Spectrophotometric Determination of Iron(III)–Glycine Formation Constant in Aqueous Medium Using Competitive Ligand Binding

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In his seminal work Ramette described spectrophotometric determination of the formation constant of monothiocyanatoiron(III) *(1),* which, in presence of another weakly coordinating ligand, L^-, is likely to exist in equilibrium with $Fe(L)^{2+}$. In such a mixed-ligand environment the color-developing Fe(III)–SCN^- reaction can be used as an indicator reaction to determine the formation constant of $Fe(L)^{2+}$. This competitive binding forms the basis of this study. The formation constants of metal–ligand interactions play decisive roles in the speciation of metal ions in the environment as well as their selective uptake, transport, and storage by the living organisms. Various complex-forming agents compete for binding with the soluble iron(III) present in extremely low concentration in the living cell as well as outside in the environment *(2–4).* The degree of iron complexation with a particular ligand critically depends on its formation constant, which in turn depends on the oxidation state of iron, the pH of the medium, and the nature of the ligand itself.

Amino acids form complexes with Fe(III) that are known to exist in equilibrium with the free metal ions. Although quantitative information about the iron–amino acid formation constants is desired in many studies, the complexes are less studied due to inherent complications arising from formation of oxo-bridged polynuclear complexes *(5–8)* and also from hydrolysis of the metal ion. Thus even the formation constant of iron(III)–glycine, $Fe(Gly)^{2+}$, the simplest α-amino acid, has not been extensively studied *(9–13).* Polarography and pH-metric methods employed relatively high iron(III) concentrations and highly acidic conditions to avoid hydrolysis and to suppress the formation of polynuclear complexes *(10).* Because of high protonation constants, glycine was often used in large excess *(11, 12).* Recently, differential pulse cathodic voltammetry (DPCV) on a static mercury drop electrode (SMDE) was used to evaluate overall formation constants, β_2 and β_3, of the iron(III)–glycine system that employed low iron(III) concentration (25 μM) and moderately alkaline pH (6.36–9.05) *(13).*

Since these methods require sophisticated experimentation and advanced computational methods that are difficult to emulate in undergraduate laboratories, we herein report a simple and convenient spectrophotometric method for the laboratory determination of the iron(III)–glycine formation constant via competitive ligand binding. Solver, Excel's suite of analysis tools, was used for the dynamic optimization of the molar absorption coefficient and the equilibrium constant. The program finds a requested optimal value in the target cell, by adjusting the values in the adjustable cells, as specified by the user, while subjecting the model to a number of restrictions or constraints. This method was tested in the class and was found to work satisfactorily. It can easily be extended to determination of formation constants of other non-phenolic amino acids and organic bases with iron(III) and gives good quantitative comparisons.

Materials and Methods

Equipment

All absorbance measurements were made on a PerkinElmer Lambda 16 UV–vis spectrophotometer. The Fe(III)–SCN^- complex exhibits maximum absorption in the visible region between 450 and 460 nm, hence inexpensive glass or PVC cuvettes were used. pH of the solutions was measured with a glass electrode connected to an Orion pH meter. The molar absorption coefficient and formation constant of the Fe(III)–SCN^- complex were calculated using the Solver tool in Excel on a Dell PC and were subsequently used to calculate the formation constant of the Fe(III)–glycine complex.

Chemicals

The common chemicals anhydrous ferric chloride, potassium thiocyanate, glycine, potassium permanganate, nitric acid, sulfuric acid, and granular zinc used were purchased from Ajax Finechem (Australia). Stock solutions of ferric chloride in 0.2 M HNO_3 and glycine and potassium thiocyanate in distilled deionized water were prepared (see the online material for details).

Experimental Procedure

Standardization of Iron(III)

The Fe(III) concentration in the stock solution was determined titrimetrically using the Byars and McCreary method *(14).* The Fe(III) was initially reduced to Fe(II) with zinc granules in dil H_2SO_4 and was subsequently titrated with standard $KMnO_4$ until a faint pink color persisted.

Determination of Formation Constants

Standard solutions were prepared by mixing 1.0–6.0 mL of Fe(III) and 1.0 mL of KSCN stock solutions and diluting to 25 mL with 0.2 M HNO_3. Similarly the test solution was prepared by adding 2.0 mL Fe(III), 1.0 mL KSCN, and 1.0 mL glycine solutions and diluting the mixture with 0.2 M HNO_3 to 25 mL. All solutions were found to have pH between 0.7 and 0.8. UV–vis spectra of the solutions were subsequently recorded between 350 and 600 nm against a blank solution containing 1.55×10^{-3} M Fe(III) in 0.2 M HNO_3. The absorbance values of the solutions were recorded at λ_{max} of 457 nm. Five replicate analyses were carried out independently by five different groups of students.

Hazards

Potassium thiocyanate causes skin irritations, iron(III) chloride, potassium permanganate, and acids (oxalic, sulfuric and nitric) are corrosive and may cause burns to skin and other body tissues.

Results and Discussion

For simplicity sake, it was assumed that in the mixed-ligand systems only these two equilibria existed:

$$\mathrm{Fe^{3+} + SCN^{-} \rightleftharpoons Fe(SCN)^{2+}} \quad (1)$$

$$\mathrm{Fe^{3+} + Gly^{-} \rightleftharpoons Fe(Gly)^{2+}} \quad (2)$$

Hence, the molar absorption coefficient, ε, and formation constant, $K_{\text{Fe-SCN}}$, for the Fe(III)–SCN⁻ complex were calculated from the absorbance data, which were subsequently used to calculate the formation constant for the Fe(III)–glycine complex, $K_{\text{Fe-Gly}}$.

Formation Constant of $Fe(SCN)^{2+}$

In very dilute solutions of low pH (<1.0) the 1:1 complex $Fe(SCN)^{2+}$ is likely to be present as the dominant species as shown in eq 1 *(1)*. However, at higher concentrations of SCN^- additional complexes such as $Fe(SCN)_2^+$, $Fe(SCN)_3$, and $Fe(SCN)_6^{3-}$ are also likely to be present *(15)*. They all are blood red colored and have intense absorption bands between 400 and 500 nm. The UV–vis spectra of $Fe(SCN)^{2+}$ at different Fe(III) concentrations are shown in Figure 1.

Although the Fe(III)–SCN⁻ formation constant, $K_{\text{Fe-SCN}}$, is a function of ionic strength of the solution *(16)*, it can be written in terms of concentrations, as shown in eq 3, if ionic strength is kept constant:

$$K_{\text{Fe-SCN}} = \frac{\left[\mathrm{Fe(SCN)^{2+}}\right]}{\left[\mathrm{Fe^{3+}}\right]\left[\mathrm{SCN^{-}}\right]} \quad (3)$$

From the ICE table for the equilibrium reaction, the formation constant for Fe(III)–SCN⁻ can thus be written

$$K_{\text{Fe-SCN}} = \frac{\left[\mathrm{Fe(SCN)^{2+}}\right]}{\left\{C_{\mathrm{M}} - \left[\mathrm{Fe(SCN)^{2+}}\right]\right\}\left\{C_{\mathrm{L}} - \left[\mathrm{Fe(SCN)^{2+}}\right]\right\}} \quad (4)$$

where C_M and C_L are the total Fe(III) and the total SCN⁻ concentrations, respectively. Since in the dilute solutions, aquated Fe(III) and SCN⁻ ions show negligible absorption between 400 and 500 nm, only $Fe(SCN)^{2+}$ is present as an absorbing species.

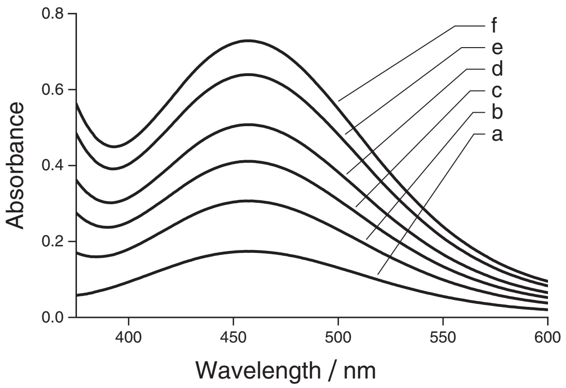

Figure 1. Absorbance spectra of $Fe(SCN)^{2+}$ solutions containing 3.02×10^{-4} M SCN⁻ and increasing concentrations of Fe(III): (a) 7.75×10^{-4} M, (b) 1.55×10^{-3} M, (c) 2.32×10^{-3} M, (d) 3.10×10^{-3} M, (e) 3.87×10^{-3} M, and (f) 4.65×10^{-3} M in presence of 0.2 M HNO_3.

From the Beer–Lambert law

$$A = \varepsilon l \left[\mathrm{Fe(SCN)^{2+}}\right] \quad (5)$$

where A is the absorbance at $\lambda_{\max}$, ε is molar absorption coefficient at $\lambda_{\max}$, and $l = 1$ cm. Equation 4 can be rewritten as

$$K_{\text{Fe-SCN}} = \frac{\left(\dfrac{A}{\varepsilon l}\right)}{\left[C_{\mathrm{M}} - \left(\dfrac{A}{\varepsilon l}\right)\right]\left[C_{\mathrm{L}} - \left(\dfrac{A}{\varepsilon l}\right)\right]} \quad (6)$$

Equation 6 can be solved in Excel using the Solver optimization tool or it can be expanded and rearranged *(15)* to

$$\frac{C_{\mathrm{M}} C_{\mathrm{L}}}{A} = \frac{C_{\mathrm{M}} + C_{\mathrm{L}}}{\varepsilon l} + \frac{1}{\varepsilon l K_{\text{Fe-SCN}}} \quad (7)$$

The left-hand side of eq 7 contains only experimental data that can be plotted against $(C_M + C_L)$ to obtain a straight line. Values of ε and $K_{\text{Fe-SCN}}$ are thus obtained from the slope and intercept of the straight line, respectively. The experimental values of C_M, C_L, and absorbances for the different solutions along with the calculated parameters obtained from the graphical method and by using Solver optimization are listed in Table 1.

Table 1. Experimental Values and Calculated Data for the Fe(III)–SCN⁻ Complex

C_M/ (10^{-3} mol L⁻¹)	C_L/ (10^{-4} mol L⁻¹)	Absorbance	($C_M + C_L$)/ (10^{-3} mol L⁻¹)	($C_M C_L/A$)/ (10^{-6} mol² L⁻²)	ε/(L mol⁻¹ cm⁻¹)		$K_{\text{Fe-SCN}}$/(L mol⁻¹)	
					Graph	Solver	Graph	Solver
0.775	3.02	0.1617	1.08	1.45	4830	4690	179	183
1.55	3.02	0.3076	1.85	1.52				
2.32	3.02	0.4252	2.63	1.65				
3.10	3.02	0.5053	3.40	1.85				
3.87	3.02	0.5880	4.18	1.99				
4.65	3.02	0.6242	4.95	2.25				

Note: The shaded area designates the calculated data.

Calculation of Formation Constant of Fe(Gly)$^{2+}$ Using the Graphical Method

The plot of ($C_M + C_L$) versus $C_M C_L/A$ for the Fe(III)–SCN$^-$ complex (Figure 2) gave a slope of 2.069 × 10^{-4} mol L^{-1} and intercept of 1.161 × 10^{-6} mol^2 L^{-2}. These values when substituted in eq 7 gave ε = 4830 L mol^{-1} cm^{-1} and $K_{\text{Fe-SCN}}$ = 179 L mol^{-1}. Then, assuming that only two equilibria, eqs 1 and 2, existed in the mixed-ligand solution, concentrations of uncoordinated Fe(III), Fe(Gly)$^{2+}$, and uncoordinated glycine were calculated. The mixed Fe(III)–glycine–SCN$^-$ solution, having initial [Fe(III)] of 1.55 × 10^{-3} M, [SCN$^-$] of 3.02 × 10^{-4} M, and [glycine] of 5.05 × 10^{-4} M in 0.2 M HNO$_3$, exhibited an absorbance of 0.2969 at $\lambda_{\max}$ 457 nm. This corresponded to [Fe(SCN)$^{2+}$] of 6.15 × 10^{-5} M. Thus the concentration of uncoordinated SCN$^-$ was calculated to be 2.41 × 10^{-4} M. Subsequently using the equilibrium constant $K_{\text{Fe-SCN}}$ of 179 L mol^{-1}, eq 4 gave free [Fe(III)] of 1.43 × 10^{-3} M. By subtracting the combined concentration of free Fe(III) and Fe(SCN)$^{2+}$ from the total Fe(III) concentration, the concentration of Fe(Gly)$^{2+}$ was calculated to be 6.08 × 10^{-5} M and that of the uncoordinated glycine to be 4.44 × 10^{-4} M. Substituting these concentrations into eq 8 gave the formation constant of Fe(III)–glycine, $K_{\text{Fe-Gly}}$, 95.9 L mol^{-1}.

$$K_{\text{Fe-Gly}} = \frac{\left[\text{Fe(Gly)}^{2+}\right]}{\left\{C_{\text{M}} - \left[\text{Fe(SCN)}^{2+}\right] - \left[\text{Fe(Gly)}^{2+}\right]\right\} \times \left\{C_{\text{Gly}} - \left[\text{Fe(Gly)}^{2+}\right]\right\}} \quad (8)$$

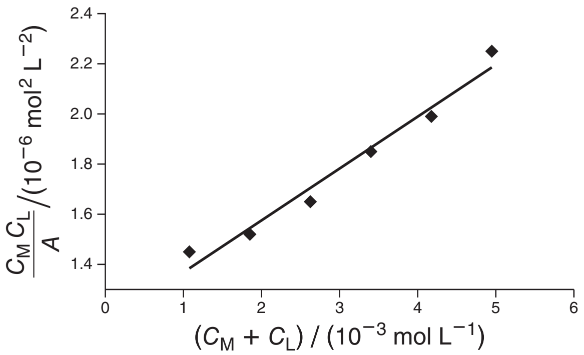

Figure 2. ($C_M + C_L$) versus $C_M C_L/A$ for the Fe(III)–SCN$^-$ complex.

Using Solver in Excel

The Solver tool in Excel facilitates direct calculation of ε and $K_{\text{Fe-SCN}}$ from the absorbance data of the solutions. Initially an arbitrary value of ε was first assigned to the data and the corresponding formation constant values, $K_{\text{Fe-SCN}}$, for different sets were calculated from their respective absorbance data using eq 6. Four other cells were designated to calculate average and standard deviation values of ε and $K_{\text{Fe-SCN}}$. At first the target cell in Solver was set to minimize the standard deviation in the calculated $K_{\text{Fe-SCN}}$ values, while the ε values in the column (see the online material) were set for changing. Later on the target cell was changed to minimize the standard deviation in ε, while retaining the ε values in the column for changing. After two such iterations constraints were introduced to keep both standard deviation values equal and to <5% of their respective calculated average ε and $K_{\text{Fe-SCN}}$ values. Again both standard deviations were sequentially minimized. After two iterations average values of ε = 4690 ± 80 L mol^{-1} cm^{-1} and $K_{\text{Fe-SCN}}$ = 183 ± 3 L mol^{-1} were obtained. As seen in Table 2 both standard deviations are <1.7% of the calculated mean values of ε and $K_{\text{Fe-SCN}}$.

Using the optimized average value of ε = 4690 L mol^{-1} cm^{-1} for the Fe(III)–SCN$^-$ complex in the mixed-ligand Fe(III)–glycine–SCN$^-$ solution and eq 5, the concentration of Fe(SCN)$^{2+}$ was calculated to be 6.32 × 10^{-5} M. Thus with initial concentrations Fe(III) of 1.55 × 10^{-3} M, SCN$^-$ of 3.02 × 10^{-4} M, and glycine of 5.05 × 10^{-4} M in 0.2 M HNO$_3$, the concentrations of uncoordinated SCN$^-$ and Fe(III) were calculated to be 2.39 × 10^{-4} M and 1.44 × 10^{-3} M, respectively. From the difference of combined concentrations of uncoordinated Fe(III) and Fe(SCN)$^{2+}$ from total [Fe(III)], the Fe(Gly)$^{2+}$ and uncoordinated glycine concentrations were calculated to be 4.24 × 10^{-5} M and 4.63 × 10^{-4} M, respectively. Substituting these concentration values in eq 8 the formation constant for Fe(Gly)$^{2+}$, $K_{\text{Fe-Gly}}$, of 63.3 L mol^{-1} was obtained.

Conclusion

The results presented in Table 1 show a fairly good agreement between the calculated values of $\varepsilon_{\text{Fe-SCN}}$ and $K_{\text{Fe-SCN}}$ by the two methods and yield a consistent value of $K_{\text{Fe-Gly}}$. Depending upon the expertise of the students in use of Excel software either of these methods is equally suitable for the undergraduate

Table 2. Solver Calculated Parameters from Concentration and Absorbance Data or the Fe(III)–SCN$^-$ Complex

C_M/ (10^{-3} mol L^{-1})	C_L/ (10^{-4} mol L^{-1})	Absorbance	ε/ (L mol^{-1} cm^{-1})	$K_{\text{Fe-SCN}}$/ (L mol^{-1})	ε/(L mol^{-1} cm^{-1})		$K_{\text{Fe-SCN}}$/(L mol^{-1})	
					Average	SD	Average	SD
0.775	3.02	0.1617	4590	179	4690	80	183	3
1.55	3.02	0.3076	4730	185				
2.32	3.02	0.4252	4780	187				
3.10	3.02	0.5053	4710	184				
3.87	3.02	0.5880	4750	185				
4.65	3.02	0.6242	4600	181				

Note: The shaded area designates the calculated data.

laboratory demonstration. This method was tested in two classes of 22 students each and was found to work satisfactorily across different groups of students. The calculated values of $K_{\text{Fe-Gly}}$ were mostly between 1.0×10^2 and 3.0×10^2 L mol^{-1}. It is easily extendable to determination of formation constants of other non-phenolic amino acids and organic bases with iron(III) and gives reproducible quantitative comparisons.

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