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Development of high performance and facile to pack molecularly imprinted particles for aqueous applications

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ABSTRACT

Different kinds of molecularly imprinted particles were synthesized and compared, aiming at the development of materials combining high molecular recognition capabilities and facile use as column packing materials for chromatographic aqueous applications. Solution, inverse-suspension and precipitation polymerization were considered and two different model molecules (5-fluorouracil and caffeine) were used to highlight the effect of the interaction between the template molecule and the functional monomer on imprinting efficiency. Particles synthesized through the proposed inverse-suspension process exhibit facile use for packing columns, allow the stable running of chromatographic systems and present a high performance in drug uptake and release in aqueous media. Frontal analysis measurements highlight these key features of the synthesized particles. Drug sorption capabilities of 0.890 μmol/g and 5.774 μmol/g were measured for 5-fluorouracil and caffeine, respectively, using frontal analysis with eluents containing the target molecules at concentration 0.1 mM. Due to the lower amount of solvent required than with precipitation polymerization, the developed inverse-suspension process presents high synthesis yields, which can be exploited for the large-scale manufacture and commercialization of molecularly imprinted materials. The combined features of the particles makes possible their direct use in bioseparations or in the development of assays and pharmacokinetic studies concerning the presence of drugs in biological fluids.

1. Introduction

Molecular imprinting is based upon the creation in a polymer network of tailor-made cavities having high specificity and affinity with respect to a target molecule. This goal can eventually be achieved through the promotion of an efficient target/template interaction during the polymer network formation. Generically, the molecular imprinting process comprises three different stages: (i) template-functional monomer self-assembly, (ii) polymerization with creation of a polymer network and (iii) template removal. Finally, the generated three-dimensional cavities should ideally match the template molecule in their size, shape and the arrangement of functional groups. The major purpose is to get molecular 'memory' in a polymer network, which should be able to recognize the specific target molecule in several reversible cycles (e.g. uptake/release of the target molecule).

Nowadays, molecularly imprinted polymers (MIPs) play the role of artificial antibodies and find many applications in different fields due to their chemical stability and to the straightforward method of synthesis typically used for their production [1]. Emergent applications of MIPs encompass different kinds of separations, such as chromatographic and bio-separations [2–5], solid-phase extraction (SPE) systems [6–10], different kinds of assays [11,12], development of sensors [6,12–17], membranes [18,19], catalysis [20,21], and many biological processes (e.g. in biomedicine for controlled drug delivery or other biological functions [22,23]). A surge of scientific papers devoted to molecular imprinting systems was observed in the last two decades as reported in recent literature surveys on this field [24,25]. Many other works describing the use of MIPs in the above-mentioned applications can be found in reviews such as in references [24,25]. Some current challenges in MIP development are related with the imprinting of macromolecules [26] and the development of MIP particles with controlled size (e.g. nanoparticles) and format (e.g. core-shell architectures) as well as the extension of their synthesis processes to large scale manufacturing [27]. Production of MIPs using reversible-deactivation

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radical polymerization (RDRP) [28–31], aiming at the control of the network formation process (leading to tailor-made molecular architectures), introduction of sensitivity to external stimuli (such as the changes of temperature, pH, ionic strength exploited for controlled drug delivery) and compatibility with aqueous systems [32–36] are other important research lines in MIPs development.

This work reports a comparative study concerning the synthesis and evaluation of MIP particles aiming at the targeting of 5-fluorouracil (5FU) and caffeine (CAF) template molecules. 5FU is a drug used in cancer chemotherapy (e.g. with gastrointestinal tract or brain tumors) but presents harmful toxicity effects due to the high doses often needed in the treatments. Driven by these adverse consequences, the development of MIP materials for 5FU recognition and drug controlled release has been reported in a few recent research works [32–41]. Caffeine belongs to the group of methylxanthines found in many natural beverages (coffe, tea, and so on). Caffeine can have beneficial effects on health (it is used in several medicines for stimulation of central nervous system and of the overall metabolism) but can also generate many unwanted effects (fast heartbeat, nervousness, and so on). Triggered by the important properties and applications of caffeine, the MIP based extraction, selective separation and molecular recognition of this molecule was developed in the last years in several researches [7–9,15–17,42–57]. The selective molecular recognition of other methylxanthines, such as theobromine and theophylline, has also been addressed in other research papers [8,50,54,58–61].

In the research here reported, the two different template molecules above described (5FU and CAF) and some of their structural analogues (uracil and thymine with 5FU, theobromine and theophylline with CAF) were considered together in combined studies aiming at the development and evaluation of MIPs with optimized performance. Different combinations between template (T), functional monomer (FM) and crosslinker (CL) were used to obtain insights on the effects of T/FM/CL interactions on imprinting efficiency and performance of the produced materials in molecular recognition.

The preparation of MIPs with different morphologies, namely monoliths and spherical particles, was also considered in this research in order to assess the effect of particle shape on the materials performance (e.g. molecular recognition and drug sorption/desorption capabilities). Solution polymerization and inverse suspension polymerization were alternatively used to obtain materials with different morphologies. For comparison purposes, a few syntheses using precipitation polymerization in acetonitrile were also considered in this research. Changes in the morphology of the different products obtained have been highlighted using SEM.

Performance tests of the prepared MIPs in molecular recognition and the quantification of retained amounts of drugs which were further released from these materials have been carried out using different techniques, namely solid phase extraction (SPE), batch equilibrium binding sorption isotherms, chromatographic elution and frontal analysis. Imprinting factors (*IF*), selectivity factors (*SF*) and amount of drugs retained and released per unit of mass of adsorbent were measured through these different techniques. A comparative and absolute evaluation of the effect of the synthesis conditions on the MIPs performance for 5FU and caffeine molecular recognition was thus obtained.

At last, it was here shown that facile to pack molecularly imprinted particles with high molecular recognition capabilities for caffeine and 5FU can be produced using the proposed inverse-suspension technique, which presents also a high synthesis yield. These materials exhibit good drug uptake/release in aqueous solutions making feasible their use in biotechnological and biomedical applications.

2. Materials and methods

2.1. Materials

Functional monomers acrylic acid (AA), methacrylic acid (MAA), crosslinkers ethylene glycol dimethacrylate (EGDMA), trimethylolpropane triacrylate (TMPTA), thermal initiator azobisisobutyronitrile (AIBN), solvent *n*-heptane, template molecule 5-fluorouracil (5FU), structural analogues uracil (UR) and thymine (THY) were purchased from Sigma-Aldrich (Germany). Template molecule caffeine (CAF) and the structural analogues theophylline (THP) and theobromine (THB) were purchased from Acros Organics (Belgium). Analytical reagent grade dimethylformamide (DMF), acetonitrile (ACN) acetic acid (AcOH), methanol (MeOH) and acetone were bought from Fisher Chemical (UK). Surfactant sorbitan mono-oleate (span 80) was purchased from Panreac (Spain). The functional monomer 2,6-*bis*(acrylamido)pyridine (BAP) was synthesized following the procedure reported in the literature [37]. Millipore water (Milli-Q quality) was used in all the experiments unless otherwise mentioned.

2.2. Synthesis of imprinted (MIP) and non-imprinted (NIP) materials

2.2.1. Solution polymerization

Typically, the template molecule (5FU or CAF) was mixed in DMF with the functional monomer (AA, MAA or BAP) and the template – functional monomer (T-M) interaction was promoted with an ultrasounds bath during 30 min. Then, a DMF solution containing the crosslinker (EGDMA or TMPTA) and the initiator was added to that mixture. The final solution was transferred to a glass vessel and purged with a flow of dry argon for 10–20 min. The vessel was then sealed and polymerization was started, under vigorous stirring, in a thermostatic oil bath pre-set at 70 °C, proceeding during 24 h.

2.2.2. Inverse-suspension polymerization

In a thermostatic oil bath pre-set at 70 °C, span 80 was dissolved in *n*-heptane under vigorous stirring. In parallel, a DMF solution containing the required amounts of template, functional monomer crosslinker and initiator was prepared following a procedure similar to the above described solution polymerization runs. Afterward, under vigorous stirring, this solution was drop wise fed to the reaction vessel containing the oil phase (*n*-heptane/span 80) and polymerization was carried out during 24 h.

2.2.3. Precipitation polymerization

A similar procedure to the above described solution polymerization runs in DMF was adopted for precipitation polymerization but using ACN as solvent at a large excess (96 wt%).

Non-imprinted materials were prepared in parallel, following the same experimental procedure as for imprinted, eliminating the presence of the template molecules (5FU or CAF). Details on the synthesis runs performed in this research are presented in Tables 1 and 2. The following definitions apply to the parameters used to describe these polymerization runs:

- Y_m (%): Mass fraction of monomer + crosslinker in the solution.
- Y_I (%): Mole fraction of initiator comparatively to monomer + crosslinker.
- Y_{CL} (%): Mole fraction of crosslinker in the mixture monomer + crosslinker.
- $Y_{CL/T}$: Mole ratio between crosslinker and the template molecule.
- $Y_{M/T}$: Mole ratio between functional monomer and the template molecule.