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Cyclodextrin/Diacerein Inclusion Complex: a Tool for Therapeutic Drug Delivery

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Diacerein (DAR) is a pro-drug that undergoes hydrolysis into Rhein (Rh); complexation with Cyclodextrins has been suggested to modulate DAR degradation rate. In this work, the effect of hydroxypropyl derivate of β-Cyclodextrin (HPβCD) complexation on the DAR hydrolysis has been investigated. As for DAR stability in cyclodextrin complexes, a decrease in the hydrolysis rate constant (K_c) with increasing cyclodextrin concentration has been observed (0.23 h^{-1} vs 0.11h^{-1} for free drug and for 100 mM HPβCD, respectively). A stability constant (K_{st}) of DAR/HPβCD complex of 50 M^{-1} was obtained by the kinetic data. Such a value was compared with those obtained by Benesi–Hildebrand equation applied to spectroscopic measurements, HPLC method and Phase Solubility studies. The influence of complexation technique on aqueous solubility and dissolution rate profiles was also assessed. For this purpose solid complexes are prepared by freeze drying, co-evaporation, and kneading techniques. The results, obtained by UV-Vis spectroscopy analysis, have been compared with those obtained for the physical mixture. Eventually, selective physicochemical determinations based on differential scanning calorimetry (DSC) and Fourier Transform Infrared Spectroscopy (FT-IR) were used to characterize the complexes.

1. Introduction

Diacerein (4,5-diacetyloxy-9,10-dioxoanthracene-2-carboxylic acid, DAR) is an anthraquinone derivative used as a slow acting symptomatic drug in osteoarthritis, metabolized to active Rhein (Rh) (Fidelix et al., 2006). Recently, DAR attracted increased attention because of its potent pharmacological activities including anti-oxidative, anti-inflammatory, anti-leishmanial and prevention of alcohol-induced liver disease (Tamura et al., 2001). Since DAR low water solubility limits further pharmacological exploitation and practical application, the inclusion complexes with cyclodextrins have been recently investigated (Patrekar et al., 2009; Maski et al., 2009). Cyclodextrins (CD) are polysaccharides made up of six to eight D-glucose monomers

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connected at one and four carbon atoms. They have the property of forming inclusion complex with various guest molecules with suitable polarity and dimension because of their special molecular structure/hydrophobic inner cavity and hydrophilic external surface. The cyclodextrins are also known to affect the chemical stability of drug molecules; in this work, the influence of cyclodextrin on hydrolytic rate of DAR degradation, solubility and dissolution rate have been assessed. Degradation rate constants of the drug in aqueous cyclodextrin solution were used to evaluate the K_{st} of the DARh/HPβCD complex by non-linear fitting of the kinetic data. Generally, the apparent stability constant for a drug/cyclodextrin complex would normally be obtained from the slope of the phase/solubility diagram. Unfortunately, such a method does not take into account the hydrolysis of a drug and the complexation of the hydrolysis products with CD. As far as the influence of complexation technique on dissolution improvement of DAR/HPβCD complex, solid complexes are also prepared by freeze drying, co-evaporation, and kneading methods. Selective physicochemical determinations based on differential scanning calorimetry (DSC) and Fourier Transformed-IR (FT-IR) were used to characterize the complexes and to confirm complexation also at the solid state.

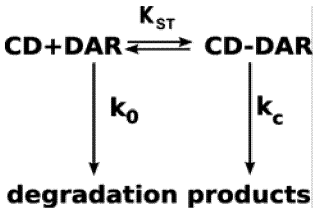
2. Materials and Methods

2.1 Materials

DAR (lot n. F07198901.0.01, TRB Chemedica, Vouvry, Switzerland); HPβCD (Kleptose HPB, lot n. 813447, Roquette, France) All other materials and solvents used were of analytical grade or purer.

2.2 Hydrolityc degradation studies

A stock solution of DAR ($1x10^{-3}$M) was prepared in DMSO. A volume of 2.5 ml of the stock solution was added to the pH 7.2±0.1 HPβCD phosphate buffer solutions (range 0-100 mM) to make a final volume of 25 ml ($1x10^{-4}$M DAR). Samples were kept in the dark at 25±0.1 °C and the hydrolysis reaction of DAR to Rh was monitorated *vs* time by Uv-vis spectra. The freely available plotting software GNUplot was used for data storage and processing and different calibration curves, for each cyclodextrin concentration, were used to calculate [Rh] formation in solution. The observed first-order rate constant (K_{obs}) for the degradation was obtained from a non linear regression analysis of $[DAR]/[DAR]_0$ concentration plotted *vs* time. The K_{st} and the DAR hydrolysis constant in the complex (k_c) values where obtained by non linear fitting of K_{obs} data, according to the scheme below. All studies were carried out in triplicate.



2.3 Solid State Complex Characterization

DAR and HPβCD either as pure materials or inclusion complexes were evaluated by both DSC and FT-IR. Physical mixture (PM), kneading (KN), coevaporation (COE) and freeze drying (FD) methods have been performed to prepare solid inclusion complexes. Briefly, DSC measurements were carried out using a Setaram DSC 131, heating from 50°C to 350°C for the drug and from 50°C to 400°C for the CD and for all binary system tested. All measurements are made under a nitrogen flow of 20 ml/min at the scan rate of 10° C/min. The FT-IR spectra were performed on Perkin Elmer Paragon 1000. The samples were prepared by the potassium bromide disc method and scanned for absorbance 4000-400 cm^{-1}.

2.4 In vitro Dissolution Studies

The dissolution rate of DAR alone and of its inclusion complexes were studied using USP XXIII dissolution rate test apparatus with a paddle stirrer (stirring speed = 100 rpm, temperature 37°C ± 0.5°C). Samples were withdrawn at various time intervals, filtered analyzed by spectrophotometer at λ=344 nm. The dissolution experiments were conducted in triplicate and for a maximum time of 60 min. After this time, at pH 7.2 a massive hydrolysis of DAR to Rh is obtained.

2.5 HPLC-method

The chromatographic experiments were performed using a Perkin Elmer series 200 LC controller Pump and a Perkin Elmer series 200 UV-Vis detector (detection: 344 nm for DAR). A reversed phase column Kromasyl 100 C18, 5µm, 250x4.6mm Higgins Analytical was employed. The mobile phase used for these studies was methanol–water (30/70 v/v), in which HPβCD was dissolved at different concentrations (0-100mM).

2.6 UV-Vis and Fluorescence spectroscopy

A Perkin Elmer Lambda 25 UV-Vis spectrophotometer with matched 10-mm quartz cells and a Perkin Elmer LS50 spectrofluophotometer (Perkin Elmer, USA) were used to measure the absorbance and fluorescence, respectively. All the spectra were recorded using Perkin Elmer FL WinLab and UV WinLab version 2.85.04 software packages for fluorescence and visible spectroscopy, respectively.

3. Results

3.1 Hydrolysis kinetics

In all the tests the DAR UV-Vis spectra showed a time decreasing peak at λ =345 nm (corresponding to the maximum DAR absorbance), with the appearance of a time raising absorbance peak at λ=435nm, consequent to the Rh production also confirmed by the appearance of the distinctive fluorescence peaks at 511 nm and 580 nm (data not shown). No significant hydrolysis was detected before 60 min. For the non catalyzed reaction (in the absence of HPβCD), hydrolysis followed a first-order kinetic, and the kinetic constant was determined from the DAR concentration data vs time by a non-linear least square fitting. The presence of HPβCD had given an evident stabilizing effect on DAR, with apparent kinetic constants K_{obs} decreasing with increasing CD concentration. According to Fig. 2 the rate constant for the hydrolysis of 10^{-4} M DAR

decreased from 0.23 h^{-1} to 0.11 h^{-1} in the presence of 100 mM HPβCD in solution. The experimental data of K_{obs} as a function of the different HPβCD concentration in solution can be used to derive the k_c values and the stability constant value K_{st} of the complex. K_c of 0.07 h^{-1} and a K_{st} value of the complex of 50 M^{-1} was obtained by non linear fitting of the K_{obs} data. Fig. 1 shows that the agreement between the experimental data and the model is quite good, confirming the formation of 1:1 complex and the proposed degradation scheme.

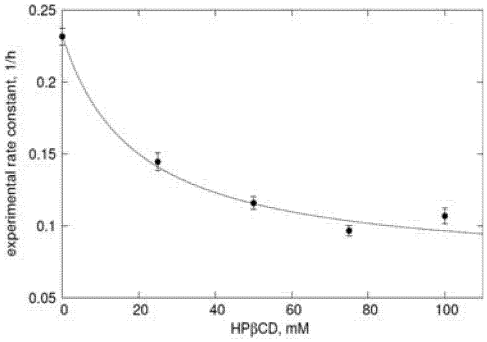


Figure 1: Effect of complexation on DAR hydrolytic degradation constant

K_{st}, was also determined in triplicate, using Equation 1 applied to HPLC method. k' is the capacity factor at each cyclodextrin concentration [CD], and k_s' is the solute capacity factor in absence of CD. The linear relationship between $1/k'$ and the CD concentration with correlation coefficient higher than 0.98 indicates that the behavior of DAR is well described by the model, assuming a 1:1 stoichiometry between the guest and CD (data not shown). A K_{st} of 30 M^{-1} was obtained for the 1:1 complex.

$$\frac{1}{k'} = \frac{1}{k_s'} + \frac{K_{st}}{k_s'}[CD^n] \quad (1)$$

By the addition of HPβCD, the absorption maximum at λ=345 nm was shifted to a longer wavelength with a simultaneous increase in the molar absorption coefficient. Based on these modifications, the formation constant was also determined from the Benesi-Hildebrand equation (Equation 2):

$$(A - A_0) = (\varepsilon_1 - \varepsilon_0)[DAR]_0 + (\varepsilon_1 + \varepsilon_0)[DAR]_0 K_{st}[CD^n]_0 \quad (2)$$

where ε_0 and ε_1 denote the absorption coefficients of the free guest and the complex one. According to Equation (3) from a $(A-A_0)$ *vs* $[CD]_0$ plot, the ratio of the intercept to the slope gives the value of the formation constant equal to 45 M^{-1}. The K_{st} 1:1 values derived from kinetic, HPLC and UV-Vis spectroscopy methods are significantly lower than that obtained in the literature (Patrekar et al. 2009; Maski et al. 2009) from phase solubility studies according to the Higuchi/Connors method, where hydrolysis of DAR to Rh is not taken into account because only the absorbance in the UV region (258 nm) has been measured (194 and 300 M^{-1}, respectively). On the contrary, such values are close to 253 M^{-1} that represents the K_{st} determined for Rh/HPβCD system (Petralito et al. 2009), confirming the hypothesis that DAR is completely hydrolyzed to Rh, and that

solubility and binding of Rh/HPβCD instead of DAR/HPβCD system have been erroneously reported.

3.2 Thermal analysis

PM and KN systems have indicated a partial reduction of dehydration of HPβCD and a decreasing and a shift toward higher temperature of the melting endotherm of the pure DAR, but not its complete disappear. This is a proof of the presence of the pure DAR in the PM and in the KN products that has not completely interacted within the CD. Furthermore when the endothermic peak of pure DAR (at onset 232°C) disappears, as it been observed in the COE and in the FD binary systems, it is confirmed the strong interaction between CD and host investigated molecules (data not shown).

3.3 FT-IR spectroscopy

FT-IR results confirm the host-guest interaction; all DAR/HPβCD systems displayed frequency shifts and/or the disappearance of characteristic IR bands of the drug, indicating alterations in the drug. While the PM and the KN product showed approximately the superposition of drug and CD spectra, the FTIR spectra of FD and COE complexes showed the disapperence of carbonyl absorption band assigned to carboxyl carbonyl stretching suggesting the inclusion of the guest in the hydrophobic environment of the HPβCD cavity (data not shown).

3.4 Dissolution rate

The dissolution values were plotted as % of DAR dissolved from binary systems and pure DAR *vs* time (Fig. 2). From the obtained profiles, it can be observed that dissolution rate was significantly improved by DAR complexation with HPβCD, showing that all the examined binary systems exhibit a drug dissolution rate higher than that of the pure DAR, with a dissolution time scale of a few minutes. Moreover, the increase in the dissolution rate observed for PM might be mainly attributed to the hydrophilic effect of CD which increase the drug solubility in the solid-liquid interface and can reduce the interfacial tension between DAR and the dissolution medium, thus leading to a higher dissolution rate. Eventually, during the dissolution tests, no significant hydrolysis was detected while, if the tests were prolonged for more than 60 min, a significant Rh concentration appears in the dissolution medium.

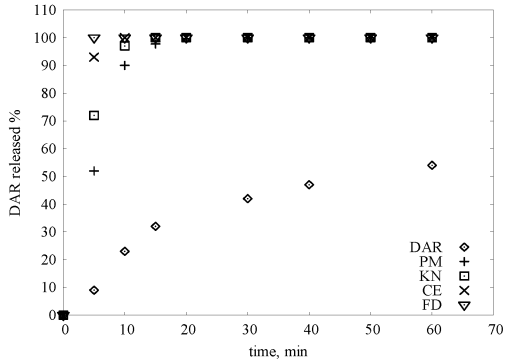


Figure 2: Dissolution profile of DAR and binary systems.

4. Conclusions

The interaction between DAR and HPβCD resulted in a stabilization effect on DAR against hydrolysis. Such an aspect could represent an advantage of using DAR/CD complex that, reducing the hydrolytic rate in the stomach into rhein, potentially limits its laxative side effect, and therefore ameliorates the poor tolerability of oral diacerein therapy. The obtained K_{st} values by different evaluation methods appear to be almost different from data present in literature where the phase solubility studies have been erroneously used to determine the equilibrium constant for the formation of the DAR complex with cyclodextrins.

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