Green strategies on the development of biopurification antibody-like materials using supercritical CO_2

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1. Introduction

The antibody purification market is expected to reach EUR 1.8 billion by 2030, growing at an annual rate of ~10%.[1] However, antibody purification has strong limitations, such as the use of inefficient production methods, a lack of efficacy and issues of cost-effectiveness.[2] Researchers have been questioning the quality and reproducibility of the antibodies and are looking for alternative materials that can mimic the biorecognition process of these natural molecules. Molecular Imprinted Polymers (MIPs) have high potential for biorecognition applications.[3] Molecular Imprinting Technique (MIT) is a synthetic way to obtain crosslinked polymers within specific affinity sites complementary in terms of size, conformation and functionality of the target molecule.[4] MIPs mimic natural molecules in their molecular recognition ability, with significant advantages if compared to natural antibodies, since are cost-effective, robust, stable under harsh conditions, have a long lifetime, and are easily stored.[3] The MIP synthesis using biomolecules as targets normally use organic solvents with a small fraction of water to increase their solubility in the organic solvent.[4] The water molecules could interfere with the interactions between the template and the functional monomer, and negatively affect the stabilization of the template-monomer complex. In addition, in the use of conventional strategies MIPs are obtained as blocks that need to be further crushed, which can destroy the affinity cavities created during the molecular imprinting process.[4] Therefore, the combination of MIT with supercritical dioxide carbon ($scCO_2$) overcomes the conventional MIP drawbacks and brings new features to the materials compared to conventional ones. CO_2 is an abundant, non-toxic, non-flammable and inert gas with a very accessible critical point, which is easily removed at the end of the polymerization step without additional energy input.[5] MIPs have been produced in a synthetic supercritical route yielding molecular recognition materials for several applications.[6] Herein, a MIPs for biopurification using small biomolecules as target molecules were developed using $scCO_2$ technology.

2. Materials and Methods

Polymers were developed using the $scCO_2$ - assisted molecular imprinting method[7]with recognition of L-leucine (LEU), L-arginine (ARG) and L-lysine (LSY) by dual-templating (LEU+LSY and LEU+ARG) and one-templating (LEU) approaches. For the syntheses, the amino acids were used as templates in small fraction of organic co-solvent, the functional monomer, the crosslinker, and the thermal polymerization initiator were placed in a 33 mL high-pressure cell at 45 ºC and 200 bar under stirring for 24 h. Non-molecularly imprinted polymer (NIP) was synthesized following the same procedure, with the exception that no template was added to the cell.

Figure 1. MIP synthesized using $scCO_2$ technology.

The template desorption was performed packing each MIP on a stainless-steel column coupled to a 33 mL high-pressure cell containing 1% (v/v) of ethyl acetate at 40 ºC and 200 bar in continuous flow for 3 h. All polymers were obtained in the form of a white, dry and ready-to-use powders (**Figure 1**). Several static binding tests were carried out for different amino acid aqueous solutions. In a static binding experiment, 20 mg of polymer samples were placed into snakeskin dialysis membrane bags and introduced in a 25 mL of 0.5 mg/mL amino acid aqueous solution, for 24 h, under stirring at 100 rpm. After this period, the solutions were analyzed using a spectrophotometer UV-VIS at 569 nm through adapted ninhydrin colorimetric

method from literature.[8] The following calculated parameters, binding capacity (*Q*) and imprinting factor (*IF*), were used to evaluate the binding performance of the polymers:

$$(1) \qquad Q = \frac{(C_0 - C)V}{W} \qquad\qquad (2) \qquad IF = \frac{Q_{MIP}}{Q_{NIP}}$$

where C_0 and C are the template concentrations (mg amino acid/mL) in the solutions measured initially and after sorption, respectively, V (mL) is the volume of the solution and W (mg) is the sample polymer weight. The imprinting factor (*IF*) measures the imprinting effect and was calculated using equation 2, where Q_{MIP} is the binding capacity of the MIP and Q_{NIP} is the binding capacity of the NIP.

3. Results and discussion

MIPs present higher *Q* compared to the NIPs (**Figure 2**). The best results were achieved for the dual-template MIPs using the solution with both target biomolecules. Comparing the results between individual amino acid solutions, it could be inferred that $MIP_{(LEU\text{-}LSY)}$ has higher recognition ability to the LSY than LEU, since *Q* and *IF* were higher in LSY solution test. The $MIP_{(LEU\text{-}ARG)}$ has higher recognition to the LEU since *Q* was higher in LEU solution test, although the imprinting effect is lower in this solution.

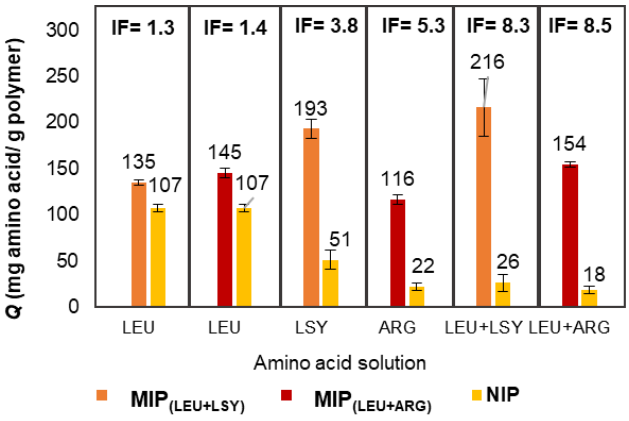

Figure 2. Static binding tests results.

4. Conclusions

The developed MIPs are cost-effective since high-affinity materials are obtained by commercially and low-cost starting materials using a sustainable technology. The preliminary binding results are quite promising, revealing quite stable polymeric materials for a wide range of biorecognition processes, including biopurification, being very attractive for bio-industrial applications.

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