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ORIGINAL PAPER

Extraction of nucleobases, nucleosides and nucleotides by employing a magnetized graphene oxide functionalized with hydrophilic phytic acid and titanium(IV) ions

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Abstract

A magnetite@graphene oxide nanocomposite was first coated with polyethylenimine and then modified with phytic acid and titanium(IV) ions. The high loading with Ti(IV) and the good hydrophilicity of PEI and PA result in a material that can be applied to the efficient extraction of highly polar nucleobases, nucleosides and nucleotides. The physicochemical properties of the composite were investigated by scanning electron microscopy, transmission electron microscopy, energy dispersive X-ray spectroscopy, Fourier transform infrared spectroscopy, water contact angle measurements, thermogravimetric analysis, and vibrating sample magnetometry. A series of parameters that affect extraction and elution under the conditions of immobilized metal affinity chromatography (IMAC) and hydrophilic interaction liquid chromatography (HILIC) were examined. The analytes were eluted from the nanocomposites using 10 mM trisodium phosphate as the elution solution in the IMAC mode, and 50% methanol-water as elution solution in the HILIC mode. Figures of merit include (a) an intra-day precision of 0.1–1.0% in the IMAC mode; (b) an intra-day precision of 0.4%–0.8% in the HILIC mode; (c) detection limits between 1.8–2.8 ng mL^{-1} in the IMAC mode; and (d) detection limits of 4.0–10.5 ng mL^{-1} in the HILIC mode. The method was applied to the extraction of the nucleotides cytidine-5′-monophosphate (CMP), uridine-5′-monophosphate (UMP), guanosine-5′-monophosphate (GMP), and adenosine-5′-monophosphate (AMP), and the nucleobases and nucleosides hypoxanthine, adenosine, cytosine, inosine and cytidine from *Cordyceps sinensis*, *Lentinus edodes* and plasma samples.

Keywords Immobilized metal ion chromatography · Hydrophilic interaction liquid chromatography · Magnetic solid phase extraction · Nucleobase-based compounds

Introduction

Nucleobases-based compounds (viz. nucleobases, nucleosides and nucleotides) are ubiquitous in cells and play essential roles in various biological functions [1]. Apart from in field of biology [2–4], nucleobases-based compounds such as monophosphate nucleotides are nutrients of special importance during periods of rapid growth or after injury, and are indispensable supplementary nutrients in neonatal feeding [5]. Nucleobases, nucleosides and nucleotides also exist in some medicinal herbs [i.e. *Ganoderma lucidum*, *Cordyceps sinensis* (*C. sinensis*)] as important bioactive compounds related to multiple pharmacological activities [6–8]. To date, several analytical methods, including reverse phase-high performance liquid chromatography (RP-HPLC) [9], ultra-high performance liquid chromatography [10], ion-pairing RP-HPLC [11], hydrophilic interaction liquid chromatography (HILIC) [12], capillary electrophoresis or capillary electrochromatography [13, 14], have been developed for the quantification of nucleobases-based compounds and their analogs in natural plants, foods and biological fluids. However, sometimes these analytical methods still show limitations to analyze all nucleobases-based components directly when they are

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in low abundance. Therefore, effective and selective pretreatment approach for these compounds from complex samples before chromatographic analysis is necessary.

The number of established extraction methods of nucleobase compounds are very limited. The most conventional strategy is solvent-based extraction, and aqueous solution was always employed since the highly polar characteristic of the compounds [9–11, 15]. Solid phase extraction (SPE) methods, mainly commercial materials, such as OASIS WAX and Oasis MCX cation exchange cartridges [16, 17], are also applied occasionally for the efficient extraction of polar nucleobases-based compounds. However as the nature of needing several steps for column activation and equilibrium procedure, the whole extraction process of commercial sorbents is a little time-consuming. On the other hand, considering the *cis-diol* structure in the cyclic ribose on nucleosides, new SPE materials based on the boronate affinity are considered as a selective extraction technique for them [18–20]. Similarly, the metal-based affinity strategy, mainly including immobilized metal ion affinity chromatography (IMAC) and metal oxide affinity chromatography, are employed for enrichment of *cis-diol* nucleosides [21–24]. While except for the affinity with *cis-diol* structure, the metal-based affinity materials present the metal electrostatic interaction and/or chelation effect on phosphate-containing compounds. However this characteristic was mainly employed for phosphoproteins and phosphopeptides analysis [25–28], and never applied for nucleotides. In reality, boronate modified sorbents and metal-based affinity materials have some drawbacks. Firstly, because the external shell of the boronate modified sorbents is an organic species, the secondary hydrophobic interactions and aromatic interactions between sorbents and interferes may suppress selectivity for *cis-diol* compounds. Secondly, chemical cleavage of the bonded boric acid groups may be induced by intense conditions such as copper salts, silver salts, or hot water. The most troublesome problem is, both boronate modified sorbents and metal-based affinity materials cannot be used to extract compounds that had neither a *cis-diol* nor a phosphate structure (i.e. nucleobases and deoxynucleosides related compounds). Therefore, it will be highly attractive to prepare a kind of SPE material with adsorption capacity for nucleobases, nucleosides and nucleotides simultaneously.

HILIC is a promising solution for highly polar and hydrophilic compounds, displayed outstanding performance in the separation of polar molecules. Various hydrophilic-based materials for sample pretreatment have received much attention to academic researchers [29, 30]. It is mainly focused on the enrichment of glycan, glycopeptide and glycoprotein, and as far as we know have not yet been applied to enrichment of hydrophilic nucleobases-based compounds in complicated matrix.

In order to achieve simultaneous pretreatment of nucleobases, nucleosides and nucleotides in complex samples, the phytic acid-Ti^{4+} modified magnetite@ graphene oxide nanocomposites was synthesized with polyethylenimine (PEI) as a kind of post functionalization candidate (magGO@PEI@PA@Ti^{4+}). The material possesses metal coordinated and hydrophilic dual functions. It can extract nucleobases-based compounds with two distinct mechanisms, i.e. IMAC for nucleotides and HILIC for nucleobases and nucleosides. The uniqueness of the present approach is mainly in the utility of the characteristics of all involved monomers on the sorbent to maximize the adsorption capacity for target compounds. Specifically, 1) the feature of Fe_3O_4 ensures the easily and directly isolation of target analytes; 2) the high density of amino groups on the PEI makes it a modifiable and hydrophilic intermediate; 3) GO provides ultrahigh specific surface area for increasing compound binding sites; 4) as a natural compound composed of six phosphate groups, PA possesses excellent hydrophilic and metal ion coordination ability; and 5) immobilization of Ti^{4+} on the surface of the magGO@PEI@PA@Ti^{4+} composite makes the material bind with phosphate-containing compounds. The physicochemical properties of synthesized materials were thoroughly characterized and some important parameters involved in extraction/elution process were carefully optimized. Finally, the synthesized sorbent was successfully applied for the extraction of nucleobases, nucleosides and nucleotides in medicinal mushroom *C. sinensis*, natural foods *Lentinus edodes* (Berk.) sing (*L. edodes*) and biological plasma samples.

Experimental

Reagents and samples

Iron(III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), polyethyleneimine (PEI, 50%, relative molecular mass (Mr) = 70,000), sodium hydroxide (NaOH), sulfuric acid (H_2SO_4), hydrochloric acid (HCl), acetic acid (AC), 30% hydrogen peroxide, ammonium hydroxide ($NH_3 \cdot H_2O$), potassium permanganate ($KMnO_4$), ammonium acetate (NH_4AC), disodium hydrogen phosphate (Na_2HPO_4), and potassium dihydrogen phosphate (KH_2PO_4), sodium acetate, sodium citrate, ethanol, ethylene glycol, were all of analytical grade (>97%) and from Chron Chemicals (Chengdu Chron Chemicals Co., Ltd., China, http://www.chronchem.com/en/). Titanium sulfate [$Ti(SO_4)_2$] was purchased from DiBai Biotechnology Co., Ltd. (Shanghai, China, http://www.chemxyz.com/index.php). Graphite powder (2000 mesh) and phytic acid (PA) were from Aladdin Chemical Technology Co., Ltd. (Shanghai, China, https://www.aladdin-e.com). Water used for all the experiments was purified by a water purification system (ATSelem 1820A, Antesheng Environmental Protection Equipment Co., Ltd., Chongqing, China, http://www.atshb.com/). All the solvents used in the HPLC analysis such as

methanol (MeOH) and acetonitrile (ACN) were of HPLC-grade and purchased from Adamas-beta (Adamas Reagent Co., Ltd., Shanghai, China, http://www.adamas-beta.com/).

The reference compounds, namely, cytidine-5′-monophosphate (CMP), uridine-5′-monophosphate (UMP), adenosine-5′-monophosphate (AMP), guanosine-5′-monophosphate (GMP) (>98%, determined by HPLC) were purchased from Adamas-beta, hypoxanthine, adenosine, cytosine, inosine and cytidine were all purchased from Sigma (St Louis, MO, USA, www.sigmaaldrich.com), their chemical structures were shown in Fig. S1. Natural *C. sinensis* was from Langxian, Tibet province and was compared with the features described in the Chinese Pharmacopoeia (2015 edition). The dried *L. edodes* were purchased from the local supermarket in June 2018. Samples were deposited at the Pharmaceutical Engineering Laboratory in the School of Chemistry and Chemical Engineering, Chongqing University, Chongqing, China.

Apparatus and instruments

A field-emission scanning electron microscope (FESEM) (Quanta 650, FEI, Hillsboro, OR, https://www.fei.com/) and an energy dispersive X-ray spectroscopy (EDX) were used to study surface morphology at 20.0 kV and elemental content of the nanocomposite using Cu Kα radiation. Transmission electron microscope (TEM) image was taken using a JEM 2100 (JEOL Ltd. Tokyo, Japan, https://www.jeol.co.jp/en/) transmission electron microscope working at 200 kV. Fourier transform infrared (FTIR) spectra was collected between 4000 and 400 cm^{-1} on a Bruker Tensor 27 FTIR Spectrometer (Bruker, USA, https://www.bruker.com/) in KBr media. The water contact angle was recorded using an OCA40 optical contact angle measurement instrument (Dataphysics, Germany, https://www.dataphysics-instruments.com/). Thermal gravimetric analysis (TGA) was carried out on Mettler TGA/DSC1/1600LF (Mettler-Toledo AG, Analytical, Switzerland, https://www.mt.com/cn/zh/home.html) from 25 °C to 800 °C at a heating rate of 10 °C min^{-1} in N_2 gas flow. The magnetic property of the material was measured by a vibrating sample magnetometer (VSM) model AGFM/VSM 3886 (Kashan, Iran) at room temperature (about 25 °C) in a magnetic field strength of 2 T.

Preparation of phytic acid and Ti(IV) (PA-Ti^{4+}) modified magnetite@GO nanocomposites

The preparation process of PA-Ti^{4+} modified magnetite@GO composites included three main steps: 1) preparation of GO and Fe_3O_4 magnetic nanoparticles (MNPs) separately; 2) functionalization of Fe_3O_4 MNPs with PEI and deposited it on the surface of GO; 3) grafting of the magnetic graphene oxide polyethyleneimine composite (magGO@PEI) with PA and Ti^{4+}, the scheme is shown in Fig. 1. GO was prepared by Hummers method with some modifications, Fe_3O_4 MNPs were prepared by a solvothermal reaction method. The specific preparation procedure of GO, Fe_3O_4, and Fe_3O_4@PEI were described in Electronic Supplementary Material (ESM). PA and Ti^{4+} were immobilized on magGO@PEI through electrostatic interaction. magGO@PEI was prepared according to the previous reports with minor modification [31] and the details were described in ESM. Next, the above magGO@PEI

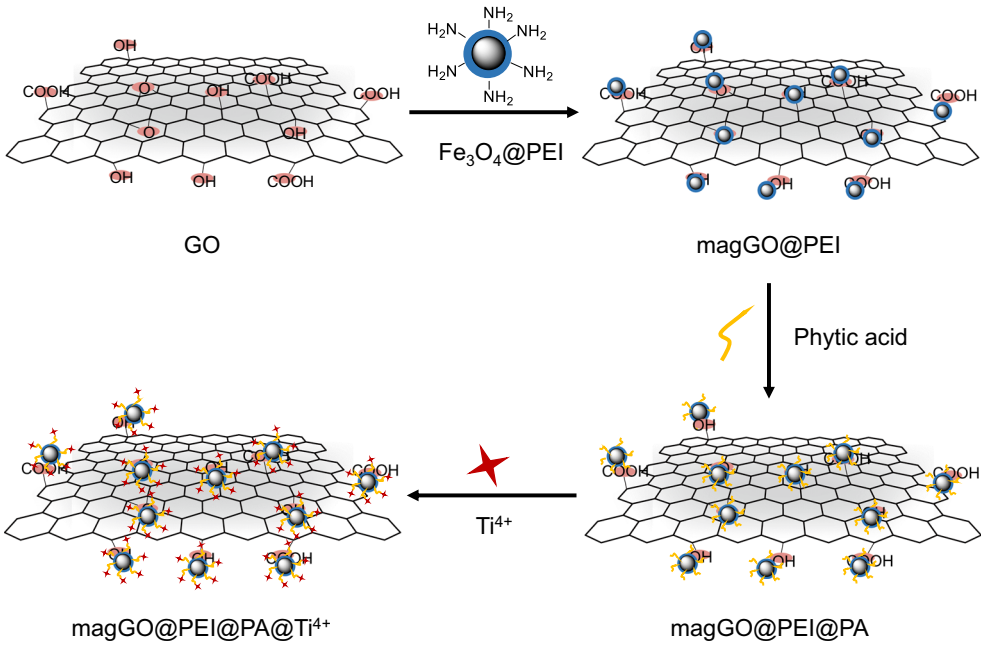

Fig. 1 Schematic illustration for the preparation of magGO@PEI@PA@Ti^{4+}

composite was dispersed in 100 mL PA (7 mg mL^{-1}) and stirred for 6 h at room temperature (about 25 °C) to form magGO@PEI@PA. Finally, the magGO@PEI@PA was incubated with 100 mM $Ti(SO_4)_2$ solution (100 mL) for 2 h to immobilize Ti^{4+}. The product magGO@PEI@PA@Ti^{4+} composite was rinsed with water for three times and dried at 45 °C under vacuum for overnight.

Magnetic solid-phase extraction (MSPE) procedure

In the investigations of extraction conditions for nucleobases, nucleotides and nucleosides, the extraction rate was selected as the evaluation index to compare the influence of different extraction conditions on the result. The extraction rate is defined as $(C_0\text{-}C)/C \times 100\%$, where C_0 and C are the initial and equilibrium concentrations of each compound, respectively.

Firstly, 2.5 mg magGO@PEI@PA@Ti^{4+} was added to 1 mL sample solution in a glass vial. The mixture was oscillated on a temperature-controlled air bath shaker (SHZ-82, Jintan Zhengrong Experimental Instrument Factory, Jiangsu, China, http://www.zenroelab.com/). Then, the magGO@PEI@PA@Ti^{4+} composites were isolated from the solution phase by employing a magnet under the bottom of the vial, and the supernatant was decanted and subjected to HPLC analysis to determine C for calculating extraction rate. After the solution being totally removed, the same volume of desorption solvent was added, and the mixture was oscillated for another period of time. The resulting desorption solution was subjected to HPLC analysis.

Preparation of sample solutions

The solutions of four nucleotides were separately prepared in distilled water at a concentration of about 500 μg mL^{-1}. The five nucleobases and nucleosides were prepared in acetate buffer at a concentration of about 300 μg mL^{-1}, respectively. All of the solutions were kept in the dark under 4 °C. The stock solution was prepared by mixing the same volume of the analyte solution, and a final concentration of about 10 μg mL^{-1} for each analyte was used in the process of MSPE parameters optimization.

C. sinensis and *L. edodes* material were oven-dried at 35 °C for 48 h. All dried materials were pulverized and griddled through 50 mesh sieves before extraction. A total of 0.5 g *C. sinensis* and 1.0 g *L. edodes* fine powder were accurately transferred to a glass-stoppered conical flask, respectively. Then 10 mL boiling ultrapure water (95–100 °C) was added and ultrasonic extracted under 75 °C for 30 min. The obtained solution was centrifuged at 5000×*g* for 10 min; the upper solution was collected and used for MSPE.

Rabbit plasma was also used for MSPE and its preparation procedure was provided in ESM.

HPLC analysis

HPLC analysis was performed on an Agilent 1260 Series liquid chromatography system(Agilent Technologies, USA, www.agilent.com), which was equipped with a vacuum degasser, a quaternary pump, an auto-sampler, and a diode array detector (DAD) and was controlled with the Agilent Chem Station software. The details of HPLC analysis were described in ESM.

Results and discussion

Characterization of magnetic nanomaterials

The morphology and configuration of the magnetic particles were presented in both SEM and TEM images. As shown in Fig. 2a, some spherical shape, even size, and rough surface particles are presented after gradually modifying Fe_3O_4 with PEI, PA and Ti^{4+}. Moreover, it is also clearly observed that the Fe_3O_4 particles are spread on some lamellar structure (area in the blue circle), confirming the successful synthesis of magnetic graphene nanocomposite. Furthermore, TEM was employed to further evaluate the size and surface morphology of the magGO@PEI@PA@Ti^{4+} particle. As shown in Fig. 2b, c, the magGO@PEI@PA@Ti^{4+} possesses uniformly spherical shape with typical core-shell structure and a mean diameter of ~340 nm, and the further modification results in the formation of a layer (~28 nm) on the surface of the Fe_3O_4 nanoparticles. Similarly, the lamellar GO structure can also be observed in the TEM image (area in the red circle). In addition, the composition of the composites was analyzed by EDX, and the results are illustrated in Fig. 2d, e. As compared with magGO@PEI, new P and Ti elements are appeared attributed to the abundant P and Ti existed in PA ($C_6H_{18}O_{24}P_6$) and $Ti(SO_4)_2$.

FTIR were used to characterize the functional groups, and the spectra are presented in Fig. 3a. The characteristic band of Fe_3O_4 appeared at 585 cm^{-1} (Fe-O vibration), which is observed in all synthesized composites. The strong adsorption peak in the region of 3500–3200 cm^{-1} is assigned to O-H stretching vibration for hydroxyl and carboxyl arising from GO in Fig. 3a (curve b, c). Most importantly, bands at 1125 and 880 cm^{-1} in Fig. 3a (curve c), which confirm the successful decoration of PA on magnetic materials, are separately assigned to the P=O and P-O-H stretching vibration in phosphateester group. As one of the significant characteristics, the water-compatibility of the composites was evaluated. One drop of water was deposited on the surface of magGO@PEI@PA@Ti^{4+} slice, where it rapidly spread over the contact area (Fig. 3b). The water contact angle approaches 10 within 1 s, which indicates the hydrophilicity of the surface of the material.

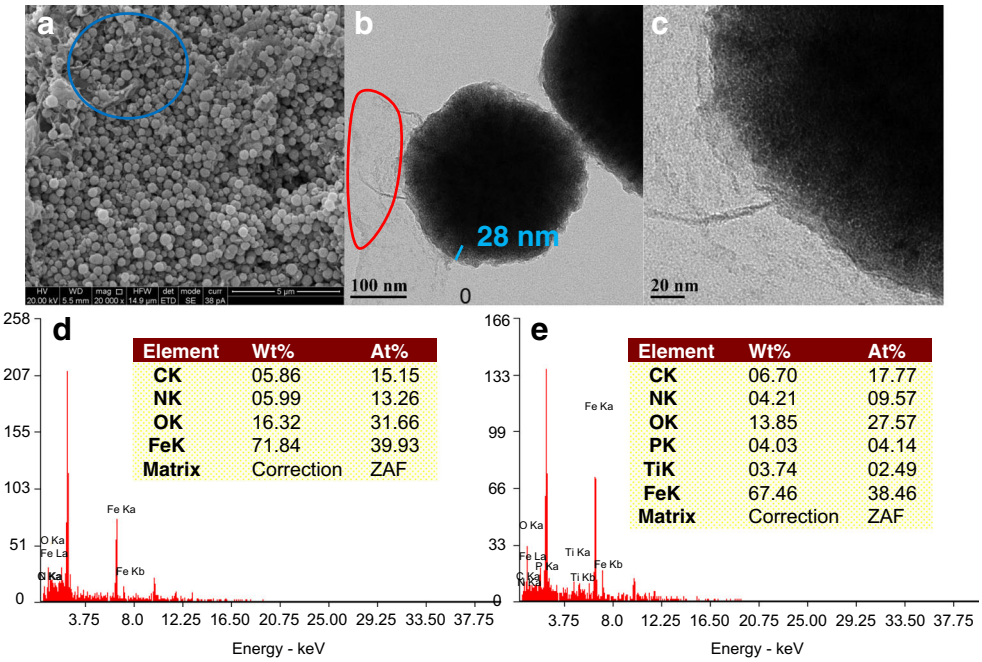

Fig. 2 Characterization of synthesized materials by SEM, TEM and EDX. **a** SEM images of magGO@PEI@PA@Ti^{4+} at 20.0 kV × 20,000 amplification; **b** and **c** TEM images of magGO@PEI@PA@Ti^{4+}; **d** Chemical compositions of magGO@PEI analyzed by EDX; **e** Chemical compositions of magGO@PEI@PA@Ti^{4+} analyzed by EDX

Thermogravimetric analysis was used to compare the thermal behavior of Fe_3O_4, magGO@PEI and magGO@PEI@PA@Ti^{4+}. As illustrated in Fig. 3c, the mass decrease of Fe_3O_4 is evidently less than that of magGO@PEI, proves the stability of the magnetic core. The curve of magGO@PEI shows the weight loss of 17.45%, while the total loss of magGO@PEI@PA@Ti^{4+} after decorating is 17.83% and the value is continuing falling. This demonstrates that new substances had been coated on the basis of magGO@PEI. Specifically, the TGA curve of magGO@PEI@PA@Ti^{4+} indicates a 5.58% weight loss between 25 and 180 °C, which is related to the evaporation of surface-adsorbed water and water in the crystal structure. The same weight loss is also observed for Fe_3O_4 and magGO@PEI. An increasing weight loss down to 400 °C is observed for magGO@PEI@PA@Ti^{4+} compared with magGO@PEI, which can be attributed to the decomposition of adsorbed groups (PA and Ti^{4+}) on the surface.

The magnetic properties of Fe_3O_4 and magGO@PEI@PA@Ti^{4+} were measured by VSM in the field ranges from −20,000 to +20,000 Oe at room temperature (about 25 °C). As shown in Fig. 3d, the magnetization curves of Fe_3O_4 and magGO@PEI@PA@Ti^{4+} do not show hysteresis, indicating that these two materials exhibit superparamagnetic behavior. The magnetization saturation values of Fe_3O_4 and magGO@PEI@PA@Ti^{4+} are 55.6 emu g^{-1} and 45.9 emu g^{-1}, respectively. Because the surface of Fe_3O_4 is grafted, the magnetization saturation value of magGO@PEI@PA@Ti^{4+} is declined slightly. Also, the magGO@PEI@PA@Ti^{4+} nanocomposites can be dispersed uniformly in water via sonication to form a homogeneous solution. Under an external magnetic field, the nanocomposites are quickly aggregated in 5 s (NdFeB magnet, size 8 × 4 mm, coercive force 876 kA m^{-1}, remanent 1.17 T, intrinsic coercivity 20 kA m^{-1}, maximum energy product 36 kJ s^{-1}, density 7.5 g cm^{3-1}, operating temperature 150 °C). Therefore, owing to the very good superparamagnetism, magGO@PEI@PA@Ti^{4+} can be rapidly and easily separated from the solution, which can facilitate the SPE process.

Extraction and desorption of nucleotides in the immobilized metal affinity chromatography (IMAC) mode

In order to achieve the maximal extraction efficiency of the magGO@PEI@PA@Ti^{4+} for four nucleotides, the following parameters in MSPE protocol were optimized: extraction time, kind of acids and ionic strength of extraction solution, as well as type and concentration of eluent. All parameters were examined in three independent experiments ($n = 3$), the results and respective data are given in the ESM and Fig. S2. After being systemically optimized, the following experimental conditions were found to give the best results: (a) an extraction time of 15 min; (b) deionized water as loading buffer; (c) no ion was added in sample; (d) 10 mM Na_3PO_4 as elution solution for 10 min desorption.

Extraction and desorption of nucleobases and nucleosides in the hydrophilic interaction liquid chromatography (HILIC) mode

D.V. McCalley investigated the effect of various conditions on the selectivity of the separation of a set of neutral, acidic and basic solutes in HILIC mode, providing a practical guide in the selection of suitable conditions for establishing a separation. The magnitude of the effect of changes produced from

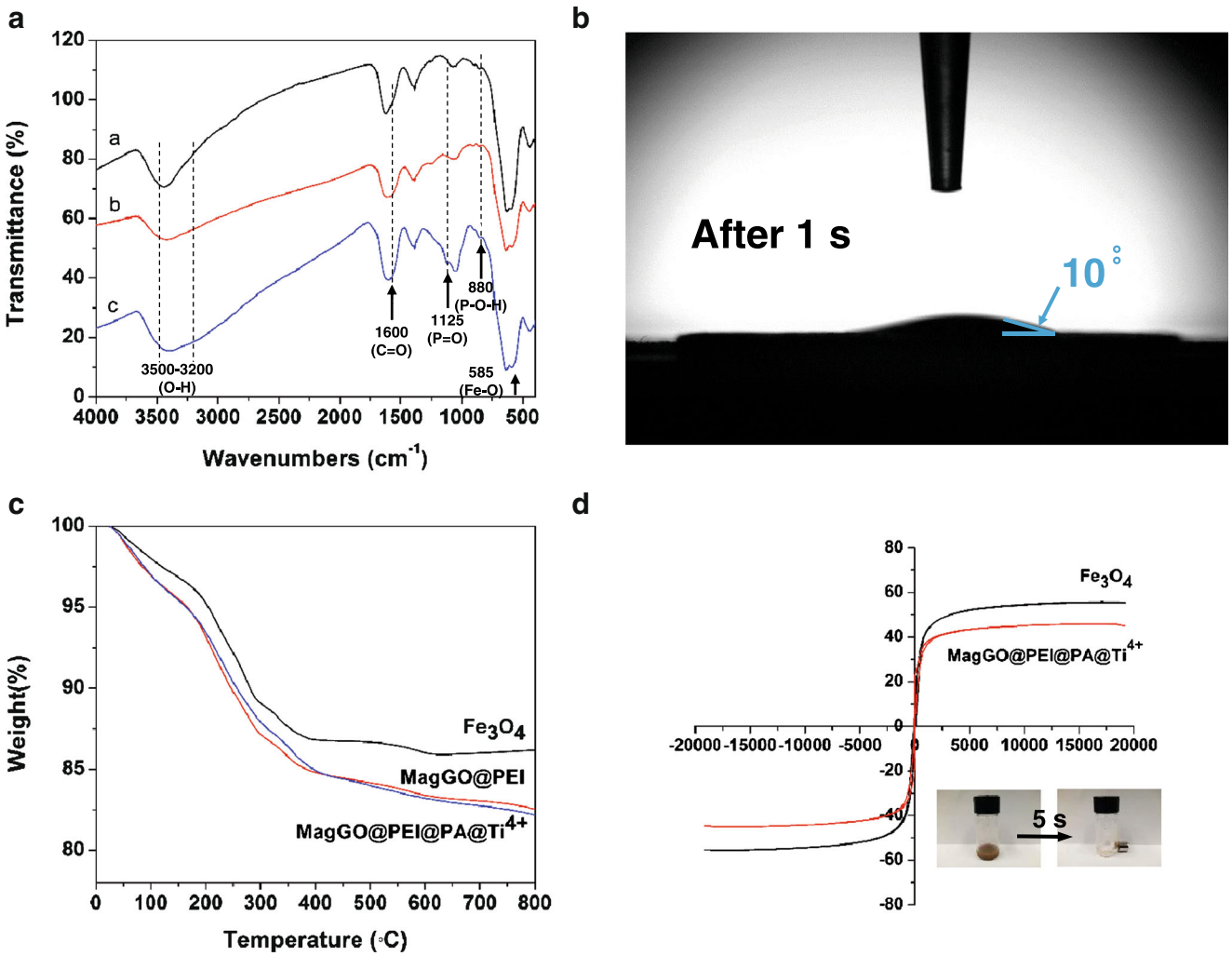

Fig. 3 Characterization of synthesized materials. **a** FTIR spectra of Fe_3O_4@PEI (curve a), magGO@PEI (curve b), magGO@PEI@PA@Ti^{4+} (curve c); **b** The profiles of a water drop on the films of magGO@PEI@PA@Ti^{4+}; **c** TGA curves of Fe_3O_4 (curve a) and magGO@PEI@PA@Ti^{4+} (curve b); **d** VSM curves and photo of magnetic separation (inset) of Fe_3O_4 and magGO@PEI@PA@Ti^{4+}

the correlation of retention factor was estimated as: stationary phase > mobile phase pH > organic solvent concentration > buffer concentration > column temperature [32]. Accordingly, a systemic optimization of conditions for the extraction of nucleobases and nucleosides were performed, and the elution effects of various solvent systems were also compared. Each experiment was repeated for three times ($n = 3$). The results are shown in ESM and Fig. S3. Finally the following optimum conditions were adapted: (a) pH of extraction solution was 7.5; (b) 99% ACN was added into the loading buffer; (c) the ion strength was controlled at 2.5 mM with NH_4AC buffer; (d) extraction under 35 °C; (e) an extraction time of 20 min; (f) desorption with 1 mL 50% MeOH for 20 min under 25 °C.

Analytical performance

The MSPE-HPLC method was systematically validated by determining the linearity range, limits of detection (LOD) (S/$N = 3$), limits of quantification (LOQ) (S/$N = 10$), inter- and intra-day precision under two different mode, respectively. The determination was performed through extracting and analyzing reference solutions spiked with different concentrations of analytes under the optimized extraction and desorption conditions. As it can be seen in Table S2 and Table S3, good linearity are obtained for all nine compounds in the range of 0.098–6.25 μg mL^{-1} and with a relatively high coefficients ($R^2 \geq 0.9990$). The LOD for four nucleotides are between 1.8 and 2.8 ng mL^{-1}, while LOQ are between 8.1 and 13.1 ng mL^{-1}; The LOD for five nucleobases and nucleosides are between 4.0 and 10.5 ng mL^{-1}, and LOQ are between 6.1 and 32.8 ng mL^{-1}. For precision test, the 6.25 μg mL^{-1} mixed references solutions were analyzed for six times within one day ($n = 6$) and the inter-day reproducibility was examined in duplicates for consecutive three days (n = 6). Intra-day RSD and inter-day RSD values are 0.1%–1.0% and 1.6%–3.4% for nucleotides, 0.4%–0.8% and 2.3%–4.3% for nucleobases and

Table 1 Comparison of the method/sorbents with previously reported methods/SPE sorbents

Analytical method	Analyte	Sample	Sorbent (mg)	Extraction solvent (mL)	LOD (ng mL^{-1})	LOQ (ng mL^{-1})	Ref.
PLE-LC-MS	16 nucleosides, bases and their analogues	*Cordyceps*	–	>10	0.7–26.6	1.5–53.1	[33]
Commercial Varian PBA (Affi-Gel boronate gel)-SPE-LC-MS	Adenosine, cytidine, uridine	Urinary samples	–	>4	80–180	240–540	[34]
3-aminophenylboronic acid-GO-SPE-CE-UV	Adenosine, guanosine, inosine, uridine	TCM injection samples (gingko and ginseng)	–	1	11–17	36–55	[18]
Zr-Fe_3O_4-MSPE-LC-UV	Adenosine, cytidine, guanosine, uridine	Urinary samples	50	10	5–17	16–52	[35]
ZrO_2-NCP-SPME-nESI-MS/MS	Adenosine, cytidine, guanosine, uridine	Urinary samples	10		13.6–1258	45.4–4194	[21]
Phytic acid-Ti^{4+} magGO nanocomposites-MSPE-HPLC-DAD	CMP, UMP, GMP, AMP, hypoxanthine, adenosine, cytosine, inosine, cytidine	*Cordyceps*, *Lentinus edodes* and plasma sample	2.5	1	1.8–2.8; 4.0–10.5	8.1–13.1; 6.1–32.8	This study

PLE, Pressurized liquid extraction; *NCP-SPME-nESI-MS/MS*, nanocoating cellulose paper based microextraction and nanoelectrospray ionization-tandem mass spectrometry

nucleosides, respectively. The satisfactory precisions illustrate the achievement of good reproducibility using the MSPE-HPLC method under both IMAC and HILIC modes.

Comparison of the method with previous reports

The extraction performance of magGO@PEI@PA@Ti^{4+} based MSPE-HPLC analysis is compared with other previous reports involving the pretreatment of nucleobase-based compounds (Table 1). The merits of the present sorbent can be concluded as following aspects: a) relatively better specificity than traditional solvent extraction methods; b) the simpler and shorter extraction process compared with commercial sorbents as their nature of needing several steps for column activation and equilibrium procedures; c) saves solvent and sorbents compared with traditional liquid-liquid extractions and commercial SPE sorbents; d) proposes a new material employing MSPE method for those characteristic nucleobase-based compounds including nucleobases, nucleosides and nucleotides which have rarely been explored with SPE sorbents before; e) to extract nucleobases-based compounds from the same sample by two distinct mechanisms, IMAC for nucleotides while HILIC for nucleobases and nucleosides, and achieves good results with relatively low LOD and LOQ values. In addition, the synthesized materials have a wide range of application potential. Considering the mechanism of the magGO@PEI@PA@Ti^{4+}, some other species can also be extracted employing this material. Compounds contained amino or hydroxyl group (i.e. catecholamines, histidine-tagged proteins etc.) can coordinate with the metal ions on the surface of the adsorbent under IMAC mode, while some highly polar drugs (metformin, aminoglycoside antibiotic) which cannot be well retained on the common reverse phase SPE column can also be pretreated by the magGO@PEI@PA@Ti^{4+} under HILIC mode.

Application to real samples

The practicality of the magGO@PEI@PA@Ti^{4+} based MSPE method was investigated and applied to determine nucleobases, nucleosides and nucleotides in real samples, including medicinal mushroom *C. sinensis*, edible mushroom *L. edodes* and animal plasma. Three concentrations (low, medium and high) of reference compounds were spiked in sample, and the accuracy of the present method was validated by calculating the recovery. The relative recovery (RR%) was acquired from the equation below:

$$\mathrm{RR}(\%) = \frac{C_{found} - C_{real}}{C_{added}} \times 100\% \quad (1)$$

wherein, C_{found}, C_{real} and C_{added} were the concentration of the analyte after addition of a known amount of

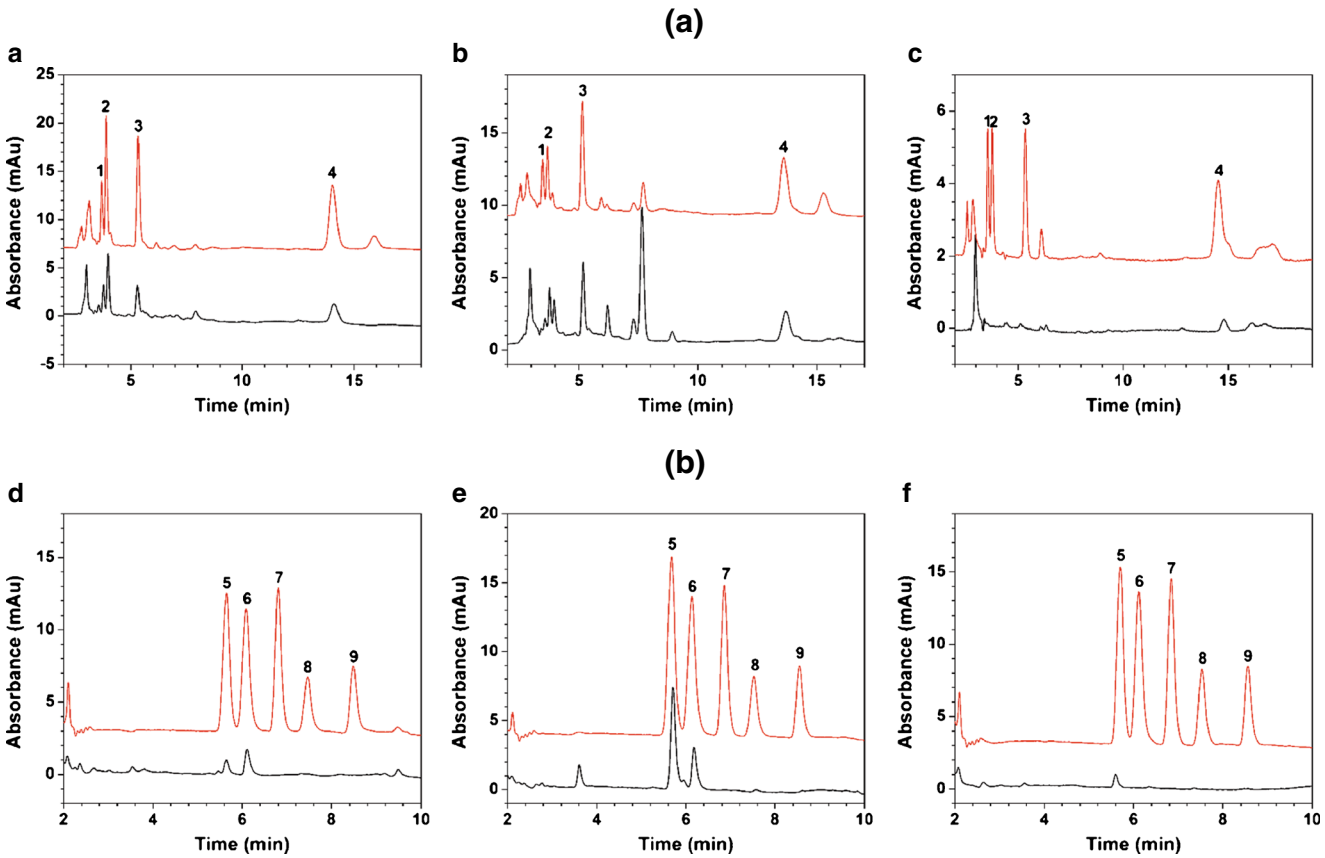

Fig. 4 Typical chromatograms for blank (black line, lower) and spiked (red line, upper) samples. **a** Sample after MSPE under IMAC mode: *C. sinensis* (a), *L. edodes* (b) and rabbit plasma (c); **b** Sample after MSPE under HILIC mode: *C. sinensis* (d), *L. edodes* (e) and rabbit plasma (f). All chromatograms were present as samples spiked with medium level concentration of reference compounds, the specific concentrations were consistent with Tables 2 and 3. (1) CMP; (2) UMP; (3) GMP; (4) AMP; (5) Hypoxanthine; (6) Adenosine; (7) Cytosine; (8) Inosine; (9) Cytidine

reference compound into the real sample, the concentration of analyte in the real sample and the concentration of a known amount of the reference spiked into the real sample, respectively. All measurements were replicated three times ($n = 3$), Fig. 4 presents representative chromatograms of determining nucleotides, nucleobases and nucleosides in the different samples after pretreatment by magGO@PEI@PA@Ti^{4+} under IMAC and HILIC mode, respectively. The mean values of the results are summarized in Tables 2 and 3.

The recoveries of CMP, GMP, AMP, cytosine, inosine and cytidine are all in the range of 71.6%–106.3% with RSD between 0.4%–12.6%. The complex natural and biological matrix have some substantial interference on the determination of these six important ingredients in the real samples, but the values are comparable with

Table 2 Analysis of four nucleotides in different samples by magGO@PEI@PA@Ti^{4+} based MSPE-HPLC-UV method

Analyte	Spiked ($\mu g\ mL^{-1}$)	CMP		UMP		GMP		AMP	
		Recovery (%)	RSD (%, n = 3)	Recovery (%)	RSD (%, n = 3)	Recovery (%)	RSD (%, n = 3)	Recovery (%)	RSD (%, n = 3)
Cordyceps sinensis	0.5	91.7	11.6	86.3	4.8	80.6	1.9	77.6	6.1
	1.0	73.5	11.2	66.7	12.3	79.0	1.8	78.0	10.4
	2.5	79.3	10.2	71.4	5.6	93.8	10.1	77.1	9.9
Lentinus edodes	0.25	95.7	5.5	58.6	12.4	96.5	6.7	81.1	1.0
	0.5	81.2	9.4	58.8	12.9	83.7	2.5	87.3	3.0
	1.0	88.5	6.6	53.1	10.1	83.7	9.0	84.3	4.8
Plasma	0.1	100.0	6.8	81.6	13.2	95.3	10.3	86.3	6.3
	0.5	86.9	12.6	55.3	10.4	83.1	9.4	78.8	4.8
	1.0	85.6	6.8	54.5	12.8	85.1	7.5	77.4	4.2

Table 3 Analysis of five nucleobases and nucleosides in different samples by magGO@PEI@PA@Ti^{4+} based MSPE-HPLC-UV method

Analyte	Spiked (μg mL^{-1})	Hypoxanthine		Adenosine		Cytosine		Inosine		Cytidine	
		Recovery (%)	RSD (%, n = 3)	Recovery (%)	RSD (%, n = 3)	Recovery (%)	RSD (%, n = 3)	Recovery (%)	RSD (%, n = 3)	Recovery (%)	RSD (%, n = 3)
Cordyceps sinensis	0.3	70.6	7.9	53.1	6.2	84.3	6.3	97.5	12.4	106.3	0.6
	1.0	64.2	1.6	50.7	1.4	71.6	1.0	75.3	3.2	96.6	9.2
	3.0	67.7	1.7	56.9	9.2	77.2	5.3	73.5	5.5	107.3	4.3
Lentinus edodes	0.3	65.7	1.2	56.5	2.9	90.5	2.6	95.5	5.9	88.4	5.5
	1.0	64.6	6.4	59.8	2.6	87.1	3.9	88.4	8.6	93.6	1.2
	3.0	62.1	1.4	53.6	2.3	84.0	0.7	67.3	1.6	79.5	2.7
Plasma	0.3	78.1	2.7	62.0	4.4	83.3	3.0	91.8	10.7	104.8	7.2
	1.0	78.0	0.9	64.6	2.3	84.0	0.4	89.5	0.9	79.4	5.1
	3.0	70.0	0.7	60.5	1.2	79.1	0.9	79.7	0.5	95.3	0.9

some reported methods toward nucleosides [18, 21]. The recovery of the other three compounds, UMP, hypoxanthine, and adenosine, are not quite satisfactory with a RR value between 50.7%–86.3%. The possible reason for this result might be due to the relatively low extraction rate of the material for these three compounds: (a) the dissociation of UMP under the extraction condition causing some interference for the chelation of the compound with the metal; (b) the insufficient hydrophilicity of adenosine and hypoxanthine; and (c) the strong matrix interference in complicated natural products and biological matrix.

Conclusions

A metal coordinated and hydrophilic dually functional sorbent based on PA-Ti^{4+} modified magnetite@GO nanocomposites was prepared. Because of the high loading amount of Ti^{4+} and hydrophilicity of PEI and PA, large surface area and good magnetic property contributed from GO and Fe_3O_4, the magGO@PEI@PA@Ti^{4+} nanocomposites were employed to extract typical nucleotides under IMAC mode and nucleobases and nucleosides under HILIC mode. The satisfactory precision and low detection limits facilitate its implementation in the determination of some nucleobases, nucleosides and nucleotides in complicated spiked *C. sinensis*, *L. edodes* and animal plasma samples. This is the first attempt to extract these three kinds of highly polar compounds from a complex matrix with one SPE material under two distinct mechanisms. Although the enriched nucleobase-based compounds from the complex samples are very limited due to the limitations of the extraction efficiency of the material, the synthesis and application of the combined properties of IMAC- and HILIC- material would be of potential value in the SPE field to pretreat and determine more kinds of polar nucleobase-based compounds from real matrices. Further efforts are required for the development of the enrichment materials owing high capacity under two different modes, and the strategy of combination of different metal ions or hydrophilic modifying materials can be taken into consideration for enhancing enrichment efficiency in the future.

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Compliance with ethical standards The author(s) declare that they have no competing interests.

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