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Boc-L-tryptophan imprinted polymeric microparticles for bioanalytical applications

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ABSTRACT

Molecularly imprinted polymer (MIP) microparticles with good chromatographic characteristics were synthesized via the suspension polymerization process in a single preparative step. Initially, the effects of process parameters (i.e., porogen concentration, polymerization temperature, types and concentrations of functional monomer and cross-linker) on the particle size distribution and particle morphology were experimentally investigated. Subsequently, various MIP microparticles were synthesized in the presence of an amino acid derivative (i.e., boc-L-tryptophan), acting as template molecule. Batch-wise guest binding experiments were then performed to determine the rebinding capacity of the synthesized MIP microparticles towards the template molecule. Competitive binding experiments were also carried out with boc-D-tryptophan (i.e., the enantiomer of boc-L-tryptophan) to assess the selectivity of the imprinted polymer microparticles towards the two enantiomers. Finally, a quantitative description of the experimentally measured rebinding isotherms was obtained using the well-known Freundlich–Langmuir models. The present results clearly demonstrate the potential application of the synthesized MIP microparticles for bioanalytical separation of peptides and proteins since the amino acid templates employed in this study are the building units of larger biomolecules.

1. Introduction

Molecular imprinting is a process where a functional monomer and a cross-linker are co-polymerized (i.e., via a free-radical polymerization mechanism) in the presence of a target molecule (i.e., the so-called imprint molecule) that acts as a molecular template. Initially, the monomers are self-assembled around the template molecule through covalent or non-covalent molecular interactions of the functional groups attached to both the template and the functional monomer. This is followed by the copolymerization of the functional monomer with the bifunctional or trifunctional cross-linker. Subsequent removal of the imprint molecules reveals specific binding sites that are complementary in size and shape to the template molecule [1]. Thus, molecular imprinting is an efficient method for the synthesis of polymers with highly specific recognition sites [2–5].

In general, non-covalent imprinting is easy to achieve and applicable to a wide range of template molecules since many of practically important molecules (e.g., pharmaceuticals, herbicides, biologically active substances, and environmental contaminants) possess polar groups (e.g., hydroxyl, carboxyl, amino and amide) that can non-covalently interact with the functional groups of the monomer. The advantage of non-covalent imprinting is that the procedure is relatively simple and the synthesis of covalent conjugates is not required prior to polymerization. Furthermore, the template is easily removed from the polymer under very mild conditions since it is only weakly bound to the polymer matrix via non-covalent molecular interactions. Additionally, the guest binding-release, which takes advantage of the non-covalent interactions, is fast. Therefore, non-covalent molecular imprinting has been extensively studied due to its simplicity and versatility [1].

In principle, any kind of non-covalent molecular interactions, including ionic, hydrogen bonding, π–π interactions and hydrophobic interactions, can be effectively employed in molecular imprinting. However, hydrogen bonding is the most appropriate interaction for selective molecular recognition since this type of non-covalent force is highly dependent on both distance and direction between the functional monomer and the template molecule. Thus, monomers that bear the required functional groups (e.g., carboxyl, amino, pyridine, hydroxyl, and amide groups) complementary to the template molecule are commonly chosen for molecular imprinting [6,7].

Up to now, biomolecules of relatively low molecular weight (e.g., amino acid derivatives and oligopeptides) have been employed as templates in molecular imprinting. Commonly, the free-radical copolymerization of the functional monomer with the cross-linker is carried out in bulk. The cross-linked polymer is subsequently ground and sieved to obtain the final product in particulate form [8–12]. The free-radical bulk polymerization process is well established and is especially suitable for bioanalytical applications of MIPs. However, the process involves a number of steps (i.e., polymerization, grounding and sieving) that result in a low overall process efficiency due to the formation, during the polymer grounding step, and subsequent removal of a large amount of fine polymer particles. Commonly, the final polymer yield is less than 50%. Thus, there is a need for the development of alternative methods for the preparation of MIP microparticles with well-defined morphological characteristics [13–15].

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In principle, suspension polymerization offers an attractive alternative to the bulk polymerization process since it results in a high yield of spherical polymer particles. The polymer particles, produced by suspension polymerization, are suitable for different applications, including analytical chromatography, solid phase extraction, and other flow-through applications, since columns and cartridges packed with uniform spherical particles exhibit better flow characteristics than those packed with irregular particles [16].

Molecularly imprinted polymer microparticles were prepared by the inverse suspension polymerization method using a non-aqueous medium (i.e., liquid perfluorocarbon). However, the latter method suffers from a number of disadvantages, including the high cost of the perfluorocarbons and the need of a suitable fluorinated stabilizer that, usually, has to be synthesized. Alternatively, MIP microparticles can be prepared by the inverse suspension polymerization using mineral oil as the continuous phase. Key to the success of this method is that the dispersed liquid phase should not be soluble in the continuous oil phase. This sets some constraints regarding the selection of solvent employed as porogen. Thus, the relatively non-polar solvents (e.g., chloroform, dichloromethane and toluene) that generally favor the development of non-covalent interactions cannot be used with mineral oil. Additionally, MIP microparticles can be prepared by a multi-step process including the grafting of the imprinted polymer to preformed spherical particles (e.g., silica or acrylates) [16,17].

In the present study, the suspension polymerization method, in an aqueous continuous phase, was employed for the synthesis of molecularly imprinted polymer porous microparticles for bioanalytical applications. In particular, MIP microparticles with well-defined morphological characteristics and optimum rebinding properties were prepared using two types of functional monomers (i.e., methacrylic acid (MAA) and methacrylamide (MAm)) and two types of cross-linkers (i.e., ethylene glycol dimethacrylate (EGDMA) and trimethylopropane trimethacrylate (TRIM)). The protected amino acid (boc-L-tryptophan) was used as template molecule. Finally, chloroform dissolved in the dispersed liquid droplets was used as porogen. The effects of process parameters (i.e., porogen concentration, polymerization temperature, types and concentrations of functional monomer and cross-linker) on the particle size distribution and particle morphology were experimentally investigated. Batch-wise guest binding experiments were carried to determine the rebinding capacity of the synthesized MIP microparticles towards the template molecule. Competitive binding experiments were also carried out with boc-D-tryptophan (i.e., the enantiomer of boc-L-tryptophan) to assess the selectivity of the imprinted polymer microparticles towards the two enantiomers.

2. Materials and experimental methods

2.1. Materials

Methacrylic acid (MAA), ethylene glycol dimethacrylate (EGDMA), acetonitrile and methanol were purchased from Merck. Chloroform was purchased from Riedel and acetic acid from Carlo Erba. Trimethylopropane trimethacrylate (TRIM) and 2, 2′ azobis-(2-isobutyronitrile) (AIBN) were purchased from Aldrich. Boc-L-tryptophan (boc-L-trp), boc-D-tryptophan (boc-D-trp), methacrylamide (MAm), methanol and poly(vinyl acetate) (PVA) of 100,000 MW and degree of hydrolysis of 86–89% were purchased from Fluka.

2.2. Preparation of the molecularly imprinted microparticles

The polymerization experiments were carried out in a laboratory scale, water-jacketed glass reactor of 100 ml working volume, equipped with a six-blade impeller, an overhead condenser and a nitrogen purge line. The reaction mixture was thermostated to within ±0.05 °C with the aid of a constant temperature bath. Initially, the template molecule (i.e., boc-L-Trp), the functional monomer, the cross-linker and the initiator (2% w/w on the total monomers mass) were dissolved in chloroform. In Table 1, the experimental conditions selected for the preparation of the different types of MIP and NIP microparticles are reported. In all the experimental runs, the molar ratio of the cross-linker to the functional monomer was equal to 5:1 for EGDMA and 3.3:1 for TRIM so that the total double bonds concentration, for both types of the cross-linker, was the same [12]. Accordingly, the organic phase (15 ml in volume, containing the functional monomer, the cross-linker, the porogen, the amino acid derivative and the initiator) was dispersed into an aqueous PVA solution (35 ml in volume, 1% w/w) under the action of a mechanical agitator and a nitrogen atmosphere. Subsequently, the polymerization was carried out, at the specified temperature (i.e., 60 °C or 80 °C), for 24 h. The template molecules were then removed from the polymer microparticles by means of successive washing cycles with a methanol–acetic acid solution (9:1 v/v). The template removal was monitored via UV spectroscopy. Finally, the washed polymer microparticles were conditioned in methanol. Non-imprinted polymeric (NIP) microparticles were also prepared under the same polymerization conditions.

Table 1
Selected experimental conditions for the preparation of MIP and NIP microparticles.

Polymer	Template	Monomer	Cross-linker	Chloroform	Temperature
MIP 1	553 mg	MAA, 0.62 ml	EGDMA, 6.88 ml	7.5 ml	60 °C
NIP 1	–	MAA, 0.62ml	EGDMA, 6.88ml	7.5ml	60 °C
NIP 2	–	MAA, 0.62 ml	EGDMA, 6.88 ml	7.5 ml	80 °C
MIP 3	332 mg	MAA, 0.37 ml	EGDMA, 4.13 ml	10.5 ml	60 °C
NIP 3	–	MAA, 0.37ml	EGDMA, 4.13ml	10.5ml	60 °C
MIP 4	497 mg	MAA, 0.56 ml	TRIM, 6.94 ml	7.5 ml	60 °C
NIP 4	–	MAA, 0.56ml	TRIM, 6.94ml	7.5ml	60 °C
MIP 5	555 mg	MAm, 0.62 g	EGDMA, 6.82 ml	7.5 ml	60 °C
NIP 5	–	MAm, 0.62g	EGDMA, 6.82ml	7.5ml	60 °C

2.3. Characterization of the molecularly imprinted microparticles

The size distributions of NIP and MIP microparticles were measured with the aid of a Malvern mastersizer 2000 light scattering instrument. The surface morphology of the microparticles was determined by a JSM-6300 scanning electron microscope. The pore size distribution and the specific surface area of the washed microparticles were analyzed by Brunauer–Emmett–Teller (BET) analysis, using a Quantachrome Autosorb Automated Gas Sorption apparatus.

2.4. Binding experiments

Equilibrium batch-wise guest binding experiments were conducted to evaluate the rebinding isotherms of both NIP and MIP microparticles. For each rebinding isotherm, five polymer samples of the same weight (i.e., 0.15 g) were employed. The polymer samples were equilibrated for 24 h, under mild agitation conditions, in respective analyte solutions (5 ml each) of known concentrations, using either chloroform or acetonitrile as solvent. Subsequently, the solid polymer microparticles were separated from the solvent, using a membrane filter with a pore diameter of 0.45 μm, and the concentration of the free analyte (F) in the solution was measured by a Lamda 35 UV/VIS spectrometer from Perkin Elmer. Accordingly, the corresponding bound analyte concentration (B) was calculated from the difference of the initial minus the final free analyte concentration. The quantification of boc-L-trp and boc-D-trp was realized at 280 nm.

3. Results and discussion

In the suspension polymerization process, the organic phase, containing the functional monomer, the cross-linker, the solvent, the template molecules and the chemical initiator, is initially dispersed, with the aid of an agitation system, in the continuous aqueous phase containing the surface-active agent. Subsequently, the temperature is