Journal of Chromatography A, 1474 (2016) 8–13

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Boronate affinity-based surface molecularly imprinted polymers using glucose as fragment template for excellent recognition of glucosides

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ARTICLE INFO

Article history:
Received 18 September 2016
Received in revised form 24 October 2016
Accepted 24 October 2016
Available online 25 October 2016

Keywords:
Surface molecularly imprinted polymers
Boronic acid
Segment template
Glucoside
Selective extraction

ABSTRACT

Rapid and efficient extraction of bioactive glycosides from complex natural origins poses a difficult challenge, and then is often inherent bottleneck for their highly utilization. Herein, we propose a strategy to fabricate boronate affinity based surface molecularly imprinted polymers (MIPs) for excellent recognition of glucosides. D-glucose was used as fragment template. Boronic acid, dynamic covalent binding with D-glucose under different pH conditions, was selected as functional monomer to improve specificity. Fe_3O_4 solid core for surface imprinting using tetraethyl orthosilicate (TEOS) as crosslinker could control imprinted shell thickness for favorable adsorption capacity and satisfactory mass transfer rate, improve hydrophilicity, separate easily by a magnet. Model adsorption studies showed that the resulting MIPs show specific recognition of glucosides. The equilibrium data fitted well to Langmuir equation and the adsorption process could be described by pseudo-second order model. Furthermore, the MIPs were successfully applied for selective extraction of three flavonoid glucosides (daidzin, glycitin, and genistin) from soybean. Results indicated that selective extraction of glucosides from complex aqueous media based on the prepared MIPs is simple, rapid, efficient and specific. Moreover, this method opens up a universal route for imprinting saccharide with *cis*-diol group for glycosides recognition.

1. Introduction

Glycosylation is a frequent and crucial tailing reaction in plants, microbes, and insects, which greatly alters the physicochemical and pharmacological characteristics, such as water solubility, chemical stability, intra- and intercellular transport, pharmacokinetic parameters and biological activities [1,2]. Natural products and functional foods, including countless glycosides, remain a prime source for medicinal chemistry and molecular pharmacology. It is therefore obvious that quantitative and qualitative analysis of glycosides from complex natural products or functional foods is considerably important. Chromatographic methods, especially for high performance liquid chromatography (HPLC), are considered as the powerful analytical techniques for complex system [3,4]. However, because of the complexity of sample matrix, some sample preparation procedures are often needed to enrich low-abundant glycosides and get rid of interferences prior to HPLC determination. The conventional used liquid–liquid extraction and solid-phase extraction using C_{18} or C_8 material suffer from low acceptable selectivity and specificity, which makes the subsequent analysis of glycosides difficult. Thus, developing highly effective techniques for selective extraction of glycosides has become the urgent issues.

Molecularly imprinted polymers (MIPs), containing specific recognition sites complementary in size, shape, and chemical functionality to the template, have attracted wide attentions and attained significant applications in selective extraction of target components from complex matrices (e.g. natural product, food, biological and environmental samples) [5–10]. Notably, surface imprinted magnetic materials possess high supermagnetism for easily and rapidly separation and highly dense recognition sites located on the surface of magnetic solid support for remarkable facilitation of mass transfer, adsorption and desorption [7,9,10]. Among the surface imprinted technology, sol-gel based MIPs, formed by acid/base catalyzed hydrolysis/condensation of silane monomers, have draw increased attention for their excellent physical rigidity, chemical inertness, thermal stability, hydrophilic properties, and controllable size with excellent specific adsorption and avoidable non-specific adsorption [11]. As a consequence, it is important to emphasize that sol-gel based MIPs layer coated on the surface of magnetic solid supports could be proven to have

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http://dx.doi.org/10.1016/j.chroma.2016.10.059
0021-9673/

overwhelming superiorities. Although some achievements have been made, fabrication of MIPs for highly selective extraction of glycosides from natural products or functional foods is limited [12,13]. The main limitation is the hard preparation and high price of natural glycosides.

Dummy template (a structural analogue of template) and segment template (partial structure of template) could solve the problem of expensive and rare template and at the same time the template leakage [14–16]. In addition, MIPs using segment template have a higher density of recognition sites than those using full-size template, which results in higher adsorption capacity [17]. Glucose could then be selected as segment template for preparation of MIPs for selective recognition of glucosides (e.g flavonoid glucosides, terpene glucosides, alkaloid glucoside, etc.) from natural products or functional foods.

Boronic acid moieties could bind with *cis*-diol containing compounds through dynamic covalent bonds, and then boronate affinity has been considered as a unique means for the selective capture of *cis*-diol containing compounds (e.g. sugar, glycoprotein, nucleoside or catecholamine) [18,19]. In order to make an unambiguous differentiation between *cis*-diol containing compounds, fabrication of boronate affinity based MIPs have been presented in selective recognition and detection of glycoprotein or carbohydrate [11,20–24]. It is noted that monosaccharide (e.g. sialic acid and glucuronic acid) has been used as segment template in boronate affinity based MIPs to differentiate or image cells and tissues [25–28].

Herein, we describe the synthesis of boronate affinity based surface MIPs using D-glucose as fragment template for excellent recognition of glucosides from complex matrices. For this purpose, boronic acid functionalized magnetic Fe_3O_4 nanoparticles are firstly used as functional cores, and then a thin layer of MIPs is formed using D-glucose and tetraethyl orthosilicate (TEOS) as segment template and crosslinker, respectively. Subsequently, the adsorption properties are evaluated using a model flavonoid glucoside (genistin), and its selectivity is evaluated using three other flavonoid glycosides (daidzin, glycitin, rutin) and their aglycones (genistein, daidzein, glycitein, quercetin). Finally, the efficiency of the prepared MIPs is evaluated for selective extraction of flavonoid glucosides from soybean extract.

2. Experimental

2.1. Chemicals and reagents

Iron (III) chloride hexahydrate ($FeCl_3{\cdot}6H_2O$), 1,6-hexanediamine, TEOS, 3-formyl-phenylboronic acid (FPBA), sodium borohydride and HPLC grade acetonitrile were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Anhydrous methanol and ethanol, $NH_3{\cdot}H_2O$ (28 wt%), and ethylene glycol were obtained from Kemiou Chemical Reagent Co., Ltd (Tianjin, China). Genistin, daidzin, glycitin, rutin, genistein, daidzein, glycitein, quercetin, and D-glucose were acquired from Sigma–Aldrich Chemicals (St. Louis, MO, USA). Other reagents were of analytical grade.

2.2. Preparation of boronate affinity-based surface MIPs

The schematic route for preparation of boronate affinity-based surface MIPs was shown in Fig. 1. At first, amino-functionalized Fe_3O_4 nanospheres were synthesized according to our previous report [29]. Then, amino-functionalized Fe_3O_4 was immobilized with boronic acid moieties. Typically, amino-functionalized Fe_3O_4 nanospheres (200 mg) were dissolved in anhydrous methanol solution (40 mL), and then FPBA (300 mg) were added and

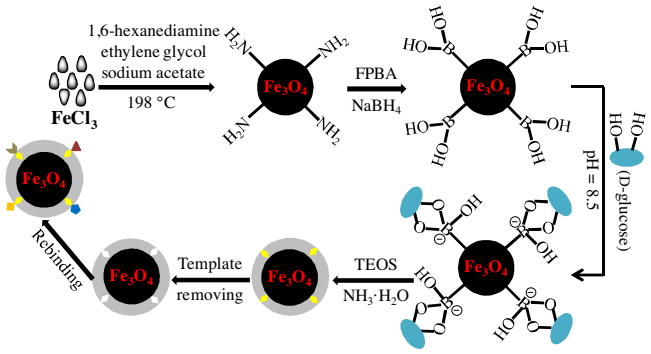

Fig. 1. The synthetic procedure for preparing boronate affinity-based surface MIPs.

mechanically stirred at room temperature for 12 h. After that, sodium borohydride (1.2 g) was added, and the resulting mixtures were continuously stirred at 4 °C for 24 h. The FPBA functionalized Fe_3O_4 nanospheres were collected magnetically, washed with water and ethanol for three times each, and then dried at 50 °C overnight. Subsequently, FPBA functionalized Fe_3O_4 nanospheres (200 mg) were dispersed in ammonium bicarbonate buffer solution (40 mL, 50 mM, pH = 8.5). Then D-glucose (0.8 g) was added and the resulting mixtures were shaken at room temperature for 3 h. The D-glucose-FPBA immobilized Fe_3O_4 nanospheres were obtained magnetically and washed with ammonium bicarbonate buffer solution (50 mM, pH = 8.5) three times, then dried in air at room temperature. To imprint D-glucose on the surface of Fe_3O_4, the collected D-glucose-FPBA immobilized Fe_3O_4 nanospheres were dispersed in ethanol/water/TEOS (200.1 mL, 160/40/0.1, V/V/V) solution, and then aqueous ammonia (3 mL) was added and stirred for 15 min. Finally, D-glucose imprinted Fe_3O_4 nanospheres were collected magnetically, and then eluted with 10% acetic acid (pH = 2.3) to remove D-glucose absolutely.

As a control, the corresponding non-imprinted polymers (NIPs) were prepared with the same procedures as described above in the absence of D-glucose.

2.3. Characterization of MIPs

Fourier transform infrared spectrometer (FT-IR) (Nicolet 6700, Thermo Nicolet Co., Waltham, MA, USA) were used to collect infrared spectra (4000 – 400 cm^{-1}). Transmission electron microscopy (TEM) (JEM – 2100F, JEOL, Japan) was used to observe the size, structure and morphology. Brunauer-Emmett-Teller (BET) surface area was acquired from a Micromeritics ASAP 2020 device (Micromeritics, Norcross GA, USA). Magnetization was measured at room temperature in a vibration sample magnetometer (VSM7407, Lake Shore, USA).

2.4. HPLC analysis

HPLC analysis was operated on an Agilent 1260 HPLC system. Separation was conducted on an Agilent ZORBAX SB-C_{18} column (150 mm × 4.6 mm i.d., 5 μm, Agilent, Santa Clara, CA). Gradient mobile phase consisted of A (0.4% acetic acid in water) and B (0.4% acetic acid in methanol) with a flow rate of 0.8 mL/min was programmed as follows: 0–20 min, 23–60% B; 20–25 min, 60–80% B. The temperature was controlled at 25 °C, while spectra were acquired at 254 nm.

2.5. Adsorption experiments

The adsorption experiments of MIPs/NIPs were evaluated using genistin, a flavonoid glucoside, as test compound.