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RESEARCH PAPER

Valproic Acid-Hydrophilic Cyclodextrin Complexes and Valproic Acid-Solid Dispersions: Evaluation of Their Potential Pharmaceutical Use

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ABSTRACT

The purpose of this study was to evaluate the potential use of two novel solid formulations of valproic acid (VPA) prepared by complexation with hydrophilic cyclodextrins (CDs) as hydroxypropyl-β- and sulfobutylether-β-cyclodextrin and by solid dispersion (SD) in hydrophilic carriers as polyethylene glycol 6000 (PEG 6000) and polyvinylpyrrolidone K-30 (PVP K-30). The corresponding cyclodextrin-based complexes were prepared by the freeze-drying method while the solid dispersions were obtained by the solvent method. Valproic acid solubility improved by CDs complexation and solid dispersion techniques. Comparison of dissolution profiles with that of VPA sodium salt (NaVP) was made by using release parameters such as dissolution efficiency, percent of drug dissolved after 60 min, and difference and similarity factors. Based on difference and similarity factors, it can be concluded that all the VPA formulations possess dissolution profiles essentially equivalent to those of NaVP at pH 6. However, this conclusion is not confirmed by using the analysis of variance (ANOVA) approach, indicating some significant differences between some SD-based formulations and NaVP at that pH value. Preliminary pharmacological studies in the pentylenetetrazole test in rats showed some important differences among the SD-based formulations, NaVP, and VPA as oil/water emulsion. Some implications and limitations of the investigated formulations are discussed.

Key Words: Valproic acid; Inclusion complexation; Solid dispersion; Dissolution rate.

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INTRODUCTION

Valproic acid (VPA) is an anticonvulsant agent structurally unrelated to other currently marketed antiepileptic agents.[1] It remains in clinical use as the favored anticonvulsant for controlling myoclonic seizures. Valproic acid occurs as a colorless to pale yellow, slightly viscous liquid, sparingly soluble in water. Administration of VPA is often associated with all the pharmaceutical problems involved in the manipulation of liquid material as well as with side effects such as gastric irritation, hepatotoxicity, and teratogenicity.[1] Moreover, VPA shows low ability to cross the blood-brain-barrier because it is more efficiently transported from brain-to-blood; this effect is mediated by efflux transporter systems involving P-glycoprotein.[2] As a consequence, high doses of VPA are required to achieve effective concentrations in the brain tissues. In terms of its biopharmaceutics classification, VPA is a class II drug since it is characterized by low solubility and high intestinal permeability.[3,4] For drugs belonging to this class, dissolution is important because it changes the actual drug concentration in solution over time.[5] For oral use, VPA is formulated as an oily solution in soft capsules or as a sodium salt (NaVP) in enteric-coated or crushable tablets.[6] Soft gelatin capsules, however, are very useful but expensive dosage forms and their production requires the control of several process variables. On the other hand, excessive moisture uptake is the major drawback for NaVP and it may cause handling and manufacturing difficulties, chemical instability, and variability in dissolution rates and bioavailability. Moreover, in the gastric tract the reconversion of the salt into the respective acid (VPA) may occur. Therefore, the development of an alternative VPA solid dosage form, which may exhibit a favorable in vivo dissolution performance, is desirable. Unlike classical approaches for converting a liquid material into the solid aggregation state, such as salt formation with an appropriate acid or base, chemical modification leading to solid prodrug, or microencapsulation, our strategy was to evaluate the potential of employing inclusion complexation with cyclodextrins (CDs) and solid dispersions (SDs). In recent years, CDs have widely been utilized in the pharmaceutical field due to their ability to entrap entirely, or partially, a variety of drugs, and this may lead to improvement of drug properties, including enhancements of solubility of poorly water-soluble drugs, dissolution rate, chemical stability, and bioavailability.[7,8] Similarly, the SDs may lead to the formation of systems in which the drug is dispersed almost to a molecular level, exhibiting an increased dissolution rate and potentially higher bioavailability.[9,10] An additional practical advantage offered by both CDs and SDs is that the matrix may prevent contact of the drug with biological surfaces and thereby may decrease tissue irritation.

Complexes of VPA/β-CD have been prepared, but due to the low drug content,[11,12] large amounts of VPA/β-CD complexes would be equivalent to the daily drug dose. To our knowledge, however, the VPA complexation capability of hydrophilic neutral and anionic cyclodextrins [i.e., hydroxypropyl-β- and sulfobutylether-β-cyclodextrin (HP-β-CD and SBE-β-CD, respectively)] was not previously investigated. These cyclodextrins, in fact, have the distinct advantage of being more soluble and safer than β-CD, due to their minimal toxicity profiles.[13]

As part of our ongoing research program aimed at the identification of improved formulations for traditional anticonvulsant,[14] hypnotic,[15] and anesthetic agents,[16] we have prepared novel solid formulations of VPA by complexation with hydrophilic neutral and anionic cyclodextrins and by dispersion in some hydrophilic carriers such as polyethylene glycol 6000 or polyvinylpyrrolidone K-30. The specific aim of the present study was, therefore, to evaluate the potential pharmaceutical use of these new solid VPA formulations.

MATERIALS AND METHODS

Chemicals

Valproic acid (VPA) and sodium valproate (NaVP) were purchased from Sigma-Aldrich S.r.l. (Milan, Italy). Polyethylene glycol 6000 (PEG 6000) and polyvinylpyrrolidone (PVP) K-30 were purchased from Fluka (Milan, Italy). 2-Hydroxypropyl-β-cyclodextrin was obtained as a gift from Roquette (Cassano Spinola, Italy) and its substitution degree (5.88) calculated by means of ^{1}H-NMR.[17] Sulfobutylether-β-cyclodextrin sodium salt $(SBE)_{7m}$-β-CD [Captisol, degree of substitution 6.4 (determined by the supplier) referred to in the following text SBE-β-CD] was kindly donated from Cydex Inc., Overland Park, KS. Reagents used for the preparation of the buffers were of analytical grade. Fresh deionized water from all glass apparatus was used in the preparation of all the solutions.

High-Performance Liquid Chromatography (HPLC) Analysis

High-performance liquid chromatography (HPLC) analyses were performed using a Waters Associates

Model 600 pump equipped with a Waters 990 (Waters, Milan, Italy) variable wavelength UV detector and a 20 μL loop injection valve or an autosampler (Water model 712 WISP) with 40 μL vials. For analysis, a reversed-phase Simmetry (25 cm × 4.6 mm; 5 μm particles) column in conjunction with a precolumn module was eluted by using a mixture (60:40) of acetonitrile and deionized water containing 0.1% trifluoroacetic acid solution. The flow rate of 1 mL/min was maintained. The column effluent was monitored continuously at 205 nm. Under these conditions, the retention time of VPA was 7.8 min. Quantification of the compounds was carried out by measuring the peak areas in relation to those of standards chromatographed under the same conditions. Standard curves were prepared at a wavelength of 205 nm using acetonitrile as the solvent and were linear ($r^2 > 0.998$) over the range of concentrations 0.015–0.599 mg/mL, the former value being the lower limit of quantification.

Preparation of Solid VPA-CDs Complexes and -PEG 6000, and -PVP K-30 Solid Dispersions

In preparing the solid VPA-HP-β-CD inclusion complex by the freeze-drying method, VPA was equilibrated with equimolar amount of HP-β-CD (0.66 mmol) or SBE-β-CD (0.46 mmol) in 10 μL of deionized water. The mixtures were stirred at room temperature for 5 days, filtered through a 0.22-μm membrane filter, and the clear filtrate subjected to freeze-drying (Cinquepascal, Lio 5P model freeze-drier, Milan, Italy). Thus, the samples obtained were stored in a desiccator until their further manipulation. The degree of incorporation of complex (i.e., the solid composition in terms of mg of drug/g of solid complex) was obtained by dissolving 50 mg of the complex in 5 μL of 0.05 M potassium phosphate buffer pH 6 and the resulting content of VPA determined by HPLC. The results are expressed as mg of VPA per g of complex and are means of three determinations.

Dispersions of VPA in PEG 6000 or PVP K-30 containing two different weight ratios (1:5, and 1:10) and denoted as SDPEG 1/5, 1/10, SDPVP 1/5, and 1/10, respectively, were prepared by the solvent method as follows. To a solution of VPA (200 mg) in ethanol 80° (25 mL) the appropriate amount of PEG 6000 or PVP K-30 was added. Next, the solvent was evaporated under reduced pressure at 40° C and the resulting residue, dried under vacuum for 3 h, was stored for at least overnight in a desiccator. The samples prior to be used for the subsequent analysis were pulverized using a mortar and pestle and the powders were passed through a 280 mm sieve. No trace of ethanol was shown by ^{1}H-NMR spectroscopy in the case of VPA/PEG-based formulations while the residual ethanol level was ≤2% for VPA/PVP K-30 systems.

Spectroscopic Studies

^{1}H-NMR and ^{13}C-NMR spectra of VPA and corresponding complexes with HP-β-CD and SBE-β-CD were recorded at 25° C using a Bruker (Milan, Italy) AM 300 WB (300 MHz) spectrometer or a Varian (Milan, Italy) 300 MHz instrument. The samples for NMR measurements were prepared by dissolving 50 mg of VPA or VPA/HP-β-CD or VPA/SBE-β-CD complexes in 0.6 mL of D_2O. This solvent allows a suitable solubilization of the free drug and complexes for these experiments. ^{1}H-NMR and ^{13}C-NMR chemical shifts were referred to DHO (4.670 ppm) and dioxane (67.800 ppm) as internal standards, respectively. The 1D ^{1}H NOE experiments were carried out by using the same solutions used for ^{1}H-NMR. Samples were deaerated by bubbling N_2 directly in the NMR tube. The spectra were recorded in the following conditions: number of scans, 128; acquisition time, 2.72 sec; pulse width, 2.90 μs; time domain, 32 K; spectral width, 4500 Hz; decoupling power, 30 dB. The NOE measurements were performed by irradiation of the H-3 and H-6 signals of HP-β-CD at 25° C. The increments of the signals (NOE %) were evaluated with the NOE difference program.

Fourier transform IR (FT=IR) spectra were obtained on a Perkin–Elmer 1600 FT-IR spectrometer. Samples were prepared in KBr disks (2 mg sample in 200 mg KBr). The scanning range was 450–4000 cm^{-1} and the resolution was 1 cm^{-1}.

Thermal Analysis

Differential scanning calorimetry (DSC) curves were obtained by a Mettler Toledo DSC 822e Star e 202 System (Mettler Toledo, Switzerland) equipped with a thermal analysis automatic program.

Aliquots of about 5 mg of each sample were placed in an aluminium pan of 40 μL capacity and 0.1 mm thickness, press-sealed with a perforated aluminium cover of 0.1 mm thickness. An empty pan sealed in the same way was used as reference. Conventional DSC measurements were performed by heating the sample to 220° C at a rate of 5° C /min, under a nitrogen flow of 50 cm^3 /min. The starting temperatures were −25° C, −10° C, and 25° C for PEG 6000, PVP K-30, and CDs, respectively. The DSC measurements were conducted also on quenched samples. Quenching was achieved by

cooling the crucible after the heating cycle at room temperature (time required about 10 min) and holding the sample for further 10 min at this temperature. Then, it was reheated to 220° C at a rate of 5° C/min. Indium was used as the standard for calibrating the temperature. Reproducibility was checked running the sample in triplicate.

Solubility and Dissolution Studies

Solubility measurements of VPA were carried out at 37° C using HP-β-CD or SBE-β-CD in 0.05 M potassium phosphate buffer pH 6. A large excess of the antiepileptic agent was added to 2 μL of the appropriate CDs solution in screw-capped test tubes. The mixtures were vortexed for about 5 min and shaken in a thermostatically controlled water bath shaker for 4 days. After this time, the pH of the medium was checked and a lower value (i.e., pH 4.5) resulted, likely due to the fact that VPA is an acid drug. Then, an aliquot of aqueous phase of each mixture (collected by a pasteur pipette having the tip covered with cotton and introduced in the water phase by bubbling air) was transferred (after cleaning of the external wall of the pipette) to a 10-mL glass syringe preheated at the appropriate temperature and filtered through a 0.22-μm membrane filter (Millipore® cellulose acetate) in thermostated test tubes. The filtrates were allowed to stand at the appropriate temperature until analyzed by HPLC. Samples were analyzed directly or diluted when needed with mobile phase. The injection volume was 20 μL. The apparent 1:1 stability constant (K_c) was estimated from the slope of the straight line of the phase-solubility diagram according to the following equation: Kc=slope/S_0 (1-slope).[18] The solubility value (S_0) of VPA was determined directly in 0.05 M potassium phosphate buffer pH 6 at 37° C and resulted in good agreement with that reported in literature (i.e., 1.27 mg/mL in water at room temperature).[19]

Dissolution experiments were carried out in triplicate with an Erweka DT dissolution test in 0.05 M potassium phosphate buffer pH 6, in HCl/KCl buffer pH 2 and in HCl 0.1 N/NaCl 0.2% pH 1.2 at 37° C using the paddle method at a rotation speed of 60 rpm. Samples of each preparation equivalent to 80 mg of VPA were added to 300 μL of dissolution medium. Such conditions were chosen taking into account the limit of VPA quantification by the analytical procedure used. At appropriate time intervals, 2 μL of the mixture was withdrawn and filtered through a 0.22-μm membrane filter (Millipore®, cellulose acetate) in thermostated test tubes. Samples were withdrawn from a zone roughly midway between the surface of dissolution medium and the top of the rotating blade. The initial volume was maintained by adding 2 mL of dissolution medium. About 1 μL of the clear filtrate, after appropriate dilution, was allowed to stand in a bath at 37° C until analyzed by HPLC. Samples were analyzed directly or diluted when needed with mobile phase. The injection volume was 20 μL. The results were computed with a standard calibration curve of the drug. Each test was repeated three times (CV<3%). Dissolution efficiency (DE) was calculated from the area under the dissolution curve up to a certain time, t (measured using the trapezoidal rule) and expressed as a percentage of the area of the rectangle described by 100% dissolution in the same time.[20] The difference factor (f_1) is the average % difference over all time points in the amount of test batch (i.e., SDPEG 1/5, SDPEG 1/10, SDPVP 1/5, and SDPVP 1/10) dissolved as compared to the reference batch i.e., NaVP). The f_1 value is 0 when the test and the reference profiles are identical and increases proportionally with the dissimilarity between the two profiles. The similarity factor (f_2) value is between 0 and 100. The value is 100 when the test and the reference profiles are identical and approaches zero as the dissimilarity increases. Values between 0 and 15 for f_1 and between 50.8 and 100 for f_2 indicate equivalent dissolution profiles. These factors are defined as follows respectively:[21]

$$f_1 = \left\{ \frac{\sum_{t=1}^{n} |R_t - T_t|}{\sum_{t=1}^{n} R_t} \right\} \times 100 \quad (1)$$

$$f_2 = 50 \log\left\{ \left(1 + \frac{1}{n} \sum_{t=1}^{n} (R_t - T_t)^2 \right)^{-0.5} \times 100 \right\} \quad (2)$$

where n is the number of dissolution sample times, and R_t and T_t are the individual or mean percent dissolved at each time point, t, for the reference (NaVP in this study) and the test dissolution profiles, respectively. The similarity factor has been adopted by Food and Drug Administration (FDA) and by Human Medicines Evaluation Unit of the European Agency for the Evaluation of Medicinal Products (EMEA), as a criterion for assessment of similarity between two in vitro dissolution profiles. The statistical significance of differences in dissolution efficiencies were analyzed

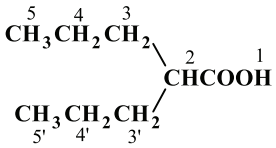
also utilizing the analysis of variance (ANOVA) test followed by Tukey post test.

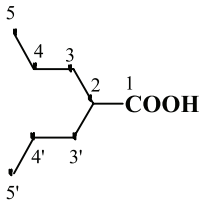
Pharmacological Studies

Adult male or female Sprague–Dawley CD rats (Charles River, Como, Italy) with body masses of 200–250 g at the beginning the experiments, were maintained under an artificial 12-h-light/dark cycle (light on 08:00 to 20:00 h) at a constant temperature of 23°±2°C and 65% humidity. Food and water were freely available, and the animals were acclimatized for >7 days before use. Experiments were performed between 08:00 and 14:00 h. Animal care and handling throughout the experimental procedure were performed in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). The experimental protocol was approved by the Animal Ethical Committee of the University of Cagliari.

VPA/HP-β-CD and VPA/SBE-β-CD complexes, SDPEG 1/10 and SDPVP1/10, and powdered NaVP were dissolved in distilled water by sonication for 10 min. Valproic acid was dispersed in distilled water with 0.5% (w/v) of Tween 80. Rats (five per each group) were food-deprived for 12 h before the experiment and received by an appropriate needle an intragastric administration of the drugs at the equivalent dose of 260 mg/Kg in a volume of 3 μL. Drugs were administered 60 minutes before pentylenetetrazole (55 mg/kg) injection intraperitoneally. Control rats received an equivalent volume of vehicle. The animals were observed for 60 min after injection of pentylenetetrazole during which time the onset, duration of seizures, and death were recorded. For all the animals the following seizure pattern was observed: at the onset, only clonic convulsions of the anterior legs, followed by the clonic seizures of all four legs, and next by the tonic/clonic ones. Data are presented as

Table 1. Proton and carbon chemical shifts corresponding to VPA in absence and presence of HP-β-CD and SBE-β-CD.





Proton number	δ_{free}	$\delta_{complex}$ [a]	$\Delta\delta$ [b]	Peak multiplicity	Carbon number	δ_{free}	$\delta_{complex}$ [a]	$\Delta\delta$ [b]
2	2.431	2.385	−0.046	Multiplet	1	183.56	181.28	−2.28
3 and 3′	1.502	1.542	0.040	Multiplet	2	46.31	45.96	−0.35
4 and 4′	1.298	1.350	0.052	Multiplet	3 and 3′	36.66	35.47	−1.19
5 and 5′	0.883	0.944 and 0.932	0.061 and 0.049	Triplet	4 and 4′	21.75	21.41	−0.34
					5 and 5′	14.90	14.80 and 14.74	−0.10 and −0.16

Proton number	δ_{free}	$\delta_{complex}$ [c]	$\Delta\delta$ [b]	Peak multiplicity	Carbon number	δ_{free}	$\delta_{complex}$ [c]	$\Delta\delta$ [b]
2	2.431	2.390	−0.041	Multiplet	1	183.56	181.15	−2.41
3 and 3′	1.502	1.539	0.037	Multiplet	2	46.31	45.92	−0.39
4 and 4′	1.298	1.335	0.037	Multiplet	3 and 3′	36.66	35.45	−1.21
5 and 5′	0.883	0.920 and 0.918	0.037 and 0.035	Triplet	4 and 4′	21.75	21.42	−0.33
					5 and 5′	14.90	14.86 and 14.79	−0.04 and −0.11

[a]Chemical shifts corresponding to VPA in the presence of HP-β-CD.
[b]$\Delta\delta = \delta_{complex} - \delta_{free}$ VPA.
[c]Chemical shifts corresponding to VPA in the presence of SBE-β-CD.

means±SEM and were analyzed utilizing analysis of variance (ANOVA) test followed by Tukey post test.

RESULTS AND DISCUSSION

NMR Measurements

Evidence of inclusion complex formation for the VPA/HP-β-CD and VPA/SBE-β-CD systems was obtained by ^{1}H- and ^{13}C-NMR spectroscopy. The chemical shifts for the protons of VPA both in the absence and presence of HP-β-CD and SBE-β-CD are summarized in Table 1 as δ free and $\delta_{complex}$, respectively. As can be seen, significant shifts were observed for all protons of VPA in the presence of these CDs. For the guest molecule, the 3 and 3′, 4 and 4′, and 5 and 5′ protons showed downfield shifts (Table 1), while the H-2 proton shifted in the opposite direction. Hence, the environment of the latter proton should be different from the formers. The ^{13}C chemical shifts and carbon assignments made for VPA as well as the ^{13}C chemical shift modifications upon complexation are also shown in Table 1. For the guest molecule, all the carbons showed upfield shifts (Table 1), with the greater Δδ (i.e., $\delta_{complex} - \delta_{free}$) being observed for the C-1 and C-3 and C-3′. These findings indicate that the propyl moieties of the VPA molecule should be located partially inside the HP-β-CD cavity, while the carboxylic group should be located outside the CD cavity. To further support this suggestion, 1D NOE experiments on the VPA/HP-β-CD complex were performed. As for the proton resonances of HP-β-CD, we examined only the effects of irradiation of the protons located inside the cavity, both close to the large rim (H-3) and to the narrow side (H-6). These proton resonances of HP-β-CD were identified as reported in literature.[22] Thus, a significant NOE effect was observed between the 5 and 5′ protons of VPA and the H-3 proton of HP-β-CD. As shown in Fig. 1, indeed, irradiation of H-3 proton of HP-β-CD [i.e., the multiplet resonating at about 4.3 δ,[22]] had a significant effect on the integral of methyl group protons of VPA. In contrast, irradiation of H-6 proton of HP-β-CD had no effect on the methyl group protons of VPA. Hence, on the basis of ^{1}H- and ^{13}C-NMR spectroscopy, it may be hypothesized that the methyl group protons of VPA are located partially inside the hydrophobic HP-β-CD cavity.

Solid State Studies

The purpose of these studies was to characterize the solid state of both VPA/HP-β-CD and VPA/SBE-β-CD complexes, and VPA/PEG 6000 and VPA/PVP K-30 solid dispersions. The FT-IR spectrum of pure VPA showed a strong absorption band at 1709 cm^{-1} attributable to the C═O group stretching. In all the infrared spectra of CD-based formulations the characteristic absorption band of the pure drug at 1709 cm^{-1} is maintained, even though an evident reduction of intensity was observed. On the other hand, all VPA/PVP K-30 solid dispersions showed a large absorption band at 1660 cm^{-1}, which presumably masked possible shifts of the drug carboxyl group stretching. It would be consistent with the formation of a hydrogen bonding interaction between the drug carboxyl group and the carbonyl function of PVP. The VPA/PEG 6000 systems displayed reduced bands at 1726 cm^{-1}, in a drug-concentration dependent manner. The observed shift of the carbonyl stretching band in the VPA/PEG 6000 systems may suggest an interaction between VPA and carrier. In Fig. 2 FT-IR spectra of selected systems are shown.

The DSC profiles of VPA/PVP K 30, VPA/PEG 6000, and VPA-CD systems are shown in Fig. 3. As can be seen, for the VPA/PVP K-30 system, a broad endotherm was observed in the range 50–120° C, which disappeared in the reheating cycle after cooling. This endotherm peak should be due to the contemporary loss of water and part of VPA. This suggestion is based on the composition (determined by HPLC) of the sample recovered after thermal analysis. For this sample, indeed, a 73% decrease in VPA content was observed. Moreover, as shown in Table 2, no significant difference was noted between the glass transition temperature (Tg) of the PVP-based solid dispersion and that of the polymer alone (i.e., 160.6°±4° C and 164.8°±2.2° C, respectively). Meanwhile a marked decrease in Tg (i.e., 115.7°±2.2° C) was observed in the reheating cycle of the dispersed system. At first glance, this plasticizing effect is not obvious to be accounted for, but it can be speculated that the VPA/PVP K-30 system, stressed from the first heating cycle, is characterized by a new phase with a greater and intimate interaction between the two components.

The DSC profiles of the PEG-based systems exhibited a melting endotherm. It occurs at 61.7°±0.5° C for the pure polymer and at 58.7°±0.5° C for the dispersed system. Furthermore, in the reheating cycle, an additional endothermic peak is clearly seen at 58.0°±0.5° C or 51.6°±0.5° C for the pure polymer and the dispersed system, respectively. It may likely due to the melting of the polymeric carrier, PEG 6000, in two different conformations (folded and extended) produced in the first heating cycle. For this sample a decrease of 60% in VPA content was observed. The two CD-based formulations also showed

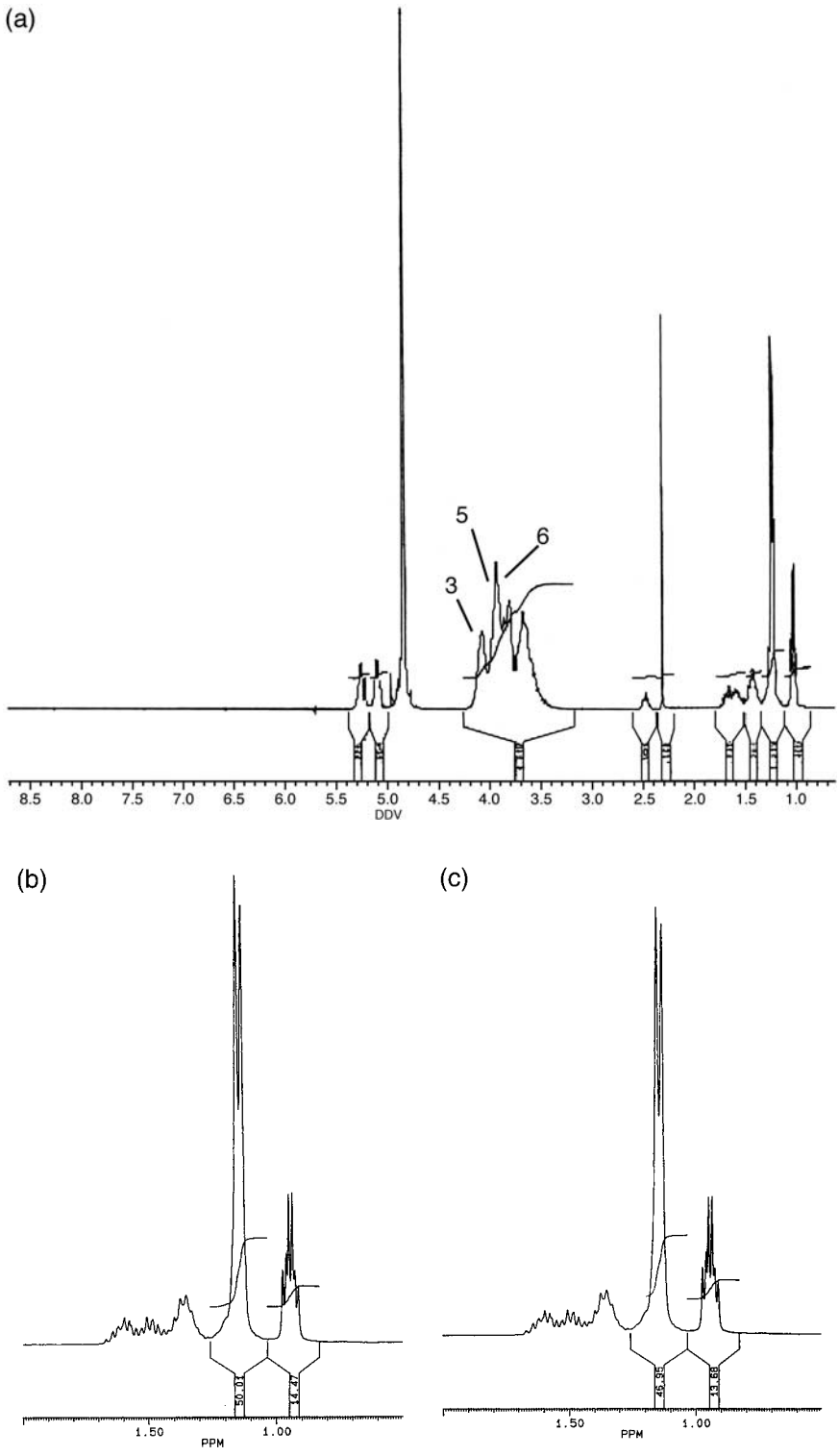


Figure 1. 1D NOE experiments on the VPA/HP-β-CD complex. (a) 1D ^{1}H-NMR spectrum of VPA/HP-β-CD complex showing the resonances of the H-3, H-5 and H-6 protons of the CD; (b) 1D 1 H-NMR spectrum of VPA/HP-β-CD complex in the range 0.8–1.7 δ; and (c) Effect on the integral of methyl group protons of VPA by irradiation of the H-3 proton of HP-β-CD.

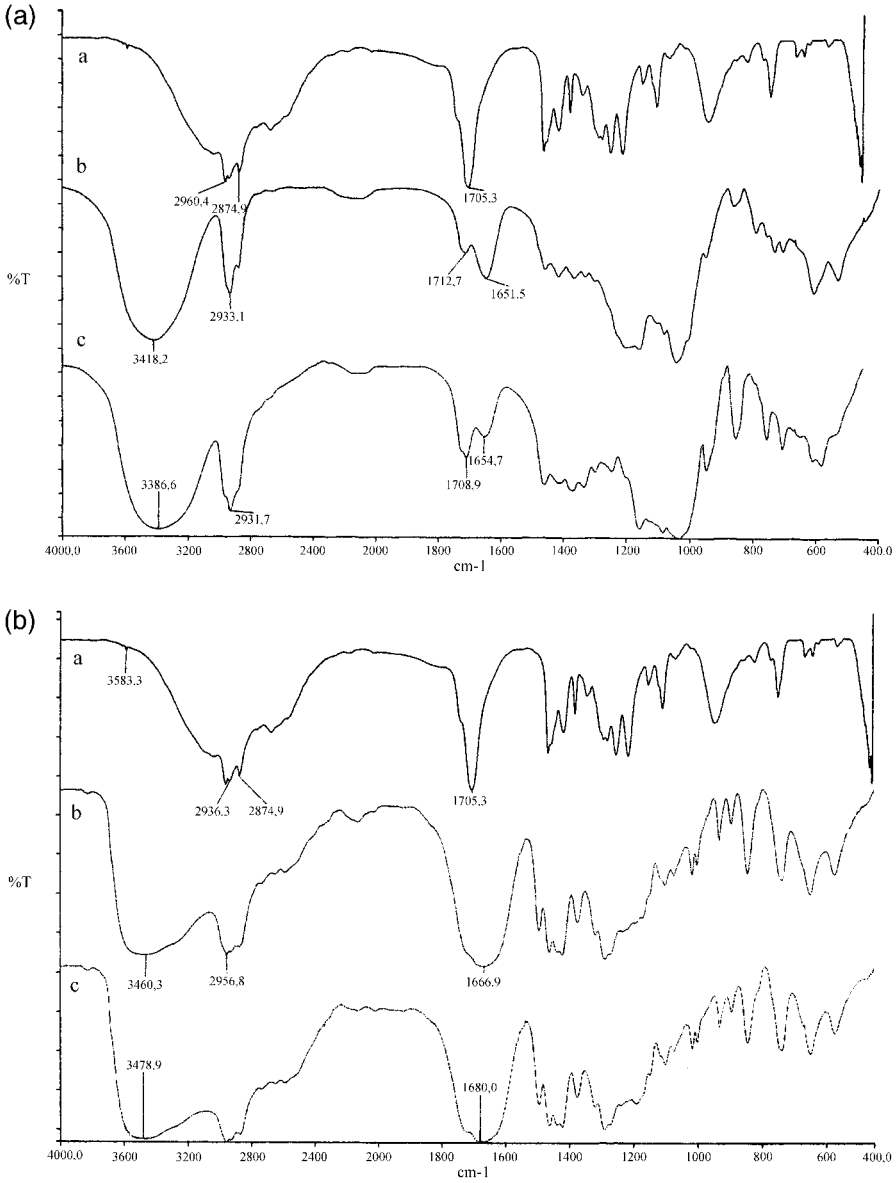


Figure 2. FT-IR spectra of (a), VPA (a), VPA/SBE-β-CD (b), VPA/HP-β-CD (c); (b) VPA (a), SDPVP 1/10 (b), SDPVP 1/5 (c).

a broad endotherm in the range 50–120° C (data not shown), which disappeared in the reheating cycle. In both cases a decrease of more than 90% in VPA content was observed.

These findings, taken together, indicate that VPA/CD and VPA/SD systems should correspond essentially to amorphous materials.

These suggestions were further supported by the analysis of x-ray diffraction patterns of VPA/CD and VPA/SD systems (data not shown). Indeed, the x-ray diffraction spectra of both CD complexes and solid dispersions were characterized only by very large diffraction peaks and were identical to those of the corresponding carriers.

Solubility and Dissolution Studies

Valproic acid VPA is a weak acid with an apparent pKa of 4.6 and is poorly soluble in water (its solubility in phosphate buffer pH 6.0 at 37° C was

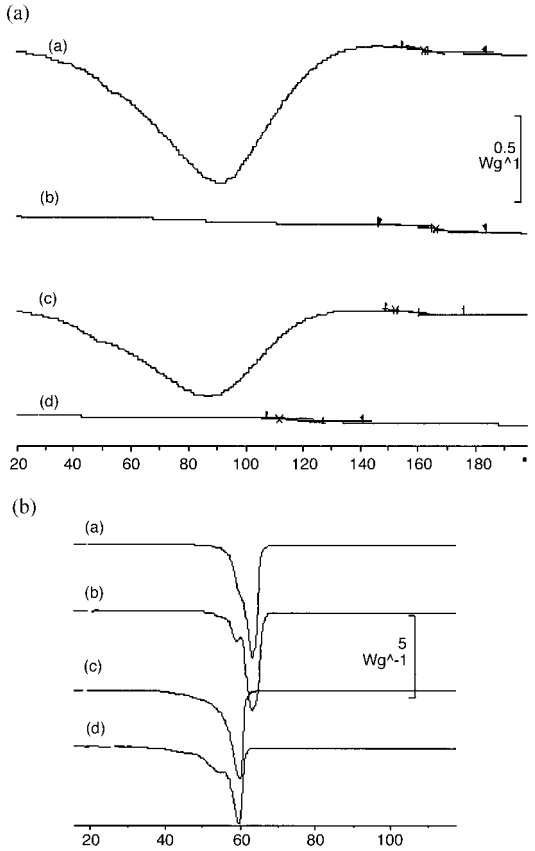


Figure 3. DSC thermal profiles of (a) a) heating cycle of PVP K-30; b) reheating cycle of quench-cooled PVP K-30; c) heating cycle of SDPVP 1/10; and d) reheating cycle of quench-cooled SDPVP 1/10. (b) a) heating cycle of PEG 6000; b) reheating cycle of quench-cooled PEG 6000; c) heating cycle of SDPEG 1/10; and d) reheating cycle of quench-cooled SDPEG 1/10.

Table 2. Thermal effects observed for CD- and SD-based formulations.

Formulation	Tm (°C)	Tg (°C)	Loss of VPA (%)
PVP K-30		164.8±2.2	
SDPVP 1/10		160.6±4.0[a]	73
		115.7±2.2[b]	
PEG 6000	61.7±0.5[a]		
	58.0±0.5[b]		
SDPEG 1/10	58.7±0.5[a]		60
	51.6±0.5[b]		
VPA/HP-β-CD			90
VPA/SBE-β-CD			90

[a]Heating cycle.
[b]Reheating cycle after cooling.

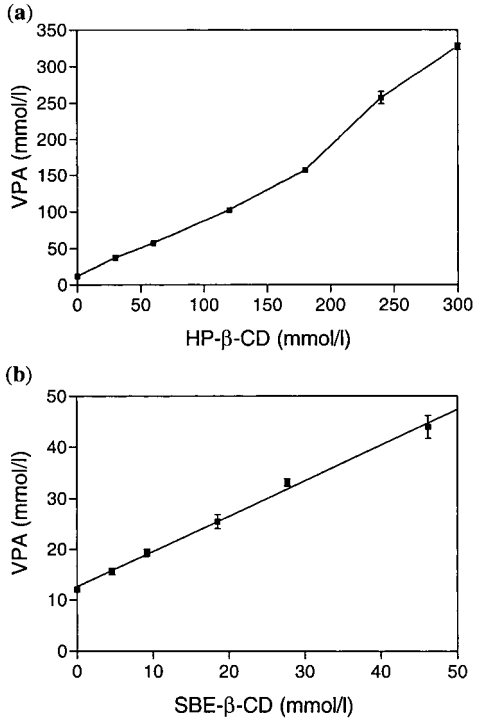


Figure 4. Phase-solubility diagrams of VPA with (a) HP-β-CD; (b) SBE-β-CD. Apparent 1:1 formation constant ($K_{1:1}$) were calculated from the slope of the straight lines.

found at 1.75 mg/mL). Solubility studies showed that the concentration of VPA at 37° C and pH 6.0 is affected by the presence of CDs. Thus, a 45% w/v HP-β-CD solution provided for a 47.21 mg/mL content of VPA corresponding to a 27-fold increase in the concentration of VPA. On the other hand, a 10% w/v SBE-β-CD solution provided for only a 6.34 mg/mL content of VPA. Figure 4(a) shows the equilibrium phase-solubility diagram observed for VPA with increasing concentrations of HP-β-CD. This solubility curve can be classified as type Ap, as defined by Higuchi and Connors[18] suggesting the formation of higher-order complexes. In this regard, however, it should be remembered that, as pointed recently out by Loftsson et al.,[23] the stoichiometry of drug/cyclodextrin complexes cannot be derived from simple phase-solubility studies. The value of the apparent 1:1 stability constant ($K_{1:1}$) of the complex has been calculated from the slope of the initial straight line portion of the phase-solubility diagram according to the following equation: $Kc = slope/S_0$ (1-slope),[18] where S_0 is the solubility value of VPA in phosphate buffer pH 6.0 (Table 3). The $K_{1:1}$ value so obtained resulted

Table 3. Stability constant and degree of incorporation by complexation between VPA and HP-β-CD or SBE-β-CD in phosphate buffer (ph=6.0).

Cyclodextrin	Apparent stability constant $K_{1:1}$ (M^{-1})[a]	Degree of VPA incorporation (mg/g complex)[a]
HP-β-CD	241 (3.9)	75 (3)
SBE-β-CD	192 (12)	39.5 (3)

[a]Mean of three determinations. Relative standard deviation (CV%) values are reported in parentheses.

241 M^{-1}. The value of the apparent 1:1 ($K_{1:1}$) and 1:2 ($K_{1:2}$) stability constants of the system were also deduced by a curve fitting procedure with a quadratic model[18] expressed by the equation:

$$S_{tot} = S_0 + K_{1:1}S_0[CD] + K_{1:1}K_{1:2}S_0[CD]^2 \quad (3)$$

The corresponding values so derived were 2.23 mg/mL, 34 and 3.3 M^{-1} for S_0, $K_{1:1}$ and $K_{1:2}$, respectively. As can be seen, the ($K_{1:1}$) stability constant obtained by curve fitting is about a magnitude order lower than that measured by the slope of the initial straight line portion of the phase-solubility diagram. This discrepancy of the stability constant values may be due to the fact that to single out the initial straight line portion is not unambiguous. Furthermore, the $K_{1:1}$ value (34 M^{-1}) was larger than the $K_{1:2}$ value (3.3 M^{-1}), indicating that the 1:1 complex should be more stable than the 1:2 complex. The enhancement of the VPA solubility observed with SBE-β-CD solutions is linear and characterized by a slope of less than 1, and it was assumed that the solubility increase was due to the formation of a 1:1 complex. Again, the apparent stability constant values ($K_{1:1}$) (Table 3) were estimated from the slope of the straight line [Fig. 4(b)]. The stability constant value with the neutral HP-β-CD was slightly higher than that observed with the anionic SBE-β-CD (e.g., 241 and 192 M^{-1}, respectively). These stability constant values are indicative of a shallow inclusion. Table 3 also shows the VPA incorporation degrees in the solid cyclodextrin complexes. As can be seen again due to the low drug content, large amounts of VPA/HP-β-CD or VPA/SBE-β-CD complexes would

Table 4. Dissolution efficiency (DE), percent of drug dissolved after 60 min (DP), difference factor (f_1), and similarity factor (f_2).[a]

Formulations	pH	DE[b]	DP	f_1[b]	f_2[b]
NaVP	1.2	86	89		
	2	89	92		
	6	93	98		
SDPEG 1/5	1.2	86	92	9.27	41.41
	2	85	89	7.51	46.55
	6	89	89	5.25	62.63
SDPEG 1/10	1.2	85	90	5.38	56.75
	2	78[d]	83	16.09	36.55
	6	100[d]	102	6.37	59.66
SDPVP 1/5	1.2	87	90	2.96	74.48
	2	87	91	3.99	64.75
	6	87[c]	94	3.83	65.65
SDPVP 1/10	1.2	87	89	2.38	74.80
	2	91	93	5.88	52.81
	6	89	94	6.25	58.65

[a]Data are the mean of three determinations, CV<3%.
[b]Difference factor (f_1) for reference (NaVP) vs. test products. Last point for dissolution is at 180 min. The time to dissolve 50% of drug ($t_{50\%}$) was in all cases <5 min.
[c]$p<0.01$ Similarity factor (f_2) for reference (NaVP) vs. test products. Last point for dissolution is at 180 min.
[d]$p<0.001$ significantly different from reference (NaVP).

Table 5. Evaluation of anticonvulsant effects in rats in the pentylenetetrazole test for the VPA/CDs- and VPA/SDs-based formulations.

VPA formulations	Number of animals protected vs. total number of animals tested (% protection)	Onset (sec)	Duration (sec)
Controls	0/5	125±25	[a]
SDPEG 1/10	2/5 (40)	219±89[b]	33.7±10
SDPVP 1/10	1/5 (20)	82±23	27.0±7
NaVP	1/5 (20)	157±29	22.2±4
VPA as oil/water emulsion	3/5 (60)	133±16	30±5

[a]The death of all the animals occurred.
[b]$p<0.05$ significantly different from control (NaVP).

be equivalent to the daily drug dose. Therefore, they were not further pursued in this study.

Comparison of dissolution profiles between NaVP and SD-based formulations was made by using release parameters such as dissolution efficiency (DE), percent of drug dissolved after 60 min (DP), difference factor (f_1), and similarity factor (f_2). The results of these studies are shown in Table 4. As can be seen from the reported data, high DEs were observed in any case, being all the values in the range 78–100%.

Comparing NaVP and VPA/SDs dissolution profiles again the f_1 values obtained were in any case less than 15 except for the dissolution of SDPEG1/10 in strong acidic conditions (pH 2). The f_2 values were higher than the limit value of 50 except for dissolutions of SDPEG 1/5 and 1/10 in strong acidic conditions (pH 1.2–2). Based on difference and similarity factors, it can be concluded that NaVP and VPA/SDs dissolution profiles were essentially similar at pH 6. In contrast, utilizing the ANOVA approach, the dissolution profiles of SDPEG 1/10 and SDPVP1/5 resulted significantly different from that of NaVP at pH value corresponding to the small intestine (i.e., pH 6). It is noteworthy that in such conditions, SDPEG 1/10 showed 100% in DE.

Pharmacological Studies

To verify whether there are any differences in terms of pharmacological effects between the fast-issolving SD-based solid formulations and the reference one (NaVP), we decided to explore their anticonvulsant effects in the pentylenetetrazole test, following oral administration and given in equimolar doses to rats. In this preliminary pharmacological study, percent of protection as well as onset and duration of convulsions were recorded for the following formulations: SDPEG 1/10 and SDPVP1/10, powdered NaVP. For the sake of comparison, a liquid formulation of VPA as an oil/water emulsion was also included. The results obtained are summarized in Table 5. The protection observed for the examined formulations was in the order: VPA as an oil/water emulsion>SDPEG1/10>SDPVP1/10, NaVP. With the exception of the SDPEG 1/10, no significant differences of onset and duration of convulsions were noted among the formulations. It should be pointed out that SDPEG 1/10 give rise to a better protection than VPA and it may be related to its very high DE. The greater protection showed by the oil/water emulsion may be the result of the presence of the surface-active agent Tween 80.

In conclusion, this study has demonstrated that complexation with HP-β-CD and SBE-β-CD cannot be used for preparing useful powders containing VPA due to the fact that the doses as CD complex would be unacceptably high. In contrast, SDPEG and SDPVP with high drug content (e.g., SD 1/5 and presumably also at higher content, SD 1/4 or SD 1/3) have potential for the development of quick dissolving VPA solid formulations, likely with minimal tissue irritation and nondeliquescent in nature. However, the in vivo performance of these SD-based formulations should be investigated in detail to fully evaluate their biopharmaceutical properties and quality.

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