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Mixed hemimicelles solid-phase extraction of cephalosporins in biological samples with ionic liquid-coated magnetic graphene oxide nanoparticles coupled with high-performance liquid chromatographic analysis

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ABSTRACT

A novel mixed hemimicelles solid phase extraction based on magnetic graphene oxide (Fe_3O_4/GO) and ionic liquid (IL) was developed for the simultaneous extraction and determination of trace cephalosporins in spiked human urine. The high surface area and excellent adsorption capacity of the graphene oxide after modification with1-hexadecyl-3-methylmidazoliumbromide(C_{16}mimBr) were utilized adequately in the solid phase extraction(SPE) process. A comprehensive study of the parameters affecting the extraction recovery, such as the zeta-potential of magnetic graphene oxide, amounts of magnetic graphene oxide and surfactant, pH of solution, ionic strength, extraction time, and desorption condition were optimized. A comparative study on the use of different surfacant-coated Fe_3O_4/GO NPs as sorbents was presented. Good linearity (R^2> 0.9987) for all calibration curves was obtained. The LODs were ranged between 0.6 and 1.9 ng mL^{-1} for the cephalosporins and the LOQs were 1.5 to 5.5, respectively. Satisfactory recoveries(84.3% to 101.7%)and low relative standard deviations from 1.7% to 6.3% in biological matrices were achieved. The mixed hemimicelles magnetic SPE (MSPE) method based on ILs and Fe_3O_4/GO NPs magnetic separation has ever been successfully used for pretreatment of complex biological samples.

1. Introduction

Solid-phase extraction (SPE) continues to be the leading technique for the extraction of analytes in the separation and analysis [1–5]. It has many obvious advantages including high preconcentration factors, low consumption of organic solvents and the operation is simple. In recent years, a new type of SPE based on hemimicelles and admicelles (mixed hemimicelles) has been developed. In this method, the sorbents were produced by the adsorption of ionic surfactants such as sodium dodecyl sulfate (SDS) [6] or ionic liquid on the surface of mineral oxides [7–10]. Hemimicelles consist of monolayers of surfactants adsorbing head down on an oppositely charged mineral oxide surface [11]. The outer surface of the hemimicelles is hydrophobic whereas that of the admicelles is ionic [12], which provides two kinds of mechanisms (hydrophobic and electrostatic interactions) for the retention of analytes. The SPE based on mix hemimicelles has many advantages, such as high extraction yield, easy elution of analytes and high breakthrough volume. Moreover, the degree of hydrophobicity and the charge of the sorbent using the surfactants can be easily modified according to the nature of analytes [13]. Thus, it is a good tool for rapid and sensitive analysis of biochemicals and drugs in many complex matrices.

However, because of a relatively small surface area of the microparticle sorbents used, the reported mixed hemimicelles assembly SPE method may lead to a comparatively low extraction capability and be time-consuming, especially for large volume samples. To overcome the problem, magnetic carrier technology has attracted more interest [14,15]. Magnetic materials as adsorbents have several advantages in comparison with traditional adsorbents. The magnetic nanoparticles can readily be isolated from sample solutions by the application of an external magnetic field. There-

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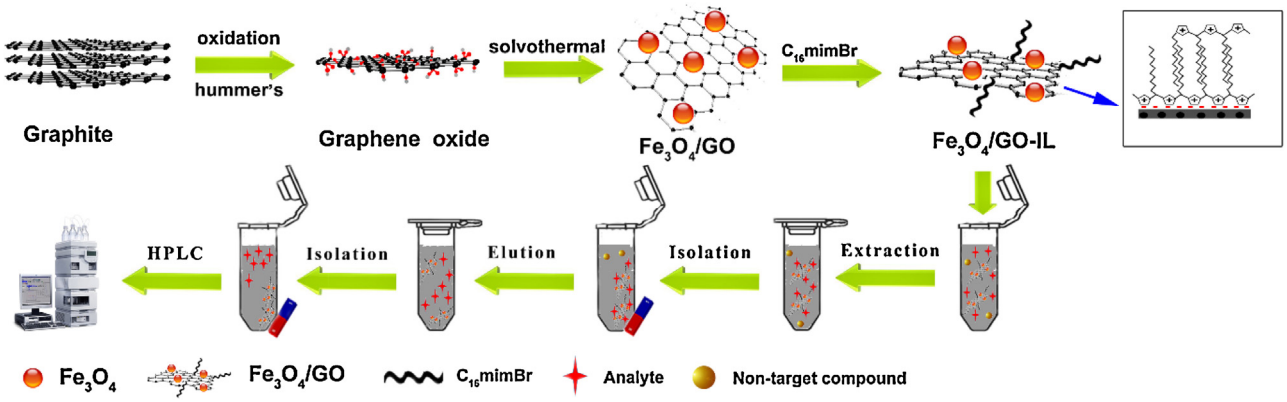

Fig. 1. Scheme of the preparation of surfactants-coated Fe_3O_4/GO NPs and its application as SPE sorbents for extraction and preconcentration of analytes.

fore, the enrichment time can be shortened greatly. In order to keep the advantages of magnetic nanoparticles and to obtain better extraction efficiency, the use of Fe_3O_4/GO for mixed hemimicelles SPE is a promising alternative. Nowadays, Fe_3O_4/GO have attracted much attention in the research community in virtue of their unique size and physical properties. Fe_3O_4/GO possesses a large surface area and superparamagnetism [16–21], which provide a nanosized platform for hemimicelles/admicelles formation, leading to high extraction efficiency [7,22]. Although substantial progress has been made, the further development of mixed hemimicelles on the surface of Fe_3O_4/GO for SPE of trace amounts of analytes in complex matrices samples remains a challenge.

Ionic liquids (ILs) are formed by organic cations and inorganic or organic anions (molten salts) [23]. In recent years, RTILs have attracted great attention due to their unique chemical and physical properties such as nonvolatility (non- flammable), excellent solvation qualities, non-flammable and high thermal stability [24,25]. Herein ILs are regarded as promising "green" solvents to replace the traditional toxic solvents in many fields. Some research groups have also studied the aggregation and micelles formation of ionic liquids in aqueous solution [26–28]. The self-aggregation behavior of ILs makes their successful application as surfactants to couple with magnetic nanoparticles in mixed hemimicelles solid phase extraction, which combines the advantages of both ILs and magnetic materials [7]. The results showed that hydrophobic analytes tend to exhibit high distribution coefficients with monocationic IL-aggregates. Herein, the extraction efficiencies obtained with ILs were good.

Cephalosporins in general showed instability in solution and in solid state [29]. The wide use of these antibiotics in modern antimicrobial therapy [30]. They are among the safest and the most effective broad spectrum bactericidal antimicrobial agents [31,32], therefore, they are the most prescribed [33,34]. In order to probe the potential benefits, or adverse effects of different cephalosporins consumption more efficiently, it is necessary to develop analytical methods capable of sensitively, accurately and simultaneously quantifying of a number of trace amounts of flavonoids in human fluids.

In this work, we established a mixed hemimicelles SPE procedure based on the combination of an IL, 1-dodecyl-3-methylimidazolium hexafluorophosphate (C_{16}mimBr) with Fe_3O_4/GO NPs. The new adsorbent possesses the advantages of both the ILs and the magnetic graphene oxide. Five cephalosporins were selected as model compounds to determine the feasibility of this method and the significant experimental factors affecting the extraction recoveries were examined with cefoperazone and cefotaxime. The results of analyzing five cephalosporins in real biological samples were proven to be applicable. We found that, for the analysis of cephalosporins in biological samples, the mixed hemimicelle based nanosized SPE method is a sensitive method.

2. Experimental

2.1. Chemicals and materials

All reagents were of analytical grade and used as supplied. Five cephalosporins (cefoperazone, cefotaxime, cefuroxime, cefuroxime and cefaclor) were supplied by National Institutes for Food and Drug Control. Their chemical structures shown in Fig. S1 (Supporting Information). Flake graphite with high purity was obtained from theNanjing XFNANO Materials Tech Co. Ltd (Nanjing, China). H_2SO_4 (98%), H_2O_2 (30%), $KMnO_4$, $FeCl_3.6H_2O$, sodium acetate, ethylene glycol (EG), diethylene glycol (DEG) and all other chemicals in analytical purity were purchased from Sinopharm Chemical Reagent Co. Ltd (Beijing, China). The cationic surfactant cetyltrimethylammonium bromide (CTAB) and sodiumdodecyl sulphate(SDS)was obtained from Beijing Chemical Reagents Company (Beijing, China). 1-hexadecyl-3-methylmidazoliumbromide (C_{16}mimBr) and other four ionic liquids were purchased from Lanzhou Greenchemistry ionic liquids (Lanzhou Institute of Chemical Physics, Chinese Academy of Science, Lanzhou, China). HPLC-grade methanol was supplied by Tedia Company Inc (Fairfield, USA). Deionized water was acquired from Milli-Q50SP Reagent system (Millipore Corporation, MA, USA).

2.2. Instruments

The transmission electron microscope (TEM) image was performed on a FEI Tecnai G2 F20 (Tokyo, Japan). X-ray diffraction (XRD) measurements were carried out using a Rigaku D/max-rB diffractometer (Rigaku, Tokyo, Japan) with Cu Kα radiation (40 kV, 60 mA). FT-IR spectrum was collected by using 8400 s FTIR spectrometer in KBr pellet at room temperature (Shimadzu Corporation, Japan). The magnetic properties were studied by an LDJ 9600-1vibrating sample magnetometer (VSM, LDJ Electronics Inc., USA) operating at room temperature with applied fields of up to 10 kOe. Zeta-potential measurements of Fe_3O_4/GO NPs were performed with ZetaPlus Zeta Potential Analyzer (Brookhaven, USA).

2.3. Sample preparation

Standard stock solutions of cephalosporins (1 mg ml^{-1}) were prepared with methanol and then diluted to the desired concentration. Blank urine samples were collected from volunteers in China Pharmaceutical University (Nanjing, China). Appropriate milliliters

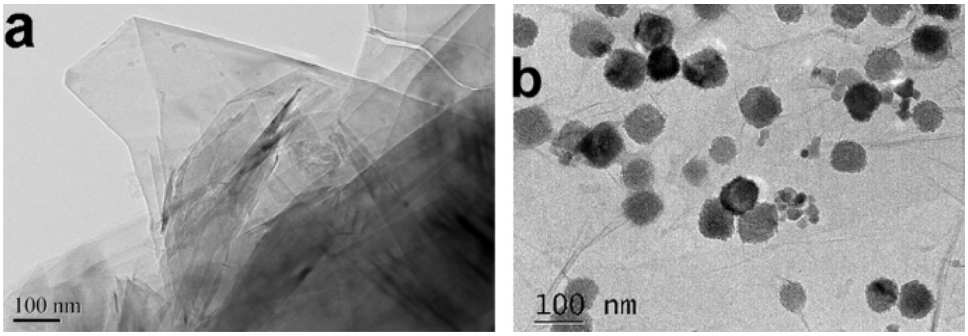

Fig. 2. TEM images of GO (a) and Fe_3O_4/GO (b).

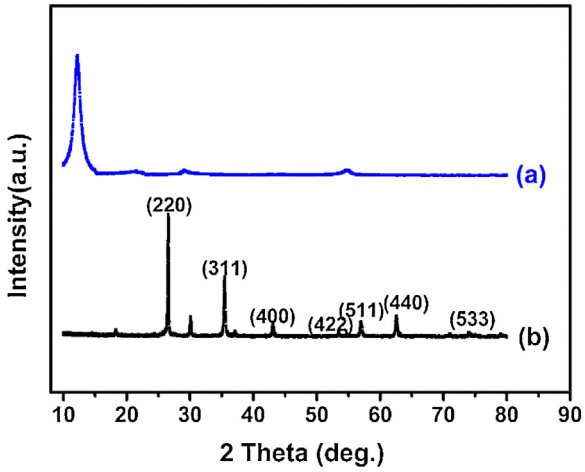

Fig. 3. XRD patterns of GO (a) and Fe_3O_4/GO (b).

of stock solutions of cefoperazone and cefotaxime were added to the blank urine solutions. All solutions were stored at 4 °C.

2.4. Preparation of Fe_3O_4/GO NPs

2.4.1. Synthesis of graphene oxide

Graphene oxide was synthesized according to the modified Hummers method [35]. Briefly, graphite powder (300 meshes, 1.0 g) and sodium nitrate (1.5 g) were mixed in sulfuric acid (75 mL, 98 wt%) under stirring and cooled by using an ice bath. Then $KMnO_4$ (6.0 g) was added gradually under stirring and the temperature of the mixture was kept to be below 10 °C by cooling. The reaction mixture was maintained at approximately 4 °C in an ice bath for 4 h. Next, 100 mL of distilled water was added and the temperature was increased to 90 °C in an oil bath. As the reaction progressed, the mixture gradually became pasty. Afterwards, the reaction was cooled to room temperature and poured onto ice (150 mL) with 30% H_2O_2 (10 mL) until gas evolution ceased. The colour of the mixture turned to yellow. Finally, the mixture was filtered and washed with 10% HCl aqueous solution to remove metal ions followed by water until the pH was nearly neutral. The solid was obtained by centrifugation and washed thoroughly with deionized water, dried in a vacuum at room temperature.

2.4.2. Synthesis of Fe_3O_4/GO NPs

Fig. 1 shows the illustration of the whole procedure of the preparation of surfactants coated Fe_3O_4/GO NPs and its application as SPE sorbents for simultaneous extraction and preconcentration of analytes. The Fe_3O_4/GO NPs were prepared by a solvothermal method. Typically, GO (0.2 g) was introduced into a mixture of EG (45 mL) and DEG (45 mL) in 250 mL beakerflask, followed by ultrasounding for 2 h. Then 0.68 g $FeCl_3.6H_2O$ was dispersed into the above solution. Subsequently, 8.10 g sodium acetate and 2.25 g polyethylene glycol (PEG) were added, followed by stirring for 30 min. Finally, the mixture was sealed in a Teflon-lined stainless steel autoclave and maintained at 200 °C for 10 h. The product was washed several times with water and ethanol, and was dried at 45 °C in a vacuum oven.

2.5. MSPE procedure based on mixed hemimicelles

The extraction procedure depicted in Fig. 1 was as follows. Firstly, 4 mg Fe_3O_4/GO NPs, 2 mg C_{16}mimBr and 2 mL phosphate buffer solution (pH 7.0) were added into a centrifuge tube in sequence. The mixture was sonicated for 5 min, and then C_{16}mimBr was adsorbed on the surface of Fe_3O_4/GO NPs immediately to form mixed hemimicelles. Subsequently, a strong magnet was placed at the side of the tube and the C_{16}mimBr-coated Fe_3O_4/GO NPs were isolated from the solution. After 2 s the suspension became limpid and was decanted. Secondly, 2 mL urine samples were added to the above-mentioned tube. The mixture was equilibrated for 15 min in an oscillator. After equilibrium, a magnet was deposited outside the bottom of the centrifuge tube for 1 s to separate the sorbents from the sample solution. The collected sorbents adsorbing the target analytes were eluted with 1 mL acetone containing 1% acetic acid (0.5 mL every time and washed two times) to desorb the analytes. The eluent was dried with a stream of nitrogen at 50 °C and redissolved in 0.5 mL of methanol. Finally, after filtration through 0.45 μm membrane, 10 μL of the solution was injected into the HPLC system for analysis.

2.6. HPLC-UV analysis of urine samples

High performance liquid chromatography (HPLC, Agilent, USA) was used for determining five cephalosporins. The analytical column was a ZORBAX Eclipse XDB-C_{18} column (4.6 mm × 150 mm, 5-Micro). The mobile phase consisted of triethylamine- glacial acetic acid buffer solution (pH 3.5) as solvent A and acetonitrile as solvent B. The ratio of solvent A to solvent B was 85:15(v/v), and the flow rate of the mobile phase was set at 1.0 mL min^{-1}. The oven temperature was set at 35 °C and the analytes were detected at 285 nm.

3. Results and discussion

3.1. Characterization

The morphology of the prepared graphene oxide and Fe_3O_4/GO NPs was characterized by TEM. As shown in Fig. 2a, the GO consisting of ultrathin, semi-transparent and crumpled nanosheets with lateral size ranged from dozens of nanometers to several micrometers. Fig. 2b shows the TEM images of Fe_3O_4/GO. It can be seen

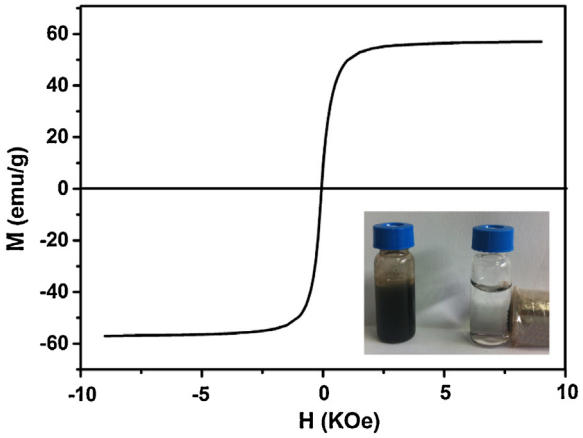

Fig. 4. Magnetization curves of Fe_3O_4/GO NPs obtained by a vibrating sample magnetometer at room temperature.

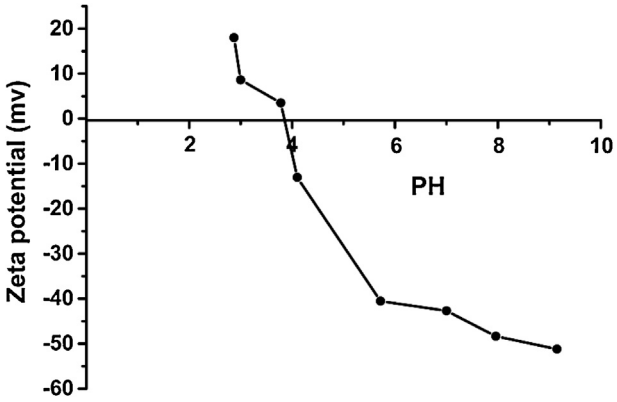

Fig. 5. Zeta-potential of Fe_3O_4/GO NPs at different pH.

that the Fe_3O_4 nanoparticles with a size of about 50–60 nm are well adsorbed on the surface of the graphene oxide. Some Fe_3O_4 nanoparticles are slightly aggregated due to their extremely small size and π–π coupling.

The phase and crystalline structure of the GO and Fe_3O_4/GO were characterized by XRD, as shown in Fig. 3a.The graphite oxide presents a very sharp diffraction peak at 2θ=10.74°, which indicates that the (002) inter-planar spacing increased due to the oxide treatment. For Fe_3O_4/GO (Fig. 3b), the position of all significant diffraction peaks matched well with data from the JCPDS card for Fe_3O_4 and can be assigned to the (111), (220), (311), (400), (422), (511), and (440) of crystal planes of Fe_3O_4. The XRD pattern of Fe_3O_4/GO shows a broad peak corresponding to the (002) reflection of graphene at 23.6°, suggesting that the samples are exceedingly poorly ordered along the stacking direction. Furthermore, the diffraction pattern confirmed the formation of the Fe_3O_4/GO NPs.

The magnetic characteristics of the synthesized Fe_3O_4/GO were evaluated by VSMat room temperature and the magnetization curve is illustrated in Fig. 4. Neither remanence nor coercivity was observed, indicating that the synthesized Fe_3O_4/GO magnetic particles are superparamagnetic, which ensures recycling of the magnetic adsorbent conveniently. The saturation magnetization was found to be 57.068 emu g^{-1}, high enough for magnetic separation using an external magnet.

The evidence for the successful adsorption of C_{16}mimBr onto the surface of Fe_3O_4/GO is provided by FT-IR spectra. The infrared spectrum of Fe_3O_4/GO and C_{16}mimBr-coated Fe_3O_4/GO are presented in Fig. S2. Many absorption signals were matched between the two spectra, such as the typical peaks around 1060 cm^{-1} corresponding to characteristic band of C-O. However, the stretching vibration of C-H band at 2986, 2850 cm^{-1} and the vibration of N-H in the imidazole ring at 1490 cm^{-1} appeared in the spectrum of the C_{16}mimBr-coated Fe_3O_4/GO, as shown in red line, indicating that C_{16}mimBr was successfully coated on the surface of Fe_3O_4/GO.

Surface area measurement of the nitrogen gas absorption yielded a Brunauer Emmett and Teller (BET) surface area of 325.9 m^2 g^{-1} for GO and 115.7 m^2 g^{-1} for GO/Fe_3O_4 (Fig. S3). Both are also higher than MCNTs and Fe_3O_4 nanoparticles. Furthermore the large π-electron system of GO can provide a strong affinity for carbon-based ring structures, which are ubiquitous in drugs, pollutants and biomolecules.

3.2. Isoelectric point

As a significant characteristic of metal oxides, the isoelectric point (IEP) of Fe_3O_4/GO was measured by zeta-potential NPs under different pH (Fig. 5). The IEP was found to be about 3.85 which is similar to the previously reported data for Fe_3O_4/GO [36]. This indicates that at pH< IEP, the Fe_3O_4/GO exhibits positive surface charge, while at pH>IEP, the surface charge is negative.

3.3. Optimization of the extraction conditions.

3.3.1. Effect of the amount of Fe_3O_4/GO NPs

Fe_3O_4/GO NPs have been used as better sorbents for their high surface areas and strong magnetism. Therefore, fewer amounts of nanoparticle sorbents can achieve satisfactory results. To find the optimized amount of adsorbent for the extraction,amounts of Fe_3O_4/GO NPs ranging from 0 to 8 mg were tested. As can be seen from Fig. 6A, the maximum extraction efficiency of the analytes was achieved when only 4.0 mg of Fe_3O_4/GO was used, and then decreased in recoveries when the amount of the adsorbent was above 4.0 mg. Therefore, 4.0 mg of Fe_3O_4/GO NPs was used in the next experiments, which was much less than the literature-reported amount of the commonly used adsorbents.

3.3.2. Effect of the amount of ionic liquid

During the extraction process, the amount of ionic liquid surfactant is one of the important factors in the present method because the formation of the mixed hemimicelle/admicelle depends on this condition directly. Both hemimicelles and admicelles were formed on the surface of Fe_3O_4/GO NPs and the adsorption was driven by both hydrophobic interactions, electrostatic attraction and π–π interactions. The influence of surfactant content was studied by adding different amounts of C_{16}mimBr, ranging from 0 to 5 mg for 4 mg Fe_3O_4/GO NPs. As shown in Fig. 6B, both cephalosporins were hardly adsorbed on the surface of Fe_3O_4 NPs in the absence of the C_{16}mimBr while its adsolubilization increased remarkably with the addition of ionic liquid. However, when the quantity of C_{16}mimBr exceeded 2 mg for Fe_3O_4/GO NPs, recoveries of the target analytes decreased gradually which may be attributed to the fact that the micelles had formed and they caused the analyte redistribution into solution again. Given the findings, 2 mg of C_{16}mimBr per 4 mg of Fe_3O_4 NPs was used in the next experiments.

3.3.3. Effect of sample pH

In the mixed hemimicelles based SPE, value of pH plays a critical role in the hemimicelles formation and the target compound extraction. The surface charge density of Fe_3O_4/GO NPs is a main factor affecting the extraction of analyte and is pH-dependent. The IEP of Fe_3O_4/GO NPs was 3.85. When the pH is above the IEP, the Fe_3O_4 NPs surface has negative charge and efficient interactions can occur between Fe_3O_4 NPs and the ionic liquid. In the present study, the effect of pH was examined by varying the pH values from

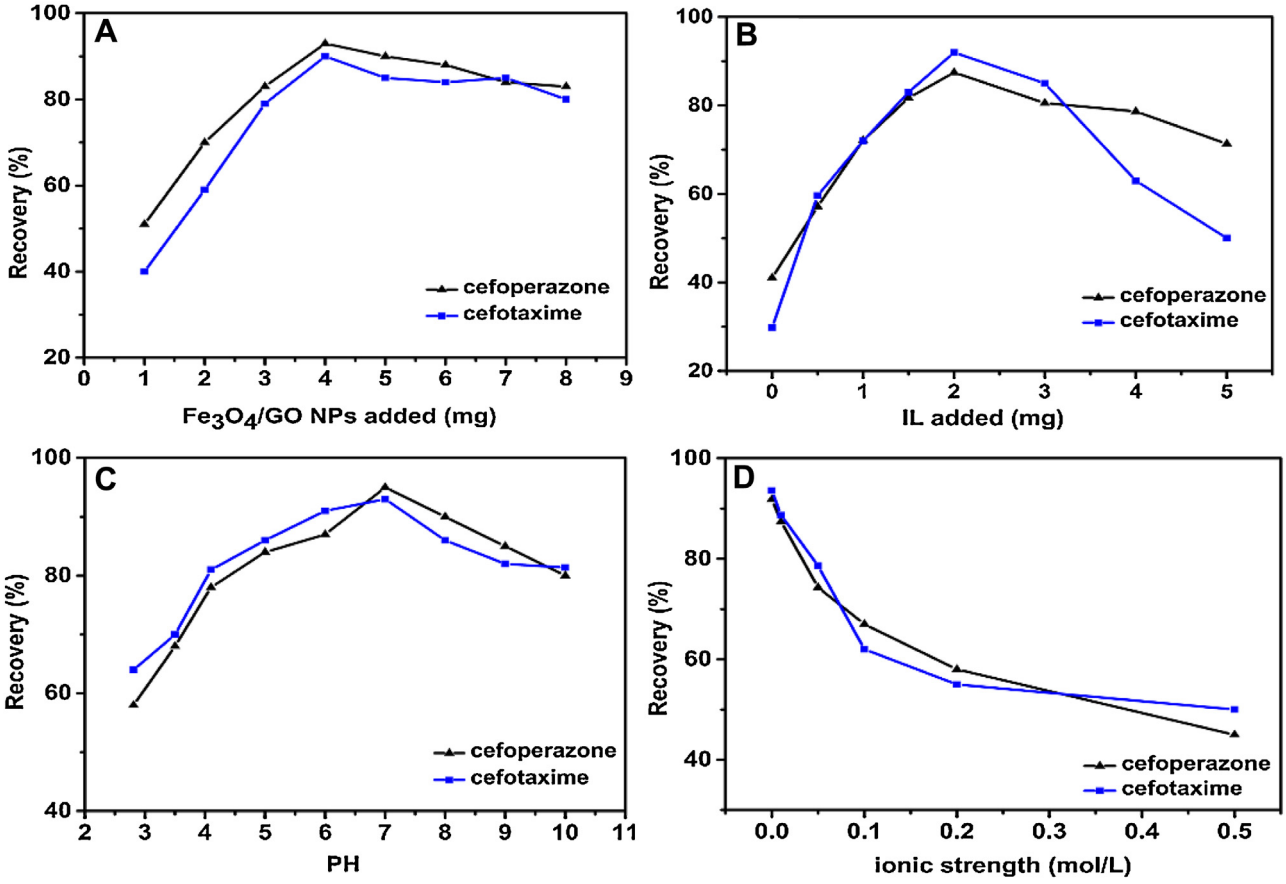

Fig. 6. (A)Effect of the amount of adsorbent on the adsorption of cefoperazone and cefotaxime. (B) Effect of amount of surfactants on the adsorption of cefoperazone and cefotaxime. (C) Effect of pH on the adsorption of cefoperazone and cefotaxime. (D) Effect of the ionic strength on the adsorption of cefoperazone and cefotaxime.

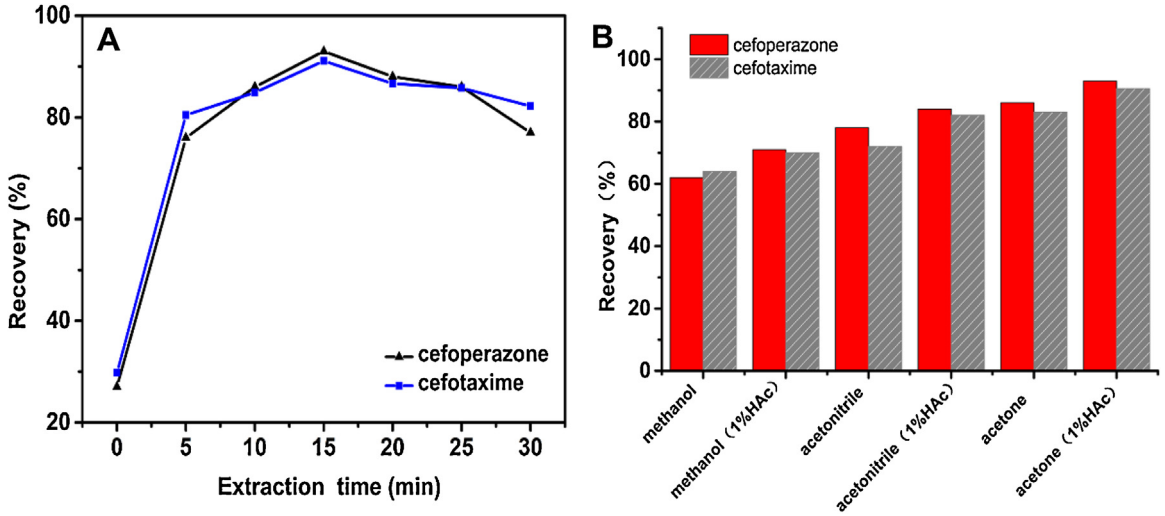

Fig. 7. (A) Effect of the extraction time on the adsorption of cefoperazone and cefotaxime. (B) Effect of desorption solvents on the extraction efficiency of cefoperazone and cefotaxime.

2.8 to 10.0. As shown in Fig. 6C, IL-coated Fe_3O_4/GO NPs exhibited low adsorption for analytes when the pH was between 2.8 and 5.0. With the increase of pH, the adsorption amount obviously increased and reached the maximum value at pH 7.0. This can be explained by the fact that the Fe_3O_4/GO NPs surfaces became negatively charged, and this action led to strong electrostatic attraction between C_{16}mimBr and the charged Fe_3O_4/GO NPs surface. However, the extraction recovery decreased slightly when the pH was above 7.0. This may be explained assuming that that negatively charged Fe_3O_4 NPs surface and negative ions (OH^-) in the bulk solution compete for adsorption of positive ions of C_{16}mimBr, which influences the mixed hemimicelles formation and decreases the recovery of analytes. Therefore, pH 7.0 was selected for the next studies.

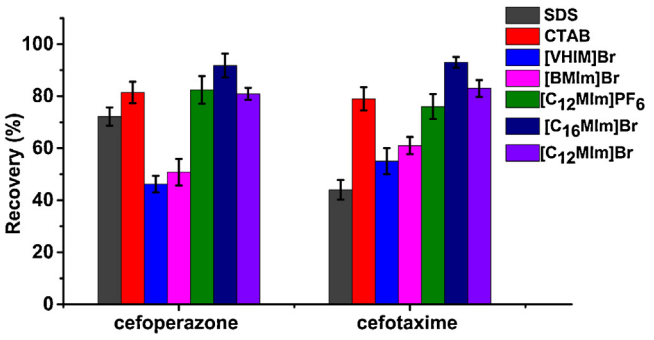

Fig. 8. Comparison of the types of surfactants on the extraction efficiency of cefoperazone and cefotaxime.

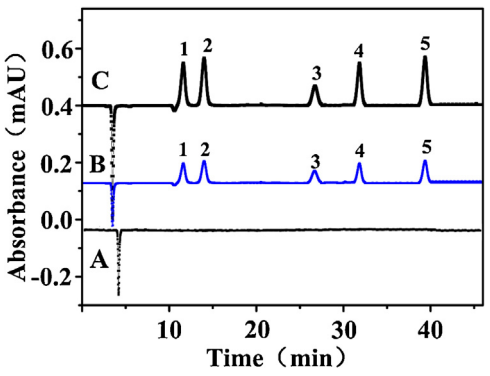

Fig. 9. HPLC–UV chromatograms of samples with SPE procedure: (A) blank urine sample; (B) urine sample spiked with 10 ng mL^{-1} standard solution of five cephalosporins;and (C) urine sample spiked with 50 ng mL^{-1} standard solution of five cephalosporins, respectively. (1. cefadroxil; 2. cefaclor; 3. cefuroxime; 4. cefotaxime; 5. cefoperazone).

3.3.4. Effect of ionic strength

In order to investigate the effect of the ionic strength on the adsorption of the analytes, NaCl was added to the solution over the concentration range of 0.005–0.5 M. As shown in Fig. 6D, the recoveries of the two analytes decreased with the increase of ionic strength. The results indicate the importance of electrostatic attraction in the adsorption process and the competition between sodium ions and [C_{16}mim] $^{+}$ for the Fe_3O_4/GO NPs substrate. Therefore, absence of salt was more suitable for extraction process and all the experiments were accomplished without salt addition.

3.3.5. Effect of extraction time

In the SPE process, standing time had an obvious effect on the adsorption of analytes. The effect of extraction time on the adsorption was examined by changing the time from 0 to 30 min. The results in Fig. 7A indicate that 15 min was sufficient for the highest extraction recoveries of the two analytes. When the extraction time was more than 15 min, recoveries of the two analytes decreased. The phenomenon indicated that with an increase in the extraction time, a part of the ILs returned to the solution, resulting in the decrease of recovery. Therefore, an extraction time of 15 min was chosen for the subsequent evaluation.

3.3.6. Desorption conditions

Organic solvents can easily disrupt the mixed hemimicelle structures and therefore make elution of analytes easy. The desorption of cephalosporins from the IL-coated Fe_3O_4/GO NPs was studied to use different organic solvents including methanol, acetonitrile, acetone, in the absence and presence of 1% acetic acid. As show in Fig. 7B, we found that the desorption ability of acetone containing 1% acetic acid is superior to other two organic solvents, and 1 mL (0.5 mL every time and washed two times) was enough to obtain the complete desorption. For achieving the more efficient desorption condition, desorption time was investigated in the range of 1–10 min. A duration time of 5 min appeared to be sufficient for complete desorption.

3.3.7. Comparison with different surfactants

The surfactant and mineral oxides used in this work were selected based on the interactions they could establish with the target analytes. So selecting a suitable type of surfacant for the preparation of dispersants is of greatest importance. To this end, a comparative study on the use of seven types of surfacant (SDS, CTAB, [VHIM]Br, [BMIm]Br, [C_{12}MIm]PF_6, [C_{16}MIm]Br, [C_{12}MIm]Br) containing different inorganic anions was presented. As shown in Fig. 8, a significantly higher extraction efficiency was obtained by mixing long-chain ILs ([C_{16}mim]Br and [C_{12}mim]Br) in the magnetic functionalization process. This may be attributed to the fact that the imidazolium-based ILs with longer alkyl side chains strengthened the directionality of hydrogen bonds and other weak forces (e.g., van der Waals) [37], which provided a greater interaction with the mixed hemimicelles and the hydrophobic regions of target analytes. The results indicated that [C_{16}mim]Br employed in functional Fe_3O_4/GO NPs indeed had a significant effect on the extraction of analytes. Therefore, [C_{16}mim]Br was selected as the optimal IL and used in this study.

Table 1
Analytical parameters of the proposed method.

Analytes	Linear range (ng/ml)	correlation coefficient R^2	LOD (ng/ml)	LOQ (ng/ml)
cefoperazone	1.5–500	0.9987	0.6	1.5
cefotaxime	3–500	0.9991	1.2	3.2
cefuroxime	1.5–500	0.9985	1.5	4.2
cefadroxil	1.5–500	0.9989	1.1	3.4
cefaclor	3–500	0.9994	1.9	5.5

3.4. Regeneration of the Fe_3O_4/GO NPs

The reuse of solid adsorbents is of great importance for both economic and environmental standpoints. The Fe_3O_4/GO NPs could be conveniently regenerated by rinsing the NPs sequentially with water and methanol for several times. The results indicated that the amounts of the Fe_3O_4/GO NPs slightly decreased after six cycles of SPE procedure. However, no significant loss of the sorption capacity were observed(RSD% = 7.3), suggesting that the intrinsic stability of nanoparticle components allowed the Fe_3O_4/GO NPs to be repeatedly used as adsorbents.

3.5. Analytical performance and application

3.5.1. Linearity, LOD and LOQ

To evaluate the accuracy and feasibility of the method developed, quantitative parameters of the method, such as linear range, correlation coefficient, limit of detection (LOD), limit of quantitation (LOQ) and recovery were evaluated.

The linearities of the analytes were studied in urine samples. All the parallel experiments were repeated three times. Limits of detection (LODs) of the investigated compounds were estimated as the minimum concentration determined with a signal-to-noise ratio of 3 and the LOQ values taken by signal-to-noise ratio of 10. The linear range, slope, intercept, correlation coefficients (R^2) and LODs are listed in Table 1. The results show the linear ranges of analysis were 1.5–500 ng mL^{-1}. R^2 ranged from 0.9985 to 0.9994. The LODs were ranged between 0.6 and 1.9 ng mL−1 for the cephalosporins and the LOQs were 1.5 to 5.5, respectively.

Table 2
Precision and accuracy for detection of analytes in urine sample.

Analytes	Intra-day(R% ± RSD),n = 5			Inter-day (R% ± RSD),n = 3		
	10 ng/mL	100 ng/mL	200 ng/mL	10 ng/mL	100 ng/mL	200 ng/mL
cefoperazone	89.4 ± 4.6	95.2 ± 3.8	94.3 ± 3.4	93.1 ± 5.2	90.5 ± 4.9	90.2 ± 5.4
cefotaxime	91.7 ± 4.1	93.6 ± 3.7	96.8 ± 2.9	90.6 ± 5.1	92.4 ± 5.5	94.4 ± 4.7
cefuroxime	92.1 ± 4.7	93.8 ± 6.2	91.1 ± 5.8	90.4 ± 3.4	91.6 ± 4.1	90.8 ± 4.6
cefadroxil	82.4 ± 3.6	83.6 ± 3.6	83.9 ± 2.7	82.1 ± 4.7	82.8 ± 2.9	84.9 ± 3.1
cefaclor	90.5 ± 4.2	88.7 ± 2.2	89.5 ± 3	89.9 ± 6.3	87.4 ± 3.3	86.4 ± 3.8

The samples were treated with the same SPE before injection to HPLC. Fig. 9 shows the chromatograms obtained from urine sample analyses with the method. It can be seen that no interferences such as the proteins and endogenous components in urine samples were observed.

3.6. Precision and accuracy

The intra-day and inter-day precision and accuracy of the method were evaluated by assaying five replicated spiked urine samples at three different concentration levels (low, middle and high concentrations) of the targets in the same day and in three consecutive days, respectively. The relative standard deviations (RSDs) of the intra-day and inter-day precision and accuracy values for spiked biological samples are summarized in Table 2. The RSDs of the intra-daily tests are less than 6.2%, and the RSDs of inter-daily tests (three consecutive days) are less than 6.3%. These results indicate that the present method has good accuracy and precision.

3.6.1. Analysis of urine samples

In order to validate the suitability of the developed method, the method was applied to analyze the analytes in urine samples. The recovery of the method was validated by the standard addition method. The reference standards were added at three different concentration levels (low, middle and high concentrations of the matrix) with five parallel experiments at each level. All the parallel experiments were repeated three times and the results are tabulated in Table 3. It can be seen that recoveries ranging from 84.3% to 101.7% for the five target analytes was obtained. Thus it is suitable for analyzing the biological samples.

3.7. Comparison with other methods

In the previous work, many methods were successfully developed for the analysis of cephalosporins in some matrices. In order to further demonstrate the superiority of our proposed method, we compared our method with previous reported [38–44]. As seen from Table 4, our method had higher extraction ability. Moreover, our method provided a relative wider linear range and a comparable LOD. The results revealed that the proposed method for the analysis of cephalosporins in different matrices was simple, rapid and sensitive.

Table 3
Recoveries obtained for the determination of the analytes in urine sample.

Analytes	Spiked (ng mL^{-1})	Mean accuracy (%)	RSD%
cefoperazone	10	92.4	4.9
	100	87.2	6.3
	200	91.4	4.5
cefotaxime	10	101.7	4.1
	100	94.6	4.9
	200	92.8	2.6
cefuroxime	10	88.2	4.7
	100	100.8	6.1
	200	92.6	4.8
cefadroxil	10	84.3	5.6
	100	84.9	3.1
	200	88.3	5.2
cefaclor	10	90.8	2.3
	100	87.9	1.7
	200	94.5	3.2

4. Conclusion

A rapid, sensitive and simple SPE method which combines the advantages of mixed hemimicelles and magnetic graphene nanoparticles was applied in extraction and preconcentration of cephalosporins in biological samples. The π–π, hydrophobic and electrostatic interactions between the mixed hemimicelles and analytes made this SPE capable of high extraction efficiency and capacity. In the present experiment, only small amount of Fe_3O_4/GO NPs (4 mg) and C_{16}mimBr (2 mg) were needed to achieve good results, indicating that the method was very efficient and sensitive. Moreover, a comparative study on the use of different surfactants were investigated, and C_{16}mimBr was found to be more appropriate for SPE. An important aspect should be pointed out that the reagents and materials used in this study were low-cost and environmentally friendly. The results indicated that the developed method can be used as a simple and efficient extraction and preconcentration technique for trace cephalosporins in urine samples prior to HPLC

Table 4
Comparison of the proposed method with other methods for the determination of cephalosporins.

Sample	Extraction method	Determination method	Linear range	LOD	RSD (%)	Recovery (%)	Ref.
Photo-degradation products	–	spectrophotometric	5–40 μg mL^{-1}	2.64 μg mL^{-1}	0.63	99.62	[29]
Pharmaceutical formulations	–	spectrophotometric	0.15–1.44 μg mL^{-2}	40–100 ng mL^{-1}	2	97.38–100.39	[30]
Beef muscle	SPE	LC–MS/MS	4–50 μg kg^{-1}	0.1–10 μg kg^{-1}	15	>85	[31]
Plasma	SPE	HPLC-UV	2.5–60 μg mL^{-1}	0.024–0.033 μg mL^{-1}	0.9–12.2	93.2–107.1	[32]
Milk sample	Ultrasound-assisted matrix solid-phase dispersion	HPLC-UV	–	5.0–47.3 μg kg^{-1}	<15.3	93.8–101.9	[33]
Plasma	Oasis HLB cartridges(SPE)	HPLC-UV	5–300 μg mL^{-1}	0.1 μg mL^{-1}	<10	>90	[34]
Pharmaceutical formulations	SPE	HPLC-UV	0.1–200 μg mL^{-2}	0.538 μg mL^{-1}	–	–	[38]
Biological samples	MSPE	HPLC-UV	1.5–500 ng ml^{-1}	0.16–1.2 ng mL^{-1}	3.41–5.36	89.4–96.8	Present work

analysis and the material may have a great application potential for the preconcentration of other trace antibiotics in different samples.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chroma.2016.05.071.

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