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Self Association and Cyclodextrin Solubilization of NSAIDs

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Abstract

The phase solubility profiles with HPβCD of the sodium salt of the NSAIDs (non-steriodal anti-inflammatory drugs) ibuprofen and diflunisal were studied. The slopes of the phase solubility diagrams were determined for the sodium salt of ibuprofen at pH 6.1, 6.3 and 6.7, and for the sodium salt of diflunisal at pH 6.1 and 8.4. In all cases the slope of the phase solubility diagram was greater than unity. These results suggested that the stoichiometry of the complex formed was greater than unity with respect to the drug. However molecular modeling, NMR and UV studies clearly showed that the complex stoichiometry was 1:1. These conflicting results can be explained by applying the theory developed for micellar forming compounds. Thus the solubilization of the drugs is due partially from inclusion complex formation and partially from solubilization by aggregation. We have therefore demonstrated that the solubility of drugs in a cyclodextrin solution is explained not only by inclusion complex formation but also by non-inclusion association of the uncomplexed drug with the complex.

Introduction

Cyclodextrins (CDs) are cyclic oligosaccarides with glucopyranose units (7 for β-CD) that form truncated cone with a hydrophilic outer surface and a hydrophobic cavity. CDs can increase the water solubility of hydrophobic molecules, i.e., drugs, by forming water-soluble inclusion complex with them [1]. Phase solubility diagrams show how the cyclodextrin concentration influences the solubility of a drug or other type of a hydrophobic molecule. These diagrams have been used to investigate complexation stoichiometry and complexation constants. According to theory the formation of a drug·CD complex, with a 1:1 stoichiometry, will result in a linear phase solubility diagram. The slope, expressed in drug to CD molar ratio, is then less or equal to unity. A slope greater than unity is thought to indicate formation of a higher order complex with respect to drug (i.e., 2:1 drug·CD complex) and a slope that has positive deviation from linearity is thought to indicate formation of higher order complex with respect to cyclodextrin (i.e., 1:2 drug·CD complex) [2, 3].

In the present work we will present some results to show that these interpretations of the phase solubility diagram may not always be sufficient. In addition to inclusion complex formation other types of interaction, between the drug and CD or the complex, must also be considered. We have investigated, through theoretical and experimental work, the nature of this interaction.

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Experimental

Materials

Ibuprofen and alprazolam were kindly donated by Delta (Iceland) and 17β-estradiol by Pharmatech (USA). Diflunisal was purchased from ICN Biomedicals (USA), diethylstilbestrol from Norsk Medisinaldepot (Norway) and cholesterol from Sigma Chemical Co. (USA). 2-Hydroxypropyl-β-cyclodextrin of molar substitution (MS) 0.64 (HPβCD) was purchased from Janssen Biotech (Belgium). All other chemicals and solvents used in this study were commercial available products of analytical or special reagent grade.

Methods

Preparation of sodium salts

Sodium salts of ibuprofen and diflunisal were prepared by adding 0.05 moles of the drug into 0.5 liters of aqueous 0.1 M sodium hydroxide solution. Then 1 L of distilled water was gradually added to the solution and stirred over night. The solid drug powder dissolved almost completely during this process. Finally the filtrated solution was yophilized (Snijders Scientific 2040 lyophilizer, Holland) and the lyophilized product sieved through a 500 μm sieve.

Phase-solubility profiles

The phase-solubility of the sodium salts of ibuprofen and diflunisal, and that of alprazolam, 17β-estradiol and diethylstilbestrol (DES), was determined in pH 6.0 aqueous 0.1 M

phosphate buffer containing from zero to 0.1 M HPβCD. The phase-solubility of diflunisal sodium salt, 17β-estradiol, alprazolam and DES was also determined in aqueous unbuffered solutions containing from zero to 0.1 M HPβCD. An excess of the drug was added to the aqueous solutions and the suspensions formed were heated, in tightly sealed vials, in an autoclave (121 °C for 20 minutes) [1]. After equilibration at room temperature (22–23 °C), the vials were opened, small amount of solid drug added to each vial and the closed vials then allowed to equilibrate at room temperature for additional 6 days. Finally the aqueous suspensions were filtered through 0.45 μm nylon membrane filter and the filtrate analyzed by HPLC. The effect of ionic strength on the solubilization of ibuprofen sodium salt was investigated by adding up to 12 mg/ml sodium chloride to 5% (w/v) solution of HPβCD in pH 6.0 aqueous 0.1 M phosphate buffer. These solutions were saturated with ibuprofen sodium salt as previously described.

The effect of a second drug

Aqueous HPβCD solutions, which previously had been saturated with the sodium salts of either ibuprofen (in pH 6.0 phosphate buffer) or diflunisal (in water), were saturated with either 17β-estradiol, DES or alprazolam through the previously described heating and equilibration process. After filtration the concentration of dissolved drugs were determined by HPLC.

Quantitative determinations

Quantitative determinations were performed in a high pressure liquid chromatographic (HPLC) system from Merck-Hitachi (Germany) consisting of L 4250 UV-Vis detector, L 6200 A Intelligent pump, AS-2000A Autosampler, D-2500 Cromato-Integrator and Phenomex Luna 5μ C18 reversed phase column (150 × 4.6 mm). The composition of the mobile phases and wavelengths, respectively, used for quantitative determination of the various drugs were as follows. Ibuprofen: Acetonitrile (AcN), acetic acid (AcOH) and water (H_2O) (60:1:39), 265 nm. Diflunisal: AcN, AcOH and H_2O (65:2:33), 254 nm. Alprazolam: Methanol (MeOH) and H_2O (70:30), 254 nm. 17β-Estradiol: AcN, AcOH and H_2O (55:1:44), 280 nm. DES: AcN, Ethanol and H_2O (56:1:43), 280 nm.

Job's plots

The continuous variation (Job's) plots of diflunisal and ibuprofen were determined from ^{1}H-NMR (ibuprofen) or ^{19}F-NMR (diflunisal) and UV data obtained in buffered solutions. The total molar concentration (i.e., the combined concentration of drug and HPβCD in moles per liter) was kept constant, but the mole fraction of HPβCD (i.e., [HPβCD]/([drug] + [HPβCD])) varied from 0.1 to 0.9 [8]. The buffer salts were dissolved in 30% (v/v) D_2O in water and appropriate amounts of drug and HPβCD dissolved in the buffer solution. The total concentration of diflunisal sodium salt and HPβCD was kept at 0.05 M for the ^{19}F-NMR and 5 × 1^{-5} M for the UV studies. The total concentration of ibuprofen sodium salt and HPβCD was kept at 0.01 M for the ^{1}H-NMR and 1 × 10^{-4} M for the UV studies. The NMR spectra were recorded at 297 K on a Bruker AZ250P 250 MHz spectrometer (USA). The UV-detector used was a Perkin-Elmer Lambda 3A Spectrophotometer (USA).

Molecular modeling

Space filling docking study with Sybyl 6.6 (Tripos Inc., USA) was performed on the ibuprofen and diflunisal complexes with HPβCD. The HPβCD structure was derived from neutron diffraction by the courtesy of Dr. Peter Buckwald. [9]

Results and discussion

Theoretical considerations

Phase solubility diagrams are useful tools to study the interaction between drug and cyclodextrin. The interpretation of phase solubility diagrams is based on the following theoretical considerations:
In a solution containing cyclodextrin and drug the following equilibrium will be established.

$$m\mathrm{CD} + n\mathrm{D} \underset{K_c}{\leftrightarrow} \mathrm{D}_n \cdot \mathrm{CD}_m,$$

where D is the drug, CD is the cyclodextrin, $D_n \cdot CD_m$ is the drug cyclodextrin complex and K_c is the stability constant for complex formation. The stability constant, K_c, is determined by the following equation.

$$K_c = \frac{[\mathrm{D}_n \cdot \mathrm{CD}_m]}{[\mathrm{D}]^n[\mathrm{CD}]^m}. \tag{1}$$

Phase solubility diagrams are obtained by determination of the total drug solubility $[D]_{tot}$ at different cyclodextrin concentrations. At saturation conditions [D] will be equal to the intrinsic solubility of the drug S_0. Equation (2) can then be derived to describe $[D]_{tot}$

$$\begin{aligned}[\mathrm{D}]_{\mathrm{tot}} &= S_0 + n[\mathrm{D}_n \cdot \mathrm{CD}_m] \\ &= S_0 + nK_cS_0^n([\mathrm{CD}]_{\mathrm{tot}} - m[\mathrm{D}_n \cdot \mathrm{CD}_m])^m \\ &= S_0 + nK_cS_0^n([\mathrm{CD}]_{\mathrm{tot}} - \frac{m}{n}([\mathrm{D}]_{\mathrm{tot}} - S_0))^m, \end{aligned} \tag{2}$$

where $[CD]_{tot}$ is the total cyclodextrin concentration. It is clear from this equation that relationship between two variants in the phase solubility diagram, $[D]_{tot}$ and $[CD]_{tot}$, is non-linear when m is larger than unity. What is actually observed is a positive deviation from linearity. However when $m = 1$ the following equation can be derived.

$$\begin{aligned}[\mathrm{D}]_{\mathrm{tot}} - S_0 &= nK_cS_0^n([\mathrm{CD}]_{\mathrm{tot}} - \frac{1}{n}([\mathrm{D}]_{\mathrm{tot}} - S_0)) \\ \Rightarrow \frac{[\mathrm{D}]_{\mathrm{tot}} - S_0}{[\mathrm{CD}]_{\mathrm{tot}}} &= n\frac{K_cS_0^n}{1 + K_cS_0^n}. \end{aligned} \tag{3}$$

From (3) it is clear that the slope of the phase solubility diagram (($[D]_{tot} - S_0$)/$[CD]_{tot}$) will be linear and that the slope is ≤ 1 if $n = 1$ and ≤ 2 if $n = 2$. Complexes with 1:1

stoichiometry are most commonly observed and linear phase solubility diagrams with slopes less than unity is usually thought to indicate formation of 1:1 complex. However (3) shows that this is not necessarily the case and higher order complexes with respect to the drug may also be present.

Phase solubility diagrams are also used to study non-inclusion interactions between a solubilizing agent and a drug or other type of poorly soluble compound. This is for example well known method to study solubilization of hydrophobic molecules with surfactants in aqueous solution. In this case $[D]_{tot}$ will be described by the following equation.

$$[D]_{tot} = S_0 + \kappa([Surf] - CMC), \tag{4}$$

where [Surf] is the concentration of the surfactant, CMC is the critical micellar concentration (the concentration of surfactant where micelles start to form) and κ is the molar solubilization capacity or the number of moles of the poorly soluble compound which can be solubilized per mole of surfactant:
At surfactant concentration much higher than the CMC value, (4) can be simplified to:

$$[D]_{tot} = S_0 + \kappa[Surf] \tag{5}$$

and a linear phase solubility diagram will be obtained where the slope is equal to the κ value.

$$\frac{[D]_{tot} - S_0}{[Surf]} = \kappa. \tag{6}$$

There are no theoretical limitations on the κ value but in practice the value is often smaller than one [4].

Now it is possible that non-inclusion interaction of a drug with the cyclodextrin complex is partially responsible for the solubilization of drugs in cyclodextrin solutions. Equation (7) can then be derived, by combining (2) and (5), to describe the total solubility of the drug.

$$[D]_{tot} = S_0 + [D \cdot CD] + \kappa[D \cdot CD]. \tag{7}$$

Here we assume that a 1:1 complex is formed and that CMC is much smaller than the D · CD concentration. (8) can then be derived by the same procedure as before.

$$\text{Slope} = \frac{[D]_{tot} - S_0}{[CD]_{tot}} = \frac{S_0 K_c}{1 + S_0 K_c}(1 + \kappa) = \text{slope}'(1 + \kappa), \tag{8}$$

where Slope is the slope in a phase solubility diagram and slope′ is the slope resulting from solubilization by inclusion. Thus a linear phase solubility does not necessarily suggest that the drug is only solubilized through inclusion complexation and slopes greater than unity do not necessarily indicate that higher order inclusion complexes, with respect to the drug, are formed.

Phase solubility of HPβCD

Linear phase solubility diagrams were obtained for the sodium salts of the NSAIDs, ibuprofen (Naib) and diflunisal (Nadif) (Table 1). The slopes are significantly greater than unity with $p < 0.01$ in the pH ranges investigated. These results might suggest formation of 2:1 (drug · CD) complex. However this is not consistent with the previously reported stoichiometry. Only 1:1 (or 1:2) stoichiometry has been reported for cyclodextrin complexes ibuprofen and diflunisal [10–12].

Table 1. The statistical properties of the phase solubility of diflunisal sodium salt (Nadif) and ibuprofen sodium salt (Naib) with HPβCD

Drug	PH	df[a]	slope	S_0 (mM)	r^2	s.e.[b]	p[c]
Ibuprofen	6.1	3	1.12	10	0.998	0.0292	<0.05
Ibuprofen	6.3	6	1.17	20	0.999	0.0133	≪0.01
Ibuprofen	6.7	3	1.12	40	0.998	0.0312	<0.02
Diflunisal	6.1	3	1.34	7.8	0.999	0.0302	<0.01
Diflunisal	8.4	2	1.19	54*	0.999	0.0269	<0.05

* y-intercept.
[a] Degrees of freedom.
[b] Standard error of the line.
[c] Statistical probability that the slope is not greater than unity according to student's distribution.

We investigated the possibility that small pH variations caused an increase in the slopes of the phase solubility diagrams. Table 1 shows that phase solubility diagrams for Naib had essentially identical slopes in the pH range from pH 6.1 to 6.7. In each case the pH of all solutions was adjusted to be within 0.05 pH unit from the average pH value and there was no correlation between drug concentration and the small pH variations between solutions. Two phase solubility studies for Nadif showed that the slope was significantly greater than unity at pH 6.1 and 8.4.

A general cosolvent effect from the added HPβCD could possibly affect the slope of the phase solubility diagram. However a general cosolvency effect would have a non-linear effect rather than increasing the slope of the phase solubility diagram. Further more our own investigations have shown that cosolvency does not occur in HPβCD solution up to concentrations of 60% [13].

When the salts of the NSAIDs are dissolved the ionic strength of the solution will increase. This may affect the solubility of the drug and the slope of the phase solubility diagram. However, increased ionic strength is known to decrease the solubility of many NSAIDs rather than increasing it [14]. In the present investigation the solubility of Naib in buffered aqueous solutions with a fixed HPβCD was not affected by increased NaCl concentration.

Slopes greater than unity could not be explained by supersaturation. When a small amount of drug was added to solutions that had already been saturated and filtered, no additional precipitate was formed.

The stoichiometry of the complexes was further investigated as sufficient explanations for the slope greater than unity had not been obtained. Continuous variation plot (job's plot) was preformed for Naib and Nadif. It showed that the complex is 1:1 (Figure 1). The peak from both NMR and UV data is at a mole fraction of HPβCD of 0.5 for both drugs. That is, complexation is at maximum when the drug:cyclodextrin molar ratio is 1:1. Docking study with space filling for HPβCD complexes of ibuprofen and diflunisal showed that

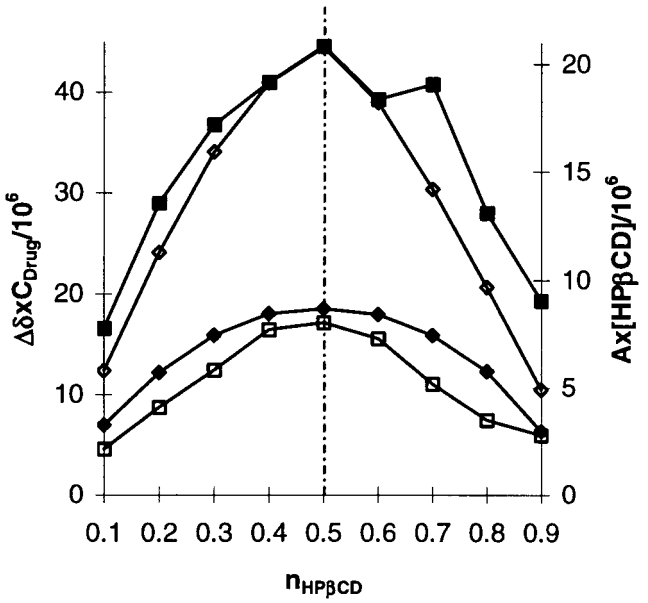

Figure 1. Job's plots for diflunisal sodium salt obtained from UV (♦), ^{19}F-NMR (◊ and ibuprofen sodium salt) obtained from UV (■) and ^{1}H-NMR (□) investigations. The scale for UV result is on the right and the scale for NMR results on the left.

one drug molecule filled the cyclodextrin cavity (Figure 2) so that no space was available to fit another drug molecule in cavity. These studies confirm that the inclusion complex stoichiometry must be 1:1.

The complex itself could also be responsible for the increased solubility. If most of the drug is solubilized through 1:1 and 2:1 complex formation (linear phase solubility) then very little free cyclodextrin should be present in the solution if the slope of the phase solubility diagram is greater than unity, as for Naib and Nadif. One way to estimate the effective concentration of free cyclodextrin in such solutions is to measure the ability of the solution to solubilizes a second drug (D2). This is based on the following theoretical considerations.

For first drug (D1) and the second drug we have:

$$K_c^{\mathrm{D1}} = \frac{[\mathrm{D1}_n \cdot \mathrm{CD}]}{[\mathrm{D1}]^n[\mathrm{CD}]}, \tag{9}$$

$$K_c^{\mathrm{D2}} = \frac{[\mathrm{D2} \cdot \mathrm{CD}]}{[\mathrm{D2}][\mathrm{CD}]}. \tag{10}$$

If the cyclodextrin solution is saturated with respect to both drugs then we have:

$$\begin{aligned}[\mathrm{D2}]_{\mathrm{tot}} &= S_0^{\mathrm{D2}} + [\mathrm{D2} \cdot \mathrm{CD}] \\ &= S_0^{\mathrm{D2}} + K_c^{\mathrm{D2}} S_0^{\mathrm{D2}}[\mathrm{CD}] \\ &= S_0^2 + K_c^{\mathrm{D2}} S_0^{\mathrm{D2}}([\mathrm{CD}]_{\mathrm{tot}} - [\mathrm{D2} \cdot \mathrm{CD}] \\ &\quad -[\mathrm{D1}_n \cdot \mathrm{CD}]),\end{aligned} \tag{11}$$

where $[\mathrm{D2}]_{\mathrm{tot}}$ is the concentration of the second drug, $[\mathrm{D2} \cdot \mathrm{CD}]$ is concentration of the 1:1 second drug complex, $[\mathrm{CD}]$ is the free cyclodextrin concentration and $[\mathrm{D1}_n \cdot \mathrm{CD}]$ is the combined concentration of first or higher order complexes of the first drug. From (11) the following equation, for the slope of the phase solubility diagram can be derived

$$\begin{aligned}&\frac{[\mathrm{D2}]_{\mathrm{tot}} - S_0^{\mathrm{D2}}}{[\mathrm{CD}]_{\mathrm{tot}}} \\ &= K_c^{\mathrm{D2}} S_0^{\mathrm{D2}} - \frac{K_c^{\mathrm{D2}} S_0^{\mathrm{D2}}([\mathrm{D2}]_{\mathrm{tot}} - S_0^{\mathrm{D2}})}{[\mathrm{CD}]_{\mathrm{tot}}} - \frac{K_c^{\mathrm{D2}} S_0^{\mathrm{D2}}[\mathrm{D1}_n \cdot \mathrm{CD}}{[\mathrm{CD}]_{\mathrm{tot}}} \\ &= \frac{K_c^{\mathrm{D2}} S_0^{\mathrm{D2}}}{1 + K_c^{\mathrm{D2}} S_0^{\mathrm{D2}}} \left(1 - \frac{[\mathrm{D1}_n \cdot \mathrm{CD}]}{[\mathrm{CD}]_{\mathrm{tot}}}\right) \\ &= \frac{K_c^{\mathrm{D2}} S_0^{\mathrm{D2}}}{1 + K_c^{\mathrm{D2}} S_0^{\mathrm{D2}}} \left(1 - \frac{[\mathrm{CD}]_{\mathrm{tot}} - (\mathrm{D2}]_{\mathrm{tot}} - S_0^{\mathrm{D2}}) - [\mathrm{CD}]}{[\mathrm{CD}]_{\mathrm{tot}}}\right) \\ &= \frac{K_c^{\mathrm{D2}} S_0^{\mathrm{D2}}}{1 + K_c^{\mathrm{D2}} S_0^{\mathrm{D2}}} \left(\frac{[\mathrm{D2}]_{\mathrm{tot}} - S_0^{\mathrm{D2}}}{[\mathrm{CD}]_{\mathrm{tot}}} + \frac{[\mathrm{CD}]}{[\mathrm{CD}]_{\mathrm{tot}}}\right).\end{aligned} \tag{12}$$

Thus the $[\mathrm{CD}]/[\mathrm{CD}]_{\mathrm{tot}}$ ratio can be described by:

$$\begin{aligned}&\frac{[\mathrm{CD}]^{\mathrm{D1,D2}}}{[\mathrm{CD}]_{\mathrm{tot}}^{\mathrm{D1,D2}}} \\ &= \frac{\dfrac{[\mathrm{D2}]_{\mathrm{tot}}^{\mathrm{D1,D2}} - S_0^{\mathrm{D2}}}{[\mathrm{CD}]_{\mathrm{tot}}^{\mathrm{D1,2}}} \left(1 - \dfrac{K_c^{\mathrm{D2}} S_0^{\mathrm{D2}}}{1 + K_c^{\mathrm{D2}} S_0^{\mathrm{D2}}}\right)}{\dfrac{K_c^{\mathrm{D2}} S_0^{\mathrm{D2}}}{1 + K_c^{\mathrm{D2}} S_0^{\mathrm{D2}}}} \\ &= \frac{\mathrm{Slope}_2^{\mathrm{D2}}(1 - \mathrm{Slope}_1^{\mathrm{D2}})}{\mathrm{Slope}_1^{\mathrm{D2}}},\end{aligned} \tag{13}$$

where $\mathrm{Slope}_2^{\mathrm{D2}}$ is the observed slope for D2 when the solution is saturated with the first and second drug and $\mathrm{Slope}_1^{\mathrm{D2}}$ is the slope in the phase solubility diagram of the second drug when no competing molecule is present.

If the complexation efficacy is very high for the first drug (i.e., the slope is large), then we can assume that the second drug will not have a significant effect on the complex concentration of the first drug. Thus

$$[\mathrm{D1}_n \cdot \mathrm{CD}]^{\mathrm{D1,D2}} \approx [\mathrm{D1}_n \cdot \mathrm{CD}]^{\mathrm{D1}}, \tag{14}$$

where $[\mathrm{D1}_n \cdot \mathrm{CD}]^{\mathrm{D1}}$ is the concentration of drug on complex in solutions saturated only with D1 and $[\mathrm{D1}_n \cdot \mathrm{CD}]^{\mathrm{D1,D2}}$ is the concentration of the complex in solution saturated with both D1 and D2.

These calculations show that if the conditions are such that the drugs are only solubilized through inclusion complex formation and if there is no interaction between complexes then phase solubility studies with second drug, D2 can be used to estimate the free cyclodextrin concentration in solutions saturated with D1. Then the following equation can then be used to calculate free cyclodextrin concentrations in solutions that are only saturated with D1.

$$\begin{aligned}\frac{[\mathrm{CD}]^{D1}}{[\mathrm{CD}]_{\mathrm{tot}}^{\mathrm{D1}}} &\approx \frac{[\mathrm{CD}]^{\mathrm{D1,D2}} + [\mathrm{D2} \cdot \mathrm{CD}]^{\mathrm{D1,D2}}}{[\mathrm{CD}]_{\mathrm{tot}}^{\mathrm{D1,D2}}} \\ &= \frac{\mathrm{Slope}_2^{\mathrm{D2}}(1 - \mathrm{Slope}_1^{\mathrm{D2}})}{\mathrm{Slope}_1^2} + \mathrm{Slope}_2^{\mathrm{D2}}.\end{aligned} \tag{15}$$

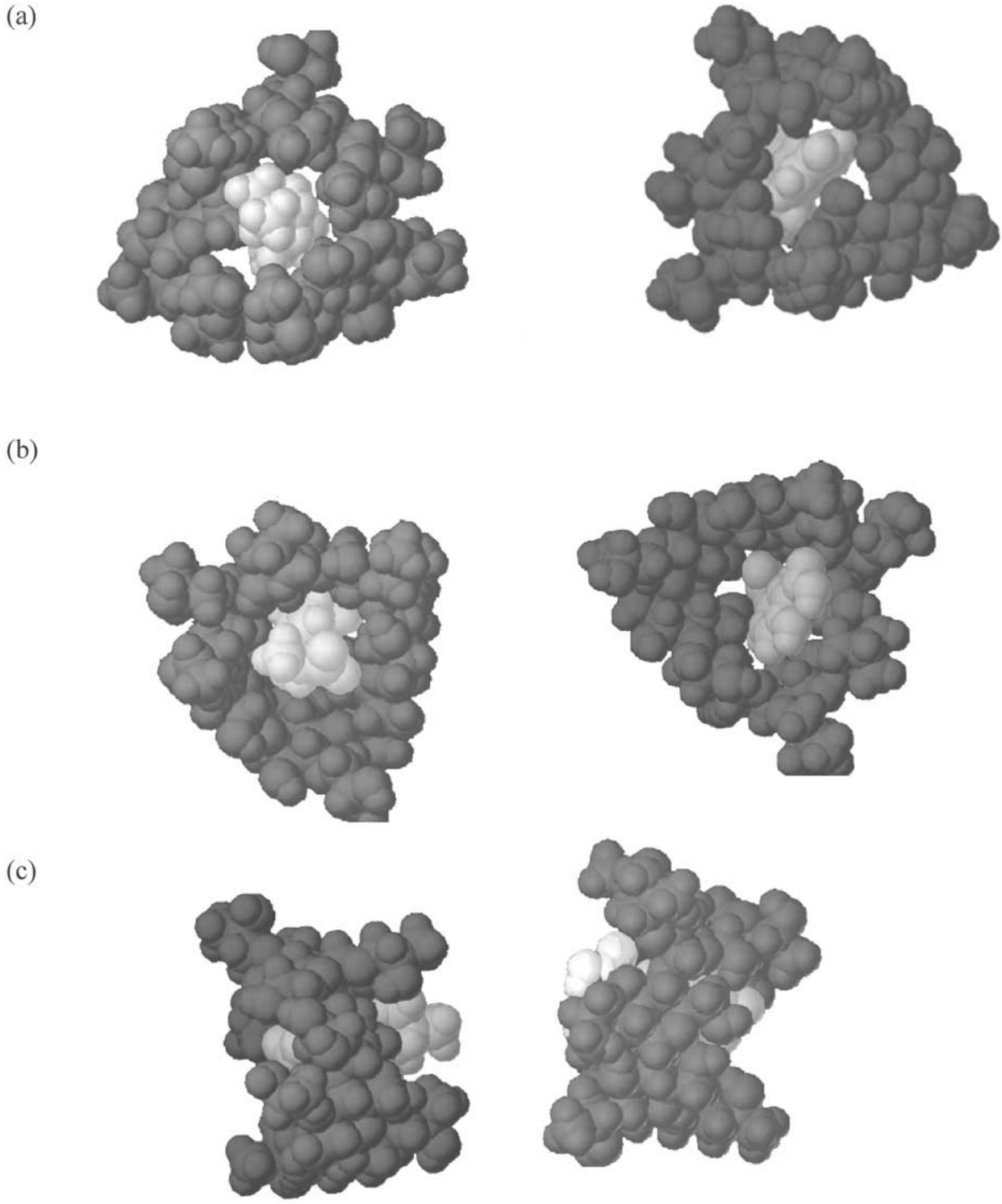

Figure 2. Molecular Modeling of Diflunisal (right) and Ibuprofen (left) in HPβCD cavity in the gas phase seen from the rear/tighter end (A), the wider end (B) and from the side (C) of the cyclodextrin.

Three second drugs (D2s): alprazolam, estradiol and DES, were used in the present study. All have linear phase solubility diagrams with HPβCD. HPβCD solutions that had previously saturated with either Naib or Nadif (D1) were saturated with the D2. The concentration of Naib or Nadif did not change after treatment with D2.

The slopes of the phase solubility diagrams for D2, in presence and absence of the D1, were used to calculate free vs. total cyclodextrin concentration ratio according to (15). The calculated values are presented in Table 2. The calculated free to total cyclodextrin ratio should be approximately the same for all the D2s tested if it is assumed that drugs were solubilized through inclusion complex formation and that other types of interaction did not affect the solubility. The calculated $[CD]_{free}/[CD]_{tot}$ ratios vary from 0.013–0.98. This inconsistency can only be explained by assuming that

Table 2. Results of experiments with a second drug (D2) when the HPβCD solutions saturated with one or two drugs. The slope and intrinsic solubility for the D2 in a solution with and without the first drug (D1). The calculated free HPβCD concentration relative to the total HPβCD concentration in saturated solution of D1

D1[a]	D2[a]	Type[b]	S_{01} (mM)	S_{02} (mM)	Slope 1	Slope 2[c]	$[CD]_{free}/[CD]_{tot}$[d]
Naib	Alp.	A_l	0.20	0.21	0.024	0.0041	0.17
Naib	DES	A_p/bl	0.02	0.01	0.71	0.076	0.11
Naib	Estr.	A_p/bl	0.11	not det.	0.31	0.009	0.029
Nadif	Alpr.	A_l	0.23	0.90	0.025	0.025	0.98
Nadif	DES	bl	0.01	0.01	0.72	0.009	0.013
Nadif	Estr.	A_p/A_l	not det.	0.044	0.31	0.007	0.023

[a] D1 and D2: The CD solutions were first saturated with the first drug (D1) and then with the second drug (D2).
Alp. = Alprazolam, DES = Diethylstilbestrol, Estr. = Estradiol.
[b] A_l stands for a linear phase solubility, A_p stands for positive deviation from linearity, and b_l stands for a bilinear diagram.
[c] The slope of a phase solubility for D2 in a saturated solution of D1, calculated from the last four data points in the phase solubility diagram if it was not linear.
[d] The free cyclodextrin vs. total cyclodextrin concentration ratio in the saturated D1 solution without D2, calculated from Equation (15).

non-inclusion phenomena affect the solubility. Theory that only accounts for inclusion complex formation is therefore not sufficient to describe this system. However, (8) can be used here as the κ value can vary depending on the properties of the D2 and complex taking part in the non-inclusion interaction.

Surface tension measurements were carried out to determine if the complexes of Nadif·HPβCD and Naib·HPβCD behaved like typical surfactants. All molecules that have a polar and nonpolar part well isolated from each other can be amphiphillic and surface active [4]. This is the case with many compounds such as drugs, modified starches and cyclodextrins. CDs that have hydrophobic groups covalently linked to the ring have been reported to be highly surface active [5] and complexes of βCD·β-carotene form aggregates [6, 7].

Nadif and Naib in HPβCD solutions lowered the surface tension of water or buffer considerably, but neither Naib·HPβCD nor Nadif·HPβCD complexes had typical surfactant-like properties. Apparently the complexes do not accumulate at the surface. This may be explained by dissociation of complex, at the surface, to release the hydrophobic moiety. Although these complexes are not surface active their behaviour in solution can resemble that of a typical surfactant i.e., they can from aggregate, which then can solubilize poorly soluble compounds by non-inclusion interaction.

Conclusion

Phase solubility diagrams are not as informative as commonly believed. We have shown that non-inclusion phenomena may also contribute to the solubilization of a drug in a cyclodextrin solution. We have shown with second drug experiments that the complex itself may have a solubilizing effect, which is not based on inclusion interaction. It is common to use phase solubility diagrams to determine stoichometry and complexation constants for cyclodextrin complexation. The present study shows that reported values may not always be accurate.

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