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Spectrophotometric study of competitive complexation equilibria involving overlapped spectral responding species: Determination of the stability constant of bismuth-pyrophosphate complex

Research Article

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Abstract: Spectrophotometric study of competitive complex formation equilibria involving overlapped spectral responding species applying a simple and versatile algorithm was carried out. The algorithm involves multivariable regression for calculation of equilibrium concentrations from multiwavelength data and mass action law for the stability constant calculation. The used regression functions are part of common statistical software. Stability constants and complex stoichiometry of competing equilibria were simultaneously determined. The species concentration profiles at several spectral overlapping and α-coefficient of competing reaction were obtained. Non-absorbing bismuth – pyrophosphate (PPh) system was studied as a competitive reaction of bismuth - 4-(2-Pyridylazo) resorcinol (PAR) complex. The formation of Bi-PPh complex with 1:1 stoichiometry was proved in the studied concentration region (C_{Bi} = 1×10^{-5} mol L^{-1}; C_{PPh} = 5×10^{-6} – 1×10^{-4} mol L^{-1}). The stability constant of the complex at pH 1 and μ= 1.0 have been determined: logβ = 4.2±0.2.

Keywords: *Stability constant determination • Overlapping spectra • Competitive spectrophotometry • Bi-PAR • Bi-pyrophosphate*

1. Introduction

The estimation of stability (binding) constants is of interest in many areas of chemistry. Quantitative data for acid-base, metal-ligand or enzyme-substrate equilibria are invaluable for analytical chemistry, industrial chemistry, biochemistry, *etc*. A wide range of methods have been applied for determination of stability constants: potentiometry with pH or ion-selective electrodes [1,2], voltammetry [3,4], spectrophotometry [1,5-9], NMR [9] or their combinations with chromatography [10,11] and electrophoresis [12]. Spectrophotometric measurements are widely used not only for the availability of equipment used, but also for the possibility to show how many equilibria exist in solution studied as well as to discriminate between different models of equilibrium [13].

Combination of spectrophotometry and chemometrics is well recognized as a powerful technique for studying complex formation equilibria with overlapping spectral responses [5-8]. Meloun *et al*. [8] have introduced a specially designed computer program for the determination of the number of light-absorbing species in the recorded spectra data matrix. The calculation of equilibrium concentrations and stability constants (β) involves many iteration steps starting with the equilibrium constants of chemically similar system, and proceeding with calculating equilibrium constants using least squares methods [6,7]. Computer programs SQUAD [14], EQUISPEC [5,15-17], SPECFIT [18] or modified MATLAB [19-21] have been specially created for processing the absorbance data from multicomponent systems. Equilibrium constants of non-adsorbing chemical systems have been also

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determined from competitive spectrophotometric data [19-26]. Methods of competing/displacement reactions follow two approaches: a) the known equilibrium of the system metal – ligand (M-L) is affected by second metal M' [27] and b) known equilibrium of M-L system is affected by second ligand L' [19,22,23,26,28]. The wide range of experimental approaches has produced a wide range of experimental data and modes of calculations. Although excellent powerful computer programs providing detailed information about complex formation equilibria are available, for the occasional user or for fast tackling of non-standard situations an algorithm using common software functions would be good choice. Microsoft Excel functions have been thoroughly described for binding constant calculation [29]. Recently we have proposed a simplified algorithm for the stability constant determination of 1:1 complex from overlapping spectra of the complex and the ligand using multivariable regression of common statistical software for calculation of equilibrium concentrations and the mass action law for stability constant calculation [30].

This report presents the results of studying complex formation equilibria of non-absorbing Bi-Pyrophosphate (PPh) system by monitoring the spectra of competitive system Bi^{3+} - 4-(2-Pyridylazo) resorcinol (PAR) and using a modified version of the early proposed algorithm. Although the high biological and medical impact of Bi and pyrophosphate compounds as well as the intensive research on bismuth compounds [31] or on pyrophosphate complexes [32], quantitative data about Bi-pyrophosphate system have not been published so far.

2. Theoretical details

2.1. Stability constant determination in a system with overlapping spectral responses of two chemical species (one step complex formation)

Consider an equilibrium $M+L \Leftrightarrow ML$, the stability constant *β* of ML complex is calculated by the equations [33]:

$$A = b(\varepsilon_{ML}[ML] + \varepsilon_L[L]) \qquad (1)$$

$$C_M = [M] + [ML] \qquad (2)$$

$$C_L = [L] + [ML] \qquad (3)$$

$$\beta = \frac{[ML]}{[M]\,[L]} \qquad (4)$$

where C_M and C_L are the known total concentrations of the metal and the ligand; [M], [L] and [ML] are the equilibrium concentrations of the metal, the ligand and the complex; A is the additive absorbance of the complex and the ligand measured at the selected wavelengths; b is the width of the absorbing layer. ε_L is the molar absorptivity of the ligand, determined by measuring the absorbance of a series of ligand solutions at different concentrations. ε_{ML} is the molar absorptivity of the complex, determined in solutions in which the ligand is totally transformed into ML.

Regression of two variables (orthogonal regression) is applied to Eq. 1 for the calculation of the equilibrium concentrations of metal and ligand. The spectral data are obtained from measuring the absorbance of a series of solutions at C_M=const and varied C_L at different wavelengths chosen applying a criterion dA/dλ≈0. The stability constant is calculated at every molar ratio of M and L and a reliable mean value is presented. The equation for calculation is derived from Eqs. 2–4:

$$\beta = \frac{[ML]}{[L](C_M - [ML])} \qquad (5)$$

2.2. Studying a complex equilibrium of non-absorbing species (Competitive complex formation)

2.2.1. Choice of chemical model

The choice of a chemical model in a dual ligand - one metal system is based on the absorbance spectra of the systems M-L and L'-M-L where two ligands compete for the same metal. The spectra of the system L'-M-L at different concentrations of L' are used. The second competing reaction can be supposed, if the absorbance changes when the concentration of second ligand (L') is varied. These changes are directly related to the equilibrium concentrations of the absorbing ligand (L) in the system.

2.2.2. Estimation of equilibrium concentrations of components of dual ligands-one metal system

The equilibrium concentrations of the metal in the system in the presence and in the absence of the second ligand (L') can be different due to the completion of the competing reaction. The equilibrium concentration of M when two competing reactions proceed is estimated from equations [34]:

$$A = b(\varepsilon_{ML}[ML]_{L'} + \varepsilon_L[L]_{L'}) \quad (6) \qquad (6)$$

$$C_M = [M]_{L'} + [ML]_{L'} \quad (7) \qquad (7)$$

$$C_L = [L]_{L'} + [ML]_{L'} \qquad (8)$$

where $[M]_{L'}$ is the equilibrium concentration of M non-participating in the ML formation reaction, the subscripts L' stands for an equilibrium with second ligand L'. The equilibrium concentrations can be calculated applying regression of two variables to the spectral data obtained in a dual ligands-one metal system.

2.2.3. Estimation of the stability constants of the non-absorbing complexes

The ratio between the equilibrium concentrations of M in the system with and without second ligand (L'), known as α-coefficient, is used for calculation of the stability constants and the stoichiometry of the competing non-absorbing complexes [35]:

$$\alpha = \frac{[M]_{L'}}{[M]} = 1 + \Sigma\beta_{ML'_j} . [L']^j \quad (9) \qquad\qquad (9)$$

The procedure involves:

a) calculation of the equilibrium concentrations of L' from the changes of absorbance of L supposing that the differences in L concentration are equal to the sum of formed M-L' complexes:

$$[L'] = C_{L'} - \sum [ML']_j = C_{L'} - \Delta[L].$$

b) finding the best fit equations for the function from Eq. 9 using the values of the correlation coefficient as a fit criteria. The order and the coefficients of the polynomial correspond to the stoichiometry and the stability constants of formed complexes, respectively.

3. Experimental procedure

The working solutions of the system PPh-Bi-PAR were prepared mixing Bi^{3+} and PAR solutions in equal concentrations (1×10^{-5} mol L^{-1}) at pH 1 (0.1 mol L^{-1} HNO_3) and μ 1.0 (1 mol L^{-1} KNO_3) and adding volumes of pyrophosphate solution to achieve concentrations from 5×10^{-6} to 1×10^{-4} mol L^{-1} $P_2O_7^{4-}$. A dual-beam Perkin-Elmer spectrophotometer with 4-cm cuvette was used for spectra recording. Distilled water was used as a reference. All solutions were prepared with distilled water. All reagents were of analytical grade. All calculations were performed using MATLAB 6.5 software.

4. Results and discussion

In competitive spectrophotometric experiment two systems were included. One was so called "unknown" non-absorbing Bi-PPh system and second was "known" system Bi-PAR. The choice of known system was based on some prerequisites: (1) a well-defined complex of moderate stability with composition 1:1 was formed [30,36] and (2) the equilibrium was effected in the presence of second ligand [37].

4.1. Stability constant determination in Bi-PAR system with overlapping spectral responses of the ligand and the complex

The equilibrium concentrations of the BiPAR and the PAR have been determined using a regression of two variables applied to the data from the measurement of the absorbance of solutions at different molar ratios C_{Bi}/C_{PAR} [30]. The regression equation is a multicomponent Beer's law expression: $A = K_{PAR}[PAR] + K_{BiPAR}[BiPAR]$. In this equation $K_{PAR}=b\varepsilon_{PAR}$ and $K_{BiPAR}=b\varepsilon_{BiPAR}$ were determined as described previously [30]. The calculated equilibrium concentrations of Bi(III), PAR and BiPAR are presented in Fig. 1. Additionally, a pure spectrum of the complex can be obtained using the calculated equilibrium concentrations of BiPAR. The calculated stability constant of BiPAR complex at μ=1.0 and pH=1 is log β=6.00±0.03. The initial hypothesis for 1:1 complex formation is proved using a function

$$C_{Bi(III)} \frac{[PAR]}{[BiPAR]} = \frac{1}{\beta} + [PAR],$$

derived from the Eq. 5. The function is linear in the range (1÷6) $\times10^{-6}$ M PAR with a regression equation:

$$C_{Bi(III)} \frac{[PAR]}{[BiPAR]} = (0.87\pm0.08)\times10^{-6} + (1.04\pm0.04)[PAR]$$

and a regression coefficient r=0.98 (P=95%; n=9).

4.2. Stability constants of complex formation equilibria with non-absorbing species

4.2.1. Chemical model

The absorbance spectra of Bi-PAR system with and without added pyrophosphate (PPh) are presented at Fig. 2. Pyrophosphate causes decrease in the absorption measured at λ=516 nm (wavelength where BiPAR complex absorbs). In contrast, at λ=403 nm (wavelength of maximum absorption of PAR) an increased absorbance was observed. Because of the absorbance variations a transformation of a part of the complex BiPAR into the complex BiPPh is assumed. As a result of the competing reaction the concentration of free PAR is increased. Both competing equilibria can be described by the following scheme:

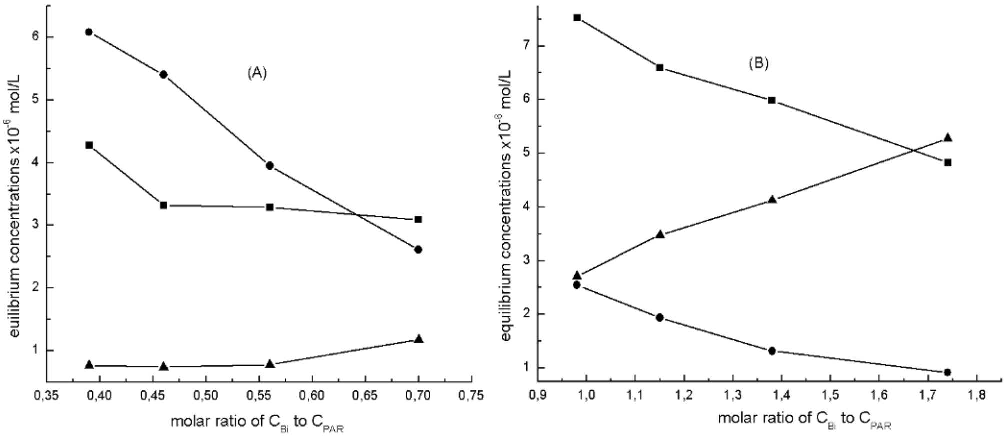

Figure 1. Equilibrium concentrations of BiPAR(■), Bi^{3+} (▲) and PAR (●) at different molar ratios of Bi/PAR and two concentrations of Bi(III): (A) $C_{Bi}=4\times10^{-6}$ mol L^{-1} and (B) $C_{Bi}=1\times10^{-5}$ mol L^{-1}.

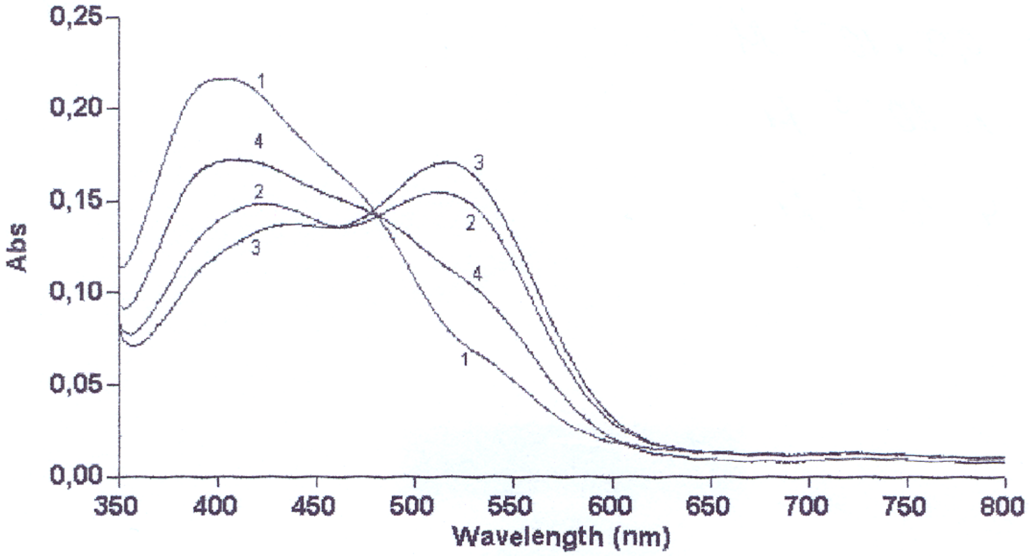

Figure 2. Absorption spectra of PAR-Bi-Pyrophosphate system: (1) pure 9.0×10^{-6} mol L^{-1} PAR; (2) 9.0×10^{-6} mol L^{-1} PAR and 1×10^{-5} mol L^{-1} Bi^{3+}; (3) 9.0×10^{-6} mol L^{-1} PAR and 1.05×10^{-4} mol L^{-1} Bi^{3+} ; (4) 9.0×10^{-6} mol L^{-1} PAR, 1.05×10^{-4} mol L^{-1} Bi^{3+} and 4×10^{-5} mol L^{-1} $P_2O_7^{4-}$ at b=1 cm, pH=1 and μ=1.0.

$$
\begin{array}{ll}
Bi^{3+} + PAR \Longleftrightarrow BiPAR & - \text{ main reaction} \\
+ & \\
iP_2O_7^{4-} & - \text{ side reaction} \\
\uparrow\downarrow & \\
Bi(P_2O_7)_i &
\end{array}
$$

Both ligands participate additionally in protolytic equilibria. The last have been omitted in the scheme for simplicity.

4.2.2. Equilibrium concentrations of the components of PAR-Bi-PPh system

The absorbance of the system PAR-Bi-PPh in the solutions containing Bi^{3+} and PAR at constant concentrations and PPh at varied concentrations (1÷10)×10^{-5} mol L^{-1} was measured. The equilibrium concentrations of Bi^{3+}, PAR and BiPAR were calculated from the Eqs. 6-8 applying multivariable regression to the multiwavelength data. The results are presented in Table 1.

4.2.3. Stoichiometry and stability constants of Bi-PPh complexes

The α-coefficient of the side reactions was estimated as a ratio between equilibrium concentrations of Bi^{3+} in the system with and without added PPh (Eq. 9). The equilibrium concentrations of PPh were calculated from the changes of absorbance of PAR at different PPh concentrations: $[PPh]=C_{PPh}-\Delta[PAR]$. The results are presented in Table 1. The concentration profiles of Bi, PAR, BiPAR and PPh are presented in Fig. 3. The function from Eq. 9 was analysed using linear regression.

Table 1. Equilibrium concentrations of the components of the competing system PAR-Bi-pyrophosphate and estimated α-coefficients at different concentrations of $P_2O_7^{4-}$ (PPh) and C_{Bi}=1.01×10^{-5} M; [PAR]= 2.64×10^{-6} M at μ=1.0

C_{PPh}, ×10^{-5} M	$[BiPAR]_{PPh}$ ×10^{-6} M	$[PAR]_{PPh}$ ×10^{-6} M	Δ[PAR]^a ×10^{-6} M	[PPh] ×10^{-5}M	$[Bi]_{PPh}$ ×10^{-6} M	$\alpha = \frac{[Bi]_{PPh}}{[Bi]}$
C_{PAR}=1.05×10^{-5} M; [Bi]=2.64×10^{-6} M						
1.03	7.09	3.33	0.69	0.961	3.01	1.56
2.41	6.53	3.99	1.35	2.28	3.57	2.40
3.44	6.21	4.49	1.85	3.26	3.89	3.10
4.13	5.76	5.10	2.46	3.88	4.34	4.23
5.13	4.74	5.51	2.87	4.84	5.36	6.87
6.84	4.23	6.22	3.60	6.48	5.87	9.56
10.35	3.25	7.23	4.60	9.89	6.85	16.8
linear regression: α=(1.03±0.2) + (1.7±0.3)×10^4[PPh] (r=0.9795; n=7; P=95%)						
C_{PAR}=9.00×10^{-6} M; [Bi]=3.50×10^{-6} M						
2.41	4.48	4.00	2.06	2.20	5.60	1.60
3.44	4.10	4.25	2.31	3.21	6.00	3.21
4.13	3.33	4.91	2.97	3.83	6.80	3.83
5.13	2.99	5.21	3.27	5.10	7.11	5.10
6.84	2.33	5.77	3.83	6.80	7.70	6.80
linear regression: α=(1.2±0.2)+(1.6±0.4)×10^4 [PPh] (r=0.9879; n=5; P=95%)						

^{a} *Δ[PAR]= $[PAR]_{PPh}$ - 2.64×10^{-6}*

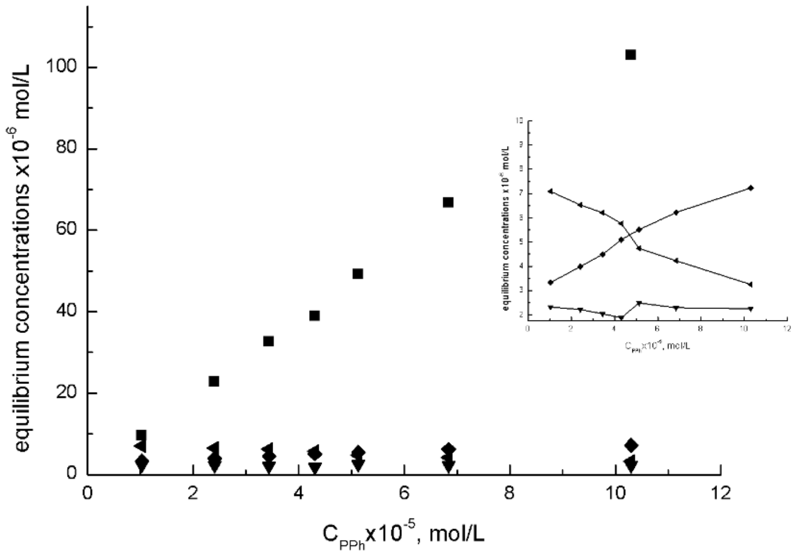

Figure 3. Equilibrium concentrations of BiPAR complex (◀), Bi^{3+} (▼), PAR (♦) and pyrophosphate (PPh)(■) at different total concentrations of PPh. In-set: enlarged profiles of BiPAR, Bi^{3+} and PAR.

The regression equations at total concentrations of PAR 1.05×10^{-5} M and 9×10^{-6} M are given in the Table 1. Good linear fit is observed (r=0.98). The formation of one complex between Bi^{3+} and PPh with stoichiometry 1:1 was obtained. The estimated value of stability constant of BiPPh complex is (1.7±0.4)×10^4 (P=0.95; f= 6).

4.3. Polarographic study of complex formation equilibria of Bi-PPh system

Complex formation equilibria of Bi-PPh system were studied also by differential pulse polarography (static mercury dropping electrode; amplitude -50 mV; U.Step 6 mV; T.Step 0.6 s) using the method given in [38]. The analysis were performed with the 646 VA Processor and the 647 VA Stand from Metrohm (Switzerland). The experiments were carried out in solutions with pH 1 (0.1 M HNO_3) and μ = 1 (1 M KNO_3). The temperature of the solutions was 20°C. Bi(III) concentration was 1×10^{-5} mol L^{-1} and the total pyrophosphate concentration was varied from 1.4×10^{-4} to 1×10^{-3} mol L^{-1}. The quasireversible reduction of Bi(III) in studied solutions was proved from the width of the left half of the Bi(III) peak and a modified Leden's function was applied:

$$F_0 = \exp\frac{zF}{RT}\Delta E_p = \sum_{j=0}^{n}\beta_j^{'}C_{PPh}^{j}$$

where $\Delta E_p = E_p^c - E_p^s$; E_p^c and E_p^s are the peak potentials of the complex (c) and simple (s) metal ions, respectively, at reversible or quasireversible reduction; C_{PPh} is the total ligand concentration; $\beta_j^{'}$ is the formal stability constant of $Bi(PPh)_j$ complex. The regression equation of F_0-function was 0.9 + 6.2×10^3 + 1.8×10^7. Hence, two complexes BiPPh and $Bi(PPh)_2$ were observed from 10 to 100 molar excess of PPh against Bi(III). The obtained stability constants were β_1= (6.2±1.2)×10^3 and β_2= (1.8±0.5)×10^7 (P=0.95; f=5), respectively. The value of the stability constant of the first complex BiPPh (logβ = 3.8±0.2) is close to the value (log β = 4.2±0.2) obtained by the proposed algorithm in solutions, containing PPh and Bi(III) at molar ratios from 0.5 to 10.

5. Conclusion

An algorithm for spectrophotometric study of competitive complex formation equilibria involving overlapped spectral responding species has been proposed. The simplified algorithm allows the overlapped spectrophotometric data to be mathematically treated using multivariable regression function of common statistical software. Stability constants, complex stoichiometry of competitive equilibria and equilibrium concentration profiles of involved species can be determined. Additionally, the stability constants of the main and the side reactions can be simultaneously determined under unchanged experimental conditions. The stability constant of potentially biologically active bismuth – pyrophosphate complex at pH 1 and μ= 1.0 have been estimated.

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