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Alfaxalone: Effect of Temperature on Complexation with 2-Hydroxypropyl-β-cyclodextrin

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Abstract

Phase solubility analysis is used to investigate the complex formation of alfaxalone with various cyclodextrins (2-hydroxypropyl-β-cyclodextrine [HPBCD], β-cyclodextrin [BCD] and 2-hydroxypropyl-γ-cyclodextrin [HPGCD]). The complexation with HPBCD was studied in more detail by looking at the effect of temperature on the stability constants using phase solubility analysis. HPLC-analysis was used to measure the dissolved amount of alfaxalone. The solubility of alfaxalone increases linearly with increasing concentration of cyclodextrin, suggesting the formation of a 1 : 1 complex. For the parent BCD the complex starts precipitating out of solution when the solubilizer concentration exceeds 0.25% making the unsubstituted BCD less useful for the preparation of solutions of alfaxalone. Substituted cyclodextrins do not form insoluble complexes with alfaxalone. The complexation constant for BCD and HPBCD are comparable in magnitude, but for HPGCD, the constant is substantially lower.

The effect of temperature on the complexation constant was also studied at elevated temperature. Increasing the temperature results in an increased S_0 (solubility without HPBCD) and a decrease in the value of the complexation constant. The net effect results in minor changes of the solubility of alfaxalone as a function of temperature. Based on regression analysis, the change in enthalpy for complex formation between alfaxalone and HPBCD is calculated as -4610 cal/mol.

The results indicate that substituted cyclodextrins are useful in the preparation of solutions of alfaxalone. Since 1 : 1 complexes are formed there is no theoretical danger for precipitation on dilution, e.g., after injection.

Introduction

Cyclodextrins are useful excipients that can modify the physico-chemical properties of active pharmaceutical ingredients by complexation. The parent cyclodextrins as well as the modified cyclodextrins find wide application in oral pharmaceutical dosage forms. For parenteral application the use is restricted to two modified cyclodextrins (2-hydroxypropyl-β-cyclodextrin and sulfobutylated-β-cyclodextrin).

Alfaxalone (3α-hydroxy-5α-pregnane-11,20-dione) is a steroid anesthetic which is characterized by a high therapeutic index and, a rapid induction of anesthesia [1]. The compound is insoluble in water and therefore is formulated using a non-ionic surfactant (cremophor). The use of the surfactant has been associated with allergic reactions resulting in the withdrawal of the formulation for human use from the market [2]. An aqueous formulation for alfaxalone which does not contain surfactants may therefore be useful.

This work presents results on the complexation of alfaxalone with the parent β-cyclodextrin [BCD], with 2-hydroxypropyl-β-cyclodextrine [HPBCD] and with 2-hydroxypropyl-γ-cyclodextrin [HPGCD]. Special attention was given to the effect of the temperature on the complexation of alfaxalone with HPBCD. The complexation of alfaxalone with HPBCD was already studied without looking at the effect of temperature [3, 4]

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Experimental

Materials

Hydroxypropyl-β-cyclodextrin (HPBCD) and hydroxypropy;-γ-cyclodextrin (HPGCD) were obtained from Chinoin. β-cyclodextrin was obtained from Janssen Chimica. Alfaxalone (micronised) was a gift from Pitman-Moore (Harefield, UK). Other materials were commercially obtained.

Methods

Solubility was studied according to the method of Higuchi and Connors [5]. An excess of alfaxalone was added to 25-ml glass vials containing the solvents. The closed vials were sonicated for 30 min. For studies at ambient temperature the vials are rotated on a mechanical spindle top to bottom for at least 24 hours. For the studies, performed at other

temperatures, the vials were kept at the desired temperature for at least one week in order to obtain equilibrium conditions. All materials (glassware, flasks, filters, ...) used at low and high temperature were pre-equilibrated at the temperature of investigation and, the samples were processed immediately. After equilibration the excess solute was removed by filtration over a 0.45 μm membrane (Millipore). The clear solution was analysed by HPLC for alfaxalone content. The HPLC systems configuration included a Varian Vista 5500 chromatograph equipped with UV200 detector, a Varian autosampler 9090 and a Varian 4270 integrator. Samples were eluted on a RP18 Hypersil ODS column (10 cm $\times$ 4.0 mm i.d., 3-μm particle size) using a flow rate of 1.5 mL/min and a mobile phase composition of water and acetonitrile (50 : 50). Alfaxalone was measured at λ = 205 nm.

Calculations

Stability constants are calculated using the following formula:

$$K_{1:1} = \frac{\text{slope}}{S_0(1 - \text{slope}}),$$

where the slope is obtained from the least squares linear regression of the molar concentrations of alfaxalone in solution versus the molar concentration of cyclodextrin in the solvent and S_0 is the intrinsic solubility of alfaxalone in the absence of cyclodextrin.

The change in free energy (ΔG), the change in enthalpy (ΔH) and, the change in entropy (ΔS) is calculated using the standard formulae:

$$\Delta G = -2.303RT \log K$$

$$\log K = -\frac{\Delta H}{2.303R}\frac{1}{T} + Cst$$

$$\Delta G = \Delta H - T\Delta S$$

where T is the temperature in degree Kelvin, K is the stability constant and R is the gas constant [6].

Results

The results of the solubility study in aqueous BCD solutions, in HPBCD solutions and in HPGCD solutions are presented in Figures 1, 2, 3, and 4. The solubility isotherm of alfaxalone with BCD is of the BS-type, indicating limited solubility of the complex in water (Figure 1). The complex precipitates out of solution when the concentration of BCD exceeds 0.25%. The solubility of the alfaxalone-BCD complex is limited to about 0.5 mg/ml. Analysis of the residues indicates the presence of BCD in the precipitates.

The complexation constant, calculated on the linear portion of the solubility isotherm, is given in Table 1.

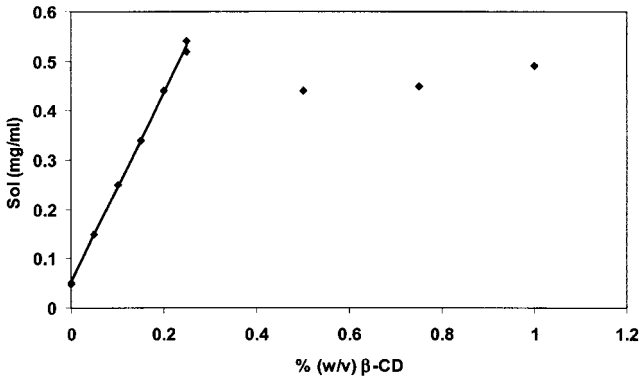

Figure 1. Solubility of alfaxalone in aqueous BCD solutions at ambient temperature. The full line is the regression line for concentrations of BCD up to 0.25% (w/v).

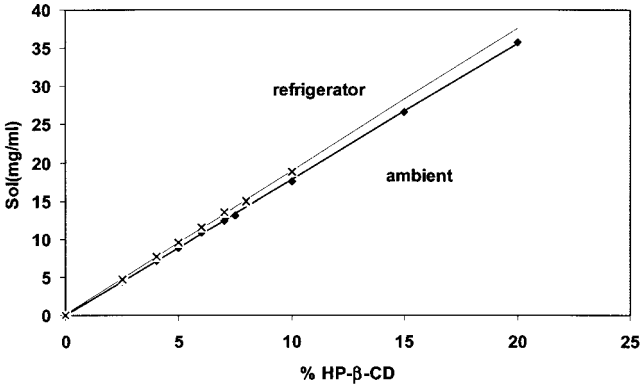

Figure 2. Solubility of alfaxalone in HPBCD at ambient temperature and in the refrigerator at 4 °C. The dots represent the experimental point and the lines are the regression lines calculated using least squares linear regression analysis.

The solubility isotherms for HPBCD and HPGCD are all of the A_L-type (Figure 2, 3 and 4), indicating linear increase in solubility for alfaxalone with increasing concentration of the modified cyclodextrin. A first glance at the effect of temperature is given in Figure 2. The solubility of alfaxalone is slightly higher at low temperature than at ambient temperature. The same trend is observed in Figure 4, where the solubility of alfaxalone decreases as the temperature increases. As the solubility in water without cyclodextrin

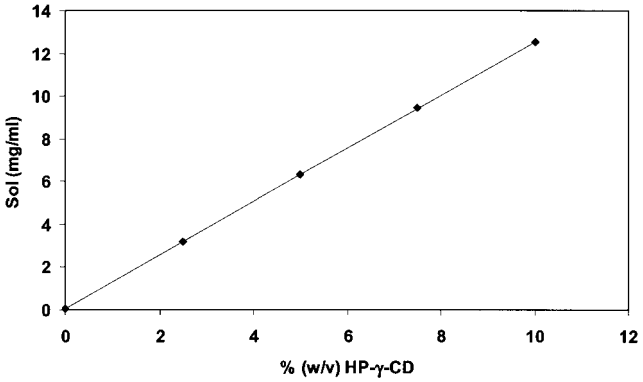

Figure 3. Solubility of alfaxalone in HPGCD at ambient temperature. The dots represent the experimental points and the line is the regression line calculated using least squares linear regression analysis.

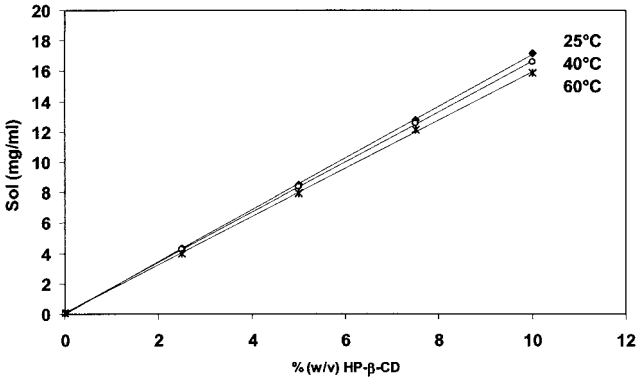

Figure 4. Solubility of alfaxalone in HPBCD at different temperature (25 °C, 40 °C and 60 °C). The dots represent the experimental point and the lines are the regression lines calculated using least squares linear regression analysis.

Table 1. Stability constants for the complexation of alfaxalone with the studied cyclodextrins. S_0 is the solubility in water without cyclodextrin

CD type	Condition	S_0 (mg/ml)	$K_{1:1}$ (l/mol)
BCD	Ambient	0.05	12150
HPBCD	Ambient	0.047	21370
HPBCD	4	0.040	31340
HPBCD	25	0.046	18240
HPBCD	40	0.065	14400
HPBCD	60	0.10	8510
HPGCD	Ambient	0.047	6760

increases with increasing temperature, the stability constant for the complexation decreases with increasing temperature. The decrease in the value of the stability constants with rising temperature points to the exothermic nature of the complexation reaction. All the stability constants calculated from the solubility isotherms are collected in Table 1.

Using the stability constants at the different temperatures the change in enthalpy can be calculated with the van 't Hoff equation (Figure 5). The calculations result in a free energy change (ΔG) of -5810 cal/mol, a change in enthalpy

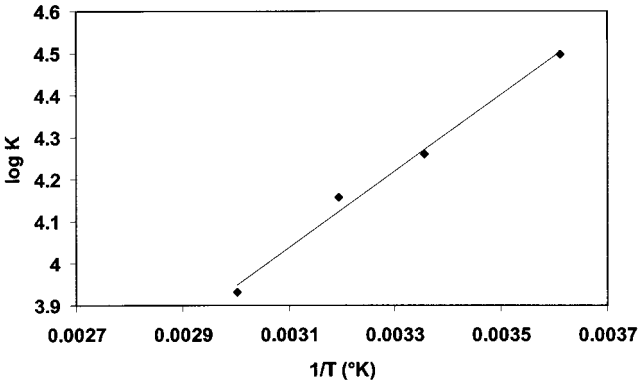

Figure 5. Plot of the logarithm of the stability constants against the inverse of the temperature to calculate the change in entropy according to the van't Hoff equation. The dots are the actual log K's and the line is the regression line based on the linear least squares analysis.

(ΔH) of -4160 cal/mol and a slightly positive change of 5.5 cal/mol° in entropy (ΔS). In a study of the effect of HPBCD on the solubility of danazol, a synthetic steroid, Badaway [7] found similar values for these terms (ΔG: -6930 cal/mol; ΔH: -2370 cal/mol and, ΔS: 14 cal/mol°). In the case of danazol, however, the solubility increases as the temperature increases. As Szejtli pointed out the inclusion complex formation proceeds by an energetically favoured interaction of a relatively non-polar guest molecule with an imperfectly solvated hydrophobic cavity and this results in small changes in enthalpy and entropy [8]. However, it should be taken into account that the values, derived for alfaxalone, are related to the over-all process of solubilization and complexation. This explains the increase in disorder (positive ΔS) after complexation as the compound leaves the highly ordered crystalline phase.

Conclusions

Modified cyclodextrins, especially HPBCD, are useful excipients to resolve formulation problems for insoluble compounds. Alfaxalone can be solubilized using relatively low concentrations of HPBCD. Moreover, the thermodynamics of complexation of alfaxalone with HPBCD are favorable since the solubility shows minor changes with temperature. This allows the storage of the formulation at low temperature to improve the stability without any risk of precipitation. Since the solubility isotherm is linear the risk of precipitation at the injection site in minimal as linear dilution will not result in precipitation. HPBCD as a nonionic excipient has the advantage that the solubility is not influenced by the presence of NaCl (data not shown) and that solutions for injection can be easily made isotonic.

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