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Fabrication of magnetic microsphere-confined graphene for the preconcentration of some phthalate esters from environmental water and soybean milk samples followed by their determination by HPLC

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ABSTRACT

In this study, a magnetic microsphere-confined graphene (Fe_3O_4@SiO_2-G) was prepared as a novel adsorbent for the preconcentration of some phthalate esters in environmental water and soybean milk samples prior to high performance liquid chromatography analysis. The properties and morphology of the Fe_3O_4@SiO_2-G were characterized by transmission electron microscopy and X-ray diffraction. This novel graphene-based magnetic nanocomposite showed great adsorptive ability toward the analytes. The method, which takes the advantages of both the high adsorption capacity of G and easy phase separation of the magnetic adsorbent from the sample solution, could avoid the time-consuming experimental procedures related to the traditional solid phase extraction. Various experimental parameters that could affect the extraction efficiencies, such as the amount of Fe_3O_4@SiO_2-G, the extraction time, the pH of sample solution and the desorption conditions, were investigated. Under the optimum conditions, the limits of detection ($S/N=3$) of the method for the compounds were between 0.07 and 0.10 ng mL^{-1} in water samples, and between 0.15 and 0.30 ng mL^{-1} in soybean milk samples. The relative standard deviations (RSDs) varied from 2.7% to 6.1% ($n=5$). The recoveries of the method were in the range between 87.2% and 109.0% for environmental water and soybean milk samples. The method is suitable to determine the five phthalate esters (diallyl phthalate, di-*n*-propyl-phthalate, benzyl butyl phthalate, dicyclohexyl-phthalate and diethyl-hexyl-phthalate) in environmental water and soybean milk samples.

1. Introduction

Graphene (G)-related material is a kind of novel and interesting carbon material and has enjoyed tremendous research attentions in recent years because of its exceptional electronic, thermal, optical, adsorption, mechanical properties and very high specific surface [1]. The chemical versatility of the graphene-based materials make them attractive for a wide range of applications, such as eletronic and photonic devices, energy research, targeted drug delivery, bioimaging, catalysis, adsorption and sensors [2].

Since the large delocalized π-electron system of G may form a strong hydrophobic and π-stacking interaction with some organic molecules [3], it might be a promising candidate as an adsorbent for some kinds of compounds. Recently, G has been used as the adsorbent in solid-phase microextraction (SPME) [4] and solid-phase extraction (SPE) [5].

Trace analysis of the analytes in environmental samples generally requires a sample pretreatment step to isolate and enrich the target analytes before an instrumental analysis. Up to now, various sample pretreatment techniques have been developed. Recently, a new mode of SPE termed as magnetic solid-phase extraction (MSPE) has been developed [6]. It has several advantages in comparison with traditional SPE. The separation process in MSPE can be performed directly in crude samples containing suspended solid materials without the need of additional centrifugation or filtration, which makes separation easier and faster. Due to these advantages, MSPE has been widely used in many fields [7–10]. So far, in MSPE, Fe_3O_4 nanoparticles are most widely used for their low price and low toxicity. Various materials have been used to modify and functionalize Fe_3O_4 nanoparticles, such as decanoic acid [11], sodium dodecyl sulfate (SDS) [12], ionic liquid [13], some carbon materials [14,15] and various polymers [16–18]. However, these magnetic adsorbents have been mainly applied for the analysis of water samples and their applications for more complex matrix samples are still very few [19–21].

The introduction of magnetic properties into G would combine the high adsorption capacity of G and the separation convenience

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of magnetic materials. Magnetic G nanocomposites have been fabricated for the preconcentration or removal of different environmental pollutants. Chandra et al. [22] have prepared the magnetite-reduced graphene oxide (GO) composites for arsenic removal. Li et al. [23] have synthesized a magnetic $CoFe_2O_4$-functionalized G sheets nanocomposite by hydrothermal treatment of inorganic salts and thermal exfoliated graphene sheets and applied it to remove methyl orange from water samples. Ai et al. [24] have synthesized the G nanosheet/magnetite (Fe_3O_4) composite by a facile one-step solvothermal method for removal of methylene blue in water. Our group has also synthesized G-coated Fe_3O_4 magnetic nanoparticles for the removal and preconcentration of some organic contaminants [25,26] from water and beverages. However, the magnetic particles (Fe_3O_4 or $CoFe_2O_4$) of the above G-based magnetic nanoparticles were exposed out, and therefore they were easily oxidized and could lose their magnetism under harsh conditions and long-term use. Moreover, plenty of the adsorption sites of G were occupied by magnetic particles and became unavailable for analyte adsorption. To solve the above problems, a suitable protective coating on a magnetic core could be used and G could be confined on the surface of the magnetic microsphere. Silica has been considered as one of the most ideal shell materials since it is not only abundant and inexpensive but also has reliable chemical stability, biocompatibility and versatility in surface modification [27]. Recently, Feng's group has prepared $Fe_3O_4@SiO_2$/graphene by physical adsorption of G onto silica-coated magnetic microspheres and applied it for the extraction of sulfonamide antibiotics from environmental water samples [28]. Jiang's group [29] has also prepared G-encapsulated magnetic microspheres ($Fe_3O_4@SiO_2@G$) for the enrichment of proteins and peptides for mass spectrometry analysis. In their preparation methods, GO was assembled on the amino-functional silica-coated spheres through electrostatic interactions and then the GO was reduced to G with hydrazine. The $Fe_3O_4@SiO_2@G$ nanomaterial has a multilayered core–shell structure and thus can avoid the above-mentioned problems. The G shell endows the material with a high sorption capacity, and the silica shell provides a good protection for Fe_3O_4 core. However, such composites formed by simple physical or electrostatic adsorption might not be stable enough for repeated extraction applications.

Phthalate esters (PAEs) are well-known polymer additives that are used in formulations of pesticides, paints and poly (vinyl chloride) plastics. They may pose reproductive and developmental impacts on animals and humans because of their endocrine-disrupting effects [30]. They can be released easily from products, migrate into environment, and consequently, pollute water, soil, air and food products since they are only physically bound to the polymers [31]. The United States Environmental Protection Agency (US EPA) has listed PAEs as the priority contaminants [32]. In Europe, New EU Regulation (EU) no. 10/2011 on plastic materials and articles intended to come into contact with food was published on January 15, 2011. In this regulation, it limits that the specific migration limits (SML) for benzyl butyl phthalate, diethyl-hexyl-phthalate were 30 mg kg^{-1} and 1.5 mg kg^{-1}, respectively. When there are no SMLs or other restrictions, a generic specific migration limit of 60 mg kg^{-1} shall apply [33]. And the total tolerable daily intake (TTDI) per person of total phthalate esters has been estimated to be 0.3 mg kg^{-1} body weight [34]. Therefore, the determination of trace level of PAEs in different matrix samples is desirable.

In this report, a more stable and effective silica-coated magnetic graphene composite with surface grafting by G ($Fe_3O_4@SiO_2$-G) was synthesized via a chemical bonding method for the first time. The performance and the stability of this magnetic microsphere-confined graphene for the enrichment of the five PAEs (diallyl phthalate (DAP), di-*n*-propyl-phthalate (DPP), benzyl butyl phthalate (BBP), dicyclohexyl-phthalate (DCP), and diethylhexyl-phthalate (DEHP)) in environmental water and soybean milk samples were evaluated.

2. Experimental

2.1. Reagents and materials

Graphite powder (50 meshes) was purchased from Boaixin Chemical Reagents Company (Baoding, China). GO was prepared from the graphite powder according to the previously reported method [25]. Ammonium ferrous sulfate, ammonium ferric sulfate, NH_4OH, ethylsilicate (TEOS), 3-aminopropyltriethoxysilane (APTES), trichloroacetic acid, dimethylformamide (DMF), 1-(3-dimethylaminopropyl)3-ethylcarbodiimide hydrochloride (EDC), *n*-hydroxysuccinimide (NHS) and polyvinylpyrrolidone (PVP) were obtained from Chengxin Chemical Reagents Company (Baoding, China). Acetonitrile, acetone, ethanol, hydrochloric acid (HCl), sodium hydroxide (NaOH), isopropanol and all other reagents were purchased from Beijing Chemical Reagents Company (Beijing, China). The water used throughout the work was double-distilled on a SZ-93 automatic double-distiller purchased from Shanghai Yarong Biochemistry Instrumental Factory (Shanghai, China).

Standards of the PAEs (DAP, DPP, BBP, DCP and DEHP) were purchased from Aladdin-Reagent (Shanghai, China). A mixture stock solution containing each of DAP, DPP, BBP, DCP and DEHP at 50.0 μg mL^{-1} was prepared in methanol. A series of standard solutions were prepared by mixing an appropriate amount of the stock solution with double-distilled water in a 10 mL volumetric flask. All the standard solutions were stored at 4 °C and protected from light.

Reservoir water was collected from Angezhuang reservoir (Baoding, China); river water was collected from Tang River (Baoding, China); two kinds of soybean milk packed in plastic cups were purchased from local supermarket.

2.2. Apparatus

HPLC was carried out on a LC-20AT liquid chromatography (Shimadzu, Japan) with two LC-20AT VP pumps and a SPD-20A UV/vis detector. A Centurysil C_{18}-BDS column (200 mm × 4.6 mm I.D., 5.0 μm) from Dalian Johnsson Separation Science Technology Corporation (Dalian, China) was used for separations. The mobile phase was further optimizated based on the reference [35], and finally acetonitrile–water was selected as the mobile phase with the following gradient elution: starting at 70% acetonitrile; 0–15 min, from 70% acetonitrile to 95% acetonitrile; 15–30 min, keeping at 95% acetonitrile; 30–35 min, from 95% acetonitrile to 70% acetonitrile. The flow rate of the mobile phase was 1 mL min^{-1}. The UV wavelength for monitoring all the analytes was set at 225 nm.

The size and morphology of the magnetic nanoparticles were observed by transmission electron microscopy (TEM) using a JEOL model JEM-2011 (HR) at 200 kV. 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).

2.3. Synthesis of $Fe_3O_4@SiO_2$-G

First, the magnetic Fe_3O_4 microsphere was synthesized by the following chemical coprecipitation method [36]. 3.4 g (8.66 mmol) $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$ and 5.02 g (17.32 mmol) $NH_4Fe(SO_4)_2 \cdot 12H_2O$

were dissolved in 400 mL water at 50 °C under N_2 atmosphere. After the solution was sonicated (200 W, 40 kHz) for 10 min, 20 mL of 8 mol L^{-1} NH_4OH aqueous solution was added dropwise to precipitate the iron oxides while the mixture solution was sonicated. The pH of the final mixture should be in the range from 11 to 12. To promote the complete growth of the nanoparticle crystals, the reaction was carried out at 50 °C for 1 h under constant mechanical stirring. Then, the precipitate was isolated in the magnetic field, and washed with double-distilled water until the pH became 7. To prevent the microsphere from agglomeration, the obtained precipitate was dispersed in 20 mL of solution containing 1.7 mL (25.6 g L^{-1}) PVP. The mixture was stirred at room temperature for 1 day. The resulting Fe_3O_4 microspheres were collected by an external magnetic field, then washed with water and ethanol, respectively, and finally air-dried.

Fe_3O_4@SiO_2-NH_2 was prepared according to the literature method [29]. 1.4 g Of the above obtained Fe_3O_4 microspheres was dispersed in the solution containing 280 mL ethanol, 70 mL water and 5 mL $NH_3 \cdot H_2O$. Then, the mixture was stirred at 30 °C for 15 min followed by the additionof 4 mL TEOS in the dispersion, and then, the resultant mixture was further stirred at room temperature for 8 h. The product was collected by an external magnetic field and then washed with ethanol. The obtained Fe_3O_4@SiO_2 was then dispersed in 140 mL isopropanol to obtain a solution of Fe_3O_4@SiO_2. 40 mL of the above Fe_3O_4@SiO_2 solution was diluted with 100 mL isopropanol followed by the addition of 0.2 mL of APTES. The mixture was sonicated (200 W, 40 kHz) for 30 min under nitrogen atmosphere and then stirred at 70 °C for 6 h to obtain amino-functionalized Fe_3O_4@SiO_2-NH_2. The product was first washed with water and then with ethanol, and finally dried under vacuum.

To prepare Fe_3O_4@SiO_2-GO, the condensation reaction between amine moieties of APTES and carboxyl group of GO was referred from the reported method [37] with some modifications. 0.2 g GO was dispersed in 50 mL of DMF with the aid of ultrasonication. Then, 0.1 g NHS and 0.2 g EDC were added in the dispersion. The pH of the mixture should be in the range of 4–6 and the mixture was stirred vigorously for 2 h at room temperature. Subsequently, 0.5 g Fe_3O_4@SiO_2-NH_2 was added and stirred for 12 h. The final Fe_3O_4@SiO_2-GO product was then centrifuged, washed with water, and finally air-dried.

Fe_3O_4@SiO_2-G was synthesized via chemical reduction of Fe_3O_4@SiO_2-GO by hydrazine. 0.2 g of Fe_3O_4@SiO_2-GO was dispersed in 100 mL of water, and then 200 μL of hydrazine (85%) was added. The pH of the mixture should be about 10 and the reaction was carried out at 95 °C for 2 h. The final product Fe_3O_4@SiO_2-G was washed with water for several times and dried at 80 °C.

2.4. Sample preparation

Water samples were directly used for the analysis. For soybean milk samples, 100 mL of the soybean milk was taken into a centrifuge tube and 0.5 mL trichloroacetic acid was added [38]. The solution was vortexed for 1 min and then centrifuged for 10 min at 3000 rpm to isolate proteins. Then, the supernatant was poured into a beaker. After that, 4.0 mL acetonitrile was added to the sedimentation and the tube was vortexed for 1 min. The solution was further centrifuged and the supernatant solution was collected using a micropipette. The same procedures for the acetonitrile extraction were performed two more times. All the supernatants were combined together and stored at 4 °C for the following experiments. Prior to the following MSPE procedures, the pH of either the water samples or the extraction solutions from soybean milk samples was adjusted to about 4–5 with hydrochloric acid and sodium hydroxide solutions.

2.5. MSPE procedures

First, 15 mg Fe_3O_4@SiO_2-G was added into 100 mL aqueous sample or the above obtained soybean milk supernatants. Then, the mixture was shaken on a slow-moving platform shaker for 10 min. Secondly, the adsorbent Fe_3O_4@SiO_2-G was isolated from the solution by placing a strong magnet at the bottom of the beaker and the supernatant was discarded. Then the residual solution and Fe_3O_4@SiO_2-G were totally transferred to a 10 mL centrifuge tube. The Fe_3O_4@SiO_2-G was aggregated again by positioning a strong magnet to the outside of the tube wall so that the residual solution could be removed. Finally, the adsorbed analytes were desorbed from the isolated Fe_3O_4@SiO_2-G adsorbent with 0.2 mL acetone by vortexing for 30 s. After positioning the magnet to the outside of the centrifuge tube, the supernatant desorption solution was collected using a micropipette. The same desorption procedures were repeated for another two more times. The desorption solutions were combined together and 20.0 μL was injected into the HPLC system for analysis.

3. Results and discussion

3.1. Characterization of the Fe_3O_4@SiO_2-G nanoparticles

The typical TEM image of the Fe_3O_4@SiO_2-G is shown in Fig. 1a. As can be seen from Fig. 1a, G sheets were confined to the Fe_3O_4@SiO_2 nanoparticles with a size of about 15–40 nm. The details of Fe_3O_4@SiO_2-G were further examined by HR-TEM (Fig. 1b). The HR-TEM image allowed us, by means of the DigitalMicrograph software, to estimate the planar space of lattice fringes to be 0.31 nm.

X-ray diffraction (XRD) was used to determine the crystal structure of the superparamagnetic nanoparticles. The XRD patterns of the Fe_3O_4@SiO_2-G and Fe_3O_4@SiO_2-GO are shown in Fig. 2. The image of Fe_3O_4@SiO_2-G in Fig. 2 shows that there is a broad peak at ca. 23°, indicating the poor ordering of G sheets along their stacking direction and reflecting that the G powder is composed of few-layer stacked G sheets. The diffraction pattern of both Fe_3O_4@SiO_2-G and Fe_3O_4@SiO_2-GO is close to the standard pattern of crystalline magnetite (Fe_3O_4). It shows diffraction peaks at $2\theta = 30.12°$, 35.54°, 43.22°, 53.52°, 57.14° and 62.68°, which correspond to crystal indexes of (220), (311), (400), (422), (511) and (440), respectively. All the significant diffraction peaks can be indexed to the JCPDS card (19-0629) for Fe_3O_4. The interplanar distances (400) and (220) of the magnetic nanoparticle were estimated by the Bragg equation to be 0.2244 and 0.3131 nm, respectively, which were in good agreement with those measured by HR-TEM.

To investigate the reproducibility of the synthesis, Fe_3O_4@SiO_2-G was prepared under the same conditions at different

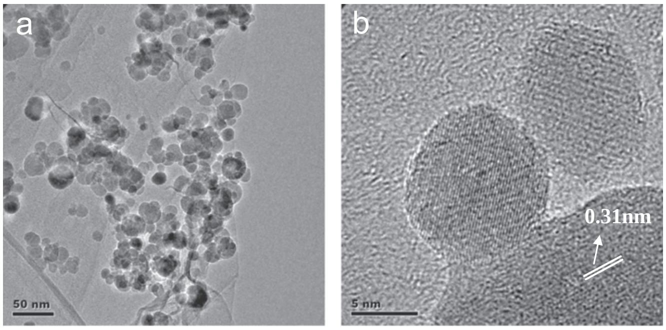

Fig. 1. TEM image (a) and HR-TEM image (b) of the Fe_3O_4@SiO_2-G composites.

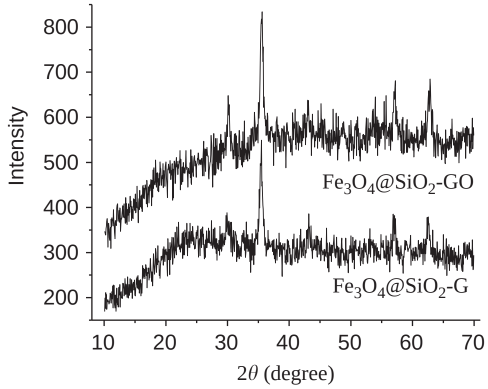

Fig. 2. X-ray diffraction pattern of the Fe_3O_4@SiO_2-GO and Fe_3O_4@SiO_2-G composites.

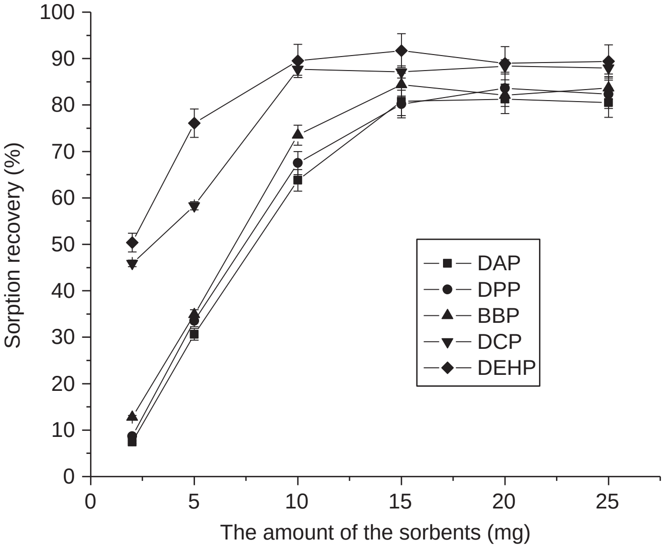

Fig. 3. Effect of the amount of the sorbents on the extraction recoveries of the PAEs.

times. The results indicated that the extraction performances of the different batches of the material for PAEs are similar.

3.2. Optimization of extraction conditions

In order to select the optimum MSPE conditions, 100 mL double-distilled water spiked with 20.0 ng mL^{-1} each of the five PAEs was used to study the extraction performance of the MSPE under different experimental conditions. The following experimental parameters, including the amount of the nanoparticle sorbents, extracting time, sample salinity and pH, and desorption conditions were investigated. All the experiments were performed in triplicate and the means of the results were used for optimization.

3.2.1. Effect of the amount of nanoparticle sorbents

In order to choose the optimum amount of the adsorbent (Fe_3O_4@SiO_2-G) for the adsorption of the PAEs, the amounts of the Fe_3O_4@SiO_2-G in the range from 2 to 25 mg were investigated. Fig. 3 shows that the adsorption of DCP and DEHP could reach the maximum plateau when the amount of Fe_3O_4@SiO_2-G was increased to 10 mg, while the maximum adsorption efficiencies for DAP, DPP and BBP were achieved when the amount of Fe_3O_4@SiO_2@G was increased to 15 mg. Therefore, 15 mg Fe_3O_4@SiO_2-G (0.15 mg mL^{-1}) was selected.

3.2.2. Extraction time

In MSPE process, the extraction time is one of the main factors that influence the extraction efficiency. The effect of the extraction time on the extraction efficiency of the PAEs was investigated in the range from 1 to 20 min. As shown in Fig. 4, when the extraction time was increased to 10 min, the extraction recoveries for all the analytes reached their maxima, indicating that the extraction equilibrium could be achieved in a short time. Therefore, an extraction time of 10 min was selected.

3.2.3. Effect of solution pH

Sample solution pH plays an important role for the adsorption of the analytes by both affecting the existing forms of the analytes and their charges and densities on the sorbent surface. Considering that the PAEs are not neutral compounds (the pK_a of BBP and DEHP was 10.6 and 9.4, respectively; for the other three PAEs, no pK_a values are available), the effect of sample solution pH was investigated in the pH range between 2.0 and 10.0 by adjusting the sample solution pH with hydrochloric acid and sodium hydroxide solutions. The results (Fig. 5) showed that the extraction recoveries for the PAEs were almost unchanged when the pH was changed from 2 to 8; when the sample solution pH was further increased, except that the extraction recovery for DCP remained almost unchanged, the extraction recoveries for all the other four PAEs were deceased to a different degree. Based on the above result, the pH of either the water samples or the extraction solutions from soybean milk samples was adjusted to about 4–5 for the experiments.

3.2.4. Effect of sample salinity

The effect of sample salinity on the extraction recoveries of the compounds was investigated through the addition of different concentrations of NaCl into the sample solution (i.e. 0, 1.0, 3.0, 5.0, 10, 15 and 25% (w/v)). The results showed that the addition of NaCl had a negligible effect on the extraction recoveries of all the analytes in the concentration range investigated. Therefore, no addition of salt to the sample solution was selected.

3.2.5. Desorption conditions

The analytes adsorbed on the Fe_3O_4@SiO_2-G particles should be desorbed completely for their further sensitive HPLC–UV

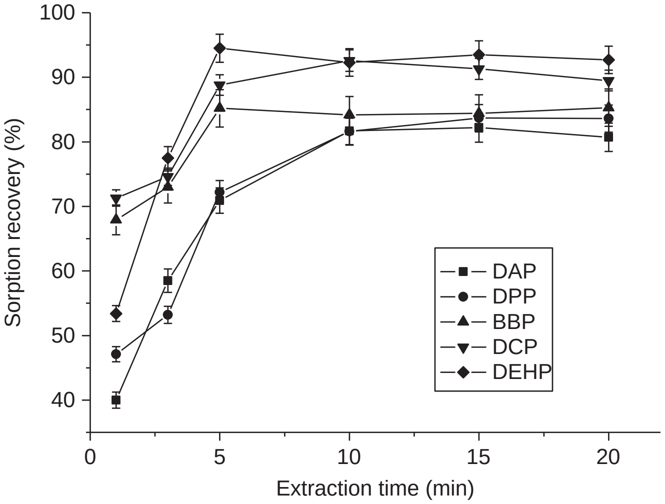

Fig. 4. Effect of extraction time on the extraction recoveries of the PAEs.

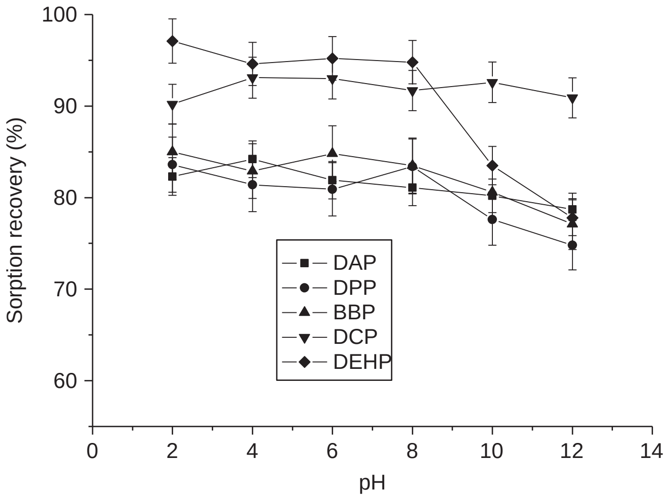

Fig. 5. Effect of sample pH on the extraction recoveries of the PAEs.

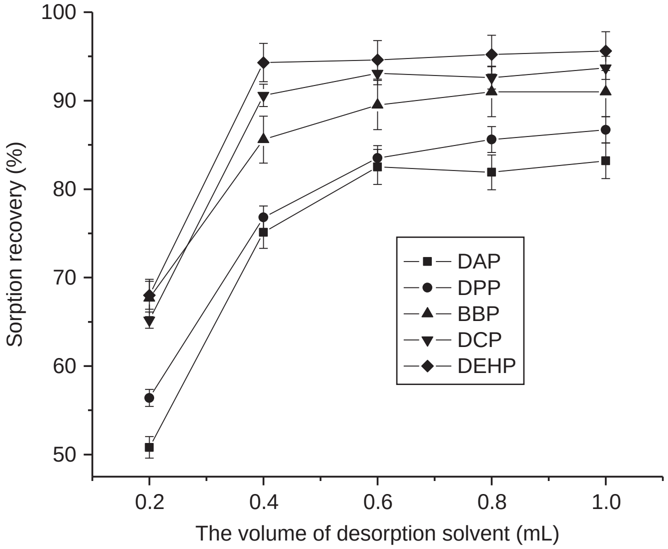

Fig. 6. Effect of the volume of desorption solvent on the extraction recoveries of the PAEs.

analysis. In this work, acetonitrile, methanol and acetone were tried as the desorption solvent for the desorption of the analytes from the magnetic adsorbents. The results showed that the desorption power of acetone was much stronger than either methanol or acetonitrile. Thus, acetone was selected as the desorption solvent. The adsorbed analytes were desorbed from the isolated Fe_3O_4@SiO_2-G particles with 0.2 mL acetone each time by vortexing for 30 s. As shown in Fig. 6, after desorption for three times (each time with 0.2 mL acetone), the desorption recoveries for all the analytes were higher than 82.0%. Giving a compromise between the desorption recovery and the concentration of the analytes in the desorption solution, 0.6 mL acetone (0.2 mL × 3) was selected for the desorption.

3.3. Adsorption isotherms

The adsorption behavior of Fe_3O_4@SiO_2-G was investigated with BBP as a model analyte. Adsorption isotherms were studied at the concentration of 0.15 mg mL^{-1} Fe_3O_4@SiO_2-G with the concentrations of the BBP being varied in the range from 7.5 to 200 mg L^{-1}. The Fe_3O_4@SiO_2-G was removed from the solution by magnetic separation after shaking for 30 min, and then the concentration of the BBP in the resultant solution was analyzed.

In order to quantitatively describe the adsorption capacities of the BBP by the G-Fe_3O_4 nanocomposite, the commonly used adsorption isotherms, Langmuir and Freundlich ones, were applied. The Langmuir adsorption equations is expressed as Eq. (1):

$$\frac{C_e}{q_e} = \frac{C_e}{q_{max}} + \frac{1}{q_{max} b} \tag{1}$$

and the Freundlich model equation is expressed as Eq. (2):

$$\ln q_e = \frac{1}{n} \ln C_e + \ln K_F \tag{2}$$

where q_{max} is the maximum adsorption at monolayer coverage (mg g^{-1}), q_e is the BBP concentration on the G-Fe_3O_4 nanocomposite at equilibrium (mg g^{-1}), C_e denotes the concentration of the BBP in solution at equilibrium (mg L^{-1}), b is the Langmuir adsorption equilibrium constant (L mg^{-1}), reflecting the energy of the adsorption, K_F and $1/n$ are the Freundlich characteristic constants, indicating the adsorption capacity and the adsorption intensity, respectively. The values of b and q_{max} can be obtained from the intercept and slope of the linear plot of C_e/q_e versus C_e and the values of K_F and $1/n$ can be obtained from the intercept and slope of the linear plot of ln q_e versus ln C_e, respectively.

The results indicated that the linear correlation coefficient for the Langmuir model (0.983) was higher than that for the Freundlich one (0.912). This indicates that the Langmuir model fitted the adsorption data better than the Freundlich one. The q_{max} and b values for the adsorption of BBP by the magnetic nanocomposites calculated from the Langmuir isotherm model were 413.0 mg g^{-1} and 0.056 L g^{-1}.

3.4. Reusability of the sorbent

The reusability of the Fe_3O_4@SiO_2-G sorbent was investigated in this study. After each use of the magnetic adsorbent, it was washed with acetone twice (each time with 3 mL). In such way, no carry-over of the analytes was detected on the adsorbent. Then, the adsorbent was reused for the next MSPE for the PAEs. The results showed that the Fe_3O_4@SiO_2-G sorbent can be reused at least 30 times without a significant loss of the sorption capacity. The results also indicated that the chemical bonding of G onto the surface of the magnetic microsphere could guarantee the stability of the Fe_3O_4@SiO_2-G sorbent.

3.5. Analysis of environmental water and soybean milk samples

3.5.1. Selection of extraction solvent for the soybean milk samples

For water samples, there is no need for any pre-treatment before their MSPE procedures as described in Section 2.5. However, for soybean milk samples, after sample centrifugation, some proportion of the PAEs went into the supernatant, but most of the PAEs were adsorbed on the soybean proteins and therefore would remain in the sediment. Therefore, further solvent extractions of the analytes from the sediment were necessary to obtain a good extraction recovery. In this work, acetonitrile, methanol and acetone were tried as the extraction solvent. As a result, acetonitrile was the best and selected as the extraction solvent. In the experiment, the supernatant from the acetonitrile extraction of the sediment was combined with the first supernatant from soybean samples for the subsequent MSPE. In such case, an increased volume of acetonitrile could be favorable for the extraction of the analytes from the sediment, but on the other hand, the resultant increased proportion of acetonitrile in the final overall supernatant could be unfavorable for the MSPE of the analytes by the $Fe_3O_4@SiO_2$-G sorbent. Therefore, the influence of the volume of the acetonitrile on the extraction efficiency of the analytes was further investigated. Fig. 7 shows that the highest extraction efficiency for DAP and DPP was achieved when the volume of the acetonitrile was 8 mL (4 mL × 2) while BBP, DCP and DEHP reached their maximum extraction efficiency when the volume of the acetonitrile was 12 mL (4 mL × 3). Giving an overall consideration of the extraction efficiency for all the analytes, 12 mL (4 mL × 3) of acetonitrile was selected for the experiments.

3.5.2. Linearity and limits of detection (LODs) of the method

A series of double-distilled water and the PAEs-free soybean milk samples spiked with each of the PAEs at seven concentration levels of 0.5, 1.0, 5.0, 20.0, 50.0, 100.0 and 200.0 ng mL^{-1} were prepared respectively for the establishment of the calibration curve. For each level, five replicate extractions and determinations were performed under the optimized experimental conditions. The characteristic calibration data obtained are listed in Table 1. Good linearity was observed over the concentration range of 0.50–200.0 ng mL^{-1} in water sample and 1.0–200 ng mL^{-1} in soybean milk sample for the PAEs with the correlation coefficients (r) ranging from 0.9817 to 0.9987. The LODs ($S/N=3$) of the method were between 0.07 and 0.10 ng mL^{-1} for water sample and 0.15 and 0.30 ng mL^{-1} for soybean milk sample, respectively. The enrichment factors (*EF*), defined as the ratio between the analyte concentration in 0.6 mL acetone and the initial analyte concentration in the 100 mL samples, were between 136 and 150 for water sample, and between 58 and 92 for soybean milk sample.

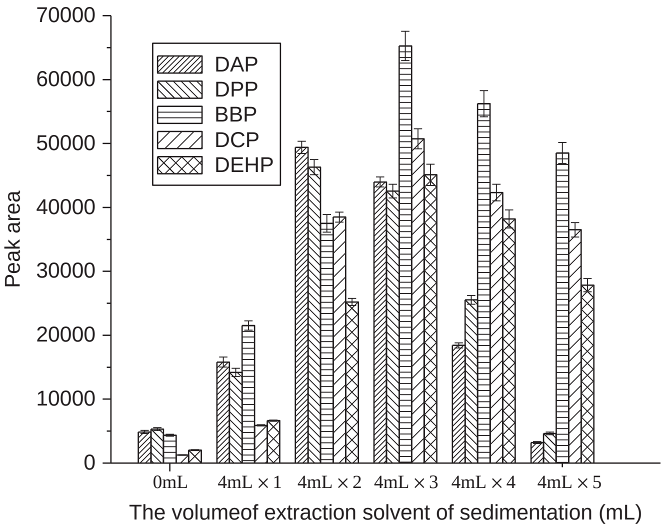

Fig. 7. Effect of the volume of acetonitrile on the extraction of the soybean milk sediment.

3.5.3. Sample analysis and recoveries of the method

In order to test the applicability of the developed method, the method was applied to analyze the PAEs in 100 mL of soybean milk samples and 100 mL (100 g) of different environmental water samples, including reservoir and river water samples. The results are shown in Table 2. As a result, no residues of the PAEs were detected in either river water sample or soybean milk B; 0.34 ng mL^{-1} of DAP and 0.40 ng mL^{-1} of BBP were found in Angezhuang reservoir water sample; 0.52 ng mL^{-1} of DPP, 0.67 ng mL^{-1} of BBP and 1.1 ng mL^{-1} of DEHP were found in soybean milk sample A. To determine the recoveries of the method, the water and soybean milk samples were spiked with the PAEs standards at the concentration levels of 5.0 and 50.0 ng mL^{-1}, respectively. For each concentration level, five replicate experiments were performed. Consequently, the recoveries for the five PAEs in water samples fell in the range from 87.2% to 109.0% and the repeatabilities expressed as the relative standard deviations (RSDs) varied from 2.7% to 6.1%. For soybean milk samples, the recoveries were ranged from 87.2% to 103.4% with the RSDs varying from 3.4% to 6.2%. Fig. 8 shows the typical chromatograms of the PAEs for the reservoir water and soybean milk sample A.

The above results show that the method had a good accuracy and repeatability, and is sensitive enough for the analysis of the phthalate esters at the level of the permissible limits for environmental water and soybean milk samples. Compared with our previous report for the preconcentration of some phthalate esters (PAEs) with graphene-coated Fe_3O_4 magnetic nanoparticles prepared by the chemical precipitation method [26], it seems that a lower sensitivity was achieved for the current method with $Fe_3O_4@SiO_2$-G. However, for the method in reference [26], 300 mL of water sample was used and the desorption solutions were evaporated to dryness and the residue was redissolved in 100.0 μL solvent for HPLC analysis. For the present method, only 100 mL of sample was required and the desorption solution was directly used for HPLC analysis without the need of desorption solution evaporation and reconstitution procedures. The $Fe_3O_4@SiO_2$-G is more stable than the graphene-coated Fe_3O_4 and can be reused many times.

4. Conclusions

In this research, a magnetic microsphere-confined graphene was fabricated by the chemical bonding of G onto the surface of magnetic microspheres and used as an effective adsorbent for the preconcentration of some PAEs in environmental water and soybean milk samples for the first time. The results indicated that the G bonded on the surface of the magnetic microspheres endows the material with a high adsorption capacity. Because the silica shell could provide a good protection for Fe_3O_4 core, the

Table 1
Analytical performance data for the PAEs by the MSPE technique.

PAEs	Water sample					Soybean milk sample				
	LR[a] (ng mL^{-1})	r	RSD (%)	LOD (ng mL^{-1})	*EF*[b]	*LR* (ng mL^{-1})	r	RSD (%)	LOD (ng mL^{-1})	*EF*[b]
DAP	0.5–200.0	0.9911	3.3	0.10	136	1.0–200.0	0.9858	6.2	0.20	58
DPP	0.5–200.0	0.9930	4.8	0.07	140	1.0–200.0	0.9913	3.8	0.15	63
BBP	0.5–200.0	0.9927	3.6	0.09	141	1.0–200.0	0.9817	4.3	0.30	75
DCP	0.5–200.0	0.9945	2.8	0.10	147	1.0–200.0	0.9905	5.1	0.20	87
DEHP	0.5–200.0	0.9987	2.1	0.07	150	1.0–200.0	0.9960	4.5	0.20	92

[a] *LR*: linear range.
[b] *EF*: enrichment factors.

Table 2
Recoveries obtained in the determination of PAEs in spiked water and soybean milk samples.

PAEs	Spiked (ng mL^{-1})	River water ($n=5$)			Reservoir water ($n=5$)			Soybean milk A ($n=5$)			Soybean milk B ($n=5$)		
		Found (ng mL^{-1})	*R*[b] (%)	RSD (%)	Found (ng mL^{-1})	*R*[b] (%)	RSD (%)	Found (ng mL^{-1})	*R*[b] (%)	RSD (%)	Found (ng mL^{-1})	*R*[b] (%)	RSD (%)
DAP	0	nd[a]			0.34			nd[a]			nd[a]		
	5.0	4.53	90.6	5.1	5.42	101.6	4.8	4.75	95.0	5.9	4.50	90.0	5.5
	50.0	49.1	98.2	2.9	48.8	96.9	2.7	47.8	95.6	4.3	51.5	102.0	3.7
DPP	0	nd[a]			nd[a]			0.52			nd[a]		
	5.0	5.45	109.0	4.2	4.71	94.2	4.2	4.88	87.2	3.6	4.38	87.6	3.1
	50.0	48.3	96.6	3.4	47.3	94.6	3.6	49.3	97.6	4.4	49.3	98.6	4.5
BBP	0	nd[a]			0.20			0.67			nd[a]		
	5.0	4.36	87.2	4.1	4.81	92.2	3.4	5.72	101.0	4.1	4.72	94.4	4.9
	50.0	50.4	100.8	3.3	53.6	106.8	4.7	46.1	90.9	6.2	46.2	92.4	4.2
DCP	0	nd[a]			nd[a]			nd[a]			nd[a]		
	5.0	4.61	92.2	5.3	4.87	97.4	5.6	5.17	103.4	5.6	5.17	103.4	4.1
	50.0	53. 8	107.6	2.9	50.1	100.2	4.0	50.9	101.8	5.5	50.9	101.8	3.7
DEHP	0	nd[a]			nd[a]			1.10			nd[a]		
	5.0	5.21	104.2	4.7	4.56	91.2	6.1	5.82	94.4	4.5	5.02	100.4	5.8
	50.0	48.5	97.0	3.0	44.3	88.6	3.3	47.5	92.8	5.2	45.5	91.0	3.4

[a] nd: not detected.

[b] *R*: recovery of the method.

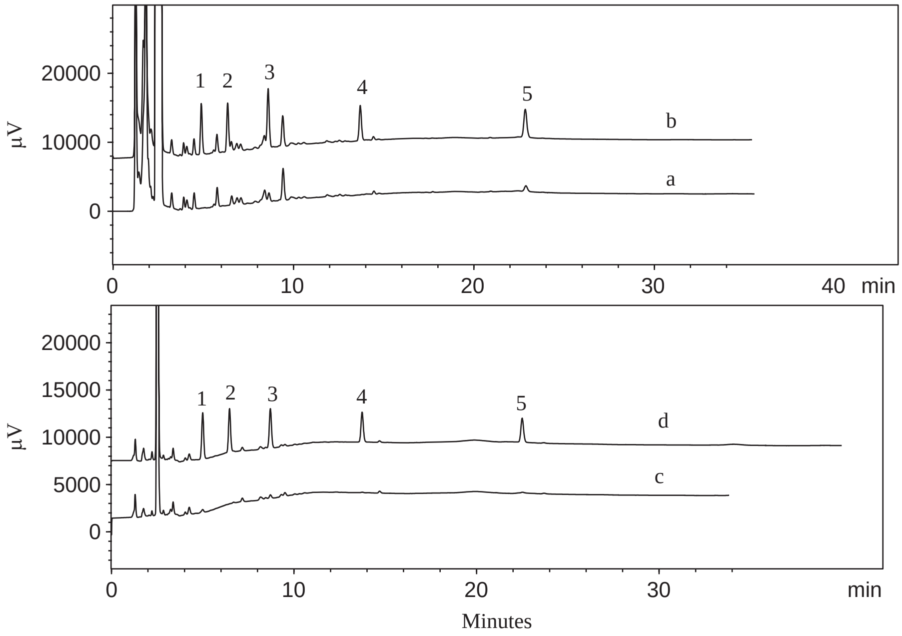

Fig. 8. The typical chromatograms of soybean milk sample (a) and the soybean milk sample spiked with PAEs at each concentration of 15.0 ng mL^{-1} (b), and water sample (c) and the water sample spiked with PAEs at each concentration of 5.0 ng mL^{-1} (d). Peak identification: (1) DAP; (2) DPP; (3) BBP; (4) DCP; and (5) DEHP.

Fe_3O_4@SiO_2-G sorbent was stable when it was used as the adsorbent. It suggests that Fe_3O_4@SiO_2-G could be a promising adsorbent for MSPE with great application potentials.

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