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Cyclodextrins and the liquid-liquid phase distribution of progesterone, estrone and prednicarbate

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Abstract The purpose of the present work was to investigate the interaction of drugs and octanol with hydroxypropyl β- (HPβCD) and γ- (HPγCD) cyclodextrin, sulfobutyl ether β-cyclodextrin (SBEβCD) and randomly methylated-β-cycoldextrin (RMβCD) and to describe the interaction by theoretical models. The poorly soluble steroid drugs progesterone, estrone and prednicarbate were used as model compounds in this study. Hexane and chloroform were also investigated in combination with HPβCD. Octanol formed a complex with all cyclodextrins and the saturation of the aqueous solution with this solvent therefore had a significant effect on the solubilization and extraction potential of cyclodextrins. Hexane had less affinity for cyclodextrins, but the drugs were poorly soluble in this solvent and it could therefore not be used in phase-distribution investigations. Previously we have derived equations that can be used to account for the competitive interaction between two guest compounds that compete for space in the cyclodextrin cavity. These equations were rearranged to calculate the complexation efficacy from phase-solubility data. An equation was derived that obtains intrinsic solubility (S_0) and intrinsic partition coefficient (P) from the slopes of the phase-solubility and phase-distribution profiles. Investigation of the data showed that the results could not

be sufficiently explained by the "classical" drug/cyclodextrin complex model that recognizes the possibility of competitive interactions but ignores any contribution from higher order complexes or aggregation of the cyclodextrin complexes. Relative difference in solubilization potential of different cyclodextrins cannot be translated to relative differences in extraction efficacy. Thus, for these three steroid compounds, RMβCD and SBEβCD gave the best solubilization potential whereas the best extraction efficacy was observed with HPγCD.

Keywords Liquid-liquid partitioning · Steroids · Octanol-water · Extraction · Complexation

Introduction

Liquid-liquid distribution methods have been used to determine the stability constants for the metal ions complexes [1], benzoic acid-caffeine complexes [2] and pKa for acid-base equilibria [3]. Most commercially available cyclodextrin derivatives are readily soluble in aqueous solutions but have very limited solubility in organic solvents. Cyclodextrins have therefore been used, in analytical applications, for selective extraction of lipophilic guest compounds, which are capable of forming inclusion complexes with cyclodextrins, from organic phase into aqueous phase. The efficacy of the extraction will depend on affinity of the lipophilic guest molecule for the cyclodextrin cavity. Thus it should also be possible to determine stability constant (K) for the complex from investigation of the relationship between the cyclodextrin concentration and the equilibrium distribution

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of the guest between the two phases. However, in any theoretical description of such system it must be considered that lipophilic organic solvent molecules can also be included in the cyclodextrin cavity and this will affect the complexation of other guest compounds. Recently we have proposed method to determine the stability constant from the slope of a phase-distribution diagrams, i.e. diagrams of the reciprocal of the apparent partition coefficient ($1/P_{app}$) vs. the cyclodextrin concentration [4]. This method was used to determine K for drug/hydroxypropyl-β-cyclodextrin complexes. In this investigation the K-value of 8 moderately lipophilic drugs were determined and the values compared to values obtained from phase-solubility investigations. The values were generally consistent but there was up to two-fold difference in the values obtained from phase-solubility and the phase-distribution investigations. Such difference between K values obtained with two different methods is not uncommon [1, 5] and could be due to experimental error, especially errors in the determination of the intrinsic octanol/water partition coefficient (P), which is used in the calculation when K is determine from the slope of the phase-distribution diagram, or the intrinsic solubility (S_0), which is used in the calculation when K is determined from the slope of the phase-solubility diagram. However, multi-component complexes involving more than one guest compound [6], non-inclusion complexes and complex aggregates can also be formed [7]. The contribution of such complex forms is not taken into account in theoretical treatment of phase-solubility and phase-distribution data. This could therefore also explain the difference in the values obtained with the two methods.

In the present work further studies were performed to compare the results from phase-distribution and phase-solubility investigations, and assess the possible contribution of cyclodextrin aggregates, non-inclusion and multi component complexes. The lipophilic steroid drugs; progesterone, estrone and prednicarbate were selected as model compounds in this study. Three organic solvents and four cyclodextrin derivatives were used in this investigation.

Experimental

Materials

2-Hydroxypropyl-β-cyclodextrin (HPβCD, molar substitution 0.62, MW ~1400) was obtained from Roquette (Letrem, France) randomly methylated-β-cyclodextrin (RMβCD, degree of substitution 1.8, MW ~1310) and 2-hydroxypropyl-γ-cyclodextrin (2HPγCD, molar substitution 0.6, MW ~1560) from Wacker chemie (Munich, Germany) and sulfobutyl ether-β-cyclodextrin (SBEβCD, MW ~2163) from CyDex Inc. (Kansas City, USA), estrone (MW = 270), progesterone (MW = 314) and n-octanol (99%) from Sigma (St. Luis, USA), chloroform (99.8%, HPLC grade) from Riedel-de Haën (Germany), hexane (95%, HPLC grade) from Rathburn (Walkerburn, Scotland). Prednicarbate (MW = 488) was donated by Stiefel (UK). All other reagents were of analytical or special regent grade.

Analytical methods

Instrumentation for HPLC consisted of a ConstaMetric 3,200 solvent delivery system (LDC Analytical, USA) operated at 1.5 ml/min, a SpectroMonitor 3,200 variable wavelength detector (LDC Analytical, USA), an AS-2000A Intelligent Autosampler (Merck-Hitachi, Germany). The column used was a Luna(2) C18, 5 µm, 150 × 4.6 mm reverse phase column (Phenomenex, UK). The mobile phase was a methanol:water in a 70:30 volume ratio. The retention times and detection wavelengths were as follows: Estrone: 280 nm, 3.2 min; progesterone 254 nm, 6.3 min; prednicarbate 242 nm, 6.7 min.

A Hewlett Packard 5890 Series II GC connected to a Hewlett Packard Chemstation (Hewlett Packard, USA) was used for the gas-chromatography investigations. The column used was a CP-Wax 57, 50 m, 0.32 mm, 0.2 µm. The heating gradient used was as follows: 5 min. at 40 °C, then 20 °C/min to 200 °C and final temperature held for 1 min. The retention times were 3.05, 6.5 and 12.02 min. for hexane, chloroform and octanol respectively.

Phase-solubility and phase distribution investigations

Aqueous complexation media, containing cyclodextrin in the concentration range 0–15% w/v, were prepared. Complexation media was saturated with organic solvent by adding excess of organic solvent and shaking the mixture on a mechanical shaker for 1–2 h. In the case of hexane and octanol the excess solvent was then removed, leaving only few droplets of solvent on the surface to ensure continued saturation. Removal of excess chloroform was not necessary as chloroform will sink below the aqueous phase. Aliquots were drawn from these solutions for phase-solubility, phase-distribution investigations for determination of organic solvent content.

The solubility of drugs was determined in aqueous complexation media, which contained no organic solvent or had been pre-saturated with organic solvent. The solutions were saturated with drug by adding excess of drug to vial containing complexation media. The vials were sealed and the drug suspension heated in an ultrasonic bath (70 °C for 60 min). This was done to promote dissolution of the drug and complexation with cyclodextrin. After equilibration at room temperature (22–23 °C) over night the vials were opened, small amount of solid drug added to each vial and the aqueous drug suspensions mechanically shaken for additional 5–6 days to obtain full equilibrium.

Finally the suspensions were allowed to settle and the aqueous drug suspensions filtered through a Millipore-Millex-HN 0.45 μ filter (Millipore, USA) before analysis by HPLC to determine the concentration of dissolved drug. Phase-solubility profiles were obtained by plotting the solubility of drug versus the cyclodextrin concentration.

Phase-distribution investigations were done by first preparing a 2 mg/ml solution of the drug in the organic solvent. Three aliquots of these solutions were then transferred to 10 ml vials containing 3 ml of complexation media. The vials were shaken, with a mechanical shaker, for 24 h at room temperature, to equilibrate the phase-distribution. One ml samples were then taken from the aqueous phase and the organic phase. The samples of the aqueous phase were centrifuged for 20 min at 15,000 rpm to fully separate the two phases. Samples from the aqueous and the organic phase were diluted into methanol and analyzed by HPLC to determine the drug concentration. The apparent partition coefficient was calculated as the concentration in the organic phase divided by the concentration in the aqueous phase. Phase-distribution profiles were obtained by plotting the reciprocal of the apparent partition (P_{app}) coefficient of the drug versus the cyclodextrin concentration.

Determination of organic solvents in aqueous cyclodextrin solutions

Octanol was extracted from the cyclodextrin solution with chloroform. Cyclodextrin solution (20 ml), which had been pre-saturated with octanol, was centrifuged at 5,000 rpm for 20 min to separate any undissolved octanol from the aqueous phase. Ten ml samples, from the aqueous phase, were transferred to a separation funnel. The octanol was extracted by three extractions with 10 ml chloroform. The chloroform extracts were diluted with methanol and the n-octanol quantity determined by gas-chromatography. Concentration of octanol in the original sample was then calculated from the combined quantity of octanol in the three extracts. More than 99% of the octanol was removed in the first two extractions.

Results and discussion

Theory

The solubility of a drug guest compound (*D*), which can form 1:1 complex with cyclodextrin (CD), can be described by the equation [1]:

$$[D]_{total} = S_{0(D)} + \frac{K_{(D)}S_{0(D)}[CD]_{total}}{1 + K_{(D)}S_{0(D)}} \quad (1)$$

where $S_{0(D)}$ is the intrinsic solubility of the drug, $K_{(D)}$ is the stability constant for the formation of the D/CD complex, $[D]_{total}$ the total concentration of dissolved drug and $[CD]_{total}$ the total cyclodextrin concentration. The phase-solubility diagram, a plot of $[D]_{total}$ against $[CD]_{total}$ will therefore be linear (A_L type) with intercept $S_{0(D)}$ and the slope (Slope $_{[D]total}$) will be:

$$Slope_{[D]total} = \frac{K_{(D)}S_{0(D)}}{1 + K_{(D)}S_{0(D)}} \quad (2)$$

The $K_{(D)}S_{0(D)}$ product is a unit less term, which sometimes is referred to as the complexation efficiency for the CD complex. Rearranging (2) gives an equation that can be used to calculate the complexation efficiency from the slope of the phase-solubility diagram:

$$K_{(D)}S_{0(D)} = \frac{Slope_{[D]total}}{1 - Slope_{[D]total}} \quad (3)$$

The $K_{(D)}$ for the Drug/Cyclodextrin complex can be obtained by dividing this value by $S_{0(D)}$. However, the S_0 value is usually very inaccurate for compounds with $S_0 < 0.1$ mg/ml [8] and in this case the solubilizing potential of cyclodextrins can be more accurately described by complexation efficiency.

Cyclodextrin solutions can also be saturated with more than one guest compound. One guest compound could for example be a drug and the second guest compound could be a small organic solvent (OS) molecules. In this case, and if the cyclodextrin cavity can only be occupied by one type of guest compound, then the slope of the phase solubility diagram for the drug will be [4]:

$$\text{Slope}_{\text{[D]total}}^{\text{OS satur.}} = \frac{K_{(\text{D})}S_{0(\text{D})}}{1 + K_{(\text{OS})}S_{0(\text{OS})} + K_{\text{D}}S_{0(\text{D})}} \qquad (4)$$

where $K_{(\text{OS})}$ is the stability constant for the OS/CD complex and $S_{0(\text{OS})}$ is the intrinsic solubility of the organic solvent in aqueous solution and $S_{0(\text{OS})}\,K_{(\text{OS})}$ the complexation efficiency for this complex.

Rearranging this equation will give:

$$K_{(\text{D})}S_{0(\text{D})} = \frac{\text{Slope}_{\text{[D]total}}^{\text{OS satur.}}}{1 - \text{Slope}_{\text{[D]total}}^{\text{OS satur.}}} \times \left(1 + K_{(\text{OS})}S_{0(\text{OSt})}\right) \qquad (5)$$

The distribution of a drug in a two phase system where one phase is an organic solvent and the other phase an aqueous cyclodextrin solution can describe with the apparent partition coefficient ($P_{\text{app}(\text{D})}$).

$$P_{\text{app}(\text{D})} = \frac{[D]_{\text{total in the organic phase}}}{[D]_{\text{total in the aqueous phase}}} \qquad (6)$$

Phase-distribution diagram for the cyclodextrin is a plot of the $1/P_{\text{app}}$ vs. $[\text{CD}]_{\text{total}}$. The aqueous phase in such two-phase systems, used in liquid-liquid extractions and phase-distribution investigations, will be saturated with the organic solvent and OS/CD complexes can therefore be formed. If the previous assumption that cyclodextrin cavity can only be occupied by one type of guest compound is valid, and when the drug concentration in the aqueous phase is $<<$ $[\text{CD}]_{\text{total}}$, then slope of the phase-distribution diagram will be [4]:

$$\text{Slope}_{1/P\text{app D}} = \frac{K_{\text{D}}\left(1/P_{(\text{D})}\right)}{1 + K_{(\text{OS})}S_{0(\text{OS})}} \qquad (7)$$

where $P_{(\text{D})}$ is the intrinsic partition coefficient for the drug when no cyclodextrin is present in the aqueous solution.

And thus:

$$K_{\text{D}}\left(1/P_{(\text{D})}\right) = \text{Slope}_{1/P\text{app D}} \times \left(1 + K_{(\text{OS})}S_{0(\text{OS})}\right) \qquad (8)$$

Dividing Eq. (5) with Eq. (8) will give:

$$\begin{aligned}
\frac{K_{(\text{D})}S_{0(\text{D})}}{K_{(\text{D})}\left(1/P_{(\text{D})}\right)} &= S_{0(\text{D})}P \\
&= \frac{\text{Slope}_{\text{[D]total}}^{\text{OS satur.}}}{\text{Slope}_{1/P\text{app D}}\left(1 - \text{Slope}_{\text{[D]total}}^{\text{OS satur.}}\right)}
\end{aligned} \qquad (9)$$
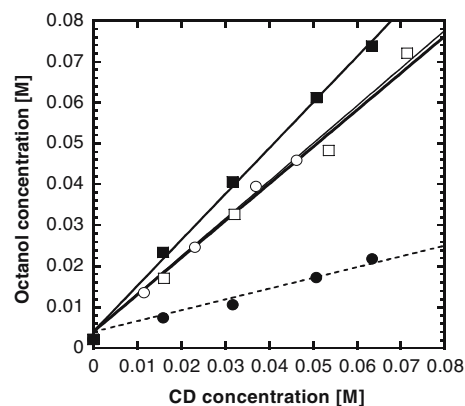

Fig. 1 Phase-solubility diagrams for ocatanol in HPβCD ($\square$), RMβCD ($\blacksquare$) , SBEβCD ($\bigcirc$) and HPγCD ($\bullet$) solutions

This equation can be used to calculate value of the product $S_{0(\text{D})}P_{(\text{D})}$, directly from the slopes phase-solubility and phase-distribution diagrams, without relying on any additional parameters. The product of these two intrinsic parameters should not be dependent on the cyclodextrin used in the study or the affinity of the drug or organic solvent molecules for the cyclodextrin cavity. Thus, we can use these calculations to check the validity of the previous assumptions about the cyclodextrin complexes. If there were no significant contribution from cyclodextrin aggregates, non-inclusion and multi component complexes then these calculations should give a constant value independent of the cyclodextrin used. However, if the calculated values vary significantly from one cyclodextrin to another, then the system cannot be described sufficiently without regard to these higher-order complex forms.

Experimental results

Three solvents, chloroform, hexane and octanol, which are commonly used in separation science and the commercially available cyclodextrin derivatives HPβCD, RMβCD, SBEβCD and HPγCD were used in this study.

Organic solvent in water can be determined by gas-chromatography, but direct injection did not give satisfactory results with cyclodextrin solutions. Concentrated cyclodextrin solutions tended to clog the injector and the results were not reproducible, which may also be due to binding of the organic solvent to cyclodextrin after injection. Octanol was therefore extracted with chloroform before injection.

Figure 1, shows the phase-solubility diagram for octanol in cyclodextrin solutions. Octanol had least affinity for the HPγCD. Slopes of the phase-solubility diagram was only 0.26 (Table 1) for HPγCD, which is consistent with 1:1 complex formation. The slope was greater than 1 for RMβCD and close to unity for SBEβCD and HPβCD. Thus it is likely that complex stoichiometry is mainly 2:1. The complexation efficiency for these complexes was therefore calculated according to the Eq. [1]:

$$K_{(OS)} S_{0(D)} = \frac{\text{Slope}_{[OS]\text{total}}}{2 - \text{Slope}_{[OS]\text{total}}} \qquad (10)$$

The HPβCD phase-solubility diagrams octanol/ HPβCD-solution phase-distribution diagrams for estrone and progesterone were linear (Fig. 2) and less than unity, which is consistent with 1:1 complex formation. Saturation with hexane had little effect on the phase-solubility diagrams, whereas significant

Fig. 2 Phase-solubility (A. C and E) diagrams for phase-distribution diagrams (B. D and F) for the three drugs in HPβCD media. Investigations were done for pure complexation media (□) and complexation media saturated with octanol (■). chloroform (○) and hexane (●)
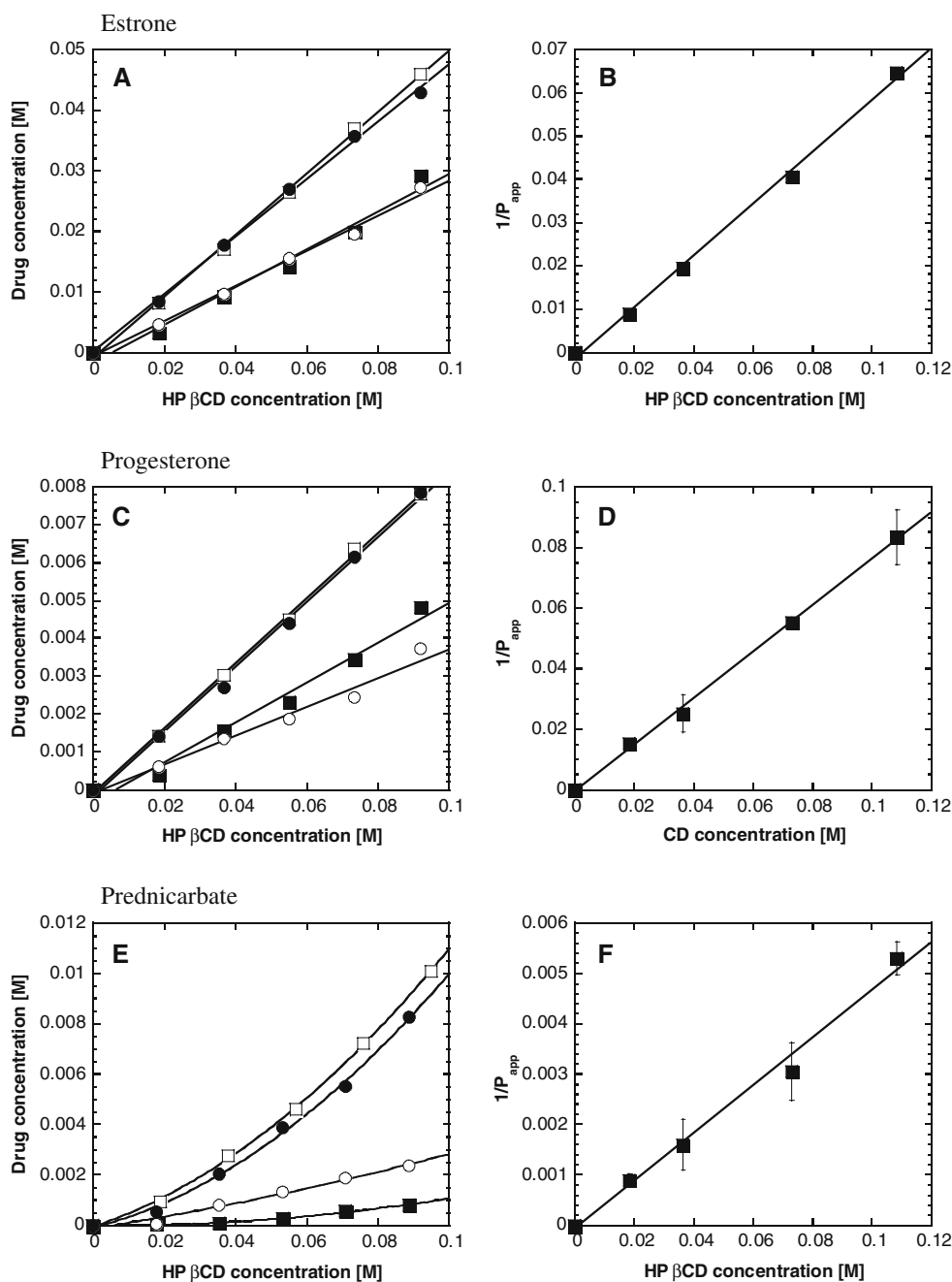
Table 1 Results from phase-solubility and phase-distribution diagrams for complexation media pre-saturated with organic solvents

Guest/cyclodextrin	Solvent and organic phase	Diagram type	Phase-solubility Slope$_{[D]total}$	Complexation efficacy	Phase-distribution Slope$_{1/P_{app}}$ [M^{-1}]	$P_{(D)}S_{0(D)}$
				$K_{(OS)}S_{0(OS)}$		
Octanol/						
HPβCD	Octanol	A$_L$	0.90	0.82		
RMβCD	Octanol	A$_L$	1.12	1.27		
SBEβCD	Octanol	A$_L$	0.92	0.85		
HPγCD	Octanol	A$_L$	0.26	0.35		
				$K_{(D)}S_{(D)}$		
Progesterone/						
HPβCD	No solvent	A$_L$	0.53	1.14		
HPβCD	Octanol	A$_L$	0.34	0.94	0.618	0.84
RMβCD	No solvent	A$_L$	0.52	1.06		
RMβCD	Ocatnol	A$_L$	0.51	2.41	0.898	1.18
SBEβCD	No solvent	A$_L$	0.69	2.26		
SBEβCD	Ocatnol	A$_L$	0.60	2.72	0.733	2.01
HPγCD	No solvent	A$_L$	0.51	1.05		
HPγCD	Ocatnol	A$_L$	0.48	1.24	2.391	0.38
Estrone						
HPβCD	No solvent	A$_L$	0.092	0.10		
HPβCD	Octanol	A$_L$	0.059	0.11	0.774	0.08
RMβCD	No solvent	A$_L$	0.163	0.19		
RMβCD	Ocatnol	A$_L$	0.137	0.36	0.675	0.24
SBEβCD	No solvent	A$_L$	0.121	0.14		
SBEβCD	Ocatnol	A$_L$	0.098	0.20	1.032	0.10
HPγCD	No solvent	A$_L$	0.037	0.04		
HPγCD	Ocatnol	A$_L$	0.036	0.05	1.811	0.02
Prednicarbate						
HPβCD	No solvent	A$_p$	non-linear			
HPβCD	Octanol	A$_p$	non-linear		0.053	
RMβCD	No solvent	A$_p$	non-linear			
RMβCD	Ocatnol	A$_p$	non-linear		0.109	
SBEβCD	No solvent	A$_L$	0.072	0.08		
SBEβCD	Ocatnol	A$_L$	0.008	0.01	0.021	0.37
HPγCD	No solvent	A$_L$	0.126	0.14		
HPγCD	Ocatnol	A$_L$	0.013	0.02	0.381	0.03

solubility reductions were observed in solutions that had been pre-saturated with chloroform or octanol. The HPβCD phase-solubility diagrams for prednicarbate had positive deviation from linearity and therefore consistent with 1:2 complex formation. The effect of the organic solvent to reduce the complexation is also greater with this drug, which is as expected when the complex concentration is proportional to the square of the free cyclodextrin concentration ($[CD]^2$). However in this case the phase-distribution diagrams were linear, which would be consistent with 1:1 complex formation.

The phase-solubility investigations were also done for the other cyclodextrins in both pure aqueous solutions and solutions that had been saturated with octanol, and the phase-distribution investigations were done with octanol as the organic solvent. The phase-distribution investigations could only be done with octanol since the steroids were poorly soluble in hexane and only minute quantities of the drugs were

extracted from the chloroform phase into the aqueous cyclodextrin phase. All phase-solubility and phase-distribution diagrams for estrone and progesterone were cyclodextrin were linear. The prednicarbate phase-solubility diagrams were non-linear for HPβCD and RMβCD. The SBEβCD and HPγCD have less tendency to form 1:2 complex due to the anionic charge and large cavity size, respectively. The phase-solubility diagrams were therefore linear and consistent with 1:1 complex formation. Interestingly the HPγCD was most efficient in extracting the drugs into the aqueous phase whereas RMβCD and SBEβCD where the best solubilizing agents. This could partially be explained by the low affinity of n-octanol for the HPγCD cavity. There was significant difference in the $K_{(D)} S_{0(D)}$ values as determined in pure aqueous or octanol saturated solutions (Table 1). In general the calculated $K_{(D)} S_{0(D)}$ values were larger for the octanol saturated solution than those obtained for pure aqueous solutions. Thus the organic solvent interfered less

with the complex formation than expected. The "true" $K_{(OS)} S_{0(OS)}$ values for the system when drug is present may therefore be less than the values determine from phase-solubility study when drug is not present. This would be the case when 2:1 complexes are formed when 1:1 are expected or when 3:1 complexes are formed when 2:1 complexes are expected.

The $P_{(D)}So_{(D)}$ can be calculated according to Eq. (9) from slopes of the phase-distribution and phase-solubility diagrams. The $K_{(OS)}S_{0(OS)}$ term is eliminated in the calculation and, thus, any inaccuracy in determination of this term should not affect the result. Unless multi component complexes or aggregates are formed the calculated $P_{(D)}S_{0(D)}$ should be constant and independent of the cyclodextrin used in the investigation. Our calculations revealed a considerable variation in the calculated $P_{(D)}S_{0(D)}$ values. These results cannot be sufficiently explained if it is assumed that only simple complexes can be formed. The $P_{(D)}S_{0(D)}$ values ranged from 0.38 to 2.01, 0.02 to 0.24, and 0.03 to 0.37 for progesterone, estrone and prednicarbate, respectively. The intrinsic octanol/water partition coefficients are 7,410 and 1,350 for progesterone and estrone, respectively [9], and the intrinsic solubilities in aqueous solution are 2.5×10^{-6} M and 3×10^{-6} M, respectively [10]. The more lipophilic prednicarbate has very low aqueous solubility which could not been accurately determined. The expected $P_{(D)}S_{0(D)}$ products would then be 0.004 and 0.018 for progesterone and estrone, respectively.

Analysis of the data for these three poorly soluble steroid compounds shows that "K" will depend on the method used, and these difficulties will remain even if the considerable difficulties in accurately determining intrinsic solubilities and octanol-water partition coefficients could be overcome. The present data cannot be sufficiently explained with the "classical" model of the drug/cyclodextrin complex, which recognizes the possibility of competitive interactions but ignores any contribution from multi component complexes or the possible aggregation of the cyclodextrin complexes.

Relative difference in solubilization potential of different cyclodextrins cannot be translated to relative differences in extraction efficacy. Thus, for these three steroid compounds, RMβCD and SBEβCD gave the best solubilization potential whereas the best extraction efficacy was observed with HPγCD.

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