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ORIGINAL ARTICLE

Rigorous nonlinear regression analysis of phase solubility diagrams to obtain complex stoichiometry and true thermodynamic drug-cyclodextrin complexation parameters

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Abstract This work reports rigorous nonlinear regression procedures aimed at analyzing various types of phase solubility diagrams (PSDs) corresponding to the different soluble and insoluble complex stoichiometries, which are generally encountered in drug-cyclodextrin (CD) complexation studies. These are depicted in final equations that can be modeled to fit experimental data of measured drug solubility against CD concentration utilizing simple spreadsheet software available for all PCs (i.e., the Solver Add-in in Microsoft Excel). They cover all types of guest/host phase solubility diagrams (A-, B_S-and B_I-types) allowing accurate estimation of soluble and insoluble complex stoichiometries generally encountered in drug/CD complexes (1:1, 2:1, 1:2, 2:2, 2:3, 3:2), the corresponding thermodynamic complex formation constants (K_{11}, K_{21}, K_{12}, K_{22}, K_{23}, K_{32}) and solubility product constants (K_{sp}) of saturated complexes.

Keywords Complex stoichiometry · Complex formation constants · Cyclodextrin complexes · Guest-host complexes · Nonlinear regression analysis · Phase solubility diagrams · Stability constants

Introduction

Recurrent attempts at establishing theoretical models embodying quantitative structure activity (property) relationships (QSAR, QSPR) face the common problem of not having carefully estimated true thermodynamic equilibrium constants pertaining to interactions between drugs and model receptor sites, which are very important to successful drug design. This problem is particularly acute in attempts at modeling drug-cyclodextrin (CD) interactions, where most of the stability constants (K_{nm}; n, m ≥ 1) reported in the literature are not truly thermodynamic equilibrium constants. They are normally estimated assuming a presupposed complex stoichiometry (n:m), which may not be correct, with the result that K_{nm} estimates are labeled apparent (K), and therefore not thermodynamic. In order to obtain thermodynamic K_{nm} values, which do truly represent the actual affinity of cyclodextrins (CDs) to various drugs of different structures and hydrophobic characters, measurements of phase solubility diagrams (PSDs) [1] must be conducted under controlled conditions of pH, buffer concentration, buffer type, ionic strength, and temperature, and the resulting experimental data must be carefully analyzed using rigorous procedures. Aside from a rigorous procedure reported for A-type PSD corresponding to 1:1 complex stoichiometry [2], earlier procedures have largely been developed for A-type PSDs [3–6], some involving approximations [7], while others using linear regression [8–10], and some using iterative procedures [11, 12]; they all suffer from limited precision due to cumulative errors associated with data manipulation on linearization or iteration. However, this work reports rigorous nonlinear regression procedures aimed at analyzing various A- and B-type PSDs of drug-CD complexes corresponding to the different soluble and insoluble complex stoichiometries, which are generally encountered in CD complexation studies. These are depicted in simple equations that can be modeled utilizing simple spreadsheet software available on all PCs (e.g.,

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the Solver Add-in in Microsoft Excel). They allow the determination of accurate thermodynamic complex formation constants (K_{11}, K_{21}, K_{12}, K_{22}, K_{23}, and K_{32}); in addition to the solubility product constants (K_{sp}) of complexes that reach saturation.

Rigorous nonlinear regression analysis of phase solubility diagrams (PSDs)

Methods and terminology

Here we consider the equilibria governing the formation of various guest-host complex stoichiometries (S_nL_m, n, m ≥ 1; S = guest, L = host), which are generally encountered in aqueous solutions of cyclodextrins (CDs) as host molecules. Figure 1 depicts various components of A- and B- type phase solubility diagrams (PSDs) [1], in which the symbols and regions indicated acquire the following meanings to be used throughout:

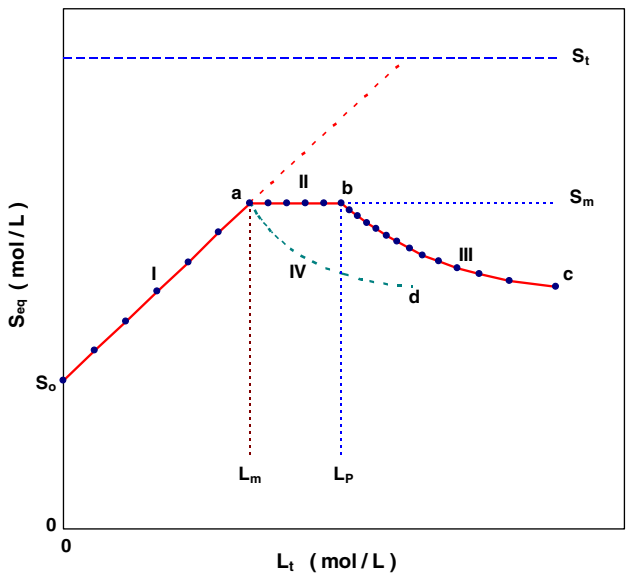

Fig. 1 General representation of phase solubility diagrams (PSDs) showing an A-type PSD (the rising portion or **Region I**: S_o- to-a plus its extension upward), the plateau (**Region II**: a-to-b), the descending portion (**Region III**: b-to-c) of a B_S type PSD, and **Region IV** (a-to-d) which corresponds to **Region III** if S_{eq} were plotted against L_{eq} instead of L_t (The meanings of symbols and regions are discussed in detail in the Methods and Terminology section. The lengths and curvatures of Regions I, III and IV vary with soluble complex stoichiometry, the relative magnitude of the complex formation constants (K_{nm}), the stoichiometry of the complex precipitate and its solubility product (K_{sp}), S_o, and L_t. The length of the plateau (Region II) is a function of the amount of guest present in excess (S_t–S_m) in addition to soluble complex stoichiometry, the stoichiometry of the complex precipitate and its K_{sp} value, and L_t. In case the solubility product of the complex precipitate is extremely low, the length of Region I becomes practically zero and the PSD is called B_I type)

S_{eq} is the equilibrium concentration (solubility) of the guest (i.e., a drug molecule) measured corresponding to a host initial concentration, **L_t** (e.g., CD molecule).
L_{eq} is the equilibrium (measured) concentration of the host corresponding to the host initial concentration (**L_t**).
S_o is the measured inherent solubility of the guest (in the absence of host).
S_m is the measured maximum solubility of the guest observed in B_S or B_I type PSDs.
L_m is the equilibrium concentration of host corresponding to the plateau (**Region II**) of the PSD (L_{eq} = L_m throughout the plateau where one of the complexes is saturated).
L_P is the initial concentration of host corresponding to the end of the plateau (**Region II**) and the beginning of the descending portion (**Region III**) of the PSD.
S_t is the total amount of the guest (mol/L) added to the solution, which is in excess of the maximum solubility (**S_m**) observed in B_S or B_I type PSDs.

Region I (S_o to a) and its extrapolation upward denotes an A-type PSD comprising only soluble complexes, which normally exhibit: (a) a positive curvature (A_P type PSD) for SL_n type complexes (n > 1); (b) a linear behavior (A_L type PSD) for S_nL complexes (n ≥ 1) and also for a strictly special case of an SL_2 complex formation where K_{11} = $1/S_o$ and the slope is exactly equal to 0.5; (c) a negative curvature (A_N type PSD) which is observed if the pH of solution changes as the solubility (S_{eq}) of an ionizable guest (e.g., acid or base) increases with an increase in the host concentration (L_t) in the absence of pH control. For example, for a basic guest in the absence of strict pH control, the pH of solution increases as S_{eq} increases with an increase in L_t, which results in a consistent decrease in S_o thus leading to the negative curvature observed in A_N type PSDs. Similarly, for an acidic guest in the absence of pH control, the pH of solution decreases as S_{eq} increases with an increase in L_t, which results in a consistent decrease in S_o thus leading to a negative curvature observed in A_N type PSD.
Region II (a to b) denotes a plateau in the PSD, which occurs if one of the soluble complexes (S_nL_m; n, m ≥ 1) reaches saturation due to its low solubility product (K_{sp}) while the guest is still present in excess of its maximum solubility (S_t > S_m). The length of the plateau (L_P – L_m) is determined by both the value of (S_t – S_m) and the stoichiometry of the saturated complex, where L_P – L_m = (m/n) (S_t – S_m).

Region III (b to c) in B_S type PSDs denotes the descending portion of the PSD, which occurs if the guest is no longer present in excess, while the saturated

complex keeps precipitating at the expense of the inherent guest solubility (S_o) as L_t increases.

Region IV (a to d) denotes **Region III** in case S_{eq} were plotted against the equilibrium concentration of host (L_{eq}) instead of L_t, where $L_{eq} = L_t - (L_p - L_m) - (m/n) \times (S_m - S_{eq})$.

The lengths and curvatures of Regions I, III and IV vary with soluble complex stoichiometry, the relative magnitude of the complex formation constants (K_{nm}), the stoichiometry of the complex precipitate and its solubility product (K_{sp}), S_o, and L_t. The length of the plateau (Region II) is a function of the amount of guest present in excess (S_t–S_m) in addition to soluble complex stoichiometry, the stoichiometry of the complex precipitate and its K_{sp} value, and L_t. In those cases where the solubility product of the complex precipitate is extremely low, the length of Region I becomes practically zero and the PSD is called B_I type.

Relevant equations used in nonlinear regression analysis of PSDs

Nonlinear regression analysis of S_{eq} against L_t data of the PSDs to obtain the best-fit estimates of the complexation parameters (S_o, K_{nm}; n, m≥ 1, and the solubility product constant, $K_{sp\ nm}$, of a saturated complex, S_nL_m) are given below. They are given for individual regions of the PSDs, which correspond to various complex stoichiometries, and types of saturated complex (complex precipitate), that are usually encountered in drug/CD PSDs.

For each of the complex stoichiometries indicated below, the most relevant relations are given to calculate the concentrations of free guest [S] and free host [L] molecules, which are to be used in calculating the predicted values of the guest solubility (S_{eq}^P) against L_t. Nonlinear regression is then used to minimize the sum of squares of differences $SSE = \sum (S_{eq}{}^P - S_{eq})^2$ by letting the input initial guesses of S_o and K_{nm} change to the best-fit estimates of S_o, K_{nm} and $K_{sp\ nm}$ on reaching a minimum in SSE. Input initial guesses of S_o and K_{nm} are also indicated. The Solver Add-in of Microsoft Excel is most suited for nonlinear regression with an automatic link to graphics.

Individual complex stoichiometries

1. SL complex stoichiometry

$$S + L \rightleftharpoons SL, \quad K_{11} = [SL]/[S][L] \qquad (1)$$

A_L type PSD or **Region I** of **B_S** type PSD:

$$[S] = S_o = \text{constant}, \quad [L] = L_t/(1 + K_{11}S_o), \quad L_{eq} = L_t,$$

$$S_{eq}{}^{Pred} = S_o(1 + K_{11}[L])$$

Nonlinear regression of S_{eq} against L_t: minimize the sum $SSE = \sum (S_{eq}{}^P - S_{eq})^2$ by letting the values of S_o and K_{11} change to reach their best-fit estimates. Input initial guesses: S_o = Intercept, K_{11} = T / (S_o(1–T)); T = slope of PSD.

Region II of **B_S** type PSD (Complex SL is saturated at a fixed concentration $[SL]_m$):

$$[S] = S_o = \text{constant}; \quad L_{eq} = L_m = \text{constant}, \quad S_{eq} = S_m = \text{constant}. [L_m] = L_m/(1 + K_{11}S_o).$$

Region III of **B_S** type PSD (Complex SL is saturated at $[SL]_m$, $K_{sp\ 11}$ = $S_o[L_m]$):

$$L_{eq} = L_t - (L_p - L_m) - (S_m - S_{eq}), \quad [SL] = [SL]_m = K_{11}S_o[L_m] = K_{11}K_{sp\,11}$$

$$[L] = L_{eq} - K_{11}K_{sp\,11}, [S] = K_{sp\,11}/[L]$$

$$S_{eq}^P = [S] + K_{11}K_{sp\,11}$$

Minimize $SSE = \sum (S_{eq}^P - S_{eq})^2$ by letting S_o and K_{11} change to obtain the best-fit estimates of S_o, K_{11} and $K_{sp\ 11}$.

2. SL_2 complex stoichiometry

$$S + L \rightleftharpoons SL, \quad K_{11} = [SL]/[S][L] \qquad (1)$$

$$L + SL \rightleftharpoons SL_2, \quad K_{12} = [SL_2]/[L][SL] \qquad (2)$$

A_L or A_P type PSD, or **Region I** of **B_S** type PSD:

$$[S] = S_o = \text{constant};$$

$$[L] = (-b + (b^2 + 4a\ L_t)^{1/2})/2a; \quad a = 2K_{11}K_{12}S_o, \quad b = 1 + K_{11}S_o$$

$$S_{eq}{}^P = S_o\{1 + K_{11}[L](1 + K_{12}[L])\}$$

Initial guesses: $K_{11} = a / \{S_o(1 + a)\}$, $K_{12} = b\ (1 - K_{11}\ S_o)/a$, where a and b are the intercept and slope, respectively, of a plot of $(S_{eq}–S_o)/Q$ against Q; $Q = L_{eq} - 2\ (S_{eq} - S_o)$.

Region II of **B_S** type PSD (Either SL_2 or SL complex is saturated):

$$[S] = S_o = \text{constant}; \quad L_{eq} = L_m = \text{constant}; \quad S_{eq} = S_m = \text{constant}$$

$$[L] = [L_m] = (-b + (b^2 + 4a\,L_m)^{1/2})/2\,a; \quad a = 2K_{11}K_{12}S_o, \quad b = 1 + K_{11}S_o.$$

Region III of $\mathbf{B_S}$ type PSD (Either SL_2 or SL complex is saturated): Two cases may be considered:

(a) $\mathbf{SL_2}$ complex is saturated ($K_{sp12} = S_o[L_m]^2$):

$$L_{eq} = L_t - (L_p - L_m) - 2(S_m - S_{eq}),$$
$$[SL_2] = [SL_2]_m = K_{11}K_{12}K_{sp\,12}$$

$$[L] = (1/2)\,\{b - (b^2 - 4ac)^{1/2}\};$$
$$b = L_{eq} - 2K_{11}K_{12}K_{sp\,12};$$
$$c = K_{11}K_{sp12}$$

$$[S] = K_{sp\,12}/[L]^2$$

$$S_{eq}{}^P = [S]\{1 + K_{11}[L](1 + K_{12}[L])\}.$$

(b) **SL** complex is saturated ($K_{sp\,11} = S_o[L_m]$):

$$L_{eq} = L_t - (L_p - L_m) - (S_m - S_{eq}),$$
$$[SL] = [SL]_m = K_{11}S_o[L_m] = K_{11}K_{sp\,11}$$

$$[L] = (L_{eq} - K_{11}K_{\,11})/(1 + 2K_{11}K_{12}K_{sp11})$$

$$[S] = K_{sp\,11}/[L]$$

$$S_{eq}{}^P = [S]\{1 + K_{11}[L](1 + K_{12}[L])\}$$

Minimize SSE = $\sum (S^P_{eq} - S_{eq})^2$ by letting S_o, K_{11} and K_{12} change to obtain their best-fit estimates, in addition to $K_{sp\,11}$. Input initial guesses for $K_{11} = T/\{S_o(1 - T)\}$, $K_{12} = 0.1\,K_{11}$; T = initial slope of PSD.

3. $\mathbf{S_2L}$ complex stoichiometry

$$S + L \rightleftharpoons SL, \quad K_{11} = [SL]/[S][L] \quad (1)$$

$$S + SL \rightleftharpoons S_2L, \quad K_{21} = [S_2L]/[S][SL] \quad (3)$$

$\mathbf{A_L}$ type PSD, or **Region I** of $\mathbf{B_S}$ type PSD:

$$[S] = S_o = \text{constant}, \quad [L] = L_t/\{1 + K_{11}S_o(1 + \delta)\};$$
$$\delta = K_{21}S_o, \quad L_{eq} = L_t,$$

$$K_{11} = T/S_o\{1 - T + \delta(2 - T)\};$$
$$S_o = \text{Intercept, while T} = \text{Slope of PSD.}$$

$$S_{eq} = S_o\{1 + K_{11}[L](1 + 2\delta)\}.$$

Minimize SSE = $\sum (S^P_{eq} - S_{eq})^2$ by letting S_o and δ change to their best-fit estimates.

Input initial guesses: S_o = Intercept, $\delta = 0.1$.

Region II of $\mathbf{B_S}$ type PSD (Either S_2L or SL complex is saturated):

$$[S] = S_o = \text{constant}; \quad L_{eq} = L_m = \text{constant};$$
$$S_{eq} = S_m = \text{constant}$$

$$[L] = [L_m] = L_m/\{1 + K_{11}S_o(1 + \delta)\}.$$

Region III of $\mathbf{B_S}$ type PSD (either S_2L or SL complex is saturated):

Two cases may be considered:

(a) $\mathbf{S_2L}$ complex is saturated ($K_{sp\ 21} = S_o^2[L_m]$):

$$L_{eq} = L_t - (L_p - L_m) - (1/2)\,(S_m - S_{eq}),$$
$$[SL_2] = [SL_2]_m = K_{11}K_{21}K_{sp\,21}$$

$$[S] = (1/2)\,\{b_1 - (b_1^2 - 4K_{11}K_{sp\,21})^{1/2}\};$$
$$b_1 = S_{eq} - 2K_{11}K_{21}K_{\,21}$$

$$[L] = K_{sp\,21}/[S]^2$$

$$S_{eq} = [S]\{1 + K_{11}[L](1 + 2\delta)\}$$

(b) **SL** complex is saturated ($K_{sp\ 11} = S_o[L_m]$):

$$L_{eq} = L_t - (L_p - L_m) - (S_m - S_{eq}),$$
$$[SL] = [SL]_m = K_{11}S_o[L_m] = K_{11}K_{sp\,11}$$

$$[L] = (1/2)\,(\{b - (b^2 - 4c)^{1/2}\};$$
$$b = L_{eq} - K_{11}K_{\,11}; \quad c = K_{11}K_{21}(K_{sp\,11})^2$$

$$[S] = K_{sp\,11}/[L]$$

Minimize SSE = $\sum (S^P_{eq} - S_{eq})^2$ by letting S_o and δ change.

Input initial guesses:

S_o = Intercept, $\delta = 0.1$.

4. $\mathbf{S_2L_2}$ complex stoichiometry ($\mathbf{A_L}$ or $\mathbf{A_P}$ type PSD)

$$S + L \rightleftharpoons SL, K_{11} = [SL]/[S][L] \quad (1)$$

$$SL + SL \rightleftharpoons S_2L_2, \quad K_{22} = [S_2L_2]/[SL]^2 \quad (4)$$

$$[S] = S_o = \text{constant}, \quad [L] = L_{eq} - (S_{eq} - S_o),$$
$$L_{eq} = L_t$$

$$S_{eq} = S_o\{1 + K_{11}[L](1 + K_{11}K_{22}S_o[L])\}$$

Minimize SSE = $\sum (S^P_{eq} - S_{eq})^2$ by letting S_o, K_{11} and K_{22} change.

Input initial guesses: S_o= a, K_{11} = a / S_o, K_{22} = b/a^2, where a and b are the intercept and slope, respectively, of a plot of (S_{eq}– S_o) / L_{eq} against L_{eq}.

5. **SL_3complex stoichiometry** (**A_P** type PSD)

$$S + L \rightleftharpoons SL, \quad K_{11} = [SL]/[S][L] \tag{1}$$

$$L + SL \rightleftharpoons SL_2, \quad K_{12} = [SL_2]/[L][SL] \tag{2}$$

$$L + SL_2 \rightleftharpoons SL_3, \quad K_{13} = [SL_3]/[L][SL_2] \tag{5}$$

$$[S] = S_o, \quad L_{eq} = L_t$$

$$[L] = \{b + (b^2 - 4a.c)^{1/2}\}/2a, a = K_{11}K_{12}$$
$$S_o, b = 1 - 2K_{11}S_o, c = L_{eq} - 3\{S_{eq} - S_o)$$

$$S_{eq} = S_o\{1 + K_{11}[L](1 + K_{12}[L](1 + K_{13}[L]))\}$$

$$\text{Minimize SSE} = \sum (S_{eq}{}^P - S)^2 \text{ by letting}$$
$$S_o, K_{11}, K_{12}, \text{ and } K_{13} \text{ change.}$$

Input initial guesses: S_o = Intercept of PSD, while reasonable initial estimates of the complex formation constants ($K_{n,m}$; n = 1, 2 , 3; m = 1), that have been found to converge to their unique best-fit estimates for guest/CD complexes of SL_3 stiochiometry, are set at one order of magnitude lower for successive higher order complexes (i.e., $K_{n+1,m}/K_{n,m}$ = 0.1; n = 2, 3; m = 1) and K_{11} = 100.

6. **S_2L_3 complex stoichiometry**

$$S + L \rightleftharpoons SL, \quad K_{11} = [SL]/[S][L] \tag{1}$$

$$L + SL \rightleftharpoons SL_2 \ , \quad K_{12} = [SL_2]/[L][SL] \tag{2}$$

$$SL + SL_2 \rightleftharpoons S_2L_3 \ , \quad K_{23} = [S_2L_3]/[SL][SL_2] \tag{6}$$

A_P type PSD, or **Region I** of **Bs** type PSD:

$$[S] = S_o$$
$$[L] = \{-b + (b^2 + 4ac)^{1/2}\}/2a; a = K_{11}K_{12}S_o;$$
$$b = 2 - K_{11}S_o, c = 2L_{eq} - 3(S_{eq} - S_o)$$
$$S_{eq} = S_o\{1 + K_{11}S_o[L](1 + K_{12}[L](1 + 2K_{23}S_o[L]))\}$$
$$\text{Minimize SSE} = \sum (S_{eq}{}^P - S_{eq})^2 \text{ by letting}$$
$$S_o, K_{11}, K_{12}, \text{ and } K_{23} \text{ change.}$$

Input initial guesses: S_o = Intercept ofPSD, $K_{11} = 100$, $K_{12} = 10$ and $K_{23} = 1$.

Region II of **B_S** type PSD (S_2L_3 complex is saturated):

$$[S] = S_o = \text{constant}, \quad L_{eq} = L_m = \text{constant},$$
$$S_{eq} = S_m = \text{constant}$$

$$[L_m] = \{-b + (b^2 + 4ac)^{1/2}\}/2a; a = K_{11}K_{12}S_o, b$$
$$= 1 + K_{11}S_o, c = L_m - 3K_{11}K_{12}K_{23}K_{sp\,23}.$$

Region III of **B_S** type PSD (S_2L_3 complex is saturated, $K_{sp\ 23} = S_o^2\ [\ L_m]^3$):

$$[L] = -1 + (1 + 4ac)^{1/2}/2a; a = K_{11}K_{12}[S], c$$
$$= L_{eq} - S_{eq} - K_{11}K_{12}K_{23}K_{sp\,23} + [S]$$
$$[S] = (K_{sp\,23}/[L]^3)^{1/2}; \quad \text{Iterate [S], a and c.}$$
$$S_{eq} = S_o\{1 + K_{11}S_o[L](1 + K_{12}[L](1 + 2K_{23}S_o[L]))\}$$
$$\text{Minimize SSE} = \sum (S_{eq}{}^P - S_{eq})^2 \text{ by changing}$$
$$S_o, K_{11}, K_{12}, \text{ and } K_{23}.$$

Input initial guesses: S_o = Intercept of PSD, and practically reasonable initial estimates of $K_{n,m}$ that have been found to converge to their unique best-fit estimates for guest/CD complexes of S_2L_3 stoichiometry are: K_{11} = 100, K_{12} = 0.1 K_{11}, and K_{23} = 0.01 K_{11}.

7. **S_3L_2complex stoichiometry** (**A_L** or **A_P** type PSD, or **Region I** of **B_S** type PSD):

$$S + L \rightleftharpoons SL, \quad K_{11} = [SL]/[S][L] \tag{1}$$

$$S + SL \rightleftharpoons S_2L, \quad K_{21} = [S_2L]/[S][SL] \tag{3}$$

$$SL + S_2L \rightleftharpoons S_3L_2, \quad K_{32} = [S_3L_2]/[SL][S_2L] \tag{7}$$

$$[S] = S_o; \quad L_{eq} = L_t$$
$$[L] = \{-b + (b^2 + 4aL_{eq})^{1/2}\}/2a; a = 3K_{11}K_{21}K_{32}S_o^3,$$
$$b = 1 + K_{11}S_o(1 + K_{21}S_o)$$
$$S_{eq} = S_o\{1 + K_{11}S_o[L](1 + K_{21}S_o(2 + 3K_{32}S_o[L]))\}$$
$$\text{Minimize SSE} = \sum (S_{eq}{}^P - S_{eq})^2 \text{ by letting}$$
$$S_o, K_{11}, K_{21}, \text{ and } K_{32} \text{ change.}$$

Input initial guesses: S_o = Intercept of PSD, and reasonable initial estimates of $K_{n,m}$ that have been found to converge to their unique best-fit estimates for S_3L_2 guest/CD complex stoichiometry are: K_{11} = 100, K_{21} = 0.1 K_{11} and K_{32} = 0.01 K_{11}.

8. **S_2L_4complex stoichiometry** (**A_L** or **A_P** type PSD):

$$S + L \rightleftharpoons SL, \quad K_{11} = [SL]/[S][L] \tag{1}$$

$$L + SL \rightleftharpoons SL_2, \quad K_{12} = [SL_2]/[L][SL] \tag{2}$$

$$SL_2 + SL_2 \rightleftharpoons S_2L_4, K_{24} = [S_2L_4]/[SL_2]^2 \tag{8}$$

This type of PSD has not yet been observed for soluble CD complexes, although solid complexes of S_2L_4 stoichiometry have been isolated (e.g., 2:4:1 terfenadine/β-CD.tarataric acid solid complex) [13]. These 2:4 drug/β-CD complexes exist in solution as two soluble 1:2 (SL_2) complexes of a protonated monobasic drug substrate (an ammonium ion, S = BH^+) binding two CDs, where the two ammonium ions are electrically attracted to two carboxylate anions of a diprotic or a triprotic carboxylic acid (e.g., tartaric or citric acids). The two soluble SL_2 fragments are completely separate in aqueous solution, and the PSD obtained is that of an SL_2 type PSD discussed in part 2 above.

Conclusion

The final equilibrium relations required to analyze phase solubility diagrams (PSDs) pertaining to different guest/host complex stoichiometries have been reported next to each PSD type (A-, B_s- and B_I-type PSDs). These relations correspond to rigorous nonlinear regression analysis of those PSDs that have been reported in the literature on aqueous guest/cyclodextrin complexes, which were obtained under controlled conditions of buffer concentration, pH, ionic strength and temperature. They allow obtaining accurate estimates of thermodynamic complexation parameters, including complex formation constants and complex stoichiometry of soluble complexes, in addition to the stoichiometry and solubility product (K_{sp}) of the complex that reaches saturation in B_s-and B_I-type PSDs. No approximations concerning soluble and insoluble complex stoichiometries were imposed in this analysis, which offers simple and suitable rigorous procedures for application by interested workers in the field.

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