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Determination of diethylstilbestrol in seawater by molecularly imprinted solid-phase extraction coupled with high-performance liquid chromatography

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ABSTRACT

An effective and highly selective molecularly imprinted material was prepared by suspension polymerization for the isolation and pre-concentration of synthetic estrogen diethylstilbestrol (DES) in seawater. The obtained MIPMs were proved to have more uniform size and porous structure, with maximum adsorption capacity of 8.43 mg g^{-1} almost two times more than NIPMs (4.43 mg g^{-1}). The MIPMs showed no significant deterioration of the adsorption capacity after five rounds of regeneration. An off-line molecularly imprinted solid phase extraction (MISPE) method followed by HPLC–DAD was proposed for the detection of DES in seawater, and recoveries were satisfactorily higher than 77%. Four seawater samples in aquaculture area were analyzed and 0.61 ng mL^{-1} DES was detected in one sample. The result demonstrated that this method can be used for the rapid separation and clean up of trace residual of DES in seawater.

1. Introduction

Diethylstilbestrol (DES) as a kind of synthetic estrogen is used as a medicine to prevent the spontaneous abortion clinically and cure the deficiency of estrogen (Giusti et al., 1995). As DES can improve the growth rate of animals and increase the protein content, it was once used as a growth promoter in livestock and aquaculture (Colborn et al., 1993; Liu et al., 2008). DES can remain in the body of human and animals longer and more stable than the natural estrogen (Tapiero et al., 2002a, 2002b), however, long-term intake of DES may lead to endocrine disorders and increase the incidence of cancer with very low concentration (Lucci et al., 2011). It has drawn extensive societal attention and many countries have banned the use of DES as growth promoter. The hazardous material DES is now considered as the endocrine disrupter reference compound in the US Protection Agency List (Judson et al., 2009). Although it had been prohibited as growth promoter for years, DES is still found in the river, fish, milk and meat of some livestock in many countries (Tang et al., 2015; Yin et al., 2016; Bravo et al., 2007; Liu et al., 2014; Xiong et al., 2014). Finding a fast and effective method to detect the concentration of DES has an important influence on avoiding unnecessary damage of excess amount of the hazardous estrogen on human.

Traditional methods such as immunoassay (Tang et al., 2015), Gas Chromatography–Mass Spectrometry (Yuan et al., 2015; Hu et al., 2014), Liquid Chromatography–Mass Spectrometry (Magrini et al., 2015), High Performance Liquid Chromatography–Diode Array Detector (Penalver et al., 2002; Zou et al., 2015) and micellar electrokinetic chromatography (Wen et al., 2013) are usually carried out for the determination of DES. Immunoassay is sensitive but lacks selectivity towards the estrogen analogues. Due to the low concentration of DES and complex compounds in environment matrix, chromatography methods mentioned above seldom meet the requirement. High efficiency clean up and pre-concentration procedures are required prior to analysis. Solid phase extraction (SPE) is the most popular method for sample enrichment due to the convenient application process and low cost. However, the lack of selectivity and the low recovery limit the spread of SPE (de Alda and Barceló, 2001). The new emerging sorbent, molecularly imprinted polymers (MIPs), possessing the characteristics of high selectivity, high binding capacity, stability, easy preparation and reusability (Stevenson, 1999), have been widely used (Chen et al., 2011). The combination of MIPMs with SPE takes advantage of the high selectivity of the sorbent and the traditional extraction for the enrichment and high efficient clean-up samples of various trace analytes in diverse matrices (Xu et al., 2014), owing to the unique imprinting and recognition properties.

Currently, MIP as SPE sorbent to pre-concentrate and separate DES in river water, pond water, tap water (Lucci et al., 2011; Xie et al., 2015), milk (Liu et al., 2010; Li et al., 2014) and fish meat (Zhao et al., 2009) have been demonstrated. However the application of molecularly imprinted solid-phase extraction (MISPE) in the marine system is rare. The complex matrix and high concentration of salt in seawater will disrupt and damage the binding force between template and functional monomer and even affect the rebinding ability of the sorbent towards the template. Finally, it

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will lead to the decrease of the binding capacity and specific selectivity (Lian and Wang, 2013b). The molecularly imprinted polymer microspheres (MIPMs) have uniform spheres which have larger specific surface area and much stable than the traditional bulk and film imprinting method with irregular shapes. Besides, the molecularly imprinted polymer microspheres (MIPMs) have higher selectivity, better monodispersity, more homogenous size distribution and easier access to the imprinting site. The desired size of molecularly imprinted polymer microspheres can be easily obtained through certain stirring rate. Based on our previous studies of MISPE (Song et al., 2009, 2012; Lian and Wang, 2012, 2013a, 2013b; Lian et al., 2014, 2015), this method reached the desired results and played an important role in sample clean-up and preconcentration prior to sample determination. To the best of our knowledge, there is no application of MIPMs for pre-concentration and determination of DES in seawater.

This paper also aims at preparing MIPMs for the selective preconcentration of DES in seawater which can be used in monitoring and preventing the adverse influence of DES on environment. The MIPMs was synthesized by suspension polymerization method using DES as the template, methacrylic acid (MAA) as the function monomer and ethyleneglycol dimethacrylate (EGDMA) as the cross-linker. FT-IR spectra and scanning electron microscopes were used for the characterization of MIPMs and NIPMs. Static adsorption experiment was used to evaluate the selectivity and binding capacity of MIPMs and NIPMs towards DES. The obtained MIPMs coupled with SPE performed good adsorption and selectivity towards DES, which provide a fast pre-concentration step for determination of DES in spiked seawater samples.

2. Experiment

2.1. Chemicals

Diethylstilbestrol (DES) was purchased from TCI (Japan) and methanol of high performance liquid chromatography grade was obtained from Merck (Darmstadt, Germany). Methacrylic acid (MAA) and 2, 2-azoisobutyronitrile (AIBN) were obtained from Kermel Chemical Company (Tianjin, China). Ethyleneglycol dimethacrylate (EGDMA) was obtained from Alfa. Polyvinyl alcohol (PVA) was obtained from Aladdin Reagent Company (Shanghai, China). All of the water used in the experiments were produced by a Millipore Milli-Q purification system (Milli-pore, Bedford, MA, USA). All reagents were at least HPLC grade or analytical grade unless specified. Stock solution of DES was initially prepared at 1000 mg L^{-1} in methanol and stored at 4 °C in the dark. Working solutions were diluted by methanol and Milli-Q water daily at various concentrations.

2.2. Synthesis of MIPMs and NIPMs

The molecularly imprinted polymer microspheres were synthesized through suspension polymerization. 1 mmol DES and 4 mmol MAA were dissolved in 15 mL chloroform by ultrasonic agitation for 15 min, then 20 mmol EGDMA and 60 mg AIBN were added to the mixture. Dispersing the mixture in the dispersant (dissolving 6 g dispersive reagent (PVA) with 150 mL water at 95 °C) in a 250 mL borosilicate glass bottle, equipped with a rubber cap. The solution was purged by dry nitrogen gas for 15 min to remove the oxygen. Finally, the bottle was set in a thermostated water bath at 60 °C for 24 h by stirring at 420 rpm in a sealed environment. The product was extracted with a mixture of methanol:acetic acid (v:v, 9:1) using a Soxhlet apparatus for 48 h. Finally, the polymer was washed repeatedly with 5 mL methanol followed by 5 mL water until no DES was detected and dried under vacuum at 40 °C. The NIPMs were obtained and washed using the same formulation in the absence of the template molecule.

2.3. Characterization of the MIPMs and NIPMs

A Hitachi S-4800 cold field-emission scanning electron microscope (SEM, Tokyo, Japan) was used to observe the surface morphology of the microspheres. Samples were fixed on the conducting resin and coated with a thin layer of gold under vacuum conditions. The FT-IR spectra was recorded on an Avatar-360 Spectrometer (Nicolet Instrument Corporation, USA) using KBr disc method in the 4000–500 cm^{-1} range with a resolution of 4 cm^{-1}. Nitrogen adsorption–desorption results were recorded using Autosorb-IQ-MP-VP volumetric adsorption analyzer (U.S.A.). The BET method was employed to determine the specific area of the obtained polymers.

2.4. HPLC conditions

The chromatographic analyses were performed using a Hitachi L-2000 series HPLC system, equipped with a binary pump, an autosampler, a column compartment and an on-line vacuum degasser. Analytical wavelength of the diode-array detector (Model L-2455) was set at 241 nm. The analytical column was a 250 mm × 4.6 mm, 5 μm LaChrom C18 column (Hitachi, Japan) with isocratic elution. The mobile phase was composed of methanol and Milli-Q water (70:30, v:v) with the flow rate of 1.0 mL min^{-1}. The injection volume was 20 μL and the temperature of the column was 25 °C.

2.5. Adsorption experiment

20.0 mg of MIPMs and NIPMs were mixed with 2.0 mL 20 mg L^{-1} DES in methanol/water (60:40, v:v) for the kinetic adsorption experiment. The mixtures were then mechanically shaken at 300 rpm for 1, 2, 4, 6, 8, 10, 20 and 24 h at 25 °C in a water bath. After binding, the mixtures were filtered through a 0.22 μm membrane and DES was determined by HPLC. The adsorption capacity was calculated by binding experiments according to Eq. (1) (García-Calzón and Díaz-García, 2007).

As to the static adsorption experiment, 20.0 mg of MIPMs and NIPMs were mixed with 2.0 mL various concentrations of DES (10–300 mg L^{-1}) in methanol/water (60:40, v:v), respectively. The mixtures were shaken and processed in the same way as the kinetic adsorption experiment, except that the shaking time is 24 h. The adsorption capacity and the equilibrium dissociation constant (Kd, mmol L^{-1}) of MIPMs and NIPMs were calculated by binding experiments according to Eqs. (1) and (2) (García-Calzón and Díaz-García, 2007):

$$Q = \frac{(C0 - Cf)v}{m} \quad (1)$$

$$\frac{Q}{Cf} = -\frac{1}{Kd}Q + \frac{Q\,\max}{Kd} \quad (2)$$

where C_0 (mg L^{-1}) and C_f (mg L^{-1}) are the initial and final concentrations of DES, v (L) is the total volume of the sample, m (g) is the mass of MIPMs or NIPMs, Q and Q_{max} (mg g^{-1}) is the amount of DES adsorbed at equilibrium and saturation, respectively.

2.6. Preparation of MISPE column

The MISPE column was prepared using a wet packing method by packing 100 mg dry MIPMs in a 1 mL glass syringe which was thoroughly cleaned and dried. Two sieve plates were attached at the bottom end and top end of the column, respectively. Prior to loading the sample, the cartridge was washed with a mixture of methanol/acetic acid (95:5, v/v) to remove the residual template, until no DES could be detected in the filtrate. After that, the cartridge was conditioned with 5 mL methanol and 5 mL water.

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