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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.

2.5. Swelling degree

The water uptake of the polymeric matrices was determined in similar conditions to those tested in the drug release experiments. Samples with 50 mg of polymer were put inside a sleeve filter of 1 μm mesh and immersed in PBS solution (pH 2.2) for 3 h, followed by immersion in a pH 7.4 solution, for 5 h. Briefly, the water content was estimated by the difference between the weight of the swollen polymer samples (W), after careful wiping with a soft tissue, and the weight of the dry polymer samples (W_0).

$$\text{Swelling} = \frac{W - W_0}{W_0} \quad (1)$$

2.6. Zeta potential

Potentiometric measurements were performed to both NIP and MIP matrices in a Zetasizer Nano ZS from Malvern. The particles were resuspended in PBS (pH 7.4), with a concentration of 5 mg/ml, sonicated for 3 min and then transferred to a disposable folded capillary cell. The zeta potential was measured using the supplied software.

2.7. Cytotoxicity assays

2.7.1. Caco-2 cell culture

Human colorectal carcinoma-derived Caco-2 cells were seeded in T-flasks of 25 cm^3 with 6 ml of EMEM (EBSS), supplemented with 2 mM L-glutamine, heat-inactivated FBS (10% v/v), NEAA (1%, v/v) and 1% antibiotic/antimycotic solution. After the cells reached confluence, they were subcultivated by a 3–5 min incubation in 0.18% trypsin (1:250) and 5 mM EDTA. Then cells were centrifuged, resuspended in culture medium and then seeded in T-flasks of 75 cm^3. Hereafter, cells were kept in culture at 37 °C in a 5% CO_2 humidified atmosphere inside an incubator.

2.7.2. Proliferation of Caco-2 cells in the presence of polymers

Dry samples, at a concentration of 20 mg/ml, were placed in 48-well plates (Nunc) and sterilized by UV exposure for at least 30 min. Then, EMEM (EBSS) was added to each well and was left in contact with the materials for 24 h.

Meanwhile, Caco-2 cells were cultured in 96-well plates (Nunc) at a density of 3×10^4 cells per well, with EBSS. After 24 h, the cell culture medium was removed and replaced by that in contact with polymers. This procedure was repeated during 3 days. Cell growth was monitored using an Olympus CX41 inverted light microscope (Tokyo, Japan) equipped with an Olympus SP-500 UZ digital camera.

The adhesion and proliferation of Caco-2 cells in the presence of polymers were also characterized by SEM. Cells (4×10^4 cells per well) were seeded with the sterilized polymers in 48-well plates, over a coverslip, for 48 h. Samples were fixed in 2.5% glutaraldehyde for 10 min. Then, they were rinsed three times with PBS for 2 min and dehydrated in graded ethanol of 70, 80, 90, and 100% during 10 min each. Finally, the coverslips were mounted on an aluminium board using a double-side adhesive tape and sputter coated with gold using an Emitech K550 (London, England) sputter coater. The samples were then analyzed using a Hitachi S-2700 (Tokyo, Japan) scanning electron microscope operated at an accelerating voltage of 20 kV and at various amplifications.

2.7.3. Cytotoxic profile of the polymers

To evaluate the cytotoxicity of the polymers, cells (3×10^4 cells per well) were seeded in a 96-well plate and cultured with EMEM (EBSS). At the same time, in another 96-well plate culture EMEM (EBSS) was added to the sterilized polymers, and left there for 24 h, 48 h and 72 h. After 24, 48 and 72 h of incubation, the cell culture medium was removed and replaced with 100 μL of medium that was in contact with the polymers. Then, cells were incubated at 37 °C in a 5% CO_2 humidified atmosphere for more 24 h for each case. Subsequently, cell viability was assessed through the reduction of MTS into a water-soluble brown formazan product ($n = 5$), by an adaptation of a method previously described in the literature (Maia et al., 2009). To do so, the culture medium of each well was removed and replaced with a mixture of 100 μL of fresh culture medium and 20 μL of MTS/PMS reagent solution. The cells were incubated for 4 h, at 37 °C, in a 5% CO_2 humidified atmosphere. Absorbance at 490 nm was measured using a microplate reader (Sanofi, Diagnostics Pauster). Wells containing cells in the culture medium without polymers were used as negative control. ETOH 96% was added to wells containing cells as a positive control (Ribeiro et al., 2009; Rosellini et al., 2010; Baoum et al., 2010).

2.8. Impregnation in $scCO_2$ environment

Impregnation experiments were performed at 65 °C and 20 MPa within 20 h, in a high-pressure cell similar to the polymerization one but with a macroporous support that divides the cell in two compartments. This prevents physical contact between the drug and the samples. Ibuprofen was placed in the bottom compartment, under the porous support with a magnetic stirrer bar, and in enough quantity to obtain medium saturation at the p, T impregnation conditions. The polymers were loaded into cellulose membranes (cutoff 3.5 kDa) which were placed on the top compartment of the cell. At the end of the impregnation period the system was quickly depressurized.

Impregnated amounts of ibuprofen were calculated upon the crush of the polymeric networks with a mortar at the end of release experiments and quantification of the release solutions.

2.9. In vitro drug release experiments

Drug release profiles were studied at sink conditions in two different experiments. In case I, the polymers were put in 200 ml of PBS at pH 7.4, for 3 days. In case II, the loaded polymers were allowed to release ibuprofen in a simulated oral administration situation. In this experiment the matrices were put in 200 ml of Glycine–Hydrochloric acid buffer solution (pH 2.2) for 3 h and then for 5 h in 200 ml of PBS (pH 7.4). In both cases the temperature was set at 37 °C and 1 ml aliquots were withdrawn at time intervals and the same volume of fresh medium was added to the solution. Quantification was performed by making calibrations curves in a spectrophotometer at 223 nm for the solution with pH 2.2 and 265 nm for PBS (pH 7.4). The total mass released was determined considering the aliquots and the dilution produced by addition of fresh buffer solution.

Ibuprofen transport through the synthesized polymeric networks in solution with different pH was modelled using a semi-empirical Korsmeyer–Peppas equation:

$$\frac{M_t}{M_\infty} = kt^n \quad (2)$$

where M_t is the absolute cumulative amount of drug released at time t, M_∞ is the total amount of drug impregnated in the polymer samples, k is the diffusion coefficient that reflects the structural and geometric characteristics of the device, and n is the release exponent, which gives an insight on the specific transport mechanism.

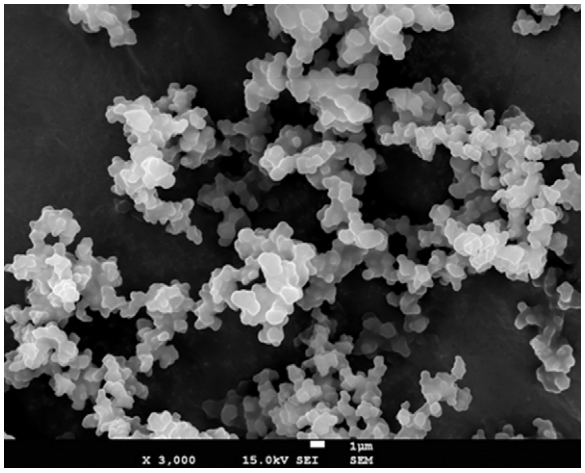

Fig. 1. SEM image of P(DMAEMA-co-EGDMA) imprinted polymer.

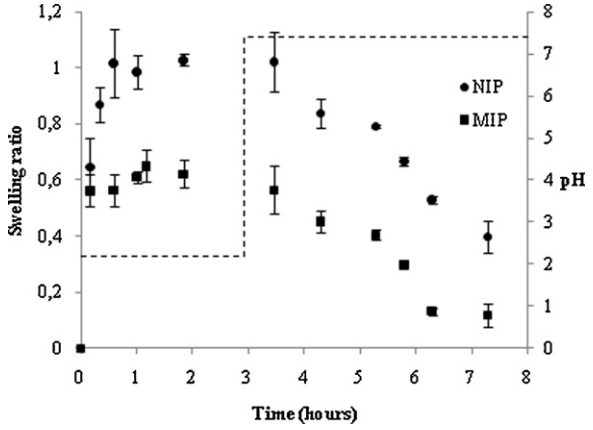

Fig. 2. Swelling ratio of NIP and MIP matrices at 37 °C and pH 2.2 in the first 3 h, and pH 7.4 in the subsequent time. Filled dots represent the water uptake from NIP and filled squares the water uptake from MIP (mean ± SD, $n = 3$). Dotted line is the pH variation.

3. Results and discussion

3.1. Synthesis and characterization of the polymers

The synthesized polymeric matrices were obtained in high yields (~85%, determined gravimetrically) as dry, free-flowing powders. This represents a major advantage over traditional MIPs that have to be grounded and sieved leading to the destruction of some interaction sites (Baggiani et al., 2005). The initial pressure–temperature conditions reactions were adjusted to assure a homogeneous system, as it could be seen through the sapphire windows.

Fig. 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 (~1 μm).

The physical characteristics concerning surface area and porosity of the synthesized polymers are shown in Table 1. The addition of the template molecule in the synthesis process does not seem to affect the nucleation of this polymerization system, with MIP and NIP presenting similar porosities and surface areas. These physical characteristics are consistent with other precipitation polymerizations in $scCO_2$ (Temtem et al., 2007).

Potentiometric measurements disclosed different zeta potential values of NIP and MIP particles. This suggests that the introduction of ibuprofen in the polymerization influences the charge distribution through the polymeric particles with the potentially ionizable amino group of DMAEMA having a different spatial orientation on the particles.

Fig. 2 shows the variation of water uptake by the synthesized NIP and MIP at acidic and neutral pH. In the first 3 h the polymers were immersed in a solution of pH 2.2 and afterwards they were immersed in a solution of pH 7.4. As it can be seen, both imprinted and blank polymers exhibit a degree of swelling in the same order of magnitude although NIP presents a slightly higher swelling ratio. At acidic pH the polymers swell more because the tertiary amine group of DMAEMA is highly protonated, which increases the charge density on the networks and leads to an income of mobile counter ions to balance the charge. At that point the internal osmotic pressure of the network increases and the matrices become swollen (Yanfeng and Min, 2001).

3.2. Cytotoxicity of the synthesized polymers

Over the last years DMAEMA has been used in the development of therapeutic drug delivery formulations. In order to study the applicability of the materials for biomedical applications, the cytocompatibility of NIP and MIP matrices was characterized through *in vitro* studies. As already mentioned, Caco-2 cells were seeded at the same initial density in the 96-well plates, with or without polymers to assess its cytotoxicity. Cell adhesion and proliferation was noticed in wells where cells were in contact with the medium, which was previously incubated with the polymer, in a concentration of 20 mg/ml (Fig. 3A and B) and in the negative control (Fig. 3C). In the positive control, no cell adhesion or proliferation was observed. Dead cells with their typical spherical shape can be observed in Fig. 3D. The observation of cell growth in the presence of materials (Fig. 3) showed that MIP and NIP are biocompatible. However, a higher number of cells were observed in the vicinity of MIP. The observed difference in the cytotoxicities of NIP and MIP can be explained by their zeta potential values. A polymeric material with positively charged particles can interact more extensively through electrostatic interactions with the negatively charged cell surface (Ding et al., 2010). It seems that the introduction of ibuprofen in the polymerization step and its subsequent removal induces a modification on the particle surface with the imprinted polymer showing less surface positive charge, 6.7 mV, against 17.5 mV for NIP. The polymeric network of NIP with higher positive zeta potential will interact more with the anionic components of the proteins of the extracellular matrix.

To further evaluate the biocompatibility of the polymers, MTS assay was also performed. To perform this assay, culture medium that was in contact with MIP and NIP samples for 24, 48 and 72 h, was added to the Caco-2 cells. The MTS assay results (Fig. 4) showed that cell viability was higher for the negative control, in which cells

Table 1
Physical characteristics of NIP and MIP particles and ibuprofen loaded in the $scCO_2$-assisted impregnation. Each result is the average of at least three independent experiments.

Polymer	BET surface area (m^2/g)	Pore volume (cm^3/g)	Pore diameter (nm)	Zeta potential (mV)	Total amount of drug impregnated (mg/g polymer)
MIP	10 ± 1	0.0106	4.1	6.7 ± 0.4	331 ± 44
NIP	9 ± 1	0.0105	4.4	17.5 ± 0.6	102 ± 25

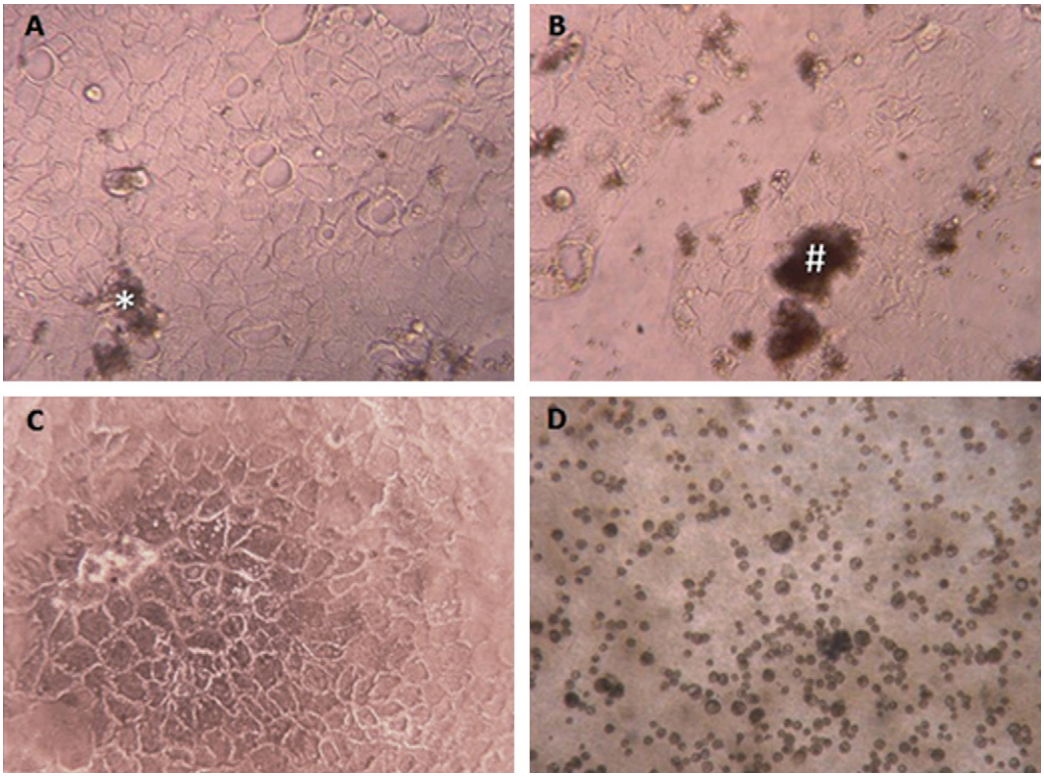

Fig. 3. Microscopic photographs of Caco-2 cells after being seeded in the presence of the culture medium that was previously put in contact with MIP* (A) and NIP# (B). The negative and positive controls are presented in (C) and (D). Original magnification 100×.

were seeded just with EMEM (EBSS), followed by those that were seeded in the presence of the test samples, MIP and NIP. As should be expected the positive control showed almost no viable cells.

To further characterize the cytotoxic profile of the polymers, SEM images were also acquired. In these images (Fig. 5), it was possible to observe the proliferation and the adhesion of Caco-2 cells to the MIP (Fig. 5A) and NIP (Fig. 5B) particles. These results confirm that the polymers are biocompatible and that they can be used for drug delivery applications.

3.3. Drug delivery experiments

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, MIP and its corresponding NIP, were impregnated in batch mode in $scCO_2$. The impregnation experiment was performed in such conditions that the amount of ibuprofen loaded in the polymers was not limited by the amount of ibuprofen present in the supercritical environment. Table 1 shows the total amount of drug impregnated in the synthesized polymers. As it can be seen, MIP was loaded with a much higher amount of ibuprofen than NIP, 33.1 wt% compared to only 10.2 wt%, which reflects the higher affinity of the imprinted polymer towards the template drug than the non-imprinted polymer. This means that the presence of ibuprofen during polymerization greatly influences the drug partition between the supercritical fluid and the polymeric phases, having a real impact in the impregnation efficiency. Impregnation in supercritical medium can lead to high amounts of drug uptaken by the polymers, as it was reported in literature, for example, in the impregnation of PVP with ketoprofen (Manna et al., 2007). The significant difference between MIP and NIP is enhanced by the fact that the polymers are loaded with the drug in the same solvent that they were synthesized. In the imprinting process, rebinding of the drug in the same solvent of the polymerization is known to improve the imprinting factor (Byrne et al., 2002). This occurs because during polymerization the binding sites that are created adjust to the solvation of the polymer by the porogen, so the binding sites integrity is optimal when the rebinding solvent is the same that is used in the polymer synthesis (Spivak et al., 1997).

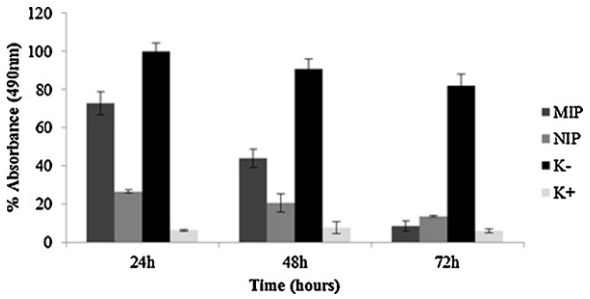

Fig. 4. Cellular activities measured by the MTS assay after 24, 48 and 72 h in contact with the polymers. MIP, NIP, K^+, positive control; K^-, negative control. Each result is the average of at least three independent experiments.

Previously impregnated NIP and MIP were evaluated as sustained drug release systems in two different experimental conditions. In case I the matrices were allowed to release the impregnated drug for 70 h, at 37 °C and pH 7.4. In case II the co-polymers were exposed to two different pHs in a total of 8 h. In the first 3 h the matrices were immersed in a solution of pH 2.2 and then moved to a solution of pH 7.4, both at 37 °C. By performing the drug release experiments at those conditions we wanted to evaluate the drug release kinetics from the polymeric networks in a situation that simulated an oral drug administration.

Fig. 6 presents the cumulative amount of ibuprofen release profiles from both MIP and NIP at pH 7.4 for 70 h. As it is shown

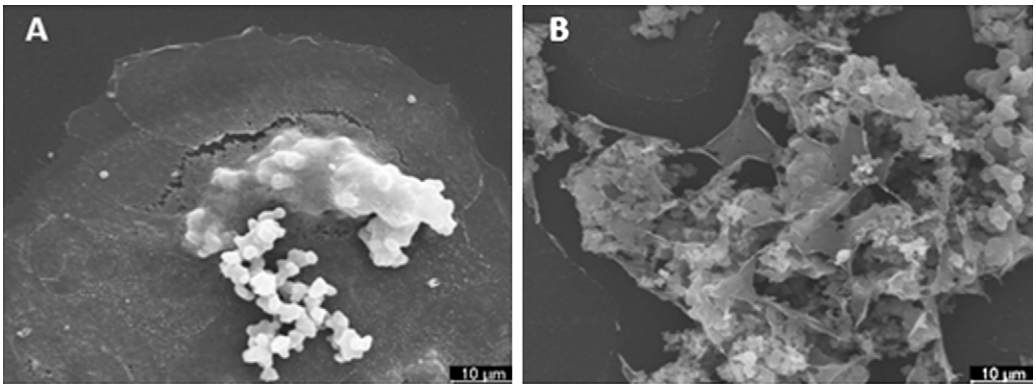

Fig. 5. Images of scanning electron microscopy. (A) MIP nanoparticles in contact with Caco-2 cells (1500×, 20 kV) and (B) NIP nanoparticles in contact with Caco-2 cells (1500×, 20 kV).

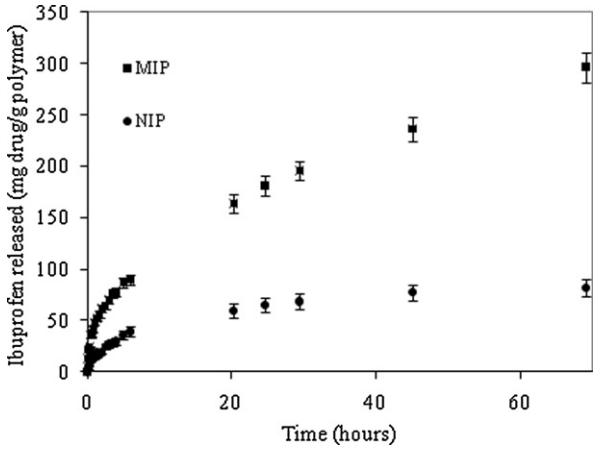

Fig. 6. Ibuprofen release profiles in phosphate buffer pH 7.4 from imprinted (filled squares) and non-imprinted (filled dots) DMAEMA–EGDMA co-polymers. Each result is the average of at least 3 independent experiments.

the imprinted polymer, which was loaded with a higher amount of ibuprofen in the impregnation step, also released much more ibuprofen than the corresponding blank polymer. The release of ibuprofen after 24 h was 181 mg/g polymer for MIP against only 65 mg/g polymer for NIP. After this time, the non-imprinted polymer almost reached the plateau of ibuprofen release whilst the imprinted polymer was still releasing the impregnated drug.

Fig. 7 shows the experimental release profiles of ibuprofen from impregnated samples of MIP and NIP as a function of time upon the matrices were put at pH 7.4 for 8 h or pH 2.2 for 3 h followed by a period of 5 h at pH 7.4. At low pH the polymers just released a low amount of ibuprofen. These results are in accordance with the release profiles of ibuprofen from cationic matrices that can be found in the literature (Rodríguez et al., 2003). The lower solubility of ibuprofen at acidic pH may contribute to sustain the release process directly and indirectly by promoting hydrophobic interactions with the polymer. When the pH was changed to 7.4 the amount of ibuprofen released increased significantly, due to its higher solubility at neutral pH. As it is possible to observe, different release kinetics are obtained as the polymers are immersed in solutions with different pH. In drug delivery systems there are several parameters that can control burst release, such as the solubility of the drug in the release medium, the drug diffusion coefficient through the materials and the initial drug distributions within the polymeric carrier (Bibby et al., 2000). As ibuprofen solubility at acidic pH is very small, the drug release is hindered with respect to the release at neutral pH.

Mathematical modeling of drug delivery from polymeric systems during the first hour at pH 7.4 and pH 2.2 was accomplished through the semi-empirical Korsmeyer–Peppas Model (Eq. (2)) that was used to determine the release constants. Fig. 8 presents the fit of experimental data to the empirical model and Table 2 summarizes the diffusional coefficient and the release exponent from the polymeric delivery systems as calculated by the theoretical equation.

Both systems NIP and MIP present the same release exponent, independently of the pH of the release solution, meaning the mechanism of drug transport through the particles is very similar. At neutral pH, release occurs by an anomalous transport ($0.43 < n < 0.85$, considering spherical particles) (Siepmann and

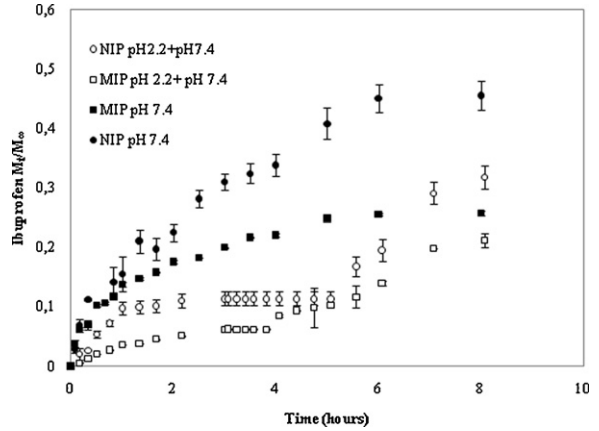

Fig. 7. *In vitro* release profiles of ibuprofen from MIP and NIP obtained at 37 °C and different pH, for 8 h. Each result is the average of at least 3 independent experiments.

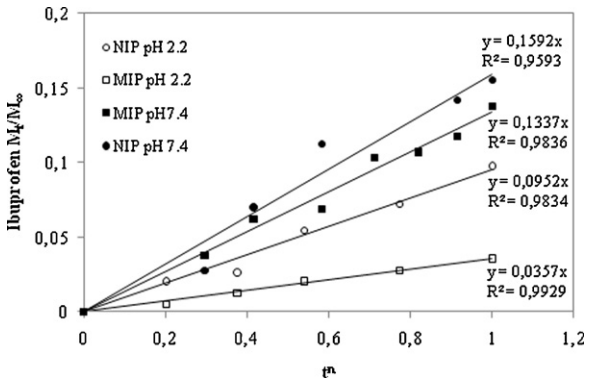

Fig. 8. Ibuprofen diffusion coefficients from NIP and MIP at 37 °C, pH 2.2 and pH 7.4 in the first hour of drug release.

Table 2
Diffusion coefficients and release exponents at different pH for MIP and NIP polymers.

Polymer	Diffusion coefficient, k (h^{-1})		Release exponent, n	
	pH 2.2	pH 7.4	pH 2.2	pH 7.4
MIP	0.0357	0.1337	0.89	0.49
NIP	0.0952	0.1592	0.89	0.49

Peppas, 2001) explained by the superposition of both diffusion and swelling, whereas in an acidic solution the release follows a case-II transport ($n > 0.85$). Diffusion coefficients of ibuprofen from the matrices to the release medium depend on the drug solubility and the affinity of the polymer to the drug. At pH 2.2 the solubility of ibuprofen is very low, which lowers the diffusion coefficients of the drug through the systems. When the release is performed at neutral pH, whereas ibuprofen has much higher solubility, the diffusion coefficients increase. At both pHs the diffusion coefficients are lower for MIP than for NIP, reflecting a more sustained drug delivery from the affinity polymers. This effect is more pronounced at acidic pH when hydrophobic interactions between ibuprofen and polymeric networks are enhanced due to ibuprofen low solubility. This suggests that not only hydrogen bonds are involved in the stabilization of imprinted polymer–drug complex but also hydrophobic interactions play a significant role.

4. Conclusion

In this work we have studied the possibility of using molecular imprinting strategy to impart drug specificity within low cross-linked matrices, synthesized and processed in $scCO_2$. We proved that it is possible to decrease considerably the crosslinking of polymeric matrices 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 template drug which was reflected in the higher drug uptake during impregnation in $scCO_2$ and consequently a higher drug release amount in the *in vitro* drug release experiments compared to the NIP co-polymer. Cytotoxicity assays demonstrated the synthesized matrices are biocompatible, with the imprinted polymer showing better results with concern to cell adhesion and proliferation. 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 using a clean technology, such as supercritical fluid technology, leading to highly pure materials with affinity towards a specific drug. We believe that the present methodology is a promising way to develop polymeric systems for drug delivery applications, with additional degrees of control over the drug release profiles, in the near future.

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