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Use of a nonlinear least-squares model for the kinetic determination of the stability constant of cyclodextrin inclusion complexes

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Abstract

The nonlinear least-squares model for the calculation of the stability constants (K_{st}) of drug:cyclodextrin complexes was used in kinetic studies. Complexation of riboflavin (R) with hydroxypropyl-β-cyclodextrin (HP:β-CD) was monitored kinetically by measuring the rate of photodegradation of R exposed to ultraviolet light in the presence of increasing concentrations of HP:β-CD. Formation of inclusion complex was confirmed in aqueous solution by proton nuclear magnetic resonance spectroscopy (^{1}H-NMR). The experimental K_{st} value (3321 M^{-1}), derived from the kinetic studies, appeared to fit well to a 1:1 drug:cyclodextrin molar ratio according to the nonlinear mathematical model. The model is particularly suitable for the study of cyclodextrin as a stabilizing system for compounds that are sensitive to light, oxygen, temperature or the media which contain them. Copyright © 1996 Elsevier Science B.V.

Keywords: Cyclodextrin; Stability constant; Kinetic studies; Photodegradation

1. Introduction

Cyclodextrins (CDs) (Saenger, 1980) are cyclic oligomers containing at least six 1→4 linked D-glucose units. They are doughnut shaped, possessing a cavity of fixed size and shape within which guest molecules can be accommodated (inclusion complexes). Essential to the preparation and the use of inclusion complexes is understanding of the mechanism of molecular association during complexation and, to that end, the stability constant (K_{st}) and the stoichiometry of the complex must be known. A number of linear procedures (Duchêne, 1987) are in use for the determination of K_{st} but most

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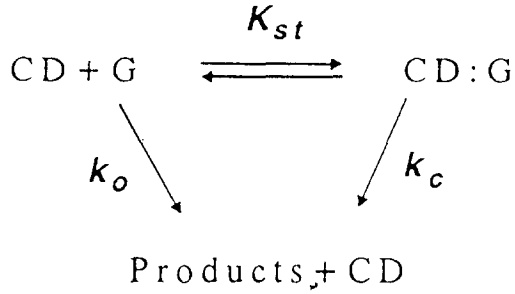
of these suffer from theoretical and practical drawbacks (Djedaini and Perly, 1993), including assumed concentrations of the interacting moieties and products, poor solubility of certain compounds, saturation binding (boundary condition) with respect to the ratio of the concentrations of the two binding partners, and the occasional formation of dimers. In contrast, nonlinear procedures are free of assumptions, have much broader applicability (Diederich, 1988) and are thus likely to displace the evaluations based on the Benesi-Hildebrand and Scott or Scatchard linear models (Diederich, 1988).

Nonlinear models can be generally worked out in three different ways (Draper and Smith, 1981): (a) linearization (or Taylor series); (b) the steepest descent method; and (c) nonlinear regression by minimizing the squared residuals of the relevant parameters. There are several algorithms for the analysis of nonlinear mathematical models (Fox, 1984) by nonlinear regression. The Levenberg–Marqardt algorithm, for instance, is one of the most commonly used for the analysis of unconstrained models (Marqardt, 1963). Recently, nonlinear mathematical models have been described for the calculation of K_{st} using potentiometry (Sideris et al., 1992) and ultraviolet spectrophotometry (Rose and Drago, 1959) during complexation. Other nonlinear models correlate the nuclear magnetic resonance (NMR) data of cyclodextrin and guest with those of the complex (Djedaini et al., 1990). Finally, the Gauss-Newton nonlinear optimization method has been applied for the analysis of solubility curves (Miyahara and Takahashi, 1982) and the Marqardt algorithm has been used for the correlation of data (obtained by high-performance liquid chromatography (HPLC)) of guest molecule retention in the presence of CD with the K_{st} (Spino and Amstrong, 1987). In the present work, we have applied nonlinear curve-fitting models for the calculation of K_{st} on the basis of the kinetics of photodegradation of the photolabile riboflavin on exposure to ultraviolet light in the presence of hydroxypropyl-β-cyclodextrin (HP:β-CD).



Scheme 1.

2. Theoretical

2.1. Calculation of K_{st} in kinetic studies

CDs often accelerate or decelerate many types of reactions (Szejtli, 1982). For example, the hydrolytic or photolytic decomposition of included compounds can be decelerated by virtue of their being protected from factors in the aqueous media or from ultraviolet light, respectively. The reaction mechanism involving the formation of a 1:1 (molar ratio) inclusion complex is illustrated in Scheme 1, where CD is the cyclodextrin molecule (HP:β-CD), G the guest molecule (riboflavin in the present work), k_0 the photodegradation rate constant for the non-catalyzed reaction (i.e. in the absence of HP:β-CD) and k_c the photodegradation rate constant of the guest in the form of the inclusion complex. Linear models which describe the kinetic behaviour of guests in the presence of CDs are usually solved according to Lineweaver-Burk (Loukas et al., 1995c) or Eadie (Loukas et al., 1994) equations.

Let us assume a solution with total concentrations of the guest (riboflavin) and cyclodextrin (HP:β-CD) equal to G_t and D_t, respectively. According to Scheme 1, the rate of disappearance of G_t is:

$$-\frac{d[G_t]}{dt} = k_0[G] + k_c[D:G] \quad (1)$$

During the decomposition of the (guest) G_t, the overall observed first-order rate constant k_{obs} can be expressed as:

$$-\frac{d[G_t]}{dt} = k_{obs}[G_t] \quad (2)$$

Combination of Eqs. (1) and (2) gives:

$$k_0[G] + k_c[D:G] = k_{obs}[G_t] \quad (3)$$

In Eq. (3), $[G]$ denotes the concentration of the free guest and can be related to the total concentration $[G_t]$ by using the mass balance:

$$[G] = [G_t] - [D:G] \quad (4)$$

Substitution of $[G]$ in Eq. (3) with the equivalent from Eq. (4) gives:

$$k_{obs}[G_t] = k_0[G_t] - [D:G](k_0 - k_c) \quad (5)$$

According to Scheme 1, the cyclodextrin inclusion complex in solution is in kinetic equilibrium with the free components. This kinetic equilibrium can be expressed as:

$$K_{st} = \frac{[D:G]}{[D][G]} \quad (6)$$

Eq. (6) can be rewritten as:

$$[D:G] = K_{st}[D][G] \quad (7)$$

Substitution of $[D:G]$ in Eq. (5) with the equivalent from Eq. (7) gives:

$$k_{obs}[G_t] = k_0[G_t] - K_{st}[D][G](k_0 - k_c) \quad (8)$$

As with the concentration of free guest $[G]$ given by Eq. (4), the free cyclodextrin concentration $[D]$ can be calculated as:

$$[D] = [D_t] - [D:G] \quad (9)$$

Substitution of $[D]$ and $[G]$ in Eq. (8) with their equivalents in Eqs. (9) and (4), respectively, gives:

$$k_{obs}[G_t] = k_0[G_t] - K_{st}([G_t] - [D:G])([D_t] - [D:G]) \times (k_0 - k_c) \quad (10)$$

Also, rearrangement of Eq. (5) gives the concentration of the complex $[D:G]$ as:

$$[D:G] = \frac{[G_t](k_0 - k_{obs})}{(k_0 - k_c)} \quad (11)$$

Finally, substitution of $[D:G]$ in Eq. (10) with the equivalent in Eq. (11) gives:

$$\Delta k_{obs} = K_{st}\left(1 - \frac{\Delta k_{obs}}{\Delta k_c}\right)\left([D_t] - \frac{[G_t]\,\Delta k_{obs}}{\Delta k_c}\right)\Delta k_c \quad (12)$$

where $\Delta k_{obs} = k_0 - k_{obs}$ and $\Delta k_c = k_0 - k_c$.

Eq. (12) is the final mathematical model which involves no approximation of concentrations of the two compounds (D and G) and correlates the initial total concentrations $[G_t]$ and $[D_t]$ with the rate constants k_0 and k_{obs}. The unknown parameters K_{st} and k_c can then be calculated according to this model (see below).

3. Materials and methods

3.1. Materials

Riboflavin-5′(dihydrogen-phosphate monosodium salt) (R) was purchased from Aldrich Chemical Company (Gilligham, Dorset, UK) and HP:β-CD with a calculated relative molecular mass (M_r) of 1300, from Janssen (Beerse, Belgium). The degree of substitution (DS) of HP:β-CD (0.4) was calculated from the ^{1}H-NMR spectrum of HP:β-CD in deuterium oxide, using digital integration (Fig. 1(b)). Deuterium oxide (99.9%) was obtained from Fluka (Poole, Dorset, UK). Double-distilled water was used throughout. All other reagents were of analytical grade.

3.2. Instrumentation

Photodegradation studies were carried out using a long-wave (365 nm) ultraviolet lamp with 6 W rating and 460 μW/cm^2 per dm intensity (model UVGL-58, UVP, San Gabriel, USA). The kinetics of riboflavin photodegradation in the presence of HP:β-CD was monitored in a Perkin-Elmer LS-3 fluorescence spectrophotometer using excitation and emission wavelengths of 445 and 520 nm, respectively. Characterization of the R–HP:β-CD complex in aqueous solution was carried out in D_2O by ^{1}H-NMR and the spectra were recorded in a Bruker AM 500 spectrometer connected to an Aspect 3000 computer. The chemical shifts were related to the residual solvent signal (HDO = 4.84 ppm at 293 K). Typical conditions

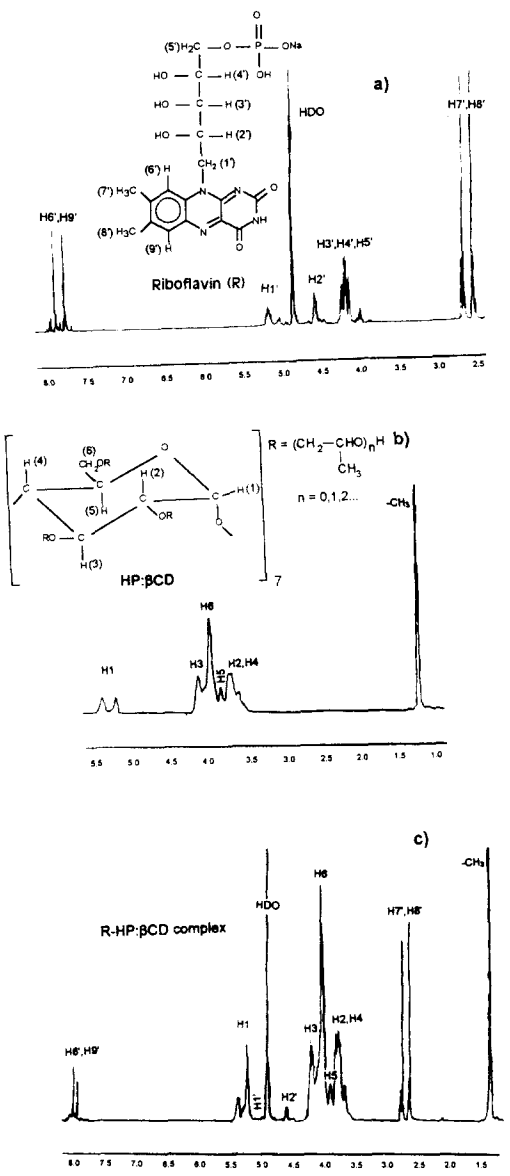


Fig. 1. 500 MHz ^{1}H-NMR spectra of (a) riboflavin; (b) HP:β-CD; (c) R–HP:β-CD inclusion complexes in D_2O. Numbers in parentheses denote protons with chemical shifts corresponding to numbered peaks in the spectra.

were 16K data points with zero filling, sweep width of 5 kHz given a digital resolution of 0.61 Hz per point, pulse width 4 μs, acquisition time 1.64 s and number of scanning 64. Statgraphics Plus version 6 (Manugistics, Rockville, MD, USA) was used for the nonlinear calculations and the illustration of graphics.

4. Results and discussion

4.1. Characterization of the R–HP:β-CD inclusion complex

Observed resonances in the NMR study of the soluble R–HP:β-CD complex were the time-averaged peaks obtained with HP:β-CD, R and its inclusion complex (fast exchange regime on the NMR time scale at 293 K) (Loukas et al., 1995a). Evidence of R–HP:β-CD complex formation in aqueous solution was based on the modification of the NMR spectrum of R. Fig. 1, showing the 500 MHz NMR spectra of the complex and the two interacting moieties as such, reveals that, under the conditions employed, only shift changes of the signals occurred. There were no new peaks that could be assigned to the complex itself, an observation suggesting that the complexation of R with HP:β-CD is a dynamic process with the R being in a state of fast exchange (relative to the NMR time scale) between the free and included forms at a rate which must exceed the reciprocal of the largest observed shift difference (in Hz) for any of the protons of the R molecule (Loukas et al., 1995a).

Results (Table 1) from the NMR study show that the internal protons (H3 and H5) of the HP:β-CD undergo greater R-induced chemical shift changes than the external H1, H2, H4 and H6 protons, indicating that the vitamin has approached the cavity. Although of low magnitude, observed H3 and H5 shifts are nevertheless indicative of inclusion occurrence (Komiyama, 1989), especially as δ values for the external protons remained essentially unchanged (Table 1). Furthermore, digital integration of the NMR signals of the phenyl protons of R (H6′–H9′; Fig. 1(a)) and of the anomeric protons of HP:β-CD in the complex (H1; Fig. 1(c)), allowed the direct estimation of the stoichiometry ratio. This was found to be approximately 1:1, implying that all of the R molecules are in the complexed form: two different signals for the R phenyl protons of

the free and complexed R would have been expected if some of the R molecules were in the free form.

4.2. Photostability of R–HP:β-CD complex

Photodegradation of R as inclusion complex with HP:β-CD followed first-order kinetics, expressed as $C = C_0 e^{-kt}$ (Eq. (13)). This equation is in the exponential form and can be transformed logarithmically into a linear form: $\ln C = \ln C_0 - kt$ (Eq. (14)), where C_0 is the initial concentration of R, C is the concentration of R after t min irradiation and k is the first-order degradation rate constant. As the graphic representation of Eq. (14) is linear, the slope of the line gives the photodegradation rate constant k from which the corresponding half life ($t_{50\%}$) of R exposed to ultraviolet light can be then calculated ($t_{50\%} = 0.693/k$).

4.3. Calculation of the K_{st}

Photodegradation of R in aqueous solution follows first-order kinetics (Loukas et al., 1995a,b) in the absence or presence of cyclodextrin (Fig. 2). It is clear that the presence of HP:β-CD has a pronounced stabilizing effect on R. For instance, according to Fig. 2, which shows the effect of HP:β-CD concentration on the photodegradation rate constant (k_{obs}) of R, the rate constant for the photodegradation of 1.7×10^{-5} M R decreased from 0.024 min^{-1} to 0.013 min^{-1} in the presence of 5.1×10^{-4} M HP:β-CD. It is also evident (Fig. 2) that k_{obs} does not relate linearly to the increasing concentration of added HP:β-CD but rather asymptotically approaches a minimum value. This so-called 'saturation behaviour' (Szejtli, 1982) is characteristic of a reaction where complex formation occurs prior to the rate-determining step and may be described by the reaction mechanism illustrated in Scheme 1.

The K_{st} value of the R–HP:β-CD complex was determined according to Eq. (12). The initial values for this model were calculated according to the following procedure: firstly, the k_0 and k_c values were calculated from the photodegradation of R and inclusion complexes, respectively, on exposure to ultraviolet light. On the basis of such k_c, k_0 and also a pair of k_{obs} values for R and HP:β-CD concentrations, the K_{st} was calculated by substituting these values into Eq. (12). Esti-

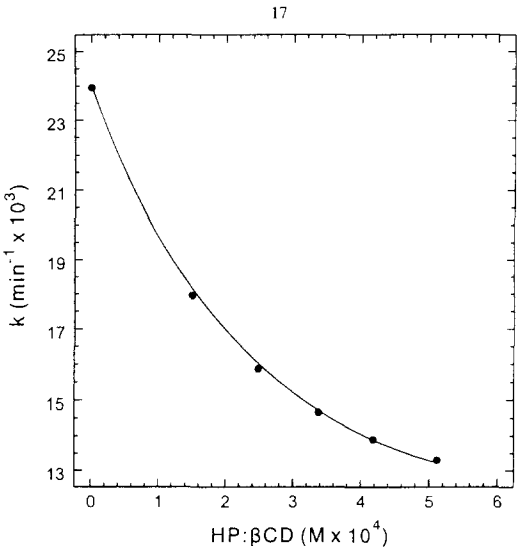


Fig. 2. Effect of HP:β-CD concentration on the first-order rate constant of the degradation of 1.7×10^{-5} M R.

Table 1
NMR chemical shifts of the protons of HP:β-CD and riboflavin in the free and inclusion complex states

Proton	δ_0 (free)	δ_c (complex)	$\Delta\delta$ ($\delta_c - \delta_0$)[a]
HP:β-CD			
H1	5.180	5.170	0.001
H2	3.732	3.729	−0.001
H3	4.040	4.020	−0.020
H4	3.588	3.585	−0.003
H5	3.828	3.819	−0.009
H6	3.969	3.970	0.001
$-CH_3$	1.256	1.252	−0.004
Riboflavin			
H6′, H9′	7.900	7.994	0.094
H6′, H9′	7.770	7.943	0.173
H7′, H8′	2.620	2.660	0.04
H7′, H8′	2.496	2.547	0.051
H1′	5.133	5.131	−0.002
H2′	4.513	4.511	−0.002
H3′, H5′	—[b]	—	—

[a] Negative values indicate upfield movement (shielding effect).
[b] Values not shown because of overlap with the chemical shifts of HP:β-CD.

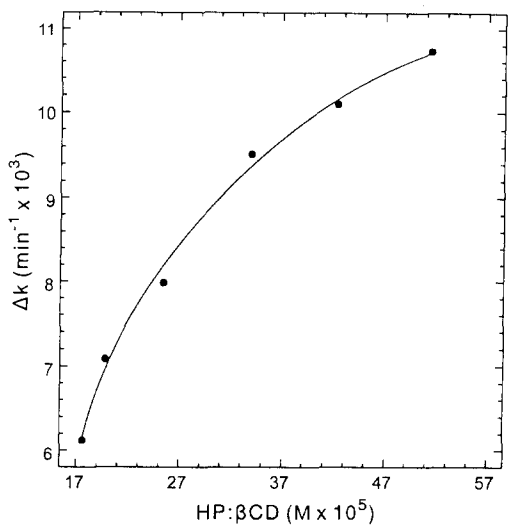


Fig. 3. Plot of the observed ΔF values for riboflavin and the estimated fitting line according to Eq. (12).

mated Δk_c and K_{st} were then used as starting values for the iterative procedure which finally resulted in a value of 3321 ± 210 M^{-1} for K_{st}. Fig. 3 represents the graphical solution of Eq. (12) and, judging from the R^2 value (0.985), the model appears to fit the observed values well.

In conclusion, the present work has shown that the nonlinear curve-fitting models described can be used for the calculation of K_{st} of drug: cyclodextrin inclusion complexes using kinetic (degradation) studies. As this model requires only the initial concentrations of the free species (drug and CD) without any limitation (e.g. excess amount of one of the two species), experimental and theoretical drawbacks are avoided. This model is particularly suitable for unstable drugs that are sensitive to external factors with sensitivity being affected by the presence of cyclodextrins.

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