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Magnetic solid-phase extraction of protein by ionic liquid-coated Fe@graphene oxide

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ABSTRACT

Amino functional dicationic ionic liquid (AFDCIL) was prepared and then coated on the surface of magnetic graphene oxide (GO) as a new magnetic adsorbent (Fe@GO@AFDCIL) for the magnetic solid-phase extraction (MSPE) of protein. The Fe@GO@AFDCIL composite was characterized by vibrating sample magnetometer (VSM), X-ray diffraction (XRD), Fourier transform infrared spectrometry (FT-IR), thermal gravimetric analysis (TGA), field emission scanning electron microscopy (FESEM) and zeta-potential nanoparticles. The bovine hemoglobin (BHb) was used as the analyte, and the extraction performance of Fe@GO@AFDCIL was investigated in the MSPE procedure. The concentration of BHb in samples was determined by a UV–vis spectrophotometer. A comparative investigation of Fe@GO@AFDCIL composite and traditional IL-coated Fe@GO composites (Fe@GO@IL) exhibited the benefits of Fe@GO@AFDCIL. The adsorbed BHb could be eluted from the Fe@GO@AFDCIL by 4% sodium dodecyl sulfate (SDS) solution. The Fe@GO@AFDCIL exhibited favorable stability which could be reused at least 15 times. Under the optimized condition, the real samples were investigated, which demonstrated that the Fe@GO@AFDCIL was able to be applied in extracting bovine hemoglobin (BHb) from real biological samples.

1. Introduction

Proteins are primary components of human body. Many of the researches have devoted to separating and purifying various kinds of protein samples (for example hormone, antibody and plasma proteins). Proteins are of large amphiphilic and intrinsically surface active [1]. They can be extracted by intermolecular forces including strong hydrophobic, π-π and electrostatic interaction. Furthermore, the protein-surface interactions were governed by the nature of protein and the surface characteristics [2].

Several separation methods have been applied for extraction of protein, such as solid-phase extraction, liquid-phase extraction [3], gel electrophoresis [4], reverses micelles extraction [5], molecular imprinting [6] and two aqueous phase extraction [7]. In addition, a novel magnetic solid-phase extraction (MSPE) method, which uses magnetic materials as the adsorbents, has great potential for extraction of proteins from mixed samples [8].

MSPE is an improved solid-phase extraction method, in which the adsorbent has a directly impact on the performance of extraction process. The magnetic adsorbent can be modified with special functionalities, thus improving the selectivity, affinity and capacity of the extraction procedures [9]. Functional materials, which consisted of ionic liquids (ILs) and magnetic composite, presented excellent properties for extraction [10].

The common magnetic particles are Fe, Fe_3O_4 and γ-Fe_2O_3. Fe particles have high specific surface area and large number of metal sites on its surface [11,12]. Based on these advantages, the Fe particles show a great prospect in MSPE. However, Fe particles are likely to aggregate and oxidize that limit its application. Modifying the surface of magnetic particles is a classical way to conquer these limitations. Generally, the modified materials are GO [13], silica [14], carbon nanotubes [15] and nanoporous carbons [16].

It is generally known that GO has excellent electrical properties, thermostability, favorable biocompatibility and superior high surface areas [17,18]. GO possesses plentiful reactive functional groups at its basal plane or sheet edge. These functional groups are inclined to establish π-π, Van der Waals interactions and hydrogen bond with proteins, which show great promise for extraction application. Modifying Fe particles with GO is not only capable of improving the specific surface area and the extraction capacity of adsorbent, but also preventing Fe particles from being oxidized and agglomerating[19]. However, previous studies have emphasized that only modified the magnetic particles with GO was deficient due to its poor dispersity in solutions [20,21].

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ILs are organic salts with a melting point of 100 °C or less, which are widely regarded as promising solvents and functional materials [22–24]. ILs possess many beneficial properties, such as high thermal and chemical stability, negligible vapor pressure, noninflammability and electroconductivity [25–27]. Besides, ILs have attracted much attention as tunable and green functional materials for MSPE [28,29]. The dicationic ILs are formed of two positively charged moieties anchored to a central core and corresponding anions. Compared with traditional monocationic ILs, dicationic ILs have many beneficial features for extraction. In addition, magnetic GO composite can be modified with ILs. It would be possible to improve the selectivity and the solubility of materials [30].

In this study, the Fe particles were covered by GO to form magnetic GO (Fe@GO), and then the Fe@GO composite was modified with AFDCIL (Fe@GO@AFDCIL). The Fe@GO@AFDCIL composite was applied as the magnetic adsorbent for extraction of protein. Bovine hemoglobin (BHb) was to serve as the model protein. The extraction performance of the Fe@GO@AFDCIL in the MSPE procedure was investigated. Subsequently, the BHb could be eluted from the adsorbent with 4% SDS solution and the concentration was probed by UV–vis spectrophotometer at 406 nm.

2. Experimental

2.1. Apparatus

All products were dried by a DZF-6051 vacuum drying oven (Shanghai, China) and a FD-1C-50 vacuum-freezing drier (Beijing, China). The MSPE procedure was conducted in a QYC200 incubator shaker (Shanghai, China). New synthesis of ILs were investigated by INOVA 400NB NMR (Varian, America) and Vario EL III (ELEMENTAR, Germany). The magnetism of sorbents was studied by EV11 Vibrating Sample Magnetometer (MicroSense, USA). The morphology of GO and magnetic sorbents were obtained using a MIRA3 LMU field emission scanning electron microscopy (FESEM, TESCAN, Czech). Infrared spectrum was recorded using a Spectrum One FT-IR spectrometer (PerkinElmer, USA). Thermal gravimetric analysis was studied by a STA 449C thermal gravimetric analyzer (Netzsch, Germany). X-ray diffraction pattern was achieved using a D/Max 2500 X-ray diffraction (Rigaku, Japan). Zeta potentials were studied by a Zetasizer Nano-ZS90 dynamic light scattering (Malvern, Britain). Ultraviolet absorption spectrum was studied by a UV2450 UV–vis spectrophotometer (Shimadzu, Japan). Secondary structure of protein was proved by a Mos-500 circular dichroism (CD) spectrometer (Biologic, France).

2.2. Chemicals and reagents

All chemical reagents in this work were of analytical grade. $FeSO_4 \cdot 7H_2O$, polyethylene glycol (PEG-4000), ethanol, graphite powder, H_2SO_4, $KMnO_4$, H_2O_2 (30%), HCl, N,N,N′,N′-tetramethylethylenediamine, sodium dodecyl sulfate (SDS), n-butyl bromideand and bovine hemoglobin (BHb) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). KBH_4, $NaNO_3$ and NaOH were supplied by Taishan Chemical Co., Ltd. (Guangdong, China). Bovine blood sample and porcine blood sample was obtained from Jiaozuo biological technology Co., Ltd. (Jiaozuo, China). Poly (diallydimethylammonium chloride, PDDA) solution (20%) was purchased from Aladdin (Shanghai, China). 2-Chloroethylamine hydrochloride, 3-chloropropylamine hydrochloride, 1,1,3,3-tetramethylguanidine and N,N-dimethylethylamine were achieved from Adamas Reagent Co., Ltd. (Shanghai, China).

2.3. Synthesis of Fe particles and GO

The preparation of Fe particles was referring to the literature [31], which was generated on the basis of following equation.

$$Fe^{2+} + 2BH_4^- + 6H_2O \rightarrow Fe + 2B(OH)_3 + 7\ h_2\uparrow$$

Firstly, 2.78 g of $FeSO_4 \cdot 7H_2O$ and 0.5 g of PEG-4000 were completely dissolved in 30 mL alcohol-water system (ethanol: ultrapurewater, 4:1), then bubbled with high-purity nitrogen and mixed with a stirring speed of 2000 rpm for 20 min. Secondly, 21 mL of KBH_4 mixed solution (20 mL 0.02 mol/L KBH_4 and 1 mL 0.5 mol/L NaOH) was added into the above mixture at the rate of 2 drops per second. Finally, after reaction for 30 min the production was washed with water and ethanol three times each respectively, and then vacuum drying.

GO were synthesized by improved hummers method [32]. 0.5 g of graphite, 0.5 g of $NaNO_3$ and 23 mL of H_2SO_4 were mechanically stirred at ice water bath for 30 min 3 g of $KMnO_4$ was added slowly then continue to stir 2 h. Raised temperature to 35 °C and mixed for further 30 min, then added 100 mL ultrapure water gradually. The reaction was kept at 95 °C for 30 min. At last, terminated the reaction by adding 3 mL of H_2O_2 (30%) to the solution which finally turned to yellow. After cooling, the yellow suspension was centrifuged. Washed with 1 mol/L HCl until the SO_4^{2-} ions were removed thoroughly. And further washed with ultrapure water to make product neutral. In the end, freeze-dried loose brown power was obtained.

2.4. Synthesis of Fe@GO composite

The Fe@GO composite was synthesized by electrostatic interaction. 0.06 g of ready-prepared Fe particles were ultrasonically dissolved into PDDA solution (2 mg/mL) for 10 min and then stood for additional 15 min. Next, the magnetic composite were ultrasonically washed by ultrapure water for 3 times. Eventually positive charged PDDA-modified Fe particles were prepared. There are lots of oxygen-containing functional groups on the surface of the ready-prepared GO, so GO was able to cover PDDA-modified Fe particles. 0.06 g of ready-prepared GO was dispersed in 120 mL ultrapure water via ultrasonication for 2 h, and then centrifuged to remove impurities. 30 mL of PDDA-modified Fe particles dispersion (2 mg/mL) were gradually added into above GO suspension with a mild stirring for 1 h. The Fe@GO composite was obtained by magnetic isolation, washed with ultrapure water for 3 times and vacuum drying.

2.5. Synthesis of ILs

In this paper, we have synthesized five kinds of ionic liquids as following: AFDCIL (1,2-Ethanediaminium, N,N′-bis(2-aminoethyl)-N,N,N′,N′-tetramethyl-, chloride (1:2)); IL_2 (Guanidine, N″-(2-aminoethyl)-N,N,N′,N′-tetramethyl-, chloride (1:1)); IL_3 (Guanidine, N"-(3-aminopropyl)-N,N,N′,N′-tetramethyl-, chloride (1:1)); IL_4 (Guanidine, N″-butyl-N,N,N′,N′-tetramethyl-, chloride (1:1)); IL_5 (1-Butanaminium,N-ethyl-N,N-dimethyl-, chloride (1:1)). Three of the amino-functional ionic liquids were prepared according to the literature [33]. Taking synthesis of AFDCIL for example (Fig. 1), 14.9 mL of tetramethylethylenediamine (0.1 mol) and 23.2 g of

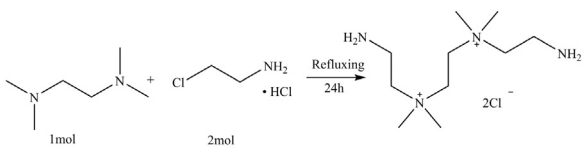

Fig. 1. Synthesis of ionic liquids (AFDCIL for example).

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2-chloroethylamine hydrochloride were mixed in 50 mL of ethanol for reflux condensation. The reaction was maintained at 80 °C oil bath for 24 h. After cooling, 11.2 g of KOH was added and stood for 30 min. By repeating suction filtration the light yellow pellucid solution was obtained. Next, the clear solution was washed with ethylacetate for several times. Finally, vacuum drying for 12 h, the product appeared thick and yellow. The structure and ^{1}H NMR spectra of the investigated ILs were shown in Supplementary material Table S1. Elemental analysis calcd (%) for new synthesis of ILs was presented in Supplementary material Table S2.

2.6. Synthesis of Fe@GO@IL composite

According to the literature [34], the ultrasonic coating method was used to synthesize the Fe@GO@IL composite. 0.1 g of ready-prepared IL was ultrasonically dissolved in 10 mL of methyl alcohol completely. And then 0.1 g of ready-prepared Fe@GO composite was added into aqueous mixtures for further reaction. Putting the whole reaction device into ultrasonic bath, and ultrasonication for 2 h intermittently. The Fe@GO@IL composite was gotten by magnetic separation and washed with methyl alcohol for 3 times, then dried in vacuum. The whole synthesis procedure was presented in Scheme 1.

2.7. MSPE of protein

In the MSPE procedure, we applied the Fe@GO@IL composite to extract BHb from the sample solution. First, 10 mg of Fe@GO@IL composite and 1 mL of BHb solution (1 mg/mL) were added into a 2 mL centrifuge tube. Subsequently, the mixture was shaken for 1 h at room temperature with the shaking speed of 200 rmp. After adsorbing in a thermostats cultivating, the magnetic adsorbent was separated from the suspension by an external magnet. Limpid supernatant was detected by UV–vis spectrophotometer to acquire the concentration of BHb whose absorption peak was around at 406 nm.

The computational formula of extraction capacity (Q) is as following:

$$Q = \frac{(C_0 - C)V}{m}$$

where Q (mg/g) is the quantity of protein adsorbed on a unit amount of adsorbent, C_0 (mg/mL) is the initial concentration of protein solution, C (mg/mL) is the concentration of protein in supernatant, V (mL) is the volume of protein solution, m (mg) is the dose of adsorbent.

The BHb was eluted from the adsorbent by 1 mL of 4% SDS solution for 1 h at 25 °C via shaking. Finally, the adsorbents were collected and reused. The whole extraction procedure was presented in Scheme 2.

3. Results and discussion

3.1. Effect of the types of ILs

In this work, we have selected five types of ILs to form magnetic adsorbents. The absorbent materials applied in MSPE should exhibit high surface area, strong magnetism and excellent extraction capacity. Fig. 2 indicated that Fe@GO@IL composites possessed higher extraction capacity when compared with Fe@GO composite, and different Fe@GO@IL composites have different extraction capacities. Obviously, Fe@GO@AFDCIL composite presented the highest extraction capacity for BHb. AFDCIL introduced plentiful amino groups on the surface of Fe@GO composite which could enhance the hydrogen bonding interactions during extraction. Based on these results, Fe@GO@AFDCIL was a promising adsorbent for MSPE of BHb.

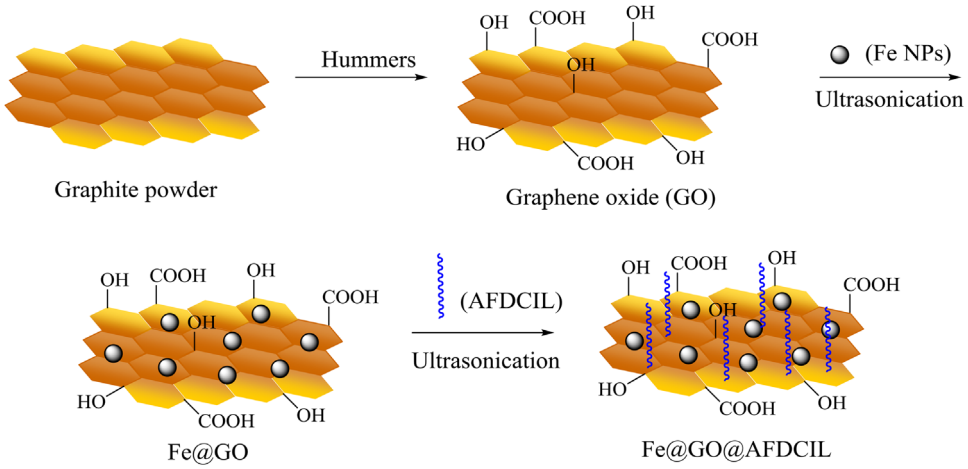
3.2. Characterization of Fe@GO@AFDCIL composite

3.2.1. Magnetic properties

The magnetic properties of Fe@GO, Fe@GO@AFDCIL were investigated by VSM at ambient temperature. Sufficient magnetic property should be obtained in order to ensure that the materials can be used in MSPE application. VSM magnetization curves of Fe@GO and Fe@GO@AFDCIL in Fig. 3a demonstrated that their saturation magnetization intensity were 20.42 and 17.83 emu g^{-1}. An obvious decrease of magnetic saturation value was observed in Fe@GO@AFDCIL. This could be mainly due to the thick non-magnetic AFDCIL layer was coated the Fe@GO composite. Although the magnetization value of Fe@GO@AFDCIL decreased after modification, it still could be separated from aqueous solution quickly once an external magnetic field was applied, as described in Fig. 3b. Therefore the present saturation magnetization value of Fe@GO@AFDCIL is sufficient for the magnetic separation.

3.2.2. XRD

Fig. 4 presented the XRD patterns of GO, Fe@GO and Fe@GO@AFDCIL. The XRD pattern of GO showed a strong peak at 10.05°, which was due to the introduction of oxygenic functional groups and trapped water molecules between the graphite layers



Scheme 1. Synthesis procedure of Fe@GO@AFDCIL composite.

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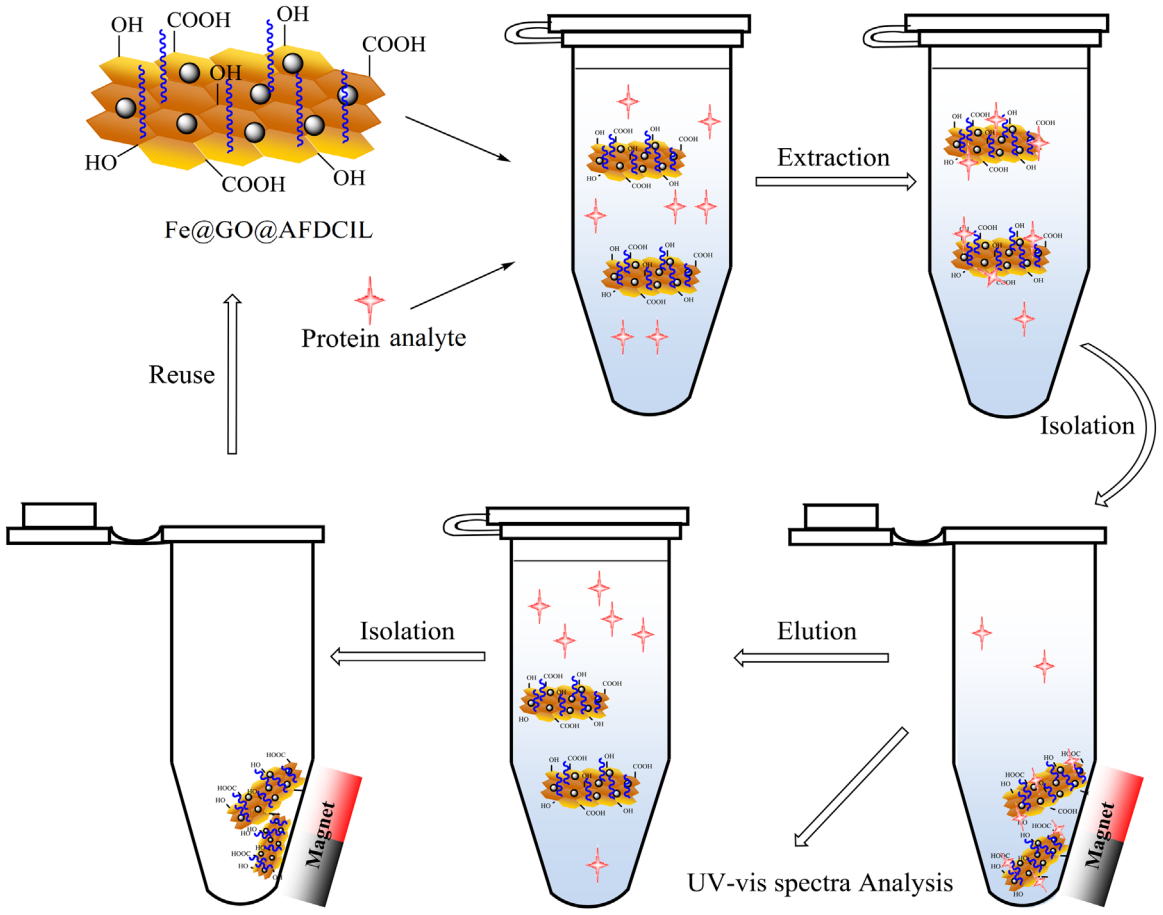

Scheme 2. The Fe@GO@AFDCIL composite was applied as the magnetic adsorbent for extraction of protein.

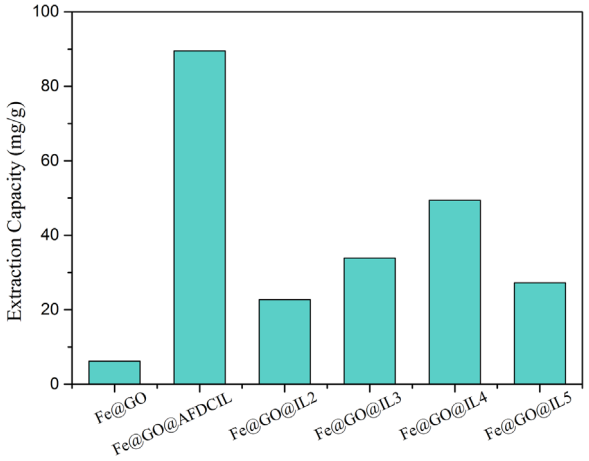

Fig. 2. Extraction capacities of Fe@GO composite, Fe@GO@AFDCIL composite and Fe@GO@IL composites for BHb.

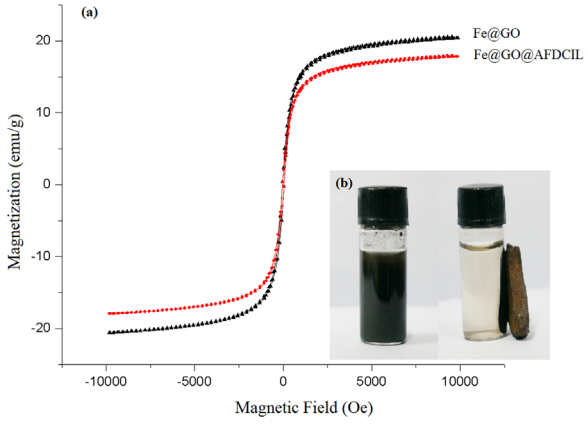

Fig. 3. Magnetic hysteresis loops of Fe@GO and Fe@GO@AFDCIL (a), and the magnetic response of Fe@GO@AFDCIL to external magnetic field (b).

[35]. A broad diffraction peak around 22.15° corresponded to the (002) planes of GO. As shown in Fig. 4b and c, it could be observed that Fe@GO and Fe@GO@AFDCIL had similar diffraction peaks. The diffraction peak of the (002) planes of GO does not disappear. And two peaks at 43.08° and 62.62°, indicating the presence of large amounts of α-Fe (JCPDS, No. 87-0722). Therefore, the XRD results confirmed that Fe@GO@AFDCIL composite was consisted of GO and Fe species, making it possible for magnetic separation.

3.2.3. FT-IR

The evidence for the successful modification of AFDCIL onto the surface of Fe@GO composite was provided by FT-IR spectra (Fig. 5). In the spectrum of AFDCIL, the peaks around 1142 cm^{-1} (C-N stretching vibrations), 1400–1650 cm^{-1} (C-N bendingvibration), double peaks at 3016 cm^{-1} and 3426 cm^{-1} (stretching vibrations of N-H) demonstrated the successful synthesis of AFDCIL. In the spectrum of GO, the characteristic peaks of GO appear at 1058 cm^{-1} (C-O-C), 1621 cm^{-1} (C-C), 1729 cm^{-1} (C=O stretching vibrations of -COOH) and broad bands around 2500–3300 cm^{-1} (O-H stretching vibrations of -COOH). Many GO absorption signals in Fe@GO and Fe@GO@AFDCIL infrared spectrums indicated the existence of GO, such as the peaks around 1058 cm^{-1}, 1621 cm^{-1} and 1729 cm^{-1}. Moreover, the vibration of C-N and amino group

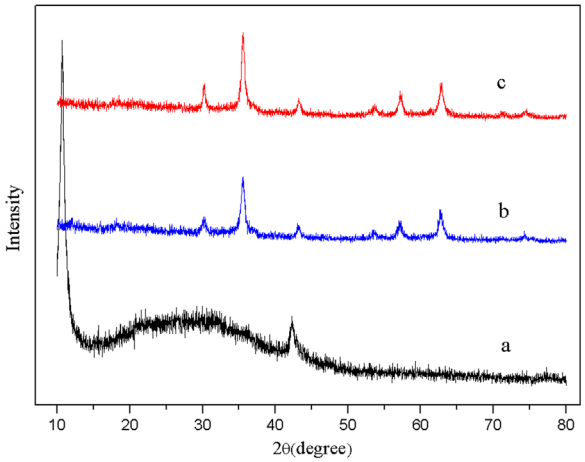

Fig. 4. X-ray diffraction patterns of GO (a), Fe@GO (b) and Fe@GO@AFDCIL (c).

absorbance in the Fe@GO@AFDCIL infrared spectrum proved that the AFDCIL coated on the surface of Fe@GO composite undoubtedly.

3.2.4. TGA

The thermal stabilities of Fe@GO and Fe@GO@AFDCIL were investigated by TGA, which were conducted in argon atmosphere at 10 K/min. The results were provided in Fig. 6, manifesting that the Fe@GO and Fe@GO@AFDCIL have different weight loss. From room temperature to 150 °C, the weight loss of Fe@GO and Fe@GO@AFDCIL were attributed to the removal of absorbed water. Further heating of composites to 800 °C, oxygen-containing groups were decomposed. However, Fe@GO@AFDCIL presented 5.9% higher weight loss than Fe@GO. This was due to the dissociation of AFDCIL from the surface of Fe@GO@AFDCIL. Based on above discussion, we could confirm that AFDCIL modified the Fe@GO successfully.

3.2.5. FESEM

The morphologies of GO, Fe@GO and Fe@GO@AFDCIL were characterized by FESEM. Fig. 7a showed a typical image of GO, which presented high transparent films and surface areas. As shown in Fig. 7b, the spherical Fe particles were covered by GO clearly. From Fig. 7c, we could observe that the surface of magnetite clusters were much rougher. The results indicated that the Fe@GO composite was modified with AFDCIL successfully.

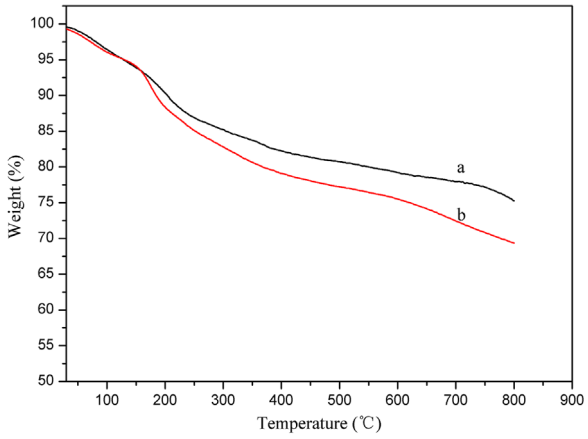

Fig. 6. Weight loss curves of Fe@GO (a) and Fe@GO@AFDCIL (b).

3.2.6. Isoelectric point

The isoelectric points (IEPs) of Fe@GO and Fe@GO were measured by zeta-potential nanoparticles at different pH values. In Fig. 8, it was found that the IEP of Fe@GO and Fe@GO@AFDCIL was 3.6 and 5.7, respectively. The surface charge of them was positive at pH value lower than IEP, and negative at pH value higher than IEP. Obviously, the IEP of Fe@GO@AFDCIL was much higher than that of Fe@GO. This might be due to the surface groups of Fe@GO were changed after being coated by AFDCIL (pKa 8.5). Thus high IEP for Fe@GO@AFDCIL was a conclusive evidence to prove that AFDCIL coated on the Fe@GO composite.

3.3. MSPE procedure

3.3.1. Effect of the pH value

The pH of the sample solution played a vital role in MSPE process, which can affect the surface charge of both analytes and adsorbent. The pH of the sample solution was conducted in 5 mmol/L phosphate buffer solution in the range of 6–11. Based on the IEP characterization of Fe@GO@AFDCIL (IEP 5.7), when the solution pH value was higher than 5.7, the surface charge of Fe@GO@AFDCIL became negative. The BHb (IEP 6.8) was protonated when the solution pH was below 6.8. Fig. 9a indicated that the extraction capacity reached its maximum at pH 6 where the surface of Fe@GO@AFDCIL composite became negative and enable to extract positive BHb. The IEPs of the adsorbent and analytes were close, which revealed that the electrostatic attraction between them wasnot strong. In addition, hydrophobic interaction and hydrogen bonding interaction were involved in the MSPE

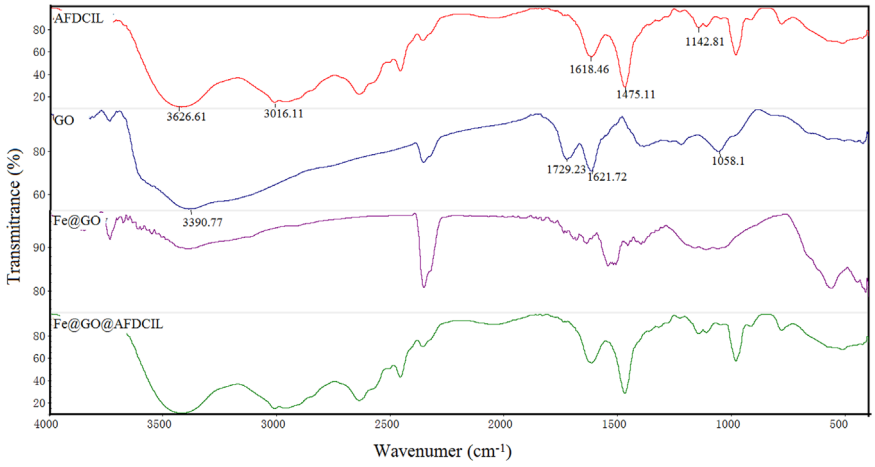

Fig. 5. FT-IR spectra of AFDCIL, GO, Fe@GO and Fe@GO@AFDCIL.

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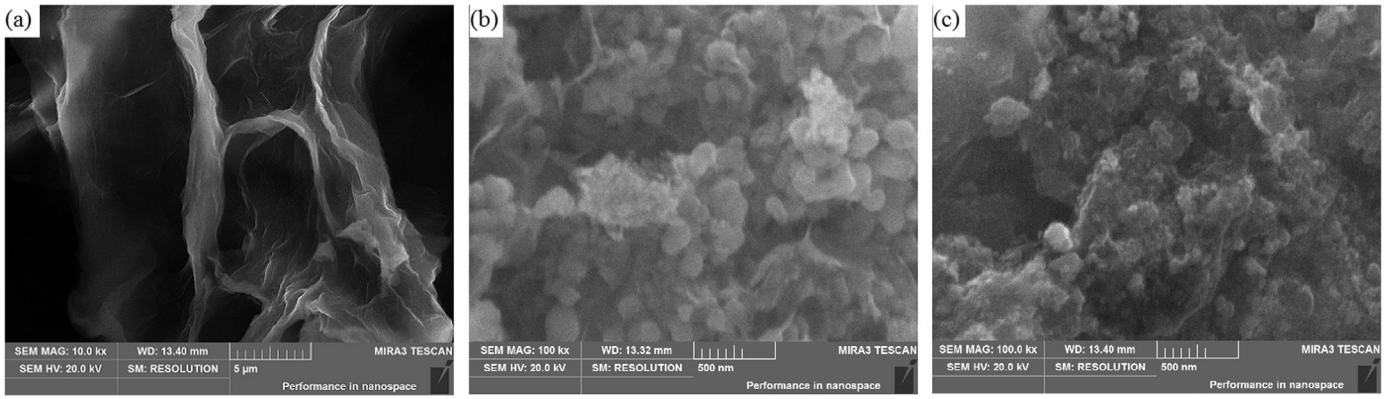

Fig. 7. FESEM images of GO (a), Fe@GO (b) and Fe@GO@AFDCIL (c).

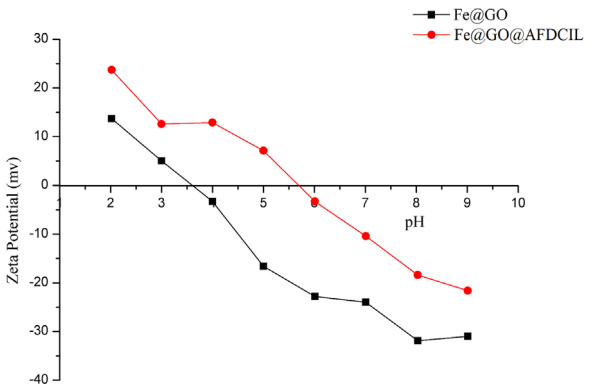

Fig. 8. Zeta potentials of Fe@GO and Fe@GO@AFDCIL in different pH solutions.

procedure, which could also affect the extraction capacity. The extraction capacities decreased with the increase of pH values mainly that the electrostatic repulsive interaction instead of the electrostatic attraction at pH > IEP of BHb. Consequently, the sample solution was set as pH 6.

3.3.2. *Effect of the extraction temperature*

To evaluate the effect of temperature on the extraction capacity, the extraction temperature was investigated. As shown in Fig. 9b, extraction capacities were increased by increasing temperature below 40 °C and the maximum extraction capacity was achieved at 40 °C. However, the extraction capacities decreased when temperature was higher than 40 °C. It might be due to that the hydrogen bonding interaction was partial broken between Fe@GO@AFDCIL and BHb. Consequently, 40 °C was chosen as the optimum temperature for subsequent experiments.

3.3.3. *Effect of the protein concentration*

Initial protein concentration plays a key role in MSPE. The effect of BHb concentration on the extraction capacity was investigated in the range of 0.5–2.5 mg/mL. The results (Fig. 9c) showed that the extraction capacities improved remarkably with the increasing concentration from 0.5 to 2.0 mg/mL, and remained unchanged when BHb concentration increased continuously. A higher initial BHb concentration provided more possibilities for Fe@GO@AFDCIL to capture analytes. However, once the sites on the Fe@GO@AFDCIL were saturated, the BHb concentration wouldnot

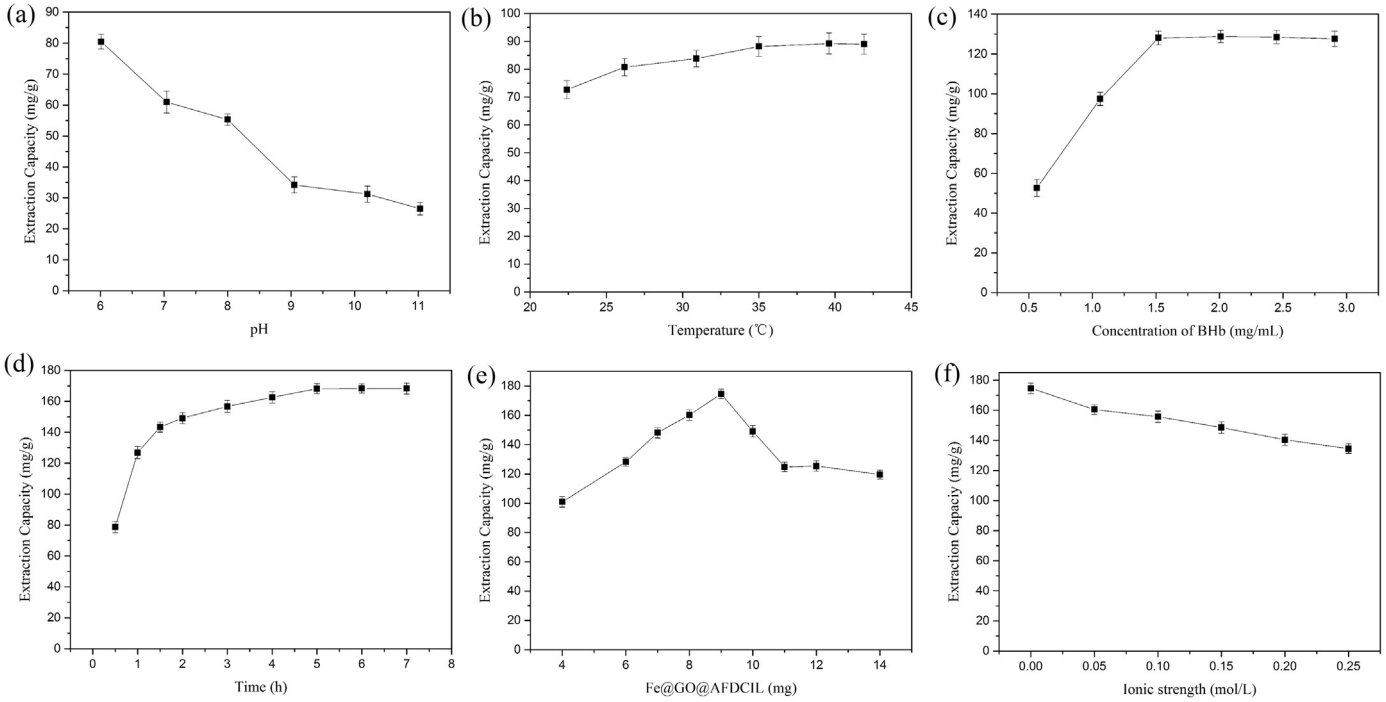

Fig. 9. Effect of pH value (a), temperature (b), concentration of BHb (c), extraction time (d), amount of Fe@GO@AFDCIL composite (e) and ionic strength (f).

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influence the extraction capacity any more. Therefore, 2.0 mg/mL BHb solution was adopted for the extraction.

3.3.4. Effect of the extraction time

To attain the equilibrium of MSPE procedure, extraction time was investigated from 0.5 to 6 h. As can be seen in Fig. 9d, the extraction capacities of BHb increased rapidly when extraction time ranged from 0.5 to 2 h and rose slowly with a further extension of extraction time from 2 to 5 h. The extraction equilibrium was achieved in 5 h, and the Fe@GO@AFDCIL indeed possessed commendable extraction capacity for analytes. However, in order to improve the efficiency of this MSPE method, 2 h was set as the extraction time for subsequent experiments.

3.3.5. Effect of the amount of Fe@GO@AFDCIL composite

To evaluate the effect of adsorbent amount for the MSPE, the amount of Fe@GO@AFDCIL was changed in the range of 4–14 mg. The results were shown in Fig. 9e, the extraction capacity reached the maximum when 9 mg of Fe@GO@AFDCIL was applied. However, the extraction capacities decreased when the quantity of Fe@GO@AFDCIL was beyond 9 mg, illustrating that excess adsorbent were helpless with such small volumes of the extraction solvent. Ultimately, 9.0 mg was the final amount of Fe@GO@AFDCIL for the extraction.

3.3.6. Effect of the solution ionic strength

To evaluate the effect of ionic strength in extraction performance, the concentration of NaCl was changed from 0 to 0.25 mol/L. As shown in Fig. 9f, the extraction capacities decreased obviously with the increase of NaCl concentration. It could be inferred that the addition of NaCl was valueless for improving extraction capacity. The phenomena could be explained as follows: (a) electrostatic interaction in MSPE process was significant, with which sodium ions and positively charged protein analytes were competitively extracted by Fe@GO@AFDCIL composite; (b) the ionic strength can influence the extraction capacity in sample matrix. When salt is added into sample solution, salting-in effect can decrease the extraction performance while salting-out effect can increased it [36]. In this experiment, salting-in effect played dominant role. The best extraction capacity can reach 174.54 mg/g in MSPE process without adding salt into sample solution.

3.4. Desorption of BHb

Desorption of analytes from adsorbents had a significant impact on the reusability of the Fe@GO@AFDCIL and the retrieve of BHb. After each extraction, the Fe@GO@AFDCIL was magnetic isolated from aqueous solution and eluted by 4% SDS solution. The suspension was shaken in a 2 mL centrifuge tube for 1 h at 25 °C. In this way, the desorption efficiency of BHb was 89.54%, indicating the adsorbed analytes could be eluted from Fe@GO@AFDCIL successfully. The eluent could weaken the hydrogen bonds between adsorbents and protein analytes. The two CD spectrums were shown in Fig. 10. In the near ultraviolet, the value of ellipticity is affected by the microenvironment of amino acid residue. Therefore the peak of BHb at about 222 nm changed was ascribed to the different solution environment. The CD spectrums showed that the characteristic ellipticity of α-helices of protein at 208 and 222 nm still exists distinctly for BHb in eluent. Thus, it could be illustrated that the secondary structure of BHb was not transformed after desorption process.

3.5. Reusability of the Fe@GO@AFDCIL composite

The reusability of adsorbent means so much to both economic and environmental ideas. The magnetic adsorbent was collected and applied to the following MSPE procedure. As shown in Fig. 11, the Fe@GO@AFDCIL could be reused at least 15 times without a significant reduction in extraction capacities ($< 12.5\%$). The good reusability testified that 4% SDS solution possessed favorable desorption ability and the Fe@GO@AFDCIL was stable enough for repeated uses.

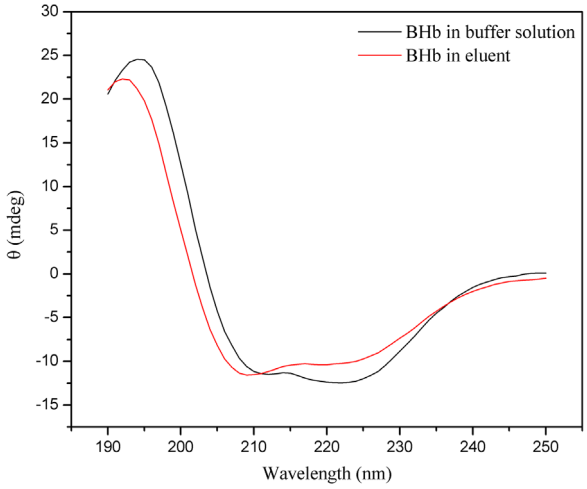

Fig. 10. The CD spectra of BHb before and after elution.

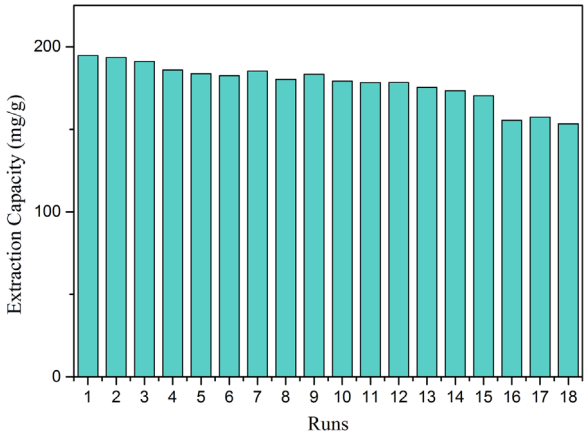

Fig. 11. Extraction capacities of BHb for different runs.

3.6. Analysis of real sample

To investigate the applicability of this magnetic extraction method for real samples, the porcine blood and bovine blood samples were analyzed under the optimized conditions as mentioned. Firstly, 9.0 mg of Fe@GO@AFDCIL and 1 mL of the blood samples which were diluted 100 times by buffer solution (pH 6) were added into a centrifuge tube. The mixture was shaken for 2 h at 40 °C, and then protein analytes were extracted by Fe@GO@AFDCIL composite. The analytical results were illustrated in Fig. 12, which was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). It could be found that the specific patterns of BHb both in the porcine blood and bovine blood samples became fade after extraction. Hence, this effective magnetic extraction method could be applied to extract BHb from real samples successfully.

3.7. Method validation

After the optimal experimental parameters were confirmed, the limits of detection (LOD), precision, repeatability and stability

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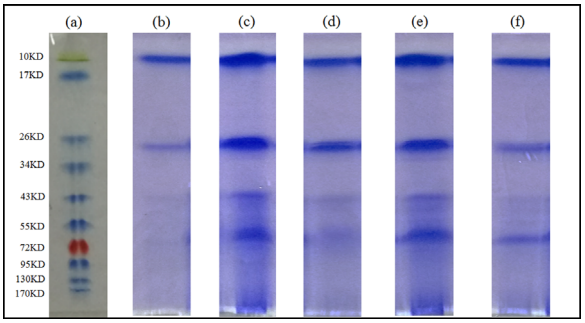

Fig. 12. SDS-PAGE of protein molecular weight maker (a), 1 mg/mL BHb (b), porcine blood sample (c), porcine blood sample after extraction (d), bovine blood sample (e) and bovine blood sample after extraction (f).

Table 1
The results of the precision, repeatability and stability experiments.

Precision measurement results (n=5)					
Repeats	1	2	3	4	5
Extraction capacities(mg g^{-1})	174.24	174.68	173.97	174.57	174.57
RSD (%)	0.15%				
Repeatability measurement results (n=5)					
Sample number	1	2	3	4	5
Extraction capacities(mg g^{-1})	172.35	174.97	171.68	174.68	175.29
RSD (%)	0.95%				
Stability measurement results (n=5)					
Day number	1	2	3	4	5
Extraction capacities(mg g^{-1})	174.42	174.06	174.25	174.46	174.85
RSD (%)	0.17%				

experiments were performed to evaluate the proposed method. The LOD can be simply defined as that concentration of analysis species which can be detected at a specified confidence level, and it was 11.87 μg/mL. The results were listed in the Table 1. The relative standard deviation (RSD) of the apparatus precision turned out 0.15%, which was examined by detecting a sample for 5 times coupled with UV–vis spectrophotometer. Five sets of parallel experiments were used to study the repeatability, and RSD was 0.95%. The stability of the proposed method was investigated by surveying the same sample for five consecutive days, and the RSD was 0.17%. The result evidenced that this method was reliable for achieving precise experimental data and existed good repeatability and stability.

4. Conclusions

In the present work, a novel magnetic adsorbent, Fe@GO@AFDCIL has been successfully synthesized and applied for extraction of BHb. Under the optimized conditions, the Fe@GO@AFDCIL composite has shown excellent adsorption for BHb. In addition, compared with conventional Fe@GO@IL composites, the Fe@GO@AFDCIL composite exhibited the highest extraction capacity for BHb. The secondary structure of BHb remained unchanged after being eluted from Fe@GO@AFDCIL by 4% SDS solution. The Fe@GO@AFDCIL exhibited good stability which could be reused at least 15 times. Besides, the Fe@GO@AFDCIL could be successfully employed in extraction of BHb from real samples. All these indicated that the developed method had great potential in extraction of BHb or other analytes from biological samples.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2016.07.031.

References

[1] K.L. Niece, J.D. Hartgerink, J.J. Donners, S.I. Stupp, J. Am. Chem. Soc. 125 (2003) 7146–7147.
[2] M.J. Higgins, P.J. Molino, Z.L. Yue, G.G. Wallace, Chem. Mater. 24 (2012) 828–839.
[3] S. Tang, H.K. Lee, Food Chem. 199 (2016) 533–540.
[4] K. Munenobu, T. Hase, T. Oyoshi, M. Yamanaka, Anal. Chem. 86 (2014) 9924–9929.
[5] C. Dumas, C.J. Meledandri, Langmuir 31 (2015) 7193–7203.
[6] Y. Liu, B. Cao, P. Jia, J. An, C. Luo, L. Ma, K. Pan, J. Phys. Chem. A 119 (2015) 6661–6667.
[7] K.J. Xu, Y.Z. Wang, Y.H. Huang, N. Li, Q. Wen, Anal. Chim. Acta 864 (2015) 9–20.
[8] D. Xiao, D. Yuan, H. He, C. Pham-Huy, H. Dai, C. Wang, C. Zhang, Carbon 72 (2014) 274–286.
[9] Q.L. Li, L.L. Wang, X. Wang, M.L. Wang, R.S. Zhao, J. Chromatogr. A 1449 (2016) 39–47.
[10] L. Vidal, M.L. Riekkola, A. Canals, Anal. Chim. Acta 715 (2012) 19–41.
[11] X. Zhou, B. Lv, Z. Zhou, W. Li, G. Jing, Chem. Eng. J. 281 (2015) 155–163.
[12] L.N. Shi, X. Zhang, Z.L. Chen, Water Res. 45 (2011) 886–892.
[13] K.J. Xu, Y.Z. Wang, X.J. Ding, Y.H. Huang, N. Li, Q. Wen, Talanta 148 (2016) 153–162.
[14] H. He, D. Yuan, Z. Gao, D. Xiao, H. He, H. Dai, N. Li, J. Chromatogr. A 1324 (2014) 78–85.
[15] Q. Zhao, F. Wei, Y.B. Luo, J. Ding, N. Xiao, Y.Q. Feng, J. Agr. Food Chem. 59 (2011) 12794–12800.
[16] L. Hao, C. Wang, Q. Wu, Z. Li, X. Zang, Z. Wang, Anal. Chem. 86 (2014) 12199–12205.
[17] O.K. Park, M.G. Hahm, S. Lee, H.I. Joh, S.I. Na, R. Vajtai, P.M. Ajayan, Nano Lett. 12 (2012) 1789–1793.
[18] B.J. Hong, O.C. Compton, Z. An, I. Eryazici, S.T. Nguyen, ACS Nano 6, 2011, pp. 63–73.
[19] L. Li, C. Luo, X. Li, H. Duan, X. Wang, Int. J. Biol. Macromol. 66 (2014) 172–178.
[20] Z. Liu, J.T. Robinson, X. Sun, H. Dai, J. Am. Chem. Soc. 130 (2008) 10876–10877.
[21] H. Yan, M. Gao, J. Qiao, J. Agr. Food Chem. 60 (2012) 6907–6912.
[22] Q. Zhang, J.N.M. Shreeve, Chem. Soc. Rev. 114 (2014) 10527–10574.
[23] W. Miao, T.H. Chan, Acc. Chem. Res 39 (2006) 897–908.
[24] T.D. Ho, C. Zhang, L.W. Hantao, J.L. Anderson, Anal. Chem. 86 (2013) 262–285.
[25] S. Koguchi, K. Izawa, ACS Comb. Sci. 16 (2014) 381–385.
[26] T. Wu, D. Wang, M. Zhang, J.R. Heflin, R.B. Moore, T.E. Long, ACS Appl. Mater. Int. 4 (2012) 6552–6559.
[27] N. Muhammad, W.N. Omar, Z. Man, M.A. Bustam, S. Rafiq, Y. Uemura, Ind. Eng. Chem. Res. 51 (2012) 2280–2289.
[28] Y.M. Wang, V. Ulrich, G.F. Donnelly, F. Lorenzini, A.C. Marr, P.C. Marr, ACS Sustain. Chem. Eng. 3 (2015) 792–796.
[29] Y. Shan, L. Qiao, X. Shi, G. Xu, J. Chromatogr. A 1375 (2015) 101–109.
[30] X. Cao, L. Shen, X. Ye, F. Zhang, J. Chen, W. Mo, Analyst 139 (2014) 1938–1944.
[31] Y. Zhang, T. Li, Z. Jin, W. Wang, S. Wang, Front. Environ. Sci. Eng. 1 (2007) 466–470.
[32] Y. Zeng, Y. Zhou, L. Kong, T. Zhou, G. Shi, Biosens. Bioelectron. 45 (2013) 25–33.
[33] S. Huang, Y. Wang, Y. Zhou, L. Li, Q. Zeng, X. Ding, Anal. Methods 5 (2013) 3395–3402.
[34] Y.H. Huang, Y.Z. Wang, Q. Pan, Y. Wang, X.Q. Ding, K.J. Xu, Q. Wen, Anal. Chim. Acta 877 (2015) 90–99.
[35] S. Choudhary, H.P. Mungse, O.P. Khatri, J. Mater. Chem. 22 (2012) 21032–21039.
[36] M. Mei, J. Yu, X. Huang, H. Li, L. Lin, D. Yuan, J. Chromatogr. A 1385 (2015), 12-1.