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An Inclusion Compound of the Anticonvulsant Sodium Valproate into α-Cyclodextrin: Physico-Chemical Characterization

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Abstract

A non-hygroscopic pharmaceutical composition was obtained following a host-guest strategy that used the anticonvulsant drug sodium valproate (VA) and α-cyclodextrin. The pharmaceutical composition was fully characterized by thermal analyses (TG/DTG, DSC), X-ray powder diffraction and by ^{1}H, ^{13}C, hydrogen relaxation times (T_1) and 2D-ROESY NMR techniques. Isothermal titration calorimetry (ITC) was used to determine the VA:α-CD 1:1 stoichiometry as well as to calculate the equilibrium constant (K) and thermodynamic energies of interaction (ΔG^{o}, ΔH^{o} and $T\Delta S^{o}$).

Introduction

Valproic acid (2-propylpentanoic acid, Figure 1), in the form of its sodium salt is one of the most used antiepileptic drugs in the clinical treatment of generalized and partial epilepsies [1–3]. Valproic acid is a liquid at room temperature and therefore not suitable for preparation of solid pharmaceutical formulations. Sodium valproate is a solid, but extremely hygroscopic, and hence difficult to manipulate [4]. Since valproic acid is completely ionized at physiological pH, its active form is valproate independently of the mode of administration [5].

Taking into consideration that the clinically used doses of valproate are normally high and that the drug presents many side effects, such as hepatotoxicity [6], the search for new pharmaceutical compositions that could lead to dose reduction is an important goal.

Cyclodextrins are cyclic non-reducing, non-hygroscopic, water-soluble oligopolysaccharides, which present a hydrophobic cavity with the appropriate size to accommodate another molecule (a drug, for example) forming inclusion compounds through host-guest interactions [7]. Molecular encapsulation of drugs into cyclodextrins has been extensively studied recently with the aim of improving characteristics of pharmaceutical interest such as stability and bio-availability [8, 9].

In the present work an inclusion compound between sodium valproate, hereafter named VA, and α-cyclodextrin (α-CD, Figure 2) was prepared, as a strategy to circumvent the problems originated by the hygroscopic character of the drug and to improve its bio-availability.

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Experimental

General

Infrared spectra were recorded on an IR-TF Galaxy 3000 Mattson spectrometer in the 4000–400 cm^{-1} range using KBr disks. NMR spectra were obtained with a Bruker DRX-400 Avance (400 MHz) spectrometer and deuterium oxide (D_2O) as the solvent. Powder X-Ray diffraction patterns were recorded on a Rigaku Geigerflex 2037 equipment using a copper tube and CuKα radiation (λ = 1.5405 nm) with a LiF monochromator. The TG/DTG curves were obtained with a Shimadzu TGA-50H balance under nitrogen flow. TG curves were measured at 10°C/min by heating the sample from 25 to 750 °C. DSC curves were obtained with a Shimadzu DSC-50 equipment using a 50 ml/min nitrogen rate flow and 10 °C/min. Isothermal Titration Calorimetry (ITC) experiments were performed in duplicate, with a Micro calorimeter VP-ITC (Micro Cal) at 25 °C. In the experiment, 1.6 ml of a 2 mM solution of α-CD was titrated with a 50 mM solution of VA, by means of a Hamilton syringe in 40 aliquots of 5 μl, with stirring, so that the VA/α-CD molar ratio varied from 0 to 5.9. Pure water (purified by a milli-Q system) was used as solvent

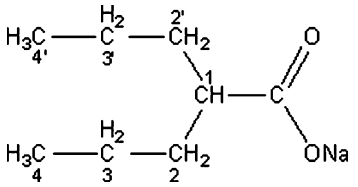

Figure 1. Structure of sodium valproate (VA).

throughout the work. The blank consisted of injection of a 50 mM solution of VA into water in the same experimental conditions described above. Calorimetric data were processed by the computer program Origin for ITC, Version 4.1 (MicroCal, Inc; Northampton, USA). The blank was subtracted from the titration curve to exclude VA/water interaction effects. The heat of dilution was so small as to be negligible, as confirmed by injection of water into a 2 mM solution of α-CD.

Preparation of drug/CD solid complex

The VA/α-CD composition was prepared by mixing VA and α-CD in water in 1:1 molar ratio with stirring for 24 h. The suspension was submitted to a freeze-drying process (Labconco Freezone model 177) during 72 h. A physical mixture (PM) of the same VA:α-CD molar ratio was obtained for comparison.

Results and discussion

The TG/DTG curves of α-CD suggest that the compound is stable until 300 °C. The curves of VA (Figure 3a, b) show a small weight loss around 40 °C, associated to the release of water molecules, but thermal stability is observable until 450 °C, indicating the presence of an extended or associated solid, through intermolecular hydrogen bonds. Upon thermal decomposition a residue of 29.6% is formed.

The TG/DTG curves of α-CD (Figure 3a, b) exhibit a weight loss (8.9%) between 33 and 93 °C, corresponding to the release of five water molecules (hydration and water from the cavity). A plateau is then observable until 313 °C, when decomposition occurs [10].

The TG/DTG curves of PM reveal thermal behaviors associated to VA and α-CD (Figure 3a, b).

The thermal events observed at 33 and 93 °C in the TG/DTG curves of α-CD and PM are absent in the curves of the VA/α-CD compound, indicating the release of water molecules and formation of a new species upon interaction between α-CD and VA (Figure 3c). Only one weight loss at 350 °C is present, which is associated to the cyclodextrin decomposition.

The DSC curve of VA shows a thermal event in the 70–130 °C range, associated to the release of water molecules, as revealed by the TG/DTG curve. In addition, a thermal phenomenon is observed at 410 °C, attributed to complete thermal decomposition of the compound (Figure 4) [11].

The DSC curve of α-CD exhibits four endothermic events at 60, 90, 140 and 310 °C. The first two correspond to the release of water molecules, the third to the cyclodextrin phase transition and the fourth to its decomposition [10].

The DSC curve of PM shows five endothermic events associated to VA and α-CD. The DSC curve of VA/α-CD shows an entirely different profile when compared to those of VA, α-CD and PM. Only one exothermic event is observable at 310 °C, attributed to the cyclodextrin decomposition, indicating the formation of a new species and reinforcing the TG/DTG results (Figure 4).

The thermodynamics of VA/α-CD interaction was analyzed by ITC at 25 °C. Figure 5 shows the signal of titration (A) and the complexation isotherm (B). Strong isothermal signals were observed for the titration of VA with α-CD. The calorimetric curve was fitted using the Wiseman Isotherm to a 1:1 stoichiometry. The coefficient obtained was $N = 1.1$, which confirms the VA/α-CD stoichiometry. The calculated equilibrium constant and thermodynamic energies of interaction were: $K = 1027.6\ M^{-1}$ and $\Delta G^{o} = -17.2$ kJ/mol,

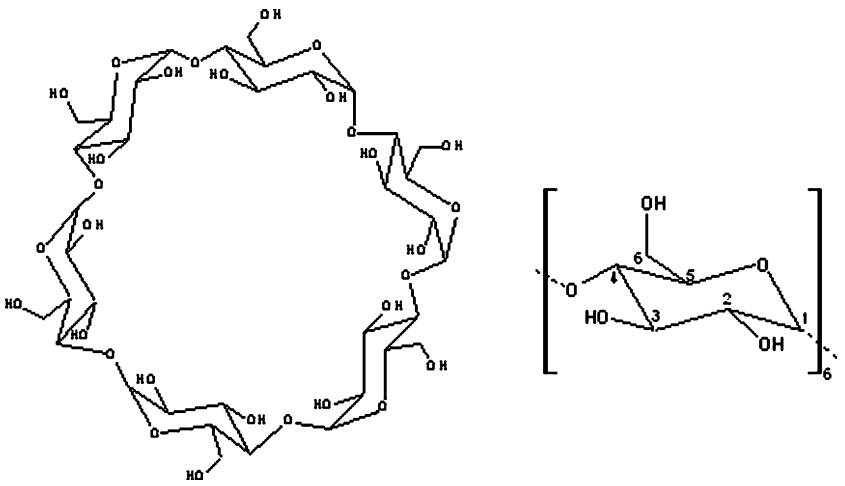

Figure 2. Structure of α-cyclodextrin (α-CD).

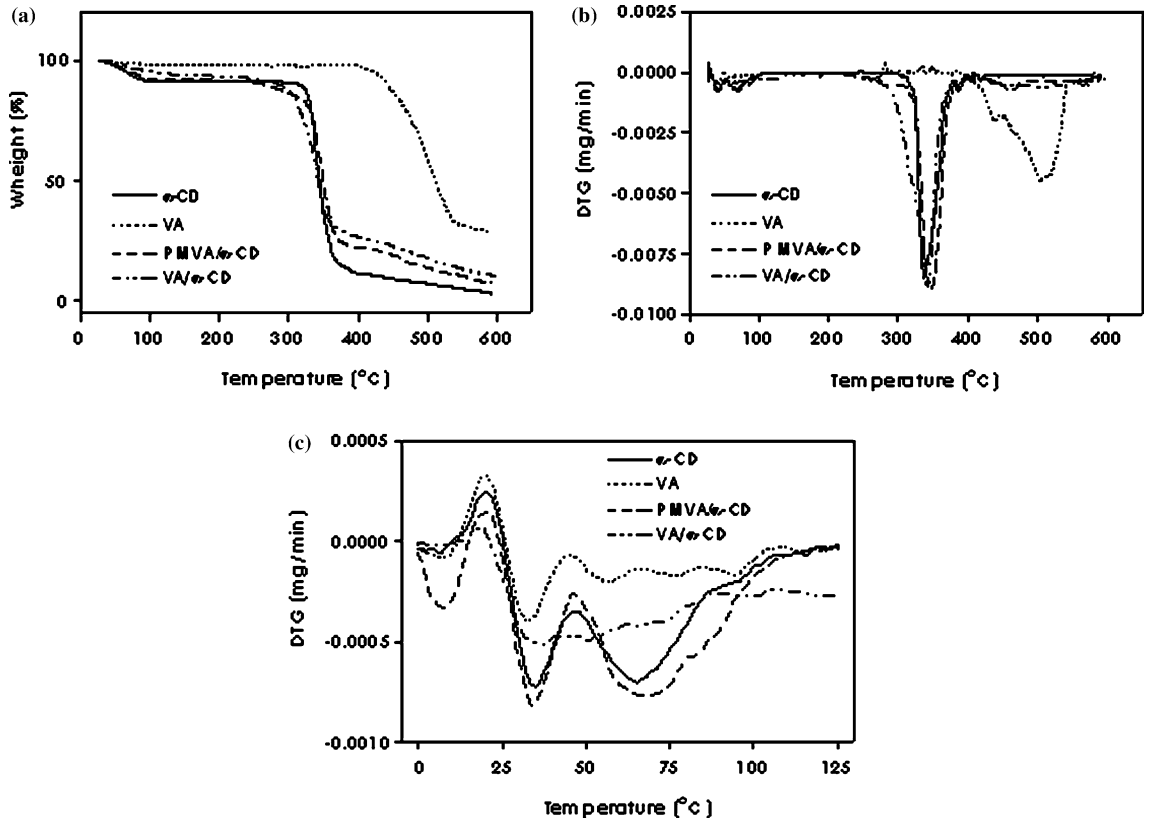

Figure 3. (a) TG curves and (b) DTG curves of VA, α-CD, PM VA/α-CD and VA/α-CD.

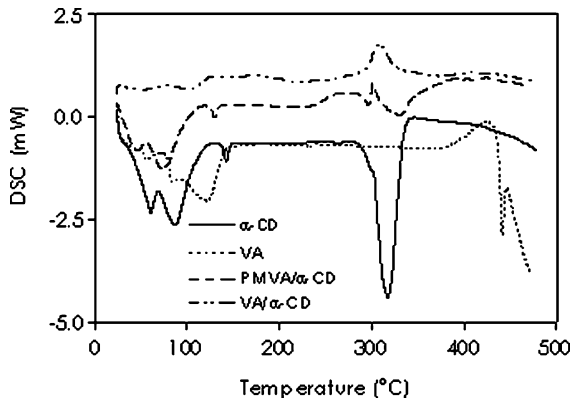

Figure 4. DSC curves of VA, α-CD, PM VA/α-CD and VA/α-CD.

$\Delta H^{o} = -11.5$ kJ/mol and $T\Delta S^{o} = 5.66$ kJ/mol [12–15]. Our results are in accordance with those obtained for other host-guest α-CD complexes related in the literature [16].

The interaction between VA and α-CD is enthalpy as well as entropy controlled. It is well known that in inclusion compounds of α-CD with small guests, the guest molecules are statistically disordered within the cavity and may occupy several positions. When water molecules occupy the cavity, the α-CD structure is somewhat collapsed. This situation causes steric strain within the macrocycle and the effect can be responsible

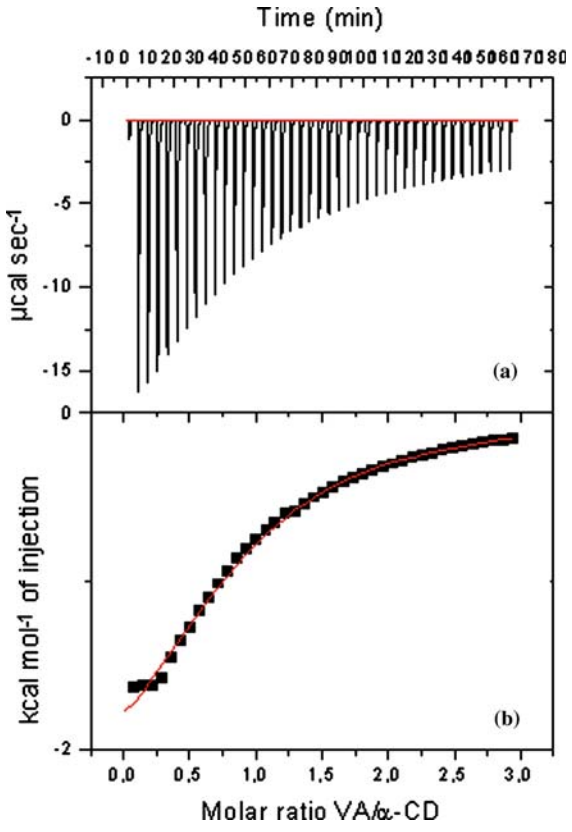

Figure 5. Isothermal titration calorimetry (ITC) for inclusion complexation between VA and α-CD at 298 K. (a) signal of titration and (b) complexation isotherm.

for a gain in entropy on complex formation with expulsion of water [17].

The enthalpy term is attributed to the ion-dipole interaction between VA and α-CD and to hydrogen bond formation between water molecules from the solvent and water released from the cyclodextrin cavity. The entropy term is attributed to modification of the cyclodextrin's conformation upon inclusion of VA and to an increase in the number of species in solution on release of water molecules [16].

Figure 6 shows the powder diffraction patterns of VA, α-CD, PM and VA/α-CD. The XRD patterns of VA and α-CD exhibit sharp peaks, characteristic of crystalline compounds. The XRD pattern of PM shows peaks characteristic of VA and α-CD.

When compared to the XRD patterns of VA and PM, that of the VA/α-CD compound shows the presence of new broader peaks, suggesting a decreasing of the particles size upon host-guest interaction. Moreover, the presence of new peaks at 6, 10 and 12° 2θ in the VA/α-CD XRD pattern indicates the formation of a new crystalline species.

Tables 1 and 2 report the ^{1}H and ^{13}C NMR signals for free VA, and for VA/α-CD, as well as the variation

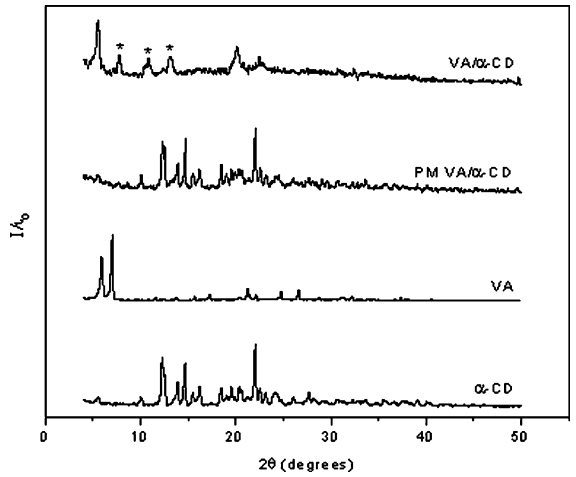

Figure 6. XRD patterns of VA, α-CD, PM VA/α-CD and VA/α-CD (*new peaks).

Table 1. ^{1}H NMR spectra of free sodium valproate (VA) and of VA/αCD (D_2O)

	VA	α-CD	VA/α-CD	Δδ
H1	2.17	–	2.23	+0.06
H2, H2′	1.20	–	1.38–1.29	
H3, H3′	1.40–1.25	–	1.54–1.39	
H4, H4′	0.82	–	0.87	+0.05
H1		5.00	5.00	0.00
H2		3.59	3.57–3.51	
H3		3.94	3.94	0.00
H4		3.54	3.57–3.51	
H5, H6		3.86–3.77	3.88–3.73	

Table 2. ^{13}C NMR spectra of free sodium valproate (VA) and of VA/α-CD (D_2O)

	VA	α CD	VA/α-CD	Δδ
C=O	187.04	–	185.72	−1.3
C1	49.26	–	49.92	+7.7
C2, C2′	35.78	–	21.47	+0.4
C3, C3′	21.07	–	36.24	+0.5
C4, C4′	14.01	–	14.65	+0.6
C1	–	101.38	102.14	+0.8
C2	–	71.69	72.35	+0.7
C3	–	73.30	73.75	+0.4
C4	–	81.21	81.80	+0.6
C5	–	72.02	72.61	+0.6
C6	–	60.43	60.75	+0.3

in chemical shift (Δ) upon inclusion. The signals in the spectra of VA and α-CD were in agreement with data reported in the literature [2, 18].

The ^{1}H NMR spectrum of VA in D_2O shows signals of H1, H2, H2′, H3, H3′, H4, H4′. The H1 signal is a quintet at δ = 2.17; the signal of H3, H3′ is a quartet at δ = 1.20; that of H4, H4′ is a triplet at δ = 0.82 and H2, H2' are in the δ = 1.40–1.25 range. The integrals are in agreement with the number of hydrogens in VA. In the ^{13}C NMR spectrum the signal of C=O is observed at δ = 187.04 and that of C1 at δ = 49.26. The signals of C2, C2′, C3, C3′and C4, C4′ are found at δ = 35.78, δ = 21.07 and δ = 14.01, respectively. The attributions were confirmed by DEPT 135.

α-CD exists as a symmetrical olygomer with six identical units and, therefore its ^{1}H NMR spectrum corresponds to that of a glycopyranose monomer. The signals of H1, H2, H3, H4, H5 and H6 are observed. The hydroxyl hydrogens are labile and undergo exchanging with deuterium from the solvent.

In the ^{1}H NMR spectrum of α-CD, the signals of H1 and H2 are found as a doublet and a double doublet at δ = 5.00 and δ = 3.59, respectively. H3 and H4 are found as triplets at δ = 3.94 and δ = 3.54, respectively, and H5 and H6 are observed in the δ = 3.86–3.77 range [18]. In the ^{13}C NMR spectrum the signals of C1–C6 are observed at δ = 101.38, δ = 71.69, δ = 73.30, δ = 81.21, δ = 72.02 and δ = 60.43. The attributions were confirmed by DEPT 135 experiments and are in agreement with the literature [18].

Table 3. ^{1}H NMR relaxation times (T_1, s) of free sodium valproate (VA) and of VA in the VA/α-CD inclusion compound (D_2O) and relaxation time ratios

	VA	VA/α-CD	$T_{1(VA/\alpha\text{-CD})}/T_{1VA}$
H1	1.737	0.752	0.43
H2, H2′	1.130	0.561	0.50
H3, H3′	0.839	0.443	0.53
H4, H4′	1.590	0.776	0.49

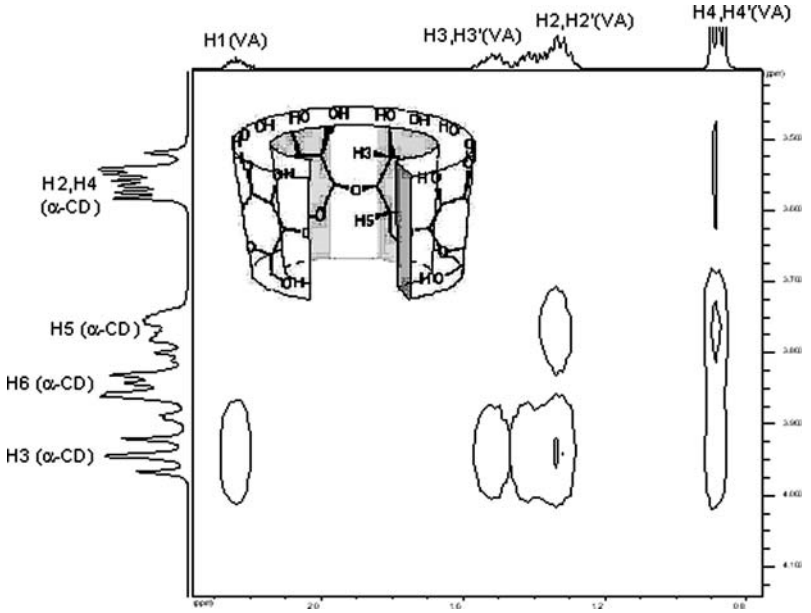

Figure 7. 2D-ROESY contours plot (expanded region) of VA/α-CD (D_2O, 400 MHz).

In the ^1H NMR spectrum of VA/α-CD small shifts to higher frequencies are observed for the VA signals (see Table 1). In the ^{13}C NMR spectrum of VA/α-CD the signal of C═O shifts to lower frequency whereas the signals of all carbons of VA and of VA/α-CD undergo a shifting to higher frequencies. The most significant shifts are observed for *C*1 ($\delta\Delta = +7.7$) and C═0 ($\delta\Delta = -1.3$), probably due to a repulsion effect between the COO^- group of VA and the electronic density of the α-CD cavity as related elsewhere [19, 20] (see Table 2).

Table 3 lists ^1H relaxation times (T_1) for free VA, and VA in the VA/α-CD inclusion compound, as well as the $T_{1(VA/\alpha\text{-CD})}/T_{1VA}$ ratio. A decreasing of around 50% was observed in all relaxation times upon interaction. To further support VA inclusion a 2D-ROESY NMR experiment of the VA/α-CD complex was performed in D_2O. The cross peaks revealed interaction of H1, H2, H2′, H3, H3′ e H4, H4′ of VA and H3 located inside the α-CD cavity. Interaction between H2, H2′ and H4, H4' of VA with H5 of the α-CD cavity was also observed, as well as interaction between H4, H4′ of VA and H2, H4 of α-CD, giving more stringent evidence for formation of the inclusion compound (Figure 7). These results are in agreement with the relaxation times' ratios and suggest inclusion of the aliphatic chains of VA into the α-CD cavity in solution.

The recent literature reports two solid formulations of valproic acid obtained by complexation with hydrophilic cyclodextrins namely hydroxypropyl-β and sulfobutylether-β-cyclodextrin [21]. In the present work the preparation and a complete physico-chemical characterization of a sodium valproate/α-cyclodextrin (VA/α-CD) inclusion compound were carried out. The obtained composition is non-hygroscopic and can improve pharmaceutical manipulation of the drug. Moreover, it can be useful for oral administration. The anticonvulsant activity of the studied compound will be evaluated in the future.

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