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Development of 2-(dimethylamino)ethyl methacrylate-based molecular recognition devices for controlled drug delivery using supercritical fluid technology

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ABSTRACT

This work reports the development of a novel potential body-friendly oral drug delivery system, which consists of a biocompatible molecularly imprinted polymer (MIP), with pH sensitive character and low cross-linking degree (20.2 wt%), synthesized and processed in supercritical carbon dioxide. The MIP is synthesized using 2-(dimethylamino)ethyl methacrylate (DMAEMA) as functional monomer and ethylene glycol dimethacrylate (EGDMA) as cross-linker, and ibuprofen as molecular recognition template. The imprinted matrix was able to show a higher affinity towards ibuprofen than its corresponding non-imprinted polymer (NIP) meaning that the molecular imprinting in $scCO_2$ was efficient even using a low crosslinking degree. MIP showed a significant molecular recognition towards the template, presenting higher drug uptake ability in the supercritical impregnation step, loading 33.1 wt% of ibuprofen compared to only 10.2 wt% for the NIP polymer. *In vitro* drug release experiments, simulating an oral administration, showed different release profiles at pH 2.2 and pH 7.4. Zeta potential measurements were performed to both MIP and NIP showing that the imprinting process has a significant influence on the charge of the polymeric particles. Cytotoxicity assays performed with human colorectal carcinoma-derived Caco-2 cells demonstrated that the polymers are biocompatible and could be potentially used in drug delivery applications.

1. Introduction

Over the last years polymeric materials have been widely used in the development of therapeutic drug delivery formulations (Alvarez-Lorenzo and Concheiro, 2004; Sumi et al., 2008; Gong et al., 2008; Kryscio and Peppas, 2009). Molecular imprinting is a synthetic approach to design molecular affinity polymeric matrices towards a specific molecule, called template (Byrne and Salian, 2008). During polymerization, the template forms a stable complex with the growing polymer, in the presence of a porogen and a cross-linker that freezes the complex within a rigid porous polymer matrix (Alexander et al., 2006). Template removal from the molecularly imprinted polymer at the end of the reaction leaves accessible chemical and sterically complementary binding sites. The synthesized materials possess very good thermal and chemical stability and high mechanical strength. These properties combined with their high affinity makes them a reliable alternative, over other materials, in different applications such as synthesis and catalysis (Lee et al., 2009), extraction (Beltran et al., 2010), chromatography (Qu et al., 2009), sensors (Guan et al., 2008) and in drug delivery (Cirillo et al., 2010). MIPs can be used as unique drug delivery systems or incorporated into other drug release systems. Moreover, the majority of the drugs act by a molecular recognition mechanism, which explains the growing interest in designing new polymers with improved performance. Recently, several attempts to obtain stimuli-responsive imprinted polymers that change their affinity towards the template molecule in response to pH (Suedee et al., 2010) temperature (Liu et al., 2011) and photo-irradiation (Gong et al., 2006) have been reported. A compromise between a rigid structure, able to maintain the integrity of the binding sites and a flexible network sensitive to external stimulus and improved drug release has to be carefully engineered, so that the matrix can successfully recognize the template drug.

Supercritical fluids are considered interesting alternative to most traditional solvents because of their physical and chemical properties. In the last years supercritical carbon dioxide ($scCO_2$) emerged as the most extensively studied supercritical

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fluid for polymerization reactions due to its high density, high diffusivity and low viscosity (Cooper, 2001). Furthermore, the matrices can have a controlled morphology and are obtained as dry powders, with no organic solvents residues, avoiding further purification and drying steps. This is a major advantage in applications where purity is key parameter, such as pharmaceutical and biomedical. $scCO_2$ has recently demonstrated to be a clean and one-step synthetic route for the preparation of affinity polymeric materials, with attested performance in chromatography (Soares da Silva et al., 2010) and drug delivery (Duarte et al., 2006). The high diffusivity and low viscosity of $scCO_2$ decrease the mass transfer limitations found in conventional synthesis. In addition $scCO_2$ is an apolar and aprotic medium so the hydrogen bonds between the template and the monomers are more stabilized than in a protic solvent, leading to more stable complexes and consequently to polymers potentially with higher affinity and selectivity (Schweitz et al., 1997). For this reason $scCO_2$ is a very attractive and promising medium for the development of these affinity materials (Soares da Silva et al., 2010,2011).

Poly(DMAEMA) is a cationic polymer widely used in biomedical applications such as in gene delivery (Dai et al., 2010) and in pharmaceutical formulations such as in EUDRAGIT®. Linear homo-polymers and co-polymers of DMAEMA have been already synthesized in $scCO_2$ (Xia et al., 1999; Wang et al., 2003).

Herein we report the development of a novel potential drug delivery polymer that consists in a cross-linked pH-sensitive MIP with recognition for ibuprofen and composed of 2-(dimethylamino)ethyl methacrylate (DMAEMA) and ethylene glycol dimethacrylate (EGDMA), synthesized using $scCO_2$ as solvent and porogen, at 65 °C and 21 MPa. The molar ratios of ibuprofen:DMAEMA:EGDMA studied were 1:5:1, respectively. As control the non-imprinted polymer (NIP) was synthesized at the same experimental conditions except that no drug template was introduced in the reactional mixture. After template desorption in supercritical environment, the matrices were loaded with the drug by impregnation in $scCO_2$. *In vitro* drug delivery experiments proved that even with low cross-linker ratio it was possible to obtain a polymeric matrix with high affinity to the drug, and with a sustained drug delivery profile. Cytotoxicity assays performed with human colorectal carcinoma-derived Caco-2 cells demonstrated that the polymers are biocompatible and therefore have the potential to be used in drug delivery applications. We thus believe that the present methodology is a promising way to develop human-body-friendly drug delivery systems, with additional degrees of control over the drug release profiles, in the near future.

2. Materials and methods

2.1. Materials

Ethylene glycol dimethacrylate (EGDMA, 98% purity) as cross-linker, 2-(dimethylamino)ethyl methacrylate (DMAEMA, 98% purity) as functional monomer (S)-(+)-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%. Amphotericin B, Eagles minimum essential medium with Earle's balanced salt solution (EMEM (EBSS), Human colorectal carcinoma-derived Caco-2 cells, L-glutamine, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphofenyl)-2H-tetrazolium (MTS), non-essential amino acids (NEAA), penicillin G, streptomycin, and trypsin were purchased from Sigma–Aldrich. Fetal bovine serum (FBS) was purchased from Biochrom AG (Berlin, Germany).

2.2. MIP and NIP 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 (Casimiro et al., 2005). In a typical procedure to produce the MIP, EGDMA (16.7 mol% with respect to the total amount of monomers), DMAEMA, AIBN (2 wt%) and ibuprofen (16.7 mol%) were loaded into the high-pressure cell. The procedure to synthesize NIP was the same except that no template was added in the reactional mixture. The cell was immersed in a thermostatted water bath set to 65 °C. Temperature control was made through an open bath circulator Julabo Ed with stability ±0.1 °C. Stirring was achieved by means of a Teflon coated magnetic bar. Carbon dioxide was added up to 21 MPa. Polymerization reactions proceeded for 24 h. 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.

2.3. $scCO_2$-assisted template desorption

To guarantee that the template used in the imprinting step was removed from the synthesized MIP, a stainless steel tubular reactor was packed with ibuprofen-imprinted polymer and mounted on an already existing supercritical fluid apparatus (Barroso et al., 2009). Briefly, the high pressure reactor was immersed in a visual thermostatted 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 °C. Then an exact flow of $scCO_2$ was added until the desired pressure was reached, 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 in order to extract any remaining reactant residues.

Each polymer was cleaned with $scCO_2$ containing 2.5% of ethanol as modifier with a flow rate of 5 ml/min, for 4 h, followed by an hour of pure $scCO_2$ stream, at 10 ml/min, in order to remove any residue of ethanol from the network.

In order to evaluate the presence of ibuprofen at the conditions used in the *in vitro* drug release experiments, 20 mg of template-desorbed imprinted polymers were finely crushed and suspended on phosphate buffer saline solution (PBS),pH 7.4, at 37 °C for 48 h. Quantification of ibuprofen in this solution was performed in a UV spectrophotometer at 265 nm. No traces of ibuprofen were detected in the solution, assuring that all the ibuprofen quantified in the release assays was proceeding from the supercritical impregnation step and not from the synthesis.

2.4. MIP and NIP characterization

2.4.1. SEM and nitrogen porosimetry

Polymers were morphologically 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 gold coated.

Specific surface area and pore diameter of the polymeric particles were determined by adsorption of N_2 according to the BET method. An accelerated surface area and porosimetry system (ASAP 2010 Micromeritics) was used under nitrogen flow.