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Highly class-selective solid-phase extraction of bisphenols in milk, sediment and human urine samples using well-designed dummy molecularly imprinted polymers☆

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ABSTRACT

Dummy molecularly imprinted polymers (DMIPs) towards bisphenols (BPs) were prepared employing 1,1,1-tris(4-hydroxyphenyl)ethane (THPE) and phenolphthalein (PP) as dummy templates. The selectivity of the resulting DMIPs was evaluated by high-performance liquid chromatography (HPLC). Both PP-DMIP and THPE-DMIP showed excellent class selectivity towards bisphenols. THPE-DMIP prepared using the template molecule with three hydroxyphenyl functionalities achieved higher imprinting factors (IF) for the bisphenols over a range of 7.9–19.8. An efficient approach based on dummy molecularly imprinted solid phase extraction (DMISPE) coupled with HPLC-DAD was developed for selective extraction of eight bisphenols in sediment, milk and human urine samples using THPE-DMIP as sorbents. The method showed good recoveries (82–102%) and precision (RSD 0.2–4%, $n = 3$) for these samples spiked at two concentration levels (25 and 250 ng g^{-1} or ng mL^{-1}). The detection limits ranged between 0.6 and 1.1 ng g^{-1} or ng mL^{-1}. Efficient removal of sample matrix and interferences was also achieved for these samples after DMISPE process. The results demonstrated great potential of the optimized methods for sample preparation in the routine analysis of trace BPs in complex samples.

1. Introduction

Bisphenol A (BPA) is an industrially important chemical that is abundantly and widely used as a primary raw material for the production of polycarbonate plastics, epoxy resins, and lacquer coatings [1]. The release of BPA into food and environment matrices [2] has drawn great attention all over the world because of its estrogenic and antiandrogenic activities. BPA has been reported to occur in various foodstuffs, environmental matrices and human samples [3–5]. Moreover, several chemicals that are structurally similar to BPA, with two hydroxyphenyl functionalities, have been used to perform the same function of BPA. The production and consumption of these bisphenol analogs such as bisphenol F and bisphenol S have increased recently [6]. Other bisphenols (BPs) like bisphenol AF, bisphenol Z, bisphenol AP bisphenol E and bisphenol B were also used in foodstuffs [7].

Several methods for quantitative analysis of bisphenols have been developed such as HPLC-UV [8], HPLC-FLD [9] and LC-MS/MS [10–13]. For the analysis of complex samples, the methods generally require a sample pretreatment step to separate and/or pre-concentrate the analyte prior to analysis. Solid-phase extraction (SPE) is an effective sample treatment technique for BPs analysis in view of its high enrichment efficiency. However, the application of traditional sorbents is to some extent limited due to their inefficient selectivities. The use of molecularly imprinted polymers (MIPs) as SPE sorbents allows not only pre-concentration and cleanup of the sample but also selective extraction of the target analyte, which are particularly important when the sample is complex and the impurities can interfere with quantification.

Generally, MIPs are obtained by polymerizing functional monomers and cross-linkers around template molecules, leading to highly cross-linked three-dimensional network polymers. The resulting imprinted polymers have high selectivity toward template molecules and are stable, robust and resistant to a wide range of pH, solvent and temperature [14]. However, at present, molecular imprinting still faces great challenges relating to its application involving molecularly imprinted solid-phase extraction (MISPE),

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such as template leakage and low binding capacity [15]. In this paper, we mainly focus on the template bleeding problem.

During the imprinting process, imprinted sites are formed not only on the surface but also deeply in the cross-linked polymer network structure, where organic solvent for removing template can hardly reach [16]. Thus, the possible leakage of template molecules even after exhaustive washing steps can happen and cause a serious impact on the accuracy of an analytical method, especially for trace analysis [14,17]. This problem can be solved by use of a dummy template [18] as any leakage will be different from the analyte [19], and the resulting MIP is defined as "dummy molecularly imprinted polymer (DMIP)". Up to now, structurally related analogs [20–24], fragments [25,26] and isotope labeled compounds [27] such as 3,3′,5′5-tetrabromobisphenol A (TBBPA), BPF, 2,6-bis(trifluoromethyl)benzoic acid (BTFB), *p-tert*-butylphenol (PTBP) and [2H16]bisphenol A (BPA-d16) have been reported for BPs imprinting. However, most of these DMIPs show much lower imprinting efficiency toward BPA as compared to the BPA-MIP, and their selectivities for other BPs were rarely investigated. Although the BPA-d16 imprinted material showed remarkable recognition ability toward BPA, it had been limited by the high cost and limited availability of mass spectrometric (MS) detection. The select of dummy template with high imprinting factors for a group of BPs as well as low cost was of great challenge due to the lack of effective screening method [28].

Previously, we proposed a simple and fast screening method for dummy templates by combing the non-imprinted polymer (NIP) column method and the computational modeling of molecular structure [29]. In that work, the selected BPS-template DMIP achieved high affinities towards BPs. And the imprinting factors (IFs) achieved were much higher than those reported in the literatures [30,31]. However, despite its ultra high selectivity for BPF, BPE and BPA, BPS-DMIP showed low efficiency for imprinting BPB and BPAF due to their larger molecular sizes. Moreover, BPS was one of the most important substitutions of BPA, and determination of BPS in environmental and biological samples should also be very meaningful. Therefore, for practical application of DMIPs in the class detection of BPs, further work still needed to be done for screening dummy templates that do not belong to BPs but guarantee higher class-selectivity for the entire BPs.

In this paper, two structural analogs of BPs named 1,1,1-tris(4-hydroxyphenyl)ethane (THPE) and phenolphthalein (PP) were selected as the dummy template molecules for BPs imprinting. The class-selectivity and binding affinity of the prepared polymers were examined using the chromatographic and binding experiments. The polymer with higher recognition ability for BPs was used as the selective extraction sorbents for BPs from sediment, milk and human urine samples. The selectivity, accuracy and precision of the developed method were also evaluated.

2. Experimental

2.1. Chemicals and reagents

1,1,1-Tris(4-hydroxyphenyl)ethane (THPE), phenolphthalein (PP), bisphenol F (BPF), bisphenol S (BPS), bisphenol E (BPE), bisphenol A (BPA), bisphenol B (BPB), bisphenol AF (BPAF), bisphenol AP (BPAP), bisphenol Z (BPZ), 2,2′,6,6′-tetrameth-yl-4,4′-sulfonyldiphenol (BS-TM), dienestrol (DIEN), diethylstilbestrol (DES), ethylene dimethacrylate (EDMA), methacrylic acid (MAA) and trifluoroacetic acid (TFA) were purchased from J&K Chemical Ltd. (Beijing, China). The initiator 2,2′-azobisisobutyronitrile (AIBN) and tetrabromobisphenol A (TBBPA) were supplied by Aladdin Chemical (Shanghai, China). 4-Vinylpyridine (4-VP) and 2,4,6-trichlorophenol (TCP) were obtained from Acros (NJ, USA). HPLC grade acetonitrile, methanol and formic acid were purchased from Fisher Scientific (Fair Lawn, NJ, USA).

2.2. Preparation of imprinted and non-imprinted polymers

Dummy molecularly imprinted polymers (DMIPs) were synthesized by the method described previously [29]. Briefly, 1,1,1-tris(4-hydroxyphenyl)ethane (THPE) and phenolphthalein (PP) were used as dummy templates, with 4-vinylpridine as functional monomer and acetonitrile as polymerization solvent. Non-imprinted polymers (NIPs) were obtained by performing the same procedure in the absence of template molecules.

2.3. Chromatographic evaluation of the prepared polymers

The DMIPs and NIP particles were slurry-packed in methanol into stainless steel HPLC columns (100 mm × 4.6 mm id) at 3000 psi using ethanol as the pushing solvent. The analyses were carried out using BPs dissolved in acetonitrile on an HPLC system which included a manual injector (Rheodyne, 7725, Park Court, CA, USA), a Waters 515 HPLC pump and a Waters 2487 dual wavelength absorbance detector (Milford, MA, USA). Acetonitrile at a flow rate of 1 mL min^{-1} was used as mobile phase and 20 μL of the analyte (20 ppm) was injected for analysis. The UV detector was set at 220 nm. Capacity factor (k) was calculated as $k = (t_R - t_0)/t_0$, where t_R and t_0 are the retention times of the analyte and the void marker (methanol), respectively. The molecular imprinting factor (IF) was calculated by the equation $IF = k_{MIP}/k_{NIP}$, where k_{MIP} and k_{NIP} are the capacity factors for the imprinted and non-imprinted polymers, respectively.

2.4. Binding experiments

The binding capacities and dissociation constants of THPE-DMIP and the corresponding NIP were analyzed by binding experiments using BPA as a model compound. BPA standard solutions with different concentrations (0.005–4.0 mM) were prepared in acetonitrile. One milliliter aliquots of each solution were mixed with 20 mg of THPE-DMIP particles in a 10 mL flask. The mixtures were incubated at 150 rpm for 24 h (25 °C) in a water bath, and then rapidly filtrated. The BPA concentration in the filtrate was measured by HPLC.

The adsorption capacity and dissociation constant K_d (mmol L^{-1}) were calculated according to the Eqs. (1) and (2) [32]:

$$Q = \frac{(C_0 - C_f)v}{m} \quad (1)$$

$$\frac{Q}{C_f} = -\frac{1}{K_d}Q + \frac{Q_{max}}{K_d} \quad (2)$$

Where C_0 (μmol L^{-1}) and C_f (μmol L^{-1}) are the initial and final concentrations of BPA, v (L) is the total volume of the sample, m (g) is the mass of DMIP, Q and Q_{max} (μmol g^{-1}) are the amount of BPA adsorbed at equilibrium and saturation, respectively.

2.5. DMISPE procedures

Solid-phase extraction cartridges with a 3 mL volume were packed with 200 mg of the THPE-DMIP and NIP sorbents.

2.5.1. DMISPE of spiked sediment sample

Sample extraction was based on the method proposed by Liao et al. [12]. In brief, 2 g of spiked sediment was extracted with 5 mL of methanol–water mixture (5:3, v/v) by shaking for 60 min. After centrifugation (4500 g for 5 min; Sorvall Biofuge Stratos,