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Development of PMMA membranes functionalized with hydroxypropyl-β-cyclodextrins for controlled drug delivery using a supercritical CO_2-assisted technology

M. Temtem, D. Pompeu, G. Jaraquemada, E.J. Cabrita, T. Casimiro*, A. Aguiar-Ricardo*

REQUIMTE/CQFB, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal

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ABSTRACT

Cyclodextrin-containing polymers have proved themselves to be useful for controlled release. Herein we describe the preparation of membranes of poly(methylmethacrylate) (PMMA) containing hydroxypropyl-β-cyclodextrins (HP-β-CDs) using a supercritical CO_2-assisted phase inversion method, for potential application as drug delivery devices. Results are reported on the membrane preparation, physical properties, and drug elution profile of a model drug. The polymeric membranes were obtained with HP-β-CD contents ranging from 0 to 33.4 wt%, by changing the composition of the casting solution, and were further impregnated with ibuprofen using supercritical carbon dioxide ($scCO_2$) in batch mode. The influence of the membrane functionalization in the controlled release of ibuprofen was studied by performing *in vitro* experiments in buffer solution pH at 7.4. The release of the anti-inflammatory drug could be tuned by varying the cyclodextrin content on the membranes.

1. Introduction

In the last decade there has been an intensive research on film polymeric delivery systems with the goal of improving efficacy and control of *in vivo* drug release (Bibby et al., 2000). The main objective of a controlled drug delivery system is to maintain a desirable concentration of drug in tissues and blood as long as possible. This concentration must be above the therapeutic limit and also lower than the upper limit of toxicity. Therefore in order to be effective, the drug release must be between these two limits for an extended period of time, avoiding large fluctuations and reducing the need of several administrations (Duarte et al., 2006). The most common mechanisms by which a drug releases from a polymeric system are dissolution, diffusion and erosion. Each of these mechanisms can be dominant but not exclusive, which makes the release of the drug a complex process to control. Depending on the chemical and physical properties of matrix, the incorporated dose and type of drug, the preparation technique, etc., many phenomena can be involved in the control of release profile such as wetting of the system's surface, water penetration through the device pores, drug and matrix solubility and degradation among many others (Siepmann and Siepmann, 2008). Problems in controlling drug release from polymeric matrixes are common, such as, burst release of the drug, lack of drug solubility in the release medium, drug distribution within the carrier.

The incorporation of cyclodextrins into the polymeric matrixes can influence the mechanism of drug release and overcome this kind of problems. Cyclodextrins and their derivatives have been frequently incorporated into polymeric drug delivery devices with the purpose of controlling drug release (Hirayama and Uekama, 1999). Cyclodextrins are cyclic oligosaccharides able to accommodate drug molecules in their cone-shaped cavity forming inclusion complexes which are stabilised by intermolecular forces such as hydrophobic interactions, van der Waals forces and hydrogen bonding. Inclusion can occur when the cyclodextrin partially or fully entraps a guest compound in its cavity (Junco et al., 2002b). These starch derivatives are useful solubilizers, can increase the bioavailability of solids through an increase in dissolution rate secondary to increasing the apparent solubility of a compound (Brewster and Loftsson, 2007). While inclusion complex formation is certainly the major mechanism associated with the solubilization potential of CDs, effects related to non-inclusion, complexation and supersaturation may be important contributors to solubilization in certain circumstances. Physically entrapped cyclodextrins in polymeric matrixes can modify drug solubility, promote erosion of the matrix or increase hydration. Bibby et al. (2000) published a good review on how cyclodextrins can modify the drug release from these matrixes. Depending on the system, type of cyclodextrin and drug, the incorporation of cyclodextrins can result either

* Corresponding authors. Tel.: +351 212 948 385; fax: +351 212 948 385.
E-mail addresses: teresa.casimiro@dq.fct.unl.pt (T. Casimiro), aar@dq.fct.unl.pt (A. Aguiar-Ricardo).

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in the increase of the release rate or in its decrease, being a complex process and with many variables able to interfere. Moreover, cyclodextrins are known to improve blood compatibility of polymeric biomaterials since they lead to an increase of the material hydrophilicity (Zhao and Courtney, 2006).

Membranes are of major importance in medical applications such as in drug delivery, tissue regeneration, artificial organs, diagnostic devices, coatings for biomedical devices and bioseparation (Stamatialis et al., 2008). Leprête et al. developed polyvinylidene difluoride membranes with fixed cyclodextrins for guided tissue regeneration, for antiseptic delivery for periodontal applications (Leprêtre et al., 2007). Due to the recent increase of environmental and human safety concerns, $scCO_2$-assisted phase inversion is an advantageous method to prepare polymeric membranes compared to conventional ones, where the use of organic solvents may pose a risk to health and environment. In addition solvent contaminations are removed by intensive post-treatment processes since totally solvent free membrane materials are required for medical and pharmaceutical applications (Temtem et al., 2007). Besides its environmental advantages, $scCO_2$ allows the production of completely solvent free polymeric matrixes since it is a gas under ambient conditions, introduces additional parameters (pressure, temperature, depressurization) to control membranes morphology, improves mass transfer (much lower viscosities) than organic liquids, and is easily adjusted with variations in pressure and temperature) and reduces the solvent recovery cost (Temtem et al., 2006). $ScCO_2$ has been applied successfully to the preparation of membranes by phase inversion method such as of nylon (Kho et al., 2001), polystyrene (Matsuyama et al., 2001), cellulose acetate (Reverchon and Cardea, 2004), polysulfone (Reverchon and Cardea, 2005; Temtem et al., 2006), polylactide (Xu et al., 2004, 2005), poly(methyl methacrylate) (Reverchon et al., 2006) and more recently chitosan (Temtem et al., 2009).

Thus $scCO_2$-assisted phase inversion method seems a promising way to prepare polymeric membranes functionalized with cyclodextrins for drug delivery. Polymethylmethacrylate (PMMA) was the chosen polymer since it is non-biodegradable, biocompatible, hydrophobic and highly biostable, thus presenting a great variety of applications in biomedicine such as in dentures, cranioplasties, orthopaedic prostheses, etc. Temporary antibiotic loaded-PMMA spacers are commonly used for the treatment of bacterial infection, the major complication that can occur in total hip replacement (Minelli et al., 2004). PMMA is also used as part of polyphasic materials used in bone filling implants (Ladrón de Guevara-Fernández et al., 2003). The controlled release of an anti-inflammatory drug from the implanted polymeric materials is an alternative to attain an effective drug concentration to fight the inflammatory response that often occur after the implantation. In addition PMMA as the majority of polymers is able to absorb CO_2 and swell in $scCO_2$ (Rajendran et al., 2005) hence it can be easily impregnated with a drug in $scCO_2$.

Supercritical fluid technology has also shown to be a greener alternative to form drug-cyclodextrin inclusion complexes (Hees et al., 2002). Natural cyclodextrins and some substituted ones such as HP-β-CDs are insoluble in $scCO_2$ (Junco et al., 2002a), although some derivatives have shown high miscibility with dense CO_2 (Potluri et al., 2002). We have recently undertaken a fundamental high-pressure NMR study on the molecular structure and dynamics of an acetylated-β-CD derivative in $scCO_2$, in order to test the feasibility of preparing inclusion complexes for controlled drug release in $scCO_2$ (Ivanova et al., 2009).

In this work, we developed highly structured and porous PMMA membranes functionalized with HP-β-CDs, using a $scCO_2$-assisted phase inversion method. The polymeric membranes were further impregnated with ibuprofen using $scCO_2$ in batch mode. Ibuprofen is a very common non-steroidal anti-inflammatory drug (NSAID), and known to form efficient inclusion complexes with HP-β-CDs (Tozura et al., 2006; Mammucari and Foster, 2008). As it is very soluble in $scCO_2$ compared to other drugs (Suleiman et al., 2005) it is suitable to be impregnated into a polymer using supercritical fluid technology without the need of co-solvents. Due to its therapeutic effect and its $scCO_2$ solubility, ibuprofen is also being used as a model drug since the process can be applied to other drugs soluble in $scCO_2$. The membranes were tested as controlled drug delivery devices by *in vitro* tests into buffer solution at pH 7.4, and the drug release data was modeled using a mathematical theory based on Fick's second law of diffusion.

2. Materials and methods

2.1. Materials

Poly(methyl methacrylate) (PMMA) (molecular weight 120,000), acetone (purity ≥99.5%) were purchased from Sigma–Aldrich. Hydroxypropyl-β-cyclodextrin (HP-β-CD) was obtained from Janssen Biotech with average molar substitution of 0.4. Methanol-d4 and water-d2 were purchased from Fluka and acetonitrile from Scharlau Chemie (both purity ≥99.8%). Carbon dioxide was obtained from Air Liquide with purity better than 99.998%. All materials were used as received without further purification.

2.2. Membrane preparation

Membranes were produced as already described in detail elsewhere. A recently developed 200 mL stainless steel cell with sapphire windows was used (Temtem et al., 2008). In a typical experiment the casting solution is prepared by dissolving 30% (w/w) of PMMA in acetone. Different contents of HP-β-CD, up to 9.2 wt%, are added to the casting solution. This solution is then loaded into a stainless steel cap (68 mm diameter and 1 mm height) and placed inside the high-pressure vessel. The cell is then immersed in a visual water bath thermostatted by means of a temperature controller (Hart Scientific, Model 2200) within ±0.01 °C. The CO_2 is added up to the desired pressure using a Gilson 305 piston pump. After reaching the desired experimental conditions, the process starts running in continuous. The supercritical solution passes through a back pressure regulator (Jasco 880-81) which separates the CO_2 from the casting solvent. The pressure inside the system is monitored with a pressure transducer (Setra Systems Inc., Model 204) with a precision of ±100 Pa.

All the experiments were performed at 20 MPa and 40 °C, with a CO_2 flow of 9.8 g/min for 2 h. At the end the system is slowly depressurized during 30 min and a thin homogeneous membrane is obtained, shown in Fig. 1.

2.3. Impregnation experiments

The membranes produced were impregnated with ibuprofen using the same apparatus as for membrane production. The cell has a porous support which divides it in two compartments. Ibuprofen was placed under the porous support with a magnetic stirrer bar, in quantity enough to obtain a saturated solution at the *p*, *T* impregnation conditions (Suleiman et al., 2005). The membrane is placed over the support. The cell is then closed and immersed in the thermostatted water bath at 40 °C. CO_2 is loaded at the desired pressure of 20 MPa. The impregnation is undertaken in a batch mode for 4 h. At the end, the cell is rapidly depressurized within approximately 1 min. Impregnated ibuprofen was calculated gravimetrically by weighting the membranes in a Sartorious balance (precision ±0.00001 g) prior and after the impregnation.

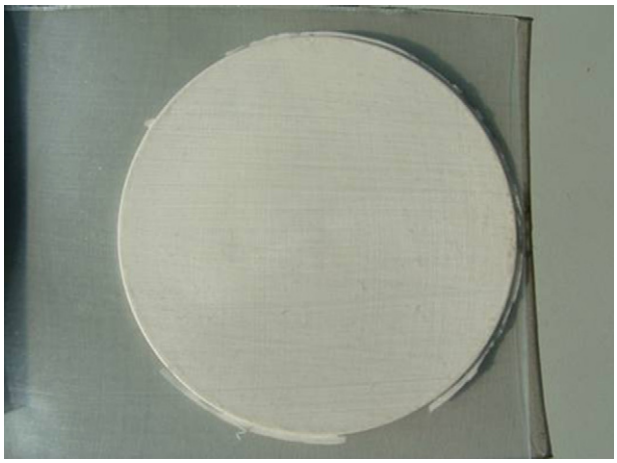

Fig. 1. Appearance of a PMMA membrane prepared with the CO_2-assisted phase inversion method.

The cyclodextrin content as well as the impregnated ibuprofen in the final membranes was confirmed by ^{1}H NMR spectroscopy using a Bruker Avance II 400 equipment (400.15 MHz). ^{1}H NMR spectra were acquired with 256 scans over a 8000 Hz spectral window with a 30° flip angle excitation pulse, the time between each repetition was set to 10 s to allow full relaxation of the proton magnetization. Solutions of the impregnated membranes were prepared in deuterated methanol using acetonitrile (4.92×10^{-7} M) as internal standard at 298 K. The quantification was done by integration of the peaks corresponding to the protons of the methyl group of acetonitrile (singlet at δ 2.04), the hydroxypropyl groups of HP-β-CD (multiplet at δ 1.14) and the methyl groups of the ibuprofen isopropyl moiety (doublet at δ 0.90). The peak corresponding to HP-β-CD has the contribution of an average of 8.4 protons (0.4 of degree of substitution).

2.4. Membranes characterization

The surfaces and cross-sections of the membranes were characterized using scanning electron microscopy (SEM) in a Hitachi S-2400 equipment, with an accelerating voltage set to 15 kV. For cross-section analysis the membrane samples were frozen in liquid nitrogen and fractured. Samples were mounted on aluminium stubs using carbon tape and were gold coated.

Pore size diameters and porosity were obtained by image analysis using SigmaScan Pro (Systat Software Inc.). EasyFit (MathWave Technologies) was used to fit statistical distributions to experimental data (Weibull, Lognormal) and to perform the Kolmogorov–Smirnov (Chakravarti et al., 1967) and Anderson–Darling (Stephens, 1974) tests to select the more adequate distribution function to each system.

Differential scanning calorimetry (DSC) measurements of the produced membranes were carried out at the REQUIMTE associated laboratory using a Setaram (Model DSC 131) equipment. The analyses were performed from 0 to 350 °C at 5 °C/min under dry nitrogen atmosphere.

2.5. In vitro controlled drug release

Small square pieces of each membrane (200 mg) were placed inside 200 mL phosphate buffer solutions (pH 7.4). Solutions were kept at 37 °C for 40 days, at sink conditions (Levis et al., 2003). 500 μL aliquots were withdrawn periodically from the solutions and collected in eppendorfs. In each sampling procedure the same volume of fresh buffer solution was added to the solution. Quantification of the released drug takes into account the dilution of the solution by adding the fresh buffer solution. Ibuprofen was quantified by UV spectroscopy at the maximum absorbance around 220 nm.

2.6. Modelling of ibuprofen release

The release of ibuprofen was modeled using a mathematical model based on Fick's second law of diffusion.

The diffusion of a drug from a thin film with negligible edge effects (high surface area:thickness ratio) is given by Eq. (1) (Siepmann and Siepmann, 2008):

$$\frac{M_t}{M_\infty} = 1 - \frac{8}{\pi^2}\sum_{n=0}^{\infty}\frac{1}{(2n+1)^2}\exp\left(\frac{-D(2n+1)^2\pi^2 t}{L^2}\right) \quad (1)$$

where M_t is the drug release at time t, M_∞ is the drug release at equilibrium and M_t/M_∞ is the fraction of drug released at time t. D is the diffusion coefficient and L the half-thickness of the film. In order to avoid the use of infinitive series of exponential functions, an approximation for this equation, described in Eq. (2), (Ritger and Peppas, 1987) can be used for $M_t/M_\infty \leq 0.6$:

$$\frac{M_t}{M_\infty} = 4\left(\frac{Dt}{\pi L^2}\right)^{0.5} \quad (2)$$

No significant porosity and swelling changes in the matrix are considered during drug release, drug release is primarily controlled by diffusion through the carrier matrix and perfect sink conditions are maintained over the release period.

3. Results and discussion

Membranes were produced with different HP-β-CD contents according to Table 1. Fig. 2 shows SEM images of the surface and cross-section of a PMMA membrane. All membranes show analogous highly uniform porous structures. Pore size distributions of the membranes produced are shown in Fig. 3. Similar pore size distributions were obtained, nevertheless an increase in the mean pore size diameter was observed, from around 14.9 to 18.4 μm, while increasing the content of HP-β-CDs in the casting solution up to 9.2 wt%. This increase could be explained by the interference of the cyclodextrin in the nucleation process of the polymer during the membrane formation as was also observed by other authors (Fontananova et al., 2003). There is an obvious compositional dependence of PMMA's T_g with the cyclodextrin content in the membrane as it is shown in Table 1, which indicates that there is a good miscibility of PMMA with the cyclodextrin. T_g increases with increasing cyclodextrin content meaning that there is an increase in the hardness of the membranes.

The morphology of the membranes is not altered during the impregnation process as it can be seen from SEM images of the membranes before and after impregnation, presented in Fig. 4. Just

Table 1
HP-β-CD content in the casting solution and in the produced membranes and corresponding glass transition temperatures of PMMA.

	wt% HP-β-CD, casting solution[a]	wt% HP-β-CD[b]	T_g/°C
Entry 1	0.0	0.0	102.07
Entry 2	1.5	5.1	102.73
Entry 3	2.6	8.7	111.72
Entry 4	4.0	13.7	114.21
Entry 5	9.2	33.4	[c]

[a] Weight of HP-β-CD/total weight (solvent + PMMA + HP-β-CD). Ratio solvent/PMMA kept constant.

[b] Weight percentage of HP-β-CD with respect to PMMA.

[c] Not possible to measure due to a more pronounced dehydration peak of HP-β-CD compared to the other membranes. T_g of PMMA used in the casting solution: 102.48 °C.

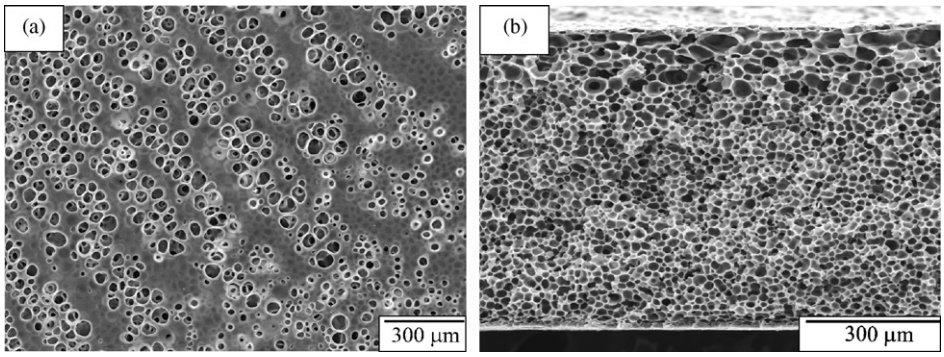

Fig. 2. Scanning electron micrographs of a PMMA membrane produced: (a) surface top view; (b) cross-section.

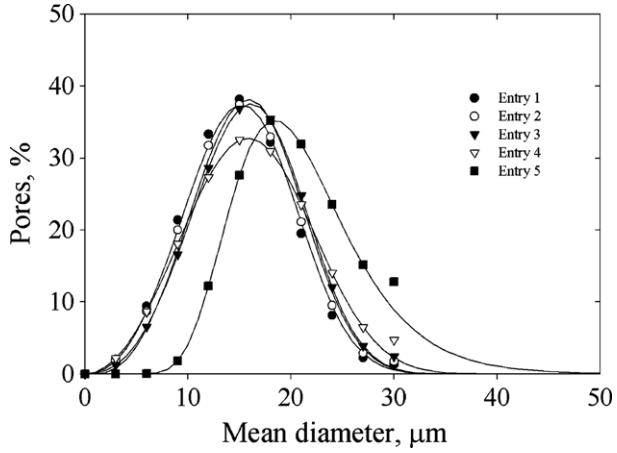

Fig. 3. Pore size distribution of PMMA membranes produced using different amounts of HP-β-CDs. Entries correspond to Table 1.

a slight pore elongation is observed, which could be a result of the plasticization and swelling of the polymeric matrix followed by rapid decompression at the end of impregnation, as it was also reported elsewhere for fast depressurizations from polysulfone membranes (Temtem et al., 2006). The percentage of impregnated ibuprofen was analogous for all membranes, around 3 wt%, therefore the cyclodextrin content does not seem to influence the impregnation step. This can be explained by the high diffusivity of $scCO_2$ into the polymer matrix including the cyclodextrin cavities. $ScCO_2$ has already shown to be very effective in impregnating drugs into polymers (Kazarian and Martirosyan, 2002) and a good medium to include ibuprofen into cyclodextrins (Mammucari and Foster, 2008). Our ibuprofen impregnation results, 30 mg/g PMMA, are in the same range of other impregnations reported in literature. Kazarian and Martirosyan (2002) reported the impregnation of ibuprofen into poly(vinylpyrrolidone) (PVP) films, achieving a higher drug uptake of 100–300 mg/g polymer (10–30 wt%). PVP and PMMA show similar CO_2 absorption about 0.20 g CO_2/g polymer. Also Diankov et al. (2007) reported the impregnation of another drug hydroxybenzoic acid, slightly less soluble in $scCO_2$ ($\sim 10^{-4}$) then ibuprofen ($\sim 10^{-3}$), into PMMA beads in $scCO_2$ attaining drug uptakes of 20–27 mg/g PMMA. The impregnation efficiency is the result of the solute's solubility in $scCO_2$ and its partition between the fluid and the polymer phases and depends on the polymer morphology and its swelling behaviour. $ScCO_2$ impregnation also enables the tuning of the drug uptake also by changing the time of the impregnation step.

In order to study the influence of the PMMA membrane functionalization with the cyclodextrins, the release profile of the ibuprofen from the films was investigated towards the content of cyclodextrin. Fig. 5 shows *in vitro* release rates of the ibuprofen from the membrane samples over time, into a 7.4 pH phosphate buffer solution at 37 °C. For all the membranes studied no burst release was observed. The initial slopes decrease rapidly and then monotonically which clearly indicates that the membranes are able to release the ibuprofen in a very controlled way including the one that does not contain cyclodextrin. The membrane with the highest content of cyclodextrin reached about 12 wt% of drug release in 40 days, six times more than the one with the lowest content, corresponding to 3.6×10^{-3} mg of ibuprofen released/mg of membrane compared to 6.7×10^{-4} mg ibuprofen/mg membrane. Fig. 5 also shows the fitting of Eq. (2) to the experimental drug release data, which follows the experimental trend and agree reasonably. As it can be seen the release rates increase with increasing HP-β-CD content. Table 2 shows the diffusion coefficients that were optimized to fit the experimental data.

Comparing the release profiles of the membranes with and without cyclodextrin we can see that the increase in drug delivery is directly related with the increase of CD content. However, all membranes were impregnated with the same percentage of ibuprofen

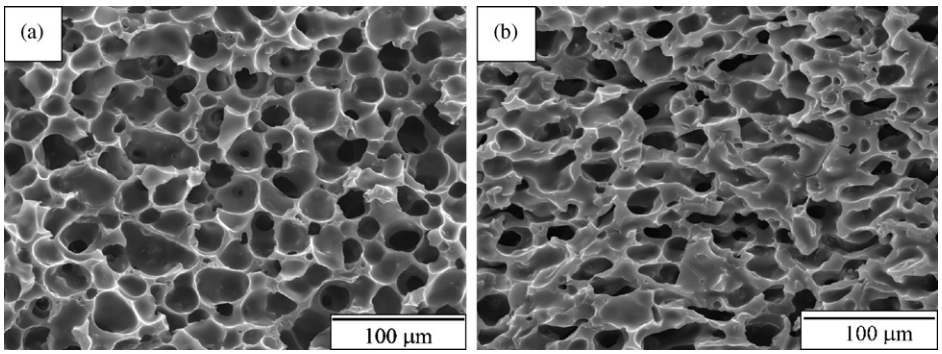

Fig. 4. Scanning electron micrographs of PMMA membranes produced with 9.2 wt% of HP-β-CDs in the casting solution: (a) before; (b) after impregnation.

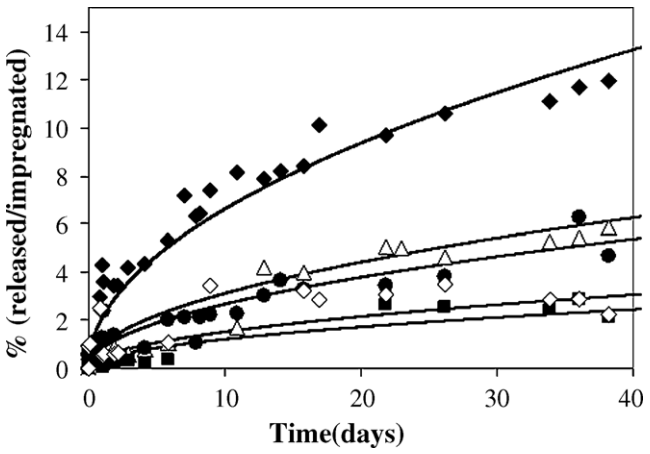

Fig. 5. Ibuprofen release profile from the different membranes into buffer solution pH 7.4: (♦) 33.4 wt% of HP-β-CD; (△) 13.7 wt%; (●) 8.7 wt%; (■) 5.1 wt%; (◇) 0 wt%.

(∼3 wt%) which means that there are no preferable impregnation/inclusion of drug in the CD cavities due to the increase of cyclodextrin content. As it was mentioned above the entire matrix is likely to be uniformly impregnated due the high diffusivity of CO_2 within the matrix and consequent swelling of the PMMA.

On the other hand when the membranes are put into the buffer solution no swelling or degradability of the PMMA membranes is observed as it was expected since this polymer is hydrophobic and highly biostable, as it was pointed out in Section 1.

The release of the drug from the membrane without cyclodextrins is mainly due to the high porosity of the matrix as observed in the SEM images, which allow the wetting within the matrix promoting the dissolution and diffusion of the drug from the membrane.

By adding cyclodextrins there is a significant increase in the drug release, which can be tuned by varying the cylodextrin content. We believe that this might have two main contributions: (i) the ability of the cyclodextrin to act as a hydrating agent, due to their hydroxyl groups, promoting diffusion of water into the matrix. HP-β-CDs are able to form hydrogen bonding, and thus interfere in the conformation of the polymeric chains during the membrane formation, turning the matrix less hydrophobic. The presence of the cyclodextrin restrains the degrees of freedom in the organization/conformation of the polymeric chains, and a less random organization is obtained, which also explains the increment of T_g temperature while increasing cyclodextrin concentration; (ii) the release of ibuprofen from the cyclodextrin cavities, which are more accessible to the release medium than the PMMA itself.

The ^{1}H NMR studies confirmed that no leaching of the HP-β-CD occurred upon the process of membrane formation nor during impregnation as it was expected since this cyclodextrin derivative is completely insoluble in $scCO_2$. NMR results are in agreement with the expected cyclodextrin content in the membrane and the impregnated ibuprofen mass determined gravimetrically (deviation lower than 5% and 11%, respectively).

Table 2
Optimized diffusion coefficients to adjust the mathematical model to drug release data.

wt% HP-β-CD[a]	D/m^2 s^{-1}
0.0	6.01×10^{-18}
5.1	2.88×10^{-18}
8.7	1.10×10^{-17}
13.7	2.08×10^{-17}
33.4	1.30×10^{-16}

[a] Weight percentage of HP-β-CD with respect to PMMA in the membranes.

However, as HP-β-CDs are hydrophilic one could expect that they would be also released to the medium alone or by forming ibuprofen–(HP-β-CD) inclusion complexes. ^{1}H NMR experiments of drug release from the membranes were performed in deuterated water at 37 °C. Although cyclodextrins are soluble in water, ^{1}H NMR spectra do not show any traces of cyclodextrin even after several weeks, which confirm that no cyclodextrin leaching from the membranes occurs. This means that the cyclodextrin is entrapped physically within the matrix.

Our results demonstrate that the drug release from the membrane is increased by incorporating cyclodextrins and that it can be further tuned by varying the cyclodextrin content, which could have potential applications as implant materials and as drug release devices.

4. Conclusions

By varying the content of HP-β-CDs in the casting solution during the $scCO_2$-assisted membrane production process, it is possible to obtain PMMA membranes functionalized with different cyclodextrin contents. The introduction of cyclodextrins physically entrapped in the PMMA matrix, leads to an increase of drug release to the buffer solution at pH 7.4 when compared to the neat PMMA membrane. Also by controlling the amount of cyclodextrin entrapped in the membrane, the ibuprofen release from the membranes can be effectively tuned. Therefore we demonstrate that the supercritical fluid-assisted membrane formation is a greener alternative to produce porous polymeric matrixes containing cyclodextrins, with improved characteristics such as enhanced drug loading, controlled drug release ability, which might have promising biomedical applications.

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