

# NOVEL SEMI-COVALENT SYNTHESIS OF MOLECULAR IMPRINTED POLYMERS IN SCCO<sub>2</sub>: MIP- SUPPORTED HYBRID MEMBRANES FOR SEPARATION BASED ON MOLECULAR RECOGNITION

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Herein we report a novel method for the synthesis and processing of molecular recognition materials using supercritical fluid technology. Semi-covalently imprinted polymers with adsorption affinity to bisphenol A (BPA) were prepared for the first time in scCO<sub>2</sub>, at 65 °C and 21 MPa. BPA hydrolysis from the polymeric matrix was also performed in supercritical environment using a specific cleavage agent. Polymeric materials were then characterized in terms of morphology, mechanical performance, transport properties and adsorption ability. Results show that the materials with BPA-affinity binding sites are able to adsorb higher amounts of this compound when compared with other structurally related compounds, showing that supercritical fluid technology is a “greener” alternative to design molecularly imprinted devices for separation purposes.

## INTRODUCTION

Molecular imprinting technique is a promising way to create specific sites for a template molecule within a polymeric matrix. Molecularly imprinted polymers have demonstrated to have high affinity and specificity, good thermal and chemical stability and high durability. These properties led to a significant increase in applications in the last decade such as in synthesis and catalysis [1], extraction [2], and sensors [3]. Traditional bulk imprinting yields hard block polymers which have to be grounded and sieved, what besides time-consuming leads to the destruction of some interaction sites and therefore reduces the molecular recognition mechanism.

Molecularly imprinted polymers can be prepared according to a number of approaches that essentially differ in the way the template interact with the functional monomer and later with the polymeric binding sites. Although the divisions among them are becoming somewhat blurred due to the emergence of more complex hybrid strategies, the main molecular imprinting approaches considered are covalent, non-covalent and semi-covalent imprinting.

Covalent imprinting, pioneered by Wulff [4], was the first strategy to introduce molecular affinity in organic polymeric networks. This imprint methodology makes use of covalent bonds both on binding site formation, by using templates with covalently bound

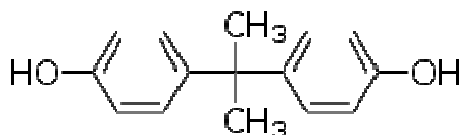
polymerizable groups, and in the rebinding process. At the end of the polymerization the template is cleaved from the matrix and the functionality left in the binding site is available for future covalent rebind. The great advantage when using this approach is that the functional groups are only associated with template sites. However, limitations found in the type of compounds that can be imprinted by this way (alcohols(diols), aldehydes, ketones, amines and carboxylic acids) and difficulties in the cleavage of the template, led to the development of other imprinting methodologies.

Non-covalent imprinting, first developed by the group of Mosbach [5], is currently the most widely applied approach to produce molecular recognition matrices. By means of this technique, template and functional monomer(s) self-assembly through non-covalent interactions such as hydrogen bonding, ion-pairing and dipole-dipole interactions, and template rebinding is achieved also via non-covalent interactions. The simplicity of this method, allied with the easy and cheap preparation, as well as the high selectivity that the polymers prepared by this approach can have, seem to overcome potential template-monomer complex stabilization issues, in which the success of this route relies.

Semi-covalent imprinting route combines both covalent interactions, by using a template with polymerizable groups and non-covalent bonds during the rebinding process. The advantages of this route are a narrower distribution of the binding sites and the inexistence of other kinetic restrictions than those encountered by diffusion.

scCO<sub>2</sub> was recently proposed as an alternative porogen in non-covalent molecular imprinting, leading to highly specific polymeric materials, with attested performance in drug delivery [6, 7, 8] and enantiomeric separations [9].

In this work we open up the possibility of using a scCO<sub>2</sub>-assisted semi-covalent imprinting approach to prepare imprinted materials to selectively adsorb environmental pollutant, such as bisphenol A, from aqueous solutions. Figure 1 shows the molecular structure of BPA.



**Figure 1.** Molecular structure of the analyte of interest in this work, BPA.

## MATERIALS AND METHODS

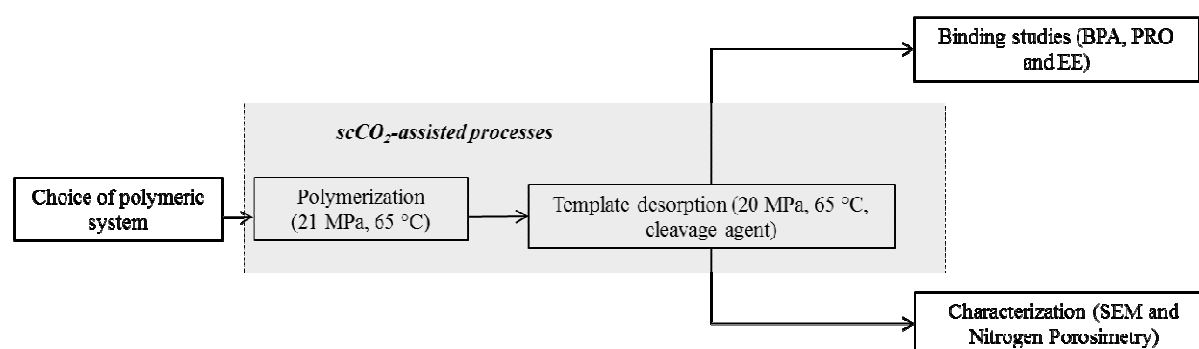
### MATERIALS

Bisphenol A (BPA, 99 % purity) as the analyte of interest, Bisphenol A dimethacrylate (BPADM, 99 % purity) as template-monomer, methacrylic acid (MAA, 99 % purity) as functional monomer and Ethylene glycol dimethacrylate (EGDMA, 98 % purity) as crosslinker were purchased from Sigma-Aldrich. Azobis(isobutyronitrile) (AIBN, 98 % purity) from Fluka was used as initiator. The cleavage agent was obtained from Sigma-Aldrich. Acetonitrile and methanol isocratic HPLC grade (99.7 % purity) from Scharlau were used. Progesterone (PRO, 99 % purity) and  $\alpha$ -Ethinylestradiol (EE, 98 % purity) were

purchased from Sigma-Aldrich. Carbon dioxide was obtained from Air Liquide with purity better than 99.998 %. All chemicals were used without further purification.

## METHODS

The experimental procedure to prepare the novel semi-covalently imprinted polymer using supercritical fluid technology is briefly represented in the scheme of Figure 2.



**Figure 2.** Scheme of the experimental procedure used to synthesize and process the polymers in scCO<sub>2</sub>.

## POLYMER SYNTHESIS

Polymerization reactions in scCO<sub>2</sub> were carried out as described elsewhere [10]. In a typical reaction to prepare the semi-covalent MIP with acetonitrile as co-solvent, BPADM as the polymerizable template, EGDMA as crosslinker and AIBN as the radical initiator were introduced in a 33 mL stainless steel high-pressure cell equipped with two aligned sapphire windows and a Teflon coated magnetic stir bar inside. The cell was immersed in a thermostatted water bath at 65 °C and temperature control was made through an open bath circulator Julabo Ed with stability  $\pm 0.1$  °C. Carbon dioxide was added up to 21 MPa and polymerization reactions proceeded for 24 hours under stirring. At these  $p, T$  conditions, a single homogeneous phase is assured, with all reactants completely dissolved in the supercritical phase. At the end of the reaction, the polymer was slowly washed with fresh high-pressure CO<sub>2</sub> for 1 hour. For the synthesis of the control polymer, NIP, the same conditions and procedure were followed, except that MAA was used instead of BPADM.

## ScCO<sub>2</sub>-ASSISTED TEMPLATE CLEAVAGE AND DESORPTION

The removal of BPA from the BPA-imprinted polymer was performed in scCO<sub>2</sub>. In a typical experiment P(BPADM-*co*-EGDMA) and the cleavage agent were introduced into a high-pressure cell with a magnetic stir bar inside. The cell was immersed in a thermostated water bath at 65 °C and pressurized with CO<sub>2</sub> until a final pressure of 20 MPa. After 24 h of reaction

the polymer was washed with fresh CO<sub>2</sub> (20 MPa) for 1 h. The polymer was then slurry packed in a high-pressure stainless steel extractor (ID 7mm, 15 cm length). This extractor was connected to a 33 mL high-pressure cell that was loaded with 5 mL of MeOH. Carbon dioxide was added up to 21 MPa and a continuous stream of CO<sub>2</sub> and methanol was used to remove the template from the copolymer.

## MORPHOLOGICAL AND PHYSICAL CHARACTERIZATION OF IMPRINTED MATERIALS

The morphology of the synthesized copolymers was characterized using scanning electron microscopy (SEM) in a Hitachi S-2400 instrument, with an accelerating voltage set to 15 kV. Samples were mounted on aluminium stubs using carbon tape and were gold/platinum coated. Specific surface area and pore diameter of the polymeric particles were determined by N<sub>2</sub> adsorption according to the BET method. An accelerated surface area and porosimetry system (ASAP 2010 Micromeritics) was used under nitrogen flow.

## BINDING EXPERIMENTS

The ability of the synthesized polymers to adsorb BPA and other related compounds from aqueous solutions was evaluated through static adsorption experiments. Given amounts of polymer were added to aqueous solutions of BPA, PRO and EE.

Equilibrium was achieved after 24 hours, as confirmed by the quantification of free analyte in solutions. The amount of substrate adsorbed by the matrices was assessed through Equation 1, where [S] corresponds to the amount of analyte bound, C<sub>0</sub> represents the initial molar concentrations of analyte, C<sub>t</sub> corresponds to the concentrations at predetermined time intervals, V represents the volume of the solution and W corresponds to the weight of the polymeric sample.

$$[S] = \frac{(C_0 - C_t) \cdot V}{W} \quad (1)$$

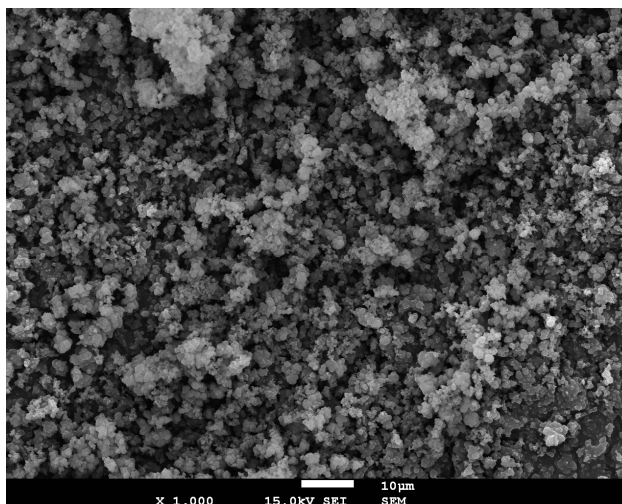
The aliquots collected were quantified by UV spectroscopy. All the experiments were carried out in duplicates.

## RESULTS

### MORPHOLOGICAL AND PHYSICAL CHARACTERIZATION

Imprinted and non-imprinted polymers were obtained as dry, free-flowing powders in high yields (80-90 %, determined gravimetrically). Figure 3 shows a SEM image of the imprinted polymer. From the SEM images obtained it was not possible to see any morphological differences between the imprinted and non-imprinted polymers. Polymers are formed by aggregates of smooth surface discrete nanoparticles.

Nitrogen porosimetry data concerning the surface area and porosity of the copolymers synthesized showed that the semi-covalent MIP has a slightly lower surface area than NIP, suggesting that the presence of BPA somehow influences the nucleation process and particles growth and precipitation.

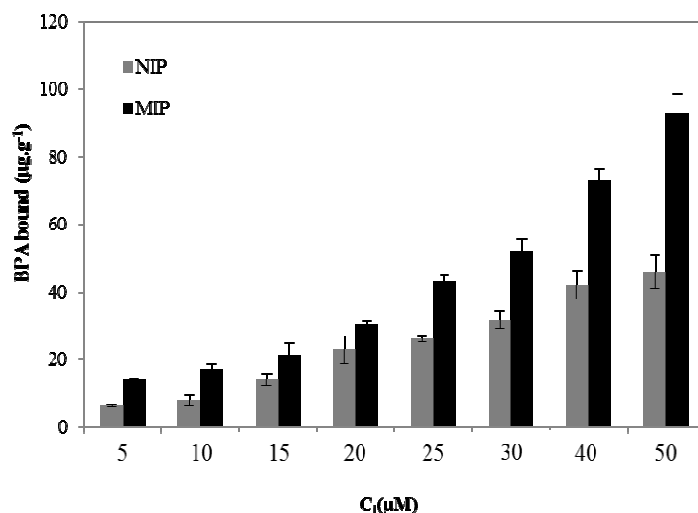


**Figure 3.** SEM image of the synthesized MIP.

## **BINDING STUDIES**

The binding affinity of BPA, PRO and EE by NIP and semi-covalent MIP in aqueous solutions was evaluated. Prior to binding experiments, the template was removed from the imprinted polymer by hydrolysis. Common procedures for BPA cleavage from imprinted polymers use conventional acid and basic conditions [11,12]. Herein, we report the use of an alternative cleavage agent to perform the in supercritical environment, in order to take advantage of the high diffusivity of  $scCO_2$ .

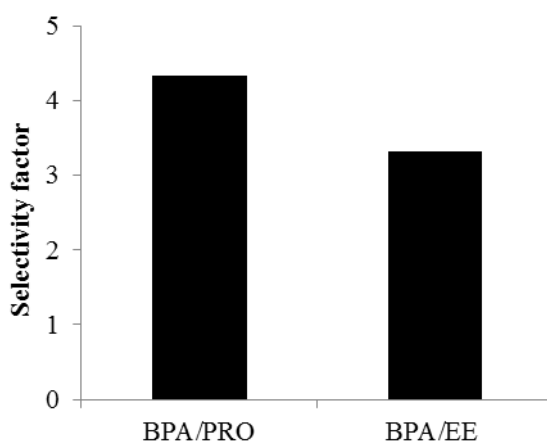
Figure 4 shows the binding isotherms of BPA by NIP and MIP. As it can be seen, the equilibrium binding of BPA increases with the initial concentration of the analyte in solution. Also, by looking at both curves it is obvious the higher adsorption ability by the MIP, which is usually indicative of the presence of affinity binding sites created by the molecular imprinting process.



**Figure 4.** Template binding studies in aqueous solutions.

The selectivity of MIP in aqueous solutions was assessed by evaluation of its capability to bind PRO and EE in comparison with BPA. The ability of imprinted polymers to selectively adsorb the template molecule in aqueous environment is one of the most challenging features of MIPs and much attention is being focused in this topic [13]. Given the known “solvent-memory effect”, some efforts have been made in the synthesis of imprinted polymers in aqueous environment, by using functional monomers that may stabilize the complexes through hydrophobic interactions, such as cyclodextrins [14] or by multi-step polymerization procedure. Nevertheless the use of water weakens other non-covalent interactions, such as hydrogen bond and electrostatic interactions and the two step procedures are time-consuming. Furthermore at the end of polymerization, energy intensive drying steps are required.

Figure 5 illustrates the results obtained in this work concerning the selectivity factor presented by NIP.



**Figure 5.** Selectivity of MIP for BPA towards PRO and EE.

The results show that the imprinted polymer with molecular recognition to BPA, introduced by molecular imprinting technique and synthesized using supercritical CO<sub>2</sub> as the porogen,

shows an adsorption capacity for the template that corresponds to 4.3 and 3.3 times the maximum adsorption of PRO and EE. This is consistent with a water-compatible molecular recognition performance.

## CONCLUSIONS

In this work we have studied the possibility of using semi-covalent molecular imprinting strategy to impart analyte specificity in scCO<sub>2</sub>-assisted preparation of MIPs. Molecular recognition of BPA, the chosen template, by MIP was evaluated in aqueous solutions showing that the designed affinity polymer was able to adsorb higher amounts of this environmental pollutant in aqueous solutions. Furthermore, selectivity experiments performed with other structurally related compounds proved that MIP selectively recognizes BPA.

The results demonstrate the feasibility of preparing MIPs capable of keeping their molecular recognition in aqueous media, using supercritical fluid technology.

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