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Nanobeads-based rapid magnetic solid phase extraction of trace amounts of leuco-malachite green in Chinese major carps

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ABSTRACT

A proof-of-concept for the use of oleic acid coated magnetic nanobeads (OA-MNBs) for the magnetic solid phase extraction (MSPE) of trace amounts of leuco-malachite green (LMG) from fish samples was developed. The OA-MNBs were prepared by covalently conjugating oleic acid on amino-modified magnetic polystyrene beads. The OA-MNBs were characterized with transmission electron microscopy, Fourier transform infrared spectroscopy and zeta-potential analyzer. The optimized parameters for MSPE with OA-MNBs of LMG from fish muscle involved a combination of pH 10.0 in 10% acetonitrile, 1.5 M sodium chloride as an adsorption solution, and an extraction procedure involving 6 mg OA-MNBs in 18 mL LMG adsorption solution. This was optimized for 0.5 g fish muscles with an incubation period of 10 min using 200 μL acetonitrile for elution. Using the optimized parameters, the performance of MSPE with OA-MNBs was evaluated by analyzing LMG-spiked fish extracts with liquid chromatography-mass spectrometry (LC-MS/MS) method. The results indicated that recoveries of LMG (from 0.1 to 2 ng/g) ranged from 71.2%–112.6% with relative standard deviations as low as 0.6%. Out of 57 field fish samples, eight LMG positive samples were confirmed using MSPE with OA-MNBs. Compared with traditional liquid–liquid extraction methods, the results showed that MSPE with OA-MNBs had a higher sensitivity for samples with low LMG concentration. Furthermore, the MSPE with OA-MNB took only 40 min to perform without the need for time consuming sample-pretreatment process. Therefore, MSPE with OA-MNBs holds promise for rapid, sensitive, and cost effective screening for LMG in fish samples.
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1. Introduction

Malachite green (MG), a triphenylmethane dye, has been extensively used in aquaculture industry for its high efficiency in prevention and treatment of external fungal and parasitic infections in fish. MG is easily adsorbed by fish tissue and rapidly metabolized into lipophilic leucomalachite green (LMG) [1–4]. LMG is a major metabolite of MG that has a long residence time ($t_{1/2}$ ~40 d) [5] in edible fish tissues. At present, the use of MG in aquaculture has been banned in the USA, Europe, China and other countries because of increased risk of carcinogenesis, teratogenesis and mutagenesis [6]. However, in spite of its many adverse effects to human health, this harmful dye is still widely used in aquaculture, aquatic transport and storage in some parts of the world because of its low cost [7–10]. In the US and internationally, there are numerous reports of MG misuse in aquaculture [11–13]. The minimum performance limit imposed by the European Commission requires methods that determine the sum of MG and LMG residues below 2 ng/g [14]. Therefore, a sensitive and convenient method for trace MG and LMG detection in aquaculture products is necessary to avoid high levels in fish products.

High-performance liquid chromatography (HPLC) coupled with visible detection or fluorescence detection, HPLC-tandem mass spectrometry and HPLC-electrospray ionization tandem mass spectrometry are general analytical methods for the confirmation of MG and its metabolite residues in fish tissues. The limits of detection (LOD) for these methods are recorded at 0.1–1.0 ng/g [15–17]. However, sample pretreatment techniques including extraction, concentration and/or clean-up procedures are necessary to remove matrix interfering compounds to improve the sensitivity and LOD of these methods. Conventional methods for concentration and clean up of LMG in aquatic animal muscle include solid–liquid extraction (SLE) followed by traditional liquid–liquid extraction (LLE), solid-phase extraction (SPE)

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[1,2,18–21] and molecularly imprinted SPE [10,22]. However, these existing methods are complicated, time-consuming and generate a large amount of organic waste in view of the complexity of the muscle tissue and trace contents of LMG. In contrast, nano or submicro superparamagnetic material based magnetic solid-phase extraction (MSPE) methods have exhibited advantages over traditional SPE method for its simplicity and acceleration of the enrichment process [23]. Due to even dispersion in solution, the magnetic adsorbent can interact homogeneously with the entire solution to achieve higher adsorption/extraction efficiency. Furthermore, the process can also be performed in suspensions containing solid or oily components without blocking the cartridges or disks that are used in traditional SPE. This method provides a relatively rapid and convenient way for withdrawal of the magnetic LMG adsorbent from sample matrices by applying an external magnetic field.

MSPE technique involving different surface functionalized magnetic particles/beads has been extensively used for preconcentration of different target analytes such as metal ions, anti-inflammatory drugs, antibiotics, analgesics, pesticides, insecticides, dyes, surfactants, carcinogens and phenolic compounds from environmental samples [24–26]. In recent years, MSPE has also been applied for food quality control, such as enrichment of tetracyclines [27,28], vitamins [29], melamine [30], manganese [31] in milk, egg or cereal. However, to date, MSPE has never been reported in the analyte extraction from complex muscle tissue.

In this study, we report the proof-of-concept for a novel sample pretreatment approach using magnetic nanobeads for the extraction and subsequent detection of LMG in fish muscle. This nanobeads-based MSPE has been explored to simplify and hasten the LMG extraction process that is confirmed with liquid chromatography-mass spectrometry (LC-MS/MS) techniques to verify the sensitivity of the MSPE using OA-MNBs.

2. Experimental

2.1. Reagents, standards and samples

Leuco-malachite green (LMG) was purchased from Merck (Darmstadt, Germany). Deuterium-labeled LMG (LMG-D_6) was purchased from Witega Laboratorien Berlin-Adlershof GmbH (Berlin, Germany) and used as the internal standard for LMG. Polystyrene/acrylic acid coated magnetic nanobeads (MNBs) were obtained from Wuxi Zodoboer Biotech. Co., Ltd. (Wuxi, China) and the density of carboxyl group on the surface of magnetic beads was 1 mmoL/g. Oleic acid (OA), ethylenediamine (EDA), 1-ethy-3-(3-dimethyaminopropyl) carbodiimide hydrochloride (EDC), *N*,*N*′-dicyclohexylcarbodiimide (DCC), *N*-hydroxy-succinimide (NHS), *N*-Hydroxysulfosuccinimide (Sulfo-NHS) and anhydrous tetrahydrofuran which were used for the synthesis of oleic acid modified magnetic beads (OA-MNBs) were purchased from Sigma-Aldrich (St. Louis, Mo, USA). All other chemicals and reagents were analytical grade and purchased from Sinopharm Chemical Reagent Co. Ltd, (Shanghai, China). Ultrapure water (18 MΩ/cm) was produced by using a Milli-Q system (Millipore, Molsheim, France).

The stock solutions of LMG and LMG-D_6 (100 μg/mL) were prepared by dissolving 1 mg LMG and LMG-D_6 in 10 mL of acetonitrile. The intermediate standard solution (1 μg/mL) was prepared by diluting the stock solution with acetonitrile. Both solutions were stored in the dark at −20 °C for less than 3 months. Working standard solutions (0–50 ng/mL for LMG) were prepared by diluting the intermediate standard solution with mobile phase (50 mM ammonium acetate buffer containing 75% acetonitrile, pH 4.5). A McIlvaine solution at pH 3.0 was prepared by mixing 18.9 mL of 0.2 M sodium hydrogen phosphate with 81.1 mL of 0.1 M citric acid.

2.2. Apparatus

JEM-2100 (HR) transmission electron microscope (JEOL, Japan), Fourier transform infrared spectrometer, FTIR, (Nicolet IR200, USA) and Zetasizer Nano ZS analyzer (Malvern, England) were used for characterization of OA-MNBs. A Waters 2489 series liquid chromatography coupled with UV/Vis detector (Milford, USA) was used for the optimization of adsorption parameters and adsorption isotherm experiments of OA-MNBs. The absorbance detector was set at 618 nm for MG detection. The chromatographic separation was performed with beta-basic C_{18} column (5 μm, 4.6 mm × 250 mm, Waters, Milford, USA). The mobile phase was an acetate buffer (50 mmol/L, pH 4.5) containing 60% acetonitrile (v/v). The injected volume was 20 μL and the separation was accomplished with a flow rate of 1.0 mL/min at 25 °C. The performance of the MSPE using OA-MNBs was confirmed on a triple quadrupole LC-MS/MS system (Agilent Corporation, MA, USA) which was composed of triple quad instrument (Agilent 6430) and LC system (Agilent 1200 series). The LC-MS/MS system was controlled by MassHunter software (Agilent Corporation, MA, USA). LC-MS/MS operation was performed according to the national standard GB/T19857-2005 (China). The chromatographic separation was performed with a Agilent Zorbax XDB-C_{18} column (4.6 mm × 50 mm, 1.8 μm) using isocratic mobile phase containing 75% (v/v) acetonitrile in aqueous ammonium acetate (50 mM, pH 4.5) at a flow rate of 0.2 mL/min. The column was maintained at 35 °C and the injection volume was 10 μL. Ionization was achieved using electrospray in the positive mode at an ionization voltage of 4000 V. Nitrogen was used as nebulizer gas at 40 psi. Ion transitions were monitored with the Multiple Reaction Monitoring (MRM) mode.

2.3. Preparation of OA-MNBs

Oleic acid was covalently conjugated to the MNBs using a diamine linker EDA. Briefly, 100 mg MNBs were dispersed in 10 mL 0.05 M MES buffer (pH 5.0) to which 38 mg EDC and 65 mg Sulfo-NHS were added to activate the carboxyl group on the MNBs. After reaction at 37 °C for 0.5 h, the MNBs were separated with a magnetic separator and transferred to 30 mL 10% EDA solution (pH was pre-adjusted to 8.0 with 6 M HCl). After reacting at 37 °C for 3 h with gentle shaking, the amino group modified MNBs were separated with magnetic separator and washed three times with ethanol. Meanwhile, 700 μL oleic acid, 0.3 g NHS and 0.27 g DCC were added to 5 mL anhydrous tetrahydrofuran and reacted at 37 °C for 1.5 h with vigorous shaking to activate the carboxyl group on the OA. The OA reaction mixture was centrifuged at 4000*g* for 5 min before adding 100 mg of the amino group modified MNBs and 500 μL triethylamine. The mixture was reacted at 37 °C for 4 h with vigorous shaking to get the OA-MNBs, and then washed four times with ethanol and stored at 4 °C for further use. Fig. 1 illustrates the protocol for the preparation of OA-MNBs. The sorbent (OA-MNBs) was characterized by TEM, zeta potential and FTIR studies.

2.4. Adsorption behaviors with OA-MNBs in fish tissues

2.4.1. Preparation of LMG matrix extract

The extraction procedure for LMG from fish tissues was performed using a modified version of a previously described method [1]. A 1.0 g fish muscle without bones and skin was homogenized with 3 mL extraction buffer (containing 1.2 mL McIlvaine buffer at pH 3.0, 1.8 mL acetonitrile). The mixture was centrifuged at 10,000*g* for 10 min and the supernatant was

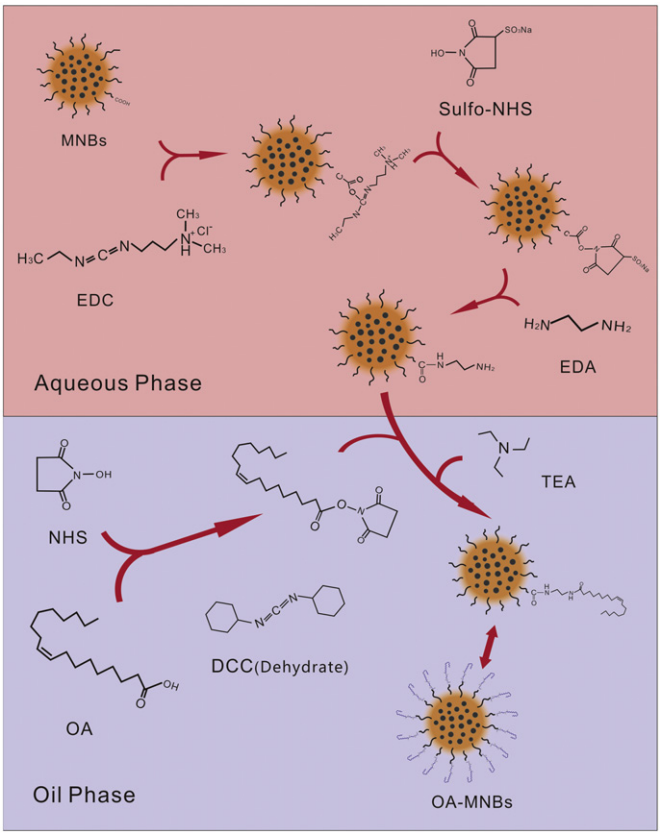

Fig. 1. Protocol for the preparation of OA-MNBs.

collected; a second extraction from the precipitate was performed with the same procedures. About 6.2 mL of supernatant solution from both extraction processes was combined and stored at −20 °C for further use.

2.4.2. Extraction of LMG from fish tissues with MSPE using OA-MNBs

The MSPE extraction method was executed by adding 6 mg OA-MNBs to the 3.1 mL LMG matrix extract from 0.5 g fish tissue. The mixture was diluted to 18 mL with 1.8 M NaCl solution and the pH of the adsorption solution was adjusted to 10.0 using 10 M NaOH to achieve a final mixture containing 1.5 M NaCl (pH of 10.0) and 6 mg A-MNBs. This mixture was shaken vigorously with a vortex mixer to form a homogeneous solution. After incubation in the dark for 10 min, the OA-MNBs were separated with a medium size magnetic separator (magnetic field strength of 0.5 T) which can hold two 50 mL centrifuge tubes. After washing with 2 mL 0.01 M phosphate buffered saline solution (pH 8.0) twice, LMG was eluted from the OA-MNBs with 200 μL acetonitrile and the OA-MNBs were separated with a small size magnetic separator (0.4 T) which can hold two 1.5 mL centrifuge tubes (Fig. 2). The eluate containing the LMG was oxidized with 50 μL 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ, 2 mg/mL) acetonitrile solution to form MG. The whole extraction process took about 40 min. The LMG extraction efficiency of MSPE using OA-MNBs was determined by HPLC using a C_{18} column followed by UV detection at 618 nm.

2.4.3. Adsorption isotherm experiments

The adsorption isotherm of LMG on the OA-MNBs was conducted at 25 °C. LMG at 45–1500 ng/mL was spiked in 3 mL adsorption solution containing 10% acetonitrile and 1.5 M NaCl (pH 10), respectively. A 2 mg OA-MNB was used to extract the LMG as described above. The equilibrium adsorption capacity was calculated as $q_e=(C_0-C_e)V/M$. Where q_e (mg/g) is the equilibrium adsorption capacity; C_0 and C_e are the initial and equilibrium concentration (mg/L) of LMG in the sample extraction supernatant; V (L) is the volume of supernatant, and M (g) is the weight of the OA-MNBs.

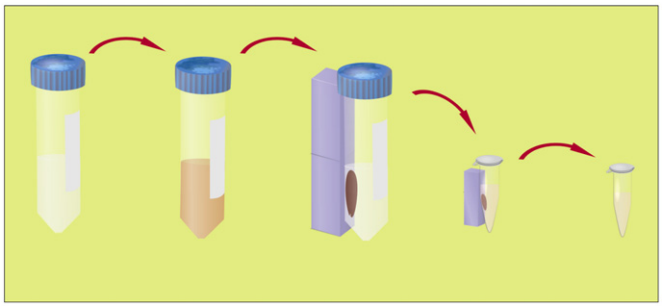

Fig. 2. Schematic illustration of the process of OA-MNBs separation.

2.4.4. Classical sample pretreatment

The classical sample pretreatment was based on liquid–liquid extraction technique (LLE) and carried out as described in the national standard GB/T19857-2005 (China) with some modifications. Briefly, 0.5 g fish flesh without bones and skin was homogenized with 4 mL extractant A (containing 0.05 M hydroxylamine hydrochloride, 0.025 M p-toluenesulfonate and 0.35 M sodium chloride), 1 mL extractant B (containing 0.04 M ammonium acetate, pH4.2) and 5 mL of acetonitrile. A 2 mL portion of CH_2Cl_2 was added and the mixture was vortexed for 2 min. The mixture was centrifuged at 4000*g* for 10 min and the organic supernatant was transferred into a 15 mL tube and dried by blowing nitrogen gas at 55 °C. The solid residue was dissolved with 2.5 mL acetonitrile and loaded to a Waters Sep-pak alumina cartridge (Waters, USA), which was previously activated with 2.5 mL of acetonitrile. The first eluate was collected; the cartridge was rinsed with 2.0 mL acetonitrile and this second eluate was collected and combined with the first. The mixed eluate was dried under a nitrogen stream. The residue was dissolved in 200 μL acetonitrile and then filtered with a 0.45 μm membrane for LC-MS/MS analysis. The entire extraction process took about 2 h.

2.5. Evaluation of the MSPE method

The performance of MSPE using OA-MNBs was evaluated in field fish samples. Fifty-seven samples from different fish species including 12 carps, 17 crucian carps, 13 bighead carps, and 15 grass carps were purchased from the local supermarket. The LMG free fish samples were deboned and homogenized and the LMG residue was first determined by LC-MS/MS using the LLE sample pretreatment method.

A 1.0 g sample of the homogenized fish flesh without bones and skin were fortified by adding LMG working standard solutions to produce spiked samples containing 0.1, 0.15, 0.2, 0.3, 0.5, 1.0, 2.0 ng/g LMG, respectively. The spiked samples were incubated in the dark for 30 min at ambient temperature. LMG-D_6 internal standard was added to 200 μL LMG eluate to produce LMG-D_6 final concentration of 1.0 ng/g. Eight fish samples that were confirmed positive to LMG positive were analyzed with the OA-MNBs based MSPE method.

3. Results and discussions

3.1. Characterization of OA-MNBs

Characterization of the MNBs and OA-MNBs was performed by TEM, zeta potential and FTIR. The carboxyl functionalized MNBs

were spherical in shape as shown in Fig. 3c with a particle size distribution of 80–120 nm and a zeta potential of −21.932 mV at pH 7.4 before OA modification. After modification, the OA-MNBs gave a zeta potential down to −0.021 mV which may have resulted from the blocking of the polar groups on the surface with non-ionizable octadecenyl group. The FTIR shown in Fig. 3b showed a characteristic signal at 1640 cm^{-1} which corresponded to the symmetric stretching vibration of C=C on that are found on the octadecenyl groups indicating successful OA coating of the MNBs.

3.2. Optimization of the extraction parameters

Various parameters such as the concentration of acetonitrile, ionic strength, pH of the extraction solution, the amount of OA-MNBs, environmental temperature and adsorption time were investigated. These were optimized to achieve the best extraction efficiency for LMG using MSPE with OA-MNBs.

3.2.1. Effect of the concentration of acetonitrile

The effect of the concentration of acetonitrile on the adsorption efficiency was performed. To achieve this goal, 3.1 mL fish extracts from 0.5 g fish tissue at 60% acetonitrile with pure water were diluted with pure water to attain a final acetonitrile concentration at 30%, 25%, 20%, 15%, 10% and 5%, respectively with a constant LMG concentration at 10 ng/mL. The results of OA-MNBs extraction of LMG (shown in Fig. 4a) indicated that extraction efficiency increased as the acetonitrile concentration decreased. At a volume of 3.1 mL fish extract with acetonitrile concentration from 10% to 5%, the extraction efficiency increased from 35% to 44%. However, when 36 mL extraction volume at 5% acetonitrile was used, MSPE was slower requiring longer extraction time. On the other hand, 10% acetonitrile at a volume of 18 mL showed optimum extraction efficiency. Thus, all succeeding studies were carried out at this volume and acetonitrile concentration.

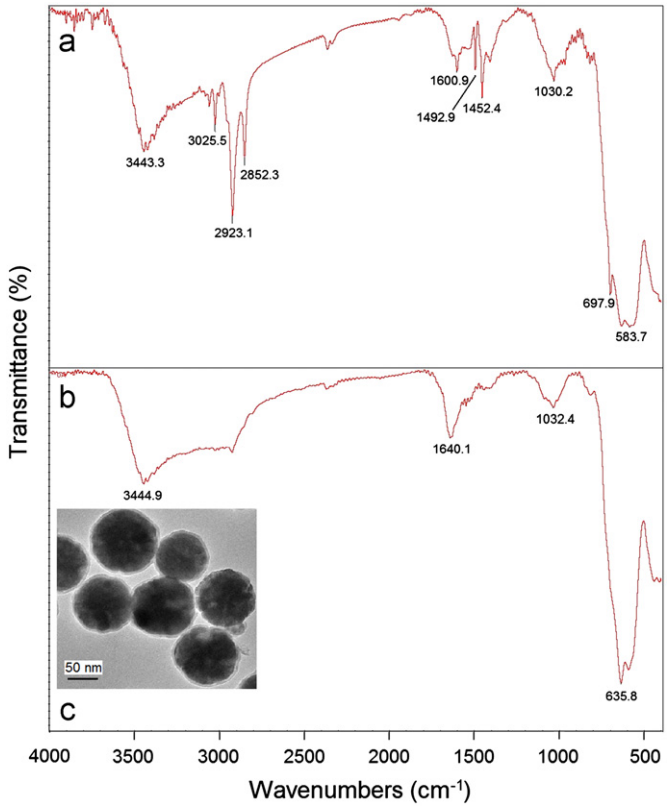

Fig. 3. Characterization of OA-MNBs. (a) FTIR patterns of magnetic beads coated with carboxyl group, (b) FTIR patterns of magnetic beads coated with oleic acid, (c) TEM image of oleic acid-coated magnetic nanobeads.

3.2.2. Effect of pH

The pH of the solution may affect the hydrophobic interaction between OA-MNBs and LMG by altering the surface charges. The surface charge of the oleic acid modified magnetic beads was almost zero and this remained constant even when the pH was changed indicating that the OA-MNBs were stable with respect to pH changes.

There was no available information on the pKa of LMG, but it is predicted to be alkaline due to the presence of two basic amines [4]. The effect of pH on the adsorption of LMG on OA-MNBs was evaluated in 18 mL extraction solution (which contained 10 ng LMG, 1.2 mL McIlvaine buffer, and 1.8 mL acetonitrile in 15 mL pure water), at a pH of 5.0–13.0 (that was achieved with dropwise addition of 10 M sodium hydroxide or 6 M hydrochloric acid). As seen in Fig. 4b, the MSPE using OA-MNBs extraction of LMG increased with increasing pH from 5.0 to 10.0, and reached a maximum at pH 10.0. Therefore, a pH of 10.0 was selected for all succeeding experiments.

3.2.3. Effect of the Ionic strength

The effect of ionic strength on the extraction efficiency of LMG shown in Fig. 4c revealed that the extraction was significantly enhanced by increasing the sodium chloride concentration from 0 to 1.5 M and remained constant from 1.5 to 3 M. It is inferred that the increase in salt concentration decreased the availability of water resulting in increased hydrophobic interaction between the LMG and the octadecenyl groups on the OA-MNBs. The sodium chloride concentration was maintained at 1.5 M in succeeding studies.

3.2.4. Effect of the amount of OA-MNBs

The effect of OA-MNBs concentration on the LMG extraction was investigated between 2 and 10 mg. The volume of the elution solvent was kept at 200 μL in order to achieve a high enrichment factor with the smallest amount of OA-MNBs. As shown in Fig. 4d, OA-MNBs from 2 to 6 mg resulted in an extraction efficiency from 56.2% to 92.8%. However, when the amount of MNBs was above 6 mg, LMG extraction showed a plateau that started at 6 mg. This indicated that the 200 μL extraction solution was maximized at 6 mg of OA-MNBs and therefore, a higher volume is required for higher amounts of MNBs. Thus, 6 mg of OA-MNBs was used with 200 μL extraction solution in succeeding studies.

Thus, optimal adsorption conditions were established for the MSPE extraction of 10 ng LMG with OA-MNBs. The optimized parameters were as follows: 3.1 mL of the 0.5 g fish sample extracts diluted to 18 mL with the extraction solution containing 10% acetonitrile and 1.5 M NaCl at a pH of 10.0 in the presence of 6 mg of OA-MNBs.

3.3. Adsorption isotherm

The adsorption of LMG on OA-MNBs was studied at 25 °C under the optimized adsorption conditions. The adsorption behavior could be described using the Freundlich equation given as

$$\ln q_e = \ln K_F + n \ln C_e$$

where K_F (mg/g) is the Freundlich constant and 'n' the Freundlich exponent; C_e is the equilibrium concentration (mg/L), q_e the amount of LMG adsorbed per gram of the OA-MNBs. KF and 'n' are constants incorporating all factors affecting the adsorption process such as adsorption capacity and intensity. Using the

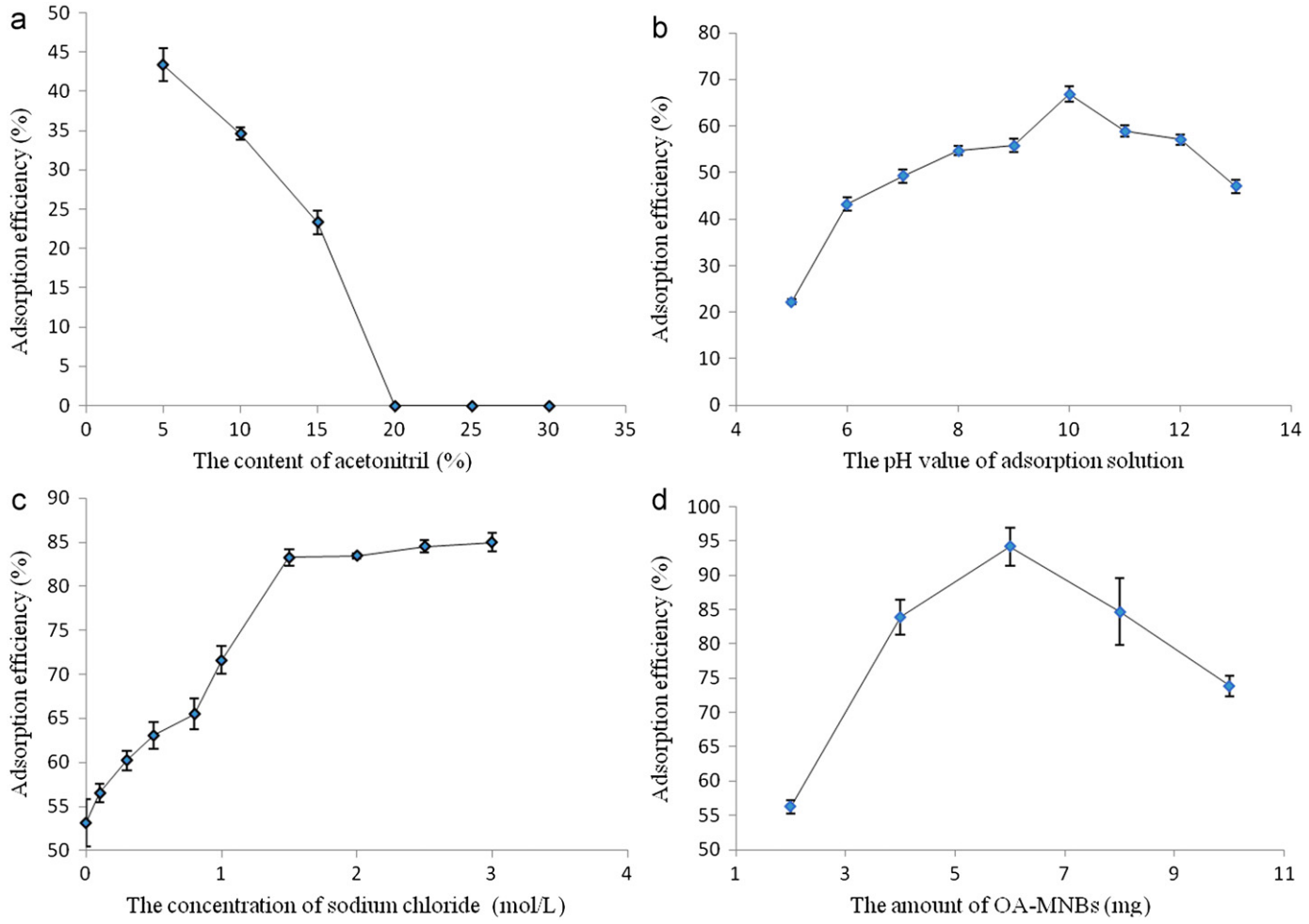

Fig. 4. Optimization of adsorption parameters of OA-MNBs for LMG from fish sample ($n=3$). (a) The effect of acetonitrile, 4 mg of OA-MNBs. (b) The effect of pH, 18 mL of adsorption solution containing 10% acetonitrile and 4 mg of OA-MNBs. (c) The effect of ionic strength, 18 mL of adsorption solution containing 10% acetonitrile at a pH of 10.0 and 4 mg of OA-MNBs. (d) The effect of the amount of OA-MNBs, 18 mL of adsorption solution containing 10% acetonitrile and 1.5 M NaCl at a pH of 10.0.

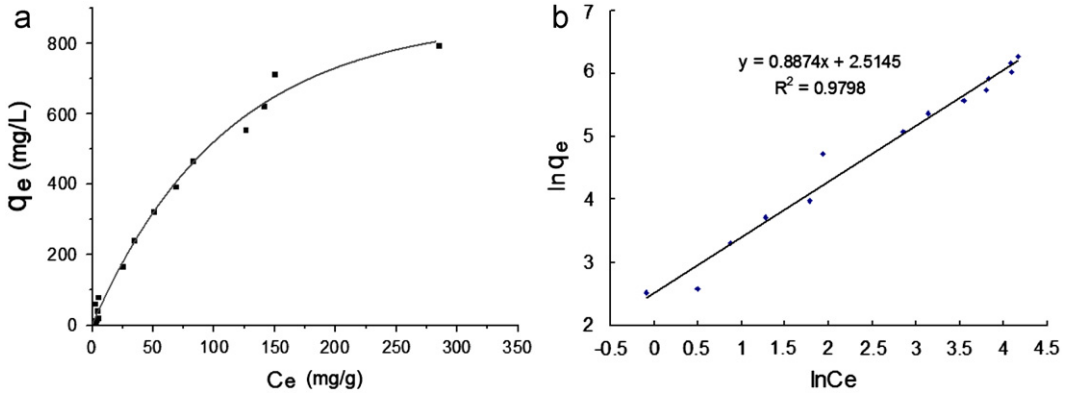

Fig. 5. Adsorption mechanism of LMG on OA-MNBs. (a) Adsorption isotherm of LMG on OA-MNBs; (b) Freundlich adsorption model for the adsorption of LMG on OA-MNBs.

equilibrium data shown in Fig. 5a, the plots of $\ln q_e$ versus $\ln C_e$ yielded a straight line (Fig. 5b) which indicated that the adsorption followed the Freundlich isotherm model. K_F and 'n' were calculated from the intercept and slope of the plot. The constants K_F and 'n' for LMG and OA-MNBs were 327 mg/g and 1.127, respectively. The values of 'n' lying between 1 and 10 indicated favorable adsorption [32].

3.4. Method evaluation

To validate the feasibility of the MSPE using OA-MNBs for the extraction of trace amounts of LMG from fish samples, LMG free fish samples were spiked with different concentrations of LMG (0.1–2.0 ng/g) to perform a comparison of % recovery with classical liquid–liquid extraction sample preparation. The LMG

extracts from both sample pretreatment methods were determined by triple quadrupole LC-MS/MS method to confirm the amount of LMG extracted. The calibration curve in the matrix blank extract given by $y=0.9636x+0.4378$ ($R^2=0.997$) for LMG concentration in the ranges of 0.1–40 ng/mL was obtained using the internal standard method. Deuterium-labeled LMG (LMG-D_6) was used as the internal standard to compensate the interfering effects from sample matrixes.

The results of both sample pretreatment methods are shown in Table 1. The average recoveries using MSPE with OA-MNBs at 0.1, 0.15, 0.2, 0.3 and 0.5 ng/g of spiked LMG ranged from 71.2% to 112.6% (RSD 0.6%–9.1%). The classical method exhibited no LMG detectable signals when the LMG was below 0.2 ng/g. Furthermore, LLE showed less than 50% recovery when the spiked LMG was at 0.3–0.5 ng/g. Overall, the MSPE using OA-MNBs exhibited an improved extraction efficiency with a recovery rate at $>70\%$ for trace amounts of LMG that was as low as 0.1 ng/g. In comparison, the traditional liquid–liquid extraction technique (LLE) was only able to achieve down to 0.5 ng/g.

3.5. Analysis of field fish samples

In order to further verify the reliability of the MSPE using OA-MNBs, 8 samples of fish that were confirmed LMG positive by triple quadrupole LC-MS/MS system using the classical sample pretreatment method, were analyzed. Fig. 6a shows the chromatogram of MG in standard solution which exhibited a good LC separation in a single run, while Fig. 6b shows the real sample containing more than one peak in the chromatogram. The eight samples analyzed showed LMG at the range of 1.17–0.22 ng/g (RSD 0.8%–4.5%) using the MSPE with OA-MNB extraction and 1.14–0.1 ng/g (RSD 1.7%–13.3%) using the classical LLE (shown in Table 2). A comparison between the measured values of the two extraction methods was performed using a Student's *t*-test with a confidence level of 95%. The results demonstrated that the measured values of the MSPE with OA-MNBs were significantly higher than those of the classical sample method when the LMG residue concentration was lower than 0.5 ng/g (samples 2, 4, 5, 6 and 8). There was no significant difference found between the measured values of the two extraction methods when the LMG residue level was higher than 0.5 ng/g (samples 1, 3 and 7). This shows that the MSPE with OA-MNB collected more analyte than the classical LLE when the LMG residue concentration in fish was less than 0.5 ng/g. This was in agreement with the results of the recovery experiments from the spiked fish samples.

4. Conclusions

The MSPE using OA-MNBs showed significant advantages compared with the classical LLE method for fish muscle samples containing trace amounts of LMG below 0.5 ng/g. In addition, this system eliminated the use of evaporator, vacuum manifold and expensive multiple organic solvents. MSPE with OA-MNBs reduced the sample processing and analysis time from 2 h to less than 40 min and the recovery was enhanced by as much as 70% for concentrations as low as 0.1 ng/g. Considering the toxic effects of LMG and by products, an improved extraction method such as the MSPE with OA-MNBs that is rapid, easy to use, efficient, and can be used to recover very low levels in fish and fish products is essential. The MSPE with OA-MNBs holds promise for rapid on-site processing of complex samples for LMG analysis.

Table 1
Recovery and precision of LMG from fish muscle spiked with different amounts of LMG ($n=3$).

spiked level (ng/g)	Recovery (%, $n=3$)	RSD (%)	Recovery (%, $n=3$)	RSD (%)
	MSPE[a]		LLE[b]	
0.1	71.2	3.6	ND	ND
0.15	81.7	1.1	ND	ND
0.2	81.0	7.2	ND	ND
0.3	86.0	0.6	21.1	5.5
0.5	112.6	9.1	48.9	2.2
1	102.3	2.0	87.7	5.9
2	102.2	2.3	95.3	0.7

ND: Not detectable.

[a] MSPE: oleic acid coated magnetic nanobeads based magnetic solid phase extraction.

[b] LLE: traditional liquid–liquid extraction described in the national standard GB/T19857-2005 (China).

Table 2
Analysis of field fishery products of LMG positive with OA-MNBs-based MSPE ($n=3$).

Sample number	LMG concentration (ng/g, mean ± S.D.)	
	MSPE	LLE
1	0.73 ± 0.02	0.76 ± 0.03
2	0.29 ± 0.01	0.15 ± 0.02
3	1.09 ± 0.04	1.07 ± 0.03
4	0.23 ± 0.01	0.10 ± 0.01
5	0.41 ± 0.01	0.25 ± 0.01
6	0.23 ± 0.01	0.12 ± 0.01
7	1.17 ± 0.01	1.14 ± 0.02
8	0.22 ± 0.01	0.10 ± 0.01

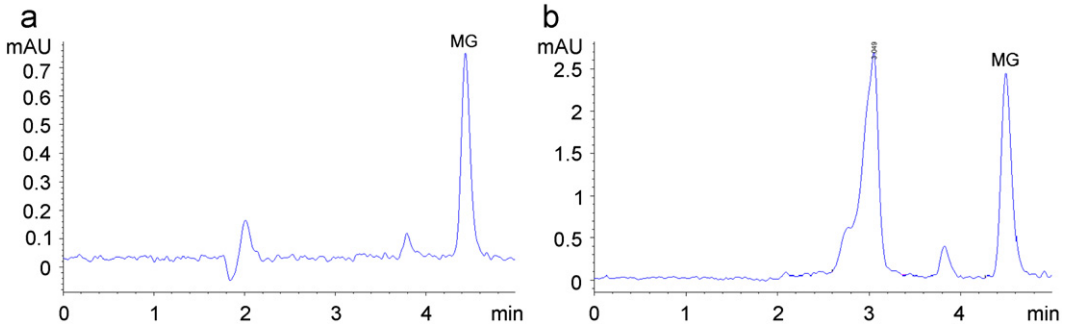

Fig. 6. HPLC chromatograms of (a) 10 ng/mL MG standard solution and (b) MSPE extract from 0.5 g blank fish sample spiked with 20 ng/g of LMG.

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