DEVELOPMENT OF CYCLODEXTRIN-HYDROGEL POLYMERIC SYSTEMS IN $SCCO_2$ FOR COLON TARGETED DRUG DELIVERY

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Herein we describe the preliminar studies on the development of new cyclodextrin-hydrogel systems in supercritical carbon dioxide ($scCO_2$) with potential applications on controlled colon-targeted drug delivery devices. β-cyclodextrin was acrylated through reaction of 6^A-*O*-toluenosulfonyl-β-cyclodextrin with the cesium salt of methacrylic acid. This β-cyclodextrin derivative was then co-polymerized with methacrylic acid, N-isopropylacrylamide and ethyleneglycol dimethacrylate, for the first time in $scCO_2$, in order to obtain a biocompatible cyclodextrin-hydrogel conjugated polymer. The objective was to combine the high drug affinity to the cyclodextrin cavity with a pH-sensitive hydrogel which can protect the drug till it reaches the colon using a green, completely solvent-free process. The synthesized polymeric materials were then impregnated with a model drug, ibuprofen, using a batch supercritical fluid impregnation process. *In vitro* experiments were carried out in order to evaluate the performance of the cylodextrin-hydrogel system as drug release device at different pHs, 2 and 7.4, which simulate the gastric and colon environments.

INTRODUCTION

Colon-targeted drug delivery devices must have a delayed release profile with a long lag-time, usually 8 hours, which is the required time to the drug reach the colon after oral administration in man. [1] If a cyclodextrin-drug complex is taken orally usually it readily dissociates in the gastrointestinal fluid with consequent release of the drug, thus it is not a suitable colon-targeted drug release device. Cyclodextrin-drug conjugates, where the drug is convalently bound to the cyclodextrin have been proposed to survive the stomach and small intestine, and the drug released in the colon through enzymatic degradation of the conjugate.[2] More recently polymer-cyclodextrin derivatives have been proposed as drug delivery devices in order to increase drug upload into contact lenses. [3]

Supercritical carbon dioxide has proved to be an excellent medium to prepare hydrogels, which are obtain as completely dry, flowing powders, with no solvent residues and with no need of intensive drying steps before further processing.[4] The incorporation of a cyclodextrin into a co-polymer allows to control the impregnation degree and the drug release, as we have observed previously. [5] The co-polymerization of a polymerizable cyclodextrin with pH sensitive monomers will allow the combination of the high affinity of the cyclodextrin cavity to the drug molecules, and the sensitivity of the polymer to different pH environments. Thus we foresee potential applications in pharmaceutical and biomedical areas for these cylodextrin-hydrogel conjugated materials developed using supercritical fluid technology.

MATERIALS AND METHODS

MATERIALS

Synthesis of cyclodextrin monomer: β-cyclodextrin and methacrylic acid were purchased from Aldrich. DMF was purified and dried by standard methods [6] before use. Analytical thin-layer chromatography TLC was performed on E. Merck Kieselgel 60, F254 silica gel (0.2 mm thick). High resolution mass spectra were recorded on an AutoSpeQ spectrometer. 1H NMR spectra recorded on a Bruker ARX 400 spectrometer (400 MHz for 1H).
Co-polymerization in $scCO_2$: Ethylene glycol dimethacrylate (EGDMA, 98% purity) as cross-linker, N-isopropylacrylamide (NIPAAMAm, 97% purity) and methacrylic acid (MAA, 98% purity) as functional monomers, ibuprofen as model drug (99% purity) and azobis(isobutyronitrile) as initiator (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%.

SYNTHESIS OF THE ACRYLATED-β-CYCLODEXTRIN MONOMER

Methacrylic acid (0.33 mL, 4.29 mmol) was added dropwise to an aqueous solution of CsOH (50%, 0.73 mL, 4.29 mmol), at room temperature. The mixture was stirred for 30 min, and concentrated under reduced pressure. The resulting salt was dissolved in dry DMF (5 mL) and 6A-O-toluenosulfonyl-β-cyclodextrin (0.56 g, 0.43 mmol) was added. The resulting mixture was heated at 90ºC for 24 hours. The reaction evolution was monitored by TLC (silica, MeCN:H2O:aqueous ammonia, 6:3:1). The mixture was allowed to cool to room temperature and washed with ethanol (2 x 38 mL) and ethyl ether (38 mL). This washing procedure was repeated twice. The 6A-O-acryloyl-β-cyclodextrin was isolated in 47% yield. Data is in accordance with literature [7].

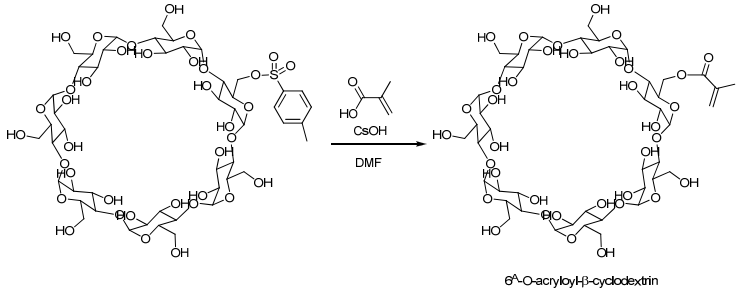

Figure 1: Synthesis of the acrylated cyclodextrin monomer.

CO-POLYMER SYNTHESIS IN $SCCO_2$

Polymerizations were carried out in a 33 mL stainless steel high-pressure cell equipped with two aligned sapphire windows as already described elsewhere.[8] The cell was loaded with reactants: monomers (75% molar NIPAAm, 1% EGDMA), acrylated-β-cyclodextrin (when used 2.74% molar), crosslinker, initiator. Two different co-polymers were formed: P(NIPAAm-MAA-EGMA) and P(NIPAAm-MAA-EGDMA-acrylβCD). The cell was then 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 polymers were slowly washed with fresh high-pressure CO_2 in order to clean any unreacted residues.

$SCCO_2$-ASSISTED IMPREGNATION

The impregnation of a model drug, ibuprofen, into the co-polymers was performed at 65ºC and 21 MPa within 20 hours, in a cell similar to that of the polymerization, with a porous 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.[9] The polymers were loaded into cellulose membranes (cutoff 3.5KDa) 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 transferred to porous membranes and put in buffer solutions (pH 2 and 7.4) at controlled temperature 37ºC. 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 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

Both co-polymers P(NIPAAm-MAA-EGDMA) and P(NIPAAm-MAA-EGDMA-acrylβCD) were obtained as white, dry, free flowing powders at the end of the reaction. Figure 2 shows SEM images of the synthesized co-polymers as well as the corresponding impregnated co-polymers. As it can be seen P(NIPAAm-MAA-EGDMA) is very homogeneous in terms of particle size, with slightly agglomerated discrete particles sizing less than 1 μm.

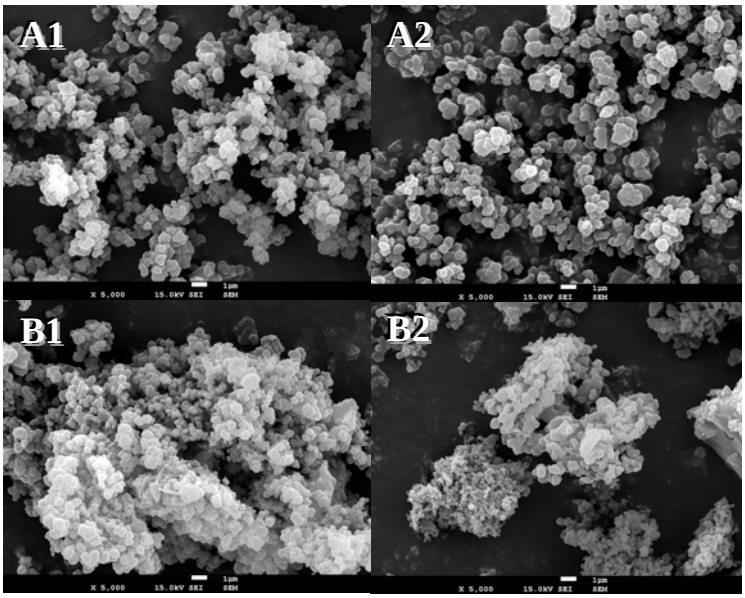

Figure 2: SEM images of: A1) NIPAAm-MAA-EGDMA co-polymer; A2) A1 impregnated; B1) NIPAAm-MAA-EGDMA-acrylβCD co-polymer; B2) B1 impregnated.

The co-polymer P(NIPAAm-MAA-EGDMA-acrylβCD) clearly shows two different particle size distributions, of much more agglomerated submicron-sized particles. No morphological differences could be observed between the co-polymers before and after impregnation.
Figure 3 shows the Maldi-TOF spectrum of a co-polymer obtained by the polymerization reaction between the polymerizable cyclodextrin monomer and MAA. This technique was used to confirm the co-polymerization evidence between the cyclodextrin monomer and an acrylated monomer in $scCO_2$. This technique can not be applied to the final cyclodextrin hydrogels since they are crosslinked thus drastically limiting solubility. The experiments were performed at Unidade de Espectrometría de Masas, Universidade Santiago de Compostela, Spain. DHB was the used matrix and AgTFA salt was used to help ionization. The intensity of the peaks is weak which may suggest difficulties in solubilizing the sample or lack of miscibility with the matrix.

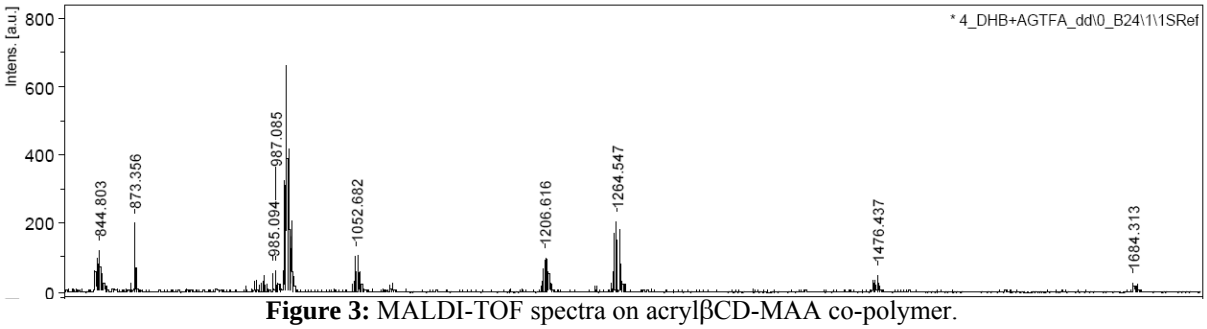

Figure 3: MALDI-TOF spectra on acrylβCD-MAA co-polymer.

The Maldi spectrum shows evidence of co-polymerization between the synthesized cyclodextrin derivative and the MAA monomer. The mass values show small shifts to the exact expected masses due to the formation of adducts with the matrix or the salt ions or even due to the release of oxygen or protons.This preliminary result shows that the co-polymer is formed but there is still some acrylated-cyclodextrin that did not react (Mw 1206). The molar mass of 1476 corresponds to CD-$(MAA)_2$ and 1684 to CD-$(MAA)_4$ oligomers. The molar masses lower than the mass of the cyclodextrin correspond to homo-PMAA where MAA oligomers of maximum 12 monomers were obtained.

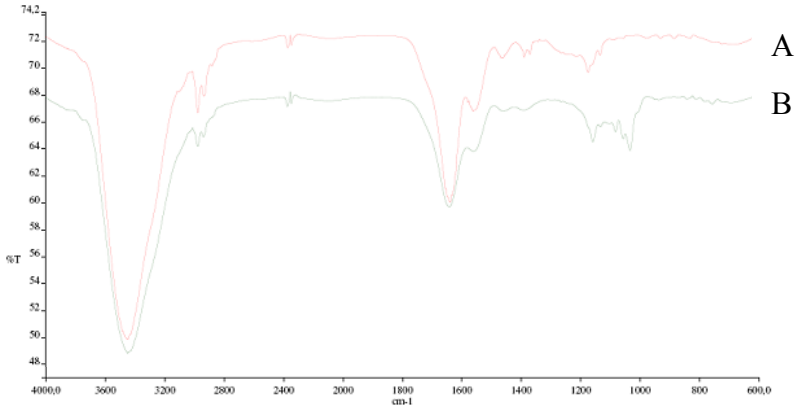

Figure 4: FTIR spectra of the synthesized co-polymers: A) Poly(NIPAAm-MAA-EGDMA); B) Poly(NIPAAm-MAA-EGDMA-acrylCD)

Figure 4 shows the FTIR spectra of the synthesized co-polymers. The co-polymer P(NIPAAm-MAA-acrylCD) shows a strong C-OH stretching vibration at 1040 cm^{-1}, which is characteristic of the cyclodextrin. The bands at 1640 cm^{-1}, 1575 cm^{-1} and 1175 cm^{-1} are

related to C=O stretching vibration, N-H deformation vibration and C-N bonds from the tertiary amide from NIPAAm.
Figure 5 shows the comparison of drug release from both synthesized co-polymers at pH 7.4, similar to colon environment. Both co-polymers were impregnated with the same percentage of ibuprofen ~30wt%.

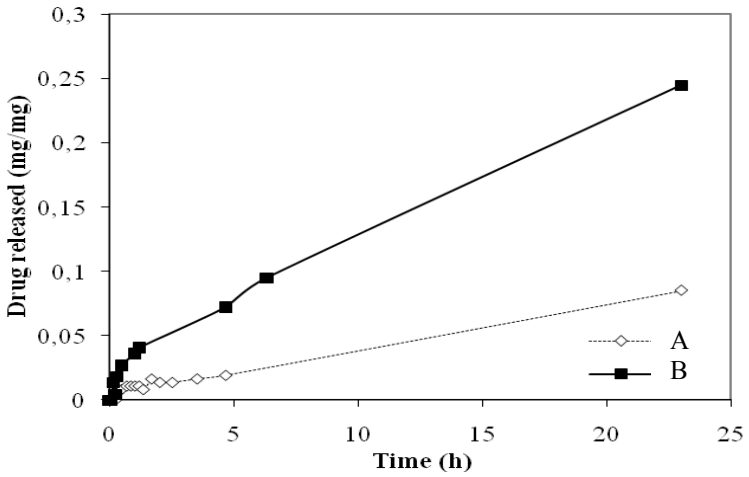

Figure 5: Drug release from the synthesized co-polymers at pH 7.4 and 37ºC: A) P(NIPAAm-MAA-EGDMA) and B) P(NIPAAm-MAA-EGDMA-acrylβCD).

The matrixes are similarly impregnated due to the high CO_2 diffusivity. But when the co-polymers are put into buffer solution there is a significant increase in the drug release from the cyclodextrin containing co-polymer both from the hydrogel and cyclodextrin cavities In addition the presence of cyclodextrin is likely to increase hydrofilicity, promoting the diffusion of water into the crosslinked matrix.

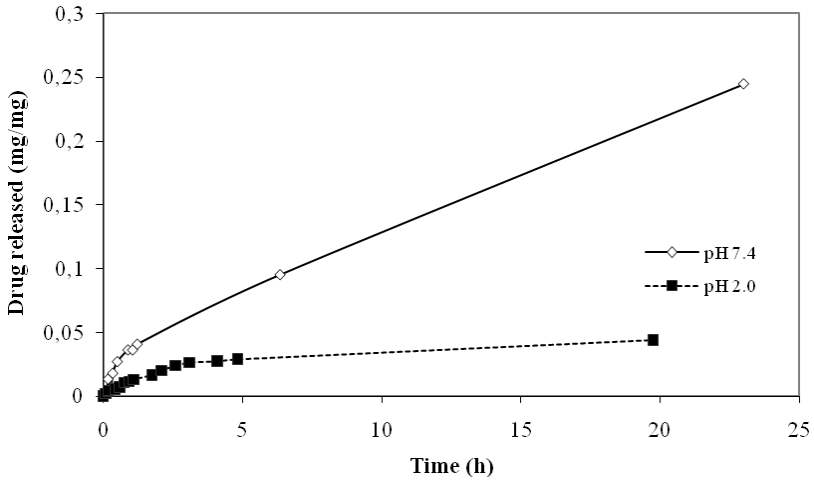

Figure 6: Drug release from the synthesized P(NIPAAm-MAA-acrylCD) co-polymer at different pHs.

The drug release experiments from the cyclodextrin-hydrogel co-polymer at different pH (2 and 7.4) buffer solutions are shown in Figure 6. As it can be seen at low pH 2 which simulates the gastric fluid, the cyclodextrin-hydrogel conjugated has a very low release to the medium, releasing less than 0.05 mg of ibuprofen per 1 mg of polymer over 20 hours of experiment. At

pH 7.4 which simulates the colon environment, there is a much higher rate of drug release, which is controlled for more than 20 hours. This means that the co-polymer P(NIPAAm-MAA-EGDMA-acrylβCD), if taken orally, would not release the drug at pH 2 at the stomach, but at higher pH 7.4 as in colon, it would start releasing the drug in a controlled manner for several hours.

CONCLUSIONS

Our preliminary results show that the cylodextrin-hydrogel co-polymer conjugate was formed and that it shows different drug release profiles depending on the environmental pH. Also compared to the co-polymer which does not contain cyclodextrin, it leads to an increased drug release which can be due to drug release from the cyclodextrin cavities and increased hydrofilicity of the matrix. Further studies are been carried out to develop these materials with potential application in colon targeted-drug delivery.

ACKNOWLEDGEMENTS

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