Carbohydrate Polymers 101 (2014) 517–523

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Synthesis of chitosan molecularly imprinted polymers for solid-phase extraction of methandrostenolone

Yun Wang[a,*], Enlan Wang[a], Ziming Wu[a], Huan Li[a], Zhi Zhu[a], Xinsheng Zhu[b], Ying Dong[a]

[a] School of Food and Biological Engineering, Jiangsu University, Zhenjiang 212013, China
[b] Zhenjiang Products Quality Supervision and Inspection Center, Zhenjiang 212132, China

ARTICLE INFO

Article history:
Received 18 March 2013
Received in revised form 13 June 2013
Accepted 24 September 2013
Available online 29 September 2013

Keywords:
Chitosan
Methandrostenolone
Molecularly imprinted polymers
Solid-phase extraction
HPLC

ABSTRACT

Chitosan molecularly imprinted polymers (CHI-MIPs) for selective extraction of methandrostenolone (MA) was synthesized by cross-linking of chitosan with epichlorohydrin in the presence of MA as the template molecule. Systematic investigations of the influences of template, functional polymer, cross-linker as well as porogen concentrations on the rebinding capacity of CHI-MIPs were carried out. Adsorption and kinetic binding experiments indicated that the synthesized CHI-MIPs had high adsorption and excellent affinity to MA. Solid-phase extraction (SPE) using the prepared CHI-MIPs as adsorbent was then investigated, and the optimum loading and eluting conditions for SPE of the MA were established. The optimized SPE procedure was used to extract the MA from several spiked samples and a good sample clean-up was obtained with the average recoveries ranged from 95.97 to 101.79%.

1. Introduction

The trace level quantification of organic contaminants in complex matrices usually requires a step of sample pretreatment before instrumental analysis. Solid-phase extraction (SPE) is one of the most popular cleanup techniques due to factors such as convenience, time saving and simplicity (Picó, Fernández, Ruiz, & Font, 2007; Pragst, 2007). Nevertheless, the main drawback of conventional SPE sorbent is lack of selectivity, leading to co-extraction of matrix interference components with the target analyte. The best way to overcome this drawback is by performing selective extraction targeted for the compound of interest. The first sorbent used for selective extraction was immuno-sorbent, which relies upon reversible and highly selective interactions between analyte and antibody (Hennion & Pichon, 2003; Senyuva & Gilbert, 2010). However, the obtainment of antibody is difficult, time-consuming and expensive, their application is to some extent limited.

In recent years, molecularly imprinted polymers (MIPs) have attracted much attention due to their outstanding advantages, such as predetermined recognition ability, stability, relative ease and low cost of preparation, and potential application to a wide range of target molecules (Alexander et al., 2006; Chen, Xu, & Li, 2011; Turiel & Martín-Esteban, 2010). MIPs are synthetic polymers possessing specific cavities designed for a target molecule. In the most common preparation process, monomers form a complex with a template molecule through covalent or non-covalent interactions and are then joined by using a cross-linking agent. After removing of the imprinting molecules by chemical reaction or extraction, binding sites are exposed which are complementary to the template molecule in size, shape, and position of the functional groups, and consequently allow their selective uptake. MIPs have been successfully used as artificial receptors in separations (López Mdel et al., 2012; Santos, Vitor, Andrade, Martins, & Figueiredo, 2012), sensors (Pardieu et al., 2009), catalysis (Volkmann & Brüggemann, 2006), and drug development and screening (Shi, Wu, Qu, Li, & Zhang, 2007).

Chitosan is produced by the deacetylation of chitin, which is known to be the most abundant natural amino-polysaccharide presenting non-toxicity, bio-degradability and bio-compatibility (Rinaudo, 2006). The presence of multiple functional groups such as amino and hydroxyl groups on its polysaccharide chain provides the flexibility for structural modifications and for preparing molecularly imprinted polymers, and it is, therefore, interesting not only as an abundant resource but also as a novel type of functional materials. The range of its applications has been enormously expanded in various fields including biotechnology, water-treatment, membranes, cosmetics, food industry, and medicine (Rinaudo, 2006; Wan Ngah, Teong, & Hanafiah, 2011). However, few works deal with the combination of chitosan in molecular imprinting (Guo, Xia, Wang, Song, & Zhang, 2005; Kyzas, Lazaridis, & Bikiaris, 2013;

* Corresponding author. Tel.: +86 511 88797059; fax: +86 511 88780201.
E-mail address: wangy1974@ujs.edu.cn (Y. Wang).

0144-8617/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2013.09.078

Monier & El-Sokkary, 2010; Nishad, Bhaskarapillai, Velmurugan, & Narasimhan, 2012; Tong, Guan, Wang, Xu, & He, 2011).

Methandrostenolone (MA) is a kind of synthetic anabolic androgenic steroids and has been strictly regulated in most countries over the world due to its adverse effects on human health. In the present study, a novel chitosan molecularly imprinted polymer (named hereafter as CHI-MIP) intended to clean-up and pre-concentration of MA from natural samples was prepared. The surface structure and the physicochemical properties of the polymers were characterized; the adsorption capacity and the selectivity toward the template in the presence of structurally related compounds were determined. The performance of the developed MIPs for the clean up and preconcentration of MA from several actual samples was also evaluated.

2. Materials and methods

2.1. Chemicals

Chitosan with an average molecular weight of 100 kD and a deacetylation degree of 93% was purchased from Golden-Shell Biochemical Co., Ltd. (Yuhuan, China). Methandrostenolone, trenbolone (TB), testosterone propionate (TP), epichlorohydrin and sodium borohydride ($NaBH_4$) were supplied by Sigma–Aldrich (St. Louis, MO, USA). High-performance liquid chromatography-grade methanol was obtained from Fisher Scientific, Inc. (Pittsburgh, PA, USA). All other chemicals were of analytical grade and supplied by Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Milli-Q water was obtained from ultrapure water system supplied by Chengdu Ultrapure Technology Co., Ltd. (Chengdu, China).

2.2. Preparation of CHI-MIPs

Chitosan was cross-linked with epichlorohydrin and molecularly imprinted with MA as the template molecule following a single-step procedure as previously described (Aburto & Le Borgne, 2004; Wang, Xu, Zhang, Wang, & Dong, 2011a), with modifications. Firstly, 0.6 g of chitosan was dissolved in 30 mL of 2% acetic acid aqueous solution (v/v). The resulted chitosan solution was mixed with 15 mL of methanol containing 100 mg of MA and the mixture was stirred for 30 min. 50 mL of liquid paraffin containing 0.3 mL of span-80 and 2 g of sucrose were added into the mixture solution and stirred at 50 °C for 10 min. Then, 1 mL of formaldehyde aqueous solution (37%, w/w) performed as amino protective solute was added into the mixture and stirred for 30 min. After adjusted pH to 9.0 with 2 M sodium hydroxide aqueous solution, epichlorohydrin was added dropwise into the mixture to the final concentration raged from 0.5% to 2.5%. The cross-linking reaction was performed by agitation at 70 °C for 3 h with the pH maintained at 9.0 throughout. To remove the template molecules and the residuals of the polymers, the prepared CHI-MIPs were extracted in a Soxhlet apparatus using 80% methanol for 24 h and then digested with 0.1% hydrochloric acid to remove the amino protective solute. Finally, the CHI-MIPs were thoroughly washed with deionized water and dried in a vacuum drier. Chitosan-based non-imprinted polymers (CHI-NIPs) were prepared with the same procedure in the absence of template molecule.

2.3. Optimization of CHI-MIPs preparation

The optimum porogen content, molar ratios of cross-linker (epichlorohydrin) and template (MA) to polymer (chitosan) were determined through the rebinding capacity of the prepared CHI-MIPs.

2.3.1. Methanol:water ratio

The volume ratios of methanol to water tested for the CHI-MIPs preparation were 1:3, 1:2, 1:1, 2:1 and 3:1. Sucrose content, molar ratios of epichlorohydrin to chitosan and MA to chitosan were 1%, 1:6 and 1:10 respectively, whereas all the other conditions were as formerly described.

2.3.2. Sucrose content

The contents of sucrose tested for the optimal preparation of CHI-MIPs were 0.5%, 1%, 1.5%, 2% and 2.5%. Volume ratio of methanol to water, molar ratios of epichlorohydrin to chitosan and MA to chitosan were 1:2, 1:6 and 1:10 respectively, whereas all the other conditions were as previously described.

2.3.3. Molar ratio of epichlorohydrin to chitosan

The molar ratios of epichlorohydrin versus chitosan (glucosamin) tested for each type of CHI-MIPs were 1:10, 1:8, 1:6, 1:4 and 1:2. Volume ratio of methanol to water, sucrose content and molar ratio of MA to chitosan were 1:2, 1% and 1:10 respectively, whereas all the other conditions were as previously described.

2.3.4. Molar ratio of MA to chitosan

Similarly, molar ratios of MA to chitosan (glucosamin) ranged from 1:20, 1:10, 1.5:10 and 1:5 were tested for CHI-MIPs preparation. Volume ratio of methanol to water, sucrose content and molar ratio of epichlorohydrin to chitosan were 1:2, 1% and 1:6 respectively, whereas all the other conditions were as formerly described.

2.4. Characterization of CHI-MIPs and CHI-NIPs

The synthesized CHI-MIPs and CHI-NIPs were characterized with scanning electron microscope (S-4800, Hitachi Ltd., Japan) and Fourier transform infrared spectroscopy (Nicolet Nexus 670, Thermo Fisher Scientific, USA). The pore size in polymers surface was measured by nitrogen adsorption porosimetry (NOVA e2000, Quantachrome, USA). The average pore diameter was calculated using the BJH analysis of the desorption branch.

2.5. Kinetic adsorption

In the kinetic experiments, 0.2 g of CHI-MIPs were placed in a conical flask and mixed with 10 mL of 800 μg/mL MA dissolved in 10% methanol. The suspensions were incubated for 5 hours at room temperature. Samples were collected at fixed intervals (30 min) and analyzed by HPLC method (Wang, Xu, Zhang, Wang, & Dong, 2011b). The same experiments were performed for the respective CHI-NIPs.

2.6. Static adsorption

The effect of initial MA concentration on the adsorption was determined by mixing of CHI-MIPs with 10 mL of MA solutions at definite concentrations (0.1–1 mg/mL). Immediately after mixing, the mixture was incubated at room temperature for 5 h. After adsorption, the residual concentration of the MA was determined by HPLC. The static adsorption capacity was calculated based on the following formula (Shaikh, Memon, Khan, Bhanger, & Nizamani, 2012):

$$Q = \frac{(C_i - C_f) \times V}{m}$$

where Q (mg/g) was the mass of MA adsorbed per gram of polymers, C_i (mg/L) was the initial concentration of MA, C_f (mg/L) was its final concentration after adsorption, V (L) was the total volume of adsorption mixture, and m (g) was the mass of polymers. The same experiments were performed for the respective CHI-NIPs.