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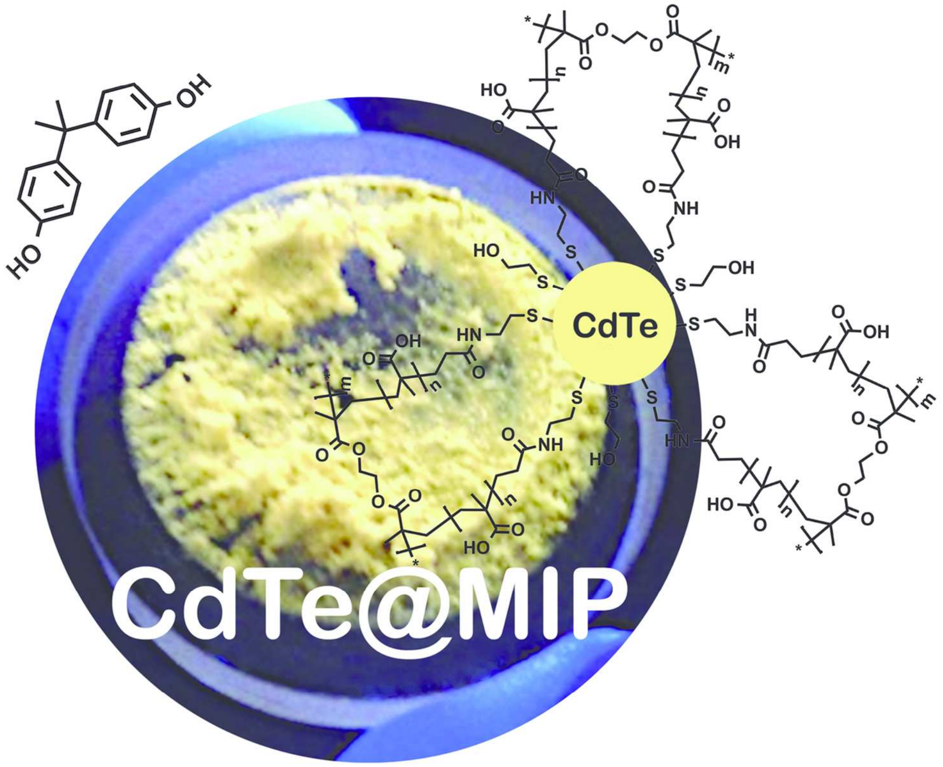

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Supercritical CO_2-assisted synthesis of an ultrasensitive amphibious quantum dot-molecularly imprinted sensor

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Molecularly imprinted polymers are simple and robust materials for the selective binding of analytes with affinities and selectivities similar to biological probes. A green supercritical CO_2-assisted molecular imprinting protocol enabled the production of smart sensory particles, incorporating quantum dots, with molecular recognition to bisphenol A at very low concentrations (4 nM). The protocol uses amphibious vinyl-coated quantum dots and enables the design of sensors for a wide range of molecules through a simple, low cost and clean technology.

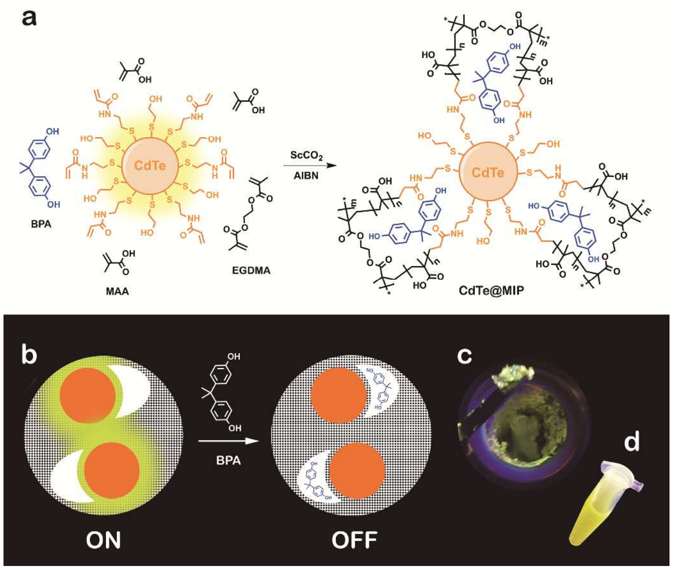
1. Introduction

In the last few years, quantum dots (QDs), a special class of photoluminescent semiconductor nanocrystals, attracted the attention as promising probes for recognition of target compounds in biology, medicine and more recently, in analytical chemistry. In comparison to conventional probes, QDs often exhibit superior optical and electronic properties, such as strongly size-depended high photoluminescence, great photostability, broad excitation spectrum and highly stable photobleaching.[1] In order to improve the selectivity of QD-based sensors, its incorporation into molecularly imprinted systems emerges as an effective technique. Molecularly imprinted polymers (MIPs) are produced by the copolymerization of selected monomers and cross-linkers, in the presence of a template (target) molecule, thus producing polymer networks with cavities that have complementary size, shape and functionality of the target molecule, leading to materials with high specificity and selectivity.[2] MIPs have been widely applied in extraction,[3] separation,[4] drug delivery,[5] and sensing,[6] due to their simple preparation, high stability, high binding affinity and capacity and low cost.[7] Several conventional methods have been reported for MIP synthesis, such as suspension, emulsion, dispersion, solution, and precipitation polymerization.[8] However, these methods usually comprise complex and multi-step procedures, being the use of organic solvents and large amounts of template common drawbacks. The production of MIPs using supercritical carbon dioxide ($scCO_2$) overcomes these limitations since CO_2 is an eco-friendly, environmentally acceptable and non-flammable solvent.[9] Moreover, the supercritical technology allows one-step production of materials with high purity, since the final depressurization and extraction that occurs after polymerization enables the removal of unreacted monomers, initiator and template from the polymer matrix. Also, it is possible to obtain dry powders with controlled morphology and porosity by a relatively simple and inexpensive process.[10] Bisphenol A (BPA) is commonly used as a plasticizer in the production of polycarbonate plastics and epoxy resins, and therefore is typically found as a contaminant in many plastics like drinking containers (polycarbonate caging and plastic water bottles are especially risky), water pipes and tableware.[11] In European waters the effluent levels of BPA reach values up to 43 parts per billion (ppb) (43 nM), and high concentration levels (~36 nM) have been also detected in amniotic fluids.[12] The impact of BPA in the immune system has been thoroughly investigated, and a huge number of toxicological studies have identified it as a promoter of estrogenic endocrine disruption and inducer of tumorigenic evolution. Its participation in the derangement of corpuscular functions, triggering the disorder of estrogenic hormone secretion and the involvement in many chronic diseases is well reported.[13] Therefore, selective and sensitive monitoring of BPA in aqueous samples, especially at very low concentrations is of utmost priority.

Herein, we describe the synthesis of amphibious (soluble in water and organic solvents) cadmium telluride (CdTe) QDs with vinyl groups on the surface, and its supercritical-assisted molecularly imprinted radical copolymerization in presence of BPA (CdTe@MIP). Through this strategy we successfully produced highly selective and sensitive fluorescent MIPs, taking advantage of both a scCO$_2$-assisted green technology and the exceptional QDs optoelectronic properties.

2. Results and discussion

The novel CdTe QDs having a tailored surface by attachment of mixed thiols, 2-mercaptoethanol (50 wt%) and N-(2-mercaptoethyl)acrylamide (50 wt%), were designed and synthesised using a modified protocol (Scheme 1a).[14,15] Surface capping with 2-mercaptoethanol was done to confer water solubility and prevent aggregation upon solvent removal. The reactive thiol monomer, N-(2-mercaptoethyl)acrylamide, was synthesised for the first time thus enabling the copolymerization of the QDs with selected co-monomers.



Scheme 1. a) Schematic synthesis of CdTe@MIP in supercritical carbon dioxide (scCO2). b) Schematic bisphenol A (BPA) detection by CdTe@MIPs. The "on" state corresponds to fluorescent CdTe@MIP particles imprinted with free BPA recognition sites. An "off" sate (fluorescence quenching) is achieved upon rebinding with BPA. c) A top view from the highpressure cell containing the CdTe@MIP powder, after synthesis and BPA removal, and (d) an aqueous solution of the functionalized QDs used in the synthesis, both pictures were taken under an UV lamp (λ_{ex}= 366 nm). AIBN= Azobisisobutyronitrile.

For the synthesis of the CdTe@MIP smart sensors, QDs were copolymerized with methyl methacrylate (MAA) using ethylene glycol dimethylacrylate (EGDMA) as the cross-linker, and azobisisobutyronitrile (AIBN) as the initiator, in the presence of BPA, and using scCO$_2$ as the polymerization medium The polymerization reactions were carried out as described elsewhere.[16] After polymerization, the efficient removal of the BPA template from the polymer matrix is crucial to free the specific sites, thus allowing successful rebinding. In this work the template desorption was also performed in scCO$_2$, taking advantage of its high diffusivity through the polymer matrix, and using DMSO as a cosolvent. It has been reported that polymer-coated QDs show enhanced stability,[17] and remarkably, CdTe@MIPs were found to be extremely stable, with unchanged optoelectronic properties after several months storage. In order to evaluate the size and shape of these novel CdTe QDs and CdTe@MIP sensors, TEM images were acquired (Fig. 1).

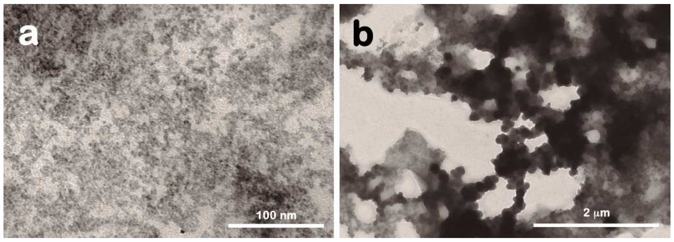


Fig. 1. TEM images of (a) amphibious CdTe quantum dots and (b) CdTe@MIP synthesized in supercritical CO$_2$.

The QDs were obtained as spherical discrete particles, with diameters ranging from 5 to 10 nm, with a low degree of aggregation. CdTe@MIPs possess larger sizes as a consequence of their surface polymer coating. The characteristic absorption bands of the CdTe QDs are observed around 421 nm and 461 nm, being the emission centered at 560 nm (see spectra in Supplementary Information). After polymerization the QDs absorption is blue-shifted 11 nm (λ_{max}= 410 nm) and the emission is red-shifted 24 nm (λ_{max}= 584 nm). The performance of CdTe@MIP sensing particles towards BPA was accessed by fluorescence spectroscopy. The experiments were performed using small amounts of polymer (2 mg) in the presence of different concentrations of BPA (up to 500 nM, data not shown) and the corresponding fluorescence spectra recorded (Fig. 2a). The quenching of the fluorescence was achieved for a well defined and extremely low range of concentrations (4-10 nM), being the detection limit 4 nM.

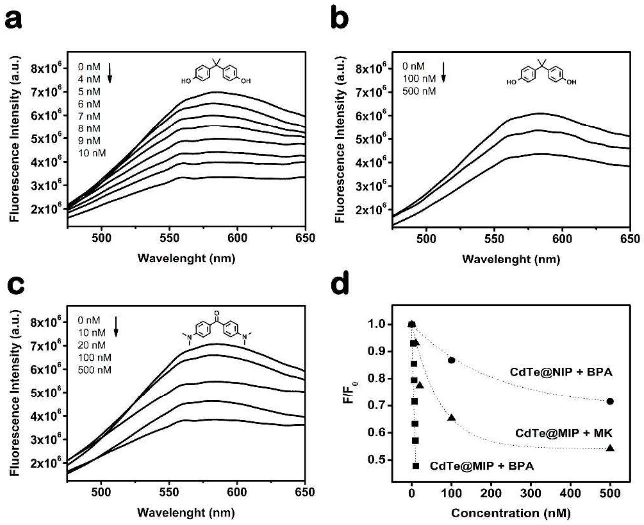


Fig. 2. Smart sensing by CdTe@MIPs and CdTe@NIPs in supercritical carbon dioxide. The spectra show the fluorescence quenching of (a) CdTe@MIP and (b) CdTe@NIP by addition of bisphenol A and (c) the fluorescence quenching of CdTe@MIP by addition of Michler's ketone. The corresponding fluorescence quenching efficiency (F/F$_0$) is plotted for comparison (d).

The effect of the imprinting on the sensitivity of the CdTe@MIP was evaluated by comparison with the non-imprinted polymer (CdTe@NIP) under the same experimental conditions. In this case the quenching of the fluorescence was observed only for a BPA concentration 50 times higher (500 nM, Fig. 2b). Furthermore, the selectivity of BPA imprinted CdTe@MIPs was examined by performing the sensing protocol using Michler's ketone (MK) [4,4'-bis(dimethylamino)benzophenone] as a structural analog of BPA (Fig. 2c). As expected, CdTe@MIPs imprinted with BPA are highly selective and do not show a significant response towards extremely similar molecules. The comparison between these three systems is depicted in Fig. 2d. The fluorescence quenching in the CdTe@MIP system can be described by the Stern-Volmer equation:

$$F_0/F = 1 + K_{SV}[C]$$

where F and F_0 are the fluorescence intensities of CdTe@MIP in presence and absence of an analyte, respectively. K_{SV} is the Stern-Volmer quenching constant and [C] is the analyte concentration. Using this equation, the Stern-Volmer constants were calculated for lower concentrations (regions with a linear behaviour). The obtained K_{SV} values for CdTe@MIP (0.162) and CdTe@NIP particles ($7\text{x}10^{-4}$) as well as the imprinting factor (IF= 231, ratio of the quenching constants of CdTe@MIP and CdTe@NIP for BPA), clearly demonstrate their outstanding selectivity which strongly support the potential use of supercritical fluid technology in the development of QD-based probe systems. This high value might be a reflection of some key features of the MIP synthesis and template desorption in $scCO_2$ due to its high diffusivity and solvent power. Polymers are obtained completely homogeneous and ready-to-use with no need of mechanical processes, which may destroy active sites. In addition, since $scCO_2$ is an apolar and aprotic solvent it can favor interactions between the functional monomers and the template during polymerization. The use of $scCO_2$ also lead to different polymer chain rearrangements compared to organic solvents, which may have a direct impact on the formation of the specific sites for the template and their accessibility.

To the best of our knowledge only one example of a related system can be found in literature,[18] where CdSe quantum dot-encapsulated molecularly imprinted mesoporous silica particles were produced for the detection of BPA ($CdSe@SiO_2@MIP$: K_{SV}= $25\text{x}10^{-4}$, IF= 13.6), which presents a lower IF, although a direct comparison cannot be made since they are completely different systems.

The fluorescence quenching mechanisms observed in QDs systems, either involving charge transfer (CT) or Förster resonance energy transfer (FRET) pathways are still not fully understood. However, a photoinduced electron transfer (PET) between an excited state QD and the template quencher, with an occupied or unoccupied state intermediate in energy, is generally accepted.[19] The binding (BPA-imprinted) cavities in the CdTe@MIP particles are closely built around the QDs shell and are surrounded by a rich polyacrylic acid environment. Since there is no overlapping between the absorption spectra of BPA (λ_{max}= 290 nm) and the emission spectra of CdTe@MIP (λ_{max}= 584 nm), a FRET mechanism can be excluded. Therefore, we postulate that a PET from the QD shell to BPA may occur when it reaches the cavity site, thus leading to an efficient quenching of the fluorescence.

3. Conclusions

In summary, we demonstrated that sensory particles could be produced incorporating specially designed CdTe quantum dots into molecularly imprinted polymers using supercritical carbon dioxide. The surface of the novel CdTe amphibious quantum dots was functionalized with mixed thiols, to confer both stability and reactivity. Molecular imprinting using BPA as the template enabled the development of BPA trace analysis (4 nM detection limit). The high sensitive and selective detection of the developed CdTe@MIP sensors envisages their use in a wide range of applications.

4. Materials and Methods

4.1 Materials

All commercial reagents were used as received unless otherwise mentioned. Carbon dioxide was supplied by Air Liquide with a purity of 99.998%.

4.1 Instrumentation

The FT-IR spectra were measured using KBr pellets on a Perkin Elmer Spectrum 1000 instrument. The NMR spectra were recorded on a Bruker ARX 400 MHz equipment using $CDCl_3$ as solvent. ^1H and ^{13}C NMR chemical shifts are reported as δ (ppm= parts per million) relative to the residual solvent peak. The UV-spectra were acquired in a PerkinElmer Lambda 25 UV/Vis Spectrometer with a slit width of 5 nm. Fluorescence measurements were performed in a Horiba Scientific Spectrofluorimeter model FL3-2iHR, equipped with a Fiber Optic Sample Acessory model 1950, with a slit width of 5 nm. The excitation wavelength was fixed at 410 nm.

4.2 Ligand synthesis

For the synthesis of N-(2-mercaptoethyl)acrylamide, cysteamine (1.33 g) was dissolved in dry acetonitrile (140 ml) and placed in an ice bath. Bis(trimethylsilyl)acetamide (6.35 ml) was added and the mixture stirred for 15 min. Then, acryloyl chloride (1.4 ml) in dry acetonitrile (5 ml) was added dropwise and the mixture was stirred at 0 ºC for 2 hours. The solution was filtrated and the acetonitrile removed under reduced pressure to give a dark yellow oil. The product was then dissolved in chloroform (30 ml) and stirred with water (20 ml) for 30 min. After extraction and three more washes with 20 ml of water, the organic phase was evaporated to give the product as colorless oil, which was stored under inert atmosphere at low temperature (-20 ºC).

4.3 Synthesis of CdTe quantum dots

N-(2-mercaptoethyl)acrylamide (40 µg) in dimethyl sulfoxide (1 ml) and 2-mercaptoethanol (40 µL) were added to a solution of cadmium chloride hemi(pentahydrate) (57 mg) in water (180 ml) previously degassed with argon, and mixed in a two-neck flask to form the cadmium precursor. Then, 1 ml of freshly prepared NaHTe aqueous solution from NaBH4 (363 mg) and Te (63.6 mg) powder were added to the reaction mixture under stirring, and the solution was refluxed for 6 hours. After this period the obtained orange solution was filtered under vacuum in order to remove unreacted telluride. The water was evaporated and QDs were obtained as a dimethylsulfoxide solution (1 mL) that was used in the next step.

4.4 Synthesis of CdTe@MIPs

Bisphenol A (0.050 g) was used as template, MAA (0.038 ml) as the monomer and EGDMA (0.414 ml) as the cross-linker with molar ratio of 1:5:25, respectively. The initiator used was AIBN (1 wt%). All reagents and the quantum dot dimethylsulfoxide solution were loaded into a 33 mL stainless steel high-pressure cell, which was sealed and immersed in a thermostated water bath at 65 ºC under stirring. The CO_2 was introduced up to 28 MPa and the reaction proceeded for 24 h. At the end of the polymerization, the resulting powder was slowly washed with fresh high-pressure CO_2 in order to clean the remaining residues of template and unreacted reagents. For the production of non-imprinted polymer (CdTe@NIP) the same procedure was followed without the addition of template.

4.5 $ScCO_2$-assisted template desorption

Template desorption from CdTe@MIPs at the end of the polymerization was performed by loading a stainless steel extractor with the pre-synthesized MIPs and 2 mL of DMSO. The cell was immersed in a thermostated water bath at 40 ºC and slowly washed for 3 h with fresh high-pressure CO_2 at 20 MPa.

4.6 Binding assays

10 µl (4 nM) of a BPA solution was added to 2 mg of CdTe@MIP and the corresponding fluorescence spectra acquired. The polymer sample was allowed to dry and the same procedure applied successively till a final concentration of 500 nM. This protocol was also followed for CdTe@NIP (up to 500 nM of BPA) and for CdTe@MIP (up 500 nM of Michler's ketone).

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Notes and references

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† Electronic Supplementary Information (ESI) available: FT-IR and NMR spectra of *N*-(2-mercaptoethyl)acrylamide; UV-Vis, Fluorescence and FT-IR spectra of amphibious CdTe quantum dots and FT-IR spectrum of CdTe@MIP. See DOI: 10.1039/b000000x/

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