Investigation and Physicochemical Characterization of Vinpocetine-Sulfobutyl Ether β-Cyclodextrin Binary and Ternary Complexes

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The purpose of this study was to investigate the interactions between vinpocetine (VP), sulfobutyl ether beta-cyclodextrin (SBEβCD) and the water-soluble polymers polyvinylpyrrolidone (PVP) and hydroxypropyl methylcellulose (HPMC). The water-soluble polymers were shown to improve the complexation efficiency of SBEβCD, and thus less SBEβCD was needed to prepare solid VP–SBEβCD complexes in the presence of the polymers. The interactions between VP and SBEβCD, with or without PVP or HPMC, were thoroughly investigated in aqueous solutions using the phase-solubility method as well as in the solid state. The amount of VP solubilized in water or aqueous polymer solution increased linearly with increasing SBEβCD concentration, demonstrating A_L-type plots. We estimated the apparent stability constant (K_c) at room temperature of VP–SBEβCD binary complex to be $340\,\mathrm{M}^{-1}$ and this value increased to $490\,\mathrm{M}^{-1}$ or $390\,\mathrm{M}^{-1}$, respectively, with the addition of PVP and HPMC, assuming a 1:1 VP–SBEβCD molar ratio. Improvement in the K_c values for ternary complexes clearly confirmed the benefit of the addition of water-soluble polymers to promote higher complexation efficiency. Solid VP–SBEβCD binary and ternary systems were prepared by physical mixing, kneading, coevaporation, and lyophilization methods and fully characterized by scanning electron microscopy, differential scanning calorimetry, and X-ray diffractometry. The results obtained suggest that coevaporation and lyophilization methods yield a higher degree of amorphous entities and indicated formation of VP–SBEβCD binary and ternary complexes.

Key words   sulfobutyl ether β-cyclodextrin; vinpocetine; water-soluble polymer; solubility study; complexation efficiency; characterization

Vinpocetine (VP) is a vincamine derivative used for the treatment of disorders arising from cerebrovascular and cerebral degenerative diseases.[1] VP is thought to increase the cerebral flow in the ischemic areas of patients with cerebrovascular disease, decrease platelet aggregability in patients with transient ischemic attack or stroke, increase red blow cell deformability in stroke patients and have neuroprotective abilities and a protective effect against brain ischemia.[2]

VP is a poorly water-soluble base-type drug and it is usually available as tablets containing 5 mg of the active ingredient. However, existing formulations exhibit poor bioavaibility (about 6.7%)[3] and poor absorption, due to low VP solubility.[4] To overcome these difficulties, several attempts have been made such as the formation of VP water-soluble citrate and phosphate salts resulting in an increased drug dissolution rate and consequent onset of pharmacological action.[5] VP salts have also been used to prepare liquid products for oral and parenteral administration. However, it is known that salts of poorly water-soluble weak bases can precipitate at pH values found in the gastrointestinal tract.[6] Complexation of VP with cyclodextrins (CDs) has been studied by Kata and coauthors.[4,7] The aim of their study was to improve the solubility and dissolution rate of VP. However, to the best of our knowledge, the solid states of the various VP–CD complexes have not previously been characterized.

Due to the potential of CDs to form reversible inclusion complexes with a wide variety of drugs, they have been recognized as a group of useful pharmaceutical excipients.[8] Formation of such complexes usually results in some favorable changes in the physicochemical characteristics of the guest molecules, such as increased solubility, improved stability, enhanced bioavaibility, and reduced side effects.[9—11] Unfortunately, the complexation efficiency of CDs is rather low and consequently a significant amount of CDs is frequently needed to solubilize small amounts of a water-insoluble drug. However, it is possible to enhance both the aqueous solubility of the complexes and the complexation efficacy by adding small amounts of a water-soluble polymer to the complexation media.[12] In this way, numerous authors have reported the synergistic effect of water-soluble polymers on the solubility enhancing effect of CDs and consequently a decrease in the CD amount required to prepare soluble drug-CD complexes.[13—20]

The objective of this work was to study the interactions of VP with sulfobutyl ether β-CD (SBEβCD) in association with or without water-soluble polymers such as hydroxypropyl methylcellulose (HPMC) and polyvinylpyrrolidone (PVP), as the respective complexing and co-complexing agents for VP. SBEβCD is a polyanionic, highly water-soluble CD derivative, with an average degree of substitution (DS) of seven and greater solubility in water than the parent β-CD (βCD), which often forms strong complexes with many water-insoluble drugs. Thus SBEβCD was selected on account of its good complexing and water-solubility properties.[21] However, the molecular weight of SBEβCD is 2160, a significantly higher value than that of βCD (MW 1135). This increase in molecular weight will increase the formulation bulk. The water-soluble polymers were added with the aim of increasing the complexation efficiency of SBEβCD toward VP and thereby reducing the formulation bulk. The formation of VP–SBEβCD inclusion complexes in solution with or

without water-soluble polymers was investigated using the phase-solubility method. The effect of brief heating in an autoclave on VP solubility in several solutions of SBEβCD and polymers was also investigated and compared with VP solubility achieved in the same solutions that were simply treated with mechanical stirring at room temperature. Three different techniques (kneading, coevaporation, and lyophilization) were applied to obtain the solid binary and ternary complexes, using tartaric acid as an acidifier of the complexation medium.[22] The confirmation of the solid-state interactions in the binary and ternary systems was performed by physicochemical characterization based on scanning electron microscopy (SEM), differential scanning calorimetry (DSC), and X-ray diffractometry (XRD), comparing these systems with the corresponding physical mixtures prepared in the same molar ratio.

Experimental

Materials   SBE-β-CD (Captisol™; DS 6.8; MW 2160) was kindly supplied by Cydex (Kansas City, MO, U.S.A.). VP (MW 350.46) was purchased from Covex (Madrid, Spain). PVP (Polyvinylpyrrolidone K-30), HPMC (hydroxypropyl methylcellulose 4000 cps) and tartaric acid were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). All other chemicals used were commercially available products of special reagent grade.

Solubility Studies   Phase-solubility studies in deionized water were carried out for both binary and ternary systems according to the method of Higuchi and Connors.[23] Excess amounts of VP were weighted into 20-ml glass flasks to which were added 10 ml of aqueous solutions containing increasing amounts of SBEβCD (0.001—0.06 M) with or without a fixed polymer concentration of 0.25% (w/v) PVP and 0.1% (w/v) HPMC. For binary systems, the glass containers were sealed and mechanically stirred at room temperature (21±2 °C) for 72 h to reach equilibrium. Preliminary studies were carried out using different equilibration periods, confirming that equilibrium was reached within 72 h since longer equilibration did not result in increased VP solubility. In the case of ternary systems, the glass containers were sealed and heated in an autoclave (Uniclave 88) at 120 °C for 20 min and then the resulting suspensions were allowed to equilibrate at room temperature (21±2 °C) for 72 h. All suspensions were filtered through a 0.45-μm membrane filter (Millipore) and analyzed spectrophotometrically (UV-1603, Shimadzu, Kyoto, Japan) at 316 nm for drug content with reference to a suitable constructed standard curve (correlation coefficient=0.9998). To nullify the absorbance due to the presence of SBEβCD and polymers, the apparatus were calibrated with the corresponding blank every assay. Three replicates were made for each experiment and the results are presented as the mean values. The changes in the solubility of VP resulting from the addition of various concentrations of SBEβCD, with or without water-soluble polymers, were used to plot phase-solubility diagrams and to evaluate the stoichiometry and stability constant of the resultant complexes. The apparent stability constants (K_c) were estimated from the straight line of the phase-solubility diagrams according to the following equation of Higuchi and Connors[23]:

$$K_c = \frac{slope}{S_o(1-slope)} \qquad (1)$$

where S_o (the intrinsic solubility) is the saturation concentration of VP in pure water, and *slope* denotes the slope of the straight line.

Another study was performed to evaluate the effect of the water-soluble polymers and heat treatment on VP solubility. An excess amount of VP was weighted into 20-ml glass flasks to which were added 10 ml of the following solutions: deionized water, 0.25% (w/v) PVP solution, 0.1% (w/v) HPMC solution, 60 mM of SBEβCD solution containing 0.25% (w/v) PVP, and 60 mM of SBEβCD solution containing 0.1% (w/v) HPMC. After sealing all flasks, the resulting suspensions were submitted to mechanical stirring at room temperature (21±2 °C) for 72 h or were heated in an autoclave at 120 °C for 20 min and then allowed to equilibrate for 72 h at room temperature. As described previously, all suspensions were filtered through 0.45-μm membrane filters and analyzed spectrophotometrically at 316 nm for drug content. The solubility data are averaged values from triplicate samples.

To verify the chemical stability of VP in the solutions exposed to auto-

claving, a stock solution of VP 1 mg/ml was made in acetonitrile. Different volumes of this solution were added to 10 ml of the following solutions: deionized water, 0.25% (w/v) PVP solution, 0.1% (w/v) HPMC solution, 60 mM of SBEβCD solution containing 0.25% (w/v) PVP, and 60 mM of SBEβCD solution containing 0.1% (w/v) HPMC. The samples, with different theoretical concentrations of VP ranging from 5 μg/ml to 100 μg/ml, were sonicated for 10 min or autoclaved for 20 min at 120 °C. After cooling at room temperature, the resultant solutions were filtered through a 0.45-μm membrane filter and appropriately diluted with an acetonitrile : 0.01 M phosphate buffer (67.5 : 32.5) solution. Drug concentrations were determined by HPLC. Briefly, quantitative analysis was performed on an HPLC chromatograph (Hewlett-Packard, model 1050) equipped with an injection valve of 20-μl sample loop (Model 7125, Rheodyne, Cotati, U.S.A.), a stationary phase of LiChrospher 100 RP18 (250 mm×4.6 mm; 5 μm) (Merck, Darmsdadt, Germany) fitted with a LiChrosper RP18 guard column (4 mm×4 mm; 5 μm) (Merck). The mobile phase consisted of a mixture of acetonitrile : 0.01 M phosphate buffer solution containing 0.07% triethylamine and 1 mM of heptane-1-sulfonic acid sodium salt adjusted to pH 3.5 with phosphoric acid (67.5 : 32.5). Twenty-microliter volumes were eluted isocratically with a flow rate of 1.0 ml/min at room temperature, and detection was carried out with ultraviolet absorption at a wavelength of 254 nm. Quantification was carried out by integration of the peak areas using the external standardization method.

Preparation of VP–SBEβCD Binary and Ternary Solid Systems   Three different methods were used to prepare the solid binary and ternary complexes. For comparison, physical binary and ternary mixtures were prepared in the same molar ratio as the complexes.

Physical Binary and Ternary Mixtures   Equimolar physical mixtures (PMs) of VP and SBEβCD were prepared by homogeneous blending in a glass mortar of exactly weighed amounts of the 63—120 μm sieve granulometric fractions of the two components, until a homogeneous mixture was obtained. For ternary PMs, 15% (w/w) of PVP or 6% (w/w) of HPMC was added. For further comparison, PMs containing tartaric acid were also prepared in the same stoichiometric ratio as the binary and ternary coevaporated (COE), lyophilized (LPh), and kneaded (KN) systems, in compliance with the preparation of the systems described below. All mixing procedures were performed adopting the geometric method.

Kneaded Binary and Ternary Products   KN products were prepared from binary and ternary PM by adding a small volume of aqueous 17% (w/v) tartaric acid solution. After wetting the PM in a ceramic mortar, the resulting systems were vigorously kneaded for 30 min to produce a homogeneous dispersion. Once homogeneous slurry was obtained, samples were dried at 40 °C for 48 h.

Coevaporated Binary and Ternary Products   Equimolar amounts of SBEβCD and VP were dissolved in water or in aqueous 1.5% (w/v) tartaric acid solution. The resulting mixture was stirred at 100 rpm and at 60 °C for 3 h, and the clear solution obtained was evaporated under a vacuum at 50 °C in a rotatory evaporator (Heidolph, Laborota). For ternary products, equimolar amounts of SBEβCD and VP were dissolved in aqueous solution containing either 0.25% of PVP or 0.1% (w/v) of HPMC, as well as in aqueous 1.5% (w/v) tartaric acid solution. The resulting solution was mixed and sonicated for 15 min and then heated in an autoclave at 120 °C for 20 min. After an equilibrium period of 72 h at room temperature, the clear solution was evaporated under vacuum at 50 °C in a rotatory evaporator. All solid residues were further dried at 40 °C for 48 h.

Lyophilized Binary and Ternary Products   Equimolar amounts of SBEβCD and VP were dissolved in water or aqueous 1.5% (w/v) tartaric acid solution. The two solutions were sonicated for 15 min and then mixed for 2 h at 50 °C. The resultant clear solution was frozen by immersion in an ethanol bath (Shell Freezer, Labconco, Freezone® model 79490) at −50 °C and then the frozen solution was lyophilized in a freeze-dryer (Lyph-lock 6 apparatus, Labconco) for 72 h. For ternary products, equimolar amounts of SBEβCD and VP were dissolved in aqueous solution containing either 0.25% (w/v) of PVP or 0.1% (w/v) of HPMC, as well as in aqueous 1.5% (w/v) tartaric acid solution. The resulting solution was mixed and sonicated for 15 min and then heated in an autoclave at 120 °C for 20 min. After an equilibrium period of 72 h at room temperature, the clear solution was frozen by immersion in an ethanol bath at −50 °C and subsequently lyophilized in a freeze-dryer for 72 h.

All resultant dried KN, COE, and LPh binary and ternary systems were sieved, and fractions smaller than 100 μm were collected for further studies.

Drug Content   To ensure no loss and degradation of VP during the preparation of VP–SBEβCD binary and ternary systems, accurately weighed samples of all binary and ternary products were dissolved in a known

amount of water. After suitable dilution of the samples, the concentration of VP in the solution was determined by the HPLC method as described above. Drug content was calculated from the following equation: Percent of drug content (%)=(practical drug content/theoretical drug content)×100. The determinations were performed in triplicate.

Scanning Electron Microscopy The surface morphology of the raw materials and of the binary and ternary systems was examined by means of a scanning electron microscope (Jeol, JSM 5310, Tokyo, Japan). The samples were fixed on a brass stub using double-sided tape and then made electrically conductive by coating in a vacuum with a thin layer of gold. The photographs were taken with a Pentax (model MZ-10) camera at an excitation voltage of 10 kV and magnifications of 200 and 2000.

Differential Scanning Calorimetry The DSC curves of pure materials and binary and ternary systems were recorded on a Shimadzu DSC-50 System (Shimadzu, Kyoto, Japan) with a DSC equipped with a computerized data station TA-50WS/PC. The thermal behavior was studied by heating all samples (1 mg of VP or its equivalent) in a sealed aluminum pan, using an empty pan sealed as reference, over the temperature range of 30—400 °C, at a rate of 10 °C/min and under a nitrogen flow of 20 cm³/min. Indium (99.98%, mp 156.65 °C, Aldrich, Milwaukee, WI, U.S.A.) was used as standard for calibrating the temperature. Results were obtained in triplicate.

X-Ray Diffractometry Powder XRD patterns of VP, SBEβCD, PVP, HPMC, and binary and ternary systems (PMs and complexes) were analyzed at room temperature with an automated Philips X'Pert (model PW 3040/00) diffractometer system equipped with Co as anode material and a graphite monochromator using a voltage of 40 kV and a current of 35 mA. The diffractograms were recorded in the 2θ angle range between 5—50° and the process parameters were set at: scan step size of 0.025 (2θ); scan step time of 1.25 s; and acquisition time of 1 h. A software package attached to the diffractometer was used to calculate peak heights of all diffraction patterns. The XRD traces of all raw materials and binary and ternary systems were compared with regard to peak position and relative intensity, peak shifting, and the presence and/or absence of peaks in certain regions of 2θ values. Crystallinity was determined by comparing some representative peak heights in the diffraction patterns of the binary and ternary systems with a reference. The relation used for the calculation of the crystallinity was the relative degree of crystallinity (RDC)=I_{SA}/I_{REF}, where I_{SA} is the peak height of the sample under investigation and I_{REF} is the peak height of the same angle for the reference with the highest intensity.[24] To identify the possible interactions among VP, SBEβCD, HPMC, and PVP, VP was used as a reference sample for calculating RDC values of all binary and ternary systems. The crystallinity of PMs and respective products was compared by considering these systems as reference samples for the corresponding binary and ternary products, as described by Veiga *et al.*[25]

Results and Discussion

Solubility Studies VP has a structure (Fig. 1) that allows at least a portion of the molecule to be accommodated inside the cavity of SBEβCD. The formation of a VP–SBEβCD inclusion complex in solution was confirmed by the phase-solubility method, since SBEβCD increases the solubility of VP in aqueous solution.

The phase-solubility profiles of VP in aqueous SBEβCD solutions with and without addition of water-soluble polymers [0.25% (w/v) PVP or 0.1% (w/v) HPMC] are shown in Fig. 2. The solubility of VP increased linearly as a function of SBEβCD concentration, giving in all cases A_L-type phase-solubility diagrams and indicating the formation of a complex where the complexing agent (SBEβCD) is present in first-order degree with respect to the drug. As the slopes of these solubility diagrams were all less than 1 it was possible to assess a 1:1 stoichiometry and calculate the apparent stability constants (K_c) of the binary and ternary complexes using the equation of Higuchi and Connors.[23] The estimated values of S_o, slopes of phase-solubility diagrams and K_c, are presented in Table 1. The 1:1 stoichiometry obtained is also strongly supported by the nature of the SBEβCD molecule, since each SBEβCD molecule carries an average of 6.5 nega-
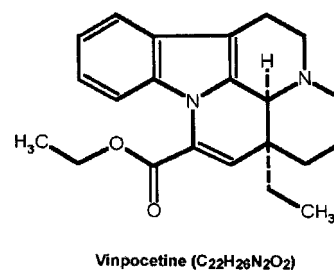
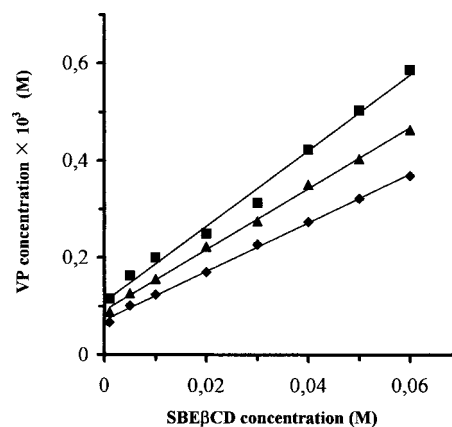

Fig. 1. Structural and Chemical Formulas of VP

Fig. 2. Phase-Solubility Diagrams for VP at Room Temperature in the Presence of SBEβCD without Water-Soluble Polymers (◆) and with 0.25% (w/v) PVP (■) or 0.10% (w/v) HPMC (▲)

Each point is the mean of three determinations.

Table 1. Effect of the Water-Soluble Polymers PVP and HPMC on VP Intrinsic Solubility (S_o), Slope of the Phase-Solubility Diagrams, and Stability Constants (K_c) of Binary and Ternary Systems

	S_o (μg/ml)	Slope[a]	r^{2a}	K_c (M⁻¹)	K_{TS}/K_{BS}
No polymer	5.14	0.0050	0.999	340	—
0.25% (w/v) PVP	8.94	0.0078	0.997	490	1.4
0.10% (w/v) HPMC	7.21	0.0063	0.998	390	1.1

K_{TS}/K_{BS} is the ratio of K_c for ternary and binary complexes. *a*) Values obtained directly from the drug phase solubility diagram.

tive charges that make formation of higher-order complexes difficult.[26] It was observed from the phase-solubility diagrams that the addition of water-soluble polymers to the complexation media followed by heating in an autoclave at 120 °C/20 min resulted in an increase in the slopes, which was reflected in VP solubility. Thus VP solubility in 60 mM SBEβCD aqueous solution was 129.3 μg/ml when no polymers were present, but increased to 205.7 and 162.5 μg/ml, respectively, with the addition of 0.25% (w/v) PVP and 0.1% (w/v) HPMC to the complexation media. Based on these results, we can conclude that the addition of water-soluble polymers resulted in a significant enhancement of SBEβCD solubilization toward VP. This was a synergistic effect with regard to VP solubility. That is, the solubility of VP was greater when both SBEβCD and the polymer were present in the complexation media than the additive solubilizing effect of SBEβCD and the polymers when used separately.[27]

Water-soluble polymers are known to interact with drug molecules in aqueous solution to form polymer–(drug)$_n$ com-

Table 2. Solubility of VP in Different Solutions Submitted to Different Processing Conditions: Mechanical Agitation at Room Temperature for 72 h and Autoclaving at 120 °C/20 min Followed by a Period of Equilibrium of 72 h

| Solution composition | | | | Mechanical agitation $[VP]_{(1)}$ (μg/ml) | Autoclaving at 120 °C/20 min $[VP]_{(2)}$ (μg/ml) | Solubilization efficiency $[VP]_{(2)}/[VP]_{(1)}$ |
Water	HPMC	PVP	SBEβCD			
×				5.14	5.69	1.1
	×			6.25	7.21	1.2
		×		7.84	8.94	1.1
			×	129.3	148.8	1.2
	×		×	113.2	162.5	1.4
		×	×	122.9	205.7	1.7

plexes, leading to a notable increase in drug solubility.[28—31] Moreover, water-soluble polymers are also known to interact with CDs by interaction with the outer surface of the CD molecule[32] and with drug–CD complexes[33] in a similar way as with micelles, forming drug–CD–polymer aggregates or a cocomplex, *i.e.*, a complex between several drug–CD molecules and a polymer chain [(drug–CD)$_n$–polymer]. These macromolecular clusters show higher K_c values than the simple binary drug–CD complexes, which accounts for their higher solubility.[18] In this study, the observed enhancement of K_c values of between 14 and 44% upon addition of HPMC and PVP, respectively, shows that the polymers are able to interact with the VP–SBEβCD complex.[17] The synergistic effect observed is indicative of the formation of a ternary (VP–SBEβCD)$_n$–polymer because otherwise drug solubility would merely be the sum of the contributions of the solubilizing action of the polymers and SBEβCD.

The results obtained from the evaluation of heating in an autoclave on VP solubility are summarized in Table 2. It was observed in all samples that VP solubility improved upon heating, when compared with the same samples submitted simply to mechanical stirring at room temperature. This effect might be a consequence of better VP solubilization at high temperatures even in deionized water. The solubility improvement ranged from 10.56 to 67.33% and was more marked when water-soluble polymers and SBEβCD were jointly present in the solution. Loftsson reported that brief heating in an autoclave and cooling to room temperature enhanced drug–CD–polymer complexation and that effect was due to the water-soluble polymers that alter the hydration of the CD molecule and thus its three-dimensional structure in aqueous solution.[22] In the light of this knowledge, we can assume that the improved VP solubilization after autoclaving could be due to facilitated fitting of the VP molecule to the SBEβCD cavity resulting from differences in flexibility and of conformational changes in SBEβCD molecules in the presence of the polymers. The formation of hydrogen bonds, Van der Walls interactions, or hydrophobic interactions between VP, SBEβCD, and the water-soluble polymers and/or the release of high-energy water molecules from the SBE-βCD cavity may also contribute to improved VP solubility.[34] Another effect that might be cooperating in the synergistic effect of polymers on VP solubility upon heating in an autoclave is the stabilizing action of the water-soluble polymers since they do not only enhance drug solubility by improving the solubility of VP–SBEβCD complexes but because of the low viscosity of polymer solutions after cooling, the water-soluble polymers can retard or hinder the precipitation of VP

from its saturated solution formed by autoclaving.

However, VP solubility in SBEβCD solutions containing water-soluble polymers in the samples stirred mechanically exhibited lower solubility values than the solutions containing SBEβCD alone. In these solutions the lower viscosity conferred by the polymers was an obstacle to VP solubilization and complexation by SBEβCD, since VP solubility values were lower than the sum of VP solubilization by the polymers and by SBEβCD.

Relative to the examination of VP chemical stability in deionized water, 0.25% (w/v) PVP solution, 0.1% (w/v) HPMC solution, 60 mM SBEβCD solution, and 60 mM SBEβCD solution containing 0.25% (w/v) PVP or 0.1% (w/v) HPMC, we did not observe the formation of degradation products either after sonication for 10 min or heating in an autoclave for 20 min at 120 °C. The concentration of VP in the various solutions remained unchanged and no new elution peaks appeared in the chromatograms of the analyzed samples, corroborating the evidence of the maintenance of VP chemical stability in the conditions studied.

To conclude, several mechanisms may contribute to the improved VP solubility in the presence of water-soluble polymers and SBEβCD upon heating in an autoclave, that is, improvement of the stabilizing, solubilizing, and complexing effects of SBEβCD; the formation of aggregates between VP–SBEβCD complexes and the polymer chains; and the stabilization of the saturated VP–SBEβCD solutions obtained by autoclaving. Complementary studies using H-NMR techniques are currently under investigation to gain insight into the mechanism.

Drug Content The HPLC analysis performed on the prepared binary and ternary systems in all cases showed a 97.2—101.7% drug content, based on the theoretical composition. No new elution peaks appeared in the chromatograms of the solubilized binary and ternary VP–SBEβCD systems. These results indicate that no loss or degradation of VP occurred during the preparation of solid binary and ternary products.

Scanning Electron Microscopy From the results of SEM analysis, it can be seen that pure drug particles appeared as small crystals (1—30 μm) of irregular shape and homogeneous size (Fig. 3a), whereas hollow spherical particles with a large size distribution (1—180 μm) were evident in the SBEβCD microphotographs (Fig. 3b).

The binary PM showed particles of SBEβCD embedded with VP particles and a comparable morphology with pure components occurring separately, revealing no apparent interaction between both species in the solid state (Fig. 3c). On
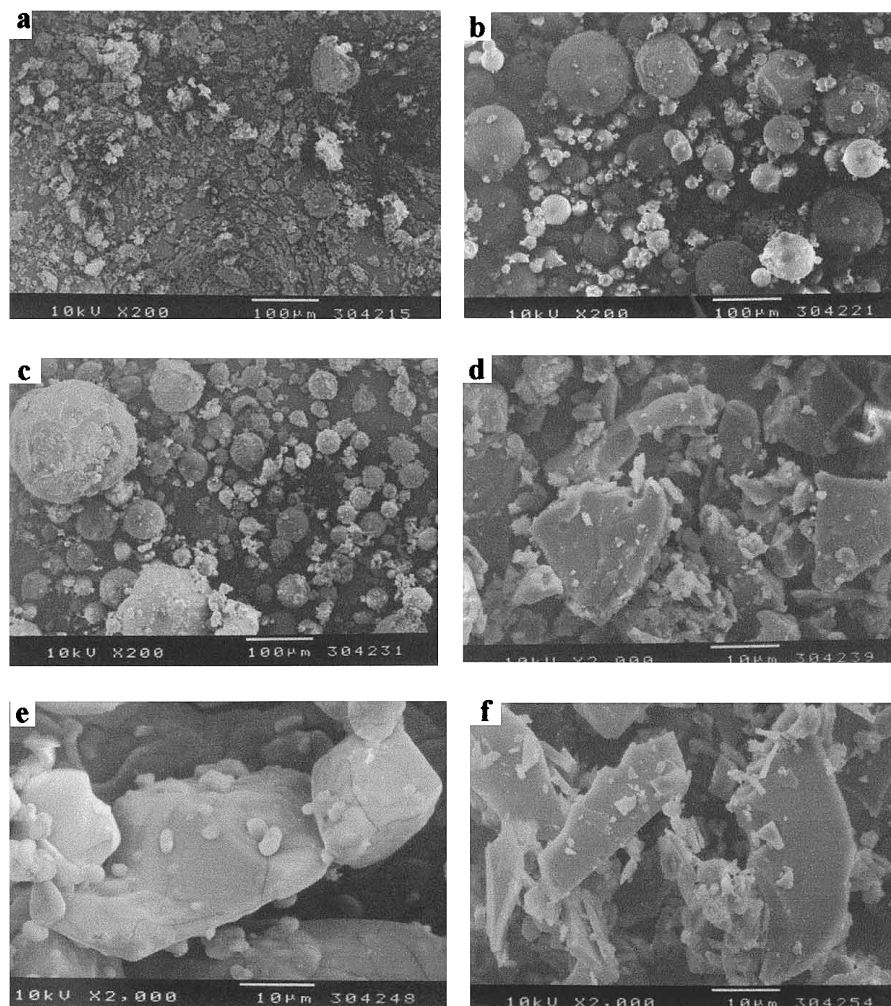
Fig. 3. Scanning Electron Micrographs of Pure VP (a), Pure SBEβCD (b), and VP–SBEβCD Binary Systems: PM (c), KN (d), COE (e), and LPh (f)

the contrary, a drastic change in the original morphology and shape of both VP and SBEβCD particles was observed in all binary and ternary products, indicating that the morphology was influenced by the selected method of preparation. The morphology of KN binary (Fig. 3d) and ternary products (Fig. 4a, d) was quite similar, showing the loss of sphericity, smooth surface, and clearly reduced size of the SBEβCD particles. VP and SBEβCD particles were observed to be irregularly shaped and tended to form aggregates, and it was difficult to discern between the two types of particle. This behavior could be explained by the partial solubilization of both components during the vigorous kneading process.

The micrographs of binary and ternary COE products (Figs. 3e, 4b, e) also revealed the formation of undifferentiated particles with a tendency to form tiny aggregates of amorphous pieces, clearly different from those of the raw materials. Finally, the lyophilization technique gave rise to amorphous pieces of irregular and small size with a lamellate aspect common to both binary and ternary products (Figs. 3f, 4c, f). In both binary and ternary COE and LPh products we could only distinguish one type of granule, probably due to total solubilization of the raw materials in the course of their preparation. This drastic change in the particle shape, aspect, and size, although scarcely conclusive, led us to estimate the existence of a single phase in COE and LPh preparations[35]

and consequently the formation of inclusion complexes.[36]

Differential Scanning Calorimetry Thermal methods are widely employed in the assessment of the solid phase aiming essentially to determine significant differences between traces obtained from scanning the untreated mixture (PM) and those from the interacted mixture (generally indicated as an "inclusion compound").[37] In this study, DSC was used to characterize VP complexes in the solid state and to obtain further supporting evidence on complex formation.

The thermograms of pure components and corresponding binary and ternary systems in the melting range of the drug and dehydration of the carrier are presented in Fig. 5. The thermal curve of pure VP was typical of a crystalline anhydrous substance with a sharp endothermic peak at $149.3 \pm 0.6\,^{\circ}C$ corresponding to the melting point of the drug. A broader endothermal effect was instead recorded for amorphous SBEβCD, PVP, and HPMC as a consequence of water loss.

The comparison of DSC curves from binary systems with those belonging to ternary systems did not result in significant differences. Both characteristic peaks of VP (drug melting) and SBEβCD (water loss) were clearly distinguishable in both binary and ternary PM. In PM with the addition of tartaric acid, the characteristic thermal profile of VP was shifted to lower temperatures at around $147.3\,^{\circ}C$ for binary
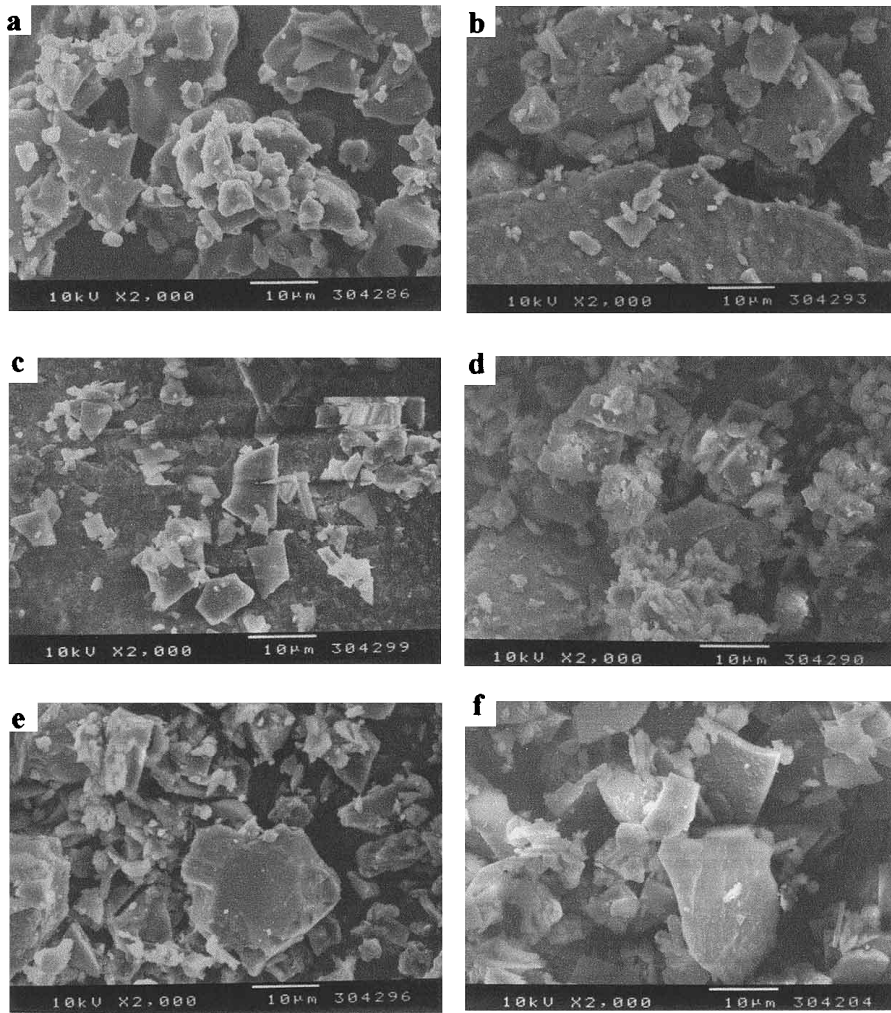
Fig. 4.   Scanning Electron Micrographs of VP–SBEβCD–PVP Ternary Products: KN (a), COE (b), and LPh (c); and VP–SBEβCD–HPMC Ternary Products: KN (d), COE (e), and LPh (f)
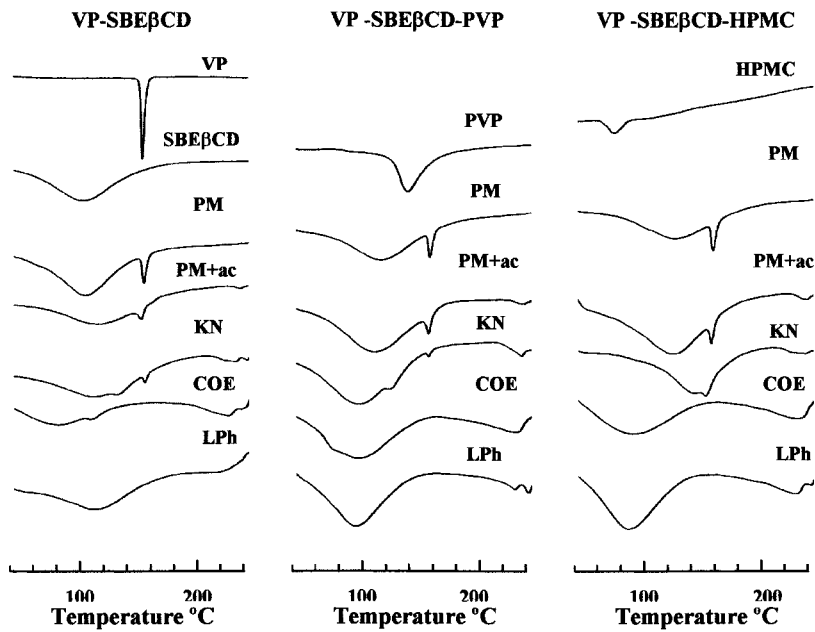

Fig. 5.   DSC Curves of VP, SBEβCD, Water-Soluble Polymers (PVP and HPMC), and Binary and Ternary Systems: Physical Mixtures (PMs), PMs with Tartaric Acid (PM+ac), KN, COE, and LPh Products

PM and 148.6 °C for ternary PM with PVP, and no change was observed in ternary PM containing HPMC. The intensity of the peak was also more reduced in binary PM containing tartaric acid. Those slight changes relative to the peak of pure VP may suggest a weak interaction between the components of the PM with tartaric acid during the mixing or heating for DSC scanning.[34,38]

Considering binary and ternary KN products, there was a substantial reduction in the VP peak intensity and a shift to lower temperatures (between 144.2 and 148.9 °C). The small peak corresponding to the melting of free drug in the DSC curves of all KN products suggests that, like for PM, there is no inclusion compound formed in either system even though a drug–CD interaction occurs. The molecular arrangement of VP in KN products can indicate the reduction of drug crystallinity or indicate that probably VP is partially dispersed at a molecular level in the solid product.[39]

The disappearance of the VP endothermic peak in all binary and ternary COE and LPh products may be a strong indication of the formation of amorphous entities and/or inclusion complexes.[40] These results suggest that only the COE and LPh products can be considered as true inclusion complexes, differing from simple PMs.

X-Ray Diffractometry Powder XRD is a useful method for the detection of CD complexation in powder or microcrystalline states. The diffraction pattern of the complex should be clearly distinct from those of the superposition of each component if a true inclusion complex exists.[41] The inclusion process may increase the amorphous character and can also be explained based on the procedure employed to obtain the complex.[42]

The XRD patterns of VP, SBEβCD, PVP, HPMC, and binary and ternary systems, in the range of 5—50° 2θ range, are shown in Fig. 6 and the RDC values and peak intensities of VP–SBEβCD systems are presented in Tables 3 and 4. The complexation products were identified by comparing their diffractograms with those of pure VP, SBEβCD and PM.

The XRD pattern of VP revealed several high-intensity reflections corresponding to the diffraction peaks 14.387°, 16.138°, 17.313°, 20.438°, 21.862°, and 27.638° (2θ), which were indicative of its crystalline character, while a hollow pattern was recorded for SBEβCD, PVP, and HPMC, demonstrating their amorphous states. In the binary and ternary PMs the presence of free crystalline drug was revealed by few broad peaks of low intensity that emerged on the diffuse background of the amorphous carriers. The XRD patterns of PMs contain the principal diffraction peaks of VP and are apparently only the superposition of each component, with a marked decrease in the intensity of the diffraction peaks. This can be attributed to the reduction in particle size as a consequence of the preparation method and to the dilution of the drug in the PM. From the RDC values of the PMs it was observed that, when pure VP is considered the reference sample, the degree of crystallinity of both binary and ternary PMs was similar, as can be confirmed by the peak height of the principal diffraction peaks of VP. For all ternary systems there were no spectroscopic differences by comparison of both water-soluble polymers selected (PVP and HPMC), probably due to similar performances, except for the COE ternary products as described below.
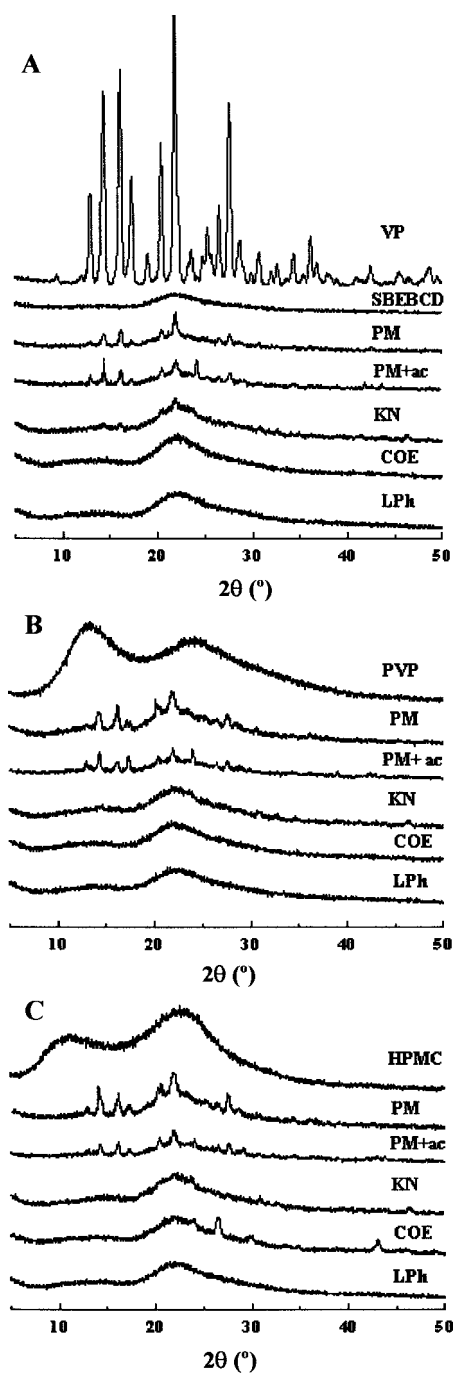

Fig. 6. Powder XRD Patterns of VP–SBEβCD Binary Systems (A), VP–SBEβCD–PVP Ternary Systems (B), and VP–SBEβCD–HPMC Ternary Systems (C)

PM, physical mixture; PM+ac, physical mixture with tartaric acid; KN, kneaded product; COE, coevaporated product; LPh, lyophilized product.

The diffractograms of ternary KN products resembled to those of the corresponding binary ones and exhibited lower crystallinity than the respective PMs, although the characteristic VP peaks were still detectable. This slight loss of crystallinity could reasonably be explained by the presence of reciprocal interactions in the solid state between host and guest, that is, the formation of mixed particles during the drying process, as observed in SEM analysis. Those observations were also in agreement with the results of DSC studies.

In contrast, complete drug amorphousness was detected in

Table 3. RDC of VP–SBEβCD Binary and Ternary Systems

VP–SBEβCD binary system	RDC	Reference used	VP–SBEβCD–PVP ternary system	RDC	Reference used	VP–SBEβCD–HPMC ternary system	RDC	Reference used
VP+SBEβCD PM[a]	0.240	VP	VP+SBEβCD+PVP PM[a]	0.238	VP	VP+SBEβCD+HPMC PM[a]	0.241	VP
VP:SBEβCD KN[b]	0.204	VP	VP:SBEβCD:PVP KN[b]	0.193	VP	VP:SBEβCD:HPMC KN[b]	0.194	VP
VP:SBEβCD COE[c]	0.195	VP	VP:SBEβCD:PVP COE[c]	0.188	VP	VP:SBEβCD:HPMC COE[c]	0.178	VP
VP:SBEβCD LPh[d]	0.170	VP	VP:SBEβCD:PVP LPh[d]	0.159	VP	VP:SBEβCD:HPMC LPhd)	0.172	VP
VP:SBEβCD KN[b]	0.851	Corresponding PM[a]	VP:SBEβCD:PVP KN[b]	0.81	Corresponding PM[a]	VP:SBEβCD:HPMC KN[b]	0.804	Corresponding PM[a]
VP:SBEβCD COE[c]	0.815	Corresponding PM[a]	VP:SBEβCD:PVP COE[c]	0.792	Corresponding PM[a]	VP:SBEβCD:HPMC COE[c]	0.741	Corresponding PM[a]
VP:SBEβCD LPh[d]	0.711	Corresponding PM[a]	VP:SBEβCD:PVP LPh[d]	0.670	Corresponding PM[a]	VP:SBEβCD:HPMC LPh[d]	0.712	Corresponding PM[a]

a) Physical mixtures; *b*) kneaded products; *c*) coevaporated products; *d*) lyophilized products.

Table 4. Peak Intensities of VP in the XRD Patterns of VP–SBEβCD Binary and Ternary Systems

Peak position ($2\theta°$)	Peak intensity (counts)												
		VP–SBEβCD binary system				VP–SBEβCD–PVP ternary system				VP–SBEβCD–HPMC ternary system			
	VP	PM[a]	KN[b]	COE[c]	LPh[d]	PM[a]	KN[b]	COE[c]	LPh[d]	PM[a]	KN[b]	COE[c]	LPh[d]
14.387	3821	836	556	540	531	844	633	590	513	827	587	565	547
16.138	4236	917	566	557	470	958	621	543	515	875	609	547	533
17.313	2179	660	516	527	475	661	621	542	513	658	578	513	510
20.438	2826	964	800	855	784	954	865	834	730	974	866	815	795
21.862	5264	1261	1073	1028	896	1253	1015	992	839	1268	1020	939	903
27.638	3578	836	619	610	558	813	607	637	569	859	606	608	573

a) Physical mixtures; *b*) kneaded products; *c*) coevaporated products; *d*) lyophilized products.

the diffraction patterns of almost all COE and LPh products, showing a typical flat behavior that confirms the strong ability of the amorphous carrier SBEβCD to induce an amorphous nature in VP as a result of the preparation technique (solvent evaporation and lyophilization). This occurrence could be attributed to an interaction between VP and SBE-βCD showing the presence of a new solid phase where a possible formation of an inclusion complex was contemplated.[43] As the loss of the crystalline character of a drug can be a consequence of the lyophilization process, the X-ray data could not discriminate whether the LPh products obtained were true inclusion complexes or homogeneous dispersed mixtures of the amorphous components. Nevertheless, the XRD patterns of the binary and ternary COE products might suggest the formation of inclusion complexes in which VP was at least partially entrapped in the SBEβCD cavity.[44] On the other hand, the diffractogram of the VP–SBEβCD COE product appeared as partially amorphous, showing the appearance of new diffraction peaks at 23.912°, 26.487°, 29.987°, and 43.238° (2θ) distinct from those observed in the respective PMs. This result demonstrated that the VP–SBEβCD–HPMC COE product is a new chemical species different from the original substances[45] and may be considered as indirect proof of inclusion complexation between VP, SBEβCD, and HPMC.

The RDC values of COE and LPh binary and ternary products were lower than those of KN products and the respective PMs. The diffraction patterns of LPh ternary systems were superimposable with the corresponding binary ones. A stronger amorphous drug character was reflected by lower RDC values for ternary COE products in comparison with binary ones, because the RDC values are calculated from the height of the most representative diffraction peak of the drug.

The degree of amorphous entity formation in the various VP–SBEβCD systems can be ranked in the following order: binary PM=ternary PM<binary KN product<ternary KN products≤binary COE product<ternary COE products≤binary LPh product=ternary LPh products. As expected, the extent of the formation of amorphous species was found to be dependent on the selected method of preparation.

Conclusions

Complexation of VP with SBEβCD and water-soluble polymers was obtained in the aqueous medium, as confirmed by the phase-solubility analysis. The values of K_c increased with the addition of PVP and HPMC to the complexation medium, demonstrating higher complexation efficiency and solubilizing effect of SBEβCD toward VP in ternary complexes, but this effect was only observed after brief heating in an autoclave. The physicochemical solid-state characterization of VP–SBEβCD binary and ternary systems suggested

the achievement of new solid phases, some of them in an amorphous state, and gave strong evidence of the formation of binary and ternary inclusion complexes between VP, SBEβCD, and water-soluble polymers in the solid state, particularly for COE and LPh binary and ternary products.

Based on these results, we believe that the interaction of VP with SBEβCD and water-soluble polymers, through the formation of inclusion complexes, can lead to important modifications in the physicochemical and biological properties of the guest molecule, which might eventually have relevant pharmaceutical potential.

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