Development of dummy artificial receptors for smart pharmaceutical purification processes

R. Viveiros,[a,b,#] V.D.B. Bonifácio,[c] W. Heggie,[b] T. Casimiro[a*]

[a]*CleanMIPTech group, LAQV-REQUIMTE, Chemistry department, NOVA School of Science & Technology, NOVA University of Lisbon, Portugal*

[b]*Hovione FarmaCiencia SA, R&D, Sete Casas, 2674-506 Loures, Portugal*

[c]*iBB-Institute for Bioengineering and Biosciences and i4HB-Institute for Health and Bioeconomy, Instituto Superior Técnico, University of Lisbon, Portugal*

[#]raquel.viveiros@fct.unl.pt

1. Introduction

Since 2006, the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA)[2] have imposed strict limits on the content of genotoxic compounds in final drug products, which has led pharmaceutical companies to seek even more efficient procedures, that could address the problem. Therefore, there is a particular interest in cost-effective processes that can efficiently remove genotoxic compounds down to levels defined by regulatory entities.

Typical purification processes such as chromatography, nanofiltration, recrystallization, resin purification, extraction, activated carbon powder treatments, distillation and hybrid processes are typically adopted[3] often with low specificity and, in some cases, at high cost.

Molecularly Imprinted Polymers (MIPs) as artificial receptors are very appealing alternatives to address this problem due to their high specificity for the removal of contaminants from crude mixtures.[4] These materials are very stable under harsh conditions, robust, reusable and be prepared in a cost-effective manner. Dummy templating approach is used when the real template can turn the preparation process hazardous or when the cleavage of the real template molecule from the matrix is difficult, thus effecting the accuracy of the analysis and quantification.[5] The analogue templating approach allows to fine tune the cavity size of the artificial receptors and produce high affinity materials for a family of compounds.

Acetamide (ACET) is a low molecular compound, well-known as potential genotoxic impurity at the final stages of drug manufacture.[1] Analogue Template Molecularly Imprinted Polymers (AT-MIPs) were developed in $scCO_2$, to target ACET by using benzamide and pivalamide, as planar- and 3D-shaped analogue template molecules using methacrylamide and ethylene glycol dimethacrylate, as the functional monomer and crosslinker. The AT-MIPs are assumed to have quasiplanar and 3D-shaped affinity cavities.[6]

2. Materials and Methods

Materials. Benzamide (BENZ, 99% purity), pivalamide, (PIV, 98% purity), acetamide (ACET, 99% purity), methacrylamide (MAM, 99% purity) ethylene glycol dimethacrylate (EGDMA, 98% purity) from Sigma-Aldrich. Azobis(isobutyronitrile) (AIBN, 98% purity) from Fluka. HPLC grade acetonitrile (ACN) from Carlo Erba was used for chromatographic separations. SPE syringes Supelclean TM LC-Ph SPE Tubes from Supelco. Trifluoracetic acid (TFA) from Applied Biosystems. Carbon dioxide from Air Liquide with purity better than 99.998%. All chemicals were used without further purification.

Development of planar and 3D-shaped affinity cavities on AT-MIPs using $scCO_2$. Typically, for MIP synthesis, 1 mmol of analogue template (BENZ or PIV), 4 mmol of MAM, 20 mmol of the EGDMA and 1 wt% of AIBN are placed inside of a 33 mL stainless steel high-pressure reactor with two aligned sapphire windows, a Teflon™ magnetic stir bar. The reactor is immersed in a water bath at 65 ˚C using an open bath circulator. Carbon dioxide is raised up to 21 MPa and the polymerization reaction proceeds during 24 hours under stirring. At the end of polymerization, the copolymer is washed with fresh stream of CO_2, slowly for

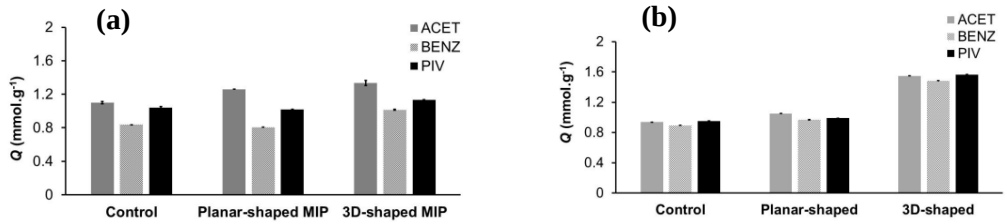
1 hour, to remove the template molecule and rinse out the reactants residues. The control polymers, non-imprinted polymers (NIPs) were produced using the same methodology without addition of template.

$ScCO_2$-assisted desorption of analogue template molecules from AT-MIP matrices. A cylindrical vessel was packed with AT-MIPs (MAM-MIP-BENZ or MAM-MIP-PIV), connected with 33 mL high-pressure vessel containing 3 mL of ACN. The carbon dioxide was loaded up to 21 MPa and the process runs in continuous mode for 3 hours to completely extract the analogue template molecules used in the polymerization process. All apparatus were submerged at 40 ˚C using a thermostatted water bath and a continuous flux of $scCO_2$ was used.

Analogue template MIP binding tests: (a) ***Static binding tests.*** 20 mg of AT-MIPs and control were weighted and placed into SnakeSkin™ dialysis membranes and were put in contact with 50 mL of 0.25 $mg.mL^{-1}$ template solution, for 24 h at 100 rpm stirring, (b) ***Dynamic binding tests.*** 20 mg of each affinity polymer planar- and 3D-shaped AT-MIP (MAM-MIP-BENZ and MAM-MIP-PIV) and the control, the MAM-NIP were packed in empty SPE columns. Columns were equilibrated/conditioned with 3 mL of ACN. Thereafter, 3 mL of 0.25 $mg.mL^{-1}$ of solution containing ACET+ BENZ+PIV were passed through the column. Between each load solution, columns were rinsed with 10 mL of ACN.

3. Results and Discussion

Higher adsorption capacity of all amide-based compounds was obtained by 3D-shaped MIP than planar-shaped MIP and control material in static binding experiments (a). This result is expected because although 3D-shaped MIP have demonstrated to have lower spatial conformation (*Molinspiration* software) than planar-shaped MIP, has a 3D cavity thus more accessible than the 1D cavity.



In dynamic binding tests (b), the 3D-shaped AT-MIP, which possess a larger cavity, was able to recognize all amides with almost the double of adsorption capacity than the planar-shaped AT-MIP, showing to be suitable for the removal of the amide family.

4. Conclusions

Versatile cost-effective AT-MIPs were produced using $scCO_2$ technology for the selective removal of the genotoxic compound ACET. The size and shape of the cavity (planar or 3D) revealed to be important parameters on the material's performance and huge influence on the type of performed experiments (static or dynamic mode). However, from an industry point of view, 3D-shaped AT-MIP has higher potential applicability for the removal of amide-based genotoxins from pharmaceutical crude mixtures since are able to capture 32 % more ACET than the planar-shaped AT-MIP, in the presence of a mixture of the amides.

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Acknowledgements: The authors thank financial support from Fundação para a Ciência e a Tecnologia, Ministério da Ciência, Tecnologia e Ensino Superior (FCT/MCTES Portugal), through project PTDC/EQU-EQU/32473/2017. The Associate Laboratory Research Unit for Green Chemistry - Clean Technologies and Processes - LAQV is financed by national funds from FCT/MCTES (UIDB/QUI/50006/2020) and cofunded by the ERDF under the PT2020 Partnership Agreement (POCI-01- 0145-FEDER-007265).