

Medium Effects on the Stability of Terbium (III) Complexes with Pyridine-2,6-Dicarboxylate

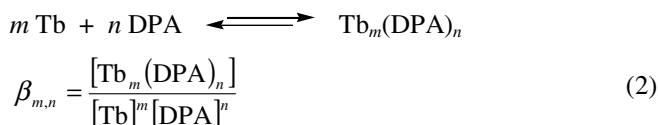
(Supporting Information)

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Complexation equilibrium models

One-step equilibrium model. A one-step equilibrium that leads to formation of a polynuclear complex containing m terbium ions and n DPA ligands, $\text{Tb}_m(\text{DPA})_n$, can be represented with the overall formation constant $\beta_{m,n}$:



The total concentrations of terbium (III) and DPA (C_{Tb} and C_{DPA} , respectively) can be expressed as sums of the equilibrium concentrations of the present species:

$$C_{\text{Tb}} = [\text{Tb}] + m[\text{Tb}_m(\text{DPA})_n] \quad (8)$$

$$C_{\text{DPA}} = [\text{DPA}] + n[\text{Tb}_m(\text{DPA})_n] \quad (9)$$

Expressing $[\text{Tb}]$ and $[\text{DPA}]$ from eq. 8 and 9, then substituting the results in eq 2, and consequently rearranging, results in the following expression:

$$\beta_{m,n} C_{\text{DPA}}^n (1-R)^n = \frac{R C_{\text{DPA}}}{n \left(C_{\text{Tb}} - \frac{m}{n} R C_{\text{DPA}} \right)^m} \approx$$

$$\approx \frac{R C_{\text{DPA}}}{n C_{\text{Tb}}^m}, \text{ if } C_{\text{Tb}} \gg C_{\text{DPA}} \quad (10)$$

where R is the portion of DPA that is ligated to terbium, i.e., $R = n \frac{[\text{Tb}_m(\text{DPA})_n]}{C_{\text{DPA}}}$, from which the following linear

logarithmic relationship between the total terbium and DPA concentrations, the overall formation constant, and the coefficients m and n is obtained:

$$\lg \left(\frac{R}{C_{\text{Tb}}^m} \right) = n \lg(1-R) + \lg(\beta_{m,n}) + (n-1) \lg(C_{\text{DPA}}) + \lg(n) \quad (3)$$

The linear relationship, described by eq. 3, is applicable to emission data with very low optical density at the excitation wavelength, so that the relations between the emission intensities and the concentrations of the luminophores are linear, i.e., $S_{\text{Tb}_m(\text{DPA})_n} \propto [\text{Tb}_m(\text{DPA})_n]$. Thus, the

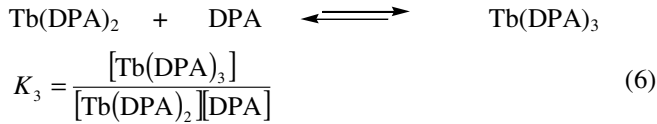
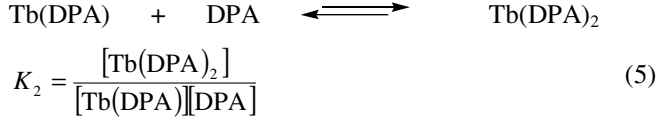
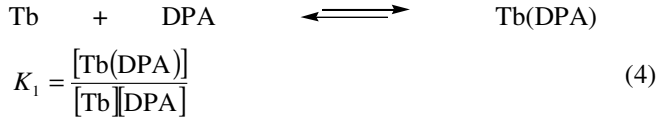
concentration R values were obtained by dividing the observed S by S_{max} , where the latter was extracted from sigmoidal fits by extrapolating S to infinity C_{Tb} , i.e.,

$$R = \frac{S}{S(C_{\text{Tb}} \rightarrow \infty)}.$$

Furthermore, the data treated with eq. 3 ought to be collected under high excess terbium concentration in comparison to C_{DPA} , in order the approximation in eq. 10 to be valid. Also, plausible results can be obtained only if the data represent one-step equilibrium. By choosing m to be a positive integer, the recorded emission intensities were converted to the corresponding logarithmic forms described by eq. 3. The slopes of the linear fits yielded the corresponding parameters n . Concurrently, the formation constants, $\beta_{m,n}$, were extracted from the intercepts of the fits. For most of the conditions (e.g., $\text{pH} < \sim 4$), m was set to unity, implying that observed ligation led to the formation of mononuclear Tb-DPA complexes.

Stepwise equilibrium model. The one-step model described by eq. 3 was used for treatment of titration data where the total DPA concentration was kept between 2 and 25 nM. For the cases of titration of micromolar concentrations of DPA, however, the one-step equilibrium model was not applicable.

It has been established that terbium (III) can ligate up to three moieties of DPA if solely the formation of mononuclear complexes is considered.¹⁷ Hence, the three discrete equilibrium steps with the corresponding stepwise formation constants, K_1 , K_2 and K_3 , are:



Apparently, the stepwise formation constants are related to the corresponding overall formation constants, i.e., $\beta_2 = K_1 K_2$ and $\beta_3 = K_1 K_2 K_3$. Furthermore, it has been reported that DPA has a propensity for self-aggregation in aqueous solution.³² A correction for this process was introduced to the equilibrium model via a DPA dimerization constant, $K_d = \frac{[(\text{DPA})_2]}{[\text{DPA}]^2}$.

Similarly to eq. 8 and 9, the total terbium (III) and DPA concentrations can be expressed as sums of the equilibrium concentrations of the present species:

$$C_{\text{Tb}} = [\text{Tb}] + [\text{Tb(DPA)}] + [\text{Tb(DPA)}_2] + [\text{Tb(DPA)}_3] \quad (11)$$

$$C_{\text{DPA}} = [\text{DPA}] + [\text{Tb(DPA)}] + 2[\text{Tb(DPA)}_2] + 3[\text{Tb(DPA)}_3] + 2[(\text{DPA})_2] \quad (12)$$

Substituting from the expression for the equilibrium constants yields two equations with two unknown:

$$C_{\text{Tb}} = [\text{Tb}] + K_1[\text{Tb}][\text{DPA}] + \beta_2[\text{Tb}][\text{DPA}]^2 + \beta_3[\text{Tb}][\text{DPA}]^3 \quad (13)$$

$$C_{\text{DPA}} = [\text{DPA}] + K_1[\text{Tb}][\text{DPA}] + 2\beta_2[\text{Tb}][\text{DPA}]^2 + 3\beta_3[\text{Tb}][\text{DPA}]^3 + 2K_d[\text{DPA}]^2 \quad (14)$$

Expressing $[\text{Tb}]$ from eq. 13 and substituting in eq. 14 results in a 5th-order equation that can be solved numerically:

$$C_{\text{DPA}} \mathbf{P} = \mathbf{P}[\text{DPA}] + K_1 C_{\text{Tb}} [\text{DPA}] + 2\beta_2 C_{\text{Tb}} [\text{DPA}]^2 + 3\beta_3 C_{\text{Tb}} [\text{DPA}]^3 + 2\mathbf{P}K_d [\text{DPA}]^2 \quad (15)$$

where $\mathbf{P} = \sum_{i=0}^3 \beta_i [\text{DPA}]^i$; $\beta_0 = 1$ and $\beta_1 = K_1$.

Solving eq. 15 yields the equilibrium concentration of DPA, consequently, eq. 13 yields $[\text{Tb}]$, and then from eq.

4 – 6 the equilibrium concentrations of all three Tb-DPA complexes can be calculated. The concentrations of the luminophores, i.e., terbium containing species, can be related to the observed emission intensity via an expression for their quantum yields (similar to eq. 1):²⁷

$$S = \sum_{i=0}^n S_{\text{Tb(DPA)}_i} = lB \frac{1 - 10^{-\sum_j A_j}}{\sum_j A_j} \times \left(\sum_{i=1}^n i \Phi_{\text{Tb(DPA)}_i} \epsilon_b [\text{Tb(DPA)}_i] + \Phi_{\text{Tb}} \epsilon_{\text{Tb}} C_{\text{Tb}} \right) \quad (7)$$

where $\sum_j A_j$ represents the total absorption of the sample at

the excitation wavelength, B is a proportionality constant that depends on the instrumental setup, n is the maximum state of ligation achieved, l is the optical excitation path-length, and ϵ_b is the absorption extinction coefficient of DPA moieties that are bound to terbium ions. The rightmost term represents the contribution to the emission from the directly excited Tb. (The purpose of substituting $[\text{Tb}]$ with C_{Tb} in the last term of eq.15 is to include the luminescence from the direct excitation of terbium (III) that is ligated with DPA.) Obviously, eq. 7 together with eq. 4 – 6, 13 and 15 represent the observed emission intensity as a function of the total terbium and DPA concentrations, and the formation constants.

The stepwise model (eq. 7) was used for treatment of titration data where C_{DPA} was kept in the micromolar range. The values for parameter n , which in this case describes the number of steps, can be determined in advance from the shapes of the emission spectra.¹⁶ For instance, for data collected at pH < 2, n was set to 2 because the luminescence that is characteristic for Tb(DPA)_3 was not observed even when the concentration of DPA exceeded the concentration of Tb^{3+} 100 fold (Figure 13 a and d). At pH = 3.7, however, at low terbium (III) concentrations, the splitting of the emission bands at 488 and 542 nm (${}^7\text{F}_6 \leftrightarrow {}^5\text{D}_4$ and ${}^7\text{F}_5 \leftrightarrow {}^5\text{D}_4$ transitions, respectively) is an indication for the presence of terbium tri-DPA complex (Figure 13b), while at higher terbium (III) concentrations, the loss of fine structure suggests predominant formation of unsaturated complexes. The described splitting of the spectral bands is even more conspicuous at pH 7 (Figure 13c). Therefore, for the data collected under the latter conditions, n (eq. 7) was set to 3.

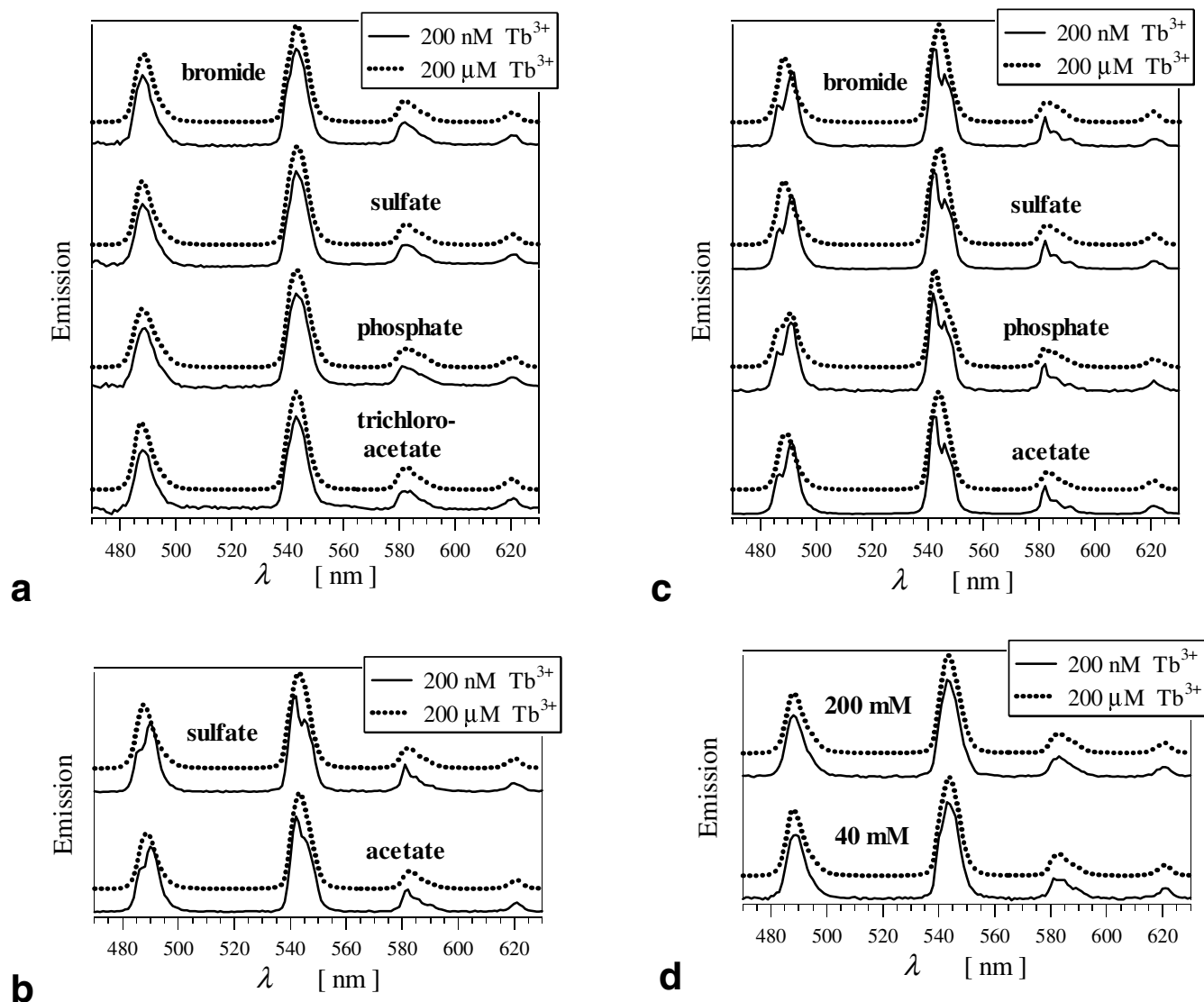
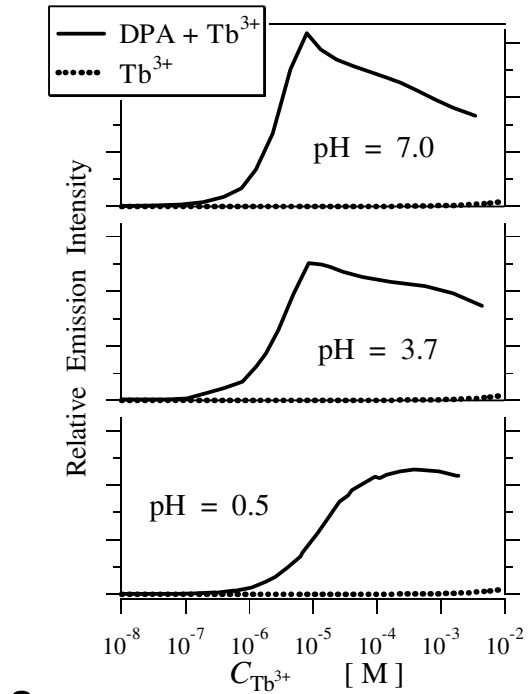
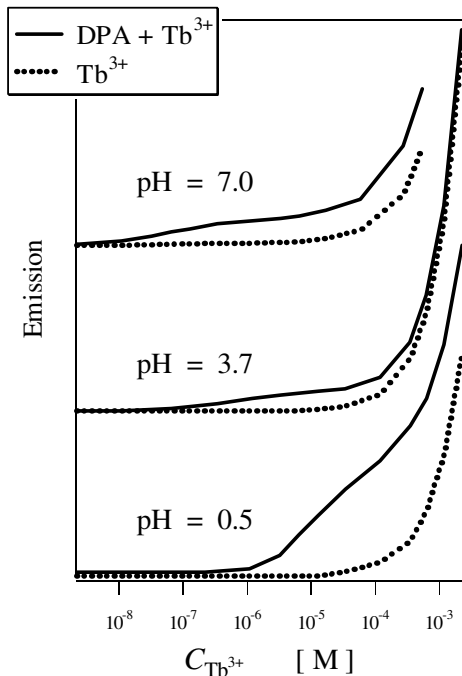


Figure 13. Emission spectra of 20 μM DPA in the presence of ten-fold excess and deficiency of terbium (III) in various 0.2 M buffers with pH equal to: (a) 0.5; (b) 3.7; (c) 7.0; and (d) in 0.2 M and 40 mM sulfate buffer, pH = 1.9 ($\lambda_{\text{ex}} = 266 \text{ nm}$).

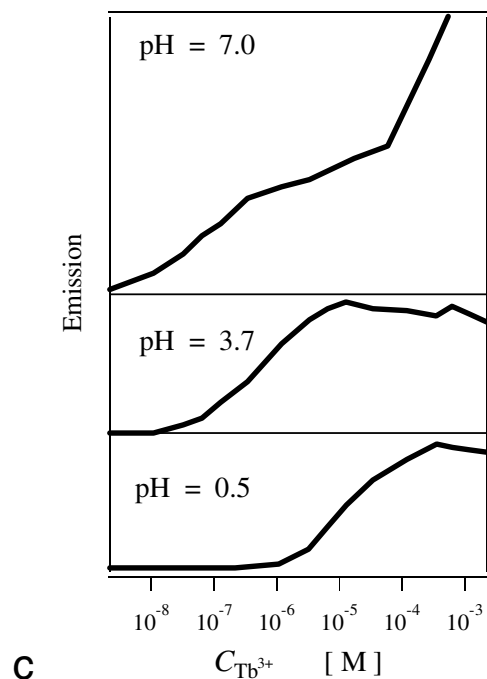


a



b

Figure 14. Comparison of the emission of terbium (III) with the enhanced emission when DPA is present in 200 mM sulfate buffer at various pH values: **(a)** 20 μM DPA, $\lambda_{\text{ex}} = 266$ nm;



c

(b) 2.3 nM DPA (for pH 3.7 and 7.0) and 23 nM DPA (for pH 0.5), $\lambda_{\text{ex}} = 278$ nm; **(c)** corrected titration curves for the corresponding nanomolar DPA concentrations, presented in b, where the emission of Tb^{3+} is subtracted from the emission for DPA + Tb^{3+} .

Correction of the titration curves

The emission enhancement was employed for monitoring the equilibrium concentrations of DPA ligated to terbium ions at various conditions. The analyses were performed on titration data that capture the alteration in the integrated emission intensity, S , when Tb^{3+} is added to samples with fixed concentrations of DPA. For experiments on micromolar amounts of DPA, the observed emission resulted solely from excitation of DPA ligated to terbium; the luminescence due to direct excitation of terbium ions (the second term in the nominator of eq. 1) was not important at $\text{Tb}(\text{III})$ concentrations below ~ 1 mM (Figure 14a). However, when nanomolar concentrations of DPA were titrated, the luminescence from the directly excited terbium ions significantly contributed to the total emission even at micromolar concentrations of Tb^{3+} (Figure 14b). Therefore, the data were corrected by subtraction of the emission measured for terbium (III) in the absence of DPA (Figure 14c).

Titration of terbium (III) with DPA

Although the spectral patterns for the titrations at pH 0.5 did not imply formation of *tris*-Tb-DPA complexes (Figure 13a), when DPA is added in about 100-fold excess to micromolar terbium (III) solutions, emission spectra with splitting of the two most intense bands were observed. Alternative titration experiments were conducted where 7 μM Tb^{3+} was titrated with increasing amounts of DPA. Figure 15 displays the results obtained in the presence of sulfate. Regrettably, the exceedingly high optical densities at the excitation wavelength (due to the high DPA concentrations attained) and possible self-aggregation behavior of the ligating moiety³² prevented reliable quantitative analysis of such type of data. However, from the alteration in the shapes of the emission spectra (which assumingly represents the transition from *bis*- to *tris*-Tb-DPA complexes) that was observed at $C_{\text{DPA}} \sim 1 \text{ mM}$, it can be deduced that the value for K_3 (eq. 6) is about 10^3 M^{-1} . Similar results were obtained for the other pH 0.5 buffer conditions, yielding values for β_3 in the range of $10^{14} - 10^{15} \text{ M}^{-4}$. However, these values are for the apparent formation constants, obtained without taking under consideration DPA self-aggregation. For instance, assuming an aggregation constant for DPA, K_d (eq. 13), of about $10^5 - 10^6 \text{ M}^{-1}$, can result in 4 – 5 orders of magnitude increase in the values for K_3 and β_3 .

When terbium (III) was titrated with DPA at higher pH the data pattern was similar to the one obtained for pH 0.5 (Figure 15).

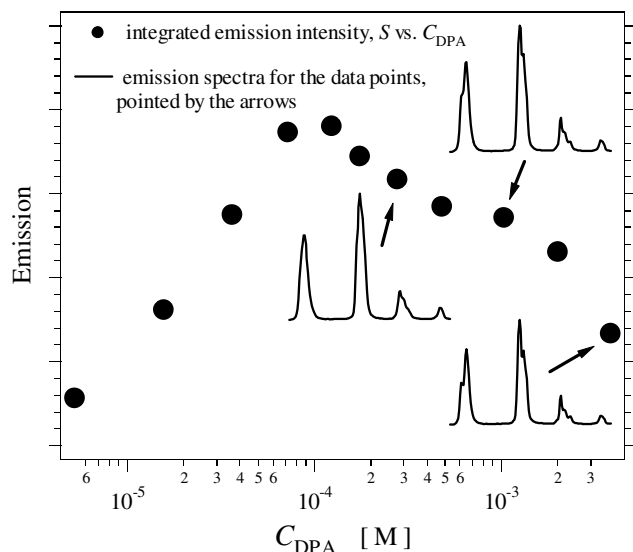


Figure 15. Emission data for 7 μM TbCl_3 titrated with DPA in 0.2 M sulfate buffer, pH = 0.5 ($\lambda_{\text{ex}} = 266 \text{ nm}$); The splitting of the two intensive bands suggests formation of a *tris* complex.

Titration of nanomolar amounts of DPA with terbium (III) at pH 7

The logarithmic analysis (eq. 3) was applied to the region of data covering the first sigmoidal alteration,³³ and logarithmic analysis (eq. 3) was applied to region of data covering the first sigmoidal alteration.³⁰ Since at $m = 1$ the treatments of the data collected for acetate and sulfate yielded integer coefficients n , each set of data was converted to a series of logarithmic functions $\lg(R / C_{\text{Tb}}^m)$, where m was varied from 1 to 4. When plotted against $\lg(1 - R)$, only the data collected for acetate buffer resulted in conspicuous linearity at only one value for m (i.e., $m = 1$) and when the coefficient m was set to 2, 3, or 4, apparent curvature in the plots suggested that the higher values for m should be disregarded (Figure 16).

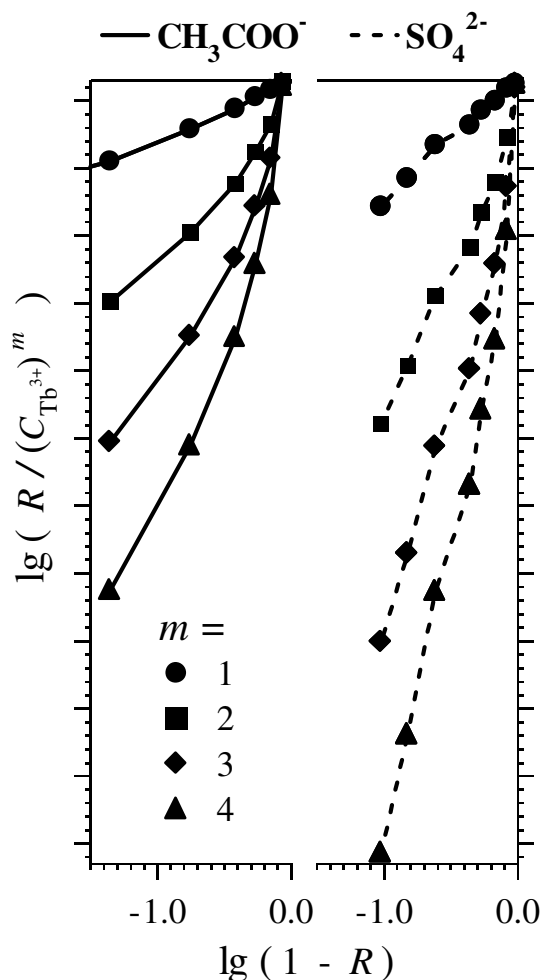


Figure 16. Emission data, converted to the logarithmic function describing the one-step complexation model (eq. 6, $m = 1 - 4$) for 2.3 nM DPA titrated with terbium (III) in 0.2 M acetate and sulfate buffers, pH = 7.0.

Unfortunately, in the rest of the cases, the analyses resulted in significant ambiguity; i.e., the plots were not only linear for all four values of m , but also the slopes of the fits yielded values for n that are close to an integer number for more than one possible value for m (Table 3). The results indicate that for neutral pH only ligation with DPA in the presence of acetate (and/or perhaps in sulfate) leads to the formation of mononuclear Tb-DPA complexes.

For the complexation in acetate the ratio between the two coefficient, m/n , is practically unity, and in sulfate it is about 0.5. From the original data curves (Figure 8, a and b) it is obvious that the steepness of the first transition step for the measurements in acetate and sulfate are smaller than steepness observed in the rest of the buffer conditions. Therefore, it can be expected that m would be larger than n in the presence of halides, perchlorate and nitrate,²⁹ i.e., insinuating that under the latter conditions the ligation process leads to the formation of polynuclear Tb-DPA complexes. Indeed, this conclusion is supported by the

results obtained from the linear logarithmic analyses (Table 3). Such a finding is in concord with previous reports on the tendency of terbium (III) to form polynuclear complexes in this particular pH range.^{15,34}

The second transition, depicted by the increase of the measured emission at C_{Tb} of $\sim 10^{-5} - 10^{-3} \text{ M}^{-1}$ (Figure 8), is most certainly a corollary of conjugating of more terbium ions to the Tb-DPA moieties producing complexes with larger emission quantum yields. Such a result should not be surprising since it has been reported that the emission intensities observed for DPA containing complexes can be substantially increased by addition of another rare-earth (or yttrium) ion.³ In this case, the titration data (Figure 8) suggest the formation of analogous highly emissive poly-homonuclear complexes. However, because of the unattainability of high enough terbium concentrations at neutral pH, the second equilibrium step could not be pushed to completion in order to obtain data needed for a reliable quantitative analysis.