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COMPLEXATION BETWEEN DARUNAVIR ETHANOLATE AND β-CYCLODEXTRIN EXPERIMENTAL AND THEORETICAL STUDIES.

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ABSTRACT

Darunavir (DRV) is a protease inhibitor used in the treatment of HIV infection, which constitutes a keystone in the therapy of patients infected with this virus. Unfortunately, DRV has low solubility in water and poor bioavailability, therefore it requires administration in relatively high doses in order to exhibit therapeutic efficacy. A commonly applied approach to increase the solubility of drugs is the formation of complexes with macromolecules, of which molecular encapsulation with β-cyclodextrin (βCD) constitutes an alternative for the development of new pharmaceutical dosage forms. Therefore, it was to evaluate by theoretical (molecular modelling) and experimental (spectroscopic) approaches the possibility of obtaining an inclusion complex between DRV and βCD. From the results obtained by the docking procedures, we found three clusters of conformations for the DRV:β-CD complex, corresponding to conformations in which the ligand moieties were buried into the β-CD hydrophobic cavities. Molecular modelling results were compared with spectroscopic studies, with ^{1}H NMR studies evidencing that DRV and βCD proton resonances were modified upon complexation, thus confirming the formation of the inclusion complex. The combination of theoretical and experimental techniques confirmed the formation of the inclusion complex between DRV and βCD.

Keywords: β-cyclodextrin, complexation, darunavir ethanolate, molecular modeling, protease inhibitor, spectroscopic studies.

1. INTRODUCTION

Darunavir (DRV, Figure 1), a protease inhibitor used in the treatment of HIV infection, is a pillar of therapy cocktail for patients with the virus. On the market are found tablets DRV ethanolate of 75, 300, 400 and 600 mg, because this is the most stable form.

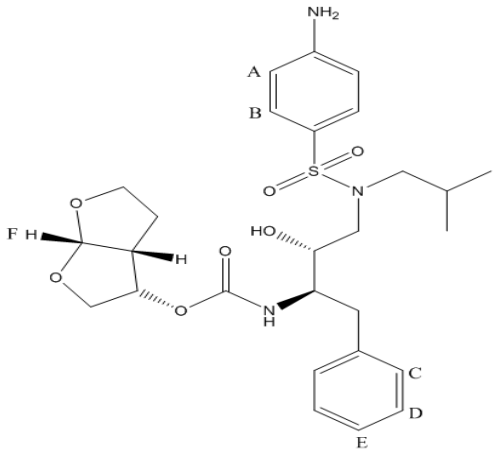

Figure 1. Chemical structure of darunavir, emphasizing the protons A, B, C, D, E and F, for chemical shifts analysis concerning the ^{1}H of darunavir.

The most recently discovered chemical entities, despite the high therapeutic activity, have low water solubility and low bioavailability, leading to poor absorption from the gastrointestinal tract [1]. The important characteristics of a molecule that needs to be considered positive for anti-HIV effects are, among others, solubility and stability in biological fluids. When these properties are unfavorable for the development of specific drugs, the formulation and processing alternatives can be employed to achieve the maximum therapeutic gain [2].

These new drugs require frequent administration in relatively high doses being the major cause of non-adherence to treatment and an obstacle to the fulfillment of pharmacotherapy [3]. DRV has low solubility in water and poor bioavailability therefore require administration in relatively high doses for a successful therapeutic effect. In this context, note the need for the development of antiretroviral drug encapsulation and delivery strategies in order to reduce the dosing frequency and improve compliance of existing pharmacotherapy [3].

Thus, there is great interest in developing efficient methods, reliable, cost effective and scalable to increase the oral bioavailability of poorly soluble drugs in water [1]. These methods are becoming complementary to the development of new drugs [3].

The commonly applied approach to increase the solubility of drugs is the formation of complexes with macromolecules, of which molecular encapsulation with β-cyclodextrin (βCD, Figure 2) constitutes an alternative for the development of new pharmaceutical dosage forms [4-6].

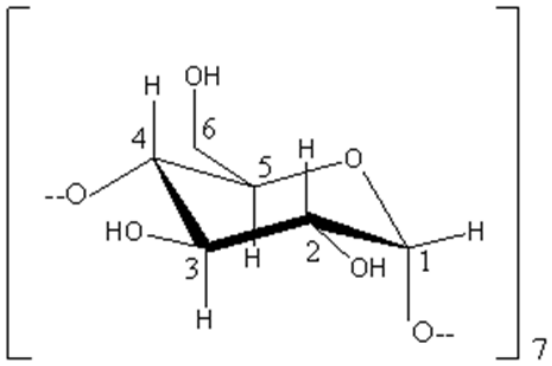

Figure 2. Chemical structure of β-cyclodextrin.

Based on those facts, the objective of the present study was to investigate the possibility of obtaining an inclusion complex (IC) between DRV and βCD.

2. MATERIALS AND METHODS

2.1 Materials

DRV, content of 98.0%, lot SRP07000d (Sequoia Research Products, UK) and βCD (MW = 1135) was kindly supplied by Roquette (France).

2.2 Preparation of the DRV:βCD system

Three curves with an aqueous solution of DRV were constructed in triplicate to obtain absorptivity coefficient (ξ). The purified water (Milli-Q Water Purification System Millipore^{TM}) was used as solvent.

The phase-solubility diagram (PSD) was performed by addition of βCD solutions, at different concentrations, in the same mass of DRV. These solutions were stored and placed in a water bath at 25°C for 4 days. After this period, the solutions were centrifuged and a dilution was

performed for reading on the spectrophotometer in the ultraviolet region. The stoichiometry of the reaction and drug-complexing stability constant (Kc) were calculated using Equation I:

$$Kc = \frac{angular\ coefficient}{linear\ coefficient \times (1 - angular\ coefficient)} \quad (I)$$

The complexation was prepared in distilled water by joining the drug in the complexant in the proportion 1:1 (molar ratio), according to the results obtained in the analysis of the stoichiometry of PSD. The resulting solutions were placed in an ultrasonic bath for 1 h and afterwards frozen at - 40ºC prior to starting the freeze-drying (Freeze Dri 4.5 Labconco Corp).

2.3 NMR Studies

NMR experiments were performed on a Bruker™ Avance II High Resolution Spectrometer, equipped with a Broad Band Inverse probe (BBI) and a Variable Temperature Unit (VTU). D_2O 99.9 atom % D was used for NMR experiments (Sigma-Aldrich™). The spectra were measured at 298 K. The operating frequency for protons was 400.16 MHz, with the chemical shift of the residual solvent at 4.8 ppm being used as the internal reference. Induced changes in the ^1H chemical shifts for DRV and βCD (Δδ), which originated due to its complexation, were calculated using the Equation II:

$$\Delta\delta = \delta_{complex} - \delta_{free} \quad (II)$$

The geometry of the inclusion complex was studied by two-dimensional rotating frame Overhauser experiments (2D ROESY), with spinlock for mixing phase sensitive, using 180x 180-x pulses for polarization transfer. The spectra were measured with a relaxation delay of 2 s, p15 pulse for ROESY spinlock of 20 ms and l4, spinlock loop, (p15/p25 * 2) = 400. Before Fourier transformation, the matrix was zero filled to 4096 (F2) by 2048 (F1), and Gaussian apodization functions were applied in both dimensions.

2.4 Fourier transform infrared spectroscopy (FT-IR)

The FT-IR spectra of pure DRV and βCD, their physical mixture (PM) of 1:1 molar ratio, as well as the freeze-dried (FD) system, were all measured by the same procedure for comparison in potassium bromide discs on an IR Prestige-21 spectrometer Shimadzu™.

2.5 Differential scanning calorimetry (DSC) and Thermogravimetric analysis (TGA)

DSC measurements of the pure materials and the binary systems were carried out using a DSC Q100 (TA Instruments™).The thermal behavior was studied by heating 1–3 mg of

samples in aluminum-pinhole pans, from 25 to 150°C at a rate of 2°C min^{-1} under nitrogen gas flow.

The TGA curves of the different samples were recorded on a SDT Q600 (TA Instruments™), using the same conditions as in the DSC studies, from 25 to 600°C. In both cases, data were obtained and processed using TA Universal Analysis 2000 software.

3. RESULTS AND DISCUSSION

3.1 Preparation of the DRV:βCD system

Table 1 presents the absorbance values of the DRV solutions in purified water obtained by the method of absorption spectrophotometry in the ultraviolet region at 268 nm.

Table 1. The absorbance values of DRV determined by the method absorption spectrophotometry in the ultraviolet region to the analytical curve in purified water

Concentration (µg mL^{-1})	Concentration (mmol mL^{-1})	Mean of absorbance	RSD (%)
10	0.168	0.293	1.29
16	0.269	0.437	1.21
20	0.337	0.553	0.55
24	0.404	0.667	1.06
28	0.472	0.765	0.86
32	0.539	0.863	0.23
36	0.606	0.962	0.52
40	0.674	1.056	0.10

Therefore, ξ to DRV solution in purified water is 16,283.93, according to Equation III.

$$\xi = \frac{average\ absorbance}{(molar\ concentration \times 1)} \qquad \text{(III)}$$

Table 2 presents the absorbance values of the DRV solutions in purified water with different amounts of βCD obtained by method of absorption spectrophotometry in the ultraviolet region at 268 nm.

The PSD is one of the most used tools in the concentration of inclusion complexes with CDs, with results obtained by the solubility of the guest molecule, darunavir, in solutions with increasing concentrations of CDs.

Table 2. Absorbance values of the DRV solutions with βCD in purified water

Absorbance	Concentration (mmol mL^{-1})		Volume of βCD (mL)
	DRV	βCD	
0.226	0.277	0	0
0.511	0.628	3.304	0.05
0.655	0.804	5.956	0.075
0.806	0.989	6.608	0.1
1.156	1.419	9.912	0.15
1.350	1.658	11.564	0.175
1.505	1.848	13.216	0.2

The stoichiometry of complexation of DRV with βCD is 1:1, due to the linear trend line (r = 0.9993, Figure 3). In addition, using the equation y = 0.1196 x + 0.0002 obtained was possible to calculate Kc, according to Equation I. Kc found was 526.48.

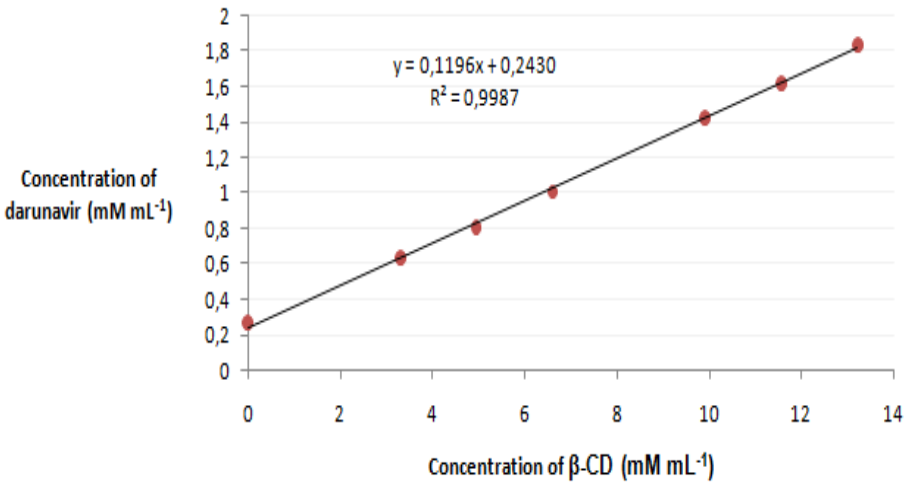

Figure 3. PSD of DRV with different concentrations of βCD solution in purified water.

When the diagram shows the conformation of linear type can calculate the stability constant (Kc, Equation I), which the ordinate of origin corresponds to intrinsic solubility of the guest molecule.

The diagram of the linear type is the most common for inclusion complexes with CDs and the calculation of Kc is done to determine the bond strength of the guest molecule with the host. Table 3 contains the values of Kc and bond strength [7].

Table 3. Bonding strength between the guest molecule and cyclodextrin according to value of Kc

Kc value (M^{-1})	Bonding strength
<500	Very poor
500–1000	Poor
1000–5000	Moderate
5000–20000	Strong
>20000	Very strong

3.2 Characterization of the IC in aqueous solution

3.2.1 Molecular modeling studies

From docking studies we found three clusters of conformations for the DRV:βCD complex (Figure 2), with the moiety of DRV being buried into the βCD hydrophobic cavity as follows: cluster-a: hexahydrofuryl moiety, cluster-b: aminobenzene ring and cluster-c: sulphonamide moiety.

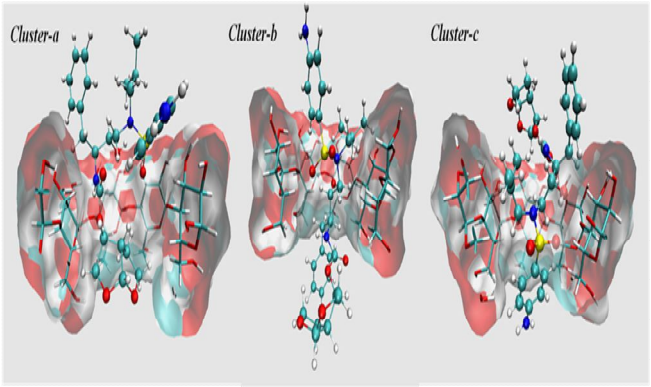

Figure 4. Clusters of conformations found for the DRV:βCD complex.

From modeling docking the most stable conformation was identified by analyzing the corresponding free energies of binding (Table 3), with cluster-b being energetically favored (-26.5 kcal mol^{-1}), and cluster-a and cluster-c exhibiting lower predicted affinities (-23.5 and -17.3 kcal mol^{-1}, respectively).

Table 4. Energetic component analysis obtained by MM-PBSA for DRV:βCD complex analyzed by molecular dynamic simulations

Component	Value (kcal mol^{-1})		
	cluster-a	cluster-b	cluster-c
Electrostatic energy	-17.4	-10.1	-7.4
Van der Waals	-36.5	-40.2	-32.4
Total gas phase energy	53.9	-50.3	-39.8
Nonpolar contribution (solvation)	-3.5	-4.0	-3.5
Electrostatic contribution (solvation)	33.9	27.9	26.0
Total solvation contribution	30.4	23.8	22.5
Estimate binding energy	-23.5	-26.5	-17.3

3.2.2 NMR and ROESY studies

By ^{1}H NMR was evidenced that DRV and βCD resonances were modified upon complexation (Table 4), suggesting the complex inclusion formation. In addition, 2D ROESY assays showed correlations between internal βCD protons and aromatic protons of DRV (Figure 5), which is in agreement with the inclusion mode described for cluster-b by modeling docking.

Table 5. Induced changes in the ^{1}H chemical shifts of DRV and βCD after complexation

Proton of DRV	Δδ (ppm)	Internal proton of βCD	Δδ (ppm)
H_A	-0.0120	H_1	-0.0001
H_B	-0.0219	H_2	0.0013
H_C-H_E	0.0605	H_3	-0.0038
H_D	-0.0746	H_4	0.0026
H_F	-0.0019	H_5	-0.0304
$H_{(CH3)2}$	-0.0066	H_6	0.0018

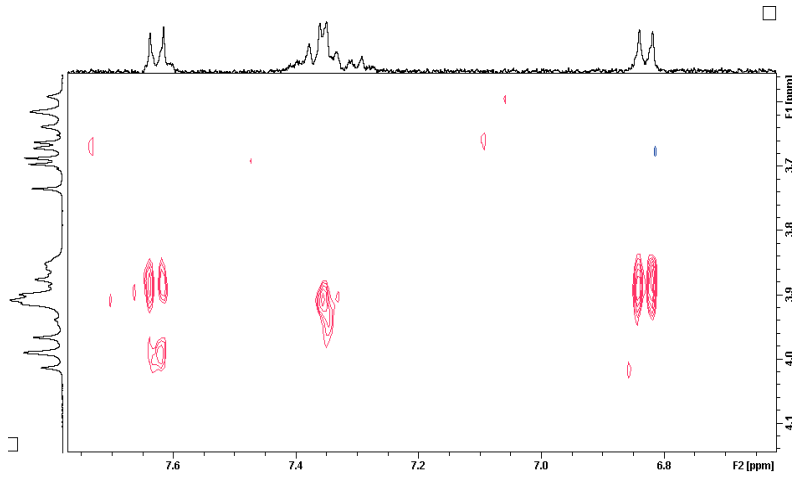

Figure 5. Expansion from the 2D ROESY spectrum of the DRV:βCD system.

3.3 Characterization of the IC in solid state

3.3.1 Fourier transform infrared spectroscopy (FT-IR)

The infrared spectra of the different samples analyzed are presented in Figure 6. As shown, the spectrum of the physical mixture (PM) is the overlap of the spectra of βCD and DRV pure. While the freeze-dried (FD) spectrum shows differences from the spectrum of the DRV and PM. The DRV pure presents characteristic bands in 3470, 3368, 3255, 3061, 2969, 1709, 1633, 1597, 1536, 1501, 1459 e 1093 cm^{-1} [7, 8] that are absent or low intensity in the spectrum of freeze-dried.

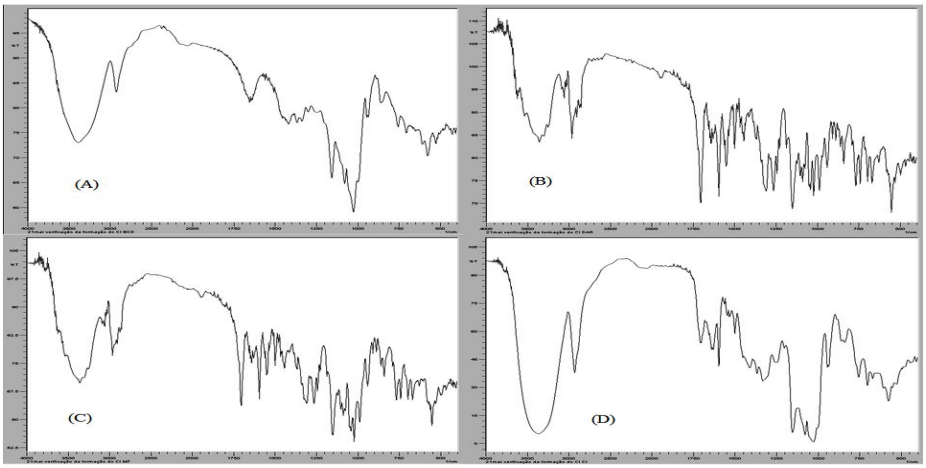

Figure 6. IR spectra of (A) βCD, (B) DRV, (C) physical mixture system (DRV:βCD PM) and (D) freeze-dried system (DRV:βCD complex).

3.3.2 Differential scanning calorimetry (DSC) and Thermogravimetric analysis (TGA)

The thermal behavior of DRV, FD and PM are shown in Figures 7 and 8. βCD lost water at temperatures between 40 and 70 ºC, with decomposition taking place above 300 ºC as

evidenced by the mass loss observed in the TG curves. DSC curve of DRV showed two endothermic peak at 80 ºC and 100 ºC, due to melting point of the hydrate DRV and ethanolate DRV, respectively [9].

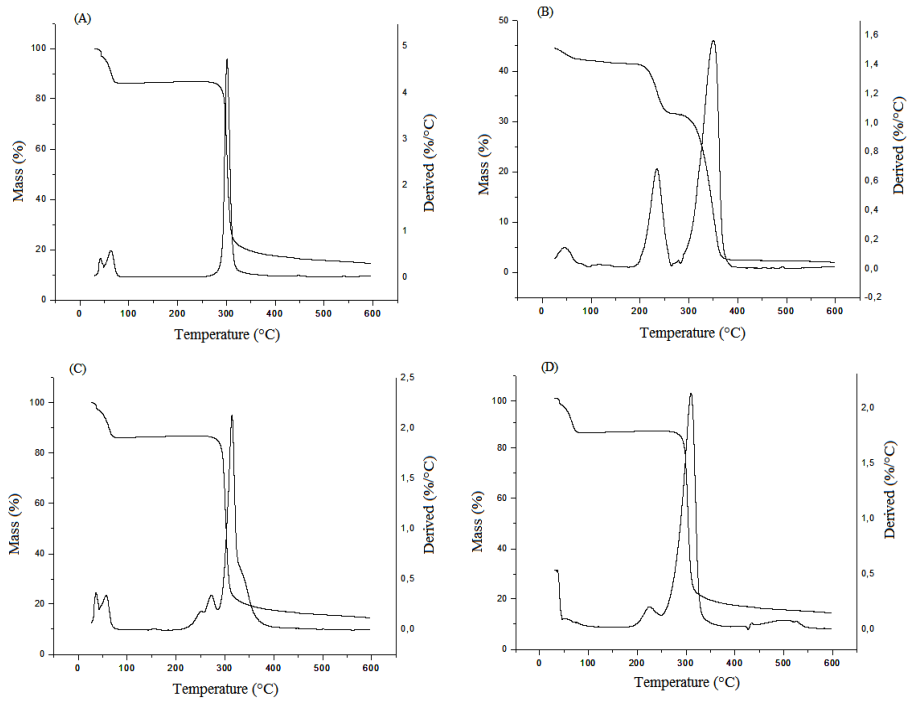

Figure 7. TG curves of (A) βCD, (B) DRV, (C) physical mixture system (DRV:βCD PM) and (D) freeze-dried system (DRV:βCD complex).

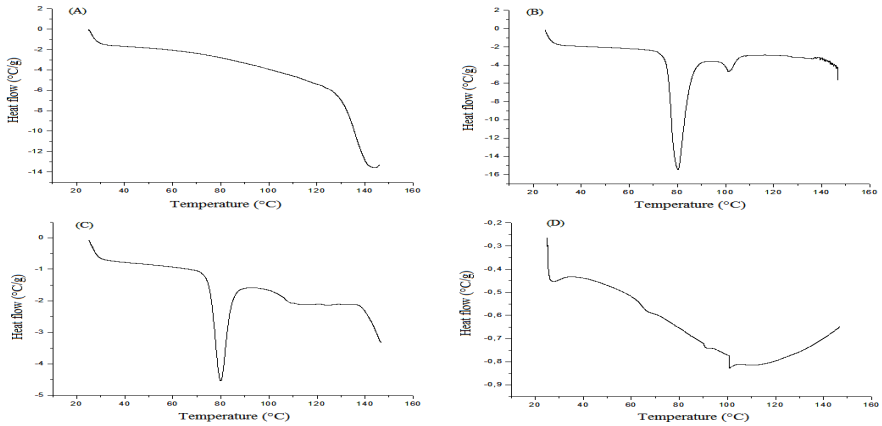

Figure 8. DSC curves of (A) βCD, (B) DRV, (C) physical mixture system (DRV:βCD PM) and (D) freeze-dried system (DRV:βCD complex).

When analyzing the DSC and TG curves of DRV:βCD PM, the characteristic events observed for the pure compounds were found. On the other hand, in the case of the system prepared by FD, the TG curve exhibits two weight loss events, the first at 50 °C and the

second event at 300°C, while the mass loss observed at 200 °C for the DRV is not present in the IC curve. In addition, by DSC was found that the endothermic peaks of pure DRV at 80 °C and 100 °C is not present for the complex, indicating that the melting event did not take place, which might be due to changes in the crystalline form of the solid, or to an inclusion complexation. These observed curve patterns are in agreement with IR spectroscopic data and thus are confirming the formation of an inclusion complex between DRV and βCD.

4. CONCLUSION

The research performed allowed the study of the complexation between DRV and βCD at a molecular level. The combination of theoretical and experimental techniques confirmed the formation of an inclusion complex between DRV and βCD.

It is valid to consider the complexation of the DRV in βCD, aiming the administration of lower doses and increasing patient adherence to the treatment.

With smaller doses, possible adverse drug reactions and drug interactions associated with antiretroviral therapy are decreased, because chronic treatments even with moderate toxicity can lead to serious complications.

We must remember that treatment failure not only affects the quality of life of patients, but also contributes significantly to the economic burden of the health system [2]. Therefore, complexation developed is extremely interesting, both from technological point of view and financial.

5. CONFLICT OF INTEREST

Authors have declared that no competing interests exist.

6. ACKNOWLEDGMENTS

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This paper is dedicated to School of Pharmaceutical Sciences on the occasion of its 90th anniversary.

7. REFERENCES

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