Affinity Polymers for Pharmaceutical Purification

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ABSTRACT

In this work molecularly imprinted polymers (MIPs) were developed in $scCO_2$ for applications in API manufacturing processes, using a model pharmaceutical impurity (PI) as template. Acrylate and acrylamide-based monomers and cross-linker were used, and the effect of using a co-solvent during polymerization was evaluated.
Produced copolymers were characterized by Morphologi G3 in order to determine particle size distribution. Binding tests were performed in order to evaluate affinity and selectivity of the matrixes towards the template, in different template concentrations and in the presence of analogous molecules. Quantification was performed by HPLC. Promisor preliminary results were obtained in terms of adsorption affinity and a strong effect on MIPs performance was observed by using co-solvent in the polymerization step. We show the high potential of these affinity polymeric materials for pharmaceutical applications.

INTRODUCTION

During production of APIs (active pharmaceutical ingredients), reactive intermediates, catalysts, acids or bases are often used. Due to their chemical structure and reactivity, some are recognized as being genotoxic and can potentially end up at trace levels in final drugs [1]. Due to increasing concerns on health risks huge efforts have been devoted to find more efficient processes to perform this purification. Expensive chromatography, nano-filtration and activated carbon powder treatments are typically used in the pharmaceutical industry to eliminate several types of impurities from APIs post reaction streams [2]. Molecular Imprinted Polymers (MIPs) appears as an alternative to these conventional strategies for use as adsorption materials in API purification processes in the pharmaceutical industry.
Molecular imprinting uses the functionality of the template, the molecule for which the affinity is wanted, to assemble its own recognition site by forming specific interactions with the matrix. During polymerization, the template forms a stable complex with the monomer, in the presence of a porogen and a cross-linker that freezes the complex within a rigid porous polymer matrix [3]. Typically synthetic MIP receptors have binding constants comparable to natural receptors. MIPs as highly cross-linked polymers, offer significant advantages such as cost, high temperature and pressure resistance, and inertia to strong bases and acids, organic solvents etc., being capable of withstanding much harsher conditions than natural molecules. These affinity materials can be applied to diverse areas such as separation sciences and purification, chemical sensors, biological antibodies, catalysis, drug delivery, etc. [4].

Nevertheless the conventional methods to prepare MIPs involve the use of organic solvents, the materials are often obtained as bulky materials which must then be crushed and ground to fine particles which destroy and greatly reduce the existing sites grinding, and there is also the need of intensive purification steps. Slow interaction kinetics and heterogeneous nature of the binding sites with respect to geometry and accessibility have also been reported. Thus MIPs developed using supercritical fluid technology can be considered emergent, greener and sustainable alternatives for APIs purification. When these materials are produced under supercritical technology presented better properties than previous strategies such as, polymer is obtained without any residues of solvent or other reactants and no need purification steps. Beyond these, polymer is obtained with controlled morphology, free-flowing powder, dry and sterile. For these reasons, these materials are strong candidates to solve problems brought by conventional materials.

MATERIALS AND METHODS

A pharmaceutical impurity (PI) was used as template, acrylate- and acrylamide-based monomer and ethylene glycol dimethacrylate (EGDMA, 98 % purity) as cross-linker were purchased from Sigma-Aldrich. Azobis(isobutyronitrile) (AIBN, 98 % purity) from Fluka was used as initiator. HPLC grade acetonitrile (ACN) from Carlo Erba was used. Carbon dioxide was obtained from Air Liquide with purity better than 99.998 %. All chemicals were used without further purification.

Particle size distribution as well as particle size diameters of the synthesized copolymers were determined using a Morphology G3 equipment from Malvern. The analysis was performed dispersing sample using foil disks with 25 μm, sample volume 13 mm^3, SDU settings (Injection pressure: 4 bar, Injection time: 40 ms, Setting time: 120 s), Optic selection 50 x. The analysis was performed in triplicated from three different dispersions with 35 000 particles.

Chromatographic analyses were carried out using a Merck L-7100 HPLC pump equipped with an L-7400 UV detector, D-7000 computer interface and a XT Maraton auto-sampler. UV detection was made at 220 nm. Column used was a VertexPlus Column 250 x 4 mm Eurospher II 100-5 C18 with pre-column. Experiments were carried out at 25 °C using a column oven (XT Maraton). The mobile phase used was was 5 % acetonitrile and 95 % Milli-Q water at a flow rate of 0.5 $mL.min^{-1}$. 5 $\mu g.mL^{-1}$ sample was injected for analysis using a loop volume of 10 μL. The solvents used were filtrated with 0.20 μm filter and degassed in a Nahita ultrasound bath model 620/10.

RESULTS

MIPs produced were based on acrylate and acrylamide monomers, EGDMA as cross-linker and a model PI as template. Copolymers were synthesized under supercritical conditions with a molar ratio of template, monomer and cross-linker (T:M:C) 1:4:20 at 65 ºC and 20 MPa for 24 h. NIPs were produced at the same conditions without template in order to calculate the imprinting factor. Polymers were obtained dry free-flowing powders in high yields (> 90 %, determined gravimetrically). All polymers present a similar morphology consistent with precipitation polymerization in $scCO_2$ [5]. $scCO_2$-assisted template desorption, a crucial step to free the specific sites to further rebinding, was performed in continuous at 40 ºC. Template residues were evaluated by

crushing a small amount of polymer and evaluate template release within 3 days by HPLC.

Results obtained by Morphologi G3 show that Acrylate-based NIP copolymers, produced with and without a co-solvent, do not present any significant differences in particle size diameter. On the other hand, in the case of MIP copolymers, Acrylate_MIP and Acrylate_MIP_solv, we could observe a slight increase in particle size diameter from 4.7 to 5.3 μm that can be related with the presence of some agglomeration.

For the Acrylamide–based polymers the same tendency of results was not verified. The addition of co-solvent did not affect the particle size, and Acrylamide MIPs presented smaller particle size diameters. Acrylamide-based NIPs presented a particle size diameter around 4.4 μm while Acrylamide_MIPs showed a particle size diameter around 3.6 μm. This might be due to specific interactions of the co-solvent with the template, slightly interfering with the overall co-solvency of the growing polymeric chains, thus precipitating sooner in the reactor.

Static binding tests were carried out in order to evaluate the affinity capacity of MIPs, using solutions containing pharmaceutical impurity with concentrations ranging from 5 to 250 $\mu g.mL^{-1}$. **Figure 1** presented the results obtained on acrylate- and acrylamide-based copolymers @5 $\mu g.mL^{-1}$.

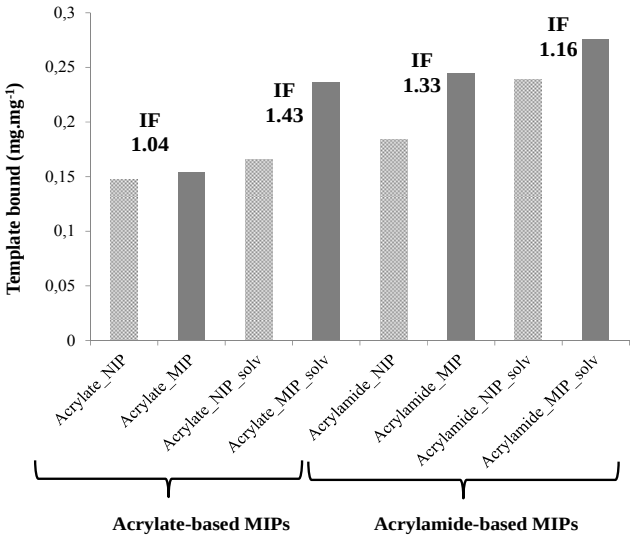

Figure 1: Template binding tests on acrylate- and acrylamide-based copolymers @5 $\mu g.mL^{-1}$.

Higher adsorption capacity of pharmaceutical impurity was attained by the imprinted material than NIP. It is interesting to observe that in all cases, imprinting factor (IF) is above 1 meaning that all MIPs have a higher ability to recognize template than NIPs. Imprinting factor is the ratio between the amount adsorbed by MIP and NIP and can give us an idea how MIP is better than the respective control. As expected the most notorious performance is obtained at very low concentrations, where applications of MIPs can have a real impact. Acrylate-based MIPs produced using a co-solvent presented the highest imprinting factor (1.43) also showing the most significant differences between MIP an NIP in all experiments.

In terms of binding performance we could also observe that Acrylate_MIP_solv adsorbs approximately the same amount than Acrylamide_NIP_solv showing the importance of the choice of the monomers in the process. This fact can be related with the acrylamide-based monomer structure which is similar to the template leading to an increased

affinity of the polymer towards the template when comparing with acrylate-based polymer. Although the lowest imprinting factor was attributed to Acrylamide_MIP_solv, it was the composition that could adsorb the highest amount of template, 0.28 $mg.mg^{-1}$, which is a promising result. This means that by simply controlling the nature of the monomers it is possible to fine-tuning the binding capacity of the polymers.

CONCLUSIONS

Highly cross-linked polymers with increased affinity to a pharmaceutical impurity were developed using molecular imprinting strategy in $scCO_2$ for pharmaceutical purification.
The synthesized polymers had a particle size diameter ranging from 3.4 and 5.3 μm.
MIPs showed better performance at lower concentrations as it was expected.
The polymer composition in terms of the nature of the monomer used and the use of a co-solvent had a strong effect on the material's affinity and thus the performance could be tuned at the synthesis step.
In conclusion, the synergy between two technologies, molecular imprinting and supercritical technology, is a promising and sustainable pathway for the development of purification devices.

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