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ScienceDirect
Biomaterials 28 (2007) 3741–3749
Biomaterials
www.elsevier.com/locate/biomaterials

Development and characterisation of molecularly imprinted polymers based on methacrylic acid for selective recognition of drugs

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Received 6 January 2007; accepted 30 April 2007
Available online 3 May 2007

Abstract

Specific molecularly imprinted polymers (MIPs) for the drug reserpine (RES) using methacrylic acid (MAA) as the functional monomer were developed and characterised for the first time in this study. Evaluation of the various polymers by binding assays indicated that the optimum ratio of functional monomer to template was 4:1. Furthermore, the imprinting effect of the MIPs was assessed by the chromatographic method, which demonstrated that the MIPs had better chromatographic behavior and selectivity than those of the corresponding NIPs. A combination of BET, NMR, UV spectroscopy, and MISPE analyses for investigation of the imprinting and recognition properties revealed that strong specific interactions between the functional monomer and RES in the prepolymerization solutions and the aqueous solutions were probably responsible for RES recognition. The preparation of RES MIPs and elucidation of imprinting and recognition mechanisms may serve as useful references for other drug MIPs.

Keywords: Molecularly imprinted polymers; Solid-phase extraction; Reserpine; Drugs

1. Introduction

The abuse of drugs in human and veterinary medicine has increased dramatically in recent decades. Some generic analytical methods, i.e. chemiluminescence [1], radioimmunoassay [2], gas chromatography (GC) [3], high-performance liquid chromatography (HPLC) [4] and combinations of GC–MS [5], or HPLC–MS [6], have been developed for drug determination. However, these methods usually require laborious procedures involving isolation, preconcentration and clean up, and liquid–liquid or solid-phase extraction, to concentrate and purify drugs before determination, and the reproducibility of these results is seriously affected by sample matrices [7]. Although specific biological recognition sorbents such as antibodies have been employed in analytical practices, they are expensive, laborious with complicated preparation procedures and labile to physical and chemical conditions [8]. Molecularly imprinted polymers (MIPs) as part of molecularly imprinted solid phase extraction (MISPE) with higher stability, selectivity and sample loading capacity can provide a new way to overcome these limitations. Some MIPs have been used for chromatographic separations, biosensors, and especially for selective recognition of templates from complicated biological matrices, such as plasma, urine and tissue samples [9,10].

MIPs are highly cross-linked porous-rich polymers with specific recognition sites complementary in shape, size and functional groups to the target molecule and capable of mimicking receptors and antibodies. MIPs can be synthesized by non-covalent procedures such as hydrogen bonding, π–π aggregation, electrostatic interactions, hydrophobic interactions, and by covalent procedures [11]. The non-covalent method is generally used due to the relatively simple experimental approach and more flexibility for different target molecules [12]. The choice of the cross-linkers and the monomers of polymerization are critical for the selectivity and adsorption capability of the

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doi:10.1016/j.biomaterials.2007.04.036

MIPs. Recent studies concern synthesis and new applications of the polymers, with less emphasis on understanding the mechanisms and interactions occurring between the template molecule and functional monomer [13]. However, a thorough understanding of the recognition mechanisms and physical parameters of corresponding polymers are very important to improve selective extraction.

UV–vis spectroscopic analysis and ^{1}H NMR study are commonly applied to characterise the nature of interactions and the extent of complex formation between functional monomers and template molecule in solution [13]. Meanwhile, scanning electron microscope (SEM) and Brunauer–Emmett–Teller (BET) analyses are used to elucidate the morphological characteristics [14]. These may provide valuable information for the synthesis and application of the MIPs.

Reserpine (RES) (Fig. 1) is a widely used medical and veterinary drug with sympatholytic, anti-hypertension and sedative hypnotics properties. However, abuse of RES can pose potentially serious threats to human beings and animals, such as severe depression, galactorrhoea, nightmares, breast cancer, Parkinsonism and gastrointestinal disturbances [15]. Hence, RES is under intense scrutiny in China and other countries.

The major goals of the present research were as follows: (i) to prepare and characterise MIPs as adsorption materials against the template molecule of RES drug; (ii) to elucidate the imprinting and recognition mechanism between the target drug and RES MIPs in the pre-polymerization and aqueous solutions; (iii) to investigate the adsorption mechanism of the MIPs in solid-phase extraction; and (iv) to achieve a reference strategy for development of MIPs for drugs and related materials.

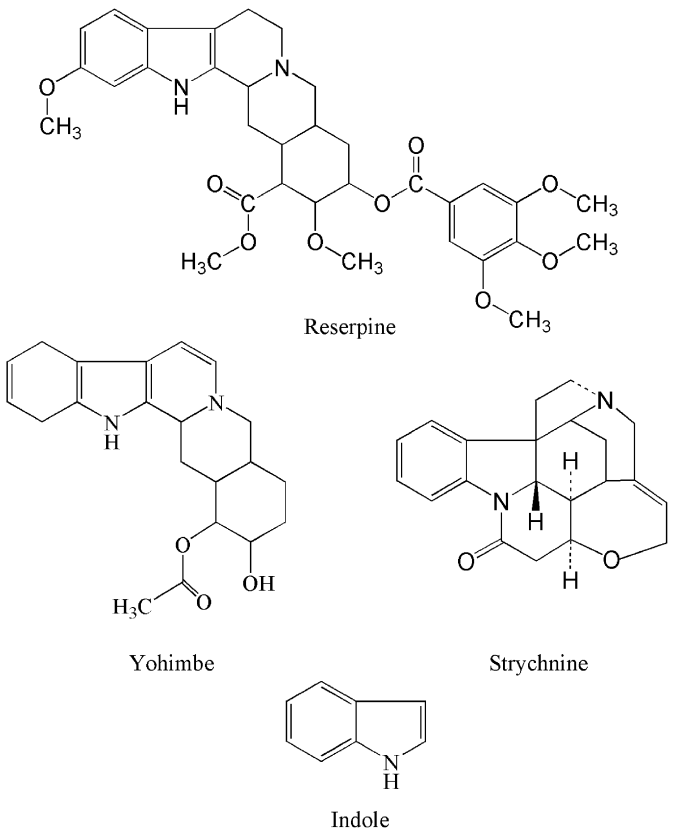

Fig. 1. The chemical structures of RES and the structural-related products.

2. Experimental

2.1. Materials and chemicals

RES, methacrylic acid (MAA), trimethylolpropane trimethacrylate (TRIM), yohimbine hydrochloride (YOH) were purchased from Sigma-Aldrich (Steinheim, Germany). Ethylene glycol dimethacrylate (EGDMA) and indole (IND) were obtained from Fluka (Steinheim, USA) and Fluka (Steinheim, China), respectively. 2,2′-azobis (2-isobutyronitrile) (AIBN) was from the China National Medicines Corporation Ltd (Shanghai, China). Strychnine (STR) was purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). HPLC grade methanol was obtained from Fisher Scientific Co. (USA). Other chemical reagents were of analytical grade from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Double distilled water and MilliQ water were obtained from local suppliers.

2.2. Preparation of imprinted polymers

The preparation of imprinted polymers was performed as follows. Typically, the template molecule RES (1 mmol) and the corresponding amounts of functional monomer MAA (Table 1) were added into chloroform in a round bottom flask, and the cross-linker EGDMA or TRIM and the initiator AIBN were added. The flask was sealed after the mixture solution was sonicated under a nitrogen atmosphere for 10 min, and then it was immersed in a 70 °C water bath and reacted for 24 h in darkness. The bulk polymers were crushed, ground and sieved to pass through a 50 μm sieve. The remaining fine particles were removed by sedimentation in acetone. The polymers were washed with methanol:acetic acid (8:2, v/v) in a soxhlet apparatus successively until no further RES could be detected by HPLC–UV analysis. Methanol was then used to remove the residual acetic acid, and the solution dried at 50 °C under vacuum for 12 h. The corresponding blank polymers as control were prepared in parallel in the absence of the template and treated in the same manner.

2.3. Binding assay

The rebinding capacity of various polymers with the target molecule RES was calculated by mixing various 50 mg polymer particles with 2 mmol/L of RES in 4 mL chloroform. The mixture was incubated for 24 h with continuous stirring at room temperature. The amount of rebound RES was calculated by subtracting the free RES from the initial amount. Polymers (20 mg) were added to a 2 mL chloroform solution of RES with various concentrations from 0.05 to 10 mmol/L and incubated for 24 h with stirring at 25 °C. After filtration, the solutions were evaporated to dryness under a nitrogen atmosphere, and re-dissolved with an appropriate volume of 6% acetic acid in water, and finally analyzed to quantify the concentration of free RES. The amount of polymer bound to the RES (B) was calculated by subtracting [RES] from the initial concentration. Each concentration was measured in triplicate for Scatchard analysis [16].

HPLC analysis of RES was carried out on a Beckman HPLC system (125 Pump, 166 Dec, USA) equipped with a UV detector, and A 7725I sample injection valve with a 20 μL sample loop and a Beckman ODS C18 column (4.6 mm id × 250 mm, 5 μm) were used. The column was kept at ambient temperature. The mobile phase consisted of methanol:water:acetic acid (70:30:0.8, v/v/v), and the flow rate was constant at 1.0 mL/min.