The Interaction of Charged and Uncharged Drugs with Neutral (HP-β-CD) and Anionically Charged (SBE7-β-CD) β-Cyclodextrins

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Purpose. The objective of this work was to determine the role that charge might play in the interaction of charged and uncharged drugs with neutral (2-hydroxypropyl-β-cyclodextrin, HP-β-CD) and anionically charged (SBE7-β-CD) modified β-cyclodextrins. SBE7-β-CD is a sulfobutyl ether, sodium salt, derivative variably substituted on the 2-, 3- and the 6-positions of β-cyclodextrin. The number seven refers to the average degree of substitution.

Methods. The binding of the acidic drugs, indomethacin, naproxen and warfarin and the basic drugs, papaverine, thiabendazole, miconazole and cinnarizine with the two cyclodextrins was determined at 25°C as a function of pH and cyclodextrin concentration by the phase-solubility method.

Results. Except for miconazole and cinnarizine (A_P-type diagrams), all other materials studied displayed A_L-type diagrams. By comparing the binding constants of both the charged and uncharged forms of the same drugs to both HP-β-CD and SBE7-β-CD, the following conclusions could be drawn. The binding constants for the neutral forms of the drugs were always greater with SBE7-β-CD than with HP-β-CD. For the anionic agents, the binding constants between SBE7-β-CD and HP-β-CD were similar while the binding constants for the cationic agents with SBE7-β-CD were superior to those of HP-β-CD, especially when compared with the neutral form of the same drug.

Conclusions. A clear charge effect on complexation, attraction in the case of cationic drugs and perhaps inhibition in the case of anionic drugs, was seen with the SBE7-β-CD.

KEY WORDS: SBE7-β-CD; cyclodextrins; charge effects; HP-β-CD; inclusion complexes.

INTRODUCTION

The objective of this work was to determine the role that charge might play in the interaction of charged and uncharged drugs with neutral (2-hydroxypropyl-β-cyclodextrin; HP-β-CD) and anionically charged (SBE7-β-CD; a sulfobutyl ether, sodium salt, derivative variably substituted on the 2-, 3- and the 6-positions of β-cyclodextrin) cyclodextrins. The number 7 refers to the average degree of substitution (1–3) of the anionically charged β-cyclodextrins (see Figure 1). The binding of the acidic drugs, indomethacin, naproxen and warfarin and the basic drugs, papaverine, thiabendazole, cinnarizine and miconazole with the two cyclodextrins was determined as a function of pH and cyclodextrin concentration by the phase-solubility method (see Table I for drug structures). In addition to understanding the role that charge on the drug and the cyclodextrin might play in inclusion complex formation (4, 5), others (6–11) and work from our laboratory (12) have shown that it is possible to enhance the solubility of weak acids and bases by the combined use of ionization, pH adjustment, and complexation with chemically modified cyclodextrins.

Specific interest in HP-β-CD and SBE-β-CD has arisen due to their greater intrinsic solubility and safety when compared to the parent material, β-cyclodextrin (13–17). SBE4-β-CD, similar to SBE7-β-CD but with an average degree of sulfobutyl substitution of four, has recently been shown to enhance the delivery of sparingly water soluble drugs orally (12), parenterally (18,19) and ophthalmically (20, 21) and can also be used to stabilize drugs (20, 22). SBE-β-CDs are safe on acute dosing and show none of the nephrotoxicity and membrane destabilizing properties of β-cyclodextrin (15,16). SBE7-β-CD is currently undergoing extensive chronic safety assessment.

EXPERIMENTAL

Materials

The synthesis and characterization procedures for SBE7-β-CD have been described previously (1,3). HP-β-CD (Encapsin™; lot EN 92-3. mw 1338; degree of molar substitution, 3.5) was a gift from American Maize-Products Co. (Hammond, IN). Indomethacin, naproxen, warfarin, thiabendazole, papaverine hydrochloride, cinnarizine and miconazole nitrate were purchased from Sigma Chemical Co. (St. Louis, MO). Papaverine and miconazole free bases were obtained by converting salts to their free base form using aqueous sodium bicarbonate. Since miconazole has three different reported polymorphs, the stable polymorph of miconazole was isolated and identified by DSC using the method of Pedersen et al. (23) after recrystallization from a mixture of *n*-hexane and ethyl acetate.

Phase-Solubility Studies

The stability constants for inclusion complex formation between the various agents and SBE7-β-CD or HP-β-CD were determined using the phase-solubility method (24). Excess of each agent was added to 0.1 M buffers at various initial pH values while the ionic strength was held approximately constant at 0.3. Citrate buffer was used in the pH range 4.0–5.0, phosphate in the range of 5.5–8.5 and carbonate in the range 9.0–10.0. The concentration of the two cyclodextrins was varied from 0–0.05 M. The suspensions were agitated at 25°C for 48 hours (equilibrium was confirmed in all cases in preliminary studies). The pH of the suspensions was determined at equilibrium and was used in the calculations of the binding constants. After equilibration, the solutions were either filtered through a 0.45 μm nylon filter (indomethacin, naproxen and warfarin) or

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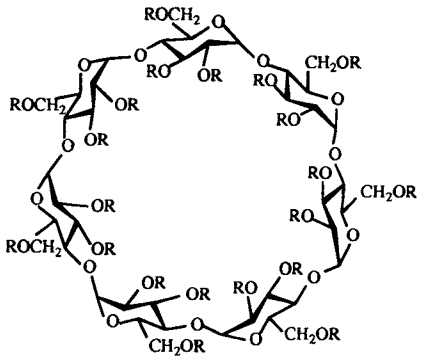


R = $-CH_2CH_2CH_2CH_2SO_3Na$ or -H
SBE7-β-CD
(average degree of sulfobutyl substitution is 7)

R = $-CH_2CH(CH_3)OH$ or $-(CH_2CH(CH_3)O)_nCH_2CH(CH_3)OH$ or -H
HP-β-CD
(average degree of hydroxypropyl substitution is 3.5)

Fig. 1. Chemical structures of SBE7-β-CD and HP-β-CD.

centrifuged (all other agents) at 10,000 rpm (used to avoid adsorption to the filters). The filtrate or supernatant was isolated and diluted with HPLC mobile phase and analyzed by HPLC.

Indomethacin was fractionated on a Hypersil ODS column with detection at 242 nm using a mobile phase of 50% acetonitrile, 50% pH 4 0.01 M acetate buffer. Naproxen was fractionated on a Hypersil ODS column with detection at 250 nm using a mobile phase of 52% acetonitrile, 48% pH 6 0.01 M phosphate buffer. Warfarin was fractionated on a Hypersil ODS column with detection at 214 nm using a mobile phase of 50% acetonitrile, 50% pH 4.7 0.01 M acetate buffer. Papaverine was fractionated on a Hypersil CPS column with detection at 254 nm using a mobile phase of 50% acetonitrile, 50% 0.02 M potassium dihydrogen phosphate buffer. Thiabendazole was fractionated on a Hypersil MOS column with detection at 254 nm using a mobile phase of 60% acetonitrile, 40% 0.01 M potassium dihydrogen phosphate buffer. Miconazole was fractionated on a Hypersil ODS column with detection at 230 nm using a mobile phase of 85% acetonitrile, 15% 0.05 M ammonium dihydrogen phosphate buffer. Cinnarizine was fractionated on a Hypersil ODS column with detection at 254 nm using a mobile phase of 80% acetonitrile, 20% pH 5.5 0.02 M phosphate buffer. The columns were all 15 cm × 4.6 mm i.d. with 5 μm packing materials. All analyses were performed at ambient temperatures. The HPLC system consisted of a Shimadzu LC-6A pump, SIL-6B autoinjector, SCL-6B system controller, SPD-6A UV detector and a CR-601 integrator (Kyoto, Japan).

Data Analysis

The pK_a values and intrinsic solubilities of each agent were calculated from the solubility of each agent in the absence of added cyclodextrin using least squares regression analysis (SuperANOVA™; Abacus Concepts, Inc., Berkeley, CA). The binding constants from the phase-solubility diagrams of each agent with SBE7-β-CD and HP-β-CD were estimated by analyzing the data of the phase-solubility studies using non-linear least squares regression analysis employing a Marquardt-Levenberg algorithm (SigmaPlot®; Jandel Scientific, San Rafel, CA) as reported by Tinwalla et al. (10).

RESULTS AND DISCUSSION

pK_a and Intrinsic Solubility of Drugs

The relationship between the dissociation constant of an agent (K_a) and intrinsic solubility (S_0) of both acidic and basic

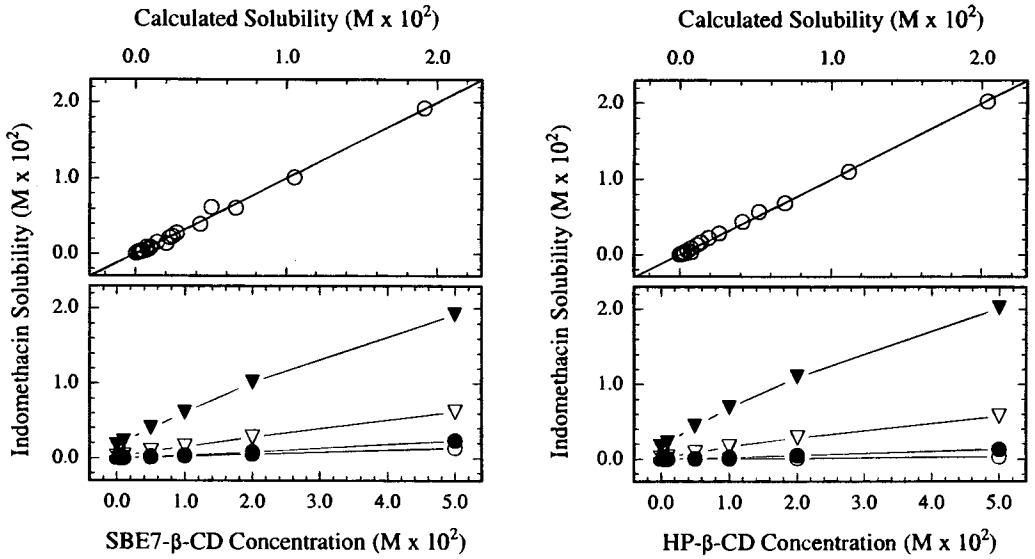
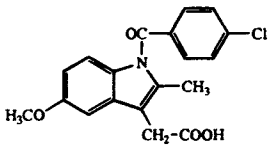
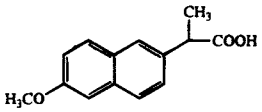
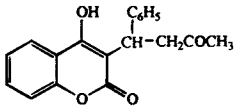
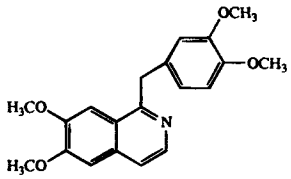
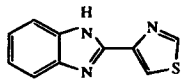
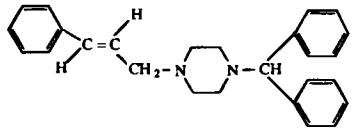
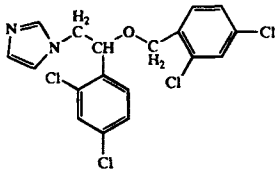


Fig. 2. Lower Plots: Phase-solubility diagrams for indomethacin in the presence of SBE7-β-CD and HP-β-CD and varying initial pH values; ○ pH 4, ● pH 5, ▽ pH 6 and ▼ pH 7. Note, pH values listed are initial pH values. Final measured pH values were used to calculate binding constants K_1 and K_2. Upper Plots: Relationship between calculated and observed values of indomethacin solubility in the presence of SBE7-β-CD and HP-β-CD and varying pH.

Table I. Intrinsic Solubilities (S_0) and pK_a Values at 25°C and Ionic Strength of 0.3

Compound	Structure	pK_a[a]	S_0 (M)[a]	SE (M)[b]
Indomethacin		4.81	8.63×10^{-6}	1.05×10^{-5}
Naproxen		4.81	1.84×10^{-4}	1.23×10^{-4}
Warfarin		4.79	6.14×10^{-6}	1.89×10^{-6}
Papaverine		6.78	1.89×10^{-5}	8.19×10^{-7}
Thiabendazole		4.72	1.24×10^{-4}	4.26×10^{-5}
Cinnarizine		7.40	4.07×10^{-8}	1.26×10^{-7}
Miconazole		6.91[c]	$< 4.80 \times 10^{-9}$[c]	—

[a] determined from linear regression according to Eq. 1 or 2.
[b] standard error.
[c] from Pedersen et al., (8).

drugs can be defined by Eqs. 1 and 2, respectively. Linear least squares regression of total solubility (S) as a function of $1/[H^+]$ for acidic drugs and $[H^+]$ for basic drugs allows for the determination of the intrinsic solubilities and the dissociation constants. The results for the current agents with their associated standard errors are listed in Table I.

$$S = S_0\left(1 + \frac{K_a}{[H^+]}\right) \quad (1)$$

$$S = S_0\left(1 + \frac{[H^+]}{K_a}\right) \quad (2)$$

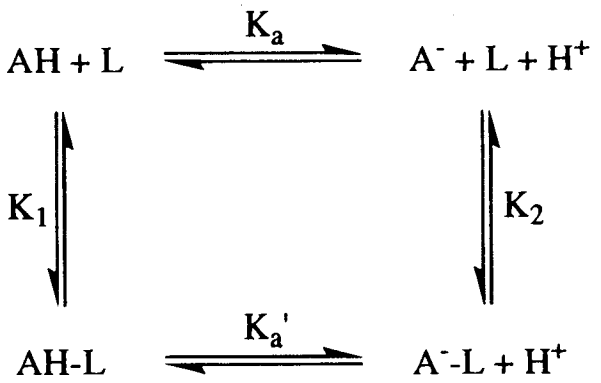
The pK_a and solubility values of all drugs were in reasonable agreement with values previously reported except for miconazole. At pH values greater than 6.0, miconazole was not detected by the HPLC method reported by Pedersen et al., (8), therefore, the intrinsic solubility estimated by Pedersen et al. (8) is reported in Table I. Also, the solubility of miconazole, which is a cationic drug, did not follow the expected behavior. The results suggested that miconazole might self-associate at lower pH values. In addition, the accuracy of the solubility determined for indomethacin is suspect since this value was smaller than its associated standard error. The measured solubility was, however, comparable to literature reports for the solubility of indomethacin (2.62×10^{-6} M) (25). Therefore, the value determined in this study was used in the subsequent binding constant determinations.

Phase-Solubility Analysis

In the presence of the modified β-cyclodextrins, the phase-solubility behavior of the agents qualitatively followed the expected behavior. That is, the apparent solubility of the agents increased with increasing cyclodextrin concentration. However, two complications were observed. First, since all the agents were either acids or bases, the final pH values at equilibrium were often different from initial values. Therefore, the use of

equations that could be linearized (9, 26) to determine the binding constants of both the neutral and charged form of an agent, knowing the intrinsic solubility and K_a for the agent, could not be used. Second, although most of the agents displayed A_L-type phase-solubility diagrams suggesting mainly 1:1 interactions between the agent and the cyclodextrin, cinnarizine displayed apparent A_P-type phase-solubility diagrams with HP-β-CD and miconazole displayed apparent A_P-type phase-solubility diagrams with both SBE7-β-CD and HP-β-CD suggesting the presence of 1:2 interactions in addition to 1:1 interactions in these instances. Because of these two complications, analysis of the phase-solubility data was performed by non-linear analysis as suggested by Tinwalla et al. (10).

Djedaïni et al. (27) and Lin et al. (28) have reported that the inclusion stoichiometry of indomethacin, an anionic drug, with HP-β-CD was 1:1 as determined by NMR and DSC methods. The lower plots in Figures 2 and 3 show the phase-solubility diagrams for indomethacin and papaverine, an anionic and cationic drug, respectively, in the presence of SBE7-β-CD and HP-β-CD. The lines in these figures only represent the joined points. The observable negative deviation from linearity in Figure 2 was a result of shifts in the final pH values with the larger shifts occurring, as expected, under conditions where higher solubility was obtained. Several qualitative observations can be made from these figures. Although the solubility of indomethacin exhibited higher solubility in the presence of SBE7-β-CD compared to HP-β-CD at pH values 4 (four times higher for SBE7-β-CD) and 5 (two times higher for SBE7-β-CD), relative differences were less pronounced at pH values greater than the pK_a (Figure 2). The solubility of papaverine in the presence of SBE7-β-CD increased more than in the presence of HP-β-CD at all pH values (Figure 3; note ordinate axis scale difference).



Scheme 1. Association and dissociation equilibria of an acidic drug (AH) with β-CD derivatives (L).

A mathematical model for 1:1 interaction between an agent and a complexing agent can be defined for Scheme 1 for an acidic agent.

In this scheme, K_1 is the binding constant for the neutral acid (AH) with the cyclodextrin, K_2 is the binding constant for the anion (A^-) with the cyclodextrin, K_a is the dissociation constant of the acid and K_a' is the effective dissociation constant of the acid in the complexed form.

In the case of the Scheme 1, the total solubility of the agent, S, in the presence of either SBE7-β-CD or HP-β-CD (species L) would be defined by Eq. 3.

$$S = [AH] + [A^-] + [AHL] + [A^-L] \quad (3)$$

Similarly, the total concentration of the cyclodextrin derivatives, $[L_T]$, is represented by Eq. 4.

$$[L_T] = [L] + [AHL] + [A^-L] \quad (4)$$

where [L] is the concentration of free cyclodextrin and

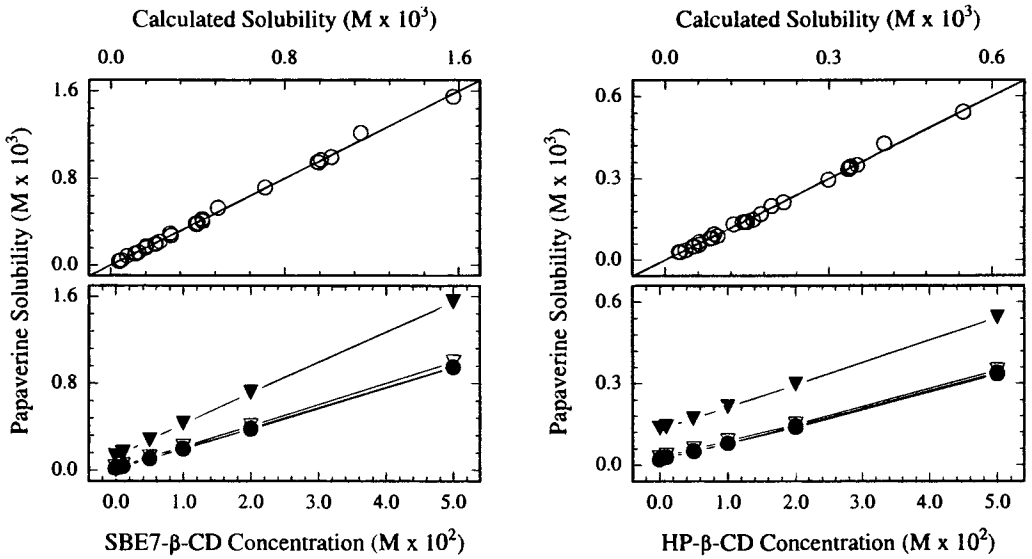


Fig. 3. Lower Plots: Phase-solubility diagrams for papaverine in the presence of SBE7-β-C and HP-β-CD and varying initial pH values; ○ pH 9, ● pH 8, ▽ pH 7 and ▼ pH 6. Note, pH values listed are initial pH values. Final measured pH values were used to calculate binding constants K_1 and K_2. Upper Plots: Relationship between calculated and observed values of papaverine solubility in the presence of SBE7-β-CD and HP-β-CD and varying pH.

$$[AH] = \text{intrinsic solubility of the acid, } S_0 \text{ in Eq. 1} \quad (5)$$

$$[A^-] = \frac{S_0 K_a}{[H^+]} \quad (6)$$

$$[AHL] = K_1 S_0 [L] \quad (7)$$

$$[A^-L] = K_2[A^-][L] = \frac{K_2 S_0 K_a [L]}{[H^+]} \quad (8)$$

$$[L] = \frac{[L_T]}{\left(1 + K_1 S_0 + \frac{K_2 K_a S_0}{[H^+]}\right)} \quad (9)$$

By substituting the equality for [L] in Eq 9, into Eqs. 7 and 8, along with Eqs 5 and 6, the total solubility of the drug as given by Eq. 3, as a function of $[H^+]$ and $[L_T]$, can be related back to the properties of the drug, that is, its intrinsic solubility (S_0), its dissociation constant (K_a) and its ability to form inclusion complexes with the cyclodextrin, defined by the association constants K_1 and K_2.

Liu et al. (9, 26) combined these various equations to produce a mathematical method for determining the binding and dissociation constants assuming that linear plots of S as a function of $[L_T]$ were obtained. Because small changes in the final pH can lead to non-linear phase-solubility plots (see Fig. 2), a non-linear treatment of the data was preferred as suggested by Tinwalla et al. (10). Basically, by determining the solubility of the acidic drug in the absence of the cyclodextrins but as a function of changes in $[H^+]$, the values of S_0 and K_a can be determined from fits to Eq. 1. By determining drug solubility both as a function of $[H^+]$ and $[L_T]$, and knowing S_0 and K_a, the values of K_1 and K_2 can be determined (10). It is also possible to determine all four parameters from the total data, however, experience suggested that this leads to less than satisfactory results in most cases.

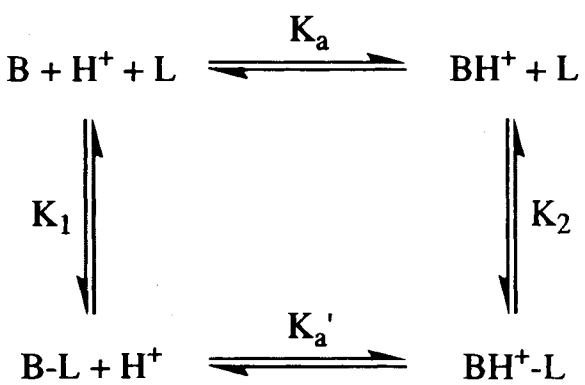
For a basic agent, Scheme 2 applies and a set of equations like those for Eqs. 3–9 can be defined. The equations are not repeated here as they appear in the paper by Tinwalla et al. (10).

The upper plots in Figures 2 and 3 contain the observed versus the calculated solubilities for indomethacin and papaverine, respectively, using the independent estimates of S_0 and K_a along with K_1 and K_2 determined from the non-linear analysis. The good linearity and randomness of the data around the slope of unity along with visual analysis of the randomness of the residual plots for the models described (data not shown) suggests that the models defined by Schemes 1 and 2 appear to adequately describe the combined effect of pH adjustment and complexing agent concentration on the solubility of these two drugs. Figures 2 and 3 are representative of the other drugs fitted to the models described in Schemes 1 and 2. A summary of the binding parameters for indomethacin and papaverine, as well as for naproxen and warfarin (two acids), and thiabendazole (a base), all of which showed A_L-phase solubility behavior, is shown in Table II. The data is both for SBE7-β-CD and HP-β-CD.

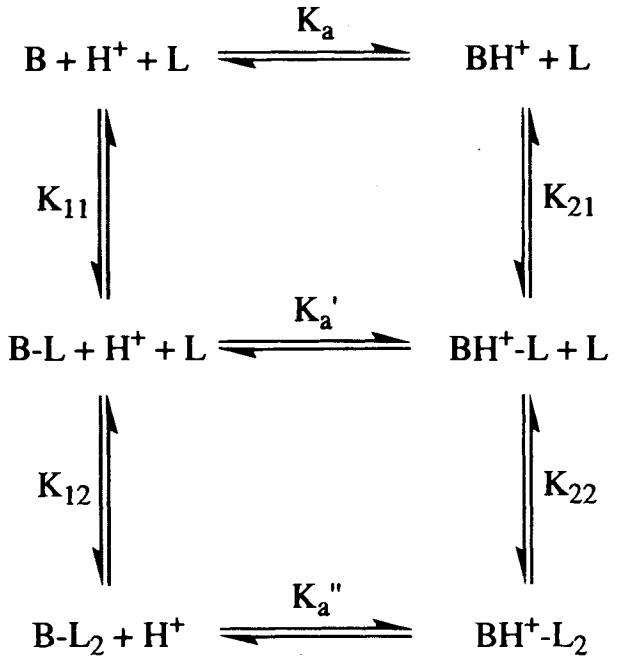
Scheme 3 illustrates both a 1:1 and 1:2 interaction between a basic agent and a complexing agent as reported by Johnson et al. (11).

For the sake of conciseness, the equations defined by Johnson et al. (11) will not be reproduced here. Analogous to the possibility of only a 1:1 interaction illustrated by Schemes 1 and 2 and defined by Eqs. 3–9 for an acid, a similar set of equations can be defined for a basic drug and the observed solubility data as a function of cyclodextrin concentration can be non-linearly fit to give the constants, K_{11}, K_{12}, K_{21} and K_{22} as defined in Scheme 3. Note that K_{11} represents the 1:1 binding constant between the base and one molecule of the cyclodextrin, K_{21} the 1:1 binding constant between the conjugate acid of the base and one molecule of the cyclodextrin, K_{12} the 1:2 binding constant between the base and two molecules of cyclodextrin, and K_{22} the 1:2 binding constant between the conjugate acid of the base and two molecules of the cyclodextrin.

The two agents, miconazole (lower plot Figure 4; note ordinate axis scale difference) and cinnarizine both displayed A_P-type phase-solubility diagrams consistent with 1:2 was well as 1:1 interactions between the agents and HP-β-CD. Similar behavior was observed for the phase-solubility behavior for



Scheme 2. Association and dissociation equilibria of a basic drug (B) with β-CD derivatives (L).



Scheme 3. Association and dissociation equilibria of a basic drug (B) with β-CD derivatives (L) with the possibility of both 1:1 and 1:2 interactions.

Table II. Binding Constants (M^{-1}) and Standard Errors (SE) for the Inclusion Complexation between Indomethacin, Naproxen, Warfarin, Papaverine and Thiabendazole with SBE7-β-CD and HP-β-CD

		K_1		K_2		
	Cyclodextrin	(M^{-1})	SE	(M^{-1})	SE	K_2/K_1
Indomethacin	SBE7-β-CD	4.71×10^3	5.71×10^2	8.19×10^2	2.76×10^1	1.74×10^{-1}
	HP-β-CD	1.59×10^3	3.12×10^2	9.55×10^2	1.66×10^1	6.01×10^{-1}
Naproxen	SBE7-β-CD	3.60×10^3	8.80×10^1	4.32×10^2	2.36×10^1	1.20×10^{-1}
	HP-β-CD	1.67×10^3	7.63×10^1	3.31×10^2	1.91×10^1	1.98×10^{-1}
Warfarin	SBE7-β-CD	1.01×10^4	4.90×10^2	2.62×10^2	1.12×10^1	2.59×10^{-2}
	HP-β-CD	2.54×10^3	4.40×10^2	5.09×10^2	1.38×10^1	2.00×10^{-1}
Papaverine	SBE7-β-CD	1.00×10^3	8.76×10^0	9.37×10^1	3.41×10^0	9.37×10^{-2}
	HP-β-CD	3.37×10^2	3.15×10^0	1.74×10^1	1.21×10^0	5.16×10^{-2}
Thiabendazole	SBE7-β-CD	4.43×10^2	2.54×10^1	5.60×10^1	5.34×10^0	1.26×10^{-1}
	HP-β-CD	1.36×10^2	1.31×10^1	7.38×10^0	1.97×10^0	5.43×10^{-2}

miconazole with SBE7-β-CD (lower plot, Figure 4) while cinnarizine displayed only 1:1 interactions with SBE7-β-CD. This was confirmed by both visual inspection of the data and the poor fit to the mathematical equations consistent with Scheme 2 or 3, respectively, as determined by visual analysis of the randomness of the residual plots for the models described (data not shown). Additionally, non-linear regression analysis for cinnarizine complexation by SBE7-β-CD according to the model described by Scheme 3 provided values for K_{12} and K_{22} which were not significantly different from zero and 1:1 constants which were not significantly different than those for the Scheme 2 model at the 99% probability level.

The upper plots in Figure 4 contain the observed versus the calculated solubilities for miconazole using the independent estimates of S_0 and K_a along with K_{11}, K_{21}, K_{12} and K_{22} determined from the non-linear analysis. Again, the good linearity and randomness of the data around the slope of unity along with visual analysis of the randomness of the residual plots for the models described (data not shown) suggests that the model defined by Scheme 3 appears to adequately describe the combined effect of pH adjustment and complexing agent concentration on the solubility of miconazole.

The chemical structures of miconazole and cinnarizine lend themselves to the possibility of 1:2 interactions because both molecules contain two possible sites of interaction and the sites are reasonably well removed from each other. Similar behavior was seen with an experimental HIV protease inhibitor, kynostatin, by Johnson et al. (11). Pedersen et al. (8) also reported A_P-type behavior between miconazole and HP-β-CD at pH 7.1 while Tokumura et al. (29) reported data on the interaction of cinnarizine with β-cyclodextrin itself.

Table III lists the binding constants for miconazole and cinnarizine which were estimated by the non-linear least squares regression analysis of the phase-solubility data. In order to estimate the binding constants, the intrinsic solubility and K_a values for cinnarizine were fixed in this analysis. The intrinsic

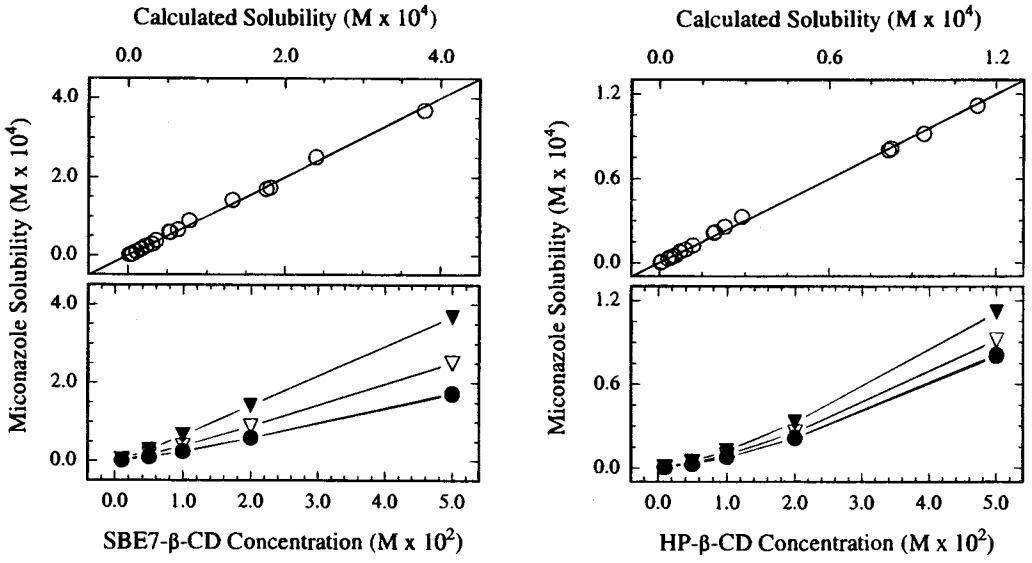


Fig. 4. Lower Plots: Phase-solubility diagrams for miconazole in the presence of SBE7-β-C and HP-β-CD and varying initial pH values; ○ pH 9, ● pH 8, ▽ pH 7 and ▼ pH 6.5. Note, pH values listed are initial pH values. Final measured pH values were used to calculate binding constants K_{11} and K_{21}. Upper Plots: Relationship between calculated and observed values of miconazole solubility in the presence of SBE7-β-CD and HP-β-CD and varying pH.

Table III. Binding Constants (M^{-1}) and Standard Errors (SE) for the Inclusion Complexation between Miconazole and Cinnarizine with SBE7-β-CD and HP-β-CD

	Cyclodextrin	Constant	Value (M^{-1})	SE(M^{-1})	K_2/K_1 or K_{21}/K_{11}
Miconazole	SBE7-β-CD	K_{11}	4.17×10^5	4.31×10^4	9.83×10^{-1}
		K_{21}	4.10×10^5	5.54×10^4	
		K_{12}	1.16×10^1	4.22×10^0	
		K_{22}	2.93×10^{-1}	3.58×10^0	
	HP-β-CD	K_{11}	1.04×10^5	1.26×10^4	4.07×10^{-1}
		K_{21}	4.23×10^4	1.32×10^4	
		K_{12}	4.53×10^1	8.71×10^0	
		K_{22}	1.07×10^1	1.98×10^1	
Cinnarizine	SBE7-β-CD	K_1	6.97×10^4	1.58×10^4	2.51×10^{-1}
		K_2	1.75×10^4	3.00×10^2	
	HP-β-CD	K_{11}	2.25×10^4	5.21×10^2	1.78×10^{-1}
		K_{21}	4.00×10^3	1.74×10^2	
		K_{12}	4.94×10^1	2.92×10^0	
		K_{22}	5.50×10^0	3.45×10^0	

solubility of miconazole was not measurable; however, an estimate of the pK_a was available in the literature (8).

All the numerical values for the binding constants listed in Tables II and III are very sensitive to the value of S_0 and somewhat sensitive to the value of K_a used during the nonlinear estimation of the binding constants. It can be seen from Eqs. 4–8 that the term S_0 appears in the numerator of each equation. This directly impacted the absolute value of the binding constants that were generated. However, since the same values of S_0 and K_a were used to estimate the binding constants for both SBE7-β-CD and HP-β-CD, the relative values of the binding constants were minimally affected.

Relative Binding Constants between SBE7-β-CD and HP-β-CD

All the binding constants reported in Tables II and III for a given drug species with SBE7-β-CD and HP-β-CD are significantly different from each other at the 99% level of probability. Significance was based on rejection of the null hypothesis (no difference in the constants) when the differences in the values plus or minus the square root of the sum of squares of the associated errors times the normal deviate at the 99% level of probability did not contain zero.

The binding of the neutral agents, as indicated by K_1 values, was always superior for SBE7-β-CD compared to HP-β-CD. Linear regression of K_1 values (K_{11} for miconazole) for SBE7-β-CD (y-axis) versus K_1 values (K_{11} for cinnarizine and miconazole) for HP-β-CD (x-axis) produces a slope of approximately four with a regression coefficient of 0.99. The linear regression slope of greater than unity confirmed the higher interaction with SBE7-β-CD while the high linear correlation suggests a common mechanism of interaction for each drug with the respective cyclodextrin. From K_2 values (K_{21} for miconazole) it can be concluded that positively charged drugs (papaverine, thiabendazole, cinnarizine and miconazole) were more strongly bound to SBE7-β-CD compared to HP-β-CD (K_{21} for cinnarizine and miconazole) whereas for negatively charged drugs (indomethacin, naproxen and warfarin) the relative binding values were similar with SBE7-β-CD having superior binding for naproxen and HP-β-CD having superior binding for indomethacin and warfarin.

This trend was reversed when the values for the 1:2 interaction constants (K_{12}) were compared for miconazole. Additionally, there is an apparent lack of 1:2 binding of cinnarizine and SBE7-β-CD at the cyclodextrin concentrations studied. This was also observed by Johnson et al. (11) in their study. A possible explanation for this observation was that SBE7-β-CD does not like to form 1:2 complexes due to charge repulsion; i.e., two SBE7-β-CD molecules interacting with different functional groups within the same substrate would be coulombically unfavorable. This same coulombic repulsion may also explain the lower solubilizing capacity of cholesterol by SBE7-β-CD compared to HP-β-CD (15).

How important is charge type and/or the position of the charge on the relative binding of the seven agents studied here to charge on the cyclodextrin derivative? Inspection of the data for the 1:1 interaction constants with the charged species, K_2 and K_{21}, clearly shows a charge effect with stronger interactions occurring between positively charged agents and SBE7-β-CD and perhaps an inhibition between negatively charged species and SBE7-β-CD. Comparing absolute values, however, might be deceiving. Figure 5 is a plot of K_2/K_1 of each drug (or K_{21}/K_{11} for miconazole) for SBE7-β-CD versus that for HP-β-CD. The actual values may be found in Tables II and III.

The ratio K_2/K_1 is effectively the relative affinity for the cyclodextrin of the drug in its charged form compared to its neutral form. Therefore, this ratio represents a within compound control. In all cases, the K_2/K_1 ratio was never greater than unity meaning the cyclodextrins would rather interact with the neutral drug than its charged form. The only case where the ratio approached unity was miconazole with SBE7-β-CD. The solid line drawn in Figure 5 is for a slope of unity. Ratios that fall above this line represent compounds where the within compound binding, charge effect was superior for SBE7-β-CD, those below the line would be superior for HP-β-CD. If a ratio value falls on the line it would indicate that binding was insensitive to the cyclodextrin type. Effectively, positively

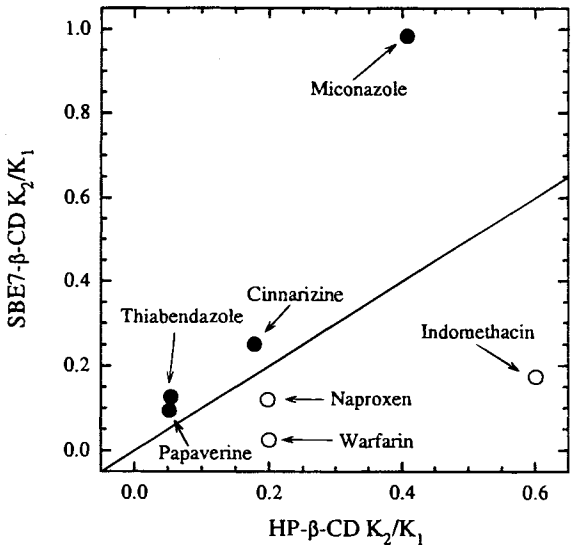


Fig. 5. A plot of K_2/K_1 (K_{2i}/K_{1i} for miconazole) for SBE7-β-CD and versus K_2/K_1 (K_{2i}/K_{1i} for miconazole and cinnarizine) for HP-β-CD; ● basic drugs, ○ acidic drugs.

charged molecules fell above the line while negatively charged molecules fell below the line, thus confirming a charge effect.

By inspection, it was not possible from this limited data to draw conclusions about the role that the site of the charge relative to the most likely binding site between the drug and cyclodextrin might play. Intuitively, however, it is reasonable that such effects are largely driven by a combination of coulombic and hydrophobic forces with regard to the binding of charged drugs to charged cyclodextrins. For systems in which the drug and cyclodextrin have opposite charge, these combinations include significant charge attraction with minimal hydrophobic interaction, significant charge attraction with significant hydrophobic interaction, and minimal charge attraction with significant hydrophobic interaction. Likewise, for systems in which the drug and cyclodextrin have the same charge, these combinations include significant charge repulsion with minimal hydrophobic interaction, significant charge repulsion with significant hydrophobic interaction, and minimal charge repulsion with significant hydrophobic interaction.

In summary, the binding constants for the neutral forms of the seven drugs studied were always greater with SBE7-β-CD than with HP-β-CD. For the anionic agents, the binding constants between SBE7-β-CD and HP-β-CD were similar while the binding constants for the cationic agents with SBE7-β-CD were significantly superior to those of HP-β-CD, especially when compared with the neutral form of the same drug. A clear charge effect, attraction in the case of cationic drugs, and perhaps some inhibition in the case of anionic drugs was seen with the SBE7-β-CD when the ratio of the binding constants of the charged species to the uncharged species were compared across compounds.

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