# INCREASING BINDING AFFINITY OF A POLYMERIC MATRIX TOWARDS A TEMPLATE BY MOLECULAR IMPRINTING IN $SCCO_2$

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Molecular imprinting is a powerful technique to create synthetic recognition sites in cross-linked matrixes. This technique uses the functionality of the target molecule (template), to assemble its own recognition site by forming specific interactions with the matrix, with widespread applications and great potential in the preparation of drug delivery systems and in separation processes [1-3]. In this work, an imprinted polymer (MIP) was synthesized in $scCO_2$ at temperatures and pressures up to 65ºC and 21MPa. The performance of the synthesized polymer as a stationary phase for affinity chromatography was investigated. Supercritical carbon dioxide appears to be an interesting alternative medium to the preparation of MIPs replacing the typical used toxic organic solvents.

## INTRODUCTION

Molecular imprinting is a self-assembly molecular recognition technique with an increasing number of applications.

During polymerization, the template molecule forms a stable complex with the monomer, in the presence of a porogen and a cross-linker agent that freezes the complex within a rigid porous polymer matrix [4]. Breaking the reversible bonds of the complex, by template removal, leaves behind the imprinted sites that are chemical and physically complementary to the template molecule. The generally accepted mechanism for the formation of MIPs is schematically presented in Figure 1.

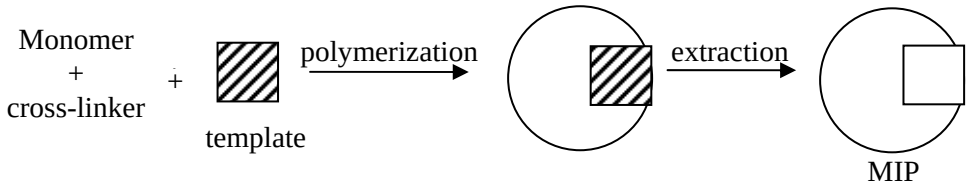


**Figure 1.** Scheme of the imprinting process

The growing need of enantiomeric separations turned MIPs into a particularly interesting material for chromatographic purposes due to their low cost, easy preparation and high enantioselective recognition. Recently MIPs for drug delivery were synthesized for the first time in $scCO_2$ [5], but no studies were made with respect to enantioselectivity of these materials.
In this work we have synthesized a NIPAAm-EGDMA copolymer in $scCO_2$ imprinted with an amino acid template molecule, which was then tested as a HPLC stationary phase.

## MATERIALS AND METHODS

Reagents were obtained from Aldrich and used as received. $CO_2$ was obtained from Air Liquide with purity better than 99.998%.
Polymerization reactions were carried out 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 with ±0.01ºC of stability and temperature control was made with a RTD probe contacting the cell, connected to a Hart Scientific PID controller.

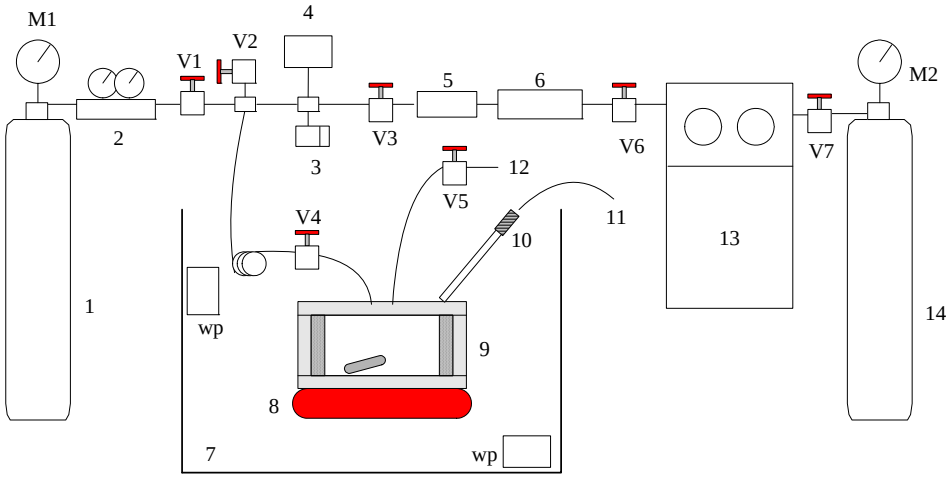


**Figure 1.** Schematic representation of the apparatus used in the polymerization reactions. 1- nitrogen cylinder; 2-gas regulator; 3-rupture disc; 4- high-pressure manometer; 5- check-valve; 6- line filter; 7-water bath; 8-immersible stirrer; 9-high pressure cell; 10- Platinum resistance RTD probe; 11-temperature controller; 12-vent; 13- pneumatic $CO_2$ compressor; 14- $CO_2$ cylinder; M1,M2- bourbon manometers; wp-water recirculation pump; V1 to V7- HIP valves.

After polymerization, the product reaction was slowly washed with fresh high-pressure $CO_2$ in order to clean the remaining residues of unreacted monomer and template. Then the polymer was packed in a blank HPLC column and tested as a stationary phase in a Merck Hitachi L-7400 equipment.

## RESULTS

A SEM image of an MIP sample can be seen in Figure 2, showing aggregates of discrete nanoparticles.

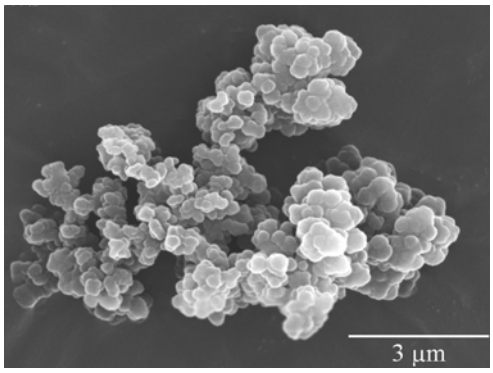


**Figure 2.** SEM image of a synthesized imprinted polymer.

For the analysis of MIP recognition properties, chromatographic conditions, such as flow rate and eluent composition, were carefully optimized. Figure 3 presents a typical HPLC chromatogram obtained, showing an enantioselectivity of alfa 1.13. The polymer matrix retains significantly more the template enantiomer, which implies a better affinity between the column and the template than with the other enantiomer.

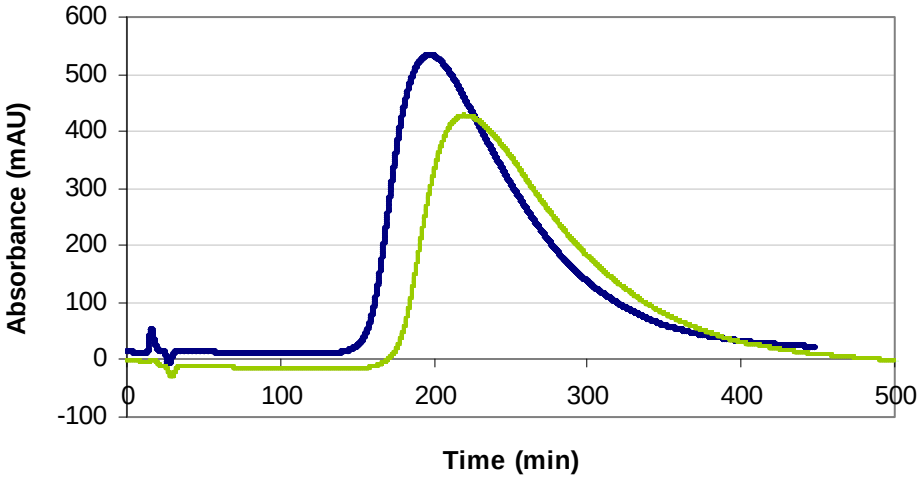


**Figure 3.** Example of the enantioselectivity obtained with the synthesized MIP polymeric material. Green chromatogram obtained for the enantiomer template.

## CONCLUSION

These preliminary results suggest that the imprinted polymeric matrix was successfully synthesized in $scCO_2$ and that it is able to recognize the template molecule. Herein we open up a new way of increasing binding affinity of polymeric matrixes by molecular imprinting in $scCO_2$, to be used as stationary phases in HPLC for enantiomeric separation.

## REFERENCES


[1]ALEXANDER A., ANDERSSON H.S., ANDERSSON L. I., ANSELL R.J., KIRSCH N., NICHOLLS I.A., WHITCOMBE M.J., *J. Mol. Recognit.* **2006**, 19, 106.

[2] ALVAREZ-LORENZO C., CONCHEIRO A., *J. Chromatogr. B* **2004**, 804, 231.

[3] SELLERGREN B., ALLENDER C.J., *Adv.Drug Deliv.* **2005**, 57, 1733.

[4] WHITCOMBE M. J., VULFSON E. N., *Adv. Mater.* **2001**, 13, 467.

[5] DUARTE A. R. C., CASIMIRO T., AGUIAR-RICARDO A., SIMPLÍCIO A. L., DUARTE C. M. M., *Journal of Supercritical Fluids* **2006**, 39, 102.