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Estimation of cyclodextrin affinity to steroids

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Abstract

A nonlinear spectrometric method for determination of the stability constant (K_S) for cyclodextrin complex with steroid was developed. The method is based on calculation of the parameters of competitive cyclodextrin complexation by simultaneous fitting of two types of curves. Those of the first type are the dependencies of absorbance of methyl orange solution on the cyclodextrin concentration, the second type being the absorption curves of displacement of the dye, by steroid, from the cyclodextrin complex. With the method proposed, K_S values were calculated with standard deviation less than 10%. This method is validated by determination of K_S values using the phase-solubility technique. For neutral steroid molecules, the effect of pH on K_S was found to be insignificant. K_S values for the cyclodextrin–dye complex were determined for randomly methylated β-cyclodextrin, 2-hydroxypropyl-β-cyclodextrin, carboxymethyl-β-cyclodextrin and sulfobutylether-β-cyclodextrin. More hydrophobic steroids were characterised by higher K_S values. Anionic β-cyclodextrins showed high affinity for the steroids studied. Simple equipment and sufficient computing allowed recommendation of the method for express estimation of cyclodextrin's affinity for hydrophobic substrates.

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Introduction

Cyclodextrins are cyclic oligosaccharides able to form host–guest complexes with hydrophobic compounds. Cyclodextrin-encapsulated steroids are widely used in drug formulation (Fromming & Szeitli 1994; Stella & Rajewski 1997; Cserhati & Forgacs 1999). Complexation of hydrophobic steroids with cyclodextrin changes their solubility significantly. Among the essential parameters of cyclodextrin–steroid affinity is the stability constant (K_S). It is usually determined by the phase-solubility technique (Higuchi & Connors 1965) based on the measurement of steroid solubility in cyclodextrin solutions. This method is a delicate and time-consuming procedure whereby phase equilibrium between crystalline and soluble steroid forms must be reached.

Spectral methods were proposed as being more promising, but their application is restricted by the extremely low water solubility of steroids. For instance, the sensitivity of nuclear magnetic resonance spectrometry is insufficient to determine the K_S of cyclodextrin–steroid complexes and the standard phase-solubility technique has been used (Uekama et al 1982; Singer et al 1991; Zia et al 1997; Ahmed 1998). Ultra-violet (UV) absorption of free and cyclodextrin-bound forms of steroids was shown to differ insignificantly and, therefore, separation of free and bound steroids was a necessary step before UV-detection. A similar approach was used in a liquid chromatography-based method for K_s determination in water–organic mixtures (Sadlej-Sosnowska 1997).

The competitive method based on the use of dyes (Szeitli 1982) seems to be convenient for K_S determination. This method is based on sequential linearisation of dye absorption curves in a series of cyclodextrin solutions and the curve of the dye competitive replacement by a colourless compound at a fixed concentration of cyclodextrin. The linear procedure for sequential computation of competitive equilibrium parameters neglects the dye contribution in mass balance equations. For hydrophobic compounds it results in unacceptable errors in K_S determination.

Nonlinear curve-fitting models have been introduced recently for complex K_S determination (Loukas et al 1996, 1997; Suzuki et al 1996). However, no nonlinear models were developed for competitive methods. We have developed an accurate and simple nonlinear procedure for determination of K_s based on the competitive absorption method which can be applied to steroids.

Materials and Methods

Randomly methylated β-cyclodextrins with a degree of substitution of 1.8 (RAMEB[1]) and 1.69 (RAMEB[2]) were obtained from Wacker-Chemie GmbH (Germany). 2-Hydroxypropyl-β-cyclodextrin and carboxymethyl-β-cyclodextrin were kindly gifted by Cerestar (USA). Sulfobutylether-β-cyclodextrin was gifted by CyDex (USA). Methyl orange, prednisolone, androsta-1,4-diene-3,17-dione and androst-4-ene-3,17-dione were purchased from Sigma (USA). 20-Hydroxymethyl-pregna-1,4-diene-3-one, 9α-hydroxyandrost-4-ene-3,17-dione (98–99% purity) were obtained from IBPM RAS (Russia).

Host cyclodextrin solutions (1×10^{-5} to 9×10^{-3} M) and those of guest methyl orange (2×10^{-5} M) were prepared using 0.1 M phosphate buffer. Guest steroid solutions (1×10^{-6} to 5×10^{-3} M) were prepared in 2.5×10^{-4} and 5.0×10^{-4} M solutions of chemically modified cyclodextrins in 0.1 M phosphate buffer and included methyl orange.

Solutions were incubated at 30°C. Absorbance measurements were performed on a double-beam spectrometer Specord-M-40 in a 1-cm^2 quartz cuvette with a thermostatted cell holder. The absorption maximum chosen to determine the binding constants was 505 nm (pH 2.67, phosphate-buffered saline 0.1 M, 30°C).

Solubility diagrams of steroids in cyclodextrin solutions were plotted according to the phase-solubility technique (Higuchi & Connors 1965). Steroids were analysed spectrometrically at 240 nm in 50% ethyl alcohol solution.

Results and Discussion

The competitive absorption method for determination of the stability constant, K_S, is based on the change in dye absorbance upon cyclodextrin complexation. In particular, cyclodextrin complexation with methyl orange is accompanied by a decrease in the dye absorbance. The addition of colourless steroid into the dye–cyclodextrin solution results in competitive equilibrium, displacement of dye by steroid from the dye–cyclodextrin complex and release of the dye from this complex.

Let us consider a system of cyclodextrin (D), steroid (S) and methyl orange (F) (Figure 1). The overall optical density of solution (E (visible spectra)) can be calculated as:

$$E = \varepsilon_{DF}[DF]+\varepsilon_F[F] \quad (1)$$

where ε_{DF} and ε_F are extinction coeffi cients of bound and free dye, respectively. It is necessary to express optical density in terms of the initial concentrations, D_0, S_0, F_0, and unknown parameters K_S and stability constant of methyl orange–cyclodextrin complex (K_F). The expressions for independent stability constants of cyclodextrin complexes can be written as:

$$K_S = [DS]/[D][S] \quad (2)$$

$$K_F = [DF]/[D][F] \quad (3)$$

where [DS], [DF], [D], [F], and [S] are the equilibrium concentrations of complexes DS and DF and unbound molecules D, F and S, respectively. Three equations for initial and analytical concentrations of free and bound forms of steroid, dye and cyclodextrin are true:

$$[S]_0 = [S]+[DS] \quad (4)$$

$$[F]_0 = [F]+[DF] \quad (5)$$

$$[D]_0 = [D]+[DS]+[DF] \quad (6)$$

To find [DF] and [F] as a function of $[S]_0$, $[F]_0$, $[D]_0$, K_S and K_F, equations 2–6 were solved. Thus, equation 1 is transformed to a function of the following parameters:

$$E = E([S]_0, [D]_0, [F]_0, K_S, K_F, \varepsilon_{DF}, \varepsilon_F) \quad (7)$$

Without steroid ($[S]_0 = 0$) the overall optical density, E*, depends on the reduced set of the parameters:

$$E^* = E^*([D]_0, [F]_0, K_F, \varepsilon_{DF}, \varepsilon_F) \quad (8)$$

The value of K_S was calculated using simultaneous fitting of the two types of experimental curves: firstly the

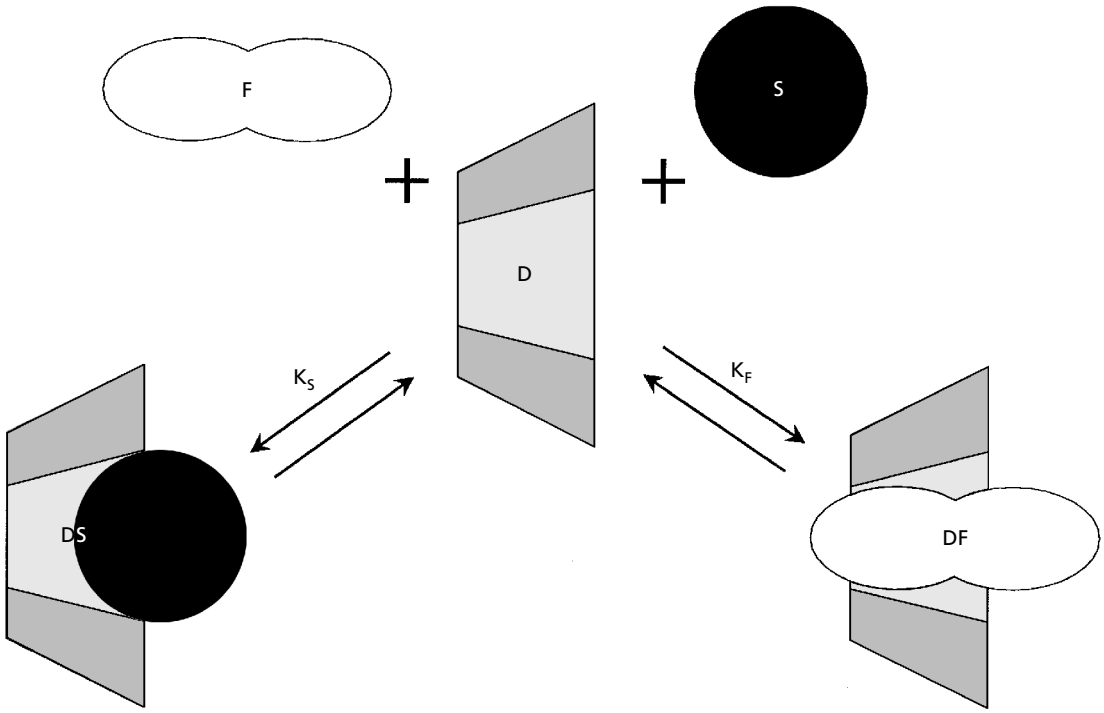

Figure 1 Competitive cyclodextrin complexation in the system steroid–cyclodextrin–dye. F is methyl orange; S is steroid; D is cyclodextrin; DS and DF are inclusion complexes; K_S and K_F are stability constants.

absorption curve of methyl orange in the presence of cyclodextrin and, secondly, the curve of release of the dye from the complex at fixed cyclodextrin concentration. To fit the model to experimental data, the least-squares method with the following objective function was used:

$$Q = \sum_{i=1}^{N} n_i \left\{ \frac{E_i - Y_i}{\sigma_i} \right\}^2 + \sum_{i=1}^{N^*} n_i^* \left\{ \frac{E_i^* - Y_i^*}{\sigma_i^*} \right\}^2 \qquad (9)$$

Here, E_i and Y_i are theoretical and experimental values of optical density for i-th; n_i and σ_i are the sample size and standard deviation of i-th experimental point, respectively; N is the number of experimental points. The asterisk denotes that experimental values were obtained without steroid. Argument of E is $[S]_0$ and argument of E* is $[D]_0$. So, simultaneous fitting of one function with different arguments to the two sets of experimental points was used and the values of parameters K_S, K_F, ε_{DF} and ε_F were calculated as a result of fitting (computation program for K_S and K_F is available free on request).

The method described was used to evaluate the ability of steroids with different hydrophobicity to form inclusion complexes with RAMEB[1]. The solubility of the steroids tested was in inverse relation to their hydrophobicity and ranged from 1.5×10^{-5} to 3×10^{-3} M in the following order: 20-hydroxymethyl-pregna-1,4-diene-3-one < androst-4-ene-3,17-dione < prednisolone < 9α-hydroxyandrost-4-ene-3,17-dione.

RAMEB concentration for methyl orange absorption curves ranged from 0 to 9×10^{-3} M providing transformation of free dye form to its cyclodextrin-complex form. For the displacement curves of the dye by steroid, the RAMEB concentrations were used to obtain a significant absorption response to small changes in RAMEB concentration. This requirement was fulfilled by RAMEB values at about the inflexion point of the methyl orange absorption curve (Figure 2).

The model fitted experimental curves for steroid series with correlation coefficient $r^2 \geqslant 0.99$. Standard deviation of K_S values calculated for a set of measurements of optical density was not higher than 10%. Standard deviation of average values of K_S (n = 6) calculated in few independent experiments was estimated as 4%.

The procedure of K_S calculation provides the possibility of simultaneous fitting of several curves of dye release from cyclodextrin complex at various fixed concentrations of cyclodextrin. In particular, due to the extremely low solubility of 20-hydroxymethyl-pregna-1,4-diene-3-one and thus the limited range of steroid

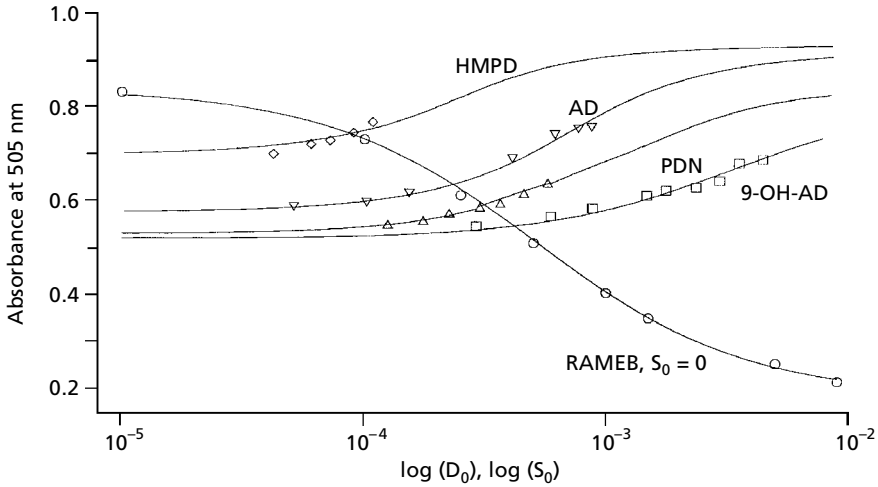

Figure 2 The dependence of absorbance of methyl orange dye (2.0×10^{-5} M) on concentration of RAMEB[1] (D_0) without steroids (○) and the curves of methyl orange replacement with steroid of different concentrations (S_0) at the following fixed RAMEB[1] concentrations (D_0): 5×10^{-4} M for 9α-hydroxyandrost-4-ene-3,17-dione (□; 9-OH-AD) and prednisolone (△; PDN); 4.5×10^{-4} M for androst-4-ene-3,17-dione (▽; AD); 2.25×10^{-4} M for 20-hydroxymethyl-pregna-1,4-diene-3-one (◇; HMPD). Experimental conditions: pH 2.67, phosphate-buffered saline 0.01 M, 30°C. The values of K_S for steroids are as follows (M^{-1}): 699, 9α-hydroxyandrost-4-ene-3,17-dione; 1490, prednisolone; 10200, androst-4-ene-3,17-dione; 16400, 20-hydroxymethyl-pregna-1,4-diene-3-one.

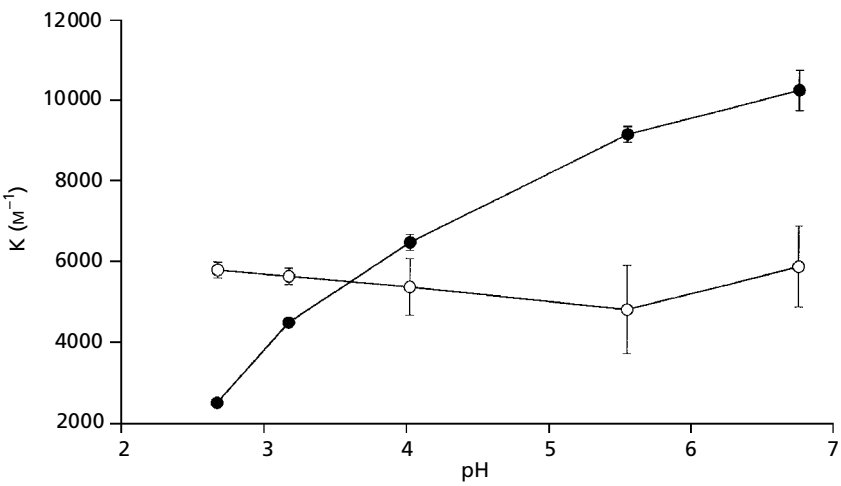

Figure 3 The effect of pH on the stability constant (K) of complexes: RAMEB[2]–methyl orange (●) and RAMEB[2]–androsta-1,4-diene-3,17-dione (○).

concentrations for the reliable determination of K_S, we had to use two curves for displacement of methyl orange by 20-hydroxymethyl-pregna-1,4-diene-3-one with respect to two fixed RAMEB[1] concentrations. One of these two curves is presented in Figure 2.

The K_S values for RAMEB[1] increased with hydrophobicity of steroids in the following order: 9α-hydroxyandrost-4-ene-3,17-dione < prednisolone < androst-4-ene-3,17-dione < 20-hydroxymethyl-pregna-1,4-diene-3-one.

The validity of these K_S values was checked by the phase-solubility technique (Higuchi & Connors 1965). Solubility diagrams for prednisolone (PDN) and 9α-hydroxyandrost-4-ene-3,17-dione (9-OH-AD) in RAMEB[2] solutions were described by linear functions: S_{PDN} (mM) = $0.87 + 0.44 \times$[RAMEB[2]] and $S_{9\text{-OH-AD}}$ (mM) = $3.41 = 0.66 \times$[RAMEB[2]], respectively. The values of K_S for prednisolone and 9α-hydroxyandrost-4-ene-3,17-dione determined from the parameters of linear regression were 1458 M^{-1} and 566 M^{-1}, respectively, and

Table 1 Stability constants for cyclodextrin–steroid complexes determined by nonlinear competitive spectrometric method.

Chemically modified cyclodextrin	K_F (M^{-1})	K_S (M^{-1})	
		9α-Hydroxyandrost-4-ene-3,17-dione	Androsta-1,4-diene-3,17-dione
Sulfobutylether-β-cyclodextrin	2040±40	1020±40	7190±170
RAMEB[1] (DS 1.8)	2200±90	699±10	5800±500
RAMEB[2] (DS 1.69)	2160±160	552±6	5840±210
2-Hydroxypropyl-β-cyclodextrin	1060±50	780±6	Not determined
Carboxymethyl-β-cyclodextrin	340±30	3060±180	29700±2600

DS, degree of substitution.

they corresponded to those determined by competitive technique: 1490 M^{-1} and 552 M^{-1}. Hence, the methods exploited different processes of cyclodextrin–steroid complexation, steroid solubilization and displacement of the dye by steroid from cyclodextrin cavity, showed the same K_S values.

Effect of pH

The effect of pH on K_S (androsta-1,4-diene-3,17-dione–RAMEB[2]) was studied within the pH range 2.6–7.0. Despite the relatively small difference in extinction coefficients for bound and free dye forms at neutral pH, the method provided a reliable estimation of K_S. The limited drop of K_S values observed was less than the error of the measurements (Figure 3, empty circles). This pointed to the absence of a considerable influence of pH on the equilibrium of non-charged molecules of steroid and cyclodextrin. Alternatively, the affinity of methyl orange to RAMEB[2] was significantly pH dependent. Deprotonation of the methyl orange (pK = 3.55) was accompanied by notable increase in the stability constant of cyclodextrin complex (Figure 3, black circles). This is consistent with the reported data that cyclodextrin complex of methyl orange base was more stable than respective acid forms. It also correlates with the effect of pH on methyl orange's affinity to native β-cyclodextrin (Suzuki et al 1996; Carrazana et al 1999).

Thus, K_S values determined at optimal pH 2.67 were acceptable for estimation of the affinity between cyclodextrin and neutral steroid.

The application of competitive method for assessing affinity between steroid and chemically modified cyclodextrin

The values of K_F and K_S were determined by the competitive method for a number of chemically modified cyclodextrins (Table 1). The cyclodextrins tested showed high affinity for steroid molecules as indicated by the relatively high K_S values obtained.

Two model steroids differing in hydrophobicity (androsta-1,4-diene-3,17-dione and 9α-hydroxyandrost-4-ene-3,17-dione) were used as guest model compounds in these experiments. As shown in Table 1, K_S values for the more hydrophobic androsta-1,4-diene-3,17-dione were one order of magnitude higher than those for 9α-hydroxyandrost-4-ene-3,17-dione. This indicates that hydrophobic interactions are important contributors to steroid–cyclodextrin complexation.

The applicability of this method was studied for a number of modified cyclodextrins widely used in drug formulations. The increase in substitution degree of methylated cyclodextrin resulted in comparative increase in K_S values, which also confirms the important role of hydrophobic interactions. Anionic cyclodextrins (carboxymethyl-β-cyclodextrin, sulfobutylether-β-cyclodextrin) revealed higher affinity for the steroids studied. The K_S values for anionic cyclodextrins as well as RAMEB[1] at neutral pH coincide with those obtained in optimal conditions (pH 2.67).

Conclusion

The new nonlinear spectrophotometric method for K_S determination is advanced. The method ensures reliable estimation of the ability of steroids to form complexes with chemically modified cyclodextrins within the K_S range 0.5×10^3 to 10^4 M^{-1}. Simplicity of equipment and the possibility of automation allows recommendation of the method for estimation of cyclodextrin complexation with hydrophobic compounds.

A wide field of application for cyclodextrins suggests synthesis of promising new cyclodextrins for use in pharmacological practice. The high affinity between chemically modified cyclodextrin and guest molecules,

as expressed by K_S value, is an obligatory condition for effective cyclodextrin usage. The developed competitive method is convenient for testing this affinity.

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