Investigation of Drug–Cyclodextrin Complexes by a Phase-Distribution Method: Some Theoretical and Practical Considerations

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The purpose of the study was to evaluate an octanol–water phase distribution method for investigation of drug/cyclodextrin (D/CD) complexes and to compare stability constant values obtained by this method to values obtained by the phase solubility method. A general equation for determination of 1:1 D/CD complex stability constant (\(K_{1:1}\)) from the slope of a phase-distribution diagram (a diagram of the reciprocal of the apparent partition coefficient vs. the total CD concentration) was derived. The equation accounted for the possible inclusion of the organic solvent in the CD cavity and the gradual saturation of the CD binding with increasing concentration of the guest compound. This method was used to determine \(K_{1:1}\) for 2-hydroxypropyl-\(\beta\)-cyclodextrin (HP\(\beta\)CD) complexes of hydrocortisone, prednisolone, diazepam, \(\beta\)-estradiol and diethylstilbestrol. These values were comparable to \(K_{1:1}\) values determined by the phase-solubility method. The phase-distribution method could also be applied to determine stability constants for the neutral and ionic forms of the weakly acidic drugs, naproxen and triclosan and the weakly basic drug lidocaine. The phase-distribution method is a very versatile and fast method and has the advantage, compared to the phase-solubility method, that it only requires very small drug samples. Thus, this method would be suitable for screening of new drug candidates.

Key words cyclodextrin; stability constant; binding constant; liquid–liquid partitioning; octanol

Cyclodextrins (CDs) are frequently used to enhance drug solubility,\(^1\),\(^2\) stability,\(^3\),\(^4\) and delivery,\(^1\),\(^4\),\(^5\) and there are now, on the market, more than 20 registered pharmaceutical formulations containing CDs.\(^5\)

The stoichiometry of drug-guest/cyclodextrin-host complexes is very often reported to be 1:1 although higher order complexes are not uncommon. In an aqueous solution the CD complex (D·CD) is in dynamic equilibrium with free drug (D) and free CD:

\[
D + CD \rightleftharpoons K_{1:1} \cdot D \cdot CD
\]

and in the case of 1:1 complexes the stability constant (\(K_{1:1}\)) can be defined as:

\[
K_{1:1} = \frac{[D \cdot CD]}{[D][CD]}
\]

were [D] is concentration of free drug, [CD] is free CD concentration, [D·CD] is the CD complex concentration and \(K_{1:1}\) is the stability constant, or binding constant, for the formation of the CD complex. The stability constant is important in any consideration of CD complexes and investigations to determine its value are an integral part of any formulation work with CDs. Information about the stability constant and intrinsic solubility may even be sufficient to calculate the utility of CDs for formulation purposes.\(^7\)

The Higuchi–Connors phase-solubility method\(^8\) is very often used to determine \(K_{1:1}\), as a part of a formulation study. This method is based on the effect of a complex forming ligand, e.g. CD, on the solubility of the drug. The intrinsic solubility (\(S_0\)) and the slope of the phase-solubility diagram are then used to calculate \(K_{1:1}\). This method is very general, requiring only some analytical procedure to measure the concentration of the dissolved drug. This method can also be used for slightly soluble drugs with intrinsic solubility in the low-\(\mu\)M or nM range.

Various other methods, such as UV titrations,\(^9\) stability studies,\(^3\),\(^10\) titration calorimetry,\(^11\) potentiometry,\(^12\) and NMR titrations\(^9\) have also been used to determine \(K_{1:1}\). However these methods can be limited to certain type of compounds where complexation significantly alters a given physicochemical parameter on which the method is based. It can also be very difficult to apply these methods when the intrinsic solubility of the guest compound is less than the detection limit. Formulation studies frequently involve slightly soluble drugs. The phase-solubility method is therefore often the most suitable method for such compounds as the only requirement is that the solubility can be determined by some analytical method.

Higuchi and Zuck showed\(^13\) that the stability constant for formation caffeine/benzoic acid complex can be determined from the effect of complexation on liquid–liquid partition coefficient of these compounds. Hydrophilic CDs do not partition into organic solvents, and have therefore been used for selective extractions of organic compounds from an organic phase into aqueous phase for sample clean-up in analytical applications.\(^14\) The effects of CDs on the octanol–water partition of drugs\(^3\),\(^5\),\(^16\) and octanol–water phase-transfer rates\(^17\),\(^18\) have also been reported. There are also a few reports where phase-distribution investigations have been used to determine stability constants for CD complexes.\(^7\),\(^9\)–\(^24\) In general it is simple to obtain the stability constant from phase-distribution investigation given the condition that only one type of guest–host complex is formed. However this is not necessarily the case. Octanol is the preferred organic solvent for phase-distribution investigations (i.e. partition coefficient determination) of drugs. The octanol–water partition coefficient (\(P\)) is reported in drug handbooks and is commonly used in various types of analysis of drug properties. Nakajima et al.\(^24\) have shown that, octanol can form an inclusion complex with \(\beta\)-cyclodextrin and that this complex formation can interfere with the inclusion of other guest compounds. They also proposed a multi-step procedure to correct for this interference in phase-distribution studies. In

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addition to the possible inclusion of the organic solvent in the CD cavity, the gradual saturation of the CD binding with increasing concentration of guest compound must be accounted for in any exact treatment of a phase-distribution system.

In the present study we investigated the general utility of octanol–water phase distribution investigations to determine the stability constants for drug/2-hydroxypropyl-β-cyclodextrin (D/HPβCD) complexes. A general equation was derived to account for the possible inclusion of organic solvent molecules, i.e., octanol, in the CD cavity and the gradual saturation of the CD binding. The method was then applied to investigate complexation of moderately and slightly soluble neutral drugs and the results were compared to results from the phase-solvability method. The possibility of using this method to determine the stability constant for the ionic and non-ionic forms of weakly acidic, weakly basic drugs was also investigated.

Experimental

Materials Diazepam and naproxen were kindly donated by Actavis (Iceland). Hydrocortisone was purchased from ICN Pharmaceuticals (U.S.A.), prednisolone, lidocaine–HCl and diethylstilbestrol from Norsk Medisinaldepot (Norway), and triclosan, n-octanol and hexanol from Sigma Aldrich (U.S.A.). 2-Hydroxypropyl-β-cyclodextrin (HPβCD) of molar substitution (MS) 0.62 (MW ca. 1400) was purchased from Roquette (France). All other chemicals and solvents used in this study were commercial available products of analytical or special reagent grade.

The moisture content of HPβCD was periodically determined and corrected for (Scaltec SMO 01 Moisture Analyzer, Germany). All water used in the experiments was purified with Milli-Q, Academic water purification unit (Millipore, U.S.A.).

Preparation of Octanol Saturated Aqueous Solutions Containing HPβCD Aqueous solutions containing up to 20% (w/v) CD were prepared by weighing HPβCD into a volumetric flask and filling to the mark with water or buffer (Table 1). Excess volume of octanol was then added and the flask was shaken for two days at room temperature to saturate the aqueous solution with octanol. Excess octanol was then removed, leaving few droplets of octanol on the surface to ensure continued saturation. Aliquots for phase-distribution investigations and determination of dissolved octanol, were drawn from these solutions, carefully bypassing the octanol droplets.

Determination of Octanol in Aqueous Solution Containing HPβCD Three milliliters of hexanol or toluene were added to 3 ml aliquots of octanol saturated aqueous solutions. These mixtures were thoroughly shaken for 1—2 h and then centrifuged at 2000 rpm for 90 min, using a Rotina 35 centrifuge from Hettich (Germany) to fully separate the two phases. One hundred microliters samples from the octanol and aqueous phase were then diluted into methanol and analyzed by HPLC to determine the drug concentration. These investigations were performed in triplicate for each HPβCD concentration.

Phase-Solvability Diagrams The phase-solvability of hydrocortisone, prednisolone, diazepam, β-estradiol and diethylstilbestrol was determined in aqueous solutions with CD concentrations up to 15% (w/v). An aqueous CD solution was added to a vial containing an excess of the drug. The vial was then sealed and the drug suspension heated in an autoclave (121 °C for 20 min) or in case of diazepam in an ultrasonic bath (70 °C for 60 min). This was done to promote dissolution of the drug and complexation with CD. The chemical stability of the drugs was also monitored during heating and equilibration period and in all cases less than 1% degradation was observed. 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 d to obtain full equilibration. In the case of hydrocortisone the phase-solvability studies were also done in octanol saturated aqueous solutions.

Finally the suspensions were allowed to settle and the aqueous drug suspensions filtered through a FP 30/0.45 μ CA-S filter from Schleicher and Schuell (Germany) as the solution sieved, and analyzed by HPLC to determine the concentration of dissolved drug.

Chromatographic Conditions The quantitative determination of drugs was performed by using a high-performance-liquid-chromatographic (HPLC) equipment consisting of a Merck-Hitachi AS-4000 injector, Merck-Hitachi L-6200A pump and a Merck-Hitachi L-4250 lamp fixed wavelength UV detector. The column used was a C18, reversed phase column (Phenomenex, U.S.A.), 150 mm, 4.6 mm 1D, 5 μm bead. The flow rate was 1.5 ml/min. The mobile phases, wavelengths and retention times are displayed in Table 2.

Results and Discussion

Theory When a CD solution is saturated with a drug (D) and a 1 : 1 D/CD guest–host complex is formed (see Chart 1A) we have the following equations:

\[
[D]_{aq} = S_0 \tag{2}
\]

\[
[CD]_{total,aq} = [CD]_{aq} + [D \cdot CD]_{aq} \tag{3}
\]

\[
[D]_{total,aq} = [D]_{aq} + [D \cdot CD]_{aq} \tag{4}
\]

where \([D]_{aq}\) is the concentration of free drug in the aqueous solution, which is equal to the intrinsic solubility of the drug \(S_0\). Mass balance (Eq. 3) requires that the total concentration of CD in the \([CD]_{total,aq}\) is equal to the sum of the concentration of the CD complex \([D \cdot CD]_{aq}\) and free CD \([CD]_{aq}\) in the aqueous solution. The total concentration of dissolved drug in the aqueous solution \([D]_{total,aq}\) can also be given by a mass balance expression (Eq. 4) or, by combining Eqs. 1, 3 and 4, as the solubility isotherm:

\[
[D]_{total,aq} = S_0 + \frac{K_1[S_0][CD]_{total,aq}}{1 + K_1[S_0]} \tag{5}
\]

The phase-solvability diagram, a plot of \([D]_{total,aq}\) against \([CD]_{total,aq}\) will therefore be linear with intercept \(S_0\) and slope:
The product $K_{1:1}S_0$ is a unitless term, which sometimes referred to as the complexation efficacy of the CD complex. Rearranging Eq. 6 then gives the equation normally used to calculate $K_{1:1}$:

$$K_{1:1} = \frac{S_0}{S_0} \times S_0 = \frac{\text{Slope}_{\text{Sol}}} {1 - \text{Slope}_{\text{Sol}}} \times S_0$$  \hspace{1cm} (7)

Phase-solubility systems where the CD solution is saturated with two guest compounds have also been studied. A representation of this systems is shown in Chart 1B where the two guest compounds are denoted as Compound A (ComA) and Compound B (ComB). In the case were only $1:1$ complexes are formed the solubility isotherm for ComA can then be derived by combining the mass balance expressions for the two compounds and stability constant definitions:

$$[\text{ComA}]_{\text{sat(aq)}} = S_{0(A)} + \frac{K_{1:1}(A)S_{0(A)}[\text{CD}]_{\text{sat(aq)}}} {1 + K_{1:1}(B)S_{0(B)} + K_{1:1}(A)S_{0(A)}}$$  \hspace{1cm} (8)

where $K_{1:1}(A)$ is the stability constant for complexation of ComA, $S_{0(A)}$ is the intrinsic solubility of ComA and $K_{1:1}(B)S_{0(B)}$ is the complexation efficacy for ComB. The slope of a phase-solubility diagram for ComA will then be:

$$\text{Slope}_{\text{Sol(A)}} = \frac{K_{1:1}(A)S_{0(A)}} {1 + K_{1:1}(B)S_{0(B)} + K_{1:1}(A)S_{0(A)}}$$  \hspace{1cm} (9)

and

$$K_{1:1}(A) = \frac {1} {S_{0(A)}} \times \left( \frac {1 + K_{1:1}(B)S_{0(B)}} {1 - \text{Slope}_{\text{Sol(A)}}} \right)$$  \hspace{1cm} (10)

Chart 1C shows system where the drug is distributed between an octanol phase and an aqueous CD phase. It assumed that both the drug and the organic solvent (octanol) can form an inclusion complex. This system is analogous to the system in Chart 1B (if the drug is considered as ComA and octanol as ComB) and Eqs. 8—10 will apply with some modifications. The main difference is that $[\text{D}]_{\text{aq}}$ is not fixed but given by the equation:

$$[\text{D}]_{\text{aq}} = \frac {[\text{D}]_{\text{sat}}} {P}$$  \hspace{1cm} (11)

where $[\text{D}]_{\text{sat}}$ is the concentration of the drug in the octanol phase. The apparent partition coefficient ($P_{\text{app}}$) can then be defined as:

$$P_{\text{app}} = \frac {[\text{D}]_{\text{sat}}} {[\text{D}]_{\text{aq}}} = \frac {[\text{D}]_{\text{sat}}} {[\text{D}]_{\text{sat(aq)}}} [\text{D} - \text{CD}]$$  \hspace{1cm} (12)

In this case $[\text{D}]_{\text{sat(aq)}}$ will vary depending on the drug concentration in the octanol phase. However, the partition coefficient of the drug will be independent of the concentration and it may therefore be more useful to consider the distribution isotherm rather than the concentration isotherm. The distribution isotherm can be derived by combing Eqs. 8, 11 and 12:

![Chart 1](image-url)

The schemes show the equilibria involving drug (D), compound A (ComA), compound B (ComB), octanol (O), CD (CD), and CD inclusion complexes (D · CD, ComA · CD, ComB · CD and O · CD).
where $K_{1:1(oct)}S_{0(oct)}$ is the complexation efficacy for octanol. The phase-distribution diagram of $1/P_{app}$ vs. $[CD]_{total(aq)}$ will therefore be linear with an intercept $1/P$. The slope of the phase distribution diagram will be:

$$\text{Slope}_{\text{Dis}} = \frac{K_{1:1}}{P} \left( 1 + \frac{[D]_{oct}}{P} + K_{1:1(oct)}S_{0(oct)} \right)$$

Rearranging this equation the stability constant for the D/CD complex can be given as:

$$K_{1:1} = P \times \frac{(1 + K_{1:1(oct)}S_{0(oct)}) \times \text{Slope}_{\text{Dis}}}{1 - [D]_{oct} \times \text{Slope}_{\text{Dis}}}$$

This general equation can then be used to determine the stability constants form the slope of a phase-distribution diagram. The complexation efficacy for the organic solvent, $K_{1:1(oct)}S_{0(oct)}$, can be determined by a phase-solubility investigation and the numerical value of this term can be used to correct for the competitive interaction of the solvent with the CD. The denominator term $[D]_{oct} \times \text{Slope}_{\text{Dis}}$ accounts for the gradual saturation of the cyclodextrin binding as the drug concentration increases. Thus when $[D]_{oct} \times \text{Slope}_{\text{Dis}} \ll 1$ then the drug concentration will not affect the slope and the phase-distribution investigation can be preformed without considering drug concentration variations. However when this is not the case then the data must be corrected for any variation in drug concentration.

**Phase Solubility Investigations for Octanol and Hydrocortisone**

Cyclodextrin solutions were saturated with octanol. The octanol was then extracted with toluene and the octanol concentration was determined by gas chromatography. The slope of the phase-solubility diagram, was 0.75 and complexation efficacy for octanol ($K_{1:1(oct)}S_{0(oct)}$) could then be calculated to be 3.0 (Fig. 1A).

The slope of the phase-solubility diagram for hydrocortisone in aqueous solution and the complexation efficacy ($S_{0}K_{1:1}$) were determined to be 0.61 and 1.5, respectively. The slope of the phase-solubility diagram in aqueous solutions, which had been pre-saturated with octanol was 0.32 (Fig. 1B). The expected slope calculated, according to Eq. 8, from the complexation efficacy for octanol and hydrocortisone was 0.28. These results were consistent with the interpretation that the interaction between octanol and hydrocortisone was competitive, and that 1:1 complexes were mainly formed. However ternary aliphatic-alcohol/guest-molecule/CD complexes have been reported and it is possible that this type of complexes were present in the aqueous phase to some extend.

**Phase Distribution and Phase-Solubility**

Figure 2 shows the phase distribution diagrams for diazepam and diethylstilbestrol. The octanol–water partition coefficient for diazepam could be determined directly with the shake-flask method, whereas diethylstilbestrol is too lipophilic for direct determination of $P$ by this method. Literature log $P$ value was therefore used for calculation of $K_{1:1}$ for diethylstilbestrol.

Hydrocortisone, prednisolone and β-estradiol, which are also non-ionic and moderately lipophilic drugs were also investigated by the phase-distribution method (Table 3). In every case the slope of the phase-distribution diagram was linear ($R>0.99$). In the case of diazepam, estradiol and diethylstilbestrol it was not necessary to account for variations in the drug concentration as the there was only limited extraction of drug from the octanol phase and the numerical value of the term $[D]_{oct} \times \text{Slope}_{\text{Dis}}$ was less than 0.01. However, in the case of hydrocortisone and prednisolone more than
50% of the drug was extracted from the octanol phase into aqueous, 15—20% w/v, cyclodextrin solutions and \([D]_{\text{oct}} \times \text{Slope}_{\text{Dis}}\) was >0.01. Although \([D]_{\text{oct}}\) was not constant the phase-distribution diagrams were linear and an estimate of \(K_{1:1}\) for each \([D]_{\text{oct}}\) could be obtained. Modified Eq. 13 could then be used to calculate the expected \(1/P_{\text{app}}\) value \((1/P_{\text{app}}^{[D]_{\text{oct}}} = \alpha)\) for the system when the drug concentration \([D]_{\text{oct}}\) approaches zero concentration.

\[
\frac{1}{P_{\text{app}}^{[D]_{\text{oct}}} = \alpha} = \frac{1}{p} + \frac{K_{1:1}}{p} \frac{[CD]_{\text{total(aq)}}}{[D]_{\text{ext}}} + \frac{K_{1:1}^{\text{dis}}}{p} \frac{[CD]_{\text{total(aq)}}}{[D]_{\text{ext}}} \tag{16}
\]

An exact \(K_{1:1}\) could then be re-calculated from phase-distribution diagram of the \(1/P_{\text{app}}^{[D]_{\text{oct}}} = \alpha\) data. The differences between the initial estimates and the final exact \(K_{1:1}\) values were less than 5%.

Given the general variability in reported \(K_{1:1}\) values for cyclodextrin inclusion complexes, depending on the method used,\(^{29}\) there was reasonably good correlation between stability constants obtained with the phase solubility \((K_{\text{sol}}^{\text{dis}})\) method and stability constants obtained with the phase-distribution method \((K_{1:1}^{\text{dis}}\), Table 3). The \(K_{1:1}^{\text{dis}}/K_{1:1}^{\text{sol}}\) ratio ranged from 0.5 to 1.5 with an average value of 1.1, for the five compounds. These observation provide further validation of the value of correction factor \((i.e. K_{1:1}^{\text{sol}}, S_{[\text{oct}]}=3.0)\) used to account for the inclusion of the octanol in the cyclodextrin cavity.

Changing the initial concentration of the drug, hydrocortisone, \(\beta\)-estradiol or diethylstilbestrol, had only minor effect on the calculated \(K_{1:1}\) (Table 3).

There is some variation in the log \(P\) values reported in literature depending on experimental or calculation method used and there are also considerable variations in the reported intrinsic solubilities, especially in the case of poorly soluble drugs.\(^{30,31}\) Variations in the reference values used will affect the \(K_{1:1}\) determination. The phase-distribution system is more complex than the phase-solubility system as the interactions between the two guest compounds, i.e. the organic solvent molecule and the drug molecule, have to be considered. However, the disadvantages of the phase-solubility method are that relatively long equilibrium time (one week or more) is required and that considerable excess of the drug must be added to the complexation media. The advantages are the phase-distribution method is that only small amount of the drug is required and the equilibration times can be very short (only a couple of hours or less). In the present study the vials containing the two phases were shaken for 5—20 h, but investigation with hydrocortisone showed that 30 min was more than sufficient time to obtain full drug equilibration between the octanol phase and the aqueous phase.

**Acidic and Basic Compounds** Two weakly acidic drugs, i.e. naproxen and triclosan, and one weakly basic drug, i.e. lidocaine, were investigated by the phase-distribution method. The apparent partition coefficient \((P_{\text{app(X+H+)}})\) for acidic and basic drugs will be the weighted average of the apparent partition coefficients for the neutral and ionic forms. For monoprotic substances the following equation will apply:\(^{32}\)

\[
\log P_{\text{app(X+H+)}} = \log(P_{\text{app(X)}} + P_{\text{app(H+)}}) = \log(1 + 10^{\text{pK}_a - \text{pH}}) - \log(1 + 10^{\text{pK}_a - \text{pH}}) \tag{17}
\]

Where \(P_{\text{app(X)}}\) is the apparent partition coefficient for the deprotonated species (deprotonated acid or neutral base) and \(P_{\text{app(H+)}}\) is the apparent partition coefficient for the protonated species (protonated base or neutral acid). Figure 3 shows log \(P_{\text{app}}\) vs. pH data for these drugs at various cyclodextrin concentrations, fitted according to Eq. 17. From these investigations the values of \(P_{\text{app(X)}}\) and \(P_{\text{app(H+)}}\) for each cyclodextrin concentration, could be determined. The \(K_{1:1}\) values for the neutral and ionic form (Table 4) could then be obtained from the slopes of the phase-distribution diagrams of \(1/P_{\text{app(X)}}\) vs. \([CD]_{\text{total(aq)}}\) and \(1/P_{\text{app(H+)}}\) vs. \([CD]_{\text{total(aq)}}\). Lidocaine is relatively hydrophilic and HP\(\beta\)CD only affected the partition of the neutral form of the drug, whereas it had insignificant effect on the partition of the ionic form. Cyclodextrin

<table>
<thead>
<tr>
<th>Drug/Conc. [mg/ml]</th>
<th>log (P)</th>
<th>Phase-distribution method</th>
<th>Phase-solubility method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(P)</td>
<td>(\text{Slope}<em>{\text{pH}}) ([\text{mg/ml}}] [\text{Slope}</em>{\text{Dis}}]</td>
<td>(K_{1:1}^{\text{dis}}) [\text{Slope}_{\text{Dis}}]</td>
</tr>
<tr>
<td>Hydrocortisone</td>
<td>0.5</td>
<td>1.59 (^{a}) 1.6 (^{b}) 1.62 (^{c})</td>
<td>38.5 \pm 1.0 12.34 0.0076 1.90 \times 10^{-6}</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1.63 (^{a})</td>
<td>42.6 \pm 4.5 11.94 0.0293 2.03 \times 10^{-6}</td>
</tr>
<tr>
<td>Pimelisone</td>
<td>2</td>
<td>1.50 (^{a}) 1.42 (^{b}) 1.6 (^{b}) 1.49 (^{c})</td>
<td>31.4 \pm 1.7 7.63 0.0403 9.58 \times 10^{-6}</td>
</tr>
<tr>
<td>Diazepam</td>
<td>2</td>
<td>2.68 (^{a}) 2.82 (^{b}) 2.7 (^{b}) 2.70 (^{c})</td>
<td>476 \pm 19 0.096 0.0007 1.84 \times 10^{-6}</td>
</tr>
<tr>
<td>Estradiol</td>
<td>0.5</td>
<td>4.0 (^{b}) 3.94 (^{c})</td>
<td>8.71 \times 10^{-3} 0.963 0.0017 3.86 \times 10^{-6}</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>5.07 (^{b}) 5.64 (^{c})</td>
<td>1.17 \times 10^{3} 0.126 0.0009 5.92 \times 10^{-6}</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.004 0.0043 5.78 \times 10^{-6}</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>0.117 0.0082 5.55 \times 10^{-6}</td>
<td>1.5</td>
</tr>
</tbody>
</table>

\(a\) log \(P\) as measured by the shake flask method. However, the values for \(\beta\)-estradiol and diethylstilbestrol could not be determined because the concentration in the aqueous phase was below the detection limit of the analytical method. In these cases literature values (bold) were used to calculate \(K_{1:1}\). \(^{b}\) From ref. 37. \(^{c}\) Clog \(P\) values calculated using the online LOGKOW/KOWWIN Program available at: http://esc.syrrex.com/interkow/kowdemo.htm. \(^{d}\) From ref. 38. \(^{e}\) The reported slopes were obtained for \(P_{\text{app, oct}}\) \((P_{\text{app, oct}}\) extrapolated to zero drug concentration.)
affected the phase-distribution of both the neutral and ionic form of naproxen and triclosan. The stability constants for the HPβCD complexes of the neutral and ionic form of naproxen have been determined by various methods. The reported \( K_{1:1} \) values for the neutral and ionic form are \( 1.67 \times 10^7 \) and \( 3.31 \times 10^3 \), \( 5.16 \times 10^3 \) and \( 6.65 \times 10^2 \), \( 6.35 \times 10^2 \) and \( 1.40 \times 10^2 \), or \( 6.52 \times 10^1 \) and \( 1.03 \times 10^2 \) M\(^{-1}\) as determined by phase-solubility, UV spectrophotometry, fluorometry or titration calorimetry, respectively. The values determined by the phase-distribution method fall well within the range of these values.

The buffer concentrations were kept at 0.2 M but the ionic strength was not strictly controlled. In general the ionic strength should have limited effect on the phase distribution of the neutral form. The ionic drug forms will partition into the organic solvent as ion pairs. This partitioning is, however, affected not just by the ionic strength in the aqueous solution but also by the concentration of the counter ion.

The investigations of D/HPβCD complexes confirmed that results were obtained with the proposed phase-distribution method were comparable to results obtained with the phase-distribution method where the stability constant is determined from the slope of the phase-solubility diagram and \( S_0 \). The proposed equation also accounts for the effect of the salt concentration on the CD complexation of the respective ion pair with the potassium cation.

**Table 4. Results from the Phase-Distribution Investigations of Weakly Acidic and Weakly Basic Drugs**

<table>
<thead>
<tr>
<th>Drug/Form</th>
<th>( \log P )</th>
<th>( P )</th>
<th>( \text{Slope}_{1:1} \text{[m}^{-1}] )</th>
<th>( R^2 )</th>
<th>( [D]_{\text{app}} \times \text{Slope} )</th>
<th>( K_{1:1}^{\text{obs}} \text{[m}^{-1}] )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naproxen</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \text{AH} )</td>
<td>3.25, a 3.2, 3.1 b</td>
<td>1.79±0.39 \times 10^3</td>
<td>0.5108</td>
<td>0.992</td>
<td>0.0044</td>
<td>3.66 \times 10^3</td>
</tr>
<tr>
<td>( \text{A}^- )</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No KCl</td>
<td>(-0.76 )</td>
<td>8.7±0.8 \times 10^{-1}</td>
<td>454 b</td>
<td>0.923</td>
<td>0.665</td>
<td>3.83 \times 10^2</td>
</tr>
<tr>
<td>0.1 M KCl</td>
<td>(-0.60 )</td>
<td>2.5±0.9 \times 10^{-1}</td>
<td>408 b</td>
<td>0.992</td>
<td>0.500</td>
<td>4.11 \times 10^2</td>
</tr>
<tr>
<td>0.2 M KCl</td>
<td>(-0.33 )</td>
<td>4.7±1.9 \times 10^{-1}</td>
<td>277 b</td>
<td>0.988</td>
<td>0.726</td>
<td>5.16 \times 10^2</td>
</tr>
<tr>
<td>Triclosan</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \text{AH} )</td>
<td>4.8, 4.6 b</td>
<td>6.31±10^4</td>
<td>0.0056</td>
<td>0.992</td>
<td>0.0000</td>
<td>1.40 \times 10^1</td>
</tr>
<tr>
<td>( \text{A}^- )</td>
<td>2.09 b</td>
<td>1.31±1.2 \times 10^2</td>
<td>0.6307</td>
<td>0.902</td>
<td>0.0043</td>
<td>3.12 \times 10^2</td>
</tr>
<tr>
<td>Lidocaine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \text{AH}^- )</td>
<td>(-1.41 )</td>
<td>3.86±0.90 \times 10^{-2}</td>
<td>No binding</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \text{A} )</td>
<td>2.29, a 2.4 b</td>
<td>1.95±0.22 \times 10^2</td>
<td>0.028</td>
<td>0.856</td>
<td>0.0002</td>
<td>2.16 \times 10^1</td>
</tr>
</tbody>
</table>

a) \( \log P \) as measured by the shake flask method. The value for triclosan could not be determined because the concentration in the aqueous phase was below the detection limit of the analytical method. In this case a literature value (bold) was used to fit the pH–log D data and to calculate \( K_{1:1} \). b) From ref. 37. c) \( \log P \) values calculated using the online LOGKOW/KOWWIN Program available at: http://esc.syres.com/interkow/kowdemo.htm. d) The reported slopes were obtained for \( P_{\text{app}}^{\text{app}} \) extrapolated to zero drug concentration.

**Conclusion**

Here we have proposed a phase-distribution method for determination of the stability constants of CD complexes from the slope of the phase-distribution diagram and the \( P \) value. In the equation we have introduced a correction factor to correct for the inclusion of octanol in the cyclosorption cavity. The proposed method is analogous to the phase-solvability method where the stability constant is determined from the slope of the phase-solubility diagram and \( S_0 \). The proposed equation also accounts for the effect of the drug concentration on \( P_{\text{app}} \).

The increase in the stability constant when KCl was added to aqueous solution but also by the concentration of the counter ion. The increase in \( P_{\text{app,X}} \) was five-fold when the concentration of the added of KCl was 0.5 M. The slope of the phase-distribution diagram was also affected. However the effect of the salt concentration on the CD complexation was much smaller with only about 70% increase at the highest salt concentration. Added salt had relatively small effect on the partitioning of the neutral form of naproxen resulting in less than 50% increase when the KCl concentration was increased from 0.0 to 0.5 M concentration and the changes in KCl concentration had no effect on CD complexation. The observed effect of the salt concentration on complexation of the neutral form naproxen was comparable to what has previously been reported.

Figure 3. pH-log \( P_{\text{app}} \) Profiles for Naproxen (3A), Triclosan (3B) and Lidocaine (3C)

Data shown for 0 ( ), 1% ( ), 5% ( ) and 15% ( ) HPβCD solutions.
solubility method. The phase-distribution method should also be applicable for other types of organic solvents and CDs, but this would require that complexation efficacy of the organic-solvent/CD complex must first be determined. Although the phase-distribution system in the is more complex than simple phase-solubility systems, the advantage of the phase-distribution method is that it can be completed in a relatively short time and the quantity of the guest compound required is relatively small. This method can therefore be considered for screening purposes. Another advantage of this method is that stability constant calculation is based on the log $P$ that, in general, is a more reliable value than $S_p$. It is also relatively straightforward to apply this procedure to obtain the stability constants for the neutral and ionic form of weakly acidic and weakly basic drugs. In the case of hydrophilic and moderately lipophilic drugs it can be used for simultaneous determination of the stability constant of the CD complex and the octanol–water partition coefficient.

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References