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Preparation of magnetic multi-functional molecularly imprinted polymer beads for determining environmental estrogens in water samples

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HIGHLIGHTS

- ▶ We design a novel MIP bead with a superparamagnetic supporter.
- ▶ We prove that MIP bead is an efficient SPE adsorbent.
- ▶ We develop method for determining many estrogens simultaneously.
- ▶ We make the determination of estrogens more economical and efficient.

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ABSTRACT

Magnetic multi-functional molecularly imprinted polymer (mag-MFMIP) beads were prepared for determining environmental estrogens in water samples. This novel material adopted a superparamagnetic microparticle as the supporter and used 17β-estradiol (E2) as the imprinting template, aiming for recognizing many kinds of environmental estrogens simultaneously. Characterization analysis and binding experiments revealed that mag-MFMIP beads had outstanding magnetic property, large adsorption capacity and high competitive selectivity to most of the commonly seen environmental estrogens. When mag-MFMIP beads were used as dispersed solid-phase extraction (SPE) adsorbents in water samples, the recoveries of estriol (E3), bisphenol A (BPA), E2 and ethynylestradiol (EE) were 72.2 – 92.1%, 89.3 – 96.0%, 93.3 – 102% and 89.7 – 95.9%, respectively with relative standard deviation (RSD) lower than 7.0%. These results indicated that mag-MFMIP beads had the potential to be the common adsorbents for many kinds of environmental estrogens, representing a novel application of MIPs in the field of environmental chemistry.

1. Introduction

There are increasing health concern about environmental estrogens as these chemicals have shown many adverse effects on the endocrine system in wildlife and humans [1,2]. Environmental estrogens include natural estrogens such as 17β-estradiol (E2) and estriol (E3), and synthetic estrogens such as bisphenol A (BPA) and ethynylestradiol (EE). E2 and E3 have been reported to induce vitellogenin production, disrupt vital systems in aquatic organisms [3–5], as well as increase the risk of cancer [6–8]. Synthetic estrogens also can interfere with hormonal functions. For example, BPA was found to disrupt thyroid hormone action [9], cause proliferation of human prostate cancer cells and block testosterone synthesis at extremely low concentrations [10,11].

Thus it is important to monitor natural and synthetic estrogen concentrations in various environmental matrixes, yet this has been shown to be a challenge task due to their low concentrations and the complexity of various matrixes. To reduce the matrix interference as well as enrich the analyte, pre-treatment processing is often necessary before instrumental analysis. Solid-phase extraction (SPE) is currently the most popular pre-treatment technique for extracting and enriching analyte from environmental samples [12]. Because SPE adsorbent directly determines the efficiency of extraction, development of new SPE adsorbent has gained increased interest [13].

Among various SPE adsorbents, molecularly imprinted polymer (MIP) is a kind of synthetic polymer that can recognize targets with high selectivity as it has specific binding sites with complementary size, shape, and functional groups to the template molecule, and has been studied intensively in recent years [14–17]. For example, a series of MIPs using 17β-estradiol or its structural analogs as the template were prepared and used as dispersive SPE adsorbents to enrich natural environmental estrogens from various samples,

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including water, plasma, dairy, meat, fishery and feeds [18–27]. As a typical synthetic environmental estrogen, BPA was of particular concern, and many recent studies reported preparation and application of BPA-MIPs [17,28–34]. Together, all these studies revealed that MIP had advantages of short separation time, high selectivity, fast binding kinetics and large adsorption capacity.

Currently, MIP materials used in most studies were limited to one target recognition and enrichment. To further improve the utilization efficiency of MIPs, a few studies recently developed multi-functional MIP (MFMIP), aiming for multiple target recognition during the pre-treatment sample process [22,23,26]. Given the fact that most environmental samples contain a mixture of chemicals, the use of MFMIP for simultaneous multiple target detection is not only necessary, but also more cost-effective than the conventional MIPs. However, the application of MFMIP for environmental estrogens has not been well explored. In this study, a novel MFMIP material that adopted a superparamagnetic microparticle as the supporter was designed and prepared (referred as mag-MFMIP beads hereafter), aiming for recognizing many kinds of environmental estrogens simultaneously. With a superparamagnetic supporter, mag-MFMIP beads can be dispersed into the solution directly and separated via external magnetic field, avoiding the process of making packed columns as in traditional SPE. Furthermore, mag-MFMIP beads, in coupled with high performance liquid chromatograph (HPLC), were applied for the selective extraction and determination of various environmental estrogens in water samples.

2. Experimental

2.1. Chemicals and reagents

17β-estradiol (E2), estriol (E3), ethinylestradiol (EE), bisphenol A (BPA), p-tert-butylphenol (BP), 3-(Trimethoxysily) propyl methacrylate (MPS), vinyltrimethoxysilane (VTTS) and 4-vinylpyridine (4-VP) were purchased from Sigma-Aldrich Co. LLC. Sodium dodecylsulfonate (SDS), iron (III) chloride hexahydrate ($FeCl_3{\cdot}6H_2O$), iron (II) chloride tetrahydrate ($FeCl_2{\cdot}4H_2O$), styrene (St), tetracethylorthosilicate (TEOS) were purchased from Xilong Chemical Plant (Shantou, China). Ethylene glycol dimethacrylate (EGDMA) was purchased from Dixiai Chemical Plant (Shanghai, China). Potassium persulfate (KPS) was purchased from United Initiators Co. Ltd (Shanghai, China). 2,2′-Azobis (2-methylpropionitrile) (AIBN) was purchased from Xiya Reagent Co. Ltd (Chengdu, China). Acetonitrile of HPLC grade was purchased from Merck (Darmstadt, Germany). All reagents were of analytical grade and all water used was double distilled.

2.2. Fabrication procedure of mag-MFMIP beads

The fabrication procedure of mag-MFMIP beads mainly included two main steps: synthesis of the magnetic supporter of MFMIP (Fe_3O_4/Poly(St-co-MPS)/SiO_2) and preparation of MFMIP at the surface of supporter (Fig. 1). The detailed procedure for Fe_3O_4/Poly(St-co-MPS)/SiO_2 synthesis has been reported previously [35]. In brief, Fe_3O_4 nanoparticles were obtained by co-precipitation of $FeCl_2$/$FeCl_3$ (1.25 g $FeCl_2{\cdot}4H_2O$, 3.2 g $FeCl_3{\cdot}6H_2O$) according to Massart's method [36], and they were subsequently modified with 0.5 mL oleic acid. Then Fe_3O_4/Poly(St-co-MPS) (2.8 mL St, 1.2 mL MPS) particles were prepared according to Yan's method [37]. Finally, Fe_3O_4/Poly(St-co-MPS) particles were coated with SiO_2 by adding 0.3 mL TEOS to produce Fe_3O_4/Poly(St-co-MPS)/SiO_2 particles, which were adopted as the supporters of MFMIP in the following procedures.

Before preparation of MFMIP, the surface of Fe_3O_4/Poly(St-co-MPS)/SiO_2 was modified chemically. In detail, 200 mg of Fe_3O_4/Poly(St-co-MPS)/SiO_2 particles, 10 mL aqueous ammonia, and 0.5 mL VTTS were sequentially dispersed in 150 mL ethanol, 10 mL aqueous ammonia, and 0.5 mL VTTS. After stirred for 8 h at room temperature, the products were collected by a magnet and washed by ethanol. The obtained material, modified Fe_3O_4/Poly(St-co-MPS)/SiO_2, was dispersed in 60 mL toluene, and then 55 mg E2 (template), 92 μL 4-VP (functional monomer), 160 μL EGDMA (cross-linker) and 16.8 mg AIBN (initiator) were added and dissolved. The above reaction was carried out at 70 °C under nitrogen for 24 h. The products were washed three times with methanol and acetic acid (9:1, v/v), and then Soxhlet extraction was carried out to remove the templates completely. The final products, mag-MFMIP beads, were neutralized with methanol and dried at vacuum for 12 h.

The fabrication procedure of non-imprinted polymer (NIP) beads was identical to that of mag-MFMIP beads but without adding template.

2.3. Characterization and binding tests of mag-MFMIP beads

Fourier transform infrared spectra (FTIR) were recorded by a Nicolet 6700 instrument. Scanning electron microscopy (SEM) experiment was performed on a Hitachi S-3000 N microscope with a voltage of 10.0 kv and a magnification of 7000, while transmission electron microscopy (TEM) experiment was performed on a FEI Tecnai G20 microscope with a voltage of 200.0 kv and a magnification of 19,500. Magnetization curve was obtained by a Lake Shore 7410 vibrating sample magnetometer at 298 K.

To obtain the adsorption isotherms, 10 mg of mag-MFMIP beads or NIP beads were dispersed in 10 mL toluene/methanol solutions (19:1, v/v) with different E2 concentrations (0.05 – 1.5 mg mL^{-1}). Similarly, the adsorption kinetic curves were obtained by determining the temporal evolution of E2 concentration (initial of 0.5 mg mL^{-1}) in the solutions. The amount of E2 binding on the mag-MFMIP beads was determined by the difference between the total and residual E2 in the solution. The measurement of E2 was carried out with an HPLC (Agilent 1200). The HPLC column was Agilent XDB-C18, and the mobile phase of HPLC was H_2O/acetonitrile (50:50, v/v) with a flow rate of 0.2 mL min^{-1}. The excitation wavelength of fluorescence detector was 229 nm, while the emission wavelength was 315 nm. The limit of detection (LOD) was calculated at three times of the signal-to-noise ratio.

The adsorption capacity Q (mg g^{-1}) was calculated according to the following equation:

$$Q = \frac{(C_o - C_f)V}{m} \tag{1}$$

where C_o (mg mL^{-1}) and C_f (mg mL^{-1}) were the initial and final concentration of E2, respectively, V (mL) was the total volume of the sample, and m was the mass of mag-MFMIP beads.

The saturated adsorption capacity was obtained by Langmuir adsorption equation:

$$Q = Q_{\max}\frac{KC_e}{1 + KC_e} \tag{2}$$

where Q and Q_{max} (mg g^{-1}) were experimental adsorption amount of E2 and saturated adsorption capacity of mag-MFMIP beads, respectively, C_e (mg L^{-1}) was the corresponding concentration of E2 in solution, and K (L g^{-1}) was the Langmuir adsorption equilibrium constant.

To evaluate the competitive adsorption capability of mag-MFMIP beads, a series of adsorption experiments were carried out in E3/BPA/E2/EE/BP solutions. The repeated bind/removal experiments were performed in E2 solution only.