Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 127 (2014) 355–360

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

journal homepage: www.elsevier.com/locate/saa

Spectroscopic characterization of both aqueous and solid-state diacerhein/hydroxypropyl-β-cyclodextrin inclusion complexes

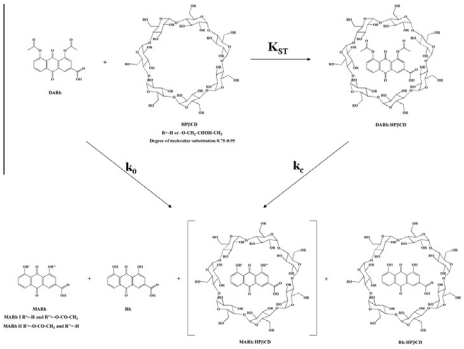
Stefania Petralito [a,1], Iacopo Zanardi [b,1], Romina Spera [a], Adriana Memoli [a], Valter Travagli [b,*]

[a] Dipartimento di Chimica e Tecnologie del Farmaco, Sapienza Università di Roma, Roma, Italy
[b] Dipartimento di Biotecnologie, Chimica e Farmacia, Università degli Studi di Siena, Siena, Italy

HIGHLIGHTS

- Diacerhein (DARh) is a prodrug of the IL-1 converting enzyme inhibitor Rhein (Rh).
- The interaction of DARh with HPβCD has been studied by molecular spectroscopy.
- Both the free and the complexed DARh can undergo hydrolysis to generate Rh.
- DARh and Rh have some coincident spectroscopic parameters.
- Accurate experimental and spectroscopic settings are essential parameters.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 22 July 2013
Received in revised form 4 December 2013
Accepted 11 February 2014
Available online 28 February 2014

Keywords:
Diacerhein
Cyclodextrins
Hydrolytic degradation
Binding constants
Molecular spectroscopy

ABSTRACT

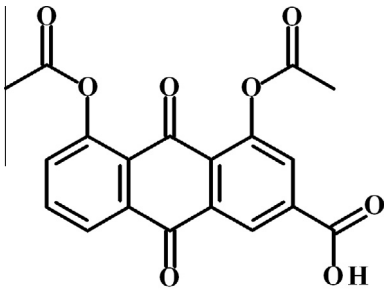
Diacerhein, a poorly water soluble antirheumatic prodrug, was spectroscopically characterized to form inclusion complexes with hydroxypropyl-β-cyclodextrin (HPβCD) in both aqueous solution and in solid phase. Complexation with the hydrophilic carriers was used to improve the solubility and dissolution rate of the compound. The kinetics of the prodrug degradation to the active rhein in aqueous buffer solution were also investigated as a function of HPβCD concentration. The solid complexes prepared by different methods such as physical mixture, kneading, co-evaporation method and freeze dried method in 1:1 M ratio, were characterized by DSC and FTIR. The dissolution profiles of solid complexes were determined and compared with diacerhein alone and their physical mixture, in the simulated intestinal fluid at 37 °C. The accurate molecular spectroscopic characterization of diacerhein in the presence of different amounts of aqueous cyclodextrins was essential to determine the correct binding constants for the diacerhein/HPβCD system. The binding constants were also validated by UV spectrometry and HPLC procedure in order to compare the values from the different methods. Higuchi–Connors phase solubility method has proved not suitable when either the free or/and the complexed prodrug degrade in aqueous solution.

Introduction

Diacerhein or diacetylrhein or diacerein (4,5-diacetyloxy-9, 10-dioxoanthracene-2-carboxylic acid, see Fig. 1) [1], hereinafter referred to as DARh, is an anthraquinonic drug used for the treatment of osteoarthritis, mainly as a prodrug after metabolic transformation to the active Rhein (Rh) [2,3]. Recently, increasing

* Corresponding author. Address: Dipartimento di Biotecnologie, Chimica e Farmacia, Università degli Studi di Siena, Via Aldo Moro 2, 53100 Siena, Italy. Tel.: +39 0577234317; fax: +39 0577234254.
E-mail address: valter.travagli@unisi.it (V. Travagli).
[1] Both the authors equally contributed to this work.

http://dx.doi.org/10.1016/j.saa.2014.02.055
1386-1425/



DARh

Fig. 1. Structural formula of DARh.

attention was devoted to the potential off-label therapeutic properties of DARh, including anti-oxidative, anti-inflammatory, anti-leishmanial and in prevention of alcohol-induced liver diseases [4–7]. DARh is practically insoluble in water at acidic and neutral pH, while its solubility increases in alkali. The latter environment, however, lead to a very fast hydrolytic degradation, with production of Rh [8]. Because of the low water solubility, further pharmacological development and practical application of the DARh itself are limited. However, the formation of cyclodextrin derivatives for achieving an enhanced aqueous solubility is experiencing a growing interest [9,10]. In addition to the enhanced solubility, another advantage for using DARh/cyclodextrin complex could rise from the control of the hydrolytic reaction into Rh, limiting its side effects and consequently improving the poor tolerability of oral DARh therapy. However, the DARh/Rh conversion should be adequately taken into account for a correct host–guest evaluation, also in light of recent information about the solubility and spectroscopic properties of Rh/cyclodextrin inclusion complex [11]. Therefore, a deepen investigation about the effect of cyclodextrins on the hydrolysis rate of DARh in aqueous solution has been reputed of particular interest. The aim of this work was to study the effect of hydroxypropyl-β-cyclodextrin (HPβCD) complexation on the solubility and hydrolytic pattern of DARh by both spectroscopic and chromatographic techniques. Moreover, for a comprehensive characterization, solid-state complexes were prepared by freeze-drying, co-evaporation, and kneading methods to confirm the occurred complexation and to verify the influence of preparation technique on improvement of DARh/HPβCD complex dissolution in aqueous media. Selective physicochemical determinations based on differential scanning calorimetry (DSC) and Fourier Transformed-IR (FT-IR) were also investigated.

Materials and methods

Materials

DARh (lot no. F07198901.0.01) was provided by TRB Chemedica (Switzerland) and HPβCD (Kleptose® HP, MW = 1400, degree of molar substitution 0.75–0.95, batch number 813447) was the generous gift from Roquette (France). All other materials and solvents used were of analytical grade or purer. Freshly distilled water was used throughout the experiments.

In solution studies

Hydrolytic degradation studies

A stock solution of DARh (10^{-3} M) was prepared in DMSO. A volume of 2.5 mL of the stock solution was added to the pH 7.2 ± 0.1 phosphate buffered saline (PBS) containing HPβCD (range 0–100 mM) to make a final volume of 25 mL (10^{-4} M DARh). Samples were kept in the dark thermostatted at 37 ± 0.5 °C and the hydrolysis reaction of DARh to Rh was monitorated by UV–vis (for both DARh and Rh) and fluorescence spectra (only for Rh) [11]. Absorption spectra were recorded on a Perkin Elmer UV–vis Lambda 25 spectrophotometer at 344 nm, corresponding to the maximum absorption wavelength of DARh, using 1 cm quartz cell. Fluorescence measurements were carried out by a Perkin–Elmer LS50B fluorimeter using 1 cm quartz cell, slit width was 5, excitation and fluorescence emission wavelength of 435 nm. In both cases, a software was used for data storage and processing. Different calibration curves, for each cyclodextrin concentration, were used to calculate Rh formation and DARh disappearance in solution. The observed first-order rate constant (K_{obs}) for the degradation was obtained from a non linear regression analysis of [DARh]/[DARh]$_0$ (where [DARh] is the concentration at a given time t and [DARh]$_0$ is DARh initial concentration) plotted vs. time [12]. All measurements were carried out at least in triplicate.

Stability constant determinations

Spectroscopic studies. The concentration of DARh in buffered solution pH 7.2 both in the presence and in the absence of HPβCD, was measured by absorbance (see above). Different calibration curves, for each cyclodextrin concentration, were used to calculate DARh concentration. Spectroscopic properties of DARh in the presence of HPβCD were also used to calculate association constant K^{I}_{st} from UV–vis data by using a linear curve fitting procedure (Eq. (1)) [13].

$$Abs = \frac{\left(\varepsilon_0 + \varepsilon_1 \times K^{I}_{st} \times [\text{HP}\beta\text{CD}] \times [\text{DARh}]\right)}{1 + K^{I}_{st} \times [\text{HP}\beta\text{CD}]} \quad (1)$$

A 1:1 inclusion complex for DARh/HPβCD interaction was assumed, because it is the most commonly claimed and usually justified stoichiometric ratio for CD–drugs complexes [14], as well as it had been also verified in the case of interaction between Rh/CDs [11].

For all absorbance measurements, aliquots of fresh DARh stock solution was poured into quartz cells of 10-mm path length (capacity about 4 mL) and then HPβCD from 0 to 100 mM was added drop wise into cuvette and diluted to the final volume and desired concentration. The solutions were stirred for 15 min and then immediately analyzed. All measurements were carried out at least in triplicate.

Kinetic determination: hydrolysis reaction. In the systems in which the 1:1 stoichiometry is assumed, it is possible to use the influence of the cyclodextrin in the hydrolysis rate of the compound to calculate the complex stability, K^{II}_{st} with the kinetic method [15,16] and Eq. (2) can be used:

$$\Delta K_{obs} = K^{II}_{st}\left(1 - \frac{\Delta K_{obs}}{\Delta k_c}\right)\left([\text{HP}\beta\text{CD}] - \frac{[\text{DARh}]\Delta K_{obs}}{\Delta k_c}\right)k_c \quad (2)$$

where $\Delta K_{obs} = k_0 - K_{obs}$ and $\Delta k_c = k_0 - k_c$, with k_0 representing the hydrolysis rate constant for the non-catalyzed reaction (i.e. in the absence of HPβCD), k_c the hydrolysis rate constant of the guest in the form of the inclusion complex and K_{obs} the experimental hydrolysis rate constant determined at the different HPβCD concentrations. The K^{II}_{st} and k_c values were obtained by non-linear fitting of the K_{obs} data using the Gnuplot software package 4.0 [17].

HPLC-method. The chromatographic experiments were carried out for the determination of the apparent association constant by using a Perkin Elmer series 200 LC controller Pump and a Perkin Elmer series 200 UV/vis detector (detection: 344 nm for DARh). A reversed phase column Chromasyl 100 C18 5 μm 250 × 4.6 mm (Higgins Analytical) was used with methanol–water (30/70 v/v),

in which HPβCD was dissolved at different concentrations (0–25 mM) as mobile phase (flow rate: 0.7 mL/min; elution time: 15 min). The chromatographic experiments were carried out at 22 ± 0.5 °C. The DARh concentration in the injected solution was 10^{-4} M and the injection volume was 20 μL in all experiments. All measurements were carried out at least in triplicate. The retention behavior of DARh was dependent by the drug partition coefficients between the mobile and stationary phases. In the presence of cyclodextrins, there is an additional contribution in the elution behavior due to the complexation process and the capacity factors for DARh were monitored in the presence of increasing concentration of HPβCD. The apparent stability constant of the complex, K_{st}^{III}, was determined using Eq. (3) [18]:

$$\frac{1}{k'} = \frac{1}{k'_s} + \frac{K_{st}^{III}[\text{HP}\beta\text{CD}]^x}{k'_s} \quad (3)$$

where k' is the capacity factor at each cyclodextrin concentration, [HPβCD], k'_s is the solute capacity factor in the absence of cyclodextrin, and the exponent x is the stoichiometry coefficient. For a 1:1 stoichiometry complex, a plot of $1/k'$ vs. [HPβCD] yields a straight line and K is obtained from the slope-to-intercept ratio.

Solid systems

Sample preparation

Solid inclusion complexes of DARh and HPβCD were prepared in 1:1 M ratios by: physical mixture (PM), kneading method (KN), co-evaporation (COE) and freeze-drying technique (FD). DARh and HPβCD were triturated in mortar with small volume of a solvent mixture of water:ethanol to obtain a kneading binary system. The thick slurry was kneaded for 60 min and then dried at 45 °C until constant weight. The dried mass was pulverized and sieved through mesh #100. Co-evaporated inclusion complex of DARh and HPβCD in 1:1 M ratio was prepared by dissolving the prodrug in a solvent such as methanol and the cyclodextrin in water, separately. The latter was then added to the former and stirred to achieve equilibrium. The resulting volume was filtered through 0.45 μm filter and then dried by a rotary evaporator (Buchi Rotavapor-KRvr 65/45). Freeze-drying inclusion complex of DARh and HPβCD in 1:1 M ratio was prepared by dissolving the drug in aqueous solution of HPβCD. The suspension was stirred and then filtered through 0.45 μm filter, freezed in N_2 liquid and lyophilized by a LIO5P apparatus (Cinquepascal, Milan, Italy) equipped with an Edwards RV5 vacuum pump, consecutively. The physical mixture (PM) of DARh and HPβCD in 1:1 M ratio was prepared by individually mixing the components.

Characterization

DSC (thermal analysis)

Differential scanning calorimetry (DSC) measurements of the pure materials (DARh and HPβCD), of the binary systems and PM were carried out using a calorimeter (Setaram Instrumentation, Caluire, France), model DSC 131. The thermal behavior was studied by heating all accurately weighed samples (6 mg) in a pierced aluminum crucible from 50 to 350 °C. All measurements are made under a nitrogen flow of 20 cm^3/min at the scan rate of 10 °C/min using an empty pan as reference. Indium (99.98%, mp 156.65 °C) was used as standard for calibrating the temperature.

FT-IR

The infrared spectra were obtained with a Fourier transform infrared (FTIR) spectrophotometer (Paragon 1000 FT-IR, Perkin Elmer). The samples of pure drug, CD, PM and inclusion complexes, were prepared by the potassium bromide disc method and scanned for absorbance 4000–400 cm^{-1}; the resolution was 2 cm^{-1} and the spectra used were the result of averaging 100 scans.

Dissolution studies

The dissolution profile of DARh alone, PM and of its inclusion complexes were studied using USP XXIII dissolution test for solid oral dosage forms test with a paddle stirrer (apparatus II type). The sample, corresponding to 50 mg of the prodrug (alone or in its inclusion complex) were placed into 1000 mL of pH 7.2 ± 0.1 phosphate buffer used as dissolution medium to simulate intestinal medium. The stirring speed of the paddle was 100 rpm, and the temperature was maintained at 37 ± 0.5 °C. The samples were withdrawn at regular time intervals, filtered through Whatman polycarbonate filter paper and analyzed by UV spectrophotometer at 344 nm to spectrophotometrically determine the amount of DARh dissolved. The volume of each sample taken out was replaced by fresh dissolution medium and the actual DARh concentration was corrected. The dissolution experiments were conducted, at least in triplicate, for a time equal to 1 h, based on preliminary experiments showing that in these experimental conditions, a significant hydrolysis of DARh to Rh at pH 7.2 would take place only after 60 min.

Results and discussion

In solution studies

Hydrolytic degradation studies

As can be seen from Fig. 2, the UV–vis spectra of 10^{-5} M DARh and Rh solutions are different for the absorption maximum at about 345 nm and 435 nm, respectively. On the contrary, they have in common the absorption maximum around 260 nm. Moreover, it is useful to recall that only Rh emits fluorescence if excited at 435 nm.

Thus, by following spectrophotometrically both the decrease of DARh peak at 343 nm and the increase of Rh peak at 435 nm vs. time, as well as fluorimetrically the appearance of the two emission fluorescent peaks at 513 nm and 580 nm due to the Rh formation with excitation at 435 nm, it is possible to study the hydrolysis reaction [11]. In fact, DARh is decomposed at basic and acid pH values to Rh. The hydrolytic degradation studies, performed at neutral pH, evidenced such a temporal pattern of DARh hydrolysis, at the experimental conditions adopted (Fig. 3). Moreover, the influence of HPβCD (0–100 mM) on the hydrolytic degradation of DARh at pH 7.2 was investigated. As previously stated, the first-order kinetic degradation of DARh can be expressed either in a direct manner by the decrease of concentration of DARh with time

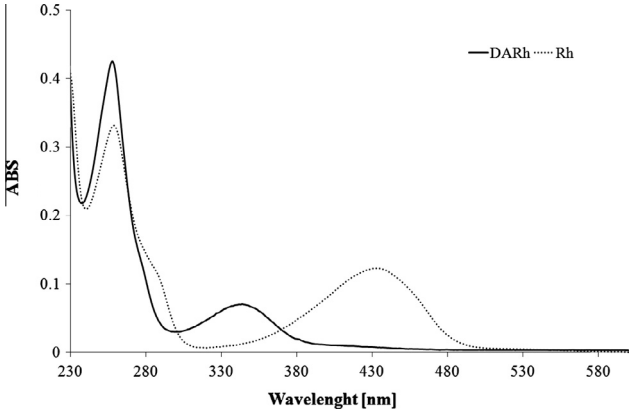


Fig. 2. Full UV–vis absorption spectra of Rh and DARh.

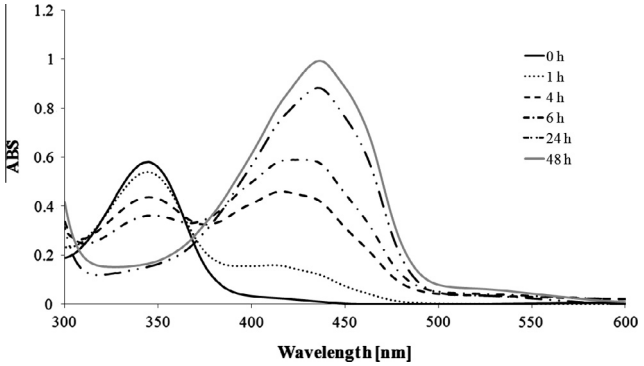


Fig. 3. UV–vis spectra of DARh vs. time: decrease of the absorbance of DARh at the maximum absorption wavelength (λ_{max} = 343 nm) and appearance of a peak corresponding to Rh formation (λ_{max} = 434 nm).

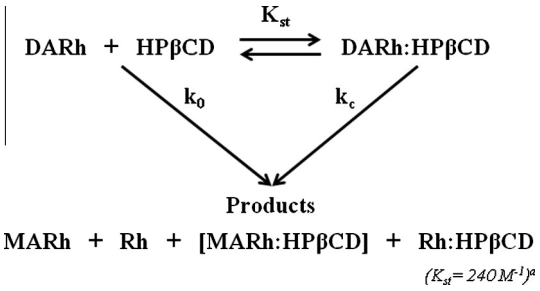


Fig. 4. Scheme of DARh/HPβCD complex formation in the presence of products derived from hydrolysis (MARh: monoacetylrhein; [a]see Ref. [11]).

($[DARh] = [DARh]_0 e^{-K^t_{obs}}$) or indirectly by using Rh spectroscopic data obtained by UV–vis spectroscopy and by fluorimetry of Rh according to the relation $[Rh] = [Rh]_0(1 - e^{-K^t_{obs}})$, where $[Rh]_0$ is the final Rh concentration corresponding to the initial concentration of DARh, [Rh] is the concentration of Rh at the t time degradation and K_{obs} is the first-order degradation rate constant. Fitting of $[Rh]/[Rh]_0$ values against t leads to the degradation rate constant values. K_{obs} values obtained by UV–vis measurements of DARh and by UV–vis and fluorescence measurements of Rh are substantially in agreement with each other. When no CD was present in solution the rate constant for the hydrolysis was 0.23 h^{-1}, while at 100 mM HPβCD an increase in stability more than 50% was observed (K_{obs} = 0.11 h^{-1}). A stabilizing effect on DARh hydrolysis reaction with increasing HPβCD concentration appears evident. Such a stabilization can be explained according with the inclusion of DARh molecule in the cyclodextrin cavity. Specifically, the hydrolysis kinetic follows the so-called "saturation behavior", consistent with 1:1 binding between DARh and HPβCD (Table 1). In detail, the rate of reaction changes with the increase of HPβCD concentration and the K_{obs} does not linearly relate to the increasing concentration of added HPβCD, but rather asymptotically approaches a minimum value [19,20]. Such a trend can be explained by the model of Fig. 4: when DARh is in solution, it comes into equilibrium with both HPβCD, to give the inclusion complex, and the hydrolysis products (monoacetylrheinate I and II, referred as MARh, and Rh), which in turn compete in the inclusion process with the HPβCD. Under the experimental conditions adopted, the DARh/HPβCD complex formation proves to be faster than hydrolysis processes.

Stability constant determinations

Spectroscopic studies. The UV–vis spectra of DARh in the presence of various HPβCD concentrations were also used to calculate the association constant, K^I_{st} (Eq. (1)). The value of K^I_{st} in the presence of HPβCD concentration (25–100 mM) in excess with respect to the guest one (DARh 10^{-4} M) is about 45 M^{-1}, resulting from the linear slope-to-intercept ratio (R^2 = 0.993). On the other hand, the same linear relationship validates the 1:1 stoichiometry of the inclusion complex. In such a condition, the Benesi–Hildebrand method [21] can also be applied to calculate the equilibrium constant of DARh/HPβCD complex. Evidence for the 1:1 complex formation was obtained by using the double reciprocal plot of Benesi–Hildebrand equation (see supplementary information as Fig. S1). For completeness' sake, in the presence of HPβCD a little absorption enhancement and a little bathocromic shift for the maximum at 344 nm have been observed (data not shown).

Table 1
Observed first-order rate constant (K_{obs}) values for the DARh degradation based on the appearance of Rh.

HPβCD concentration (mM)	K_{obs} (min^{-1}) from UV–vis spectra	K_{obs} (min^{-1}) from fluorescence spectra
0	0.2315 ± 0.0058	0.2285 ± 0.0135
25	0.1444 ± 0.0063	0.1491 ± 0.0076
50	0.1158 ± 0.0043	0.1379 ± 0.0068
75	0.0966 ± 0.0038	0.1011 ± 0.0023
100	0.1068 ± 0.0053	0.1134 ± 0.0068

Kinetic determination: hydrolysis reaction. The determination of the stability constant of the complex DARh/HPβCD can be obtained by the kinetic method [12]. In order to calculate the degradation rate constant K_c for DARh within the cyclodextrin cavity, the data for HPβCD was fitted to the Eq. (2). The same K_c value of 0.07 h^{-1} was obtained both from absorption and fluorescence data. Such an equation can be also used to get the stability constant, K^{II}_{st}, of the DARh/HPβCD complex from the same absorption and fluorescence data and K_{st} values near to 50 M^{-1} and 75 M^{-1} were obtained, respectively. The agreement between the experimental data and the model is quite good, confirming both the formation of 1:1 complex and the proposed degradation scheme (Fig. 4).

HPLC method. The retention factors of DARh in the experimental condition adopted were weakly decreased with increasing concentration of HPβCD, according to the coexistence of two physicochemical processes: the solute complexation by HPβCD, and the transfer of uncomplexed solute from the hydro-organic phase to the stationary phase. In these conditions, the interaction of the DARh/HPβCD with the stationary phase can be considered as negligible [22], so it is not taken into account and the retention factors, k, for DARh can be derived from Eq. (3). The linear relationship between $1/k$ and HPβCD concentration, with correlation coefficients higher than 0.988 (data not shown), indicates that the behavior of these equilibria is well described by the model assuming a 1:1 stoichiometry of host–guest interactions. The apparent stability constant K^{III}_{st} has been calculated from the linear slope-to-intercept ratio, resulting equal to 22 M^{-1}.

In relation to the aqueous phase investigation, the evaluation of K_{st} 1:1 apparent stability constants for DARh/HPβCD complex carried out with different method leads to approximately comparable values: UV–vis spectrophotometry: 45 M^{-1}, kinetic determination: either 50 M^{-1} or 75 M^{-1}, depending on the spectroscopic method, and HPLC: 22 M^{-1}. In particular, the latter one shows how important it is to identify the chemical species that are in competition with the formation of the inclusion compounds. In fact, the values achieved by our studies are noticeably different from the DARh/HPβCD K_{st} 1:1 value of 194 M^{-1}, previously obtained by phase solubility studies [9]. On the other hand, such an extent is close to the

values (240, 243 and 350 M^{-1}) estimated for Rh/HPβCD complex by a variety of methods [11]. This outcome can be basically justified taking into account that for a right application of the phase solubility method, the equilibrium phase must be fully achieved requiring generally long experimental times (more than a week) and therefore the complete stability of the guest molecules during the analysis must be assured. In the phase solubility methods, the presence of HPβCD makes this process slower but the value of K_{st} calculated in these conditions results in any case wrong due to the transformation of DARh in Rh. Moreover, it is noteworthy that if only the absorbance of maximum in the UV region is considered, the hydrolytic degradation of DARh–Rh cannot be evidenced (Fig. 2) and the obtained inclusion parameters can be consequently inaccurate. Such erroneous experimental conditions have been reported in recent studies that have been previously cited, where the DARh inclusion parameters in cyclodextrins were evaluated at 258 nm [9,10].

Solid systems

Thermal analysis

The DSC diagrams of the binary system are compared with those of the starting compounds (DSC diagrams are shown in the supplementary information as Fig. S2). In such analysis, the disappearance of melting peak of the guest molecule in the binary system is considered to be the evidence of the occurred complexation; besides, the decrease of the endothermic peak associated to HPβCD dehydratation in comparison with that of the pure HPβCD as well as the shift of melting peak of the guest molecule are indicative of an interaction of the drug with the cyclodextrins. As it is possible to note, the curve referred to pure cyclodextrin presents a broad endothermic peak in the interval 79–116 °C (90 °C) that may be attributed to the loss of water; all binary systems show a decrease in this dehydration endothermic peak, observed in the ΔH value, indicating an interaction drug/HPβCD. As far as curve of binary systems obtained with physical mixture and kneading are concerned, a partial reduction of dehydration peak of HPβCD and only a decrease and a shift toward higher temperature of the melting point of the pure DARh are detected. This is a proof of the presence of the pure DARh in the PM and in the KN products that has not completely interacted within the CD. Finally the disappearance of endothermic peak of pure DARh (at onset 232 °C) is observed in the diagrams related to the binary systems obtained with the FD and COE methods, it is confirmed the strong interaction between CD and host investigated molecules.

FT-IR spectroscopy

The interaction between host and guest molecule was effectively characterized by FTIR spectroscopy (Fig. 5). The principal absorption peaks of DARh were observed at 3300 cm^{-1} (O—H, stretch, broad, COOH), 3069 cm^{-1} (C—H, stretch, aromatic), 2935 cm^{-1} (C—H, stretch, aliphatic, sym), 1770 cm^{-1} (C=O, stretch, ester), 1693 cm^{-1} (C=O, stretch, ketone), 1679 cm^{-1} (C=O, stretch, COOH), 1593 cm^{-1} (C=C, stretch, aromatic), 1450 cm^{-1} (C—O, stretch, COOH), 1026 cm^{-1} (C—O, stretch, ester), 760 cm^{-1} (m substituted benzene), and 704 cm^{-1} (benzene).

While spectra of products obtained with physical mixtures and kneading showed approximately a superposition of DARh and HPβCD spectra, the intensity and the shape of bands in the region 1770 cm^{-1} and 704 cm^{-1} in the spectra of binary systems obtained with the FD and COE methods changed and band assigned to carboxyl carbonyl stretching disappears; disappearance of carbonyl absorption disappearance of carbonyl absorption band assigned to carboxyl carbonyl stretching suggesting formation of H-bonds between carbonyl group of DARh and hydroxyl group of host cavity.

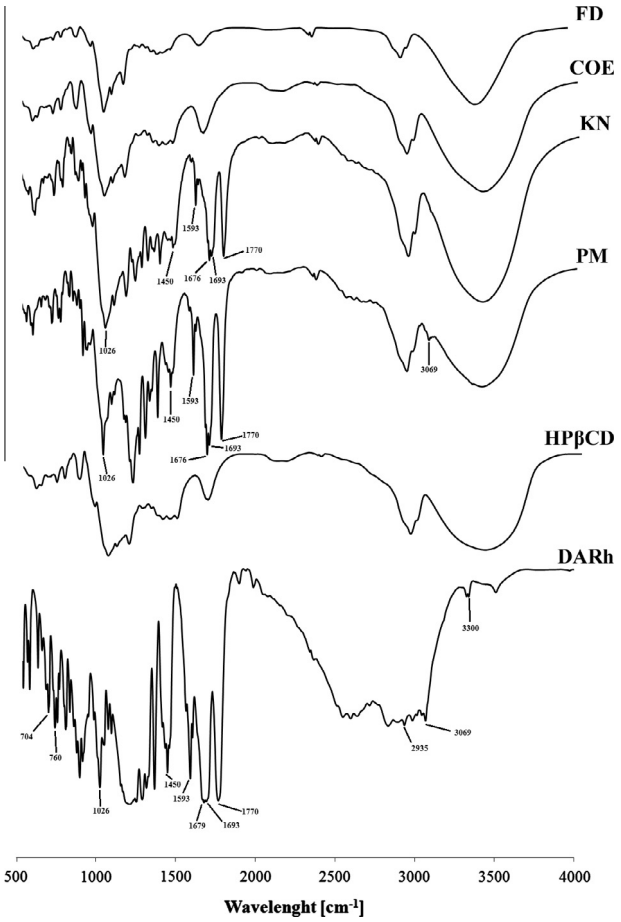


Fig. 5. Comparison of FT-IR spectra (transmittance, *T*%) of pure DARh, HPβCD, as well as solid inclusion complexes in 1:1 M ratios by physical mixture (PM), kneading method (KN), co-evaporation (COE) and freeze-drying technique (FD).

Dissolution studies

Dissolution studies have been done to study the effect of solid state complexation. The resulting dissolution profiles are given in the supplementary information (Fig. S3). All the prepared binary systems exhibit a drug dissolution rate faster than that of the pure DARh, with a dissolution time scale of a few minutes. In detail, the DARh dissolution % value at 5 min with respect to that of DARh amount was found to be approximately 100, 93.9, 72.29 and 51.98 for FD, CE, KN and PM, respectively. Furthermore, in the case of pure drug, only 55% of DARh was dissolved even after 60 min. The significant improvement in dissolution characteristics of the complexes was attributed to reduced interfacial tension between the solid particles of DARh and the dissolution medium, leading to greater rate of dissolution. The increase in the dissolution rate of DARh physically mixed with CD was possibly due to local solubilization operating in the micro environment or the hydrodynamic layer surrounding the drug particles as reported by Becirevic-Lacan et al. [23]. In situ inclusion process might have resulted in increased amount of dissolved drug in case of physical mixtures [24]. It is worth noting that during the dissolution tests, no significant hydrolysis was detected while, if the tests are prolonged for more than 60 min, a significant Rh concentration appears in the dissolution medium.

Conclusion

The use of HPβCD allows to DARh both to improve the solubility characteristics as well as to increase its hydrolytic resistance.

However, for a correct estimate of the various stability parameters is essential to adopt experimental conditions able to unequivocally discriminate the chemical species involved. In this regard, by carefully selecting the DARh maximum wavelength (433 nm) it is possible to investigate a single compound without interference from its degradation product which has the same UV absorption maximum (258 nm).

Furthermore, as expected, also the method of preparation can influence the formation of the complex in the solid state. These aspects can be highlighted by both FT-IR spectroscopic analysis and DSC. They have practical implications on the dissolution rate and, hence, on the bioavailability of the active principle.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.saa.2014.02.055.

References

[1] M. Bambagiotti-Alberti, G. Bartolucci, B. Bruni, S.A. Coran, M. Di Vaira, J. Pharm. Biomed. Anal. 49 (2009) 1065–1069.
[2] C.M. Spencer, M.I. Wilde, Drugs 53 (1997) 98–106.
[3] J. Martel-Pelletier, J.P. Pelletier, Ther. Adv. Musculoskelet. Dis. 2 (2010) 95–104.
[4] T. Tamura, T. Yokoyama, K. Ohmori, Pharmacology 63 (2001) 228–233.
[5] N. Tobar, A.G. Oliveira, D. Guadagnini, R.A. Bagarolli, G.Z. Rocha, T.G. Araújo, J.C. Santos-Silva, R.L. Zollner, L.H. Boechat, J.B. Carvalheira, P.O. Prada, M.J. Saad, Endocrinology 152 (2011) 4080–4093.
[6] K.L. Calisto, A.C. Camacho, F.C. Mittestainer, B.M. Carvalho, D. Guadagnini, J.B. Carvalheira, M.J. Saad, Crit. Care 16 (2012) R158.
[7] V. Wally, S. Kitzmueller, F. Lagler, A. Moder, W. Hitzl, M. Wolkersdorfer, P. Hofbauer, T.K. Felder, M. Dornauer, A. Diem, N. Eiler, J.W. Bauer, Orphanet J. Rare Dis. 8 (2013) 69, http://dx.doi.org/10.1186/1750-1172-8-69.
[8] M. Nebsen, M.K. Abd El-Rahman, M.Y. Salem, A.M. El-Kosasy, M.G. El-Bardicy, Drug Test. Anal. 3 (2011) 221–227.
[9] P. Patrekar, C.C. Patil, J. Pharm. Res. 2 (2009) 923–926.
[10] N. Maski, A. Kumaran, K. Girhepunje, P. Ghode, S. Randive, R. Pal, Int. J. Pharm. Pharm. Sci. 2 (2009) 121–135.
[11] S. Petralito, I. Zanardi, A. Memoli, M.C. Annesini, V. Travagli, Spectrochim. Acta A 74 (2009) 1254–1259.
[12] Y.L. Loukas, J. Pharm. Biomed. Anal. 16 (1997) 275–280.
[13] O.K. Abou-Zied, Spectrochim. Acta A 62 (2005) 245–251.
[14] A.C. Kenneth, Chem. Rev. 97 (1997) 1325–1357.
[15] Y.L. Loukas, V. Vraka, G. Gregoriadis, Int. J. Pharm. 144 (1996) 225–231.
[16] T. Loftsson, M.E. Brewster, J. Pharm. Sci. 85 (1996) 1017–1025.
[17] P.K. Janert, Gnuplot in Action, Manning Publications, Greenwich, CT, USA, 2009.
[18] R. Grillo, N.F. de Melo, C.M. Moraes, R. de Lima, C.M. Menezes, E.I. Ferreira, A.H. Rosa, L.F. Fraceto, J. Pharm. Biomed. Anal. 47 (2008) 295–302.
[19] O.S. Tee, A.A. Fedortchenko, P.L. Soo, J. Chem. Soc. Perkin Trans. 2 (1998) 123–128.
[20] J. Szejtli, Cyclodextrin Technology, Akademiai Kiado, Budapest, 1982.
[21] H. Benesi, J. Hildebrand, J. Am. Chem. Soc. 71 (1949) 1707–2703.
[22] C. Ravelet, E. Peyrin, A. Villet, C. Grosset, A. Ravel, J. Alary, Chromatographia 53 (2001) 624–628.
[23] M. Becirevic-Lacan, J. Filipovic-Grcic, N. Skalko, J. Jalsenjak, Drug Dev. Ind. Pharm. 22 (1996) 1213–1236.
[24] T. Yamada, N. Sait, T. Imai, M. Otagiri, Chem. Pharm. Bull. 47 (1999) 1311–1313.