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ORIGINAL ARTICLE

Host–guest interactions between dapsone and β-cyclodextrin (Part II): thermal analysis, spectroscopic characterization, and solubility studies

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Abstract The complex of dapsone with β-cyclodextrin was prepared by the co-precipitation/freeze–drying method. The physical–chemical characteristics of the complex were investigated by different methods and compared with those of the physical mixture and of the isolated compounds. The methods used were infrared spectroscopy, X-ray diffractometry and differential scanning calorimetry. The stability constant was calculated from phase solubility diagram (Higuchi–Connors) and fluorescence spectroscopy. The stoichiometry of the complex was confirmed by Job's plot. Fluorescence measurements at different temperatures provided the thermodynamic parameters of the complexation. The infrared spectrum showed the disappearance of the SO_2 asymmetric stretching band of the drug at 1275 cm^{-1} after complexation. The amorphization of the samples, as revealed by the X-ray diffraction patterns, was an indirect proof of the inclusion complex. The thermal analysis showed that the curves of the physical mixture are combination of the curves of both constituents (dapsone and β-cyclodextrin) while the absence of the melting peak of the drug in the DSC curve of the complex suggests the inclusion of the drug molecule in the host cavity as a 1:1 complex as indicated by Job's plot. There was a linear increase in its solubility with the increase of the cyclodextrin concentration and the complex was classified as an A_L-type. The value of the stability constant was 3,998 L mol^{-1} calculated by the Higuchi–Connors diagram and around 18,100 L mol^{-1} from the fluorescence method indicating a strong interaction between the host and the guest. Complex formation was a spontaneous and enthalpy directed process.

Keywords Dapsone · β-cyclodextrin · Thermal analysis · Fluorescence · Stability constant

Abbreviations

CD	Cyclodextrin
DPS	Dapsone
DSC	Differential scanning calorimetry
FD	Freeze–drying
FT-IR	Infrared spectroscopy
PM	Physical mixture
L	Lyophilized complex
XRD	X-ray diffraction

Introduction

Dapsone (DPS, Fig. 1) has been the main drug for treatment of leprosy. As an anti-infective agent, it is also used for treating malaria, pneumocystic carinii pneumonia in AIDS patients, Kaposi's sarcoma and dermatoses. In some cases, therapy may be continued for 3–5 years. DPS therapy may cause adverse effects such as methemoglobinemia, anorexia, hemolysis, anemia, dyspnea, nausea, tachycardia, fatigue, anorexia, headache, dizziness, exanthematous eruption, Stevens-Johnson syndrome, toxic epidermal necrolysis, and others [1, 2]. The encapsulation of DPS in cyclodextrins could improve its water solubility, bioavailability and minimize side effects. This work describes the inclusion complex formation of this drug with β-cyclodextrin (βCD).

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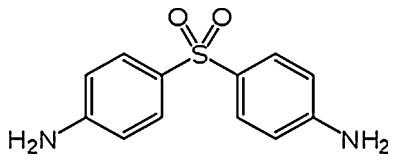

Fig. 1 Dapsone molecular structure

Cyclodextrins are cyclic oligomers of α-D-glucose connected through glycosidic α-1,4 bonds. They are crystalline, non-hygroscopic, and torus-like macrocycles, produced by the action of certain enzymes on starch. Due to their characteristics and large molecular cavity; they have the ability to include guest molecules, thus altering the physical, chemical, biological and pharmacological properties of the guest through the formation of inclusion complexes [3, 4]. Cyclodextrins are the more extensively studied host molecule in supramolecular chemistry, because of their innumerous advantages as: biocompatibility and natural production in the enzymatic degradation of starch, relatively inexpensive and non-toxic, allowing applications in several industries [5]. CDs are often used to increase the aqueous solubility, stability and bioavailability of drugs [6]. The natural CDs are: αCD (six glucose units), βCD (seven glucose units), and γCD (eight glucose units) [7]. The size of the cavity of βCD is more appropriate to interact with a great number of molecules and, therefore, this CD was used in this study. Many forces are involved in the complexation of drugs in the CD cavity, which include electrostatic interactions, van der Waals forces, hydrophobic interactions, hydrogen bonding, release of conformational strain, charge-transfer interactions and exclusion of cavity-bound water [24].

There are many techniques that can be used to study interactions between hosts and cyclodextrins. In our previous work, the inclusion compound DPS:βCD was studied by different NMR techniques, which showed a strong interaction between host and guest. In the present work, we characterize the inclusion complex of DPS and βCD by thermal analysis and spectroscopy. The solid complex was characterized by DSC, XRD and FTIR, and it was compared with the isolated compounds and their physical mixture (PM). The spectroscopic experiments were done to obtain physical–chemical parameters such as association constant, ΔG, ΔH, and ΔS.

Experimental

Materials and equipments

Dapsone was supplied by Ecofarma Farmácia Ltda., βCD was a gift from ISP Technologies, Inc. and was used as received, ethyl alcohol, 99.5% P.A., was purchased from LabSynth Ltda. Products for Laboratories, freeze–dryer FTS Systems, rotary evaporator RE111, water bath 461 and vacuum pump Büchi Labortechnik AG, UV/vis spectrophotometer Hewllet Packard–HP8453, Phoenix AP22 homogeneizer, DSC 910 DuPont Instruments calorimeter, Shimadzu-XDR-6000 X-ray diffractometer, Bomem MB Series MB102 FTIR spectrometer, and Cary Eclipse spectrofluorimeter fromVarian.

Preparation of the inclusion complex (DPS:βCD)

The inclusion complex DPS:βCD was prepared by adaptation of two methods: co-precipitation and lyophilization. βCD was dissolved in 70 mL of deionized water, followed by addition of 20 mL of DPS in ethanolic solution at molar ratio of 1:1 and 2:1. The mixtures were stirred at room temperature for 24 h. After removal of ethanol in rotary evaporator at 40 °C, the suspension was frozen in liquid nitrogen and lyophilized for 48 h.

Preparation of PMs

The PMs were prepared using the molar ratios of 1:1 and 2:1 of βCD and DPS by simply mixing the two compounds for 2 min.

Solubility studies

Excess amount of DPS were added to 10 mL of deionized water containing a range of βCD concentration (from 0 to 10 mmol L^{-1}). The tubes were shaken for 48 h at 25 ± 0.5 °C. After, the samples were filtered through a 0.22 μm membrane and the absorbance at 291 nm was measured using UV/vis spectrophotometry. Calibration curve (7 to 35 μmol L^{-1}) was used to determine the concentration of the dissolved DPS. All studies were carried out in triplicate.

Differential scanning calorimetry (DSC)

The DSC thermograms were recorded on a DSC 910 (DuPont Instruments). Samples were heated in hermetically sealed aluminum pans over the temperature range of 30–300 °C with a heating rate of 10 °C min^{-1} under an argon purge.

X-ray powder diffraction (XRD)

The powder X-ray diffraction patterns of the samples were recorded over the interval 5–50°/2θ using Cu-$\kappa\alpha$ radiation at 1.5406 Å, voltage of 40 kV and current of 30 mA, using a Shimadzu-XRD-6000 X-ray diffractometer.

Infrared absorption spectroscopy

The infrared spectra of the samples were recorded in a Bomem MB Series MB102 FTIR spectrometer using KBr pellets as support.

Method of continuous variation (Job's plot)

The stoichiometry of the inclusion complex was determined by varying the mole fraction ($r = 0$, 0.2, 0.33, 0.5, 0.67, 0.8, 1.0) of the compounds by mixing equimolar solutions of DPS and βCD. The final concentration remained constant (3 mmol L^{-1}). After stirring the solutions for 1 h, the absorption spectra in the UV/vis region were recorded at 298 K. The difference in absorbance (A) measured at 291 nm between solutions containing only DPS and the DPS/βCD mixtures, multiplied by the molar ratio (r) of DPS, was plotted as a function of the r of DPS. The point where the derivative of the curve was zero corresponded to the stoichiometric ratio for the inclusion complex. Each solution was measured in triplicate.

Fluorescence studies

To test tubes containing solutions of DAP (5 μmol L^{-1}) were added increasing amounts of βCD (0, 0.03, 0.06, 0.1, 0.3, 0.6, 1, 3, 6 and 10 mmol L^{-1}). After stirring for 1 h, the solutions were thermostated at the working temperatures (298, 310 and 322 K) for at least 1 h. Fluorescence measurements were performed using a Cary Eclipse (Varian) spectrofluorimeter coupled with its temperature controller with a precision of ±0.5 °C. The emission spectra were recorded with excitation at 295 nm, range of the 315–570 nm and scan rate fixed to 1200 nm min^{-1}. Slit widths were 5 nm for excitation and 20 nm for emission. The time of thermal stabilization of the solution in the equipment before the reading was 5 min. Each solution was measured in triplicate.

Results and discussion

Studies on solids compounds: βCD, DPS, PMs and inclusion compounds

DPS is a drug slightly soluble in water. It's solubility at 298 K is 0.16 mg mL^{-1} [8]. Therefore, in order to obtain the complex, it was first dissolved in ethanol to improve its interaction with the cyclodextrin in an aqueous solution. After evaporation of the ethanol, a white solid was obtained due to decreased solubility of the βCD in the aqueous solution. The elimination of ethanol favored the direction of water molecules into the hydrophobic cavity of cyclodextrin, promoting complex formation. The suspension was freeze–dried to remove the remaining water and obtain the final powder.

The inclusion complexes (DPS:βCD molar ratios 1:1 and 1:2) obtained by co-precipitation/lyophilization modified method were compared with individual constituents and the PM in both molar ratio. The results of all analysis are discussed below.

X-ray diffractometry

The DRX patterns of DPS, βCD, PMs and corresponding inclusion complexes are in Fig. 2.

The formation of inclusion complexes usually results in visible changes in the X-ray diffraction patterns of molecules, such as amorphization (i.e. reducing the crystallinity degree) of the diffraction pattern, the disappearance of characteristic peaks of cyclodextrins and/or the appearance of new peaks. This technique is an indirect proof of the formation of complexes [7].

Upon analysis of the X-ray patterns in the Fig. 4, it is observed that DPS and βCD present numerous sharp reflections indicating a high degree of crystallinity. The characteristic diffraction peaks of DPS and βCD crystals were detected in the PMs M11 and M12, whereas they considerably decreased or have disappeared in the corresponding inclusion complexes (L11 and L12). The XRD patterns of the complexes were more amorphous than the individual components and PMs. This is indicative that the inclusion compound was formed in the co-precipitation/freeze–drying process, as seen by other authors [8].

FT-IR spectroscopy

The infrared spectra of inclusion complexes showed changes in intensities and frequencies of some bands in

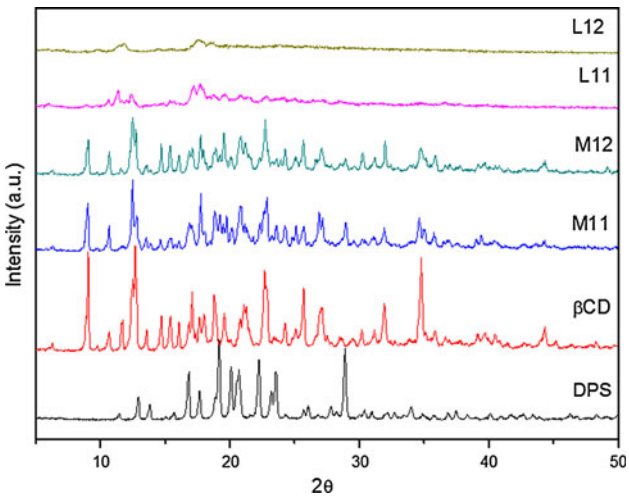

Fig. 2 X-ray diffractograms of DPS, βCD, physical mixtures (M11 and M12) and inclusion coplexes (L11 and L12)

relation to PMs and individual constituents (Fig. 3). For example, in the spectra of the complexes L11 and L12 the disappearance of the DPS band in 1275 cm^{-1} corresponding to asymmetric stretching of the group SO_2 was observed. This is also observed in the PMs (M11 e M12). The absence of this band may indicate the approach of the SO_2 group of the DPS molecule to the region of the edge of the cyclodextrin, with possible formation of hydrogen bonds with the hydroxyl groups of this edge. This is an indication of interaction between DPS and CD in the complex. The formation of hydrogen bonds of drug-βCD at the edge was also proposed by Enoch et al. [9].

Differential scanning calorimetry

The DSC curve of DPS, βCD and the binary systems are in Fig. 4. The DSC curve of DPS showed a sharp endothermic peak at 181 °C, which is attributed to the melt temperature of the drug. In the DSC curve of βCD, one wide endothermic peak was observed in the range between 100–180 °C due to the loss of hydration water. The thermal behavior of the inclusion complexes was distinct from that found for the individual components. The DSC pattern of the PMs M11 and M12 showed a presence of peaks of both pure compounds. Finally, the absence of the melting peak of the DPS in the DSC curves of the inclusion complexes L11 and L21 suggests the inclusion of DPS molecules in the βCD (absence of crystals of DPS). Also, the shift of the βCD peak to lower temperatures, which indicates a reduction of ΔH reduction and a peak broadening are all a strong evidence of disorder in the water molecules inside the cavity caused by the drug [10].

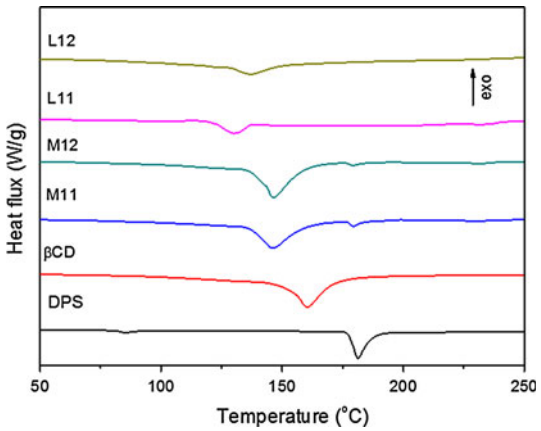

Fig. 4 DSC curves of DS, βCD, physical mixtures (M11 and M12) and inclusion complexes (L11 and L12)

Solubility studies

The phase-solubility diagram corresponding to DPS:βCD system is illustrated in Fig. 5. It was performed according to the method reported by of Higuchi and Connors [11] in order to determine the stability constant of the suggested complex and evaluate its stoichiometry. Measurements were made at 291 nm, which corresponds to the wavelength of maximum absorption of DPS solutions.

One can observe that the aqueous solubility of DPS increased linearly as a function of βCD concentration, therefore water-soluble inclusion complex was obtained using that concentration range of βCD. The phase solubility diagram behavior can be classified as A_L-type according to Higuchi and Connors. The linear host–guest correlation with slope less than 1 indicates the formation of

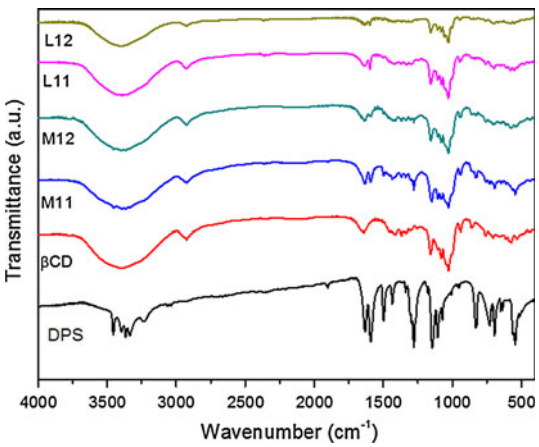

Fig. 3 FT-IR spectra of DPS, βCD, inclusion complex physical mixtures (M11 and M12) and inclusion complexes (L11 and L12)

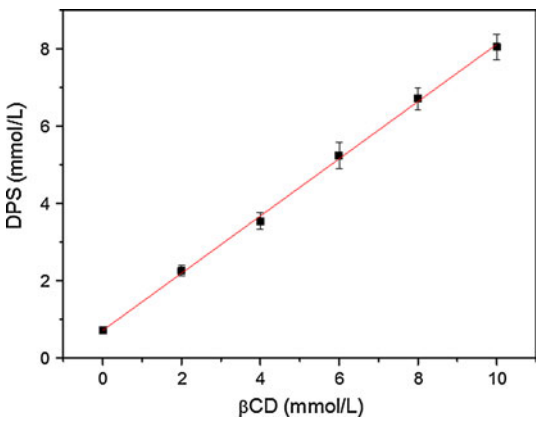

Fig. 5 Phase-solubility diagram of DPS in aqueous βCD solutions, at 298 K (mean ± SD; $n = 3$; adjusted parameters: R = 0.9993; $y = 0.71103 + 0.73979x$)

a 1:1 (DPS:βCD) complex. Although the structural formula of DPS suggests a possible 2:1 stoichiometry, possibly this complex is not favorable due to steric hindrance or the approximation of the two CD rings [12].

The apparent association constant (K) of the drug-CD complex, assuming a 1:1 stoichiometry, was calculated from the linear curve of phase solubility diagram using Eq. 1.

$$K = \frac{\text{slope}}{S_o(1 - \text{slope})} \quad (1)$$

where S_o is the apparent solubility value of DPS in the absence of cyclodextrins and the value of the slope was obtained from Fig. 6.

The calculated K_a value was 3,998 L mol^{-1} suggesting that the βCD and DPS molecules have high affinity and the inclusion complex is thermodynamically highly stable [13]. Usually the interaction constant between a drug and cyclodextrins should be at least 50 L mol^{-1} and not larger than 5,000 L mol^{-1}, since within this range the complex is sufficiently stable [14].

The apparent solubility of DPS in water (in the absence of cyclodextrin) was determined directly by the linear coefficient (0.177 mg mL^{-1} or 0.713 mmol L^{-1}). The value of solubility is close to that obtained by Porchopin et al. [15] 0.16 mg mL^{-1} in phosphate buffer pH 7.4. The solubility showed a steep increase from 0.177 to 2.013 mg mL^{-1} or 8.109 mmol L^{-1} with βCD 10 mmol L^{-1}, i.e. nearly 12 times more solubility.

Method of continuous variation (Job's plot)

This method [16] was used to determine the stoichiometry of the inclusion complex βCD:DPS. This method is one of the experimental mixing techniques used in the study of reaction stoichiometries [17]. Figure 6 corresponds to the Job's plot, which showed a maximum at $r = 0.5$, a highly symmetric plot, indicating that the complex between the drug and βCD has a 1:1 stoichiometric ratio, i.e. one molecule of βCD for one molecule of DPS. These results are in agreement with the stoichiometry suggested by the phase solubility study.

Spectrofluorimetric studies

In organic solvents, DPS exhibits intense fluorescence signals, but in aqueous solution DPS has a low fluorescence quantum yield and almost does not fluoresce, which may be due to the formation of exciplexes between the DPS excited singlet states and water molecules. However, a huge increase in fluorescence intensity is observed when solutions of βCD were added to an aqueous solution of DPS [12]. In this way, spectrofluorimetry was used in this work to estimate some parameters as apparent association constants, thermodynamic parameters, as well as the stoichiometry.

As observed by Ma et al. [12], a dramatic increase in the fluorescence signal was observed when the βCD solution was added to an aqueous solution of DPS and the signal was intensified with increasing concentrations of βCD (Fig. 7). This phenomenon can be explained by the encapsulation of a part of DPS molecule in the CD cavity, which preludes the deactivation of the excited singlet by quenching or other nonradioactive decay process occurring in the bulk aqueous solution.

A blue-shift occurred in the fluorescence signal with addition of the βCD solution, which can be explained by partial shielding of the excitable chromophore of DPS inside the βCD cavity [12]. Therefore, one can confirm the formation of an inclusion compound between the drug and βCD.

Analyzing the enhancement of fluorescence of DPS upon βCD addition, it is possible to investigate the stoichiometry of the suggested complex and to determine its apparent association constant [18, 19]. Besides, knowing this constant at three temperatures (298, 310 and 322 K),

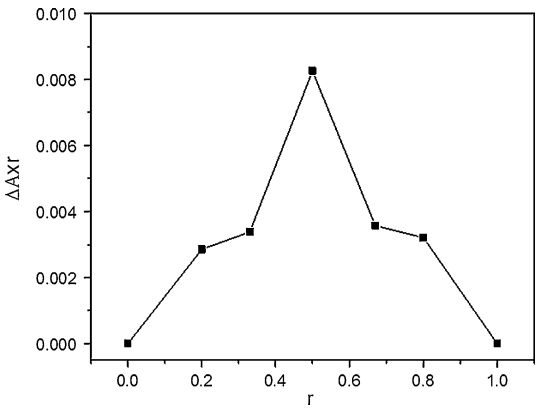

Fig. 6 Job plot determined by measurement in the region of UV/vis region

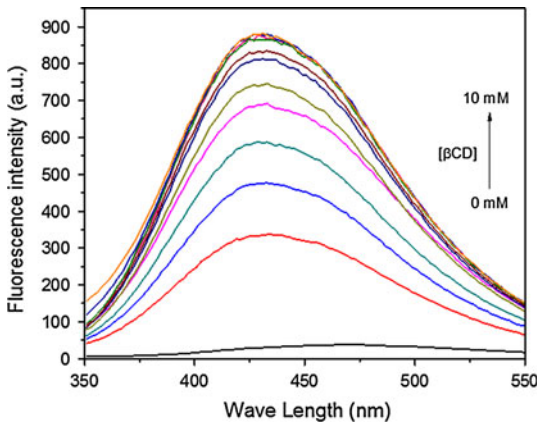

Fig. 7 Effect of addition of increasing concentration of βCD on the fluorescence emission spectra of DPS at 298 K

one can determine thermodynamic parameters by applying the van't Hoff's equation. Figure 8 shows the plot of the maximum fluorescence emission intensity of DPS with increasing concentrations of βCD. One can observe that the saturation region of DPS fluorescence started at βCD around 2 mmol L^{-1}.

Determination of the apparent association constants by fluorescence spectroscopy

The relationship between changes in DPS fluorescence, excited at 248 nm, and the concentration of βCD can provide K through the Scatchard equation (Eq. 2) [13, 20].

$$\frac{F - F_o}{[\mathrm{CD}]} = (F_\infty - F_o)\mathrm{K} - (F - F_o)\mathrm{K} \quad (2)$$

where F is the area under the fluorescence emission spectrum observed at each point of the titration, F_o is the area of DPS fluorescence in the absence of βCD, [CD] is the concentration of βCD at each point of the titration, I_∞ is the area of fluorescence when all DPS molecules have been complexed with βCD (i.e. a large excess of CD), and K is the apparent association constant (L mol^{-1}). Using this equation, a plot of $(F-F_o)/[\mathrm{CD}]$ as function of $(F-F_o)$ can give the binding constant, which is calculated from the intercept and the slope of the straight line. Eq. 2 can be rearranged in the format developed by Benesi-Hildebrand (Eq. 3) to calculate the K value [13, 21].

$$\frac{1}{(F - F_o)} = \frac{1}{(F_\infty - F_o)\mathrm{K}[\mathrm{CD}]} + \frac{1}{(F_\infty - F_o)} \quad (3)$$

Applying both equations to the data of Fig. 8, a line is obtained in the plot of $(F-F_0)/[\beta\mathrm{CD}]$ versus $(F-F_0)$ for Scatchard equation and a line in the plot of $1/(F-F_0)$ versus $1/[\beta\mathrm{CD}]$ for Benesi-Hildebrand equation, as illustrated in Figs. 9, 10 for the data at 298 K. The K values are summarized in Table 1. As one can be seen in the Fig. 9 the fluorescence intensity and, therefore, the K values decrease with increasing temperature.

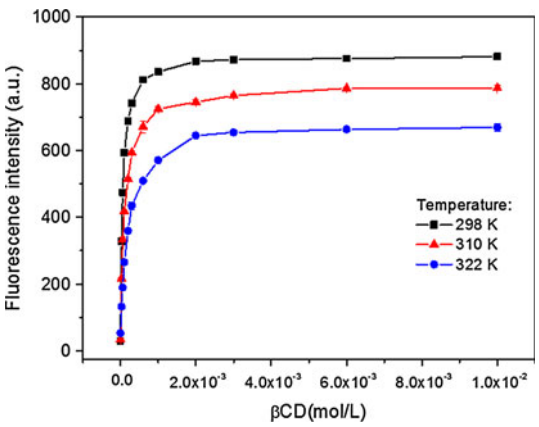

Fig. 8 Maximum fluorescence emission intensity of DPS with increasing concentrations of βCD at 298, 310 and 322 K

Determination of thermodynamic parameters

The thermodynamic parameters ΔH and ΔS for the DPS/βCD complex were determined from the temperature dependence of the constants using the van't Hoff's equation (Eq. 4). The values of ΔH and ΔS were calculated from the slope and intercept of the plot ln K versus 1/T, respectively.

$$\ln \mathrm{K} = \frac{(-\Delta \mathrm{H}^\circ)}{RT} + \frac{(\Delta \mathrm{S}^\circ)}{\mathrm{R}} \quad (4)$$

where R is the gas constant and T is the absolute temperature. The constant is related to the Gibbs free energy (ΔG) as described in Eq. 5.

$$\Delta \mathrm{G}^\circ = \Delta \mathrm{H}^\circ - T\Delta \mathrm{S}^\circ \quad (5)$$

The thermodynamic parameters of the inclusion compound were calculated based on K values obtained

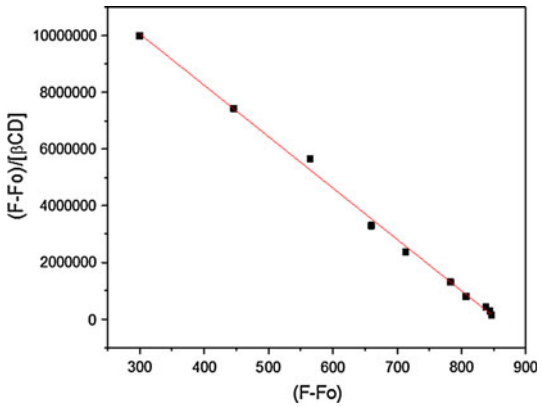

Fig. 9 Scatchard Plot for DPS at 298 K (adjusted equation: $(F-F_0)/[\beta\mathrm{CD}] = 1.5514.10^7 - 18143.76\ (F-F_0)$, R = 0.99707) and $K_{298\ K} = 18{,}144\ m^{-1}$

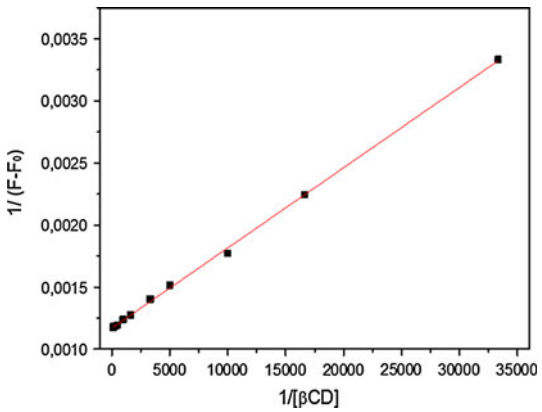

Fig. 10 Benesi-Hildebrand Plot for DPS at 298 K (adjusted equation: $1/(F-F_0) = 0.00117 - 6.46669.10^{-8}\ 1/[\beta\mathrm{CD}]$, R = 0.99707) and $K_{298K} = 18{,}093$ L mol^{-1}

Table 1 Association constants and thermodynamic parameters obtained through the Benesi-Hildebrand, Scatchard and van't Hoff equations of the inclusion compound DPS:βCD

Parameter	298 K	310 K	322 K
K (Benesi-Hildebrand) (m^{-1})	18,093	10,897	4,692
K (Scatchard) (m^{-1})	18,144	10,632	4,715
ΔG (KJ mol^{-1})[a]	−24.4	−23.6	−22.8
ΔH^o (KJ mol^{-1})[a]	−44.7	–	–
ΔS^o (J (mol K)$^{-1}$)[a]	−67.9	–	–

[a] K obtained by Benesi-Hildebrand equation

by the Benesi-Hildebrand method and they are summarized in Table 1.

In agreement with Alvariza [22] and Jullian [23], a negative ΔH° is typical of hydrophobic molecules where the complexation process is favored by van der Waals attractive interactions between host and guest and/or by the presence of intermolecular hydrogen bridges between, for example, the hydroxyls groups of the CD and the DPS molecule. Negative values of ΔS° usually describe guests that do not totally penetrate into the CD cavity or that have their movement restricted due to strong interactions with the rigid host structure, decreasing their degrees of freedom. Therefore, the formation of the complex is an enthalpically driven process. Besides, the negative value of ΔG° shows a spontaneous process for host–guest inclusion [24].

An interesting observation is the difference between the constant values obtained by the three methods. According to the method of Higuchi–Connors [11], the constant value calculated was approximately 4,000 L mol^{-1}. However, for the Scatchard and the Benesi-Hildebrand methods, the constant value was almost 18,000 L mol^{-1}. This difference can be explained by the differences in experimental technique, including the concentrations of the dissolved drug, the temperature, etc. as reviewed by Loftsson and Masson [25] and observed by Ncube et al. [26]. Besides, in the Higuchi–Connors method, the solution of DPS is undersaturated, leading to more difficulty in reach equilibrium with CD because of the interactions among the molecules in the crystal lattice, i.e. due to the intermolecular DPS:DPS attraction, whereas in the spectrofluorimetric study, the drug is totally dissolved and possibly there is no drug agglomeration.

Conclusions

An inclusion complex of DPS:βCD was successfully prepared by co-precipitation/freeze–drying methods. The results from different characterization techniques clearly indicate formation of a complex between DPS and βCD. The solubility of DPS was significantly increased in the presence of βCD. This complex is thermodynamically stable and exhibits 1:1 stoichiometry, consistent with the molecular dimensions of DPS and those of the hydrophobic cavity of βCD. Although there are differences in the values of the apparent stability constant obtained by different methodologies, all results shows a strong interaction between the drug and cyclodextrin.

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