International Journal of Biological Macromolecules 92 (2016) 451–460

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

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

Selective cholesterol adsorption by molecular imprinted polymeric nanospheres and application to GIMS

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ARTICLE INFO

Article history:
Received 6 May 2016
Received in revised form 22 June 2016
Accepted 2 July 2016
Available online 10 July 2016

Keywords:
Cholesterol
Biomacromolecule
Imprinting
Gastrointestinal mimicking solution
Selectivity

ABSTRACT

Molecular imprinted polymers (MIPs) are tailor-made materials with selective recognition to the target. The goals of this study were to prepare cholesterol imprinted polymeric nanospheres (CIPNs) and optimize their adsorption parameters and also to use CIPNs for adsorption of cholesterol (CHO), which is an important physiological biomacromolecule, from gastrointestinal mimicking solution (GIMS). Pre-polymerization complex was prepared using CHO as template and *N*-methacryloylamido-(L)-phenylalanine methyl ester (MAPA). This complex was polymerized with 2-hydroxyethyl methacrylate (HEMA). CHO was removed by MeOH and tetrahydrofuran (THF). Adsorption studies were performed after chacterization studies to interrogate the effects of time, initial concentration, temperature, and ionic strength on CHO adsorption onto CIPNs. Maximum adsorption capacity (714.17 mg/g) was higher than that of cholesterol imprinted polymers in literature. Pseudo-second-order kinetics and Langmuir isotherm fitted best with the adsorption onto CIPNs. 86% of adsorbed cholesterol was desorbed with MeOH:HAc (80:20, v/v) and CIPNs were used in adsorption-desorption cycle for 5-times with a decrease as 12.28%. CHO analogues; estron, estradiol, testosterone, and progesterone were used for competitive adsorption. The relative selectivity coefficients of CINPs for cholesterol/estron and cholesterol/testosterone were 3.84 and 10.47 times greater than the one of non-imprinted polymeric nanospheres (NIPNs) in methanol, respectively.

1. Introduction

Selective recognition of analyte is required for the extraction, purification of the target molecule from biological samples with multitude of other compounds. Immunoaffinity sorbents based on the high affinity and the selectivity of antigen–antibody interactions are developed. However, the use of immunoaffinity sorbents is quite expensive due to stability limitations [1]. Molecularly imprinted polymers (MIPs), also called synthetic antibodies [2,3], are a new class of materials representing high selectivity and good affinity for target molecules predetermined specifity to the target molecule [4]. MIPs are produced by the cross-linking of monomers pre-organized with template and cavities complementary with the shape and functional groups of target molecule are obtained after template removal [1,5]. With the ability of recognizing a specific chemical substance, molecular imprinted technology ensures selective adsorption as for the removal of different molecules such as proteins, metal ions and organic compounds [6].

In addition to their recognition abilities, MIPs possess unique properties, such as physical, chemical, mechanical and thermal stabilities. MIPs have the advantage of being more robust than antibodies and storable at room temperature for a long time [7]. In addition, they can be used in both organic and aqueous solvents, although imprinting in aqueous solutions is not straightforward [5].

MIPs can be applied for a wide range of substances such as carbohydrates, peptides, proteins, DNA, drugs, metal ions and organic compounds [4,6,8–11]. MIPs have been conformed to applications required higher selectivity such as separations [12–14], sensors [15–18], immunoassay [19] and catalysis or artificial enzymes [20–23]. Also, by combining the advantages of MIPs with those

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http://dx.doi.org/10.1016/j.ijbiomac.2016.07.007
0141-8130/

of nanostructures, MIP nanoparticles can be successfully used in these applications. MIP nanoparticles have higher surface area-to-volume ratios; thus, imprinted cavities are more easily accessible by the templates and the binding kinetics are improved [24,25].

Cholesterol, chosen as the target molecule, is an important physiological biomacromolecule and the principal sterol synthesized by human. It is an apparent marker for several cardiovascular diseases and collected as plaques in coronary arteries and causes to artherosclerosis [26,27]. A significant reduction in blood levels of cholesterol and LDL by diet and lipid reducing drugs was found to result in regression of atherosclerosis [28]. Thus, lowering high amounts of cholesterol is one of the challenging topics for human health [29]. Several CHO imprinted polymeric materials were prepared and practiced in different media. Hybrid material for improved CHO adsorption has been prepared using methacrylic acid (MAA) and tetraethoxysilane with different molar ratios [30]. CHO imprinted nanospheres prepared using MAA in different solvents were deposited into membrane system and used for selective rebinding in both buffer and ethanol [31]. Poly(glycidyl methacrylate-N-methacryloyl-(L)-tyrosine methylester) microspheres embedded into the poly(hydroxyethyl methacrylate) [32], poly(MAA) [33] and itaconate glycerol methacrylate polymers prepared by cholesteryl bearing monomer or cross-linker [34] have been used for selective CHO adsorption from GIMS. Also, β-cyclodextrin was commonly used for cholesterol imprinting with different monomer combinations [35–38].

In the current study, cholesterol imprinted polymeric nanospheres (CIPNs) were synthesized by surfactant-free emulsion polymerization method [39] and optimized for cholesterol adsorption by testing contact time, initial concentration, ionic strength, temperature effects. Adsorption studies were also performed in GIMS and competitive cholesterol adsorption was studied to show the selectivity of CIPNs.

2. Materials and method

2.1. Materials

Ethylene glycol dimethacrylate (EGDMA), sodium desoxycholate (NaDC), CHO, L-phenylalanine and methacyloyl chloride were supplied from Sigma. HEMA (99%) was supplied from Fluka. Poly(vinyl alcohol) (PVA, high molecular weight, more than 99%) and potassium persulphate (KPS) were purchased from Merck. Sodium cholate (NaC) was supplied from Applichem. Methanol, acetonitrile, tetrahydofuran (THF) and other organic solvents were chromatographic grade. All other chemicals were used at analytical grade with high purity and no purification. The water used in experiments was purified using a Millipore S.A.S 67120 Molsheim-France facility. All laboratory glassware was rinsed with deionised water and dried in a dust-free environment before use.

2.2. Synthesis of N-Methacryloylamido-(L)-phenylalanine methyl ester (MAPA) comonomer

The MAPA comonomer was synthesized according to the method of Say et al. [40]. 5.0 g L-phenylalanine, 0.2 g sodium nitrite was added to the 5% (w/v) K_2CO_3 solution (30 mL). Methacryloyl chloride (4 mL) was dripped into this solution cooled down to 0 °C under N_2 and leaved to stirring at 100 rpm for 2 h at room temperature. At the end of the reaction period, pH of solution was adjusted to 7.0. Ether and cyclohexane crystallizations were performed after ethyl acetate extraction and MAPA was produced.

2.3. Preparation and characterization of CIPNs

CIPNs were produced in accordance with the method of Akgöl et al. [39]. Primarily, pre-polymerization complex was formed between MAPA and CHO at 1:1 molar ratio. Molecular structures of CHO and MAPA were given in Fig. 1. CHO solution (19.1 mg in 638 μL THF) was mixed with MAPA solution (11.6 mg) for 3 h in dark. Pre-polymerization complex was added to stabilizer solution, 0.2775 g PVA in 25 mL water, and suspended for 5 min. HEMA (600 μL) and EGDMA (300 μL) were added as functional monomer and cross-linker, respectively. Finally, KPS (0.0198 g in 45 mL distillated water) was added as the initiator and polymerization mixture was sonicated and mixed to homogenate. Polymerization reaction was started at 70 °C after N_2 flow for 5 min and shacked at 65 rpm for 24 h in a temperature controlled water bath shaker (GFL 1092). Non-imprinted polymeric nanospheres (NIPNs) were synthesized by the same method without cholesterol in polymerization solution.

CIPNs and NIPNs were sonicated for 1 h at the end of polymerization period. For the removal of excess monomer and cross-linker, CIPNs and NIPNs were washed with water and methanol. To remove template (CHO), CIPNs were also washed with THF. Biggest amount of cholesterol (84%) was removed using methanol and THF. Several washings were perfomed with distillated water, and finally CIPNs and NIPNs were resuspended in water.

FTIR spectra of the CIPNs and NIPNs were recorded using FTIR spectrophotometer (Perkin Elmer spectrum 100 FTIR spectrometer, Waltham, USA) with a universal ATR sampling accessory. The surface morphology of CIPNs and NIPNs were examined using SEM. The dried sample was mounted on a SEM sample mount and was sputtered coated for 2 min. The sample was then mounted in a SEM (Phillips, XL–30 S FEG, Germany). The surface of the sample was then scanned at the desired magnification to study the morphology of the nanospheres. The particle size of CIPNs and NIPNs were determined by Zeta Sizer (Malvern Instruments, Model 3000 HSA, England). The specific surface area of CIPNs and NIPNs were calculated using the particle diameters by the equation below [41]:

$$N = 6 \times 10^{10}\, S/\pi\rho_s d^3 \quad (1)$$

Where; N is the nanoparticle count in 1 mL suspension; S is the percentage of solid part (S = 10 for 5 mL suspension, 0.5 g); d is the particle diameter (μm) and ρ_s is the polymer density (g/mL).

Surface areas of polymeric nanospheres were calculated from the following equation:

$$\text{Specific surface area}(m^2/g) = \text{Surface area}(m^2/\text{particle}) \times N(\text{particle/mL})/m_{\text{driednanospheres}}(g/mL) \quad (2)$$

2.4. Cholesterol adsorption studies

Effects of different parameters on the adsorption behavior of CIPNs were tested. CHO adsorption was studied over a range of time (2 min–4 h, 100 mg/L CHO, 1 mg CIPNs, 25 °C), polymer amount (0.25–2.5 mg, 100 mg/L CHO, 30 min, 25 °C, 0.5 mL), initial cholesterol concentration (5–3000 mg/L CHO, 30 min, 1 mg CIPNs, 25 °C, 0.5 mL), and temperature (5–35 °C, 100 mg/L CHO, 30 min, 1 mg CIPNs, 0.5 mL). Cholesterol soloutions were prepared in methanol in the range of 5–1000 mg/L and in THF between 1000 and 3000 mg/L. Effect of ionic strength (50–3000 mg/L NaCl, 100 mg/L CHO, 30 min, 1 mg CIPNs, 25 °C, 0.5 mL) was also examined in both methanol and gastrointestinal mimicking solution (GIMS). Adsorption capacity of NIPNs using 100 mg/L CHO at optimum adsorption conditions (30 min, 1 mg NIPNs, 25 °C, 0.5 mL) were examined to compare imprinting effect. Adsorption exper-