Sertaconazole/Hydroxypropyl-β-Cyclodextrin Complexation: Isothermal Titration Calorimetry and Solubility Approaches

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Received 2 February 2006; revised 23 March 2006; accepted 11 April 2006

Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/jps.20661

ABSTRACT: Complexation of sertaconazole (SN) with hydroxypropyl-β-cyclodextrin (HP-β-CD) was characterized by phase-solubility diagram measurements and isothermal calorimetry (ITC) in aqueous medium, and by differential scanning calorimetry (DSC), Raman spectroscopy and X-ray diffractometry in solid state. The strongest interaction was observed at pH 1.2, at which two different 1:1 complexes can be formed depending on the hydrophobic ring of the drug involved in the process. At pH 5.8 and 7.4 the likelihood of 1:2 stoichiometry increases as a consequence of the simultaneous complexation of the nonprotonized imidazolyl and the dichlorophenyl groups. In the presence of 20% HP-β-CD, SN solubility is enhanced by a factor of 116, 107, and 5 at pH 1.2, 5.8, and 7.4, respectively. Complexation enthalpy recorded by ITC showed the same tendency which confirms the practical interest of this technique for fast screening of the potential of CDs as drug solubilizers. Solubility and dissolution rate of the drug from compacts prepared with freeze-dried complexes were significantly greater than those obtained with SN powder or compacts made with physical blends. J Pharm Sci 95:1751–1762, 2006

Keywords: isothermal calorimetry (ITC); cyclodextrins; sertaconazole; solubility; complexation; inclusion compounds

INTRODUCTION

Sertaconazole (SN) is an azole drug with a broad—spectrum activity against gram-positive bacteria, yeasts, and dermatophyte and filamentous fungi, which is particularly recommended in the treatment of fungal ocular and vaginal infections.[1,2] SN is stable in aqueous media, although its extremely low hydrosolubility (<0.01% w/v)[3] makes the development of efficient liquid and solid drug dosage forms difficult. Therefore, complexation with cyclodextrins (CDs) appears to be an interesting approach to overcoming its formulation problems. Computational studies have shown that the inclusion of the dichlorophenyl group of SN into the wider side of the CD cavity is energetically favorable.[4] In simulated gastric fluid, the presence of γ-CD or HP-γ-CD at 1% w/v can increase SN solubility up to 24-fold; the affinity constant being strongly dependent on pH (values ranged from 7×10^3 to 3×10^4/M in USP gastric fluid, and from 2×10^2 to 3×10^3/M in USP enteric medium).[4] This solubilization capability of CDs should be enough for achieving antifungical effects using SN liquid or solid formulations.

The aim of this work was to gain an insight into the complexation mechanisms and properties

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Journal of Pharmaceutical Sciences, Vol. 95, 1751–1762 (2006)

of SN:HP-β-CD complexes. The information obtained could be helpful in the development of drug dosage forms for different routes, including the ocular and parenteral ones, in which HP-β-CD has been recognized as safe.[5] Thermodynamic studies can provide information on the mechanisms involved in the complexation. In general, the driving forces for inclusion are both enthalpic and entropic, but this has still not fully been understood, since a number of concomitant processes can contribute to the complexation. This combined interaction is presumed to be comprised of the following contributions: (i) van der Waals interaction between the drug and the CD cavity; (ii) entropy gain due to the destruction of the water assembly around the drug molecule; (iii) entropy loss due to the restrictions for movement of the drug in the CD cavity; (iv) enthalpy gain due to the release of water molecules from the CD cavity.[6,7] The relative importance of the entropy and the enthalpy contributions could be also altered by changes in pH and temperature.[8,9] Isothermal titration microcalorimetry (ITC) is a highly sensitive technique particularly useful for characterizing associative processes in which the energetic changes are quite small.[10–12] In the present study, ITC was used to establish the mechanisms of interaction between SN and HP-β-CD in different pH media, and to obtain information about the thermodynamic parameters, the binding constants, the stoichiometry and the possibility of multiple types (structures) of inclusion complexes (Fig. 1). In order to evaluate the potential of the SN:HP-β-CD complexation on the development of liquid and solid drug dosage forms, phase solubility diagrams were analyzed and complexes were obtained by freeze-drying or kneading. Taking into account that HPMC is an usual pharmaceutical excipient and can have a synergistic effect on the solubilizing ability of CDs when used at low concentrations,[13–16] complementary studies in the presence of this polymer were also carried out.

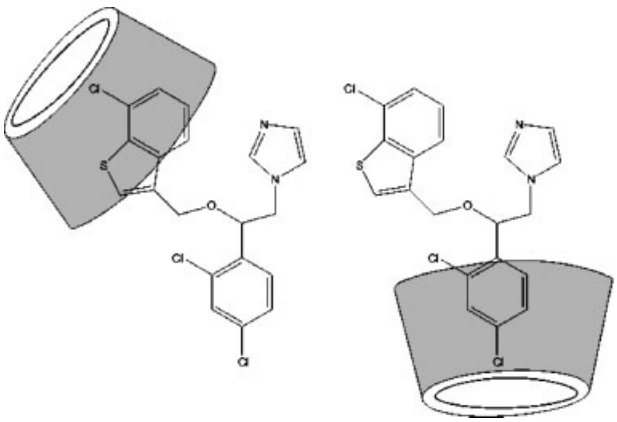

Figure 1. Scheme of the SN:HP-β-CD 1:1 complexes that can be formed at pH 1.2 depending on the hydrophobic ring of the drug involved in the process.

MATERIALS AND METHODS

Materials

SN nitrate (nitrate salt of 7-chloro-3-[1-(2,4 -dichlorophenyl)-2-(1H-imidazol-1-yl)-etoxymethyl]benzo[b]thiophene; batch 0I0695) (Fig. 1) from Ferrer International, Spain; HP-β-CD (degree of substitution of 4.6, mean molecular weight of 1300 Da; batch 102302254) from Janssen Pharmaceutische, Belgium; hydroxypropylmethyl cellulose (HPMC) Methocel® K4M (mean viscosimetric molecular weight of 84200 Da;[17] batch MM87050902K) from The Dow Chemical Company, Michigan. All other reagents were of analytical grade.

Characterization of the Complexes in Solution

Isothermal Titration Microcalorimetry (ITC)

The energetics of the interactions between SN and HP-β-CD was evaluated by ITC (VP-ITC MicroCal Inc., Northampton, MA). The experiments were carried out at 25°C by duplicate (reproducibility ± 5%) in methanol:buffer (pH 5.8 or 7.4 phosphate or USP pH 1.2 HCl buffer) 50:50 v/v medium, titrating the HP-β-CD solution onto the SN solution. A degassed aliquot (1.436 mL) of 0.8 mM SN solution was filled in the reaction cell, an identical volume of medium without SN was placed in the reference cell, and 300 μL of degassed 10 mM HP-β-CD solution were loaded in the titration syringe. The binding experiment involved 58 sequential additions of small aliquots (5 μL) of this latter solution in the reaction cell under continuous stirring (280 rpm). After each addition, the heat effect was recorded. Control experiments were carried out under identical conditions to obtain the heats of dilution and mixing involved in: (i) injection of HP-β-CD solution into the buffer, and (ii) addition of buffer to the SN solution. The injection schedule (number of injections, volume of injection, and time between injections) was set up using an

 DOI 10.1002/jps

interactive software, all data being stored in a computer. In order to estimate the net reaction enthalpy, the dilution enthalpies were subtracted from the apparent titration enthalpies (raw data).

Assuming that a guest ligand molecule (L) and a CD form several types of inclusion complexes with 1:1 molar ratio in aqueous solution as follows:

$$L + CD \leftrightarrow \sum_{i=1}^{n} (L:CD)_i \quad (1)$$

then, the heat effect of the complexation depends on the quantity of each type of complexes. In the case of $i = 1$, CD forms only one type of inclusion complex with L at a molar ratio of 1:1. If i is equal to or greater than 2, it is assumed that several types of inclusion complex are independently formed.[18] The analysis of the calorimetry data allows the determination of the affinity constant (K_i), the stoichiometry number (n_i) and the complexation enthalpy ($\Delta H^i_{\mathrm{ITC}}$) for each type of complex, using the equation:

$$Q = M_t V_0 \sum_{i=1}^{n} n_i \theta_i \Delta H^i_{\mathrm{ITC}} \quad (2)$$

where Q is the heat evolved after jth injection, M_t the total concentration of SN, V_0 the reaction cell volume, and θ_I the fraction of sites in the SN molecule that enter into the CD.

A titration curve was obtained by plotting the values of ΔQ versus the total concentration of HP-β-CD added. After completing an injection, a correction for displaced volume was made and the heat released from the jth injection can be calculated as:

$$\Delta Q(j) = Q(j) + \frac{\mathrm{d}Vj}{V_0}\left[\frac{Q(j) + Q(j-1)}{2}\right] - Q(j-1) \quad (3)$$

After subtraction of the heat of dilution, best fit values of the stoichiometry number (n_i), changes in enthalpy ($\Delta H^i_{\mathrm{ITC}}$), in entropy ($\Delta S^i_{\mathrm{ITC}}$) and binding constants (K^i_{ITC}) can be computed from the actual calorimetric data using a nonlinear least-squares Marquardt algorithm routine after successive iterations (minimization of χ^2; Origin™ software, Microcal Inc. 2001). $\Delta G^i_{\mathrm{ITC}}$ was calculated using the expression:

$$\Delta G^i_{\mathrm{ITC}} = -RT \ln K^i_{\mathrm{ITC}} = \Delta H^i_{\mathrm{ITC}} - T\Delta S^i_{\mathrm{ITC}} \quad (4)$$

in which R is the gas constant and T is the absolute temperature.

Phase-Solubility Studies

Suspensions of SN were prepared (in sextuplicate) by adding an excess of drug to HP-β-CD solutions (concentrations ranged from 0 to 20% w/v) in pH 5.8 and 7.4 phosphate or pH 1.2 HCl buffers, with or without HPMC 0.1% w/v. Three replicates were autoclaved (Raypa AES-1219, Terrasa, Spain) at 121°C for 20 min. Then, the autoclaved and nonautoclaved replicates were shaken at 25°C and 50 rpm for 5 days. The concentration of dissolved drug was determined by UV spectrophotometry (Agilent 8453, Böblingen, Germany) at 302 nm ($E_{1\%\ 1\ \mathrm{cm}} = 577.32$) in samples filtered through 0.22 μm Millipore® cellulose acetate membranes (Millipore, Spain).

The apparent affinity constant of the 1:1 SN:HP-β-CD complexes was estimated from the A_L-type diagrams using the expression:[19]

$$K_{1:1} = \frac{m}{S_0(1-m)} \quad (5)$$

where m is the slope of the plot and S_0 is SN solubility in absence of CD.[20]

To estimate the apparent affinity constants of the 1:1 and 1:2 complexes, from the A_P type diagrams, the following expression was used:[21]

$$\frac{(S_t - S_o)}{L_t} = S_o K_{1:1} + K_{1:1} K_{1:2} S_o L_t \quad (6)$$

in which S_t is the concentration of SN solubilized at each CD concentration L_t.

Kneaded and Freeze-Dried Solid Systems

Preparation

Kneading and freeze-drying techniques were applied with the aim of obtaining solid state complexes. First, amounts of SN and HP-β-CD in a 1:1 or 1:2 molar ratio were placed in a mortar, and methanol:buffer (pH 5.8, 7.4, or 1.2) 50:50 solution was slowly added to have a final solid:liquid 70:30 proportion. The systems were kneaded for 45 min and, then, dried in at 40°C for 2 days. Control SN samples were kneaded under the same conditions without HP-β-CD. To obtain freeze-dried samples, an excess of SN was added to 20% (w/v) HP-β-CD solutions prepared in pH 5.8 or 7.4 phosphate or pH 1.2 HCl buffer, with and without 0.1% w/v HPMC. The systems were autoclaved (Raypa AES-1219, Spain) at 121°C for 20 min, shaken at 25°C for 5 days and filtered using 0.22 μm Millipore® cellulose acetate membranes (Millipore, Spain). The resulting solutions

were immersed in liquid nitrogen and lyophilized in a Labconco Lyph-lock 6 apparatus (Kansas City, MO). SN content per gram of freeze-dried sample was determined, after dissolution in methanol, spectrophotometrically at 302 nm. Control SN:HP-β-CD 1:1 and 1:2 physical mixtures (with and without 0.1% w/v HPMC) were prepared by sieving the powders through 0.5 mm mesh and mixing for 10 min in a Turbula T2C (WAB, Basel, Switzerland).

Characterization

Differential Scanning Calorimetry (DSC). The experiments were carried out, in duplicate, using a DSC Q100 apparatus (TA Instruments, New Castle, DE) fitted with a refrigerated cooling accessory. Nitrogen was used as purge gas at a flow rate of 50 mL/min. The calorimeter was calibrated without pans for baseline, and with indium (melting point 156.61°C, enthalpy of fusion 29.71 J/g) and sapphire standards for cell constant temperature and heat capacity, respectively. Nonhermetic aluminium pans, in which 2–5 mg of sample were accurately weighed, and then just covered with the lid, were used to perform the experiments. The samples were heated from 30 to 162°C, afterwards cooled to 0°C, and finally heated again to 250°C, always at 10°C/min.

Raman Spectroscopy. The spectra were recorded in the 0–3500/cm range, using a spectrometer FT-Raman (FRA 106, Bruker, Rheinstetten, Germany) fitted with a Nd:YAG laser and a germanium detector refrigerated with liquid nitrogen. Lorentzian deconvolution of the band at 1540–1620/cm was performed using multi-peak curve-fitting software (Microcal Origin 7.5, Northampton, MA).

X-Ray Diffractometry. Powder diffraction patterns were recorded in a Philips X-ray diffractometer (PW1710, Eindhoven, The Netherlands) using Cu Kα radiation and 20 scans at a scan rate of 2°/min.

SN Dissolution Rate. Freeze-dried SN:HP-β-CD systems with a SN content of 1% w/w (1:40 molar ratio), obtained as explained in Preparation Section, were directly compressed in a B-MT Bonals press (Spain) equipped with 9 mm flat punches and control instrumentation of pressure (Sensing Electronics, Madrid, Spain). Piezoelectric force transducer was calibrated by Lorenz Messtechnik GmbH (Alfdorf, Germany). Compression force was set at 10000 N. Magnesium stearate solution in acetone was applied to the punches and die, before manual filling of the die with 500 mg of freeze-dried powder. Compacts of SN (5 mg) and HP-β-CD (495 mg) physical mixtures (Turbula T2C 15 min, 30 rpm) were also obtained under the same conditions. Drug dissolution from the compacts was evaluated, in triplicate, at 37°C in a 100 mL beaker containing 50 mL water under magnetic stirring (25 rpm). The concentration of the drug in periodically withdrawn samples, filtered using 0.22 μm Millipore® cellulose acetate membranes (Millipore, Spain), was determined at 302 nm (Agilent 8453, Germany). Samples of pure SN powder (5 mg) were also tested.

RESULTS AND DISCUSSION

Complexes of SN:HP-β-CD in Solution

ITC is particularly useful to quantify the heat of binding, on the order of microcalories, associated to the drug:CD interactions and to obtain information about multimodal complexation processes.[6,9,10] The energetics of the interaction of SN with HP-β-CD was evaluated by this technique at pH 1.2, 5.8, and 7.6. The methodology for poorly soluble substances was followed to perform the experiments.[9,22] SN was dissolved in 50:50 methanol/buffer mixtures to obtain a high enough concentration, and the SN solutions were placed in the cell to be titrated with the HP-β-CD solutions.

Figure 2 shows the total interaction enthalpy (raw data) and the complexation enthalpy that was estimated subtracting the SN and HP-β-CD dilution effects from the raw data. The enthalpy changes were significantly greater at pH 1.2 than at higher pH values. At pH 1.2, complexation was an instantaneous exothermic process, which indicates that the inclusion into the cavity of the HP-β-CD of the less polar aromatic rings is energetically more favorable under that pH. This fact became evident by steeply rise of enthalpy after some injections HP-β-CD. Upon further additions the binding curve progressively leveled off until saturation of the complexation. After saturation, a first decrease in the enthalpy occurs at the end point of the formation of one of the two possible types of inclusion complexes, as will be discussed further below. The additional greater decrease in enthalpy, corresponding to the tail-end of the

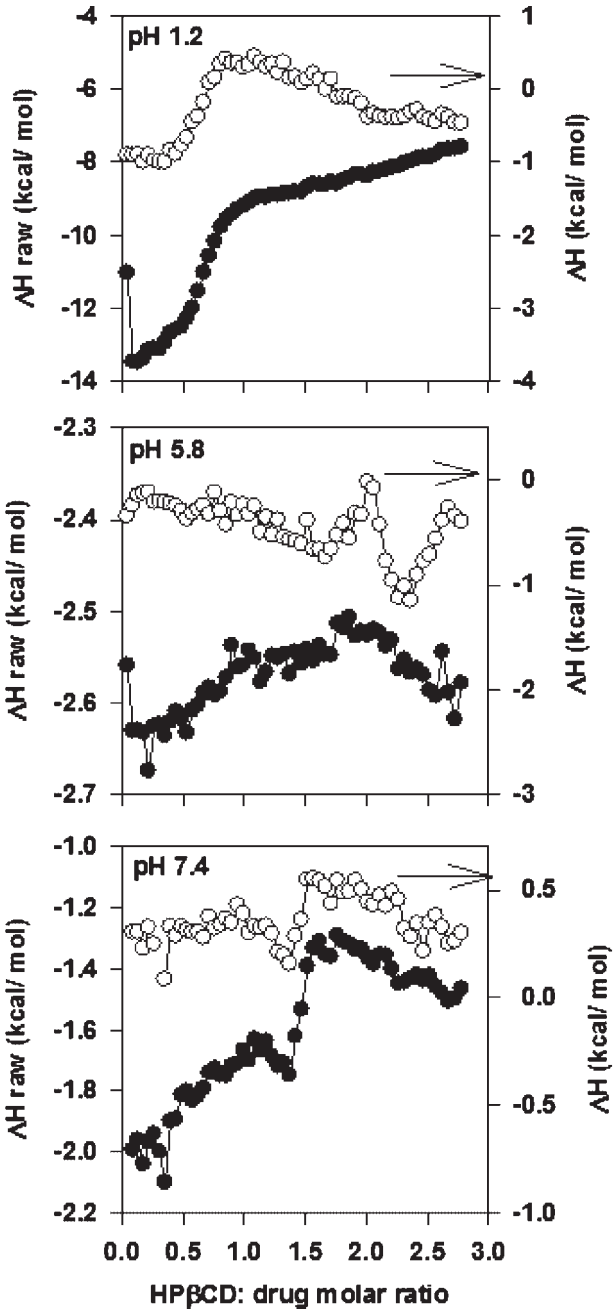

Figure 2. ITC titrations of SN with HP-β-CD in different methanol:buffer 50:50 solutions, before (solid symbols) and after (open symbols) subtraction of the controls.

titration curve with further HP-β-CD addition, can be explained by changes in the mode of drug complexation. The plot of enthalpy versus SN:HP-β-CD molar ratio clearly shows a maximum in the sigmoidal titration curve for 1:1 ratio, which indicates that the formation of complexes with this stoichiometry predominates.

When the sigmoidal titration curve was modeled to a 1:1 stoichiometry for a single type of inclusion complex (i.e., considering that only a specific group of the SN molecule enters into the HP-β-CD cavity), the fit agreed well with the experimental data up to the inflection point of the curve, although beyond that point the fit curve showed saturation behavior. However, when the titration curve was modeled assuming that SN and HP-β-CD can form several types of 1:1 complexes by inclusion of different chemical groups into the HP-β-CD cavity, the fitting of all the plot was significantly improved. Previous articles have reported the formation of several types of 1:1 complexes for systems comprising barbiturates or ampicillin and HP-β-CD, due to the inclusion of the drug through different hydrophobic groups into the CD cavity.[10,18,23,24]

SN structure contains three rings with potential affinity for the HP-β-CD cavity. At pH 1.2, imidazolyl group is protonized; the chlorobenzothiophene and the 2,4-dichlorophenyl groups being the most likely to enter into the CD cavity (Fig. 1). The greater hydrophobicity of 2,4-dichlorophenyl compared to chlorobenzothiophene (3.225 vs. 2.787),[4] makes its inclusion energetically more favorable. Previous computational studies carried out with γ-CD indicate that steric impediments prevent the simultaneous complexation of chlorobenzothiophene and 2,4-dichlorophenyl rings of a SN molecule with two CD molecules.[4] The values of stoichiometry numbers (n_1 and n_2) and of the interaction parameters (K_i, ΔH_i, ΔG_i, and ΔS_i) corresponding to each type of complex, estimated by multimodal modelization, are shown in Table 1. Two different complexes can be formed; the value close to one of the total stoichiometry number, $n = n_1 + n_2 = 0.92$, indicating that the stoichiometry is 1:1. The notable

Table 1. Thermodynamic Parameters of SN:HP-β-CD Interaction Obtained by ITC Analysis at pH 1.2 for Each Type of Complex

n_1	n_2	K^1_{ITC}	K^2_{ITC}	ΔG^1_{ITC}	ΔG^2_{ITC}	ΔH^1_{ITC}	ΔH^2_{ITC}	ΔS^1_{ITC}	ΔS^2_{ITC}
0.66	0.26	169200	977	−29.8	−17.1	0.65	−1.81	102.1	51.3

ni, stoichiometry number; *Ki*, affinity constant (M^{-1}); ΔH^i_{ITC}, complexation enthalpy (kJ/mol); ΔS^i_{ITC}, complexation entropy (kJ/mol/K); ΔG^i_{ITC}, complexation free energy (kJ/mol).

differences between n_1 and n_2 and K_1 and K_2 indicate that the formation of a type of inclusion complex is more probable than that of the other. A first type of inclusion complex involves small positive ΔH^1_{ITC} and large positive ΔS^1_{ITC}, which is typical of the occurrence of hydrophobic interactions.[25] Therefore such interactions seem to be the driving force for the formation of this complex. This should come from the inclusion of the 2,4-dichlorphenyl ring of SN into the hydrophobic cavity of the HP-β-CD. On the other hand, the small negative values of ΔH_2 and the ca. 50% lower ΔS_2 values indicate that van der Waals interactions play a more important role than the hydrophobic ones in the formation of the second type of 1:1 inclusion complexes.[26] From a structural point of view, the most likely complex of these characteristics is the one formed by the inclusion of imidazolyl ring into the HP-β-CD cavity. According to the theory of the enthalpy-entropy compensation, the gain in the enthalpy on the formation of this second complex is achieved with a loss in entropy.[27]

At pH 5.8 and 7.4, the enthalpy changes were lower and showed a shift in the stoichiometry of the SN:HP-β-CD complexes from 1:1 to 1:2. Although the heat associated to the inclusion process was too small and scattered to compute reliable thermodynamic parameters, shifts in the enthalpy profiles from exothermic to endothermic and in the maximum interaction peak were apparent. The contribution of the entropy to the complexation became more evident, which can be explained by an increase in the hydrophobicity of the drug as the pH raises and, consequently, by a higher entropy penalty of the ordered water around the drug molecules in solution. Taking into account the pKa of SN (= 6.74),[3] the percentage of nonionized molecules at pH 5.8 and 7.4 (11 and 82%, respectively) is significantly greater than that at pH 1.2 (close to 0%).

Although ITC studies are not able to quantify the solubility of a drug in a CD solution,[9] we experimentally confirmed that once a strong interaction between SN and HP-β-CD is detected, important increments in SN solubility occur both with and without HPMC (Fig. 3 and Table 2). The remarkable increase in SN solubility observed at pH 1.2 is facilitated by the protonization of the imidazolyl group of SN, which increases the value of S_0 and promotes the complexation process.[15] The solubility diagrams of the nonautoclaved systems showed, at high HP-β-CD concentrations, negative deviations from linearity that

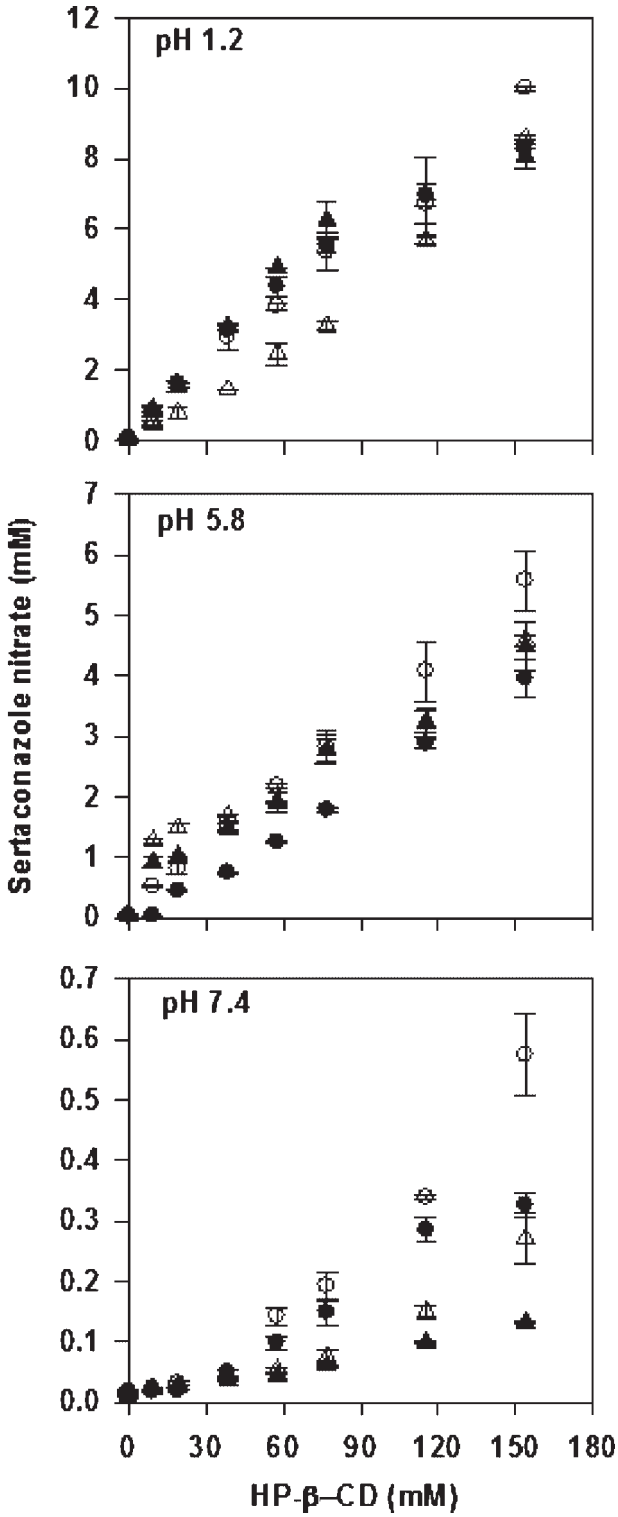

Figure 3. Phase-solubility diagrams of SN in pH 1.2 HCl and pH 5.8 and 7.4 phosphate buffers, in the presence of HP-β-CD (up triangle) or of HP-β-CD and 0.1% HPMC (circle), before (solid symbols) or after autoclaving (open symbols).

 DOI 10.1002/jps

Table 2. Solubility of SN in Different Buffers (So), in Presence of 10% w/v HP-β-CD (Sco) or 10% w/v HPβCD with 0.1% w/v HPMC (Scp)

	So (mM)	Sco (mM)	Scp (mM)	Sco[a] (mM)	Scp[a] (mM)	Scp/Sco	Sco[a]/Sco	Scp[a]/Sco
HCl buffer pH 1.2	0.054	6.257	5.544	3.253	5.344	0.89	0.52	0.85
Phosphate buffer pH 5.8	0.026	2.784	1.779	2.776	2.821	0.639	0.99	1.01
Phosphate buffer pH 7.2	0.012	0.061	0.149	0.075	0.192	2.45	1.23	3.16

[a]SN solubility was evaluated after autoclaving of the systems.

are characteristics of A_L plots.[19] These deviations are explained by the changes in the dielectric constant of the medium and/or by the occurrence of HP-β-CD self-association phenomena.[28] In order to estimate the affinity constants of the 1:1 complexes, Eq. 2 was fitted to the linear portion of the solubility diagram. On the other hand, autoclaved systems displayed A_P diagrams, which indicated the formation of 1:1 and 1:2 complexes; the $K_{1:2}$ values being markedly lower than those of $K_{1:1}$ (Table 3). These results point out that at pH 1.2, the formation of 1:2 complexes is much less probable than the 1:1 ones, which is in agreement with the results of the ITC studies. Nevertheless, the estimated values of the affinity constants were notably different than those obtained by ITC, probably because the different composition of the solvent medium (methanol was absolutely necessary for having enough drug concentration for ITC measurements) and the concentration range evaluated. The fact that the analysis of the phase-solubility diagrams combines in a unique value the complexation process of different 1:1 inclusion complexes can also contribute to these differences.

At pH 5.8 and in the absence of HPMC, A_L type diagrams were obtained whether the sample was autoclaved or not (Fig. 3). At this pH, SN:HP-β-CD 1:1 complexation still predominates, but the values of $K_{1:1}$ are significantly lower than those obtained at pH 1.2. This finding can be explained by the decrease in drug solubility associated to a lower degree of ionization of the imidazolyl group. In the presence of HPMC, A_P type diagrams were obtained. No significant effect of autoclaving on solubility was observed.

At pH 7.4, A_P type diagrams fitted all the solubility plots (autoclaved or not, with or without HPMC). The A_P model is characterized by an initial linear segment followed by a nonlinear increase in solubility, which indicate initial 1:1 complexation followed by higher order complexation as the HP-β-CD concentration increases. $K_{1:1}$ values were lower than those obtained at the other pHs, and a remarkable increase in $K_{1:2}$ was observed. This is a consequence of the greater proportion of nonionized SN molecules (ca. 82%), which decreases the solubility of the drug. This makes the overall complexation more difficult but, at the same time, facilitates the inclusion of the nonionized imidazolyl ring into the HP-β-CD cavity, enhancing the likelihood of formation of 1:2 complexes. Similar changes in the stoichiometry of the complexes (from 1:1 to 1:2) due to an increase in the pH have been previously reported for other ionic drug, levemopamil.[29] The autoclaving slightly promoted drug solubilization, especially in the presence of HPMC.

The increase in drug solubility in CD solutions by heating or adding hydrophilic polymers has been extensively reported, but the mechanisms responsible for such effects are still unknown.[28] In our case, the effect of pH on SN complexation is

Table 3. Apparent Affinity Constants (M^{-1}) of the SN:HP-β-CD Inclusion Complexes Estimated from the Phase-Solubility Diagrams Obtained Under Different Conditions

Medium	Affinity Constant	Without HPMC		With HPMC	
		Autoclaved	No Autoclaved	Autoclaved	No Autoclaved
HCl buffer pH 1.2	$K_{1:1}$	539	1711	777	2022
	$K_{1:2}$	5.8	—	3.8	—
Phosphate buffer pH 5.8	$K_{1:1}$	38.4	636	298	358.6
	$K_{1:2}$	—	—	0.02	1.6
Phosphate buffer pH 7.4	$K_{1:1}$	8.2	7.2	16.5	10.0
	$K_{1:2}$	63	60.3	83	117

remarkably greater than that caused by HPMC or autoclaving. In 10% w/v HP-β-CD solutions, SN solubility was enhanced by a factor of 116, 107 and 5 at pH 1.2, 5.8, and 7.4, respectively; these values showing the same tendency as the enthalpy values recorded by ITC. This confirms the practical interest of the microcalorimetric techniques as a tool for the quick screening of the potential of HP-β-CD in drug solubilization.

Solid State SN:HP-β-CD Complexes

SN:HP-β-CD systems prepared by kneading or freeze-drying were analyzed by DSC, X-ray diffraction, and Raman spectroscopy, and the results were compared with those obtained with powdered SN and with SN/HP-β-CD physical mixtures. Buffer solutions or mixtures of buffer with methanol were used for preparing the solutions to be freeze-dried or as solvent for the kneading process, respectively, in order to evaluate the effect of the pH of the medium on the formation of complexes to be used in solid state. The final salt proportion in the solid systems was in all cases below 0.15 %w/w. For preparing the freeze-dried systems, solutions containing SN:HP-β-CD 1.4 (acid pH) or 1:40 (alkaline pH) molar ratio were used, owing to solubility limitations.

The DSC scans of pure SN from room temperature to 250°C showed an endothermic melting peak at 160°C, which is characteristic of its polymorph A,[3] and an exothermic peak that corresponds to the decomposition at 190°C. Subsequent tests of samples of the drug alone or with HP-β-CD were carried out by first heating up to only 165°C, then cooling to 0°C, and finally heating again up to 250°C. In this way, in the first run water is evaporated and the melting energy of the SN can be quantified without reaching decomposition. In the second heating scan, the glass transition of the drug can be visualized and the possible inclusion inside the CD of the free drug melted in the first heating can be detected (Fig. 4). Applying this procedure, in the first heating of a pure drug sample the melting occurred at 160°C, and the second scan evidenced a glass transition at 34°C, a melting process at 155°C with a much lower enthalpy than that observed in the first run, and the decomposition of SN at 190°C. This suggests that after the first heating, crystalline SN is almost completely melted and transformed to the amorphous state by cooling. The Tg of SN is in the range

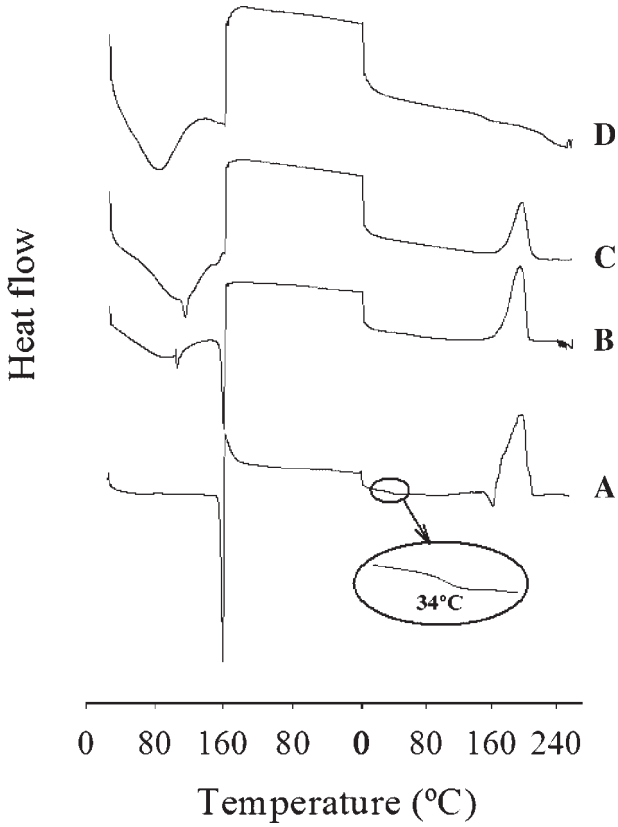

Figure 4. DSC scans of different SN:HP-β-CD solid systems: (A) SN; (B) SN:HP-β-CD physical mixture; (C) SN:HP-β-CD kneaded complex; (D) SN:HP-β-CD freeze-dried complex.

of data reported for other related drugs such as itraconazole, ketoconazole, and miconazole (59.4, 45.6, and 1.1°C, respectively).[30,31]

HP-β-CD exhibits an endothermic peak in the range of 50–150°C owing to evaporation of adsorbed water and a Tg at 250°C. The physical mixtures SN:HP-β-CD (1:1 and 1:2) showed the characteristic melting and decomposition peaks of the drug which were not seen in the freeze-dried SN:HP-β-CD systems. Samples obtained by kneading of SN:HP-β-CD 1:2 mixture did not show the melting peak either (Fig. 4C), which indicates the complete inclusion of the drug in the CD regardless of the pH of the buffer used during kneading. Control samples of pure SN after kneading showed the same DSC pattern as the original crystalline product and the X-ray patterns (Fig. 5) of the drug before and after kneading were similar. Therefore, the application of the kneading procedure itself did not justify the lack of the melting peak. In the case of the SN:HP-β-CD 1:1 kneaded mixtures, a small melting peak was recorded in the first heating run. The enthalpy

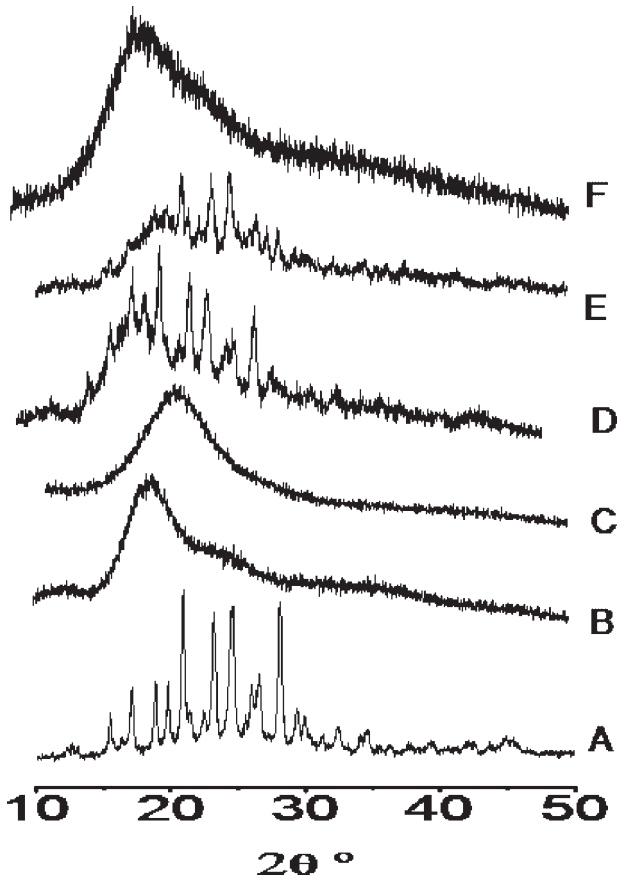

Figure 5. X-ray diffraction patterns of different SN-HPBCD solid systems: (A) SN; (B) HPBCD; (C) HPMC; (D) SN:HPBCD 1:1 physical mixture; (E) SN:HPBCD 1:1 kneaded system; (F) freeze-dried sample.

associated to this melting peak corresponded only to 8.3% of the melting enthalpy of the SN contained in the sample if all drug was at crystalline state. This suggests that, in the 1:1 kneaded mixture, this percentage of drug is not forming inclusion complexes. Therefore, a strong interaction of the drug and the HP-β-CD takes place in both the aqueous environment and the methanol:buffer solutions, which prevents its crystallization during drying. The SN decomposition peak was not observed in the freeze-dried samples, which may be due to a masking effect caused by HP-β-CD when presented in such a high proportion (SN:HP-β-CD molar ratio ranged from 1:4 to 1:40, as the pH increased). This high proportion could also contribute to the stabilization of the drug.

The SN diffraction patterns showed an intense peak at 20.96° 2θ characteristic of the polymorph A,[3] while HP-β-CD and HPMC are amorphous and only present the broad band of the poly(glucopyranose) with maxima at around 19° 2θ and 20° 2θ, respectively. The X-ray diffraction spectra of the physical mixtures corresponded to the superimposed diffractograms of SN and HP-β-CD, showing the peaks of the drug. In contrast, the SN-HP-β-CD binary and ternary (with HPMC) systems obtained by freeze-drying, at any of the pHs evaluated, only showed the broad band of the HP-β-CD, and that of HPMC when present. The kneaded samples were also markedly less crystalline than the physical mixtures. Both X-ray and DSC results indicate the formation of amorphous systems by freeze-drying, and confirms that once SN is partially included in the CD cavity (when in solution regardless of pH), the crystalline structure is not recovered during drying.

The formation of inclusion complexes can be detected by shifts in certain Raman peaks of the guest.[17,32–34] Previously reported data referred to other azole derivatives and diclofenac[34] were used to assign the SN bands (Fig. 6). The peaks at 1555 and 1587/cm are related to the stretch of aromatic C–C and C–N bonds.[3] For comparison with the

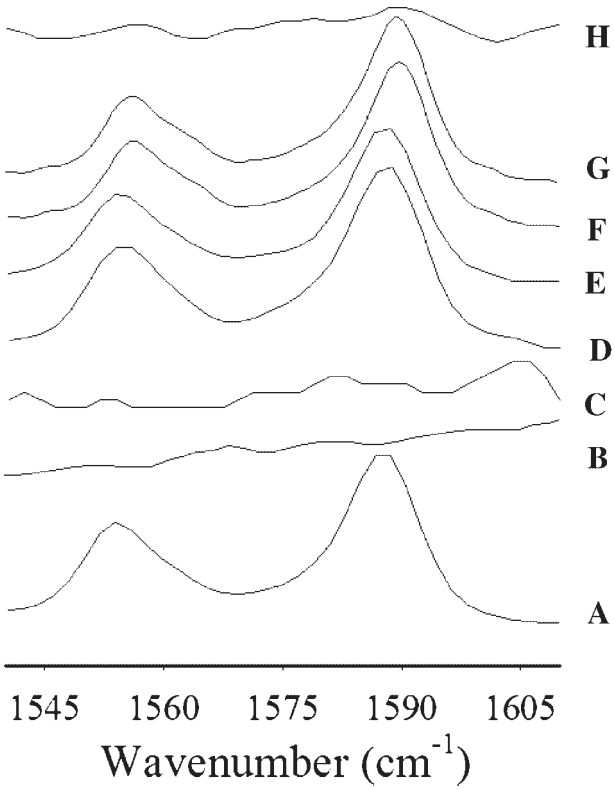

Figure 6. FT-Raman spectra of: (A) SN; (B) HP-β-CD; (C) HPMC; (D) SN:HP-β-CD 1:1 physical mixture; (E) SN:HP-β-CD 1:2 physical mixture; (F) SN:HP-β-CD 1:1 kneaded system; (G) SN:HP-β-CD 1:2 kneaded system; (H) freeze-dried system.

diclofenac spectra,[34] the 1587/cm band can be assigned to the dichlorophenyl ring stretching vibrations. The absence of HP-β-CD bands in the range 1500–2500/cm, prompted us to analyze this region in detail. SN bands remained practically unperturbed in the physical mixtures, but became broader and shifted to higher wavenumbers in the kneaded systems, these changes being characteristic of host-guest interactions. The low SN:HP-β-CD molar ratio in the freeze-dried systems (1:4–1:40) makes it hard to precisely visualize these bands. Kneaded samples analyzed by Lorentzian deconvolution showed shifts from 1555 to 1557/cm and from 1587 to 1589.2/cm at the three pHs evaluated. Despite the shifts are quite small, they suggest that the interaction between the drug and the CD mainly takes place through the 2,4-dichlorophenyl ring and probably through 1H-imidazolyl ring. These findings are in agreement with ITC and solubility studies, which highlight the ability of SN and HP-β-CD to form bimodal 1:1 and unimodal 1:2 inclusion complexes.

With the aim of evaluating the effect of SN:HP-β-CD inclusion complexes in the solubility and dissolution rate of the drug, the behavior of compacts prepared with the complexes or with a physical mixtures was compared to the dissolution profile of the same dose of solid drug. As can be seen in Figure 7, compacts prepared from freeze-dried SN:HP-β-CD complexes behaved as flash formulations releasing all drug in few minutes. In contrast, compacts prepared from the physical blend were not able to promote a complete dissolution of the drug, although the presence of HP-β-CD significantly enhanced the drug solubility.

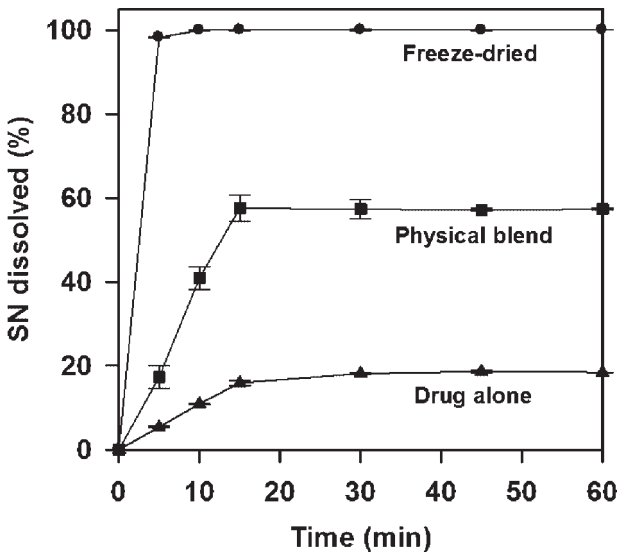

Figure 7. SN release profiles in water from compacts prepared with SN:HPBCD freeze-dried complexes or physical blends. Dissolution rate from SN powder is also shown. In all cases, the amount of drug used for the experiment was of 5 mg.

CONCLUSIONS

Both the phase-solubility diagrams and the ITC studies show the influence of pH on the complexation of SN with HP-β-CD. The protonization degree of the imidazolyl group greatly conditions the stoichiometry of the complexes and the solubilizing effect of the CD. The strongest interaction was observed at pH 1.2, at which SN is fully protonized and SN:HP-β-CD 1:1 complexes are preferentially formed. An increase in the pH decreases the ionization of the imidazolyl group and promotes the formation of SN:HP-β-CD 1:2 complexes. The main interaction between the drug and the CD takes place through both 2,4-dichlorophenyl and imidazolyl rings, as suggested by the analysis of the shifts of the SN peaks associated with those rings in the Raman spectra. Freeze-drying of the solutions and kneading of SN and HP-β-CD mixtures lead to drug complexation, as indicated by DSC and X-ray diffraction analysis. From a practical point of view, SN complexation with HP-β-CD overcomes the solubility limitations of this drug and opens interesting possibilities for the development of its dosage forms.

ACKNOWLEDGMENTS

This work was financed by the Xunta de Galicia (PGIDT01PX1203014IF, PGIDIT03PXIC20303PN, PGIDIT04BTF203011PR), Ministerio de Educación y Ciencia and FEDER Spain (RYC2001-8; SAF2005-01930). We are grateful to Janssen Pharmaceutica Products and Ferrer Grupo for providing us samples of HP-β-CD and SN, respectively.

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