Magnetic three-dimensional graphene solid-phase extraction coupled with high performance liquid chromatography for the determination of phthalate esters in fruit juice

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In this paper, a three-dimensional graphene-based magnetic nanocomposite (3D-G-Fe$_3$O$_4$) was synthesized and used as an effective adsorbent for the extraction of four phthalate esters (dimethyl(o-) phthalate, diethyl(o-) phthalate, dialky(o-) phthalate and bis(2-propylheptyl) phthalate) from fruit juice prior to high performance liquid chromatography analysis. The three-dimensional nanoporous structure of the 3D-G endows the new material with a high adsorption capacity. The properties and morphology of the 3D-G-Fe$_3$O$_4$ were characterized by transmission electron microscopy, scanning electron microscopy and infrared spectroscopy. Several experimental parameters affecting the extraction efficiencies, such as the amount of the 3D-G-Fe$_3$O$_4$, extraction time, sample pH, salt addition and desorption conditions were optimized. Under the optimum conditions, the limits of detection (S/N = 3) of the method for the analytes were between 0.04 and 0.13 ng mL$^{-1}$. The recoveries of the method for the analytes were in the range from 87.0% to 97.8%. The result showed that the 3D-G-Fe$_3$O$_4$ has an excellent adsorption capacity for the analytes.

Introduction

Graphene (G), a one-atom-thick planar carbon material patterned in a honeycomb lattice form, is a two-dimensional (2D) carbon nanomaterial and has attracted enormous research interest in recent years owing to its intriguing properties, such as high thermal and electronic conductivity, excellent mechanical strength and high specific surface area.$^{1,4}$ Because the large delocalized π-electron system of G can form a strong hydrophobic and π-stacking interaction with some organic molecules,$^3$ it has been widely used as an adsorbent to concentrate many kinds of compounds in various sample pretreatment techniques, such as solid-phase microextraction (SPME),$^{4,5}$ solid-phase extraction (SPE)$^{6-7}$ and magnetic solid-phase extraction (MSPE)$^{8-9}$.

However, due to the strong π–π stacking interaction, hydrophobic interaction and van der Waals forces, restacking and aggregation between individual graphene sheets may occur, which may greatly decrease the intrinsic specific surface area of G.$^{10,11}$ In order to overcome the above problems, very recently, Chen et al. have synthesized a three-dimensional interconnected graphene (3D-G) with a foam-like network structure by a chemical vapour deposition method.$^{12,13}$ These structures provide graphene materials with high specific surface areas, strong mechanical strengths, fast mass and electron transport kinetics due to the combination of the 3D porous structures and the excellent intrinsic properties of graphene.$^{14,15}$ 3D-G has been used in the fields of energy storage, biochemical sensing device and stretchable conductors.$^{16-19}$ Since 3D-G has a high surface area, large mesopore volume and three-dimensional nanoporous structure, it might be a promising candidate as an efficient adsorbent$^{20}$ for some organic compounds. To the best of our knowledge, the application of 3D-G as the adsorbent for the extraction or removal of organic pollutants is still very few in the literature.

Phthalate esters (PAEs) are widely used in industrial plastics, polyvinyl chloride products, cosmetics and personal care products.$^{21}$ Because PAEs are only physically bound to the polymer chains, they can easily leach, migrate or evaporate into various environments during the production, use and incineration of the polymeric materials containing these compounds.$^{22}$ Certain PAEs as well as their metabolites and degradation products can cause adverse effects on human health, especially on the liver, kidney and testicles.$^{23}$ It has been reported that PAEs are suspected to have carcinogenic and estrogenic properties.$^{24}$ So, for the sake of human health and environment protection, a sensitive analytical method is necessary for the determination of trace level of PAEs in different matrix samples.

Magnetic solid-phase extraction, as a new mode of SPE, can be carried out directly in the crude sample solution with the
magnetic solid absorbent being added to it and the phase separation can be realized simply by using an external magnet without the need of using additional filtration or centrifugation procedures, which makes separation easier and faster.25–28

In this report, a three-dimensional graphene-based magnetic nanocomposite (3D-G-Fe3O4) was synthesized to explore its application potential in MSPE. To evaluate the performance of the 3D-G-Fe3O4, PAEs were selected as the model analytes. After their preconcentration with the 3D-G-Fe3O4, the analytes in fruit juice were determined by high performance liquid chromatography-diode array detection (HPLC-DAD).

Experimental
Reagents and materials
Graphite powder (50 meshes) and FeSO4·7H2O were purchased from the Boaixin Chemical Reagents Company (Baoding, China). Acetonitrile, methanol, acetone, hydrochloric acid (HCl), sodium hydroxide (NaOH), sodium chloride, ammonium hydroxide, and all other reagents were purchased from Beijing Chemical Reagents Company (Beijing, China). Dimethyl(o)-phthalate (DMP), diethyl(o)-phthalate (DEP), diallylo(o)-phthalate (DAP) and bis(2-propylheptyl)phthalate (DPP) were purchased from Aladdin-Reagent (Shanghai, China). The water late (DAP) and bis(2-propylheptyl)phthalate (DPP) were synthesized by the method reported in our previous study.19,20 The infrared spectra (IR) were obtained using a Shimadzu 8400S infrared spectrophotometer (Japan). The electronic images were captured using a field emission scanning electron microscope (FESEM) (S-3000N, Hitachi, Japan). The transmission electron microscopy (TEM) images were collected on a TEM microscope (H-800, Hitachi, Japan). The surface area and pore size of the magnetic materials were determined through Brunauer–Emmett–Teller (BET) surface area measurement (TristarII3020, USA). The magnetic property was analyzed using a JDM-13 vibrating sample magnetometer (VSM, Jilin University, Changchun, China) at room temperature.

Synthesis of 3D-G-Fe3O4 and 2D-G-Fe3O4
The preparation process of the 3D-G-Fe3O4 is illustrated in Scheme 1. Graphene oxide was prepared from natural graphite powders by a modified Hummers’ method.29 Then, to the 50 mL aqueous suspension of GO at the concentration of 2 mg mL$^{-1}$, 0.695 g FeSO4·7H2O were added, which was sonicated for 5 min. After that, the pH of the resulting mixture was adjusted to 11 with 25% NH4·H2O, and the resultant slurry was then heated at 90 °C for 6 hours in an oil bath without stirring. Then, the formed 3D-G-Fe3O4 was separated by filtration, washed with water and then freeze-dried under vacuum to remove the absorbed water. The reduced graphene oxide sheets anchored with nanoparticles were simultaneously self-assembled into the 3D hydrogel with interconnected networks driven by combined hydrophobic and π–π stacking interactions, due to the decrease of oxygenated groups on the graphene sheets.30,31

The two-dimensional graphene nanocomposite (2D-G-Fe3O4) was synthesized according to the method reported in our previous work.22 Briefly, GO nanosheets were reduced to 2D-graphene by hydrazine solution. The magnetic (2D-G-Fe3O4) was synthesized by the in situ chemical coprecipitation of Fe$^{2+}$ and Fe$^{3+}$ in alkaline solution in the presence of 2D-G.

MSPE procedures
Firstly, 15.0 mg of 3D-G-Fe3O4 was added to a 150 mL aqueous sample solution in a conical flask. Then, the mixture was shaken on a slow-moving platform shaker for 25 min. Then, the 3D-G-Fe3O4 was separated from the aqueous phase using a magnet and the resultant supernatant was discarded. After that, the residual solution and 3D-G-Fe3O4 were totally transferred to a centrifuge tube. The 3D-G-Fe3O4 was aggregated to the bottom of the tube again by positioning a magnet to the outside of the tube wall so that the residual solution could be removed completely using a pipette. Finally, the isolated 3D-G-Fe3O4 nanoparticles were vortexed in 0.2 mL acetone for 1 min for desorption. The desorption was performed in a dry glass centrifuge tube, which has a cap to prevent the evaporation of the desorption solvent. Finally 20 μL desorption solution was injected into the HPLC system for analysis.

Prior to next use, the used 3D-G-Fe3O4 nanoparticles were washed three times with 1 mL acetonitrile and then with 1 mL water by vortexing for 1 min, respectively.

Scheme 1 Schematic illustration of the synthetic processes of the 3D-G-Fe3O4.
In order to evaluate the extraction performance of the method, the extraction recovery ($R$) was calculated according to the following equation:

$$R\% = \frac{V_{\text{rec}} C_{\text{ai}}}{C_0 V_{\text{aq}}}$$

$R\%$, $V_{\text{rec}}$ and $V_{\text{aq}}$ are the extraction recovery, and the volume of the final reconstituted solution and aqueous sample, respectively. $C_{\text{rec}}$ and $C_0$ are the concentration of the analytes in the final reconstituted solution in the extraction and the initial concentration of the analytes in the aqueous sample, respectively.

**Results and discussion**

**Characterization of 3D-G-Fe$_3$O$_4$ nanoparticles**

In the formation of 3D-G-Fe$_3$O$_4$, the Fe$^{2+}$ in FeSO$_4$·7H$_2$O was oxidized to Fe$^{3+}$ by the oxygen containing functional groups on the GO nanosheets and simultaneously, the graphene oxide was reduced to graphene by Fe$^{2+}$. Then the formed Fe$^{3+}$ ions and Fe$^{2+}$ ions coprecipitated into Fe$_3$O$_4$ nanoparticles, which were decorated onto the thin graphene sheets. Simultaneously, the reduced graphene oxide decorated with Fe$_3$O$_4$ was self-assembled into the 3D structure with interconnected networks. The reason for that could be ascribed to the partial overlapping or coalescence of the flexible reduced GO nanosheets via non-covalent interactions, such as π–π stacking, hydrogen bonding, and hydrophobic interactions.

The SEM image of 3D-G-Fe$_3$O$_4$ is shown in Fig. 1(a), from which it can be seen that the so prepared 3D-G-Fe$_3$O$_4$ had a well-defined and interconnected 3D network microstructure. The iron oxide nanoparticles were well distributed on the surface of graphene sheets. The TEM image in Fig. 1(b) illustrates one of the separated graphene sheets from the 3D network and it shows that the Fe$_3$O$_4$ nanoparticles were uniformly distributed on the graphene and no particles that were disassociated from the graphene sheets were observed.

The FTIR spectrum suggests the presence of the functional groups on GO (Fig. 1(c) (GO)). The FTIR bands at 1412, 1060 and 1750 cm$^{-1}$ are due to the vibrations of O–H, C–O and C=O, respectively. In the IR spectrum for 3D-G-Fe$_3$O$_4$, the reduction of GO to G was verified by the vanished band at 1060 and 1750 cm$^{-1}$, which indicated that the characteristic epoxy and carboxyl vibrational bands have disappeared. Moreover, the peak at 600 cm$^{-1}$ can be ascribed to the lattice absorption of iron oxide.

Fig. 1(d) shows the N$_2$ adsorption–desorption isotherms of 3D-G-Fe$_3$O$_4$. The Brunauer–Emmett–Teller (BET) specific surface area of 3D-G-Fe$_3$O$_4$ is about 308.1 m$^2$ g$^{-1}$, which is superior to the 2D-G-Fe$_3$O$_4$ (~225.0 m$^2$ g$^{-1}$ (ref. 28)).

It is important that the adsorbents possess superparamagnetic properties to realize rapid separation under a magnetic field. Fig. 2 shows the VSM magnetization curves of the 3D-G-Fe$_3$O$_4$ nanoparticles at 298 K. It exhibited typical superparamagnetic behavior. The saturation magnetization intensity of 3D-G-Fe$_3$O$_4$ was 39.5 emu g$^{-1}$, which is sufficient for its magnetic separation from a solution with a strong magnet.

**Optimization of extraction conditions**

In order to obtain the optimum conditions, 150 mL double-distilled water spiked with 40.0 ng mL$^{-1}$ each of the four PAEs was used to study the extraction performance of the 3D-G-Fe$_3$O$_4$...
under different experimental conditions. The experimental parameters investigated included the amount of 3D-G-Fe₃O₄, extraction time, sample pH, ionic strength, and desorption conditions. All the experiments were performed in triplicate and the means of the results were used for optimization.

Effect of the amount of 3D-G-Fe₃O₄ sorbents

To investigate the performance of 3D-G-Fe₃O₄, its adsorption ability for the PAEs was compared with that of both 2D-G-Fe₃O₄ and Fe₃O₄. The results shown in Fig. 3 indicated that the extraction efficiency of 3D-G-Fe₃O₄ was higher than that obtained by either 2D-G-Fe₃O₄ or Fe₃O₄ under the same conditions. This could be attributed to the high specific surface area and the three-dimensional nanoporous structure of 3D-G-Fe₃O₄. Based on the above results, the 3D-G-Fe₃O₄ was selected as the adsorbent for the extraction of the three PAEs.

In order to select the optimum dosage of the 3D-G-Fe₃O₄ for the extraction of the PAEs, different amounts of the 3D-G-Fe₃O₄ were investigated in the range from 2 to 20 mg. The results (Fig. 4) show that the adsorption for all the four PAEs could reach the maximum plateau when the dosage of 3D-G-Fe₃O₄ was increased to 10 mg and then remained almost constant. In order to ensure that the adsorbent was sufficient for the extraction, 15 mg 3D-G-Fe₃O₄ was selected.

Effect of extraction time

Extraction time is another important parameter that influences the extraction of the analytes. To investigate the influence of the extraction time on the extraction efficiency, the extraction time was varied in the range from 10 to 40 min. As shown in Fig. 5, the peak areas for all the PAEs reached their maxima when the extraction time was increased to 20 min. In order to ensure that all the PAEs can achieve their extraction equilibrium, an extraction time of 25 min was chosen.

Effect of sample pH

The pH of the sample solution is a key factor affecting the extraction efficiency, since it not only affects the existence forms of the analytes but also changes the charge type and density on the surface of the adsorbent. In this study, the effect of pH of the sample solution was investigated in the range from 2.0 to 12.0. The experimental results (Fig. 6) showed that the adsorption efficiency was almost not changed when the pH of the sample solution was changed from 2 to 6 and then decreased rapidly when the pH was further increased. The reason for this is that when the pH of the sample solution exceeds 7, the phthalates could hydrolyze to phthalate anions, which were soluble in aqueous solution. Considering that the pH of the
The pH of the sample solution was not adjusted.

**Effect of sample salinity**

In this study, the effect of salt addition was investigated by changing the concentrations of NaCl from 0% to 3.0%, 5.0%, 10.0%, 15.0%, and 20.0% (w/v), respectively. The results showed that the addition of NaCl had a negligible effect on the extraction efficiency for the analytes in the concentration range investigated. Therefore, no NaCl was added to the samples in all the subsequent experiments.

**Desorption conditions**

In this work, acetonitrile, methanol and acetone were tested as the desorption solvent. As shown in Fig. 7, acetone provided the best desorption efficiency among the three solvents. Therefore, acetone was chosen as the desorption solvent. The effect of the volume of acetone on the desorption efficiency was also investigated. The results showed that the analytes could be desorbed quantitatively when the volume of acetone was 200 μL. Therefore, 200 μL acetone was selected for desorption.

**Reusability of the sorbent**

The reusability of the adsorbent was investigated in this study. After desorption of the analytes from the adsorbent, the used 3D-G-Fe₃O₄ was washed three times each with 1 mL acetone and then with 1 mL water by vortexing for 1 min. After such washings, no carry-over of the analytes from the adsorbent was detected. The results indicated that the 3D-G-Fe₃O₄ could be reused at least 20 times without significant loss of its adsorption capacity.

**Linearity and limits of detection (LODs) of the method**

A series of PAE-free fruit juice samples containing each of the PAEs at seven concentrations (0.2, 0.5, 1.0, 5.0, 20.0, 50.0, and 100.0 ng mL⁻¹) were prepared for the establishment of the calibration curve. For each concentration level, four replicate extractions and determinations were performed under the optimized experimental conditions. Good linearity was observed over the concentration range of 0.10–100.0 ng mL⁻¹ for the four PAEs, with the correlation coefficients (r) ranging from 0.9905 to 0.9997. The LODs (S/N = 3) of the method were in the range from 0.042 to 0.060 ng mL⁻¹ for fruit juice samples. The results are listed in Table 1.

**Sample analysis and recoveries of the method**

In order to assess the precision and accuracy of the method, the method was applied to determine the PAEs in different fruit juice samples. The results are shown in Table 2. As a result, 0.34 ng mL⁻¹ of DMP was found in fruit juice sample 1 and no

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

<table>
<thead>
<tr>
<th>PAEs</th>
<th>Linear range (ng mL⁻¹)</th>
<th>r</th>
<th>RSD (%)</th>
<th>LOD (ng mL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMP</td>
<td>0.1–100.0</td>
<td>0.9996</td>
<td>3.3</td>
<td>0.042</td>
</tr>
<tr>
<td>DEP</td>
<td>0.1–100.0</td>
<td>0.9930</td>
<td>4.8</td>
<td>0.050</td>
</tr>
<tr>
<td>DAP</td>
<td>0.1–100.0</td>
<td>0.9997</td>
<td>3.3</td>
<td>0.042</td>
</tr>
<tr>
<td>DPP</td>
<td>0.1–100.0</td>
<td>0.9985</td>
<td>3.8</td>
<td>0.060</td>
</tr>
</tbody>
</table>

**Table 2** Determination of phthalate esters and recoveries in fruit juice samples

<table>
<thead>
<tr>
<th>PAEs</th>
<th>Spiked (ng mL⁻¹)</th>
<th>Fruit juice sample 1</th>
<th>Fruit juice sample 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Found (ng mL⁻¹)</td>
<td>R (%)</td>
<td>R (%)</td>
</tr>
<tr>
<td>DMP</td>
<td>0.0</td>
<td>0.58</td>
<td>nd</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>5.37</td>
<td>95.8</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>10.56</td>
<td>99.8</td>
</tr>
<tr>
<td>DEP</td>
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<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>4.88</td>
<td>97.6</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>9.60</td>
<td>96.0</td>
</tr>
<tr>
<td>DAP</td>
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<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>4.35</td>
<td>87.0</td>
</tr>
<tr>
<td></td>
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<tr>
<td>DPP</td>
<td>0.0</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>4.87</td>
<td>97.4</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>9.78</td>
<td>97.8</td>
</tr>
</tbody>
</table>

a nd: not detected. b R: recovery of the method.
Conclusions

In this report, a three-dimensional graphene magnetic nanomaterial was prepared and used as an effective adsorbent for the first time. After adsorption of the PAEs from fruit juice by 3D-G-Fe₃O₄, the PAEs were desorbed and then determined by HPLC-DAD. Because of the 3D porous structures, high specific surface area and strong magnetism of the 3D-G-Fe₃O₄, it shows an excellent adsorption capacity for the analytes. The developed method is efficient and easy to be used, without the need for additional centrifugation or filtration procedures. The results suggest that the 3D-G-Fe₃O₄ should have more applications for the effective extractions of other compounds.

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