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Physico-chemical studies of inclusion complex between hydrocortisone and cyclodextrins

Teresa Silvestri [a,1], Federica D'Aria [a,1], Fabrizio Villapiano [a], Fernando Scala [a], Laura Mayol [b,d], Danilo Aleo [c], Nunzio Cardullo [e], Sossio Fabio Graziano [a], Marco Biondi [a,d,*], Concetta Giancola [a]

[a] Department of Pharmacy, University of Naples Federico II, Via Domenico Montesano 49, 80131 Naples, Italy
[b] Department of Advanced Biomedical Sciences, University of Naples Federico II, Via Domenico Montesano 49, 80131 Naples, Italy
[c] Medivis Srl, Via Carnazza, 34/C, 95030 Tremestieri Etneo, Catania, Italy
[d] Interdisciplinary Research Centre on Biomaterials – CRIB – Università di Napoli Federico II – P. le Tecchio, 80131, Naples, Italy
[e] Dipartimento di Scienze Chimiche, Università degli Studi di Catania, V.le A. Doria 6, 95125-Catania, Italy

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ABSTRACT

Hydrocortisone (HC) is widely utilized in the pharmaceutical field for various administration routes, including oral, parenteral, and topical routes. However, its application in ocular disease treatment as a solution is limited due to its poor aqueous solubility. To address this limitation, in this study we aimed to investigate different types of cyclodextrins (CDs) to identify the most effective CD for improving the inherent solubility of HC. Results from scanning electron microscopy, rheology and differential scanning calorimetry (DSC) demonstrated the formation of inclusion complexes, while isothermal titration calorimetry (ITC) investigations accurately determined the thermodynamic parameters associated with the formation of complexes between HC and HPβCD or βCD, at 25 and 37 °C. Additionally, phase solubility studies in PBS buffer at 37 °C confirmed the formation of stable inclusion complexes. Collectively, results indicated that HPβCD was the most effective CD in enhancing the intrinsic solubility of HCs in PBS highlighting its potential for improving the solubility and formulation of HC-based pharmaceutical products.

1. Introduction

Corticosteroids are steroid hormones produced by the adrenal cortex, which are involved in several physiological functions, such as the regulation of immune response, the reaction to stress [1] and the control of the balance of sodium and water [2]. Among corticosteroids, hydrocortisone (HC) plays a major role in that it is broadly employed to treat an extensive variety of conditions, such as, allergies, asthma, autoimmune disorders, and inflammatory skin affections. HC is available in a far-ranging array of pharmaceutical dosage forms and can be used for both topical and systemic routes of administration [3–5].

HC has also been vastly utilized in the ophthalmic domain by virtue of its pronounced anti-inflammatory effect both *in vitro* and *in vivo* against dry eye disease [6], and also brings about an improvement in the symptoms of Ocular Surface Disease (OSD) in patients with and without POAG (Primary Open-Angle Glaucoma) [7].

Unfortunately, HC is sparingly soluble in water and, as a consequence, its ocular administration is challenging since the drug easily precipitates [8]. Besides, corticosteroid suspensions for ophthalmic administration suffer from some issues related to their questionable ophthalmic tolerability and possible issues of uniformity of dosage in eyedrops [9–11], making it very desirable to have the drug solubilized.

It is of great interest to investigate the possibility of administering native HC in the form of eye drops by using solubility promoters. Thus, in this work, we present the formation of inclusion complexes between hydrocortisone and four cyclodextrins as a means of improving the solubility of the drug for ocular administration. Cyclodextrins (CDs) are cyclic oligosaccharides made up of a macrocyclic ring of glucose units linked by α-1,4 glycosidic bonds, possessing a truncated cone architecture [12]. The most common CDs are α-, β-, and γ-cyclodextrin (namely α-CD, β-CD and γ-CD) which consist of six, seven, and eight glucopyranose units, respectively. All CDs expose hydroxyl functions on the exterior surface of the cone, while the central cavity is relatively

* Corresponding author.
E-mail address: mabiondi@unina.it (M. Biondi).
[1] These authors contributed equally to this work.

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lipophilic, due to its coating of skeletal carbons and ethereal oxygen of the glucose residue [13,14]. Due to these features, CDs can form inclusion complexes with poorly water-soluble molecules by taking up the molecule in part or in its entirety into its relatively hydrophobic cavity [15]. To improve solubility and complexation ability of the native β-CD, semisynthetic hydroxypropyl-β-cyclodextrin (HPβCD) is frequently used [16,17] and was studied in the formulation of aqueous ophthalmic solutions of hydrocortisone [18].

The aim of this study was to identify the most effective cyclodextrin for the formation of inclusion complexes with hydrocortisone. Additionally, a comprehensive technological and thermodynamic characterization of the complexation process was conducted. To achieve this objective, phase solubility studies were combined with Ultra-High-Performance Liquid Chromatography (UHPLC) analyses. Moreover, scanning electron microscopy (SEM), rheological measurements, differential scanning calorimetry (DSC), and isothermal titration (ITC) experiments were performed to further assess complex formation.

The solubility of HC was evaluated in aqueous solution in the presence of each CD and, to determine the optimal CD for inclusion complex formation, solubility data have been compared for each complex, which is the first step to prevent HC precipitation in eye drops.

2. Materials and methods

2.1. Materials

Hydrocortisone (HC), dibasic sodium phosphate dodecahydrate ($Na_2HPO \cdot 12\ H_2O$), sodium chloride (NaCl), potassium chloride (KCl), Posphate Buffer solution (PBS) (Tablets), ethanol (EtOH), phosphoric acid (H_3PO_4) and HPLC-grade acetonitrile (ACN) were obtained from Sigma–Aldrich (USA) while sodium phosphate monobasic dehydrate ($NaH_2PO \cdot 2\ H_2O$) from Farmalabor (Italy). 2-hydroxypropyl-b-cyclodextrin (HPβCD), α-cyclodestrin (αCD) and ɣ-cyclodestrin (ɣCD) were purchased by Wacker Chemie (Germany). β-cyclodextrin (βCD) was obtained from Cyclolab (Hungary). For UHPLC experiments ultrapure water (18 MΩ) (Direct-Pure Rephil system) was employed.

2.2. Phase solubility experiment by spectrophotometric assay

Firstly, PBS buffer was prepared by dissolving 0.201 g of KCl, 7 g of NaCl, 1.42 g of Na_2HPO_4 in 500 mL of double-distilled water (DDW). The solution was magnetically stirred for 30 min at room temperature and diluted with DDW to a final 1 L volume. Then, pH was adjusted at 7.4 by adding 0.1 M HCl. An excess amount of HC was suspended in 5 mL of PBS solutions containing CD at 3.29, 6.60, 9.89, 13.19, 16.54, 32.93, 49.46 and 66 mM. The suspensions were poured in capped vials, at room temperature for 72 h in the dark under continuous stirring 100 rpm. Thereafter, the solutions were filtered through a 0.45-μm cellulose acetate membrane (Millipore) and then analysed by spectrophotometric assay (UV-1800, Shimadzu Laboratory World, Japan; λ = 247 nm in phosphate buffer) to quantify the solubilized HC. Correspondingly, the phase diagram was obtained by plotting the molar concentration of solubilized HC against each cyclodextrin molar concentration. The constant of HC-CD complex formation, K_b, was calculated from the slope of the phase solubility diagram, with the equation (1) where S_0 is HC solubility in the absence of CD.

$$K_b = \frac{slope}{S_0(1 - slope)} \tag{1}$$

The experiments were carried out in duplicate. Recovered filtered samples were lyophilized (24 h, 0.01 atm, −60 °C; Büchi, Switzerland) and used for DSC studies.

2.3. Phase solubility experiment by UHPLC analysis

Ultra-High-Performance Liquid Chromatography (UHPLC) analyses were carried out on an Agilent 1290 Infinity instrument equipped with a refrigerated autosampler, thermostated column compartment and UV–vis detector connected with OpenLAB software.

UHPLC analyses were performed on a Poroshell Agilent C-18 column (50 mm × 2.1 mm i.d. 1.9 μm), thermostated at 25 °C, setting UV-detection at λ = 240 nm. The column was eluted at flow 0.6 mL/min with mixtures of mobile phase A (phosphate buffer, 2.7 g/L of $Na_2HPO \cdot 12\ H_2O$ and 1.6 g/L of $NaH_2PO \cdot 2\ H_2O$ aqueous solution adjusted to pH = 3 with H_3PO_4 85% w/v) and mobile phase B (ACN). CD concentrations used in this study were 3.29 mM, 6.60 mM, 9.89 mM, 13.19 mM, 16.54 mM, 32.93 mM, 49.46 mM, 66 mM. Inclusion complexes between HC and CDs were prepared as described in the section 2.2 using PBS 0.01 M solution as solvent (Tablet composition: 0.01 M phosphate buffer, 0.0027 M potassium chloride KCl and 0.137 M sodium chloride NaCl) at pH = 7.4. One tablet was dissolved in 200 mL of deionized water. Phosphate buffer was filtered with 0.2 μm filter before use. The isocratic program A:B 78:22 v/v was applied. The injection volume was of 2 μL and the run time was 3 min.

2.4. Preparation of standards for UHPLC analysis

The standard stock solution for UHPLC analysis was prepared by mixing 200 mg of HC and 2.5 g of HPβCD in 15 mL of PBS at pH = 7.4. After complete dissolution, the solution was transferred into a 20 mL volumetric flask, PBS was added up to a final volume of 20 mL. The standard stock solution was diluted with PBS to obtain standard solutions for HC. The calibration curve was obtained using standard solutions at final concentrations of 0.2, 0.5, 1.0, 5.0, and 10.0 mg/mL. For each analysis, 0.700 mL of sample was diluted to 20 mL with PBS solution.

2.5. Rheological experiments

Rheological tests were performed to investigate how complexation of HC with HPβCD affected formulation viscosity. To this aim, viscosity measurements were performed on the saturated aqueous solution of HC and the 66 mM solution of the HC-HPβCD inclusion complex. Shear experiments were carried out by means of a rotational rheometer (Malvern Kinexus), equilibrated at 25 °C, using a plate-plate geometry (PU60) and a shear rate varying in the 10 ÷ 20 s^{-1} range.

2.6. SEM analyses

The morphology of CDs, raw materials, and the inclusion complex was investigated at the scanning electron microscopy lab. of the Dept. of Earth Sciences, Environment and Resources, Federico II University, Napoli—Italy. A Field Emission Scanning Electron Microscope equipped with an Energy Dispersive Spectrometer (FESEM/EDS; Zeiss Merlin VP Compact coupled with Oxford Instruments Microanalysis Unit; Carl-Zeiss Strasse, Oberkochen, Germany) was used for observations. To perform the analyses, each sample was placed on a sample holder with a carbon tape film. Subsequently, samples were metalized by means of an automatic sputter coater (Agar Scientific ltd – Parsonage Lane, Stansed - Essex, UK) with an automatically controlled complete sequence of flush, leak, coat, and vent.

Data sets were obtained using an INCA X-stream pulse processor (Oberkochen, Germany) (15-kV primary beam voltage, 50–100 A filament current, variable spot size, from 30,000 to 200,000 × magnification, 20 mm working distance, and 50 s real-time counting) by means of INCA Energy software 5.05 (XPP array and pulse pile-up corrections).

2.7. Differential scanning calorimetry (DSC)

The formation of the complex between HC and CD has been qualitatively investigated by performing thermoanalytical tests on the lyophilized solutions (24 h, 0.01 atm, −50 °C; Buchi, Switzerland)

recovered after phase solubility experiments. The heats evolved during the thermal transitions of HC, CD and the recovered precipitate were determined by a differential scanning calorimeter (DSC; DSC Q20, TA Instruments, USA), preliminarily calibrated with a pure indium standard. Accurately weighted solid samples (approximately 5 mg) were placed in aluminium pans and heated from 10 to 300 °C in sealed aluminium pans at a constant heating rate of 20 °C min^{-1}, under an inert nitrogen flow purged at a constant 50.0 mL•min^{-1} flow rate. An empty pan was used as a reference.

2.8. Isothermal titration calorimetry (ITC)

ITC experiments were performed at 25 °C for all CD samples and 37 °C for HPβCD and βCD, in PBS solution, using a nano-ITC (TA instruments, USA). The CD solution was injected (3.4 mM) into calorimetric vessel (170 μL) containing HC solution (0.5 mM). For all titration experiments, the stirring speed was set at 250 rpm. Each titration was programmed to inject 2 μL of titrant in sample cell every 300 s. The dilution heat was evaluated in control experiments by injecting the CD solution into PBS. For all experiments, the heat flow values were plotted versus time, after correction for dilution heat. Peaks were integrated using Nanoanalyze software v 3.1.2 (TA Instruments, New Castle, DE), giving a total heat difference (Q, in μJ) between the sample and reference for each injection, as a function of CD and HC concentration in the sample cell. Using Nanoanalyze software, experimental data were fitted with the Independent Model to obtain the enthalpy change ($\Delta H°$), the binding or association constant (K_b), and the number of binding sites per hydrocortisone molecule (n). Determination of ΔH, K_b and n by fitting allowed the determination of Gibbs energy ($\Delta G°$) and entropy ($\Delta S°$) of the interaction with the equation $\Delta G° = -RT \ln K_b = \Delta H° - T\Delta S°$. The goodness-of-fit and confidence intervals were determined by Nanoanalyze software for a confidence level of 95%, 100 fitting trials and a standard deviation of 2 on the independent model. Each experiment was performed in duplicate.

3. Results and discussion

3.1. Phase solubility studies

Phase solubility studies were performed to assess inclusion complex formation between HC and CDs, at physiological temperature (37 °C). At first, phase solubility tests were carried out using a hydroalcoholic solution (70:30 water:ethanol). The aim was to enhance the apparent water solubility of the lipophilic HC molecule favouring the inclusion into CD, as shown in a recent study on quercetin and curcumin with HPβCD host–guest interaction [19,20]. Surprisingly, the outcomes showed that there was no complexation between HC and HPβCD (Fig. S1). A possible explanation for this puzzling phenomenon may be the competition between ethanol and hydrocortisone molecules for their interaction with CD cavity.

Thus, in accordance with previous literature results, a more physiological-like solvent, PBS, was used. Fig. 1 shows the plot of the HC molar concentration as a function of CD molar concentration. Indeed, according to Messner study [21] and Higuchi & Connor classification [22], HC-αCD and HC-HPβCD complexes show both an "A_L" type profile. Conversely, HC-γCD and HC-βCD complexes show a "B_S" type profile. The binding constants (K_b), estimated by equation (1), the slopes and the correlation coefficients are illustrated in Table 1. The values of K_b were found to be increasing with increasing cavity size of CDs, as reported in literature [19]. The HC-αCD and HC-HPβCD complexes show a linear trend that indicates a 1:1 stoichiometry ratio. Likewise, a linear trend was observed up 20 mM and 10 mM γ-CD and β-CD concentration, respectively. It is worth noting that a "B_S" type profile was observed for HC-γCD and HC-βCD complexes at higher CD concentration. This indicates the occurrence of complex aggregation takes place, therefore causing an overall decrease in HC solubility. In the case of HC-αCD, an "A_L" type profile with a linear trend was observed, with HC water solubility that does not increase significantly, thereby confirming a lower complexation efficiency [19]. The same results were confirmed with UHPLC measurements, as showed in Fig. 1b, thus confirming the robustness of the method.

3.2. Rheological experiments

Results of rheological experiments are displayed in Fig. 2.

Viscosity profiles of saturated HC solution and HC-HPβCD solutions at 66 mM showed a Newtonian behaviour in the shear rate range examined.

The formation of host–guest inclusion complex between HC and HPβCD resulted into a little increase in formulation viscosity. More in detail, viscosity values increased from 3.1•10^{-3} Pa s to 3.5•10^{-3} Pa s, at a fixed shear rate value of 10 s^{-1}. This is an important result since formulation viscosity can affect the volume/weight of a drop dispensed by eye drop and, consequently, drug dosage.

3.3. Results of scanning electron microscopy (SEM)

SEM is commonly employed to qualitatively assess the formation of CD/drug inclusion complexes by comparing microscopic images of CDs, the drug, and the inclusion complexes [23]. Fig. 3 showcases SEM

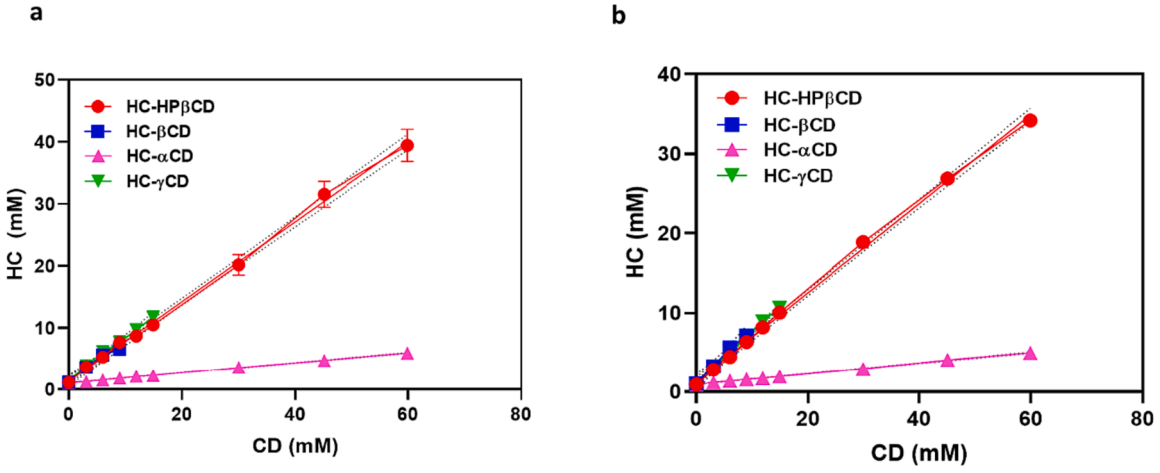

Fig. 1. Phase solubility experiments by UV (a) and UHPLC (b) analysis of HC-HPβCD, HC-βCD, HC-αCD, HC-ɣCD. CD concentration: 3.29 mM, 6.60 mM, 9.89 mM, 13.19 mM, 16.54 mM, 32.93 mM, 49.46 mM, 66 mM.

Table 1
Binding constant values, slopes, and correlation coefficients of HC interaction with HPβCD, βCD, ɣCD, αCD at 37 °C.

HC-CD	K_b (M^{-1}) ± SD	Slope UV	Correlation coefficient - UV	Slope UHPLC	Correlation coefficient - UHPLC
HC-HPβCD	1608 ± 180	0.664	0.993	0.560	0.998
HC-βCD	1590 ± 266	0.609	0.450	0.606	0.300
HC-ɣCD	1620 ± 103	0.739	0.197	0.642	0.698
HC-αCD	219 ± 2	0.084	0.998	0.067	0.998

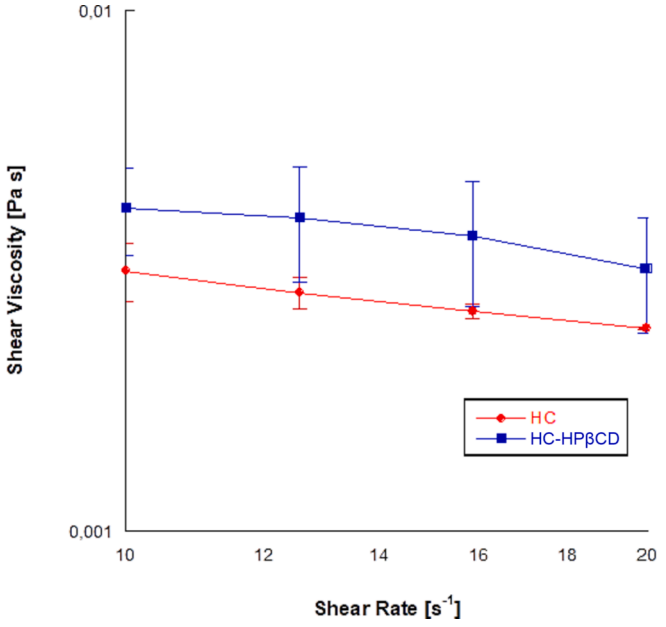

Fig. 2. Rheograms of aqueous solutions of HC and HC-HPβCD inclusion complex.

micrographs of raw materials, namely HC, βCD, HPβCD, and the inclusion complexes HC-βCD and HC-HPβCD, at CD concentrations of 33 and 66 mM. The micrographs clearly exhibit a distinct structure of HC, indicated by its sharp edges and characteristic lamellar crystalline habitus. Fig. 3C and 3G display the images of βCD and HPβCD alone, respectively. β-CD exhibits an irregular structure with blocky features, blunt edges, and rough surfaces, while HPβCD appears roughly spherical and porous. In contrast, the inclusion complexes of both cyclodextrins exhibit significant changes in shape and size, with the disappearance of the CD morphology due to the formation of inclusion complexes. Specifically, the SEM image of CD/βCD inclusion complexes indicates the formation of lamellar structures, while CD/HPβCD inclusion complexes display irregular and diverse bulk crystal structures with loose connections observed on the CD surface. These morphological alterations are attributed to the formation of the inclusion complexes at both CD concentrations [24].

3.4. DSC studies

DSC is frequently employed to evidence the differences in the physical mixtures between CDs, the drug, and the putative inclusion complex. In this work, filtered and lyophilized solutions obtained from phase solubility studies were analyzed by DSC under the conditions described in Section 2.7. HC and CDs curves, with the relative T_{onset}, ΔH and $T_{melting}$ values, have been reported in Fig. 4, Fig.S2 and Table 2. HC showed a sharp endothermic peak while CDs showed a wider peak, associated to the loss of absorbed water and water of crystallisation. Fig. 4 displays the DSC traces of the putative HC-CDs complexes. The endothermic peaks of CDs and complexes occurs between 91.6 and 144 °C, which is consistent with the literature [25]. An interesting behaviour was observed for αCD, which is characterized by two different endothermic peaks, correlated to water loss and phase change in anhydrous αCD, as observed in this study and reported in literature [26]. In particular, the cohesion of these two peaks was observed during DSC analysis of complexes between HC and αCD. This behaviour can be reasonably associated to the strong interaction between the two partners which does not allow an efficient release of HC. In conclusion, DSC studies show that inclusion complexes formation occurred since the melting peak of HC, at 227 °C, is no longer visible.

To further investigate the thermodynamic behavior of HC-loaded CDs, we have performed DSC tests on the lyophilized powder at different CD concentrations. Results evidenced that an increasing trend of absorbed heat with increasing CD concentrations (**Figure S4**). This has been ascribed to the loss of absorbed and crystallization water into the fraction of CDs which did not interact with HC.

3.5. ITC studies

ITC is the main methodology to characterize any kind of biomolecular interaction, determining the heat changes occurring upon a binding process. ITC measurements estimate with high precision the binding affinity (K_b), the binding stoichiometry (n) and the binding enthalpy change ($\Delta H°$). The other parameters ($\Delta S°$ and $\Delta G°$) can be easily calculated by the well-known thermodynamic relationships. ITC is the most widely applied methodology to explore the molecular forces in the interaction between cyclodextrins and appropriate guest molecules to identify the best inclusion complex. The results of complexation between HC and CDs, obtained with phase solubility and DSC experiments, were confirmed by ITC measurements. In particular, the affinity constant obtained from calorimetry experiments agrees with that calculated from phase solubility experiments. ITC measurements were performed for every CDs at 25 °C. Results showed that αCD and γCD did not match a defined experimental model. More precisely, for αCD no complex formation was observed; for γCD an interaction was observed but not a defined binding constant could be extrapolated. In general, the calorimetric data did not show the typical binding shape (Fig. S3), in the range of concentrations we were able to explore, and this did not allow the determination of constants for the complex formation. For these reasons, no other experiments were carried out. Thermodynamic parameters for the interaction with HPβCD and βCD at 25 °C (affinity constant K_b, standard enthalpy $\Delta H°$, entropy $T\Delta S°$ and Gibbs energy $\Delta G°$) are shown in Table 3 and Fig. 5 a,b. The interaction of HC with HPβCD and βCD matches a 1:1 pattern and ITC measurements were also obtained at 37 °C, for a comparison with K_b obtained from phase solubility experiments (Fig. 5 c,d), but above all in view of a formulation intended for ophthalmic administration. These thermodynamic parameters were quite comparable at both temperatures as better highlighted in Fig. 6. However, for the interaction of HC with HPβCD the binding constant at 37 °C was an order of magnitude higher than that at 25 °C and was higher than that of interaction of HC with βCD. Further, the binding constants determined by ITC were higher than that obtained by phase solubility measurements. This discrepancy was also found in another paper on HPβCD-host interaction [27] and was attributed to the very different concentration ranges, phase solubility works under drug saturation conditions and in non-ideal solution, both conditions could favour the aggregates, not present in the ITC measurements, performed

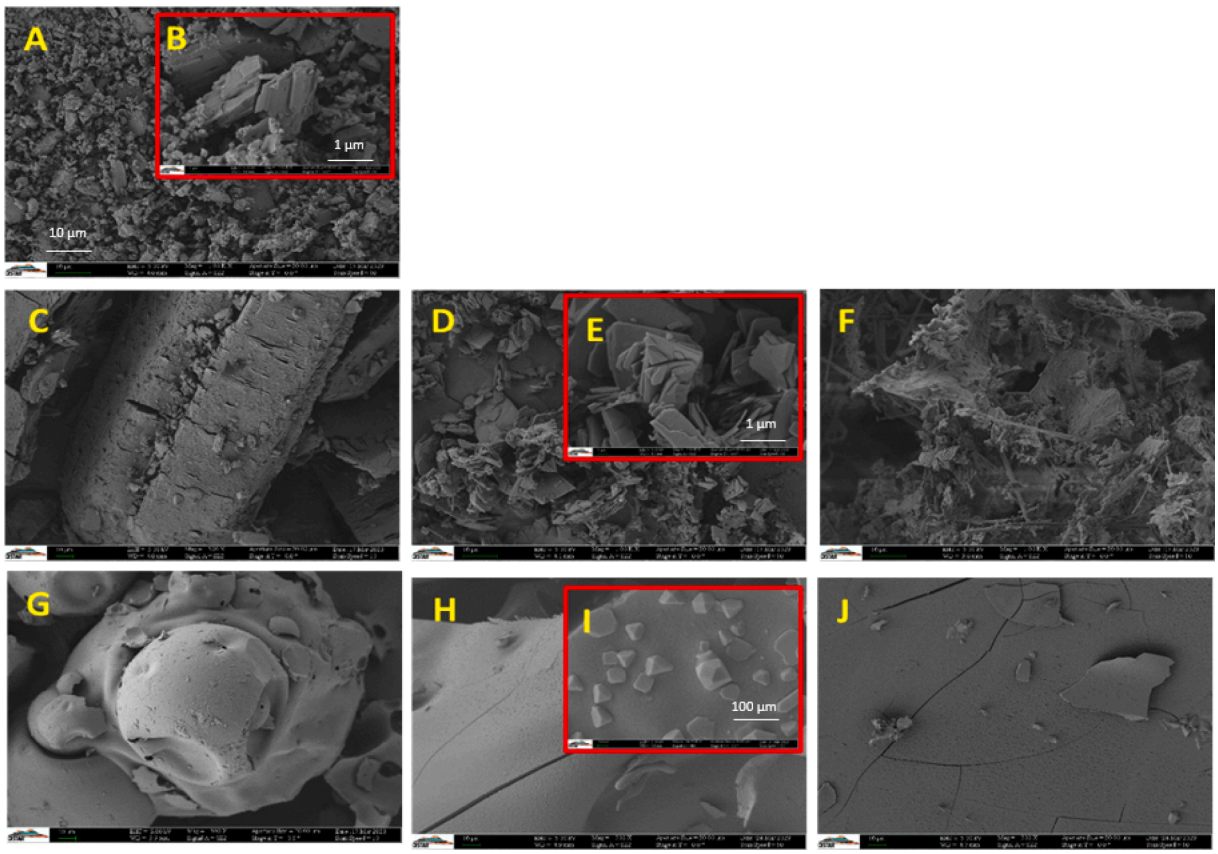

Fig. 3. Scanning electron microscopy images of HC (A and B); bare βCD (C), HC-βCD inclusion complex at 33 mM (D and E) and 66 mM cyclodextrin concentration; bare HPβCD (G) HC-HPβCD inclusion complex at 33 mM (H and I) and 66 mM cyclodextrin concentration (J). The bar is 10 μm. In Figs. (B) and (E) the bar is 1 μm, while in inset (I) it is 100 μm.

instead in dilute solution conditions. As regards the energetic of interactions, inclusion complex formation between HC and HPβCD or βCD was enthalpically (exothermic process) and entropically (increased entropy) favored, as already found in the past for other CD-drug inclusion complexes [28,29]. Inspection of Table 3 shows that enthalpy values are not very high, but in line with other host/guest ITC studies [30] and the entropic contribution (in modulus) for HC-HPβCD is greater than enthalpic one at both temperatures. The favourable enthalpic contribution ($\Delta H^\circ < 0$) to Gibbs energy is generally ascribed to the formation of new hydrogen bonds, electrostatic forces and van der Waals interactions. The entropy contribution is instead attributed to hydrophobic interactions ($\Delta S^\circ > 0$) and reduction of conformational freedom degrees ($\Delta S^\circ < 0$), following the inclusion process. However, for all types of interactions, the presence of the water as a third party is of fundamental importance. In the case of hydrophobic interactions, the release of ordered water from the hydration shell of interacting hydrophobic molecules involves the loss of hydrogen bonds that leads to an unfavourable enthalpy change ($\Delta H^\circ > 0$) and to a favourable entropy change ($\Delta S^\circ > 0$) [31]. The delicate balance of all these water-mediated interactions gives slightly but clear predominance of HC-HPβCD interaction at 37 °C and indicates HPβCD as the best cyclodextrin to encapsulate HC. Thermodynamic parameters are in very good agreement with those reported in literature for other HP-β-CD-guest interaction [30]. At 25 °C, the binding constant K_b falls within the same order of magnitude as those reported in other papers [32,33], but it exceeds by one order of magnitude the K_b value found in other studies [34,35].

Generally, the binding constants for CD-guest interaction are relatively low with respect to those of other biomolecular interactions, but this is an advantage for an effective drug release.

4. Concluding remarks

This work evaluated the inclusion of HC into the cavity of both native and semi-synthetic CDs along with the effect of ethanol on the complex formation process. The complexation of HC with HPβCD, αCD, βCD and ɣCD was more effective in water than in water:ethanol mixture (70:30) as shown by phase solubility experiments supported by UHPLC outcomes. Both HC-γCD and HC-βCD complexes in PBS exhibited a "B_S" type profile, indicating that the use of these CDs does not enhance HC solubility due to precipitation at higher CD concentrations. On the other hand, HC-αCD and HC-HPβCD complexes displayed an "A_L" type profile, suggesting that even at higher CD concentrations, saturation did not occur. Complex formation was also qualitatively confirmed by DSC and SEM results.

ITC experiments were conducted to explore the driving force behind the improved HC-CD interaction. Both HC-HPβCD and HC-βCD complex formations demonstrated a 1:1 stoichiometric ratio and were thermodynamically favored in terms of enthalpy and entropy. HC-HPβCD complex exhibited the highest binding constant at 37 °C, with a greater entropic contribution than the enthalpic one, thereby emphasizing the crucial role of hydrophobic interactions. Overall, these findings indicate that HPβCD is the most effective CD among those studied in enhancing HC solubility under pseudo-physiological conditions.

In summary, thermodynamic studies are essential for guiding slight yet essential adjustments in solution conditions to promote hydrophobic interactions and improve the affinity of HC for HPβCD. This feature is remarkably important to provide valuable insights into the fundamental interactions between HC and CDs, which can inform the design of future drug delivery systems.

The increased solubility of HC due to the formation of inclusion

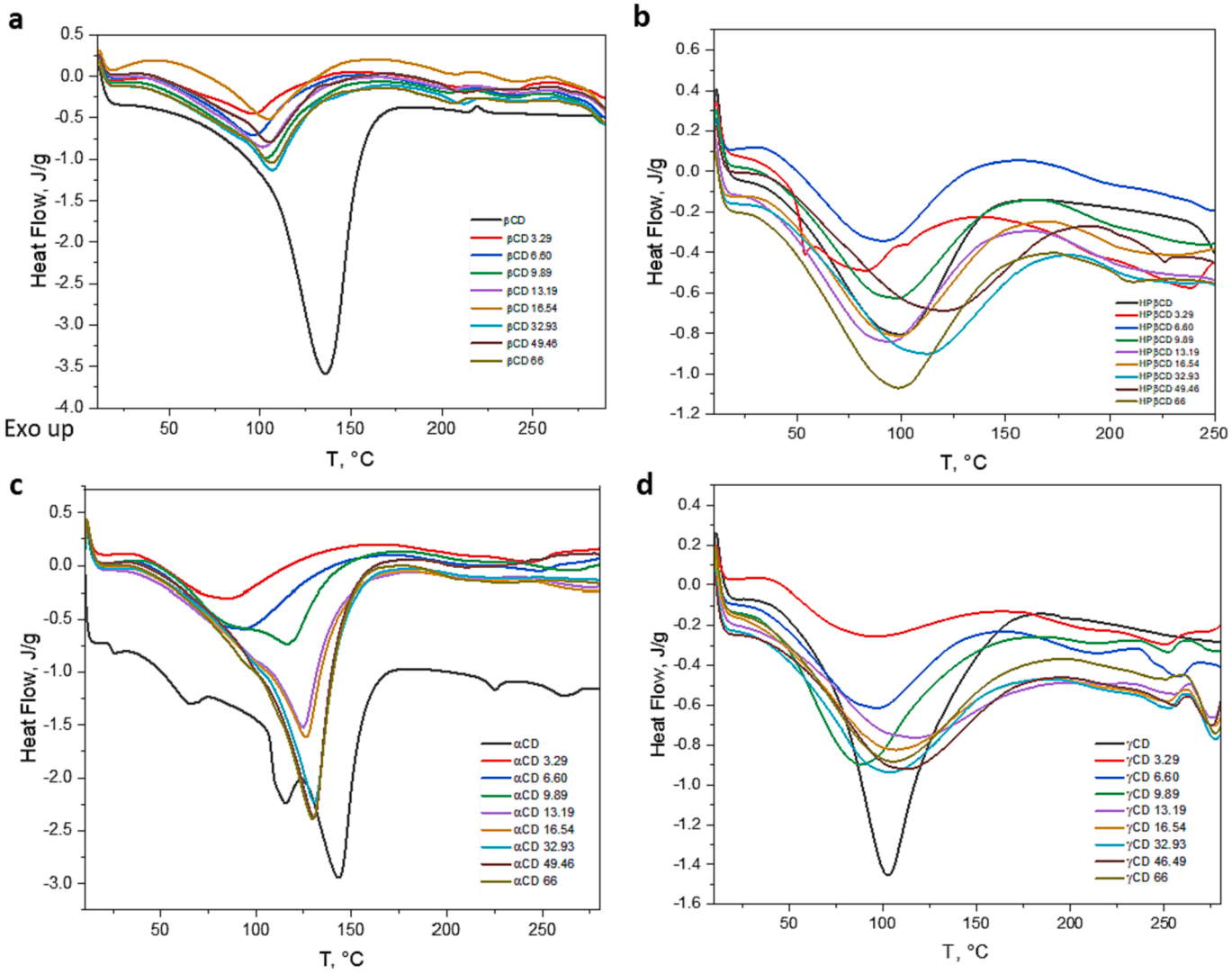

Fig. 4. DSC traces of inclusion complex between HC and βCD (a), HC and HPβCD (b), HC and αCD (c), HC and γCD (d) by employing different concentration of CDs: 3.29 mM, 6.60 mM, 9.89 mM, 13.19 mM, 16.54 mM, 32.93 mM, 49.46 mM, 66 mM. Exothermic heat flow is directed upwards.

Table 2
DSC data of HC, HPβCD, βCD, γCD, and αCD and of complex formation for HC–HPβCD, HC-βCD, HC-γCD.

Compound	T_{onset} (°C)	ΔH (kJ mol^{-1})	T_m (°C)
HC	225.0 ± 0.1	134.0 ± 0.6	227.0 ± 0.3
HPβCD	47.7 ± 2.9	125 ± 1.3	100.0 ± 4.3
βCD	102.0 ± 0.6	379.0 ± 5.2	135.0 ± 0.1
γCD	68.2 ± 5.4	205.0 ± 1.0	103.0 ± 1.2
αCD	107.0 ± 0.4	143.0 ± 3.3	144.0 ± 1.1
HC-HPβCD	41.6 ± 0.4	70.4 ± 3.4	91.6 ± 3.5
HC-βCD	56.6 ± 0.2	99.6 ± 24.9	97.2 ± 2.6
HC-γCD	41.9 ± 1.3	83.7 ± 6.5	96.1 ± 0.3
HC-αCD	44.4 ± 5.2	92.5 ± 7.6	94.3 ± 1.3

Table 3
ITC thermodynamic parameters for the association of HC with HPβCD and βCD at 25 °C and 37 °C.

HC-HPβCD	**K_b (M^{-1})**	***n***	**$\Delta H°$ (kJ/mol)**	**$T\Delta S°$ (kJ/mol)**	**$\Delta G°$ (kJ/mol)**
25 °C	(8.6 ± 2) 10^3	1	−6.0 ± 0.5	16.4 ± 1	−22.7 ± 1
37 °C	(4.9 ± 1.6) 10^4	1	−4.2 ± 0.2	23.6 ± 0.4	−27.9 ± 0.4
HC-βCD	**K_b (M^{-1})**	***n***	**$\Delta H°$ (kJ/mol)**	**$T\Delta S°$ (kJ/mol)**	**$\Delta G°$ (kJ/mol)**
25 °C	(1.9 ± 0.9) 10^4	1	−12.7 ± 0.5	11.8 ± 1	−24.5 ± 1
37 °C	(1.7 ± 0.8) 10^4	1	−11.6 ± 0.1	13.5 ± 1.2	−25.2 ± 1.2

complexes allows for eye drops with higher concentrations of the active molecule. This, in turn, is expected to favor the transport of the active ingredient to the intravitreal space, thereby improving local bioavailability of HC. These findings hold significant implications for the development of effective HC formulations for ocular delivery. The improved solubility of HC is expected to enhance the treatment of ocular inflammatory and autoimmune disorders. However, it is important to note that while CDs enhance the solubility and bioavailability of HC, long-term administration of corticosteroids can cause an elevated intraocular pressure, which is associated to an increased risk of glaucoma. Hence, in certain formulations, it is critical to prioritize the improvement in bioavailability mainly in the outermost eye regions. Future *in vitro* and *in vivo* experiments will be conducted to evaluate the safety of using the obtained complexes.

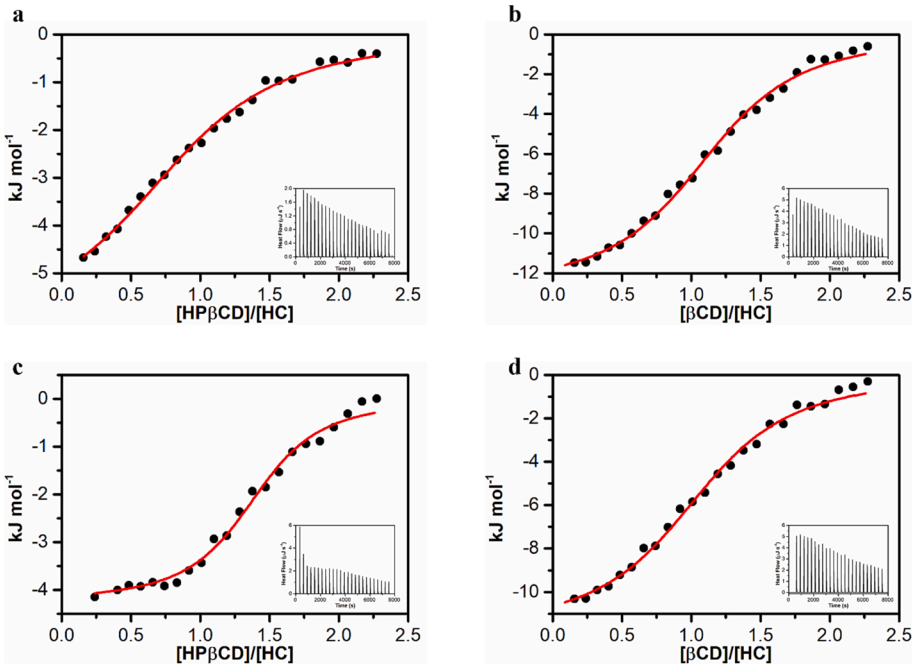

Fig. 5. ITC binding curves for HC-HPβCD (a) and HC-βCD (b) interaction at 25 °C and for HC-HPβCD (c) and HC-βCD (d) at 37 °C. In the inset the raw data are shown.

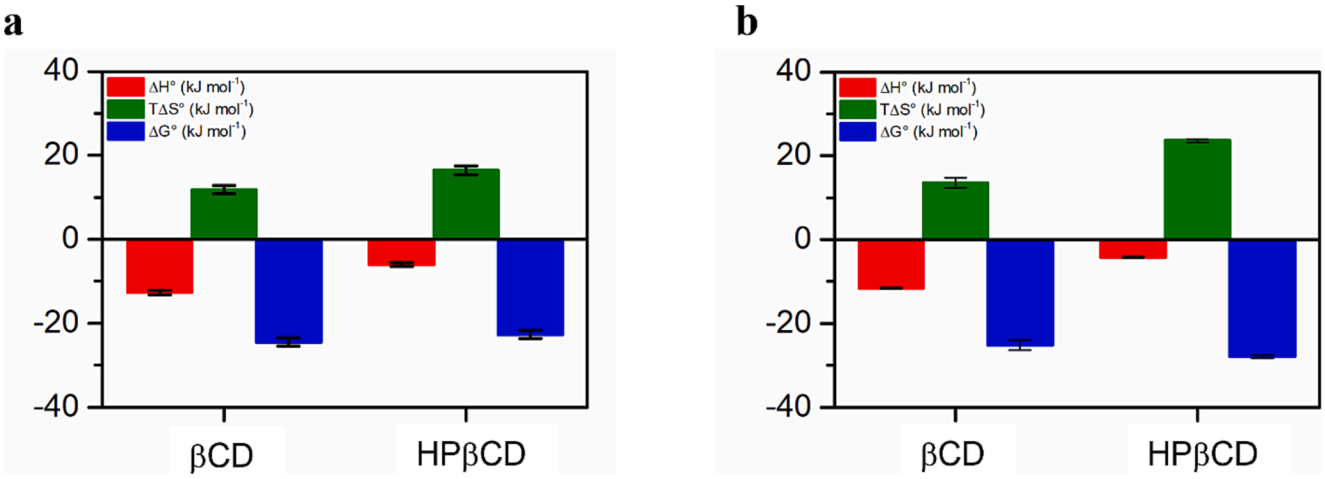

Fig. 6. Thermodynamic signature for the inclusion complex formation for HC-HPβCD and HC-βCD at 25 °C (a) and 37 °C (b).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.molliq.2023.123031.

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