and magnetic nanoparticles were dried by DZF-6051vacuum drying oven (Shanghai, China). A Thermostats cultivating shaker (Shanghai, China) was used to provided a certain temperature and rotation speed in the experiment. A UV-2450 UV-Vis Spectrophotometer (SHIMADZU, Japan) was used to determine the absorbance of the sample. NMR spectra were measured with aVarianInova-400 NMR spectrometer (Varian, USA) at room temperature by using TMS as internal standard. FT-IR spectrometer (PerkinElmer, USA) was employed to confirm the structure of Ionic liquids and magnetic nanoparticles. A STA 409 thermal gravimetric analyzer (Netzsch, D5000 X-ray Diffraction (Siemens, Japan) and EV 11 Vibrating Sample Magnetometer (MicroSense, USA) were used to further estimate the relative composition of nanoparticles. A JEM-1230 transmission electron microscope (JEOL, Japan) was employed to determine the size and morphology of the nanoparticles. A Mos-500 circular dichroism (CD) spectrometer (Biologic, France) was used to analysis of the secondary structure of lys.

2.3 Sorbent preparation

2.3.1 Synthesis of m-MWCNTs@SiO_2

Prior to use, the multiwalled CNTs need further purification. It was carried out by mixing the MWCNTs (0.5 g) with 100 ml of concentrated sulfuric acid and concentrated nitric acid mixture (mole ratio: 3/1). The mixture was sonicated for 2 h and then refluxed under magnetic stirring at 80 □ for 12 h. The dispersed solution was thoroughly washed with deionized water to neutrality, filtered and dried under vacuum at 65 □ for 24 h.

An aqueous solution of 10 mmol $FeCl_3$ and 5 mmol $FeSO_4$ in water (30 ml) was first added in a three-necked 250 ml round-bottom flask. Subsequently, a suspension of c-MWCNTs (0.5g) was added under violent stirring. 5 ml ammonium hydroxide (27%, w/v) was dropwise added into the solution and stirred for 30 min at room temperature. To make sure the pH value of the solution was 10-11, the reaction was then cured for 30 min at 80 ℃. Finally, the m-MWCNTs was isolated by a magnetic plate and washed with ultrapure water until the pH of the washing solution was 7. Finally, the particles were dried under vacuum at 70 ℃ for 24 h.

A layer of silicon coated on the surface of Fe_3O_4 nanoparticles can prevent the oxidation of Fe_3O_4. Therefore, a suspension made up by 1.0 g m-MWCNTs, 150 ml ethanol and 30 ml water was dispersed by ultrasonic dispersion for 15 min. Then 2 ml ammonium hydroxide solution(27%, w/v) and 4 ml TEOS were added under violent stirring at room temperature for 12 h. The suspension was isolated by a magnetic plate and washed with ultrapure water until the pH of the washing solution was 7. Finally, the particles of m-MWCNTs @SiO_2 were dried under vacuum at 70 ℃ for 24 h.

2.3.2 Synthesis and characterization of ILs

[OH]-Functionalized ionic liquid ([OH]-FIL) and alkyl quaternary ammonium ionic liquid (alkyl-IL) (as shown in Fig. 1) were synthesized. By way of example, 0.1 mol of N, N-dimethylethanolamine dissolved in 20 ml ethanol was firstly added to a dried 250 ml round-bottom flask equipped with a magnetic stirring bar and condenser and then 0.11mol 6-Chloro-1-hexanol was dropwise added into the flask. The mixture was stirred at 80 ℃for 24 h under nitrogen protection. The product was purified further by washing three times with 100 ml of ethyl acetate and then dried in a vacuum drying oven at 80℃for 24 h. All the synthetic ILs structures were confirmed by ^{1}HNMR and ^{13}C NMR spectra, which were shown in the Supplementary information Table S1.

2.3.3 The m-MWCNTs @SiO_2 surface modified by ILs

To modify the ILs on the surface of the m-MWCNTs@SiO_2, the [OH]-functionalized ionic liquid has been taken as the example. [OH]-FIL (1g) was dissolved in 15 ml ethanol and then 0.1 g of m-MWCNTs @SiO_2 was added. The mixture was sonicated for 30 min. The resulting solution was isolated by a magnetic plate, washed with ethanol and dried under vacuum at 70 □ for 24 h.

2.4 Magnetic solid-phase extraction procedure

The MSPE experiments were investigated batch-wise by using [OH]-FIL-m-MWCNTs@SiO_2 as the adsorbent and Lys was chosen as the model protein. Firstly, different amounts of [OH]-FIL-m-MWCNTs@SiO_2 were added into the protein solutions and shaken by a thermostats cultivating shaker at 200 rpm for an appropriated time and temperature. After the system reached equilibrium, the solid phase that contained the adsorbed protein was conveniently separated by an external magnetic field. The concentration of protein adsorbed on the [OH]-FIL-m-MWCNTs@SiO_2 were determined by a UV-vis spectrometer at 278nm after adsorption according with the concentration change of protein in supernatant solution.

The extraction rate (E) and the extraction amount (Q) are calculated based on the following equation:

$$E=(C_0-Ce)/C_0\times100\% \quad (1)$$

$$Q=(C_0-Ce)\times V/W \quad (2)$$

where C_0 is the initial protein concentration (mg ml^{-1}), Ce is the equilibrium protein concentrations (mg ml^{-1}), V(ml) is the volume of protein solution and W (g) is the weight of the adsorbent used.

3 Results and discussion

3.1 Characterization of the [OH]-FIL-m-MWCNTs @SiO$_2$

3.1.1 FT-IR studies

As is known to all, the FT-IR spectra can provide useful information for identifying the presence of certain functional groups or chemical bonds in a molecule. As shown in Fig. 2, the characteristic bands around 579 cm^{-1} corresponds to Fe–O vibrations of the magnetite core. Peaks located at 1096cm^{-1}, 953 cm^{-1} and 465 cm^{-1} which were correspond to the Si–O–Si asymmetric stretching vibration, Si–C stretching vibration and Si–O–Si bending vibration, respectively, can be observed after modified m-MWCNTs with silicon. The spectrum also displays strong bands at around 1742 cm^{-1} and 1531 cm^{-1} correspond to C-O stretching vibrations of the –COOH groups. The distinguished feature in the FT-IR spectra of m-MWCNTs@SiO$_2$ surface modified by [OH]-functionalized ionic liquid was the appearance of the new peak as follow: peaks located at 2934cm^{-1} and 2876 cm^{-1} which was assigned to the symmetric and asymmetric vibrations of methylene (CH$_2$) and methyl (CH$_3$), peak located at 3332 cm^{-1} was assigned to O–H stretching. So the FT-IR spectroscopy proved that [OH]-functionalized ionic liquid was successful modified on the surface of m-MWCNTs @SiO$_2$.

3.1.2 Vibrating sample magnetometer studies

VSM was used to evaluate the magnetic intensity of the [OH]- m-MWCNTs @SiO_2 which was important for magnetic materials to possess sufficient magnetic properties in separation from liquid medium quickly. Fig. 3(A) shows the magnetic hysteresis loops of m-MWCNTs, m-MWCNTs@SiO_2 and [OH]-FIL-m-MWCNTs@SiO_2. It was found that the saturation magnetization of them was 44.537emu g^{-1}, 19.941 emu g^{-1} and 16.365 emu g^{-1}, respectively. The saturation magnetization of m-MWCNTs@SiO_2 decreased in comparison with m-MWCNTs which resulted from the nonmagnetic silica coating shell. The saturation magnetization has a slight decrease after ionic liquids modified on the m-MWCNTs@SiO_2. Nevertheless, Fig.3(B) indicated that the saturation magnetization value of [OH]-FIL-m-MWCNTs@SiO_2 was high enough to make them easily and quickly separate from the suspension.

3.1.3 Transmission electron microscope studies

The microscopic structure of the resulting materials was observed by TEM and the images of c-MWCNTs, m-MWCNTs, m-MWCNTs@SiO_2 and [OH]-FIL-m-MWCNTs@SiO_2 particles were shown in Fig. 4. It can be seen from Fig. 4a that the diameter of c-MWCNTs were about 20-30 nm. Fig. 4b illustrated that the naked of Fe_3O_4 particles had a mean diameter of about 10-20 nm and formed on the side walls of MWCNTs. Fig. 4c showed the Fe_3O_4 particles and MWCNTs coated by silica which had uniform size about 30-40 nm. Moreover, a gray liquid surrounded about the m-MWCNTs@SiO_2 could be observed in Fig. 4d and Fig. 4e（the same sample but different point of view） after the particles 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 and monodisperse.

3.1.4 X-ray powder diffraction studies

The XRD spectra of c-MWCNTs, m-MWCNTs and m-MWCNTs@SiO_2 were investigated and shown in Fig. 5. In the 2θ range of 10-80°, at 25.96° and 42.74°, the peaks were attributed to the graphite structure (002) and (100). After modified Fe_3O_4 on the surface of the MWCNTs, the characteristic peaks of MWCNTs remain unchanged, while new diffraction peaks were identified at 30.1°, 35.5°, 43.1°, 53.4°, 57.0° and 62.6°, corresponding to the (220), (311), (400), (422), (511) and (440) planes of the standard XRD data for Fe_3O_4, which are in good agreement with the theoretical values (JCPDS Card: 019-0629). 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 [33,34]. The results indicated that the crystal phase of c-MWCNTs, m-MWCNTs and m-MWCNTs@SiO_2 had got phase stability and the integrity of the structure which were not changed during the coating process.

3.1.5 Thermal gravimetric analyzer studies

TGA analysis was employed to further study the relative composition of the amount of ionic liquid deposited onto the surface of m-MWCNTs@SiO_2. As shown in Supplementary information Fig. S1, when the temperature was changed from room temperature to 150□, the weight loss of [OH]-FIL-m-MWCNTs@SiO$_2$ was approximately 12% that is attributed to the removal of water and solvent residues. When the temperature rises to 300□, the weight loss was 23%. The reason may be due to the decomposition of ionic liquid. It can be seen that m-MWCNTs@SiO$_2$ can withstand high temperature and the weight loss is very little when the temperature rose from 300□ to the 800□. According to the TGA curves, the grafting yield of [OH]-FIL modified on the m-MWCNTs@SiO$_2$ was about 23%.

3.1.6 Isoelectric point

The isoelectric point is known to be a significant characteristic of metal oxides. Zeta potentials at different pH between m-MWCNTs@SiO$_2$ and [OH]-FIL-m-MWCNTs@SiO$_2$ were measured, and the results were given in Fig. 6. It can be found that the isoelectric point (pI) of m-MWCNTs@SiO$_2$ is pH 3.4 which was consistent with the previous findings [23]. While after the ionic liquids modified on the m-MWCNTs@SiO$_2$, pI increased to pH 6.3. It may be due to the fact that the magnetic nanoparticles coated with ionic liquids which can change the surface electrokinetics of the magnetic supports were reversed and increased after passing through a zero zetapotential [35].

3.2 Optimization of MSPE parameters

The extraction ability of the MSPE based on dual hydroxy functional ionic liquid-modified magnetic multiwall carbon nanotubes was researched by chosen Lys as the model protein. Several experimental conditions, including the amount of [OH]-FIL-m-MWCNTs@SiO$_2$, the Lys concentration, the solution ionic strength, the extraction time and the temperature were examined.

3.2.1 Effect of the amount of [OH]-FIL-m-MWCNTs@SiO$_2$

To study the extraction capacity of the proposed sorbent, different amounts of [OH]-FIL-m-MWCNTs@SiO$_2$ in the range of 4-20 mg were added to the Lys solution (1.0 mg ml^{-1} and 1 ml). The suspension was then immediately stirred for 30 min at 30 □, and the results were illustrated in Fig.7a. We can see that the extraction amount of [OH]-FIL-m-MWCNTs@SiO$_2$ decreased with the increasing of the amount of [OH]-FIL-m-MWCNTs@SiO$_2$. This is because the total extraction amount limited and further increase of the absorbent mass did not affect the adsorption of proteins at the tested condition. But the extraction rate was increased with the addition of absorbent. The reason could be that the greater the number of [OH]-FIL-m-MWCNTs@SiO$_2$, the greater number of adsorption sites would be available for protein molecules adsorbed. Hence, the optimum amount of [OH]-FIL-m-MWCNTs@SiO$_2$ for adsorption of Lys was found to be 10 mg.

3.2.2 Effect of the concentration of Lys.

The effect of the sample concentration on the extraction amount of the [OH]-FIL-m-MWCNTs@SiO$_2$ was investigated using a batch technique. A series of protein solutions (0.5-1.5 mg ml^{-1}) were examined and shaken for 30 min at 30 □ to achieve balance. As shown in Fig. 7b, it is obvious that with the increase of the Lys concentration, the extraction amount increased linearly until the concentration goes up to 1.0 mg ml^{-1} and the sample was nearly totally extracted by [OH]-FIL-m-MWCNTs@SiO$_2$ when the concentration of the Lys is 1.0 mg ml^{-1}.

3.2.3 Effect of solution ionic strength

Fig.7c shows the relationship of the solution ionic strength on the extraction of Lys by adding NaCl with concentration range of 0.0-0.5 mol L^{-1}. It can be seen that the extraction amount decreases rapidly as the concentration of NaCl increases up 0.1 mol L^{-1}. It is possible that there has a competition between Lys and the salt ions adsorbed on the surface of [OH]-FIL-m-MWCNTs@SiO_2. But, the electrostatic interaction is not the only driving forces during the extraction. There also have hydrophobic interaction and hydrogen bonding interaction for extraction of Lys, so that the extraction amount could be leveled off by adding more NaCl. It is obvious that increased the solution ionic strength is helpless during the extraction process.

3.2.4 Effects of extraction time.

In order to discuss the effect on extraction efficiency of the extraction time, a series of test were investigated by changing the shaking time and the result is shown in Fig.7d. It can be seen that the extraction efficiency of Lys increased rapidly in the first 20 min, and achieved a balance. We can speculate that there exist many absorbent sites on the surfaces of [OH]-FIL-m-MWCNTs@SiO_2 and the Lys can be easily extracted in the first 20 min. With the passage of time, the extraction efficiency had almost reached maximum, thus the speed of Lys extraction become more slowly and to be balance. So, 20 min is been chosen during the extraction process.

3.2.5 Effect of solution temperature.

To further confirm the influence of the temperature on the extraction amount of Lys, a series of experiments were performed over a temperature range of 15-60□. In light of Fig. 7e, when the temperature is below 30□, the extraction amount of Lys increase obviously and decease after reaching the top point at 30□. The possible reason for this phenomenon was that the adsorption process of Lys onto the [OH]-FIL-m-MWCNTs@SiO_2 was an endothermic process. But when the temperature continue to rise, it is enough high to destroy the hydrogen bonding interaction between [OH]-FIL-m-MWCNTs@SiO_2 and the surface water of protein's amino acid residue. Therefore, the optimum temperature of the extraction process is 30□ and the experiment temperature is easy to implement.

3.3 Methodological study.

Under the optimized extraction conditions, a series of experiments were performed to validate the precision experiment, repeatability experiment and stability experiment of the developed MSPE method (as shown in the Supplementary information Table S2). Apparatus precision was investigated three times by the analysis of the solution of Lys. The calculation of RSD was 0.37% (n=3), which indicates that the precision of the UV-vis spectra is satisfactory. Three copies of the same sample were measured under the same conditions, and the RSD obtained was 0.47%. The result indicates that this method has excellent repeatability. Stability experiment was performed by detecting a sample continuously in three-days under the same conditions. The result of the RSD was 0.52% (n=3), which explains that the sample is recoverable within three days.

3.4 Comparison of the sorbent.

In order to research the superiority of the proposed magnetic solid-phase extraction, [OH]-FIL-m-MWCNTs@SiO_2, alkyl-m-MWCNTs@SiO_2, m-MWCNTs@SiO_2 and Fe_3O_4@SiO_2 (our previous work [36]) were used to extract Lys under the same conditions, and the extraction amount of Lys was 94.6 mg g^{-1}, 73.1mg g^{-1}, 38.1mg g^{-1} and 31.7 mg g^{-1}, respectively. It is clear that the [OH] -FIL -m-MWCNTs@SiO_2 has a higher extraction amount than others, and the poorest effect is shown by the Fe_3O_4@SiO_2. Fe_3O_4@SiO_2 and m-MWCNTs@SiO_2 can extract Lys because there exist plenty of functional groups on its surface such as hydroxyl and epoxy functional groups. But the extraction capacity of m-MWCNTs@SiO_2 is a little more than Fe_3O_4@SiO_2. It is possible that the extraction amount had a positive relationship with the specific surface area of absorbent. As the size of m-MWCNTs@SiO_2 is smaller than that of Fe_3O_4@SiO_2, the specific surface area of m-MWCNTs@SiO_2 is greater than that of Fe_3O_4@SiO_2 on the same mass. In addition, after coated with ionic liquid, the extraction amount of alkyl-IL-m-MWCNTs@SiO_2 and [OH]-FIL-m-MWCNTs@SiO_2 are more than that of naked magnetic nanoparticles. This may be caused by strong hydrophobic and electrostatic interactions between the ionic liquid and Lys. However, the extraction amount of alkyl-IL-m-MWCNTs@SiO_2 is still less than that of [OH]-FIL-m-MWCNTs@SiO_2 which may be attributed to the hydrogen bond interaction between the hydroxyl of the ionic liquid cation and the aliphatic hydrocarbon residue of the protein.

3.5 Comparison of protein type.

The extraction amount of four kinds of protein (Lys, Try, OVA and BSA) was compared to investigate the effect of extraction parameters under the optimized extraction conditions. The results are showed in Table 1 and the extraction amount increases in the order: BSA< OVA < Try < Lys. The results can be explained by the isoelectric point and the size effect of proteins. In aqueous solution (pH=7), the isoelectric point of BSA and OVA are around 4.7-4.8 and have negative charges, but Lys (pI=10.8) and Try (pI=10.5) were positively charged while the surfaces of [OH]-FIL-m-MWCNTs@SiO$_2$ (pI=6.3) was negatively. Owning to the strong electrostatic attraction between negatively-charged and positively-charged nanoparticles, the extraction amount of Lys and Try is more than BSA and OVA. In addition, a smaller sized protein is easier to be extracted. In this study, the molecular weight of protein is Lys (M_W = 14 400), Try (M_W = 23 800, OVA (M_W = 45 000) and BSA (M_W = 65 000), thus the extraction amount is of Lys > Try and OVA > BSA.

3.6 Desorption and regeneration studies

In the desorption process, 0.005 mol L^{-1}Na$_2$HPO$_4$-1 mol L^{-1}NaCl were chosen as the eluent and the desorption ratios reached 91.6%. Two reasons can be explained. On the one hand, Na$_2$HPO$_4$ can destroy the hydrogen bonds between [OH]-FIL-m-MWCNTs@SiO$_2$ and Lys, on the other hand, the extraction amount of Lys decrease at high ionic strength. After desorption, a series of recycling and reusing experiments were carried out. Nearly 97.8% of [OH]-FIL-m-MWCNTs@SiO$_2$ could be recovered from each run. Finally, several consecutive adsorption–desorption cycles were performed. After five consecutive cycles revealed, the extraction amount of protein is 83.2 mg g^{-1}. A slight loss in the extraction amount of protein after five consecutive cycles revealed that the absorbent was highly stable and could endure these reaction conditions.

3.7 Analysis of the secondary structure of Lys

Far-ultraviolet CD can reflect the secondary structure of a protein. The characteristic of the a-helix structure of Lys shows a positive band centered at 192 nm and two negative bands centered at 208 and 222 nm. We can see from Supplementary information Fig. S2 that the line shape of Lys eluted from the solid extractants are similar to that of Lys before purification. The main observed spectral characteristics of Lys CD curve were almost kept the same. The result demonstrates that the secondary structure of the Lys was unchanged after eluted from the solid absorbents.

4 Conclusion

This paper systematically report the magnetic solid-phase extraction of Lys based on silica-coated magnetic multiwall carbon nanotubes surface modified by dual hydroxy functional ionic liquid. Single factor experiments illustrated that the extraction amount was influenced by the amount of [OH]-FIL-m-MWCNTs@SiO_2, the concentration of Lys, the solution ionic strength, the extraction time and the temperature.

Compared with published methods, the proposed method has many unique performances. Firstly, the magnetic solid phase extraction are convenient, time and effort saving because the magnetic absorbents are easily collected and separated from the solution with an external magnetic field. Secondly, a novel form of MSPE based on magnetic multiwall carbon nanotubes have many unique advantages that traditional magnetic materials cannot match, such as ultra-high surface area, high mechanical strength, excellent chemical and thermal stability and rich electronic polyaromatic structure. The last, dual hydroxy functional ionic liquids not only has the unique propertie of ionic liquids but also has stronger hydrogen bond and biological activity.

Moreover, MSPE based on silica-coated magnetic multiwall carbon nanotubes surface modified by dual hydroxy functional ionic liquid can combine all the advantages of the materials, such as low consumption of organic solvents, large specific surface areas, high recovery, easily separation, high extraction efficiency, stronger specific adsorption and convenience of operation. Owning to the ionic liquids can be designed so that the performances of the method also have the potential to offer new possibilities in the extraction of protein and DNA or other bio-analysis.

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Table 1 Comparison of protein type

Types of protein	M_W	pI	Extraction capacity (mg/g)
Lys	14 400	11.2	94.6
Try	23 800	10.5	73.3
OVA	45 000	4.7	39.6
BSA	65 000	4.8	31.4

Figure Captions

Sheme. 1 Preparation of sorbents and its application for the MSPE of Lys.

Fig. 1 The synthetic routes and structures of ionic liquids.

Fig. 2 FTIR spectra of c-MWCNTs (a), m-MWCNTs@SiO_2 (b) and [OH]-FIL-m-MWCNTs@SiO_2 (c).

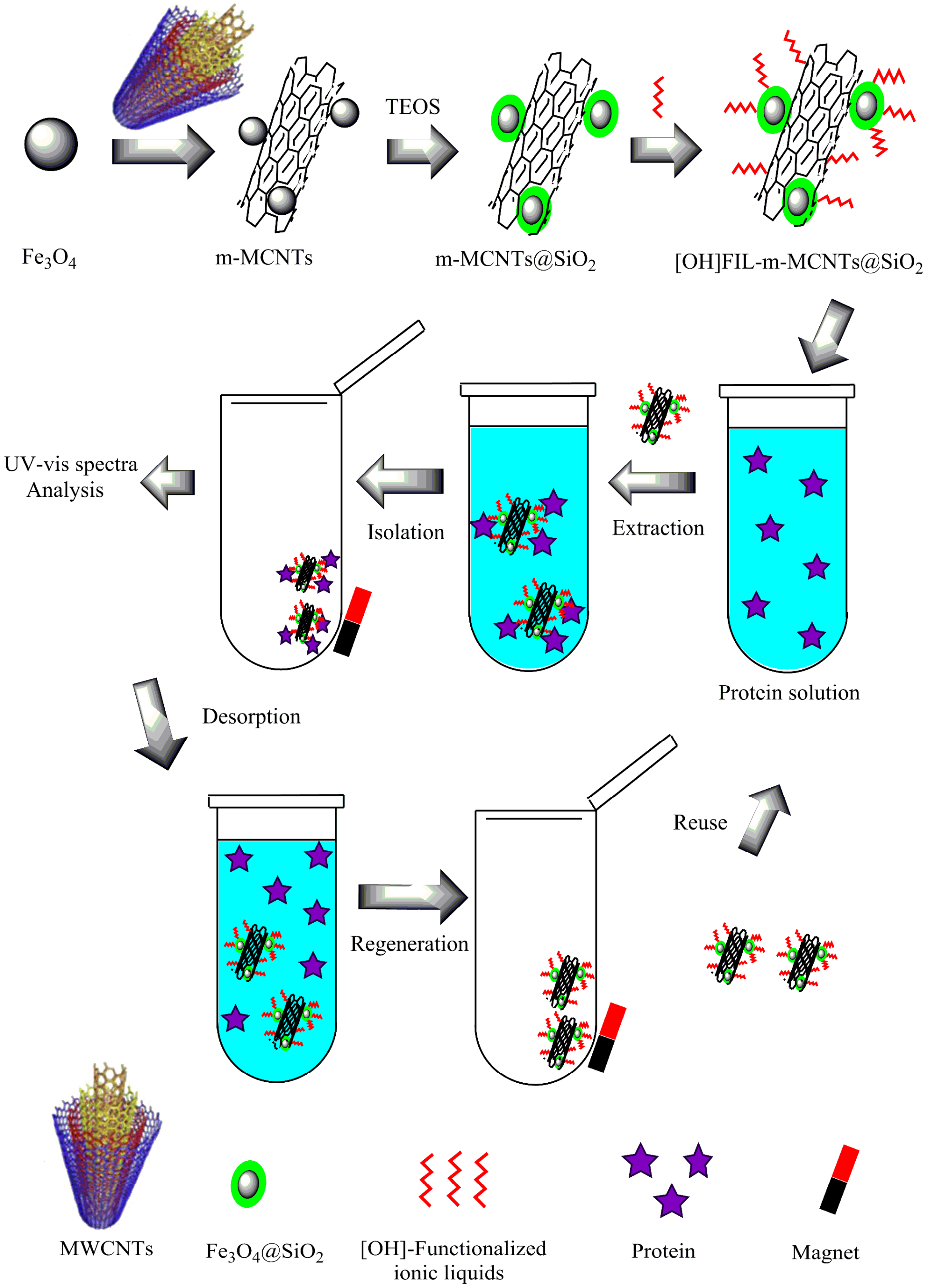
Fig.3 (A) Magnetic hysteresis loops of m-MWCNTs (a), m-MWCNTs@SiO_2 (b) and [OH]-FIL-m-MWCNTs@SiO_2 (c), and (B) the magnetic response of [OH]-FIL-m-MWCNTs@SiO_2 to external magnetic field.

Fig.4 TEM images of c-MWCNTs (a), m-MWCNTs (b), m-MWCNTs@SiO_2 (c), [OH]-FIL-m-MWCNTs@SiO_2 (d) and (e) （d and e are the same sample but different point of view）.

Fig.5 X-ray diffraction patterns of c-MWCNTs (a), m-MWCNTs (b) and m-MWCNTs@SiO_2 (c),

Fig. 6 Variation of m-MWCNTs and [OH]-FIL-m-MWCNTs@SiO_2 zeta-potential under different pH conditions.

Fig. 7 Single factor experiments (a) amount of [OH]-FIL-m-MWCNTs@SiO_2, (b) concentration of Lys, (c) solution ionic strength, (d) solution temperature and (e) extraction time.



Sheme. 1 Preparation of sorbents and its application for the MSPE of Lys.

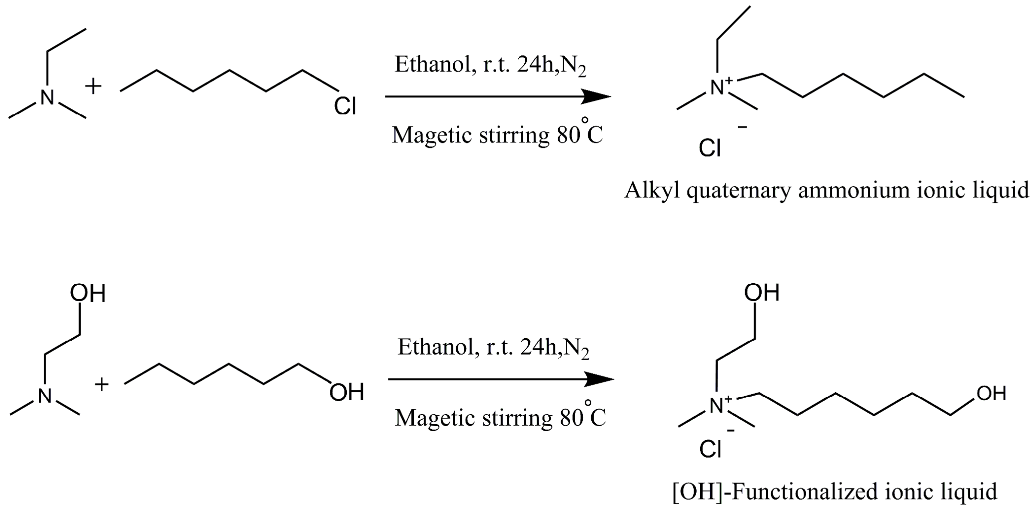


Fig. 1 The synthetic routes and structures of ionic liquids.

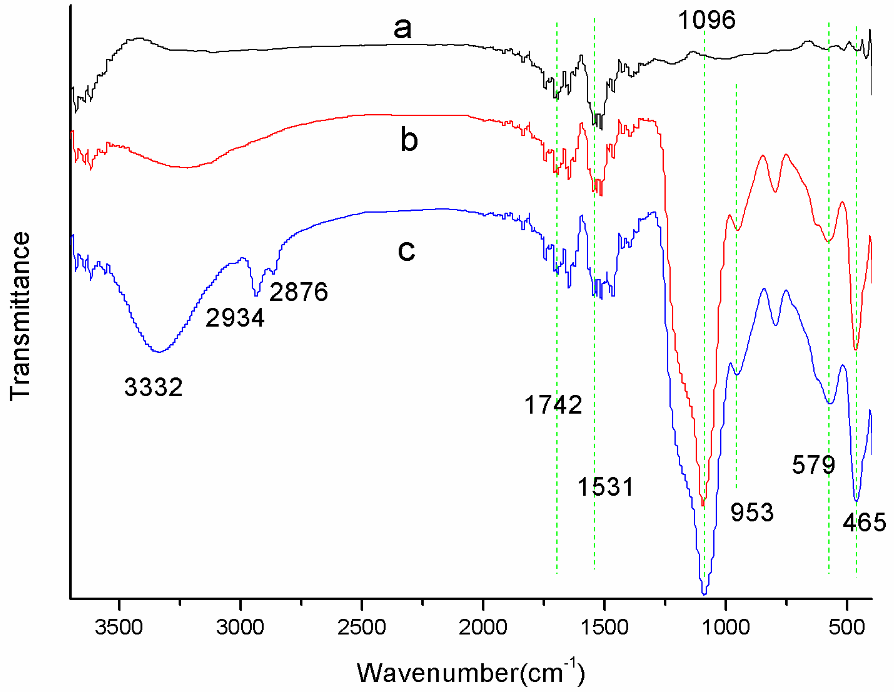


Fig. 2 FTIR spectra of c-MWCNTs (a), m-MWCNTs@SiO_2 (b) and [OH]-FIL-m-MWCNTs@SiO_2 (c).

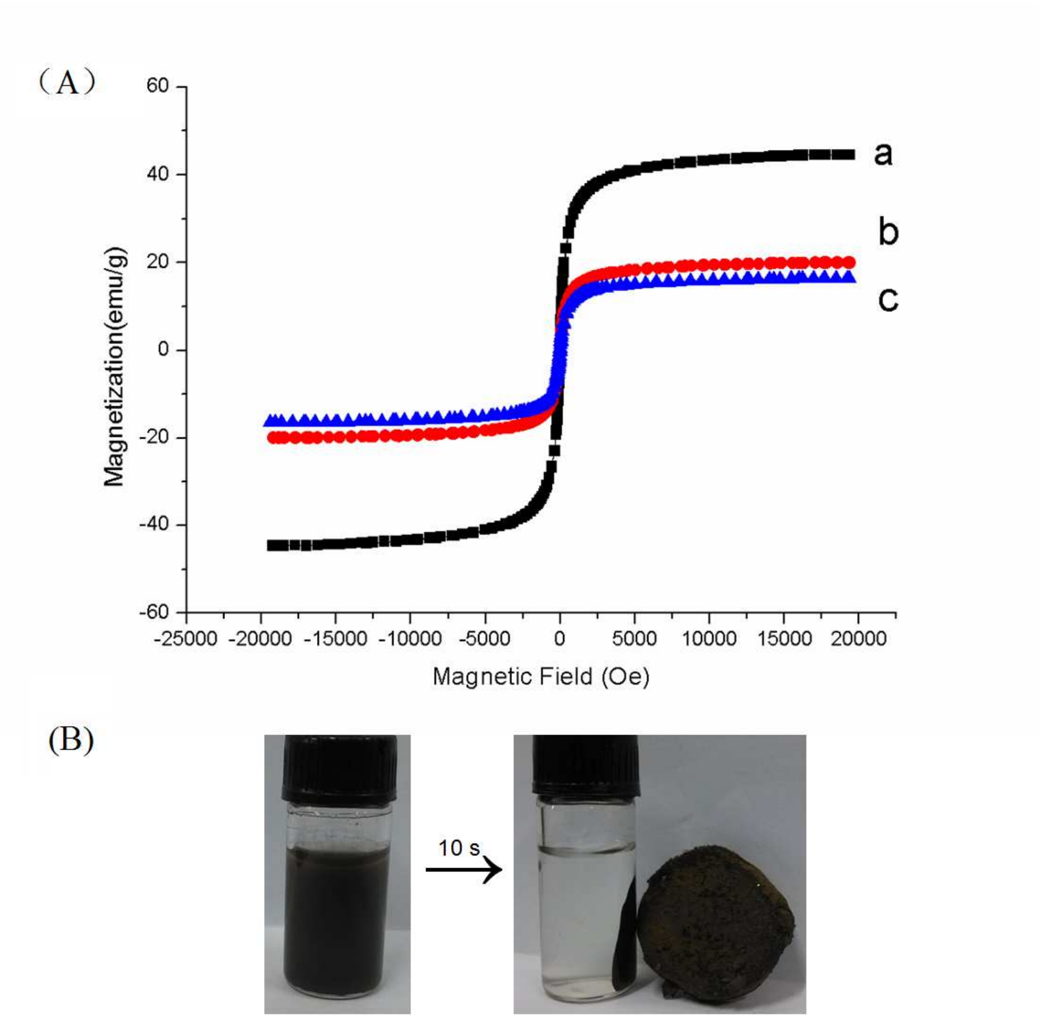


Fig.3 (A) Magnetic hysteresis loops of m-MWCNTs (a), m-MWCNTs@SiO$_2$ (b) and [OH]-FIL-m-MWCNTs@SiO$_2$ (c), and (B) the magnetic response of [OH]-FIL-m-MWCNTs@SiO$_2$ to external magnetic field.

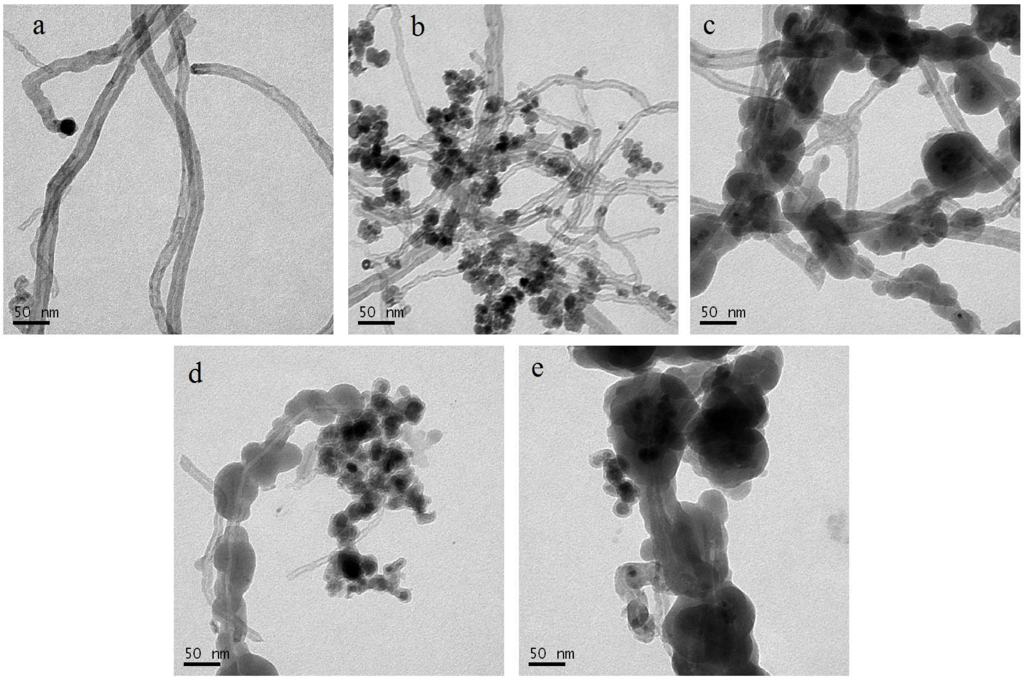


Fig.4 TEM images of c-MWCNTs (a), m-MWCNTs (b), m-MWCNTs@SiO$_2$ (c), [OH]-FIL-m-MWCNTs@SiO$_2$ (d) and (e).

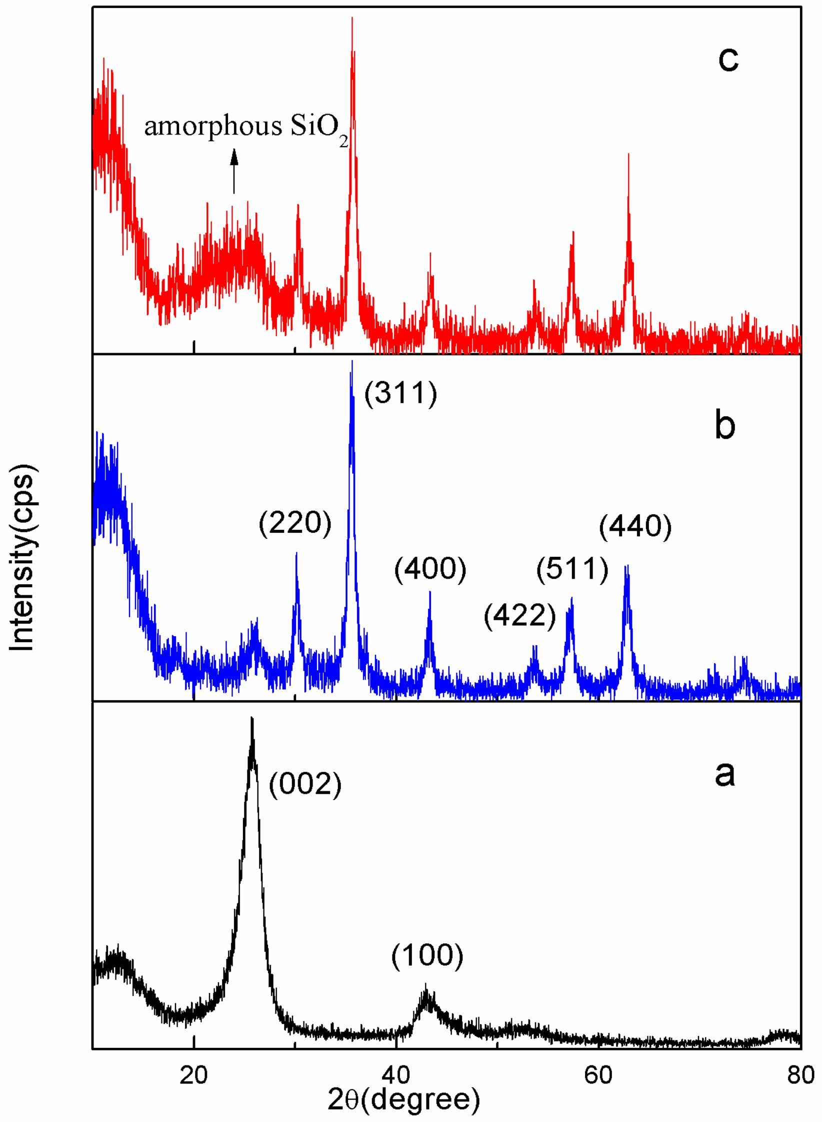


Fig.5 X-ray diffraction patterns of c-MWCNTs (a), m-MWCNTs (b) and m-MWCNTs@SiO_2 (c).

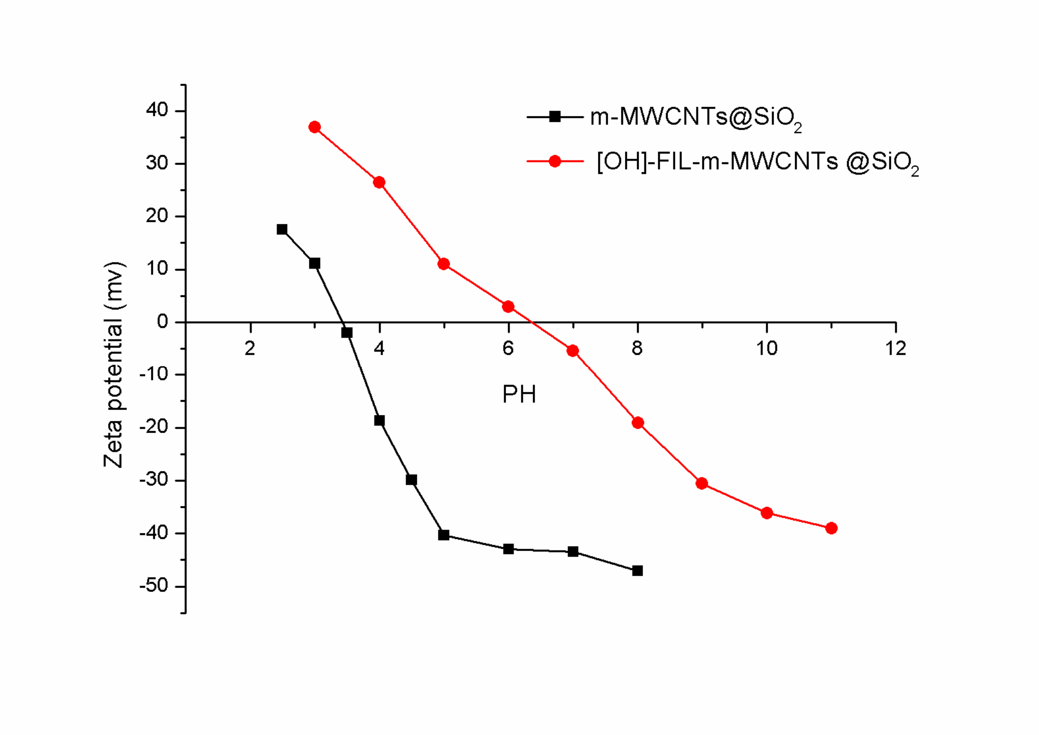


Fig. 6 Variation of m-MWCNTs and [OH]-FIL-m-MWCNTs@SiO_2 zeta-potential under different pH conditions.

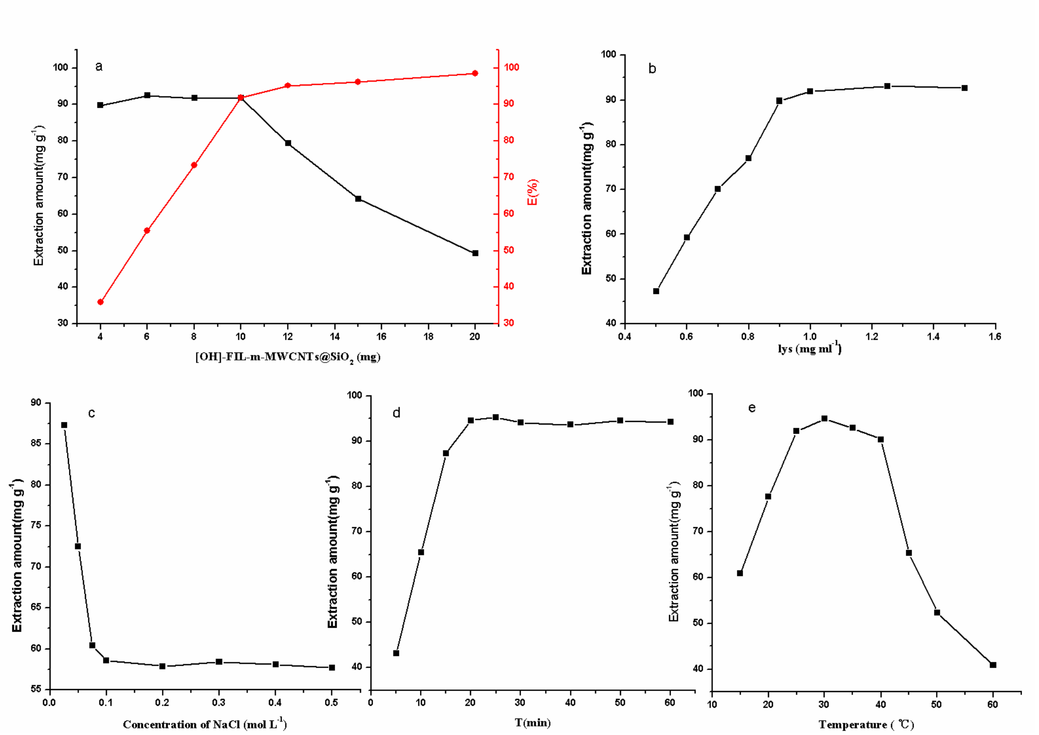


Fig. 7 Single factor experiments (a) amount of [OH]-FIL-m-MWCNTs@SiO$_2$, (b) concentration of Lys, (c) solution ionic strength, (d) solution temperature and (e) extraction time.