

# DEVELOPMENT OF A FLUORESCENT MIP SENSOR IN SCCO<sub>2</sub>

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## ABSTRACT

The aim of this study was to develop a highly sensitive fluorescent sensor based on molecular imprinting in scCO<sub>2</sub>. Water soluble CdTe quantum dots (QDs), fluorescence probes, were synthesized with reactive groups on the surface and co-polymerized in scCO<sub>2</sub> in order to prepare a molecularly imprinted polymer (MIP) with enhanced affinity to a target molecule. The obtained powder is a highly sensitive fluorescent affinity polymer, which in the presence of the target molecule leads to a significant quenching of the QDs photoluminescence. These novel QDs were characterized by TEM and FTIR, indicating successful synthesis and functionalization. MIPs were characterized by FTIR, SEM and fluorescence spectroscopy, confirming the presence of QDs in the polymeric matrix. Fluorescence experiments were performed by measuring the variation of the fluorescence in the presence of different concentrations of the target molecule. The same studies were performed for analogous molecules and for the non-imprinted polymer (NIP), in order to confirm the sensitivity and specificity of the obtained probe.

## INTRODUCTION

Bisphenol A (BPA) has toxic properties, promoting estrogenic endocrine disruption and induction of tumorigenic evolution, being typically found in many plastic consumer products like drinking containers, water pipes and tableware. A recent study suggested that BPA, even at extremely low levels, induces the derangement of corpuscular functions to make the disorder of estrogenic hormone secretion. Therefore, it is crucial to monitor trace BPA in environmental water samples, and this detection should be highly selective and sensitive<sup>[1]</sup>. Photoluminescence semiconductor nanocrystals or quantum dots (QDs) have attracted a considerable interest as fluorescence probes for detection and recognizing compounds in biology, medicine, and more recently in analytical chemistry due to their high photoluminescence. In order to improve the selectivity of QD-based sensors, the incorporation of these probes into molecularly imprinted systems is a promising technique<sup>[2]</sup>. Molecularly imprinted polymers (MIPs) includes the presence of a template molecule during the polymerization, in order to produce binding sites in the polymer matrix that are able to recognize only the target molecule, thus leading to great specificity and selectivity<sup>[3]</sup>. MIPs have been widely applied in several areas due to their advantages, such as simple preparation, high stability, high binding affinity and capacity and low cost<sup>[4]</sup>. Numerous methods have been reported for MIP synthesis, however most of them present many disadvantages. The use of supercritical fluids technology reduces the drawbacks of

conventional methods, since supercritical carbon dioxide is an eco-friendly solvent, non-flammable and with low-critical constants<sup>[5]</sup>. Also it is possible to achieve dry powders with controlled morphology, porosity, and high purity in one-step through a relatively simple and inexpensive process.

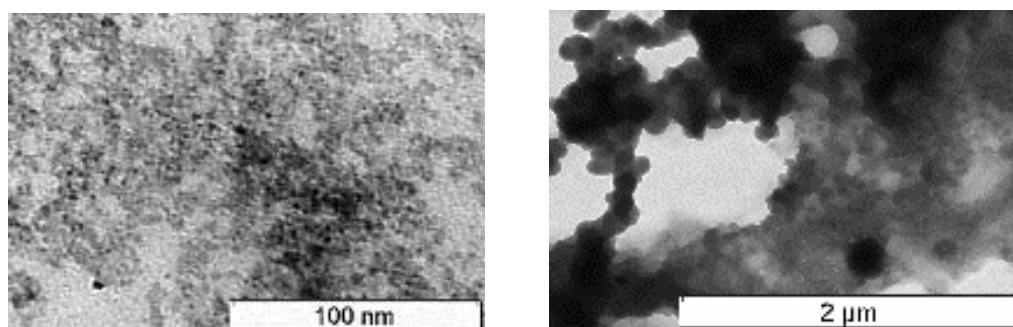
## MATERIALS AND METHODS

QDs were synthesised following a modified literature protocol. The synthesis of the fluorescent MIP, was performed in a high pressure apparatus already described elsewhere<sup>[4]</sup>. The polymerization was performed using BPA as the template, MAA as monomer, EGDMA as cross-linker and AIBN as the initiator. The reagents and the synthesised QD were loaded into a 33 mL stainless steel high-pressure cell, which was immersed in a thermostatted water bath at 65 °C under stirring. The CO<sub>2</sub> was introduced in the cell up to 21 MPa and the reaction allowed to proceed for 24 h. At the end of the polymerization, the resulting polymer was slowly washed with fresh high-pressure CO<sub>2</sub> in order to clean the remaining residues of template and unreacted monomer. For the production of non-imprinted polymer (NIP) the same procedure was followed without template addition.

The template desorption from the imprinted matrices at the end of the polymerization is a crucial step in the molecular imprinting process because the formerly created binding sites have to be emptied to become available for future rebinding. ScCO<sub>2</sub>-assisted BPA desorption was performed by loading a stainless steel extractor with the pre-synthesized polymer and 5 mL of ethanol. The cell was immersed in a thermostated water bath at 40 °C and slowly washed for 2.5 h with fresh high-pressure CO<sub>2</sub>.

## RESULTS

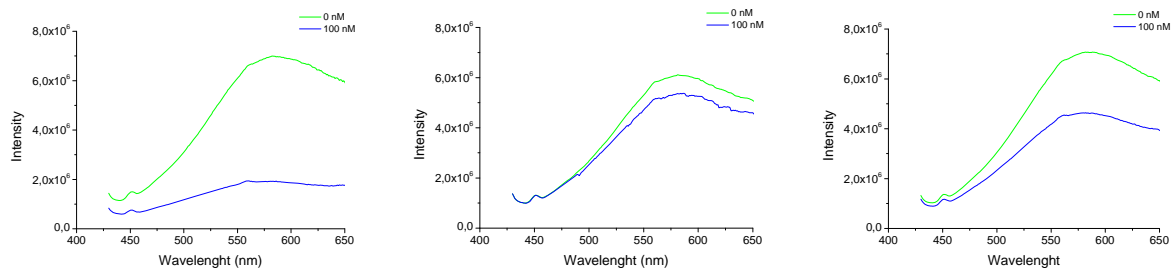
TEM images were acquired in order to study the shape and size of the QDs and MIP-coated QDs. For QDs a nano-spherical and monodisperse shape was observed. The MIP-coated QDs were larger than QDs, which evidence the successful MIP layer on the surface of the QDs.



**Figure 1:** TEM image of QDs (left) and MIP-coated QDs (right).

The analyte detection was done by the incubation of MIP-coated QD with the corresponding imprinted molecule in an aqueous media. The fluorescence emission from the MIP-coated

QDs was detected with bound template as well as in free form. Fluorescence quenching was observed upon analyte binding, thus indicating successful recognition.



**Figure 2:** Photoluminescence emission spectra of MIP-coated QDs in presence of bisphenol A (a), NIP-coated QDs in presence of bisphenol A (b) and MIP-coated QDs in presence of a bisphenol analogue (c).

Fig. 2a shows the typical MIP-QD fluorescence quenching as function of BPA concentration at 0 and 100 nM. This effect could be explained by an electron transfer process between QDs and BPA, when the later is bound to the imprinted site. The NIP-coated QDs polymer was prepared in the same way as MIP-coated QDs, but no template was added. As shown in Fig. 2b, the emission decrease for NIP-QDs in the presence of BPA was much smaller, thus proofing that binding sites were successfully created on MIP-QDs. Selectivity of the recognition sites was demonstrated by the absence of response in the presence of a BPA analogue (Fig. 2c), which strongly suggests that this structurally similar analogue is unable to bind to preformed BPA binding sites.

## CONCLUSIONS

In summary, our preliminary results demonstrate that MIP-coated QDs were successfully synthesised in supercritical carbon dioxide. Molecular imprinting seems to have a crucial role in QDs quenching, suggesting that the intensity of fluorescence emission is controlled by affinity binding of the template molecule within the MIP polymer. These new MIP-QDs materials have potential application in sensors, targeted drug delivery devices and other biomedical applications.

## ACKNOWLEDGEMENTS

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