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Preparation of amino acid-based polymer functionalized magnetic nanoparticles as adsorbents for analysis of plant growth regulators in bean sprouts

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ABSTRACT

A novel magnetic solid phase extraction (MSPE) adsorbent has been developed for enriching two plant growth regulators, including 2,4-dichlorophenoxyacetic acid (2,4-D) and 4-chlorophenoxyacetic acid (4-CPA), in bean sprouts. For preparing the MSPE adsorbent, poly(N-methacryloyl-L-phenylalanine methyl ester (P(MA-L-Phe-OMe)), amino acid-based polymer, was modified onto the magnetic nanoparticles via "grafting to" method by free radical polymerization. The resultant P(MA-L-Phe-OMe)-functionalized magnetic nanoparticles (Fe_3O_4@P(MA-L-Phe-OMe)) were characterized by Fourier transform infrared (FT-IR) spectroscopy and elemental analysis. The adsorption amount of Fe_3O_4@P(MA-L-Phe-OMe) nanoparticles to 2,4-D and 4-CPA were 39.82 mg g^{-1} and 29.02 mg g^{-1}, respectively. Moreover, the prepared MSPE adsorbents showed good selectivity towards 2,4-D and 4-CPA due to the hydrophobic interactions and electrostatic forces between the target analytes and Fe_3O_4@P(MA-L-Phe-OMe). The results demonstrated that the proposed MSPE adsorbents have high affinity to the targets 2,4-D and 4-CPA. Under the optimized conditions, the proposed materials were successfully applied to enrich 2,4-D and 4-CPA in bean sprouts samples. The recovery values of the bean sprouts solution spiked the targets were from 90.9% to 96.4% with the relative standard deviations of 2.3–3.9%. Our work proved that the novel Fe_3O_4@P(MA-L-Phe-OMe) nanoparticles were the good adsorbents of magnetic solid phase extraction (MSPE) and have good potential for the analysis of trace compound in real samples.

1. Introduction

In the process of modern agricultural production, phytohormones have great influence on the development and growth of plants [1,2]. They could promote the reproductive growth of plant and increase crop yields at low concentration, such as the growth of cell, the formation of organ and the germination of seed. Nowadays, various types of the phytohormones have been developed and exploited, such as α-naphthaleneacetic acid (NAA), gibberellic acid (GA), 4-chlorophenoxyaceticacid (4-CPA), indole-3-acetic acid (IAA) and 6-benzylamino adenine (6-BA). Among them, 2,4-dichlorophenoxyacetic acid (2,4-D) and 4-chlorophenoxyaceticacid (4-CPA) are two kinds of common phytohormones in agricultural production, which are usually used to promote cell proliferation and suppress the growth of root cells. However, they have some toxicity and are easily accumulated in bean sprouts. The excess intake have potential health hazards, such as increasing the risk of tumor formation and damage the human nervous system [3,4]. Up to date, many methods, such as high performance liquid chromatography (HPLC) [5–7], gas chromatography mass spectrometry (GC–MS) [8–10], liquid chromatography-tandem mass spectrometry (LC-MS) [11,12], enzyme-linked immunosorbent assay (ELISA) [13,14], have been employed to determine phytohormones in real samples. For example, Ma and colleagues have analyzed the phytohormones in coconut water by HPLC and liquid chromatography-tandem mass spectrometry (LC-MS/MS) based on the procedure of SPE.[1] However, the determination of 2,4-D and 4-CPA in bean sprouts are still challenge because of the low concentrations. Therefore, the efficient sample enrichment is necessary for 2,4-D and 4-CPA prior to the instrument analysis.

Solid-phase extraction (SPE), due to its strong enrichment capability, good reproducibility, simplicity of operation and low

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solvent consumption, has become one of the most commonly used sample pretreatment techniques [15–18]. Up to date, many kinds of SPE adsorbents have been developed for enrichment of phytohormones in food samples [2,19], such as conventional calixarenes and C_{18} cartridges. But most of them still suffered from the limitations of expensive materials, the strong reliance on certain conditions, complex extraction procedures, and so on. In recent decades, magnetic solid phase extraction (MSPE), as a new kind of SPE, has received great attentions for its characterization, such as time-saving, rapid and effective [20–23]. Moreover, as the substrate of MSPE adsorbents, Fe_3O_4 magnetic nanoparticles have shown their advantages in easy preparation, large surface areas, good stability, and so on.

In view of the specificity, affinity and capacity of MSPE approaches, it is vital to select a appropriate MSPE adsorbent prior to the extraction procedures [15,24]. As is well-known, amino acid-based polymers is a kind of functional materials, which have good biocompatibility, easy available. Moreover, their chain skeletons contain multiple functional groups, such as benzene ring, amino group, and so on. Therefore, we assume that the amino acids-based polymer functionalized magnetic nanoparticles should have great potential in adsorption of 2,4-D and 4-CPA via multiple interactions.

In this work, poly(N-methacryloyl-L-phenylalanine methyl ester) (MA-L-Phe-OMe)-based polymers were introduced into the fabricated MSPE adsorbent. P(MA-L-Phe-OMe)-functionalized magnetic nanoparticles Fe_3O_4@P(MA-L-Phe-OMe) were synthesized via surface free radical polymerization, and were further used as MSPE adsorbents for preconcentration of 2,4-D and 4-CPA. The resultant P(MA-L-Phe-OMe)-functionalized nanoparticles showed their good adsorption capacity and unique selectivity due to the hydrophobic interactions and electrostatic forces. Moreover, the proposed nanoparticles were further applied to enrich the desired analytes in bean sprouts.

2. Experimental section

2.1. Materials and chemicals

$FeCl_3 \cdot 6H_2O$ ($\geq$ 99%), $FeCl_2 \cdot 4H_2O$ ($\geq$ 99%), ammonia (25 wt%), tetrahydrofuran (THF), sodium hydroxide (NaOH), sodium chloride (NaCl), sodium bicarbonate ($NaHCO_3$) were supplied by Beijing Chemical Plant. γ-methacryloxypropyltrimethoxysilane (MPS, 98%) were purchased from Beijing InnoChem Science and Technology Co. Ltd. (Beijing, China). 4-chlorophenoxyacetic acid, indole-3-acetic acid (IAA), 6-Benzylamino (6-BA), L-phenylalanine methyl ester hydrochloride (L-Phe-OMe · HCl, 98%), styrene were provided by Aladdin Industrial Corporation (Shanghai, China). HPLC grade methanol was purchased from Beijing Sheng Shi Chuang Qi technology (China, Beijing). Ultra-pure water used in the experiment was deionized by Milli-Q system (Millipore, USA).

2.2. Apparatus

Fourier transform infrared (FT-IR) analysis in KBr medium was recorded on the Bruker Tensor-27 spectrophotometer. The elemental analysis for the synthesized nanoparticles was carried out using the Flash EA 1112 elemental analyzer which was produced by ThermoFinnigan (Milan, Italy). The liquid chromatographic system was an Shimadzu Prominence LC-10A HPLC system (Tokyo, Japan) with CenturySIL C18 BDC (5 μm, 150 mm × 4.6 mm ID) column (Dalian, China).

2.3. Preparation of P(MA-L-Phe-OMe)-functionalized magnetic nanoparticles

Before the Fe_3O_4@P(MA-L-Phe-OMe) nanoparticles were prepared, the functional monomer MA-L-Phe-OMe and Fe_3O_4 nanoparticles was synthesized according to the reported literature [19,25]. In order to introduce the vinyl groups onto the surface of the Fe_3O_4 nanoparticles for polymerization reaction, Fe_3O_4@MPS nanoparticles was synthesized as following steps: Firstly, 100.0 mg Fe_3O_4 nanoparticles was added into a flask (100.0 mL) containing 50.0 mL alcohol. Then 2.0 mL MPS was added and the resultant mixture was vigorous stirred at 40 °C for 12 h. Subsequently, the obtained nanoparticles were separated and washed with ultra-pure water and alcohol. Finally, the product, Fe_3O_4@MPS nanoparticles, was dried at 40 °C under vacuum.

Fe_3O_4@P(MA-L-Phe-OMe) nanoparticles were prepared by free radical polymerization, which poly(MA-L-Phe-OMe) were grafted onto the surface of Fe_3O_4 nanoparticles. The typical synthesis procedure was as follows: 150.0 mg Fe_3O_4@MPS nanoparticles, MA-L-Phe-OMe (160.0 mg, 642.6 μmol) as the functional monomer, azodiisobutyronitrile (AIBN) as the initiator (4.0 mg, 24.40 μmol) were placed in a flask (100.0 mL) with 60.0 mL THF. Then the solution was stirred and refluxed for 24 h at 80 °C. After the polymerization process, the fabricate Fe_3O_4@P(MA-L-Phe-OMe) nanoparticles were washed and dried under vacuum for 12 h at 40 °C.

2.4. Procedure for real sample pretreatment

Fresh bean sprouts were purchased from local farmers markets (Beijing, China). The bean sprouts (500.0 mg) were ground. Then, the obtained tissue fluid of bean sprouts was diluted with 25.0 mL water. The resultant mixture was added with 1.0 M NaOH solution until the pH of the solution was at 11.0 and homogeneously mixed by ultrasound for 30 min The residual solid was removed by filtration. Subsequently, the resultant filtrate was extracted with trichloromethane (25.0 mL). The aqueous phase was added with the mixture of NaCl (2.0 g), hydrochloric acid (HCl, 0.5 mL) and alcohol (1.0 mL) in order to prevent from emulsification, and then homogeneously mixed. Then, the solution was washed twice with diethyl ether and petroleum ether (V:V=1:1). The obtained organic phase was further washed twice with $NaHCO_3$ solution (0.1 M, 5.0 mL). The aqueous phase was added successively with HCl (12.0 M, 0.5 mL), diethyl ether (10.0 mL). Finally, the ether layer was subjected to distillation to remove the organic solvent. Finally, the sample solution was obtained and stored at 4 °C.

2.5. Procedure for real sample pretreatment

The adsorption experiments were performed as follows: 5.0 mg dried Fe_3O_4@P(MA-L-Phe-OMe) nanoparticles was displaced into standard 2,4-D solution (3 mL, 0.15 mg mL^{-1}). After the above mixture was stirred for 30 min, the magnetic nanoparticles were separated by a magnet. The adsorption capacity (Q) of Fe_3O_4@P(MA-L-Phe-OMe) nanoparticles to 2,4-D was indirectly calculated with the residue of 2,4-D according to the followed equation:

$$Q = (C_0 - C)V/m$$

Where C_0 (mg mL^{-1}) is the original target concentration, C (mg mL^{-1}) is the residual target concentration after adsorption with the prepared materials, V (mL) is the volume of standard target solution, m (mg) is the amount of Fe_3O_4@P(MA-L-Phe-OMe) nanoparticles.

Similarly, the Q of Fe_3O_4@P(MA-L-Phe-OMe) nanoparticles to 4-CPA was calculated. Compared with the adsorption study to 2,4-D, the incubation time for 4-CPA was 15 min.