Thermodynamic investigations on the inclusion complexation of Piroxicam with Cyclodextrin derivatives

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Abstract

Thermodynamic studies of piroxicam in aqueous solution complexed with β-cyclodextrin (β-CD), γ-cyclodextrin (γ-CD) and two β-cyclodextrin derivatives, hydroxypropyl-β-cyclodextrin (HP-β-CD) and methyl-β-cyclodextrin (Me-β-CD) were performed at different temperatures and pH values using the phase solubility method. The phase solubility diagrams of β-CD, γ-CD and HP-β-CD is of A₁-type behavior, indicating the formation of 1:1 complexes. The related stability constants range from β-CD > γ-CD > Me-β-CD > HP-β-CD, respectively. An A₂-type solubility diagram is observed for Me-β-CD, indicating the formation of 1:2 complexes at higher CD concentrations. From the temperature dependence of the equilibrium constants the reaction enthalpies and entropies have been determined. The contributions of the reaction entropies are small and no enthalpy-entropy-compensation is observed, except for γ-CD, where a very small negative reaction entropy could be estimated. Moreover, the influence of the pH value is rather high,

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because the differently charged forms of piroxicam show different solubility behavior in water.

**Keywords**

Cyclodextrin, piroxicam, inclusion complexation, solubility enhancement, reaction enthalpy, reaction entropy

**Introduction**

Cyclodextrins (CDs) are cyclic oligosaccharides derived from enzymatic degradation of starch. Natural CDs are composed of six, seven or eight D-glucose units joined by $\alpha$-1, 4-glycosidic linkages. These natural CDs are known as $\alpha$-CD, $\beta$-CD and $\gamma$-CD. The most interesting molecular structure of CDs is the donut-like shape with a hydrophobic cavity and a hydrophilic exterior. This molecular characteristic enables CDs to form inclusion complexes with a number of more or less hydrophobic guest molecules. The physicochemical and biological properties of the guests are significantly modified through the inclusion complex formation. Therefore, many applications exist for CD complexation in the fields of pharmaceutics, analytical techniques, cosmetics, agricultural products, foods and flavors, textiles and environment protection (1-4).

In pharmaceutical applications, $\beta$-CD is more commonly used than the other natural CDs ($\alpha$-CD and $\gamma$-CD) due to its appropriate cavity size for many drugs. However, the application for solubility enhancement is restricted by its low aqueous solubility (1.85g/100ml). By the mid 1970s chemically modified CDs were synthesized and introduced in order to improve the aqueous solubility, to decrease the incidence of toxicity (5) as well as to improve their complexing ability (6). According to their substituents at the rings, the modified CDs show different physico-chemical properties as solubility in water or different ability for complex formation.
One of the most important applications of these CDs is to increase the solubility and the dissolution rate of poor water-soluble drugs. Hydrophobic CD derivatives such as 2,6-diethyl-β-CD slow down the dissolution rate of water-soluble drugs. Therefore they can be used as carriers for controlled release of drugs. Ionizable CD derivatives such as O-carboxymethyl-β-CD, O-carboxymethyl-o-ethyl-β-CD, β-CD-sulphate and sulfobutylether-β-CD have been reported to improve the inclusion ability, modifying the dissolution rate as well as alleviating the local irritation of drugs.

The association constant $K$ characterizes the drug-CD inclusion complexation as it describes the stability of the complexes. On one hand the complex should be stable enough, on the other hand it should be able to dissociate to give a desirable amount of free drug when needed. Moreover, the relevant thermodynamic parameters such as the free energy change $\Delta G$, the enthalpy change $\Delta H$ and the entropy change $\Delta S$ can be calculated from the $K$ value and its temperature dependence. These parameters provide useful information about the nature of the inclusion complex, the mechanism and the driving forces for the complexation reaction.

Generally, the determination of the complexation constant $K$ can be accomplished by titration of the change in physicochemical properties of the guests as a function of the CD concentration. A number of methods have been reported, e.g. calorimetric titration (7-8), spectroscopic methods such as electron absorption spectroscopy (9-10), circular dichroism (11-12), fluorescence spectroscopy (13-14), nuclear magnetic resonance (15-16), potentiometric titration (17), gas- and liquid-chromatography (16), stability (18-19) and solubility determination (20-21). The calorimetric titration possesses the advantage, that the complexation constant $K$ and the enthalpy changes can be directly and simultaneously determined at any constant temperature. The $K$ values are also influenced by several factors, such as the temperature, the pH value of the medium and the presence of other components like cosolvents (22).
CDs have been already used to increase the solubility of piroxicam. Piroxicam (4-hydroxy-2-methyl-N-2-pyridinyl-2H-1,2-benzothiazine-3-carboxamide-1,1-oxide) is an enolic compound belonging to the oxicam group, a well-known non-steroidal anti-inflammatory drug used for treatment of rheumatoid arthritis, osteoarthritis and diverse other musculoskeletal disorders. As a number of commercial piroxicam preparations have been available for almost many years, new formulations containing piroxicam in complexation with β-CD were recently introduced in order to improve the bioavailability and the gastrointestinal tolerability (23-25). Nevertheless, reports on the thermodynamic study of this complex are rather limited. Thermodynamic studies of piroxicam and HP-β-CD or Me-β-CD have not been reported until now.

Piroxicam is an amphoteric drug containing two ionizable moieties, the enolic and 2-pyridyl group, which exerts the pKa value of 1.8 and 5.1(5.46) respectively (26). The pH of the medium has pronounced effect on its ionization, which consequently affects not only the solubility but also the binding tendency with CDs. In aqueous solution of pH 0.9-7.0 the drug exists as a zwitterion of very low solubility. In more alkaline solution (pH>7.0) the anionic species is predominant and the solubility increases significantly. Moreover, it is reported that at any appropriate pH condition, piroxicam is capable to self-associate as dimers or tetramers (16). These phenomena obviously influence the complex formation between the drug and diverse CDs.

The objective of this study is to determine the stability constant K and the thermodynamic parameters of piroxicam-CDs inclusion complexes using the phase solubility method. The focus is set on several CD derivatives and their thermodynamic parameters under consideration of the influence of pH on the stability constants to show the different phase solubility type behavior in dependence on the complexing agents.
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Experimental

Materials

Piroxicam (4-hydroxy-2-methyl-N-2-pyridinyl-2H-1,2-benzothiazine-3-carboxamide-1,1-oxide) was purchased from Sigma-Aldrich Chemie, Germany. β-cyclodextrin (Cavamax®W7 Pharma), γ-cyclodextrin (Cavamax®W8 Pharma), hydroxypropyl-β-cyclodextrin (Cavasol®W7Pharma D.S. 0.65, M.W. 1389), methylated-β-cyclodextrin (Cavasol®W7M Pharma, D.S. 1.8, M.W. 1311) were purchased from Wacker-Chemie, Germany. Hydrochloric acid, disodium hydrogen phosphate and sodium dihydrogen phosphate were of analytical grade. The used water was bidistilled.

Phase solubility studies

The phase solubility studies have been performed according to the method described by Higuchi and Connor (27). An excessive amount of piroxicam (4-10mg/10ml in dependence on the temperature) was placed into a screw-capped glass vial, to which 10 ml of aqueous solutions of CDs at varying concentrations (in the case of β-CD and γ-CD 0-15 mM and for HP-β-CD and Me-β-CD 0-100 mM) were added. The suspensions were sonicated for 10 minutes to ensure the intimate mixing. The vials were then placed on the magnetic plate kept in a thermostatic chamber of specified temperature (25°C, 30°C, 37°C and 45°C). The temperature was maintained constantly in range of ±0.1°C. The samples were magnetically stirred for 3 days to attain the equilibrium. At the end of equilibrium time, the content in each vial was filtered through 0.45μm membrane filter (Cellulose acetate filter, Sartorius, Germany). The filtrate was appropriately diluted and analyzed spectrophotometrically at 359 nm. The corresponding CD solution of the same dilution was used as reference. The pH of the suspensions and the undiluted filtrates was measured. All experiments were performed in triplicate.

To investigate the influence of pH on the complex formation, mixtures of 0.1N hydrochloric acid and phosphate buffer solution have been used instead of distilled water to obtain various pH values.
The apparent stability constants $K$ are determined using the solubility method in dependence of the phase solubility type. From the complexation constant $K$, the thermodynamic parameters have been calculated.

Accordingly, the overall equilibrium constants $K$ of the complexes can be calculated from the slope of the straight line and the intrinsic solubility of piroxicam, as described in the following relationship, where $S_o$ is the solubility of the guest molecule in pure water.

$$K_{1:1} = \frac{\text{slope}}{S_o(1-\text{slope})}$$  \hspace{1cm} (1)

The reaction enthalpies and entropies of piroxicam in aqueous solutions of CDs were obtained for different temperatures (25°C, 30°C, 37°C and 45°C) using distilled water as medium without any buffer solutions, as various buffers influence the equilibrium constants of the inclusion process. The pH value of the medium was therefore 5.5.

From the temperature dependence of the association constants, the thermodynamic parameters of the complex formation process can be calculated by the following relationships: The enthalpy change $\Delta H$ is obtained from the slope of the Van't Hoff plots,

$$\Delta \ln K = \frac{-\Delta H}{R} \cdot \frac{1}{T}$$  \hspace{1cm} (2)

where $R$ is the gas constant with 8.314 J/mol and $T$ the absolute temperature. The free energy change $\Delta G$ is calculated from

$$\Delta G = -RT \ln K$$  \hspace{1cm} (3)

The entropy change $\Delta S$ can be obtained from the following equation

$$\Delta G = \Delta H - T \Delta S$$  \hspace{1cm} (4)

**Results and Discussion**

*Solubility enhancement of piroxicam and determination of the association constant*

The inclusion of either the whole molecule or only some hydrophobic moiety of the guest’s molecule into the CDs cavity leads to an increase of the drug solubility.
Phase solubility studies therefore provide not only characterizing information about the complexation process but also support the potential of the CDs application as an useful solubilizing agent.

The phase solubility diagram of piroxicam in aqueous HP-β-CD solutions (solubility of the drug against increasing concentration of CD) at 25°C, 30°C, 37°C and 45°C are illustrated in Figure 1. This concentration dependence is characterized by a linear increase of the drug solubility with increasing CDs concentrations. Similar solubility diagrams are obtained for β-CD and γ-CD. This linear increase of the solubility with increasing concentration of the complexing agents is described as A_1 type by Higuchi and Connors (27) and 1:1 complex formation can be suggested.

![Phase solubility diagrams of piroxicam-HP-β-CD inclusion complexes at 25°C (full circle), 30°C (circle), 37°C (full triangle) and 45°C (triangle) in aqueous solution at pH 5.5.](image)

**Fig. 1.** Phase solubility diagrams of piroxicam-HP-β-CD inclusion complexes at 25°C (full circle), 30°C (circle), 37°C (full triangle) and 45°C (triangle) in aqueous solution at pH 5.5.
In contrary, the phase solubility diagram of piroxicam complexes in aqueous solution with Me-β-CD belongs to the A_p-type. A more or less linear increase of the solubility can be observed only at low concentrations (up to 20 mol/l Me-β-CD), whereas at higher concentrations a positive deviation from linearity can be observed. The so called A_p-type solubility diagram implies the formation of higher order complexes, beyond 1:1 also 1:2 piroxicam:Me-β-CD complexes exist at higher concentrations of the complexing agent. This concentration dependence is shown in Figure 2 at two different temperatures in comparison to the linear concentration dependence of HP-β-CD.

Fig. 2. Phase solubility diagrams of piroxicam-HP-β-CD inclusion complexes at 25°C (full circle) and 37°C (full triangle) and piroxicam-Me-β-CD inclusion complexes at 25°C (circle) and 37°C (triangle) in aqueous solution at pH 5.5.
From the linear plots of $\beta$-CD, $\gamma$-CD and HP-$\beta$-CD as well as from the initial slope of Me-$\beta$-CD the association constants have been determined and depicted in Table 1.

<table>
<thead>
<tr>
<th>Cyclodextrin</th>
<th>K</th>
<th>$\Delta G$</th>
<th>$\Delta H$</th>
<th>$T\Delta S$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta$-CD</td>
<td>150</td>
<td>-12.4</td>
<td>-12.1</td>
<td>0.3</td>
</tr>
<tr>
<td>$\gamma$-CD</td>
<td>135</td>
<td>-12.1</td>
<td>-13.3</td>
<td>-1.2</td>
</tr>
<tr>
<td>HP-$\beta$-CD</td>
<td>69</td>
<td>-10.5</td>
<td>-8.8</td>
<td>1.7</td>
</tr>
<tr>
<td>Me-$\beta$-CD</td>
<td>104</td>
<td>-11.5</td>
<td>-6.3</td>
<td>5.2</td>
</tr>
</tbody>
</table>

**Tab. 1.** Stability constants of piroxicam-CD inclusion complexes together with $\Delta G$, $\Delta H$ and $T\Delta S$ at 25°C in aqueous solution at pH = 5.5 (equilibrium constants in l/mol, thermodynamic data in kJ/mol)

The highest K value is obtained for the piroxicam-$\beta$-CD inclusion complex indicating the highest complexation ability of $\beta$-CD with piroxicam compared to the other investigated CDs. The somewhat lower K values observed for the piroxicam-$\gamma$-CD complexes, the piroxicam-Me-$\beta$-CD and the piroxicam-HP-$\beta$-CD complexes could be attributed to the slightly improper cavity size for a favorable fit of the drug molecule. Nevertheless, the association constants for all CDs are not very large and in the same order of magnitude. This supports that also the potential of all CDs for solubility enhancement of piroxicam is comparable. Substituents at the CD rim are generally able to exert more or less steric hindrance effects to the drug molecule. In case of the piroxicam-HP-$\beta$-CD inclusion complex, a lower association constant can be observed compared with the piroxicam-$\beta$-CD inclusion complex, which seems to be due to the steric hindrance caused by the bulky hydroxypropyl groups. This is in agreement with a previous report (15) which reasoned the same findings based on the proposed complex conformations obtained by NMR studies.
Two types of CD derivatives, HP-β-CD and Me-β-CD used in this study, exhibit different phase solubility patterns. In fact, the occurrence of the \( A_p \)-type phase solubility diagram essentially depends on the property of the drug as well as on the CD forming the inclusion complex. Although, HP-β-CD exhibits the \( A_p \)-type phase solubility profile with many drugs (28-31) it turns out that a solubility diagram from \( A_L \)-type can be observed for piroxicam up to a concentration of 0.1mol/l. In the case of Me-β-CD, an \( A_p \)-type solubility profile is observed. As a consequence, it can be seen that the solubility of the drug increases more progressively. In order to get the same amount of dissolved drug, less quantity of CD is needed, which means that at any definite amount of CD, better solubility enhancement is obtained from the \( A_p \)-type solubility profile.

**Thermodynamic studies of piroxicam-CDs complexes**

From the temperature dependence of the association constants the reaction enthalpies can be determined using Van’t Hoff plots. From the difference between \( \Delta G \) and \( \Delta H \) the contributions of the reaction entropies can be roughly estimated. The results are given in Table 1. The highest reaction enthalpy is observed for \( \gamma \)-CD followed by that for \( \beta \)-CD and significantly smaller values can be recognized for Me-\( \beta \)-CD and HP-β-CD, in accordance with the hypothesis, that the steric influence of the substitution leads to slight steric repulsion in the cavity. The contributions of the reaction entropies are throughout surprisingly rather small. They are positive, which means that complexation between piroxicam and most CDs does not show the frequently observed enthalpy-entropy compensation. Only in the case of piroxicam-\( \gamma \)-CD complexation small negative reaction entropy is detected, probably as a consequence of the higher flexibility of the larger \( \gamma \)-CD ring.

A limited number of papers exist concerning thermodynamics of piroxicam-CDs inclusion complexes. Only the thermodynamic parameters of piroxicam in complexation with \( \beta \)-CD and \( \gamma \)-CD in distilled water of slightly different pH values using
calorimetric method are reported (32). These results are qualitatively in agreement with the results obtained from the phase solubility data presented here. **The influence of pH on the stability constant of the complexes**

Piroxicam exists as a twobasic acid in different forms at various pH values. As each form has completely different association constants with CDs, caused by the charge of the molecule, the stability of the complexes and the related solubility enhancements are highly sensitive on the pH value of the solution. The effect of the pH value on piroxicam-CD complex formation is demonstrated at three different pH values. The pH values have been selected in order to obtain different ionic forms of the drug. The pKa values of piroxicam have been reported as 1.86 and 5.26 for the enolic and the protonated pyridyl group (26). The pH values chosen in this study were 2.5, 5.5 and 7.4, which introduce the totally uncharged-, amphoteric and completely negatively charged form of the drug. Different pH values resemble the pH of non-fasted gastrointestinal tract and the physiological fluid. Experiments have been conducted at 37°C for the purpose of an *in vivo* simulation. The phase solubility diagrams of the host-guest inclusion complexes are illustrated in Figure 3. Independent from the pH value, the $A_L$-type solubility diagrams are shown for all CDs, except Me-$\beta$-CD.

First of all the solubility of the drug is largest at pH values higher than 7.4, where the anionic form of piroxicam exists ($S_o = 7.85 \times 10^{-4}$ mol/l). The neutral form, which in fact exist as several tautomers, shows the lowest solubility ($S_o = 4.2 \times 10^{-5}$ mol/l). As a consequence of the higher solubilities the solubility enhancement by CDs is also rather larger for the anionic form. For piroxicam-Me-$\beta$-CD complexes at pH 2.5 and 5.5 the phase solubility diagrams are from $A_P$-type whereas at pH 7.4, the $A_L$-type solubility profile is obtained.

**Conclusion**

In order to get insight into the process of complex formation between piroxicam and diverse CDs, thermodynamic parameters have been determined from the stability
constants of the complexes obtained by the phase solubility method. The phasesolubility diagrams of β-CD, γ-CD and HP-β-CD show typical \( A_L \)-type behavior, whereas for Me-β-CD, the \( A_p \)-type solubility profile signifies that 1:1 and 1:2 complexes exist simultaneously. The equilibrium constants for the four CDs under consideration are in same order of magnitude and are sufficiently large to enable significant solubility enhancement of piroxicam. From the temperature dependence of the equilibrium constants it can be concluded that the contribution of the reaction entropies is small and shows no enthalpy-entropy-compensation, except for γ-CD, where an insignificant small negative reaction entropy could be estimated.
The influence of the pH value on the complexation reaction is rather high, because the differently charged forms of piroxicam have various solubilities in water and show therefore different solubility enhancements.

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