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Research paper

Competitive displacement of drugs from cyclodextrin inclusion complex by polypseudorotaxane formation with poloxamer: Implications in drug solubilization and delivery

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ABSTRACT

The competitive interactions between the poly-[propylene oxide] (POO)–poly-[ethylene oxide] (PEO) block copolymer poloxamer 407 (Pluronic F127) and two drugs, triamcinolone acetonide and ciclopirox olamine, by the formation of inclusion complexes with two cyclodextrin hydrophilic derivatives, hydroxypropyl-β-cyclodextrin (HPβCD; molar substitution (MS) 0.65) and partially methylated-β-cyclodextrin (MβCD; MS 0.57), were studied by means of one-dimensional ^{1}H NMR, 2D ROESY experiments, solubility studies and drug release studies. 1D and 2D NMR and solubility studies indicate that both triamcinolone acetonide and ciclopirox olamine form stable inclusion complexes with the cyclodextrin derivatives. In the case of ciclopirox olamine the complex was more stable at pH 1. Effective complexation of poloxamer with the two cyclodextrins (CDs) was also evidenced by NMR analysis, and competitive displacement of the drugs from the CD cavity by the polymer was observed. Drug solubility in CD solutions was not modified by the addition of polymers, indicating that a decrease in solubility due to the competitive displacement is probably compensated by the solubilizing effect of polymer micellization. Finally, polypseudorotaxanes formation has a significant influence on the release of the drugs studied. Changes in the release rate depend on the stability of drug–CD inclusion complex and on cyclodextrin concentration in the bulk solution; so polypseudorotaxane formation can be employed to modulate drug controlled release from thermosensitive hydrogels.

1. Introduction

The relevance of in situ-forming systems was increasing in recent years due to their effective control of drug release, easy administration and biocompatibility. These systems can be obtained by means of different mechanisms, but some of them display several drawbacks; i.e. the application of organic solvents, copolymeric agents or photoinitiators can be toxic at the gelation site [1]. Thermoresponsive systems do not require any reactant since they exhibit sol–gel transition due to hydrophobic interactions (van der Waals forces), which promote aggregation of unimers [1,2]. This phenomenon is named 'hydrophobic effect', and it is fundamentally driven by entropic forces. Temperature-sensitive hydrogels can be composed of different materials, from natural to synthetic polymers, including the amphiphilic block copolymers, such as poloxamers.

Poloxamers are synthetic triblock copolymers, formed by one poly-[propylene oxide] (POO) central block and two lateral blocks of poly-[ethylene oxide] (PEO), whose chemical formula is fitted to $HO[CH_2–CH_2O]_x\,[CH(CH_3)–CH_2O]_y\,[CH_2–CH_2]_xOH$. In drug delivery, poloxamers have received special attention because of their ability to form the so-called polymeric micelles [3] and because in aqueous solutions some varieties of Poloxamer show thermoreversible properties, which are of great interest in drug formulation for their use in different routes of administration [4].

Poloxamers whose PEO chain length exceeds the PPO chain one can form micelles in aqueous solution consisting of a core formed by the hydrophobic blocks and the shell region consisting of the hydrophilic blocks. The poloxamer micellization process has been widely studied [5,6]. Different factors can affect the micellization

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process such as salts or additives [7–9], temperature [10] or composition and molecular weight of copolymers [5,7,11].

Poloxamer 407 (Pluronic F127®, PF127), whose chemical formula is $(EO)_{95-105}$–$(PO)_{54-60}$–$(EO)_{95-105}$, forms in situ-forming systems whose suitable properties were widely studied. PF127 hydrogels show thermoreversible properties characterized by a sol–gel transition temperature, with appropriate gel strength and good adhesive and rheological properties [4,12]. Studies showed that PF127 formulations have enhanced solubilization of poorly water-soluble molecules, stabilization of drugs and prolonged release profile in many pharmaceutical formulations used in different administration routes (e.g. oral, rectal, topical, ophthalmic, nasal and injectable preparations) [4,13,14].

The thermoreversible properties of the PF127 aqueous solution show a great potential in optimizing drug delivery systems and for the past few years a large number of articles and patents related to the application of PF127 thermoreversible hydrogels has been published. Nevertheless, solubilization capacity of PF127 micelles can be not as effective for some poorly water-soluble drugs due to limited inclusion in this thermoreversible system. In this case, a possible approach is the combination of micelle solubilization capability of PF127 with other mechanisms, such as cyclodextrin complexation, which can lead to a synergistic effect. This approach has been used for different researchers to increase the dissolved drug concentration in drug delivery systems employed in ophthalmic, vaginal, nasal, rectal and topical administration [15–20].

Drug–cyclodextrin interaction is unspecific, so the presence of other molecules in the bulk (which may compete to host the cyclodextrin cavity) must be taken into account [21]. As shown in previous studies [22], interactions between PF127 and two hydrophilic derivates of β-cyclodextrins, i.e. hydroxypropyl-β-cyclodextrin (HPβCD) and methylated-β-cyclodextrin (MβCD), are sufficiently relevant to be considered. PF127-cyclodextrin interaction gives rise to a molecular complex called polypseudorotaxanes as a result of threading of the polymer through the ring cavity. These structures have been widely studied by different groups [23–25] and are very promising for a wide range of biomedical applications [26,27]. Nevertheless, formation of soluble polypseudorotaxanes has been less well studied and it is necessary to assess the consequences of their formation in the pharmaceutical properties of systems containing both CDs and Poloxamer. As demonstrated previously [22], soluble PF127-cyclodextrin polypseudorotaxane formation affects significantly the properties of solutions, being remarkably altered the PF127 micellization and sol–gel transition of the PF127 aqueous solution. The study of the methyl orange dye as a marker to reveal molecular competition showed the ability of PF127 to displace host molecules from the CD cavity. Therefore, cyclodextrin–poloxamer interactions may hamper drug solubilization, mainly driven by two molecular processes: drug molecules are displaced from CD cavity by poloxamer, reducing drug–CD complexation efficiency; and poloxamer threads through around 20–22 CDs, which overlap poloxamer hydrophobic blocks, hindering micellization and then decreasing the number of available micelles to incorporate drug molecules in their core. Based on these considerations, in this work we studied the effect of the competitive CD complexation between drugs and PF127 over drug solubilization and release. As drug models we chose an antifungal drug (olamine ciclopirox: CPO) and an anti-inflammatory steroidal drug (acetonide triamcinolone: TA). Both drugs are widely used in the treatment of topical (skin and nail diseases), ocular, vaginal and rectal pathologies, therefore these drugs formulations using reversible thermosensitive hydrogels may have a great potential to enhance their efficacy using these routes.

Olamine ciclopirox (6-cyclohexyl-1-hydroxyl-4-methyl-2(1*H*)-pyridine) is a broad-spectrum antifungal agent used in dermatological treatments. It is a weak acid (p*K*a = 7.2, log*P* = 2.03), therefore its solubility decreases in acidic medium since the unionized form is predominant; it is expected that it interacts with CDs weakly in neutral-basic medium and strongly in acidic medium. So the pH of the formulation must be borne in mind when administration routes such as the vaginal is considered or when the combination of several excipients can modify the pH of the formulation, such as in the case of some nail penetration enhancers (e.g. N-acetyl-N-cysteine) [28].

On the other hand, acetonide triamcinolone is a potent, highly lipophilic (log*P* = 2.5) steroidal anti-inflammatory drug, whose stable inclusion complexes with different CDs have been described [29–32].

In this paper, one-dimensional ^{1}H NMR and Rotating-frame Overhauser Effect Spectroscopy (ROESY) were used to analyse different drug:MβCD:polymer ratios in order to characterize the nature of the interactions and study the competitive complexation and the polypseudorotaxane formation. Furthermore, affinity constants of drug–CD complexes were determined and then drug solubility and release were studied for different solutions of drug:MβCD, drug:MβCD:polymer mixtures.

2. Experimental section

2.1. Materials

Ciclopirox olamine (CPO) was from Fagron Iberica (Spain). Triamcinolone acetonide (TA) was from Roig Farma (Terrassa, Spain). Pluronic F-127 (PF127, poloxamer 407) was from Sigma–Aldrich (Madrid, Spain). Hydroxypropyl-β-cyclodextrin (HPβCD with a molar substitution of 0.65 and Mw 1399 Da, Kleptose HPB) and methylated-β-cyclodextrin (MβCD with a molar substitution of 0.57 and Mw 1191 Da, Kleptose Crysmeb) were from Roquette Laisa (Barcelona, Spain). Potassium dihydrogen phosphate and sodium chloride were purchased from Panreac Química S.A. (Barcelona, Spain); dodecahydrate sodium dihydrogen phosphate and potassium chloride from Merck (Germany).

Ultrapure water was obtained by reverse osmosis (MilliQ, Millipore, Madrid, Spain).

2.2. NMR spectroscopy

One-dimensional ^{1}H NMR spectra were recorded at 300 K on a Bruker Avance DRX operating at a frequency of 300.13 MHz in unbuffered D_2O solutions. Acquisition parameters consisted of a spectral width of 3000 Hz, an acquisition time of 2.67 s and a relaxation delay of 1 s. The resonance at 4.7 ppm due to residual solvent (HOD) was used as an internal reference.

Rotating-frame Overhauser Effect Spectroscopy (ROESY) spectra were acquired in the phase sensitive mode using the same spectrometer and Bruker standard parameters (pulse program roesygpph19). Each spectrum consisted of a matrix of 16 K (F2) by 8 K (F1) points covering a spectral width of 3000 Hz. Spectra were obtained from the samples solutions prepared for the 1H NMR studies, using a spin-lock mixing time of 400 ms, relaxation delay 2 s and 32 scans were recorded.

2.3. Solubility determination

Solutions of different mixtures and concentrations of PF127, HPβCD and MβCD were prepared, 2 ml of the solution placed into test tubes and drug was added until saturation; then, the tubes were perfectly sealed and immersed in an orbital shaking bath Unitronic 320 OR (P-Selecta) at 301 K and 70 RPM for 7 days. After this time, saturated solutions were filtered through cellulose nitrate filters (Ø 13 mm, 0.45 μm) in order to eliminate drug in sus-

pension. Afterwards, clear solutions were diluted to determine absorbance using a spectrophotometer Diode Array (Hewlett Packard 8452A) at $\lambda = 308$ nm for CPO detection and at $\lambda = 254$ nm in the case of TA.

Solubilization capability of formulations and factors that modify drug solubility were evaluated.

2.4. Determination of the stability constant

Solubility tests were performed to determine stability constants of CPO–CD complexes. Different concentrations of CDs solutions were prepared, placing 2 ml of the solutions into sealed test tubes and adding drug until saturation. They were immersed into an orbital shaking bath Unitronic 320 OR (P-Selecta), at 301 K and 70 RPM, for 7 days in order to reach the equilibrium. Solutions were filtered (0.45 μm) and diluted to quantify their absorbance using a spectrophotometer Diode Array (Hewlett Packard 8452A) at $\lambda = 308$ nm.

The stability constant of the drug/cyclodextrin complex $K_{1:1}$ was calculated considering the 1:1 stoichiometry of drug/cyclodextrin complexes according to the phase-solubility method of Higuchi and Connors [33].

However, it has shown that cyclodextrins, as well as non-cyclic oligosaccharides, are able to form soluble non-inclusion complex aggregates, which can lead to erroneous determination of the stability constant [34]. The phase-solubility method hardly provides accurate values of stability constants since it is sensitive to formation of non-inclusion complexes, having significant differences between experimental and intercept drug solubility values. An independent parameter of non-inclusion complexes is the complexation efficiency (CE), i.e. the concentration ratio between cyclodextrin in a complex and free cyclodextrin that is not dependent on the intrinsic solubility (S_0) but exclusively on the value of the slope of the phase-solubility profile [35]. In addition, the D:CD ratio can be calculated by the CE.

2.5. Drug release studies

Experiments were carried out under stirring and temperature conditions (37 °C) using vertical Franz diffusion cells, whose receptor chamber had a total volume of 5.5 ml and available diffusion surface of 79 mm^2. Firstly, formulations containing PF127 and/or HPβCD or MβCD were prepared at different concentrations. Drug was added to each formulation until saturation and then stirred for 24 h. After this time, solutions were filtered (0.45 μm) to remove undissolved drug and placed on the donor chamber of the Franz cell (0.5 ml in the case of ciclopirox, and 2 ml for triamcinolone). Phosphate saline buffered solution (pH = 7.4) was placed into the receptor chamber, under stirring and temperature conditions ($T^a = 37$ °C) and separated from the donor chamber by a MWCO ⩾ 12,000 Da dialysis membrane. Samples were taken at scheduled times from the receptor chamber with a syringe, filtered through cellulose nitrate filters (Ø 13 mm, 0.45 μm), diluted and absorbance measured using a spectrophotometer Diode Array (Hewlett Packard 8452A) at $\lambda = 308$ nm (CPO) and at $\lambda = 254$ nm (TA).

Fick's second law was employed to estimate diffusion drug fluxes and drug diffusivity, calculated from the equation:

$$\frac{dM}{dt} = \frac{D \cdot C_0}{h} \tag{1}$$

where M is the cumulative mass of drug release per unit area; D is the drug diffusivity; C_0 is the drug concentration in the donor compartment; h is the membrane thickness and t is the time. The assumptions are that the drug is the only component diffusing out from the vehicle, sink conditions are maintained in the receptor phase and D is constant with respect to time and position in the vehicle. Only in the case of TA aqueous saturated solution due to their low aqueous solubility and PF127/MβCD solution (the final drawn sample) had little tendency to achieve sink conditions for all release assays.

The steady state flux (J) was calculated from slopes of straight lines of cumulative mass per unit area (M) versus time (t) profiles. Diffusivity coefficients were calculated from Eq. (1). Apparent permeability coefficient (P_{app}) of drugs can be obtained from D values by means of the following equation:

$$J = P_{app} \cdot C_0 \tag{2}$$

Nevertheless, according to Masson et al. [36], in the presence of CDs the drug permeability constant is not only dependent on the diffusivity of the drug but also on the diffusivity of the CD–drug inclusion complex. In these conditions, partition and diffusion coefficients cannot be directly determined in simple permeation studies and the author proposed a simplified equation to evaluate the influence of CDs on drug permeation:

$$J = \frac{(P'_M/K_{1:1}[D/CD]_d}{M_{1/2} + [CD]_d} \tag{3}$$

where P_M is the drug permeability from cyclodextrin solutions, $M_{1/2}$ the CD concentration in saturated drug solution where the flux is half of maximum flux and $[CD]_d$, $[D/CD]_d$ the cyclodextrin and complex concentration in the donor phase, respectively.

Fitting of flux data to the Eq. (3) was carried out using Solver add-in of Microsoft Excel 2010, employing the generalized reduced gradient (GRG) nonlinear algorithm to minimize the difference between experimental flux and calculated one by means of Eq. (3).

2.6. Statistical analysis

Statistical comparison of stability constants ($K_{1:1}$) of 1:1 cyclodextrin:drug inclusion complexes was made using the Kruskal–Wallis non-parametric statistical test using Dunn's Multiple Comparison Test as post hoc test.

A factorial analysis of the variance was applied (Statgraphics plus 5.1, Statistical Graphic Co. 2000) in order to evaluate the influence of the type of CD (A), CD concentration (B), PF127 concentration (C) and pH media (D) on CPO and TA solubility. ANOVA was carried out after checking normality and homogeneity of the variance.

To evaluate the effect of the type of CD (A), CD concentration (B), PF127 concentration (C) and pH media (D) on CPO aqueous solubility a $2 \times 2 \times 3 \times 2$ factorial design was employed: type of CD derivative (A) coded as (−1) for MβCD and (+1) for HPβCD, CD concentration (B) coded as (1 0) for 0% CD, (0 1) for 5% CD, and (−1 −1) for 10% CD, PF127 concentration (C) coded as (−1) for 0% and (+1) for 10%, respectively, and pH media coded as (−1) for pH = 1 and (+1) for pH = 7. A factorial analysis of the variance was applied after checking normality and homogeneity of the variance. The response surface equation that quantifies the effects of these variables on CPO solubility was obtained by regression using Design Expert software (v. 6.06, Stat-Ease Inc., Minneapolis, 2002). A stepwise regression with backward elimination was used; i.e. the four factors and their interactions terms were initially considered, and then those which provided a significant level above 0.05 were discarded. In the case of the TA, a $2 \times 3 \times 3$ factorial design was used: type of CD (A) coded as (−1) for MβCD and (+1) for HPβCD, CD concentration (B) coded as (1 0) for 0% of CD, (0 1) for 5% of CD, and (−1 −1) for 10% of CD and PF127 concentration (C) coded as (1 0) for 0%, (0 1) for 10% and (−1 −1) for 15%, respectively.

3. Results and discussion

3.1. NMR studies

Formation of polypseudorotaxanes between PF127 and HPβCD or MβCD has been previously studied using one-dimensional ^{1}H NMR spectroscopy and molecular modelling [22]. Results showed that CDs are able to include in their cavity the PF127 molecules that orientate the – CH_3 of PO to the outside of the PPO chain interacting with the cyclodextrin cavity. PF127 unimers must simultaneously interact with 20–30 CD molecules forming soluble polypseudorotaxanes that stack forming nanorods. Formation of polypseudorotaxane must induce important changes in the properties of the CDs and PF127 that should be taken into account when developing drug delivery systems based in these systems. One of the important properties that must be modified is the solubilization capability of CDs and polymeric micelles. With this aim we propose to study the competition between drug and PF127 for complexation with MβCD using one-dimensional ^{1}H NMR and 2D ROESY experiments.

We start our study evaluating the interactions between the two drugs, TA and CPO, and MβCD. Signals to the aliphatic and aromatic protons of the both drugs TA and CPO (Fig. 1) have been assigned by reports in the bibliography; however, the solubility of TA in deuterated oxide (D_2O) is very low and, therefore, its signals in the ^{1}D spectra were identified when the complex between MβCD and this compound was carried out under experimental conditions (301 K, shaking at 70 RPM for 7 days). ^{1}H NMR spectra of ciclopirox olamine, in the absence of MβCD, exhibited two groups of multiplet at δ 1.22 and 1.72 ppm, each integrating for four and five methylene protons of the cyclohexane ring attached at position 6 of the pyridinone ring. The signal at δ 2.05 ppm, integrating to three protons as singlet, was assigned to the methyl group at position 4 of the heterocyclic ring. Moreover, the singlet signals corresponding to two aromatic protons at positions 3 and 5, these protons resonate at δ 6.16 and 6.21 ppm, respectively. This is due to the deshielding effect of the near carbonyl group at position 2 of the pyridinone ring, which makes the aromatic proton appear to downfield shift. To investigate the inclusion of TA inside the MβCD in the concentrations mentioned above, resonance assignments were carried out. The signal of four methyl groups and three aromatic protons stand out as part of the chemical framework making it likely that they are included inside the MβCD. Finally the resonance assignment of MβCD protons (Fig. 1) was made on the basis of their specific shapes, ^{1}H NMR and 2D ROESY spectral data.

Fig. 2 shows the ^{1}H NMR spectra of MβCD, CPO and the mixture of both in D_2O and the mixture of MβCD:triamcinolone in 10:1 ratio (300 MHz). In both cases the most influenced protons of MβCD are H-3′ and H-5′ and show the more significant upfield shift

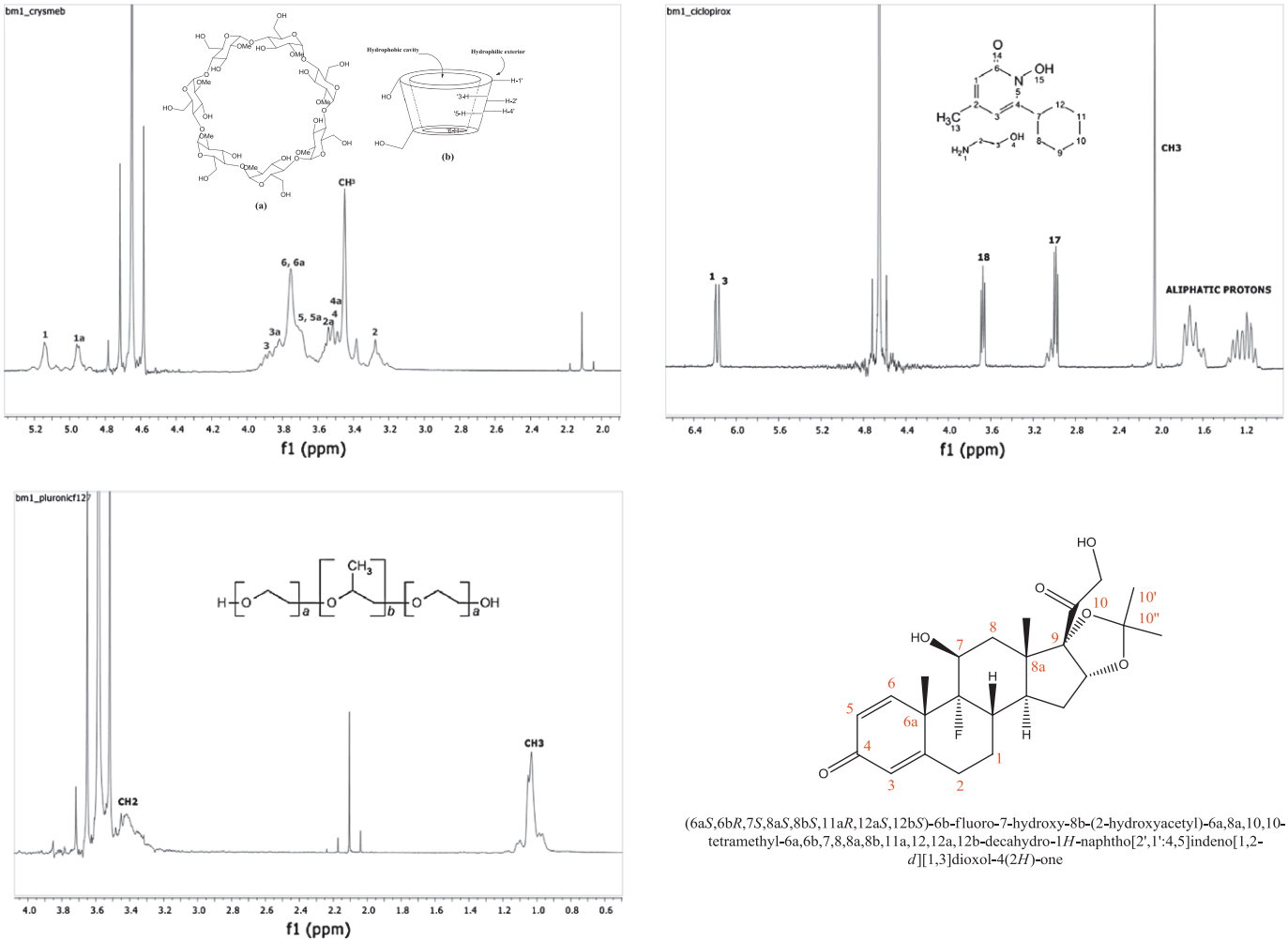

Fig. 1. ^{1}H NMR MβCD (top left), CPO (top right) and Pluronic (bottom left) spectral data in D_2O with the assignments of each proton by 2D NMR experiments, and chemical structure of TA and its chemical labels according to the IUPAC nomenclature (bottom right). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

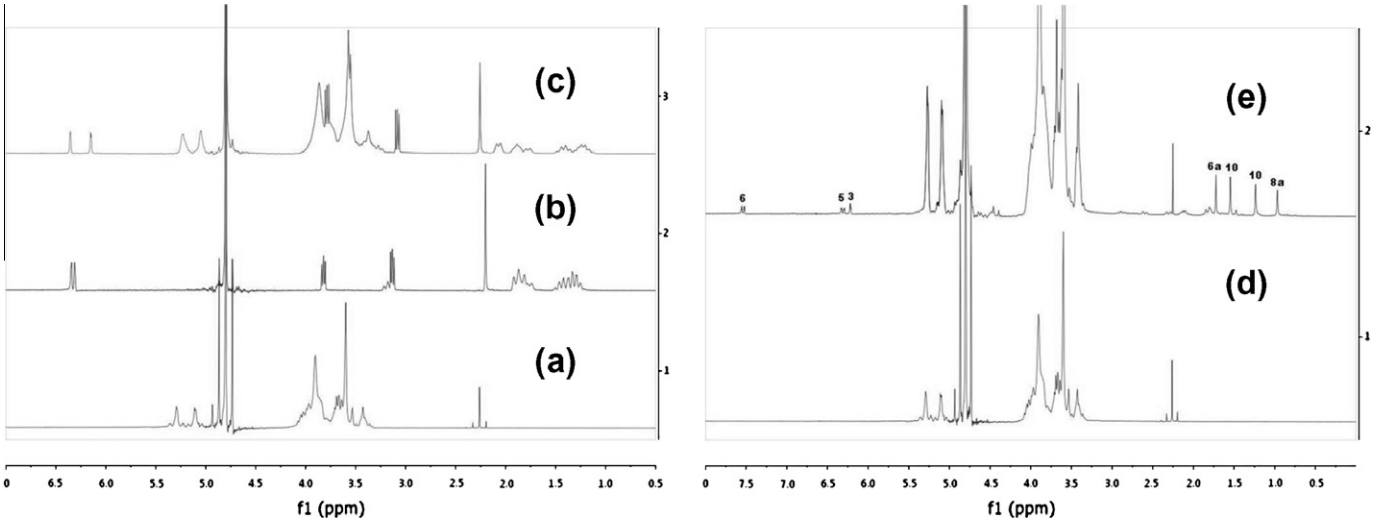

Fig. 2. ^{1}H NMR spectra data of MβCD (a and d), CPO (b), the mixture of MβCD/CPO (1:1) (c) and the solution containing MβCD/Triamcinolone in 10:1 ratio (e) in D_2O as deuterated solvent. The number labels of TA in spectrum (e) are according to the chemical structure in Fig. 1.

changes compared to other β-CD protons (H-1′, H-2′, H-4′ and H-6′). The upfield shift of MβCD cavity protons was mainly due to magnetic anisotropy affects in the MβCD cavity, arising due to the inclusion of a π electron-rich group, characteristic of aromatic rings, which indicates its inclusion in the MβCD cavity.

In the case of MβCD:CPO mixtures as can be observed, the aliphatic proton signals of the MβCD are upshielded in δ 0.06 ppm, corresponding to the protons H-1 and H-1′, and in minor scale the protons H-6 and H-6a with δ 0.04 ppm. However, it is noteworthy to see that a clear differentiation of the aliphatic protons of the cyclohexane ring in CPO, as several groups of methylene protons, is observed when MβCD is incorporated. Moreover, the resonance of the two aromatic protons, H-1 and H-3, is widely influenced by the apolar cavity, where the aromatic proton H-3 is being upshielded. All these chemical shifts, such as of the methylene and aromatic protons, probably happen by restriction in the free rotation and conformation of the cyclohexane ring when this molecule enters the CD cavity.

^{1}H NMR spectra of the mixture MβCD:TA exhibited four groups of singlet at δ 0.81, 1.08, 1.39 and 1.57 ppm, each one corresponding to three aliphatic protons (CH_3-group) at the positions 6a, 8a, 10′ and 10″ of the chemical structure, where 6a-Me-group is to lowfield due to the nuclear magnetic resonance anisotropic effect of the quinone ring attached. The other groups of signals corresponding to aromatic protons at C-3, C-5 and C-6, this last one more deshielded due to the electronic effect of the quinone ring and the conjugated double bond at the *meta* position with respect to the carbonyl group at C-4. Thus, one singlet at δ 6.07 ppm corresponding to the proton at C-3, and two groups of doublet at δ 6.15 and 7.39 ppm corresponding to the aromatic protons at C-5 and C-6, respectively.

In order to clearly establish the structure of the CPO·MβCD and TA·MβCD inclusion complexes, a ROESY experiment was performed. The results are displayed in Figs. 3 and 4. Thus, NOE intramolecular interactions of CPO are detected between the methylene protons of the cyclohexane ring and the singlet aromatic proton at C-5, which is nearest to the aliphatic ring.

A set of intermolecular interactions is observed between some aliphatic protons as CH_3-group attached at the pyridinone ring, where cross peaks are observed with CH_3-group and H-6/H-6a of the MβCD. Also, the aromatic proton at C-3 of CPO presents cross peak, with an intensified signal, at H-6/H-6a. Finally the aromatic proton at C-1 neighbour to carbonyl group of the CPO, it presents a cross peak with CH_3-group and H-6/H-6a (Fig. 3). In this sense, it was observed that the inclusion of the CPO inside the MβCD begins from the pyridinone ring, where the moiety with methyl group and protons at C-1 and C-3 are next to the methyl group and H-6/H-6a near to the narrow rim of the MβCD.

The intramolecular/intermolecular interactions of the ROESY experiment in the MβCD:TA mixture were assigned according to the NOE detected such as Me-8a with Me-10 of the molecule. The closeness of both methyl groups is due to the *axial* conformation of the molecule, through the small NOE signal to give a clear evidence of being upper 5 Å in distance. Also, the aromatic proton at C-5 shows a clear NOE signal with the proton at C-6 (Fig. 4). However, the incorporation of the TA inside the CD cavity can be observed by the intermolecular interactions between the proton groups H-5/H-5a and H-6/H-6a of the MβCD (near to the aromatic protons at C-3, C-5 and C-6 of the guest compound). These cross peaks are intense and they indicate that the incorporation of the TA is through the quinone moiety with NOE interactions close to the narrow rim of the MβCD, which is the preferred incorporation of aromatic ring inside the CD cavity, according to the literature.

As observed above, the inclusion of CPO is through the pyridinone ring and the TA through the quinone moiety, according to the 2D NMR data. Nevertheless, it is noteworthy to highlight that the presence of another compound as guest compound could compete with the stable complex. In a previous work [22] the interactions between poloxamer 407 (PF127) and HPβCD and MβCD were studied by means of surface tension measurements, π-A isotherms, isoperibol microcalorimetry, rheometry and ^{1}H NMR. The potential interactions of these three common excipients may notably modify the performance of the drug delivery systems. Thus, the addition of PF127 to the drug/CD inclusion complexes were studied by means of their NMR spectra (300 MHz) in D_2O at 295 K. The spectra of the mixtures MβCD/TA/PF127 and MβCD/CPO/PF127 are shown in Fig. 5, through the methyl group signal at C-10 corresponding to the triamcinolone at δ 1.06 ppm is overlapped with the broad signal of the methyl group in the PF127.

Analysing the 2D ROESY spectrum (Fig. 6) it is clear that the TA and the formed stable complex inside the MβCD cavity were slowly displaced by the addition of PF127, competition being observed between both compounds due to the polypseudorotaxane formation. The apolar cavity of MβCD and its solubility in aqueous medium make it possible to characterize a new complex between MβCD and PF127. The intramolecular interactions that can be visualized

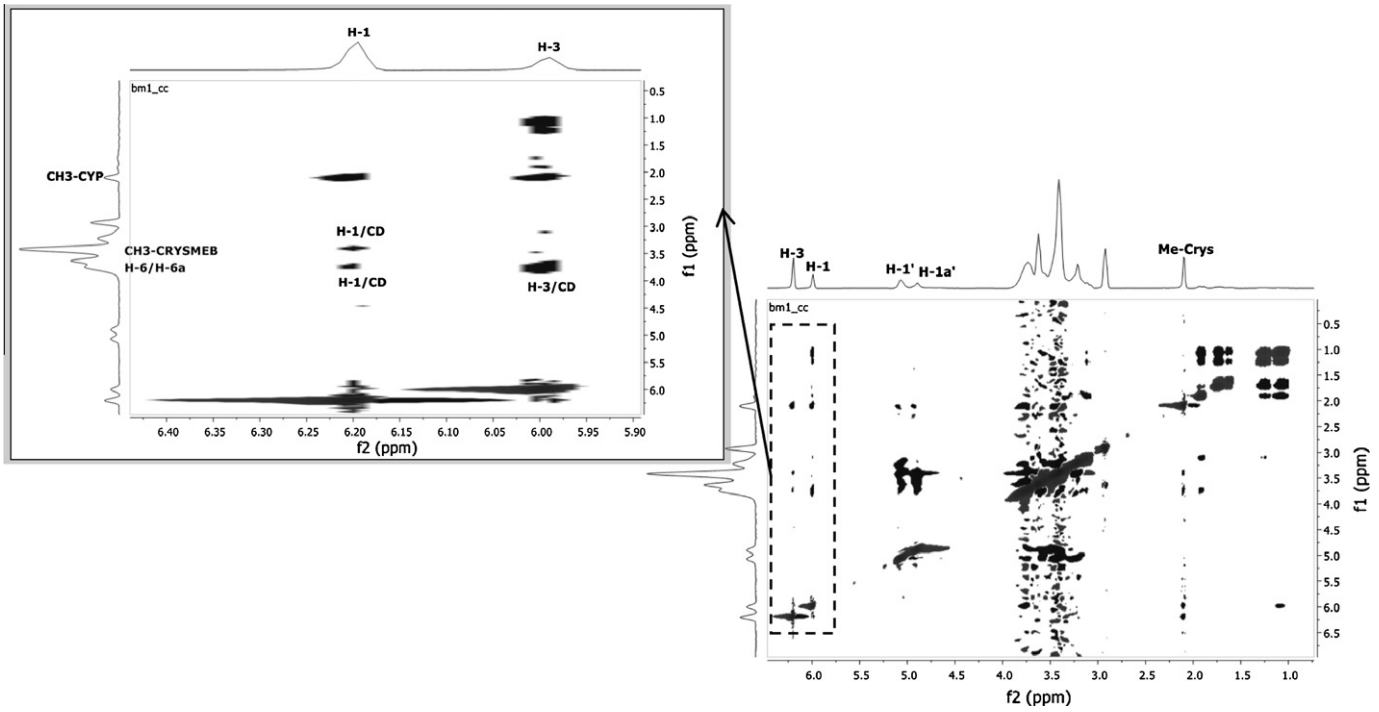

Fig. 3. ROESY spectrum of the 1:1 mixture of MβCD/CPO complex, and a section showing the ^{1}H–^{1}H NOEs between MβCD protons and aromatic protons of CPO.

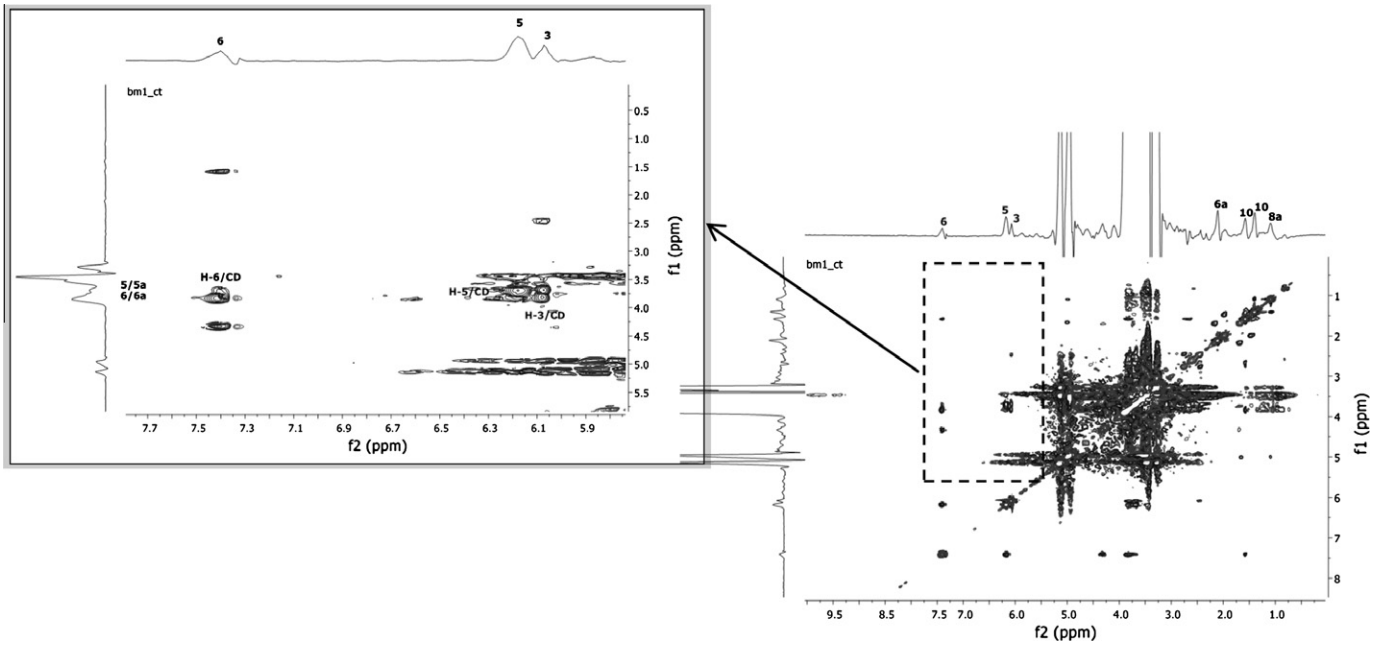

Fig. 4. ROESY spectrum of the MβCD/TA complex, 10:1 ratio and an expanded section of the 300 MHz 2D ROESY spectrum.

are aliphatic protons corresponding to H-1/H-1a with the proton from OCH_3-group of the MβCD (Fig. 6). On the other hand, the intermolecular interactions that we can visualize as a new complex is the cross peak between OCH_3-group corresponding to MβCD at position 2 and methyl group attached to the PF127 corresponding to the polypseudorotaxane formation, these results are in accordance with published data [22]. The neighbourhood of both aliphatic groups is close to the wider rim of the MβCD, and we speculate that PF127 is interacting with the protons outside of the CD cavity; it is also observed between the cross peak of CH_3-PF127/H-1/H-1a belonging to the MβCD. It is clear according to the expanded ROESY experiment that the multiple aromatic signals of the TA inside the MβCD are not present when PF127 is added. This means that the polypseudorotaxane (new complex MβCD·PF127) is more stable than the drug–cyclodextrin complex (Fig. 6). Similar displacement was obtained with CPO (see Fig. 5, 2D-RMN data not shown).

3.2. Determination of the stability constant and solubility studies

Interaction of TA with cyclodextrins has been studied previously [29–32]. TA can form inclusion complexes with the three

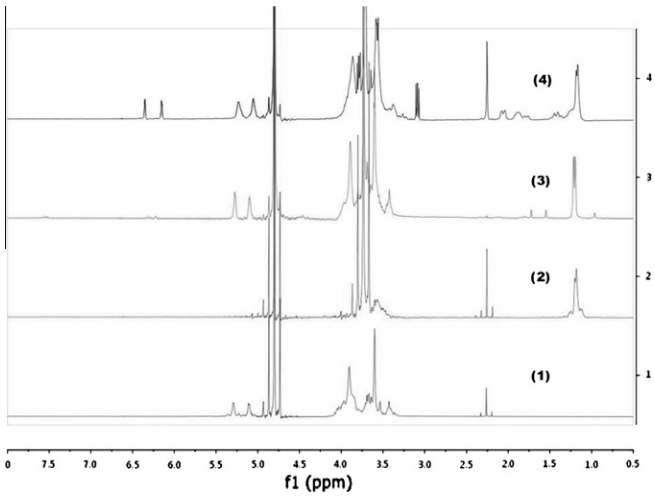

Fig. 5. ^{1}H NMR spectra data of MβCD (1), Pluronic (2), the mixture MβCD:TA:Pluronic in 20:2:1 ratio (3) and the mixture MβCD:CPO:Pluronic in 20:2:1 (4) with D_2O as deuterated solvent.

natural cyclodextrins with stability constant values of 120 M^{-1} at 25 °C for α-CD, 2370 M^{-1} at 25 °C [32] and 2800 M^{-1} at 30 °C [29] for β-CD and 9920 M^{-1} [32] for γCD. It is clear that the larger size of the γCD cavity is more appropriate to include the perhydro-1,2-cyclopentenophenanthrene ring of this corticosteroid drug, but TA also forms stable complexes with β-CD and their derivatives. Interactions of TA with β-CD hydrophilic derivatives have been examined (see Table 1); it was shown that MβCD forms more stable inclusion complexes than HPβCD.

Ciclopirox olamine solubility is highly dependent on the pH of the medium. In order to assess CD-CPO interactions at different pH media, respective CPO solubility diagrams in aqueous medium at pH 1 and 7 using HPβCD and MβCD were carried out. As CPO is a weak acid, its intrinsic solubility (S_0) decreases sharply in acidic medium compared to distilled water (0.91 mM and 53.7 mM, respectively). Determination of $K_{1:1}$ in acidic medium gives rise to notable differences if experimental intrinsic solubility (S_0) or the intercept of the straight line (S_{int}) is considered; nevertheless, a similar $K_{1:1}$ value was obtained when the drug was dissolved in a neutral solution (Table 1).

Fig. 7 shows the phase-solubility diagram of both cyclodextrins respect to CPO at different pHs. Table 1 shows $K_{1:1}$, CE and D:CD ratio values of CPO·CD complexes, in acidic and neutral solutions.

As shown in Table 1, $K_{1:1}$ values obtained (considering either S_0 or S_{int}) are strongly affected by the pH. However, statistical analysis showed that only in the case of HPβCD·CPO complex values of $K_{1:1}$ and CE increased significantly ($p < 0.05$). No differences were shown comparing MβCD·CPO complex $K_{1:1}$ or CE values determined in neutral and acidic solutions, nor between $K_{1:1}$ or CE values of HPβCD·CPO and MβCD·CPO complexes in both neutral and acidic solution.

Complex:cyclodextrin molar ratios showed that around five out of ten CD molecules form a complex with the ciclopirox in neutral solution, whereas in acidic solution around five out of six MβCD molecules form the complex and five out of seven HPβCD molecules do. Nevertheless, values of HPβCD·CPO complex molar ratio in acidic solution showed significant differences with respect to both HPβCD·CPO and MβCD·CPO complexes in neutral solution ($\alpha < 0.05$), but no differences were found between both cyclodextrins in both neutral and acidic solution.

In a previous work [22] the existence of a competitive displacement of the methyl orange dye from MβCD and HPβCD cavities was described as a consequence of the formation of PF127 polypseudorotaxanes. The stability constants of methyl orange with randomly methylated-βCD and with HPβCD have been previously reported to be 2200 and 1060 M^{-1}, respectively [37]. The capability of PF127 to displace methyl orange with such a high affinity constant from the CD cavity clearly indicates that the PF127 strongly interacts with CDs. Considering values of stability constants obtained for TA·CD and CPO·CD complexes, it is expected that PF127 can indeed displace TA and CPO molecules from the cavities of CDs, decreasing drug–cyclodextrin complexation effectiveness and so drug solubilization ability.

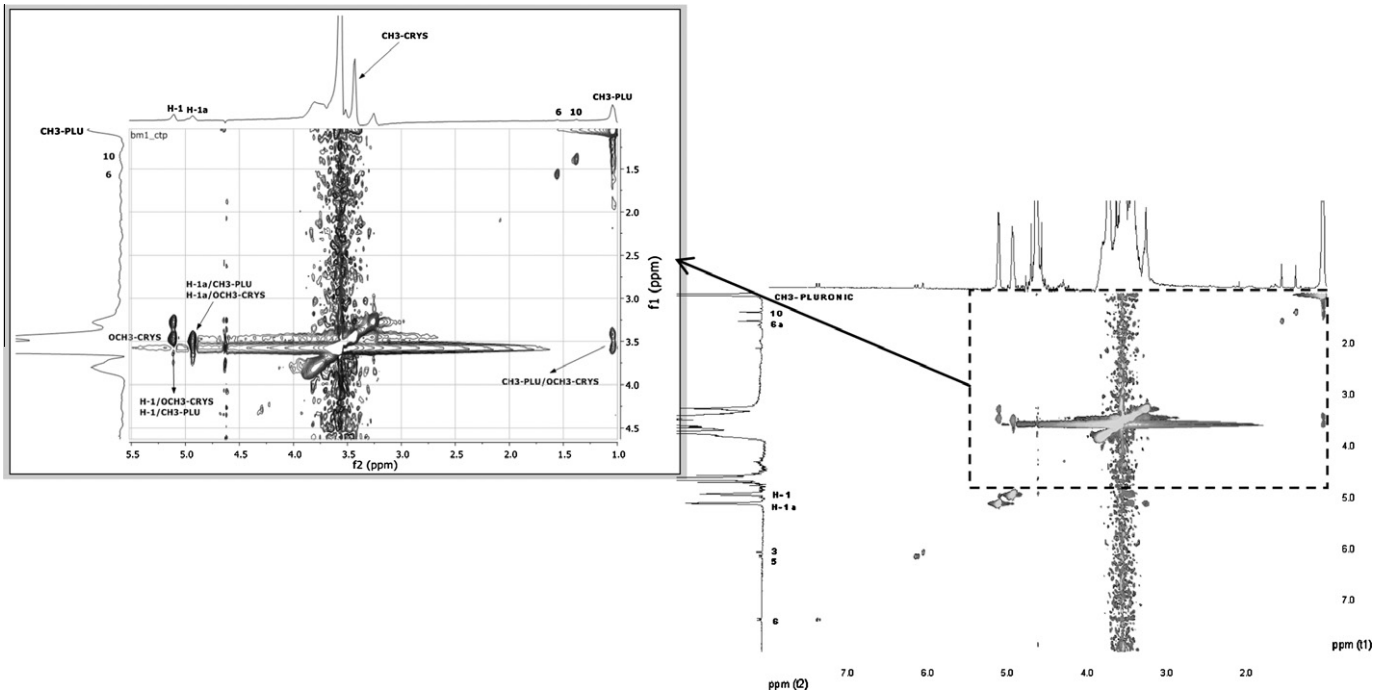

Fig. 6. ROESY spectrum of the MβCD:TA:Pluronic complex, 20:2:1 ratio, and expanded section of the 300 MHz 2D ROESY spectrum.

Table 1
$K_{1:1}$, CE and D:CD ratio values of Drug–cyclodextrin complex.

Drug	Medium	CD	S_0 (mg/ml)	S_{int} (mg/ml)	$K_{1:1}$ (M^{-1})		CE	D:CD ratio	Slope
					S_0	S_{int}			
CPO	pH 7	HPβCD	14.4	17.74	21	20	1.13	1:1.89	0.53
		MβCD	14.4	17.75	24	19	1.27	1:1.79	0.56
	pH 1	HPβCD	0.22	4.66	5956	281	4.88	1:1.22	0.83
		MβCD	0.22	6.23	3659	129	3.00	1:1.93	0.75
TA	Water	HPβCD[a]	0.11		240	640	0.063	1:16.87	0.029
		HPβCD[b]	0.114				0.241	1:5	–
		HPβCD[c]	0.017	0.013	1630	2124	0.064	1:16.70	0.060
		MβCD[c]	0.017	0.044	2774	1084	0.11	1:10.22	0.098

[a] Taken from [34].
[b] Taken from [35].
[c] Estimated using 0%, 5% and 10% of CDs (six replicates for each concentration).

Results of solubility studies of both drugs in different media are shown in Table 2. Solubilization by CD complexation is remarkably efficient, particularly in the case of TA. Statistical analysis (Table 3) revealed that solubility of CPO depends on the pH of the medium and CD concentration but it is independent of the type of CD or the presence of PF127. The regression equation obtained has a notable predictive value (91.5% of solubility variations can be explained by the model equation) and showed that CPO solubility increases with CD concentration and pH.

In the case of TA, statistical analysis showed that drug solubility is dependent on the variety and concentration of CD but it is independent on the PF127. In the case of TA solubility a lower correlation level was found (R^2 77.5%). However, although the prediction of TA solubility was worse than that obtained for CPO solubility, the proposed model was significant for a level of probability of 99.9%. The regression equation indicates that MβCD shows higher solubilization capability than HPβCD and has no influence on TA solubility due to the competitive displacement caused by the observed formation of polypseudorotaxanes.

It was described that the CMC value of PF127 in water is 0.4 mM and the addition of 5% of CDs increases CMC values around 0.5–1 mM for HPβCD and MβCD, respectively [22]. PF127 was incorporated into the solutions assayed at 10 wt.% (8 mM), at which concentration PF127 molecules aggregate forming micelles, even in the presence of cyclodextrins. In absence of drug-PF127 competition, an additive effect of the CDs inclusion complexation and micelle solubilization on the global solubility of drugs can be expected. However, no additive effect was observed in both CPO and TA solubility, probably due to the competitive displacement of the drug by the PF127 from the CDs cavity. In this situation, the incorporation of drugs into the micelles minimizes the solubility reduction due to the drug displacement from CD cavities due to the formation of PF127 polypseudorotaxanes. Therefore, use of CD complexation can be a good strategy to increase drug solubility and to incorporate high doses of poorly water-soluble drugs in thermosensitive hydrogels when PF127 micellization is not efficient enough to solubilize the complete dose, but each particular case must be carefully considered.

3.3. Drug release studies

Drug release is also an important property that can be affected by PF127-drug competition during polypseudorotaxane formation. To assess it, CPO and TA diffusion was studied from different formulations, carrying out release studies through a MWCO $\geqslant$ 12,000 Da dialysis membrane. Drug release profiles from the different mixtures of CPO:CD, CPO:PF127, CPO:CD:PF127 and TA:CD, TA:PF127, TA:CD:PF127 are shown in Figs. 8 and 9, respectively.

CD inclusion complexes significantly increase the diffusion of CPO compared with drug aqueous solutions (Fig. 8). The ability of cyclodextrins and their complexes to diffuse through semi-permeable membranes depends on the molecular weight cut-off of the membranes but it has been reported that cyclodextrins and their complexes are able to permeate membranes with MWCO > 3000 - Da [38,39]. CD·drug complexes passes through the membrane slower than drug molecules as a consequence of their higher molecular weight, but the addition of the permeability of both free drug and inclusion complex can increase the total flux of drug. Additionally, since experiments were made in vertical Franz cells the solution in the donor chamber was unstirred and so the resistance of the water diffusion layer to lipophilic drug diffusion may be important. In these conditions the inclusion of CDs in the solution can lead to an increase of the drug concentration gradient over the diffusion layer as well as a higher availability of free drug molecules next to the membrane surface, giving rise to an increase in the drug diffusion rate [40].

Incorporation of Pluronic F-127 significantly decreases the CPO release rate. Diffusion studies were made up using water as solvent and phosphate buffered saline solution (pH 7.4) as receptor medium. At this pH the stability of the CPO·CD inclusion complex is very low ($K_{1:1}$ 19–24 M^{-1}), so the addition of PF127 can decrease the release rate by three mechanisms: competitive interaction of the PF127 for the cyclodextrin cavity; incorporation of CPO into the polymeric micelles; and a notable increase of the donor solution viscosity due to the gelation of the thermosensitive system at temperatures higher than its sol–gel transition temperature [22].

Table 4 shows values of CPO fluxes (J), diffusivity (D), apparent permeability coefficient (P_{app}), $P'_m/K_{1:1}$ ratio and $M_{1/2}$ estimated for drug and drug/CDs solutions. The values of $P'_m/K_{1:1}$ and $M_{1/2}$ obtained for CPO·CD and TA·CD inclusion complexes were similar to those estimated by Masson et al. [36] using different corticosteroid drugs. As we can observe in Table 4, the incorporation of PF127 mainly affects the CPO flux through $P'_m/K_{1:1}$ ratio. PF127 incorporation into MβCD:CPO solutions increases $M_{1/2}$ and reduces the $P'_m/K_{1:1}$ ratio pointing out that formation of polypseudorotaxanes decreases the drug–MβCD complexes' affinity for the water diffusion layer and their permeation across the membrane. Nevertheless, it was observed that addition of PF127 to the HPβCD/CPO systems does not modify $M_{1/2}$ values and only slightly decreases $P'_m/K_{1:1}$ ratio. As we showed in previous work [22], PF127 exhibits less affinity for HPβCD than for MβCD and so the competition with drugs for HPβCD results in formation of fewer polypseudorotaxanes and therefore smaller drug–CD displacement.

In order to obtain a significant dose of dissolved drug, higher concentrations of MβCD must be employed for TA release studies. Fig. 9 shows TA release profiles obtained for the different mixtures

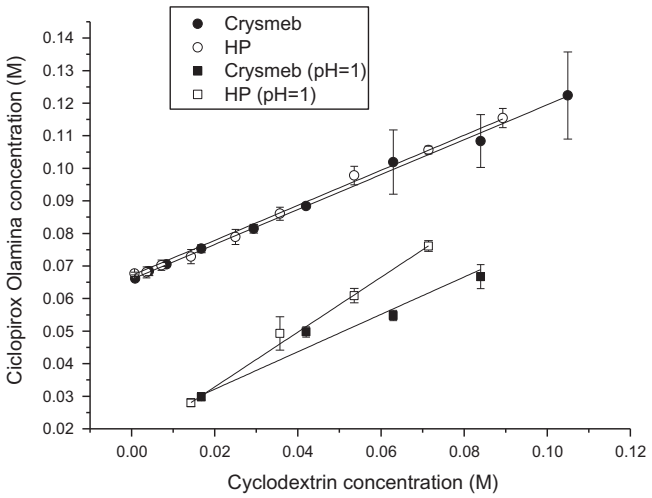

Fig. 7. Phase-solubility diagram of HPβCD and MβCD with ciclopirox olamine.

assayed. Aqueous TA solution shows a very low drug release with a total of 113 ± 18 μg of TA released per cm^2 in 48 h. This low value could be caused by the high resistance of the unstirred water diffusion layer due to the low drug aqueous solubility. Drug:PF127 solutions showed slightly slower release profiles than aqueous drug solutions with a final release of 39 ± 4 μg of TA released per cm^2 in 48 h. Since solubility studies suggested that TA is not incorporated into the polymeric micelles, lower release rate should be ascribed to marked decreases of drug diffusivity into the water diffusion layer, caused by the remarkable increase of the solution viscosity due to the PF127 gelation through effect of the temperature. As demonstrated in the previous work [22]), 10% and 15% PF127 solutions have a sol–gel transition at temperatures up to 35 and 20 °C, respectively, so at 37 °C it tends to form high viscous semisolid hydrogels. Addition of MβCD into aqueous drug solution significantly increases TA release reaching 1.8 ± 0.1 mg of TA release per cm^2 in 48 h, about 16-fold of the TA released from drug solutions. This rise of TA release rate can be attributed to the increase of the drug concentration gradient in the diffusion layer and the availability of free TA molecules next to the membrane surface, caused by the increase of TA aqueous solubility due to its inclusion in MβCD cavity. Diffusion from MβCD solutions gives rise to $M_{1/2}$ values of 0.008 M, which was smaller than CPO but the $P_m/K_{1:1}$ ratio obtained was similar.

In contrast to what occurs in the case of CPO, addition of Pluronic F127 significantly increases the release from the TA:MβCD solutions. Cyclodextrins and cyclodextrin complexes are known to self-associate forming some kind of aggregates or micelles. Loftsson et al. [38] found that in aqueous solution hydrocortisone·HPβCD inclusion complexes can lead to aggregates consisting of more than 2–8 complex molecules, which will be unable to permeate

Table 2
Solubility data of TA and CPO in presence of CD and Pluronic F127.

Drug	Pluronic concentration (%)	Type CD	CD concentration (%)	Solubility (mg/ml)	
				pH 7/water[a]	pH 1
CPO	–	–	–	14.41	0.219
		MβCD	5	30.85	13.48
			10	31.30	22.33
		HPβCD	5	20.85	13.22
			10	27.83	20.43
	10	–	–	22.18	0.546
		MβCD	5	27.48	10.99
			10	26.18	26.67
		HPβCD	5	26.00	13.52
			10	37.78	26.63
TA	–	–	–	0.017	
		MβCD	5	1.937	
			10	3.556	
		HPβCD	5	0.930	
			10	1.877	
	10	–	–	0.177	
		MβCD	5	1.290	
			10	1.559	
		HPβCD	5	0.704	
			10	1.198	
	15	–	–	0.157	
		MβCD	5	1.261	
			10	2.117	
		HPβCD	5	0.877	
			10	1.449	

[a] CPO was determined at pH 7 and TA in water.

Table 3
Dependence of drug solubility on the type of CD (A), CD concentration (B), Pluronic concentration (C) and pH media (D) (only for CPO).

Drug	Equation	F	R^2	α
Cyclopirox olamine	Solubility = 18.76–9.42$B(1)$ + 0.79$B(2)$ + 0.636D + 2.60$B(1)D$ + 0.39$B(2)D$[a]	38.45	0.91	<0.01
Triamcinolone acetonide	Solubility = 1.08 + 0.26A − 0.96 $B(1)$ + 0.086 $B(2)$[b]	15.91	0.77	<0.01

$B(1)$ and $B(2)$ represent the values of the first and second elements of the matrix, respectively.
[a] The effects of type of CD and Pluronic concentration were not statistically significant.
[b] The effect of Pluronic concentration was not statistically significant.

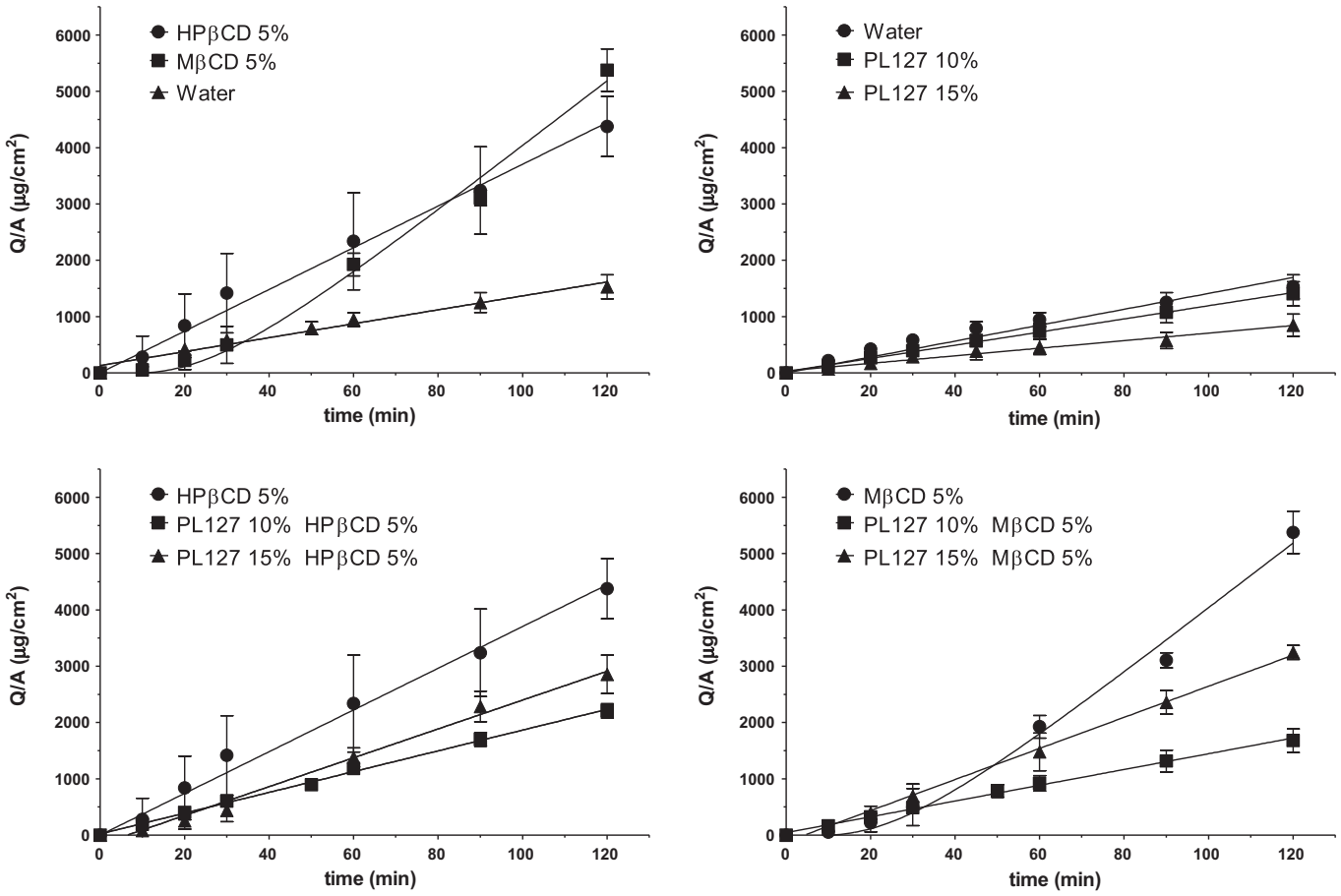

Fig. 8. Release profiles of ciclopirox olamine from different solutions.

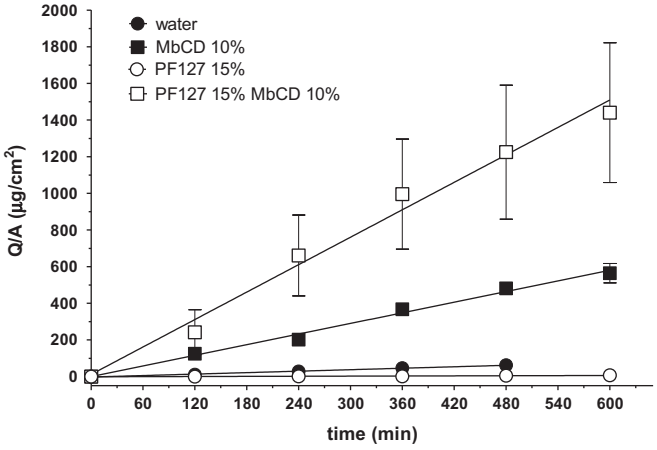

Fig. 9. Release profiles of triamcinolone acetonide from different solutions.

membranes with a low MWCO. This behaviour was also observed for other CD inclusion complexes with different corticoids and so it probably occurs as well with TA·MβCD inclusion complexes. On the other hand, TA and MβCD form stable inclusion complexes with high stability constant values, so the presence of high concentrations of CDs ($\geqslant$10%) decreases the free drug concentration and thus reduces its permeation rate across the membrane. As Table 5 shows, addition of PF127 does not modify $M_{1/2}$ values but significantly increases $P_m/K_{1:1}$ ratio value. There are two main effects that can increase the TA release rate and the drug membrane permeation: PF127 surfactant activity probably prevents the formation of aggregates; and the competitive formation of the MβCD-PF127 polypseudorotaxanes that maintains their solubilization capability of the formulation, but resulting in a lower TA-CD affinity to form inclusion complexes and so favouring the diffusion of free drug molecules across the membrane.

Table 4
Fluxes, drug diffusivity and permeability coefficients of the ciclopirox olamine from tested formulations.

PF127% (w/v)	CD% (w/v)	Flux (μg/cm² s)	D (cm²/s)	P_{app}	$M_{1/2}$ (M)	$P'_m/K_{1:1}$ (μg/cm² s)
–	–	0.1926	7.51×10^{-8}	1.50×10^{-5}		
	MβCD 5	0.7938	1.52×10^{-7}	3.05×10^{-5}	0.023	2.29
	HPβCD 5	0.5455	1.31×10^{-7}	2.62×10^{-5}	0.030	2.00
10	–	0.1927	5.34×10^{-8}	1.07×10^{-5}		
	MβCD 5	0.2291	5.62×10^{-8}	1.12×10^{-5}	0.041	1.19
	HPβCD 5	0.3043	6.34×10^{-8}	1.27×10^{-5}	0.035	1.62
15	–	0.1085	4.23×10^{-8}	6.24×10^{-6}		
	MβCD 5	0.47117	8.99×10^{-8}	1.80×10^{-5}	0.030	1.77
	HPβCD 5	0.4433	8.60×10^{-8}	1.72×10^{-5}	0.031	1.66

Table 5
Fluxes, drug diffusivity and permeability coefficients of the triamcinolone acetonide from tested formulations.

PF127% (w/v)	MβCD % (w/v)	Flux (μg/cm^2 s)	D (cm^2/s)	P_{app}	$M_{1/2}$	$P_m/K_{1:1}$
–	–	0.00236	8.46×10^{-7}	0.17×10^{-3}		
–	10	0.01638	2.80×10^{-8}	5.59×10^{-6}	0.0079	1.70
15	–	0.00022	8.71×10^{-9}	1.74×10^{-6}		
15	10	0.04114	1.15×10^{-7}	2.29×10^{-5}	0.0080	2.69

4. Conclusion

Incorporation of PF127 into the drug–CD complexes aqueous solutions involves a competition with drugs for the cavity of the CD molecule that leads to the displacement of the drug from the CD cavity and the formation of soluble polypseudorotaxanes.

Formation of polypseudorotaxanes interferes in the formation of CD–drug inclusion complexes, but the drug solubilization ability of the system is not strongly altered since reduction in drug–CDs complexation is slightly compensated by the PF127 micellization. Therefore, CDs complexation can be a suitable strategy to increase doses of poorly water-soluble drugs in thermosensitive hydrogels when PF127 micellization is not able to solubilize the whole dose.

The ability of PF127 to displace guest molecules from the CD cavities, its surfactant activity and sol–gel transitions can all effectively modify release properties of the aqueous drug–CDs inclusion complexes solutions. These changes of the drug release profile depend on properties of inclusion complexes and their aggregation, and on CD concentration. Thus, formation of polypseudorotaxanes must be borne in mind when drug delivery systems based on these macromolecules are developed; furthermore, appropriately handled they could be used to modulate release properties.

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