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Molecularly imprinted polymers for enhanced impregnation and controlled release of L-tyrosine

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ABSTRACT

L-tyrosine controlled release systems, based on imprinted polymers radically polymerized in modified organic solvents (dimethyl sulfoxide and methanol) were developed. A set of copolymers synthesized with different functional monomers and the crosslinker ethylene glycol dimethylacrylate were evaluated regarding both the imprinting and controlled release features. Those prepared in modified-DMSO were mesoporous (surface area ranging 370–690 m^2/g) but not the ones prepared in modified methanol with the exception of the imprinted polymer prepared with the monomer vinylbenzoic acid (209 m^2/g). The swelling degree ranged 32 to 64% and appeared to be mostly dependent on the functional group employed in the synthesis of the polymers. When tested in dynamic mode a clear imprinting effect has been observed in some of the imprinted polymers. However, selectivity for L-Tyr against similar compounds could not be found in these conditions. Selectivity could be observed when carrying out the isothermal analysis, with selectivity factors up to 2.7. A few polymers exhibited also other remarkable features such as high L-Tyr load capacity (up to 253 mg L-Tyr/g) and large binding strength (up to 55 $L^m mg^{(1-m)}$)/g). Therefore, imprinted polymers capable of a selective sorption of L-Tyr upon equilibration, containing stronger binding sites for L-Tyr, appeared promising vehicles for its controlled release. In fact, the release assays with L-Tyr impregnation by adsorption, showed profiles corresponding to controlled release systems, with high yield (40–50%) and continuous release (up to at least 7 h). In addition, release mechanisms recommended for controlled release systems were deduced from kinetic data fitting to the Korsmeyer and Order 0 models.

1. Introduction

The conventional pharmaceutical formats result in the fast and indiscriminate release of drugs into the blood stream, causing a plasmatic peak followed by an exponential decay. Several administrations are thus required to re-establish the therapeutic levels [1]. This inefficiency in drug administration prompted the research of controlled drug release systems that could extend the therapeutic activity to a longer period by providing the drug at a known constant rate. Similarly, in other instances of administration of chemical substances, such as the release of biocides for plague control or the release of antioxidants for food conservation, the controlled release has been the focus of extensive research [2–7]. The physical approach to the development of controlled release systems (CRS) consists in the formulation of macroscopic materials capable of complexing, adsorbing, incorporating or encapsulating the active substance, which ideally is released at a constant controllable rate.

The large majority of CRS are currently based on polymers presenting a release process controlled by diffusion [8]. Among the polymeric CRS, the imprinted polymers constitute a special category featuring a predetermined selective interaction with the active substance. The selectivity of the imprinted CRS originates at the tailored-at-synthesis strong binding sites. A sustained diffusion is thus attained due to strong polymer-substance interactions that modulate the release profile. The imprinting process (commonly known as molecular imprinting) [9] applied to the synthesis of polymeric matrices typically relies on a pre-polymerization mixture containing at least the active substance or a closely related one, a functional monomer, a crosslinker and a solvent. The functional monomer presents some non-polymerizable group(s) that forms a stable non-covalent adduct with the active substance during the polymerization process. The adduct will thus be expectedly kept in the final polymer. By extensive washing, the active substance (or template) is extracted out of the polymer, leaving, in principle, the cavities with the size, shape and chemical complementarity optimized for the rebinding of the template. The most successfully imprinted polymers achieved so far were obtained by the

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radical (co)polymerization of metacrylic, vinylic, acrylamide and similar [10] monomers.

L-tyrosine (L-Tyr) is a powerful antioxidant whose controlled release from cellulose acetate food packaging membranes has been proposed [6]. L-Tyr is also an important pro-drug involved in the synthesis of neurotransmitters in brain. In conditions of L-Tyr deficiency (a factor associated with depression) or in the treatment of Parkinson's disease, the administration of L-Tyr can bring therapeutic benefits [11, 12]. However, to the best of our knowledge, the controlled release of L-Tyr from imprinted matrices has been approached in two researches only [7, 13]. In the first one the preparation of the CRS [13] was based on the seed swelling and suspension polymerization in an aqueous medium due to the low solubility of L-Tyr in the usually low-polarity solvents required for the most common imprinting procedures. In the second one the CRS relied on a chitosan/gel copolymer [7]. Due to the scarcity of work developed in the CRS of L-Tyr based on imprinted polymers, we decided to exploit a CRS grounded on the most successful imprinting approach developed so far, the radical polymerization in organic solvent. Supported on recent literature concerning the molecular imprinting of neurotransmitters (cf. Results and Discussion), we studied a set of copolymers, synthesized in modified organic solvents, with different functional monomers crosslinked with ethylene glycol dimethylacrylate (EGDMA). These were evaluated regarding both the imprinting and controlled release features.

2. Materials and methods

2.1. Materials

For the synthesis of the polymers different monomers were tested, such us, methacrylic acid (MAA), acrylamide (AA), itaconic acid (IA) and 4-vinylbenzoic acid (VBA), all of them Sigma-Aldrich, (> 99% purity) except for the latter, VBA (ABCR, > 99% purity). The crosslinker used for the polymerization was EGDMA (Aldrich, > 99%) and the radical initiator was azobisisobutyronitrile (AIBN) (Aldrich, > 99%). L-Tyr (Aldrich, > 99%) was used as template in synthesized polymers. For the selectivity assays 3-Nitro-L-tyrosine (Nitro-Tyr), also Sigma-Aldrich, (> 99% purity) was used. All other reagents were analytical grade. Water was of Milli-Q (Millipore, Italy) purity.

2.2. Preparation of the polymers

The molecularly imprinted polymers (MIPs) were developed using two modified organic solvents. One of them (mod-MeOH) consisted of methanol mixed with water, acetic acid and trifluoroacetic acid (TFA) in the ratio of $8.75/1.25/50 \times 10^{-3}/\ 12 \times 10^{-3}$ (in volume), respectively. The second one (mod-DMSO) consisted of dimethylsulfoxide mixed with acetic acid and TFA in the ratio $5{:}50 \times 10^{-3}/\ 12 \times 10^{-3}$ (in volume), respectively. A typical synthesis comprised 20 mmol (4 mL) of crosslinker EGDMA, 1 mmol of the functional monomer (86 μL - MAA, 130 mg - IA, 71 mg - AA and 148 mg - VBA), 0.25 mmol (45 mg) of the L-Tyr template and 80 mg AIBN radical initiator agent (2% of EGDMA's mass). Table 1 condenses the constituents of all the polymerization mixtures and the acronyms used throughout the paper for the identification of the corresponding polymers.

Soon after the introduction of AIBN (the last component added to the mixture), the reaction mixtures were purged with nitrogen during 5 min, and the glass flasks were tightly closed. The polymerization took place in an oven at 50 °C, producing monoliths in about 3 h. These were then crushed and soxhlet-washed (12 cycles in average) with a MeOH/H_2O/CH_3COOH (15/55/30 v/v) solution for removal of the template and excess reagents. Non-imprinted polymers (NIPs) were synthesized in the same way, through the same synthetic steps (including the soxhlet-wash, although there was no template), but without addition of L-Tyr.

2.3. Characterization of polymers

Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) measurements were performed using a Bruker FT-IR System Tensor 27 spectrophotometer in the range of 600–4000 cm^{-1}.

The degree of swelling (S_w) in polymers was determined by keeping 30 mg (W_0) of the polymers in 1 mL of PBS buffer solution (pH 7). The increase in weight (W_t- W_0) of polymers, after 24 h of agitation, in comparison to initial weight (W_0) was used to calculate the degree of swelling (S_w) using Eq. (1)

$$S_w(\%) = \frac{W_t - W_0}{W_0} \times 100 \quad (1)$$

where W_0 and W_t are the initial and final weights of the biosorbent, respectively.

The surface area and the pore parameters were determined by a nitrogen adsorption analyzer (TriStar Plus, Micromeritics). The experiments were made with 200–500 mg of sample, which were previously dried overnight in the oven at 50 °C and were for at least 2 h, under a flow of nitrogen. The specific surface areas (*S*) were evaluated using the BET method, the specific pore volumes (*Vp*) following the Gurvitch method and the average pore diameter (*Dp*) using the BJH theory applied to the desorption branch of the isotherm.

Thermal gravimetric analysis (TGA) was conducted on a Hitachi, Model STA 7200 R*V* Analyzer from room temperature to 600 °C with a heating rate of 10 °C/min under a nitrogen flow.

A Hitachi FlexSEM1000 scanning electron microscope (SEM), operated at an acceleration voltage of 7000 V, was used to *v*isualize the surface morphology of the polymers.

2.4. Solid phase extraction

SPE cartridges were packed individually with 500 mg of each polymer. The cartridges were conditioned with 10 mL MeOH/H_2O/CH_3COOH (15/55/30 v/v) and 5 mL MeOH/H_2O (15/85 v/v). Samples (10 mL) containing 10 ppm, of each analyte (L-Tyr, Nitro-Tyr) in MeOH/H_2O (15/85 v/v) were then introduced. The percolation proceeded at a constant flow rate of 0.7 mL/min, in a Visiprep (Supelco, Bellefonte, USA) SPE station manifold. After sample loading, 6 mL of

Table 1
Composition of the pre-polymerization mixtures used in the synthesis of the imprinted-polymer set studied.

Imprinted-polymers	Solvents	Crosslinker (20 mmol)	Radical/initiator agent	Monomers (1 mmol)	Template (0.25 mmol)
AA_mod-MeOH	mod-MeOH (10 mL)	EGDMA	AIBN (2% of crosslinker mass)	AA	L-Tyr
VBA_mod-MeOH				VBA	
IA_mod-MeOH				IA	
MAA_mod-MeOH				MAA	
AA_mod-DMSO	mod-DMSO (5 mL)			AA	
VBA_mod-DMSO				VBA	
IA_mod-DMSO				IA	
MAA_mod-DMSO				MAA	