Materials Science and Engineering C 65 (2016) 400–407

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

Synthesis and characterization of imprinted sorbent for separation of gramine from bovine serum albumin

Piotr Luliński, Dorota Klejn, Dorota Maciejewska *

Department of Organic Chemistry, Faculty of Pharmacy, Medical University of Warsaw, Banacha 1, 02-097 Warsaw, Poland

ARTICLE INFO

Article history:
Received 16 December 2015
Received in revised form 11 April 2016
Accepted 14 April 2016
Available online 19 April 2016

Keywords:
Molecularly imprinted polymer
Gramine
Solid phase extraction

ABSTRACT

The aim of this study was to develop an efficient sorbent for separation of *N*,*N*-dimethyl-3-aminomethylindole (gramine) from bovine serum albumin. An imprinting technology was involved in the synthesis of polymers from nine different functional monomers in the presence of ethylene glycol dimethacrylate as a cross-linker. The analysis of binding capacities showed that the highest specificity towards gramine was achieved when 4-vinylbenzoic acid was used as the functional monomer in methanol to form the bulk imprinted polymer, MIP**1** (imprinting factor equal to 21.3). The Scatchard analysis of MIP**1** showed two classes of binding sites with the dissociation constants K_d equal to 0.105 and 6.52 μmol L^{-1}. The composition and morphology of polymers were defined by ^{13}C CP/MAS NMR, BET and SEM-EDS analyses. The recognition mechanism of MIP**1** was tested using the structurally related bioanalytes, and the dominant role of indole moiety and ethylamine side chain was revealed. A new MISPE protocol was optimized for separation of gramine. The total recoveries on MIP**1** were equal to 94 ± 12 % from standard solutions and 85 ± 11 % from bovine serum albumin.

1. Introduction

N,*N*-Dimethyl-3-aminomethylindole (gramine) is a natural indole alkaloid. This compound can be found in raw plants, mainly in Poaceae (e.g. in barley, *Hordeum vulgare* L.) and in coal tar [1,2]. The concentration of gramine in barley can reach 8 mg g^{-1} dry weight (it was suggested that gramine could be of importance for protection of barley against pests). However, gramine causes toxic side effects for grazing animals and so it is important to determine its level in selected tissues. Gramine presents a significant pharmacological activity such as the relaxation of bronchical smooth muscles and blood pressure elevation. It exerts an effect on mitochondrial energy metabolism in rat liver as well as bovine heart and is a weak inhibitor of acetylocholinesterase [3,4]. Recent studies revealed that gramine plays a role in the serotonin system as a vasorelaxation agent acting mainly as an antagonist to 5-HT_{2A} receptors [5,6]. The addition of gramine to diet was found to reduce feed intake in animal models. The estimated no-observed-adverse-effect-levels of gramine were about 0.3 g kg^{-1} of diet for rats, 0.65 g kg^{-1} for chicken and 0.5 g kg^{-1} for pigs [7,8]. The compound was found to be a component of some dietary supplements. Apart from its significant biomedical role in mammalian organisms, gramine has a radioprotective activity and effectively inhibits the growth of *Microcystis aeruginosa* [9].

The survey of literature shows that advanced analytical methods devoted to the isolation of gramine are rather scarce. Muir and co-workers [10] described the method of liquid–liquid extraction (LLE) of gramine from raw plant material followed by liquid chromatography with photo diode array detection. The chromatographic resolution was performed on C18 column. The method allowed them to determine gramine in the concentration range from 17 to 871 mg L^{-1}. However, total recoveries were moderate (76–78 %) and the chromatographic resolution between gramine and other indolealkylamines such as 3-(aminomethyl)indole, *N*-methyl-3-(aminomethyl)indole and tryptamine was low. A very similar method of the analysis of gramine from plants was proposed by Adams and co-workers [11]. Matsuo and co-workers [12] presented a modified approach in which 97 % of gramine was extracted from raw material. The obtained chromatogram revealed the presence of two peaks, one of which was identified as gramine and the other as an unknown compound. However, the resolution of peaks was insufficient. In order to improve the chromatographic resolution and to remove interfering components from the samples an additional step of solid phase extraction (SPE) was introduced after LLE. The C18 stationary phase was used in the SPE process causing a nearly 25% loss in the recovery of gramine. Hence, the investigations aimed at the preparation of a novel sorbent that could be applied for selective separation of gramine from a complex matrix are an important scientific challenge.

In this paper the imprinting technology was used to obtain a novel selective sorbent. The imprinting technology involved the creation of specific binding sites in the polymeric network after polymerization in the presence of the template molecule followed by template removal. The molecularly imprinted polymers (MIPs) formed after final template removal found various applications because of high selectivity and stability [13]. The imprinting technique was successfully applied in the

* Corresponding author.
E-mail address: dmaciejewska@wum.edu.pl (D. Maciejewska).

http://dx.doi.org/10.1016/j.msec.2016.04.051
0928-4931/

fabrication of sorbents for molecularly imprinted solid phase extraction (MISPE) [14], sensors [15], drug delivery devices [16] or nanotechnology [17]. However, the imprinting technology has never been used to produce selective sorbents for the isolation of gramine. Hence, in the present study the synthesis and characterization of the novel gramine imprinted sorbent were described. The physico-chemical analyses were used to reveal the structural and morphological differences between the imprinted and the non-imprinted polymers. The compounds of similar structures were tested in the non-competitive binding experiments to characterize the recognition mechanism. The applicability of novel material towards gramine isolation was shown in the MISPE of gramine from bovine serum albumin, a model of complex matrix. The obtained recovery of gramine was compared with the recovery after MISPE on the commercial C18 sorbent.

2. Experimental

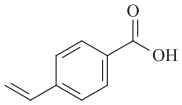
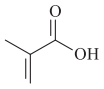
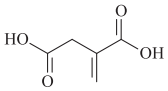
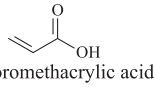
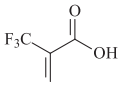
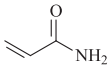
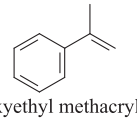
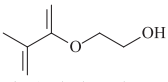
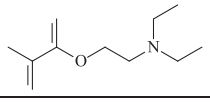
2.1. Materials

N,*N*-Dimethyl-3-aminomethylindole (gramine, the template, **A1**), 3-(2-aminoethyl)indole (tryptamine, **A2**), 3-(2-aminoethyl)-5-hydroxyindole hydrochloride (serotonin, **A3**), 2-(3,4-dihydroxyphenyl)ethylamine hydrochloride (dopamine, **A4**), (±)-1-(3,4-dihydroxyphenyl)-2-aminoethanol hydrochloride (*R*/*S*-norepinephrine, **A5**), 3,3'-diindolylmethane (**A6**), 3-indolemethanol (**A8**), 2-(3-indolyl)ethanol (tryptophol, **A9**) were purchased from Sigma-Aldrich (Steinheim, Germany), and 3-indoleacetic acid (**A7**) was from Merck (Darmstadt, Germany). The functional monomers: 4-vinylbenzoic acid (**1**) and acrylic acid (**4**) were from Alfa Aesar (Karlsruhe, Germany), methacrylic acid (**2**), itaconic acid (**3**), trifluoromethacrylic acid (**5**), isopropenylbenzene (**7**), 2-hydroxyethyl methacrylate (**8**), 2-(diethylamino)ethyl methacrylate (**9**) were from Sigma-Aldrich (Steinheim, Germany) and acrylamide (**6**) was from Fluka (Steinheim, Germany). The cross-linker, ethylene glycol dimethacrylate, EGDMA was purchased from Sigma-Aldrich (Steinheim, Germany). The solvents: methanol (pure and HPLC grade) and acetone were from POCh (Gliwice, Poland). The polymerization reaction initiator, 2,2′-azobisisobutyronitrile, AIBN, and formamide were from Merck (Darmstadt, Germany). Ammonium acetate and potassium dihydrogen phosphate were from POCh (Gliwice, Poland). The monomers were purified prior to use by standard procedures (vacuum distilled or recrystallized from the appropriate solvents). All other reagents were used without purification. Ultra-pure water delivered from a Milli-Q purification system (Millipore, France) was used to prepare the water solutions. Bovine serum albumin (≥96 %) was delivered from Sigma (Steinheim, Germany).

2.2. Synthesis of polymers

The experimental amounts of the reagents (moles, masses, and volumes) used for the preparation of different types of polymers are listed in Table 1. The molecularly imprinted polymers (MIPs) coded as MIP**1**–MIP**9** were prepared using *N*,*N*-dimethyl-3-aminomethylindole (gramine) as the template, by the radical bulk polymerization. Briefly, *N*,*N*-dimethyl-3-aminomethylindole (gramine), the appropriate functional monomer, and ethylene glycol dimethacrylate, EGDMA (the cross-linker) were dissolved in methanol (the porogen) in thick-walled glass tubes. A molar ratio of the template to the functional monomer and the cross-linker was equal to 1:4:20 and was selected on the basis of the previous analyses [18]. The role of all components of the prepolymerization mixture was intensively studied experimentally and theoretically by Yungerman and Srebnik [19]. The proposed molar ratio is in agreement with their findings. At the end, the initiator of polymerization, 2,2′-azobisisobutyronitrile (AIBN) was added. The homogeneous solutions were purged with nitrogen for ca. 5 min and then the glass tubes were sealed. Subsequently, the polymerization was carried out under a nitrogen atmosphere for 24 h at 64 °C. The bulk rigid polymers were ground in a mortar with a pestle and wet-sieved into particles below 45 μm of diameter. Fine particles were separated by repeated decantation in acetone. Gramine was removed from the polymer with continuous extraction in a Soxhlet apparatus (24–36 h, 80 mL, methanol), followed by washing with the formamide–methanol (5:95 v/v) system. Total removal of gramine as well as unreacted monomers was confirmed by UV measurements. Then, the particles were dried under vacuum at room temperature. For comparison purposes, the non-imprinted polymers, NIP**1**–NIP**9** were prepared under the same polymerization conditions but without the template molecule and were treated in the same way as the corresponding imprinted polymers.

Table 1
Amounts of monomers and porogens used in polymerization of 34.9 mg (0.2 mmol) of gramine as the template, 754 μL (4 mmol) of ethylene glycol dimethacrylate (EGDMA) as the cross-linker and 9.5 mg of 2,2′-azobisisobutyronitrile (AIBN) as the initiator.

No. of MIPs	Functional monomer (mg, mmol) chemical formula	Porogen (μL)
1	4-vinylbenzoic acid (**1**), 118.5, 0.8 	Methanol, 754
2	methacrylic acid (**2**), 68.9, 0.8 	Methanol, 822
3	itaconic acid (**3**), 104.1, 0.8 	Methanol, 754
4	acrylic acid (**4**), 57.7, 0.8 	Methanol, 809
5	trifluoromethacrylic acid (**5**), 112.1, 0.8 	Methanol, 754
6	acrylamide (**6**), 56.9, 0.8 	Methanol, 805
7	isopropenylbenzene (**7**), 94.5, 0.8 	Methanol, 858
8	2-hydroxyethyl methacrylate (**8**), 104.0, 0.8 	Methanol, 852
9	2-(diethylamino)ethyl methacrylate (**9**), 148.2, 0.8 	Methanol, 915