DEVELOPMENT OF MOLECULARLY IMPRINTED POLYMERS FOR DRUG DELIVERY APPLICATIONS USING SUPERCRITICAL FLUID TECHNOLOGY

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Molecular imprinting is an emerging field that produces precise chemical and physical architectures that can bind analytes. The technique uses the functionality of the target molecule (template) to assemble a molecular recognition site by forming specific interactions with the matrix. We have recently proposed supercritical fluid technology to replace hazardous organic solvents in the synthesis of these high affinity materials, also improving a lot the final product in terms of morphology avoiding multiple drying and sieving steps. In this work weakly cross-linked imprinted polymers with molecular affinity to ibuprofen were synthesized in $scCO_2$ at 65 ºC and 21 MPa, using DMAEMA as functional monomer and EGDMA as cross-linker agent. After impregnation at 40 ºC and 20 MPa, the polymers were tested as controlled drug release devices in PBS pH 7.4 at 37 ºC. Our results show that even with a flexible, less cross-linked structure, macromolecular memory is achieved and significant different performences in drug delivery are obtained between imprinted and non-imprinted polymers.

INTRODUCTION

Molecular imprinting is a widespread design strategy in the development of highly specific binding sites within a polymeric matrix. The technique shows great potential in chiral separations of pharmaceutical compounds [1] sample preparation using solid-phase extraction [2] and as controlled release drug delivery systems [3].

The technique uses the functionality of the target molecule (template), to assemble its own recognition site by forming specific interactions with the matrix. During polymerization, the template forms a stable complex with the monomer, in the presence of a porogen and a cross-linker that freezes the complex within a rigid porous polymer matrix [4]. Template removal, at the end of the reaction, leaves accessible chemical and physically complementary sites.

Conventional imprinting polymerizations involve excessive use of hazardous organic solvents and aqueous media which often lead to steric and conformational problems associated. Supercritical carbon dioxide has been recently proposed as an alternative solvent, capable of stabilizing the molecular interactions between monomer and template during the polymerization step. [1,6,7] Moreover, being a gas at ambient conditions, by simply depressurization of the system, the final polymer is obtained as a free flowing dry powder,

with no need of further drying, sieve and crush processes and with no solvent residues. We foresee potential application of the matrixes as substrates in medical applications and clinical analysis.

The development of low crosslinked structures with molecular affinity has been target of study in the last few years [5]. A compromise between a rigid structure, able of maintaining the integrity of the binding sites and a flexible network sensitive to external stimulus has to be carefully studied in order to sucessfully develop such matrixes. Till now the polymers developed using supercritical fluid technology are highly crosslinked [1, 6, 7], thus in this work we have develop less crosslinked imprinted matrixes and evaluate their potential application as drug delivery devices.

In this work, low crosslinked copolymers of dimethylaminoethyl methacrylate (DMAEMA) with ethylene glycol dimethacrylate (EGDMA) (1:5 molar ratio) were synthesized in $scCO_2$, in the presence of a model drug, ibuprofen, as template. This imprinted co-polymer was then evaluated as potential drug delivery device by *in vitro* experiments at pH 7.4 and compared with the non-imprinted co-polymer.

MATERIALS AND METHODS

MATERIALS

Ethylene glycol dimethacrylate (EGDMA, 98% purity) as cross-linker, dimethylaminoethyl methacrylte (DMAEMA, 98% purity) as functional monomer, ibuprofen (99% purity) as template molecule and initiator azobis(isobutyronitrile) (AIBN, 98% purity) were purchased from Sigma-Aldrich and used without further purification. Carbon dioxide was obtained from Air Liquide with purity better than 99.998%.

METHODS

POLYMER SYNTHESIS

DMAEMA-EGDMA co-polymers were synthesized in a 33 mL stainless steel high-pressure cell equipped with two aligned sapphire windows as already described elsewhere [8]. In a typical procedure to produce MIPs, cross-linker (16.7 mol % with respect to the total amount of monomers), monomer, initiator (2 wt %) and template (16.7 mol %) were loaded into the high-pressure cell. The cell was immersed in a thermostatted water bath set to 65ºC, with stability ±0.01ºC. Temperature control was made through a RTD probe contacting the cell, connected to a Hart Scientific PID controller. Stirring was achieved by means of a Teflon coated magnetic bar. Carbon dioxide was added up to 21 MPa. Polymerization reactions proceeded for 24 hours. At the end of the reaction, the polymer was slowly washed with fresh high-pressure CO_2 in order to remove the template molecule and wash any unreacted monomer residues.

$ScCO_2$ TEMPLATE DESORPTION

To guarantee that all the template introduced in the polymer during the imprinting step was removed from the resulting MIP, a stainless steel extraction reactor was packed with ibuprofen-imprinted polymer and mounted on an already existing supercritical fluid apparatus [9]. Briefly the column was immersed in a visual thermostated water bath, heated by means of a controller (Hart Scientific, Model 2200) that maintains the temperature within ±0.01 °C and temperature was set to 65 ºC. Then a $scCO_2$ stream (with or without ethanol as co-solvent) was added until the desired pressure was reached, with an exact flow, using a Gilson piston pump, model 305. After reaching the normal operational pressure, 20 MPa, the supercritical stream passed through a back pressure regulator (Jasco 880-81) which maintains the pressure constant. The pressure inside the system was monitored with a pressure transducer (Setra Systems Inc., Model 204) with a precision of ±100 Pa. Although no ibuprofen was added in the NIP synthesis, the blank polymer was also treated in the same way.

Each column was washed with supercritical carbon dioxide containing 2.5% of ethanol as modifier with a flow rate of 5 mL/min for 5 hours, followed by an hour of pure $scCO_2$ stream, at 10 mL/min, in order to remove the ethanol from the polymer.

A few milligrams of clean imprinted polymers were then finely crushed and suspended on phosphate buffer solution (pH 7.4) for 24 hours to evaluate the efficiency of template supercritical desorption. Quantification using an UV spectroscopy at 265 nm showed a residual content of ibuprofen in the imprinted polymer lower than 1.8 µg/mg.

IMPREGNATION IN $SCCO_2$ ENVIRONMENT

Impregnation experiments were performed at 40 ºC and 20 MPa within 20 hours, in a cell similar to the polymerization one but with a macroporous support which divides the cell in two compartments. This prevents physical contact between the drug and the samples. Ibuprofen was placed in the downer compartment, under the porous support with a magnetic stirrer bar, and in quantity enough to obtain a saturated solution at the p, T impregnation conditions.[10] The polymers were loaded into cellulose membranes (cutoff 3.5 KDa) which were placed in the upper compartment of the cell. At the end of the impregnation period the system was quickly depressurized. Impregnated ibuprofen was calculated gravimetrically by weighting the membranes in a Sartorious balance (precision ±0.00001 g).

IN VITRO DRUG RELEASE EXPERIMENTS

After impregnation, the polymers were put in phosphate buffer solution (pH 7.4) and 1 mL aliquots aliquots were withdrawn at time intervals and the same volume of fresh medium was added to the suspension. Quantification was performed by making a calibration curve in a spectrophotometer at 265 nm and the total mass released was determined considering the aliquots and the dilution produced by addition of fresh buffer solution.

RESULTS

Figure 1 shows a SEM image of an 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 agglomerates of well defined particles (~1μm).

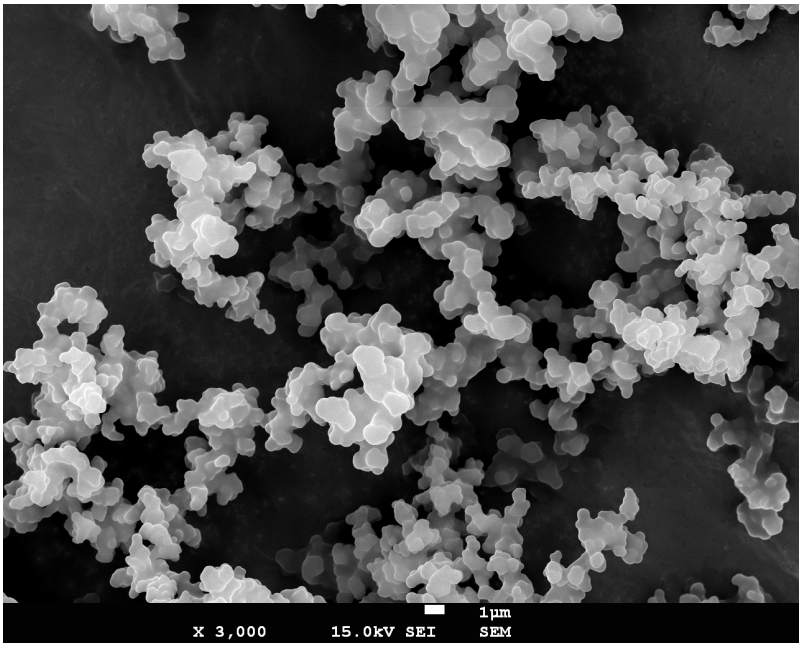

Figure 1. SEM image of the imprinted DMAEMA-EGDMA co-polymer.

In this work co-polymers with molecular recognition ability to the template molecule, ibuprofen, were developed using supercritical carbon dioxide as polymerization and impregnation medium. Both co-polymers, MIPs and their corresponding blank non-imprinted polymers (NIPs), were impregnated in batch mode in $scCO_2$, and evaluated as sustained drug release systems in PBS 7.4 at 37 ºC.

In the impregnation step the imprinted co-polymer was loaded with a much higher amount of template drug than its corresponding blank polymer (drug uptake 34 wt% for MIP and 8 wt% for NIP). As it can be seen in Figure 2, this resulted in a higher drug release of ibuprofen from the MIP co-polymers compared to the NIP. This means that the presence of ibuprofen during polymerization and thus the affinity of the polymeric matrix towards this drug molecule greatly influence the impregnation efficiency. We can also see that both co-polymers show a controlled release of the drug for more than 20 hours.

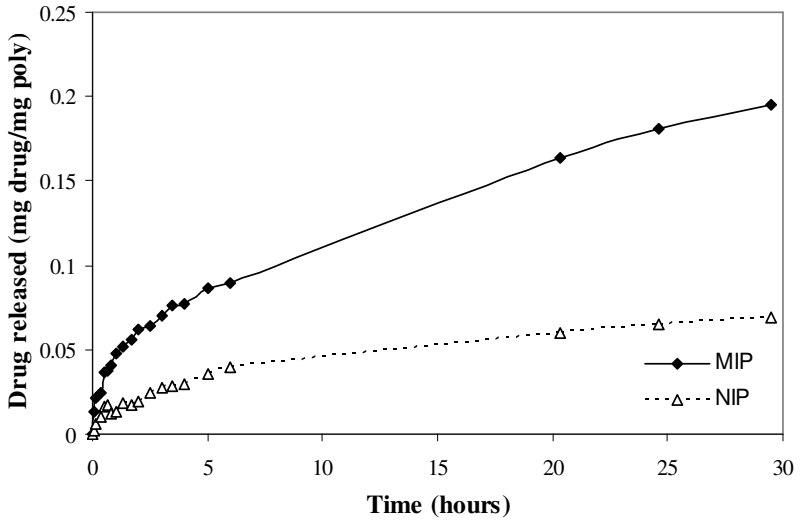

Figure 2. Ibuprofen release profiles in phosphate buffer pH 7.4 from imprinted (full line) and non-imprinted DMAEMA-EGDMA co-polymers (dotted) line.

The higher amount of ibuprofen loaded in the imprinted matrix during impregnation results from its higher affinity to the drug, promoted by the binding sites created during the polymerization reaction. This proves that careful selection of monomers and templates allow the development of low crosslinked structures with remarkable imprinting effect. This also opens up the prospect of designing stimuli sensitive polymers with recognition to target molecules.

CONCLUSIONS

In this work we have studied the possibility of using molecular imprinting strategy to impart analyte specificity and responsiveness within low crosslinked matrixes, synthesized and processed in $scCO_2$. We proved that it is possible to decrease considerably the crosslinking of polymeric matrixes and still be able to imprint them and develop co-polymers with molecular recognition to a drug molecule. Furthermore the imprinting during polymerization was able to produce co-polymers with higher affinity to the model drug which was reflected in the higher drug uptake during impregnation in $scCO_2$ and consequently a higher drug release in the in vitro drug release experiments compared to the NIP co-polymer.

These promising results show that the right balance between rigidity and flexibility of the polymeric matrix can be achieved by lowering the crosslinking degree, and that a successful imprinted co-polymer could be developed.

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