

**The Reaction of N-Methylimidazole with a Macrocyclic Ligand Complex
of Fe^{II}: An Undergraduate Experiment in the Spectrometric
Analysis of Two Successive Equilibria**

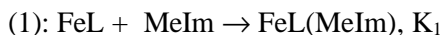
Nicholas K. Kildahl

Contribution from the Department of Chemistry, Worcester Polytechnic Institute,
Worcester, MA 01609

Introduction

The determination of equilibrium constants for adduct formation reactions using spectrometric methods is an important problem which deserves attention at the undergraduate level. Although a number of articles in this journal have dealt with 1:1 interactions (1-4), the problem of 2 successive adduct-formation equilibria has received far less attention (5-7) although it has been dealt with thoroughly in the inorganic literature (8-11). Two successive equilibria occur in a variety of situations, most notably in protonation processes and transition metal-ligand interactions. In particular, axial ligation reactions of transition metal porphyrin complexes, which commonly occur in two stages, are important model reactions for biological systems. The difficulty in the spectrometric analysis of two-step systems is that the equilibria almost invariably overlap--that is, the second step begins before the first step is complete. This complicates the mathematics sufficiently to warrant the use of computer analysis to obtain the two equilibrium constants. Computational methods for accomplishing this have been developed, most involving sophisticated non-linear least squares (NLLSQ) routines (8). It is the complexity of such calculations that has prevented this problem from being introduced at the undergraduate level. Recently, however, a comprehensive treatment of the spectrometric analysis of multistep equilibria has appeared which presents greatly simplified methods for treatment of the two-step problem (9).

In this article, I present an experiment suitable for advanced undergraduate laboratory courses in physical or inorganic chemistry. The experiment involves the determination of equilibrium constants for successive binding of 2 molecules of N-methylimidazole (MeIm) to the axial sites of the complex, $\text{Fe}(\text{Me}_4[14]\text{tetraeneN}_4)^{2+}$ (hereafter FeL, Figure 1) in acetonitrile (An) solvent using spectrometric titration techniques. The ligation reactions are shown in eqs 1 and 2, which omit solvent molecules in the axial sites.



The purposes in presenting this experiment are two. First the chemical system is ideal for several reasons: all three complexes in eqs 1 and 2 are intensely and beautifully colored, which often excites student interest; isosbestic points are observed early and late in the titration, which also interests students; the system is pushed to completion at relatively low MeIm concentration (~0.1 M); and the system is not sensitive to oxygen or to the presence of water in the solvent, which minimizes technical difficulties. Second, the two step equilibrium system is amenable to analysis by the elegant graphical methods of reference 9. Notably these methods do not involve computer analysis and are quite simple for students to comprehend. Thus they allow the student to concentrate on the chemistry of the system rather than on the black-box data analysis of the NLLSQ routines. It is my hope that this article will draw attention to the methods of reference 9, which are much under-used.

Experimental

Information for the Instructor

Reagents. Reagent grade An was obtained from Baker; reagent grade MeIm was obtained from Aldrich. Both substances may be used as received without significantly affecting results. However, if desired, An may be dried by refluxing over phosphorus pentoxide for 24 h under nitrogen followed by distillation under nitrogen. MeIm may be purified and dried by refluxing over potassium hydroxide for 2 h under reduced pressure followed by distillation under reduced pressure. All other materials used in the synthesis of the iron complex were reagent grade and were used as received.

Synthesis. FeL(An)₂(PF₆)₂ was synthesized by published procedures (12,13) and characterized by IR and electronic absorption spectroscopy (EAS) ($\lambda_{\text{max}}(\epsilon)$, nm (M⁻¹cm⁻¹): 551 (9.02 x 10³), 514 (sh)). As the synthesis requires a long reflux time, it is recommended that it be carried out by the instructor.

Physical Methods. IR spectra for characterization of FeL(An)₂(PF₆)₂ may be obtained from KBr pellets and/or Nujol mulls. We used a Perkin-Elmer 683 infrared spectrometer and PE 3600 data station. EAS may be performed with any high-quality spectrophotometer covering the visible wavelength range. The results presented here were obtained using a Shimadzu UV-2100U spectrometer system. Standard 1-cm spectrometer cells equipped with tight-fitting teflon plugs are preferable to cells with loose fitting square plastic or teflon caps, which lose solution when shaken.

Preparation of Solutions. 1) Stock Solution of FeL(An)₂(PF₆)₂. I recommend that students perform the experiment in pairs. Allow for 2 mL of stock solution per pair of students. To prepare 100 mL of a 1.25 x 10⁻³ M stock solution, weigh 0.0845g FeL(An)₂(PF₆)₂ (MW = 676.3 g/mole) into a 100-mL volumetric flask and add An to the mark. This solution is stable to air and moisture and may be prepared several days in advance if desired. 2) 0.1 M MeIm in An. Using a 50- or 100- μ L syringe, transfer 39.8 μ L of MeIm to a 5-mL volumetric flask. Add An to the mark, stopper and shake to insure uniformity. 3) 1.0 M MeIm in An. Using a 500- μ L syringe, transfer 398 μ L of MeIm to a 5-mL volumetric flask. Add An to the mark, stopper and shake.

Each pair of students will require about 30 μ L of 0.1 M MeIm and 15 μ L of 1.0 M MeIm per titration. The 5-mL volumes recommended above will therefore be sufficient for over 150 pairs of students. If desired, students may prepare their own MeIm solutions for titration. However, this will greatly increase the amounts of solutions that will have to be disposed of following the experiment.

Student Procedures.

Preparation of Solutions for Titration. Preparation and transfer of solutions should be performed in a fume hood. 1) Working solution of FeL(An)₂(PF₆)₂. Use a 1-mL pipet or a syringe to transfer 0.5 mL of iron stock solution to a 5-mL volumetric flask. Add An to the mark, stopper the flask, and shake to mix. Record the concentration of the iron stock solution and of your working solution. Note and record the color of the solution. Solutions of MeIm in An (0.1M and 1.0M) will be supplied by the instructor.

Spectrometric Titration. Using a graduated pipet or a syringe, transfer 3.00 mL of your working solution of FeL(An)₂(PF₆)₂ to a 1-cm spectrophotometer cell. Fill the reference cell with An. Record the ambient temperature. Set up the electronic absorption spectrophotometer to overlay successive spectra, then record the EAS of your working solution over the interval 750-300 nm. Measure and record the absorbances at 551, 604, and 663 nm which are the λ_{max} values for FeL, FeL(MeIm), and FeL(MeIm)₂, respectively. Using a 10- μ L syringe, add a 1- μ L aliquot of 0.1 M MeIm in An, stopper and shake the cell, and allow the solution to stand for 1 minute before recording the spectrum. The delay of 1 minute is necessary to ensure equilibration of eq 1, particularly in early stages of the titration (14). At this point and following addition of all subsequent aliquots, note and record any color changes which you observe. Record the spectrum so that it overlays the first spectrum, and read and record absorbance at $\lambda = 551, 604, \text{ and } 663$ nm. In similar fashion, add two more 1- μ L aliquots, two 2- μ L aliquots, two 4- μ L aliquots, and two 10- μ L aliquots of 0.1 M MeIm. Wait 1 minute, scan the spectrum, and record absorbance at 551, 604, and 663 nm after addition of each aliquot. Now change over to 1.0 M MeIm in An, and add a total of ~15 μ L of this solution in aliquot sizes ranging from 1 to 4 μ L. Record the spectrum and specified absorbances after each addition. Finally, change over to neat MeIm. Add a total of ~30 μ L (aliquot size ranging from 1 to 10 μ L) to the cell, recording the spectrum and absorbances after addition of each aliquot. Continue addition of MeIm until no

further spectral changes occur. The final concentration of MeIm should be ~0.125M. Again record the ambient temperature and average your two temperature measurements.

Results and Method of Data Analysis

Analysis of titration data can be accomplished using absorbance diagrams, or A-diagrams; and absorbance-difference-quotient (ADQ) diagrams (9). An A-diagram is a plot of the absorbance at one wavelength against the absorbance at a second wavelength, there being one point for each of the spectral scans taken during the titration. The diagram thus shows directly the relative absorbance changes at two wavelengths as a function of titrant concentration. For a one-step system, it can be shown (9) that the absorbance at any wavelength must be proportional to the absorbance at any other wavelength, so that an A-diagram for such a system will be linear. A single-step system is therefore quickly diagnosed from such a plot. However, if a system is governed by 2 or more equilibria, the A-diagrams will "change direction" each time a new equilibrium becomes dominant in the system. This will occur over particular ranges of titrant concentration. If successive K values differ by a factor of $\geq 10^3$ ($\text{p}K_{n+1} \leq \text{p}K_n - 3$)--that is, if the successive equilibria overlap very little or not at all--the A-diagram will consist of linear segments, one corresponding to each of the equilibrium steps. These segments are connected by curved regions where the dominance shift from one equilibrium to the next occurs. The linear segments in the A-diagram give way gradually to a smooth curve as $\Delta\text{p}K$ decreases--that is, as the amount of overlap of the successive equilibria increases. An ADQ-diagram is constructed using absorbances at 3 wavelengths, say λ_1 , λ_2 , and λ_3 . First, the value of $(A_n - A_0)_{\lambda_1}$ is calculated for each of the titration spectra. Here A_n is the absorbance at λ_1 after addition of the nth aliquot of titrant, and A_0 is the initial absorbance at λ_1 (before addition of titrant). This series of absorbance differences at λ_1 is called ΔA_{λ_1} . Corresponding series of absorbance differences are calculated at λ_2 and λ_3 , again using the initial absorbance as a reference point. These series of differences are called ΔA_{λ_2} and ΔA_{λ_3} , respectively. Two ratios (quotients) of these absorbance differences are then calculated for each titration spectrum by dividing one of the differences into each of the other two. For example, the quotients $\Delta A_{\lambda_2}/\Delta A_{\lambda_1}$ and $\Delta A_{\lambda_3}/\Delta A_{\lambda_1}$ might be calculated. Finally, one quotient is plotted against the other over the series of titration spectra. As shown in reference 9, ADQ-diagrams are linear for systems involving 2 successive equilibria, but curved if there are more than 2 steps.

To determine whether 1, 2, or more than 2 successive equilibria govern a system, it is necessary to construct first several A-diagrams, using data at a number of wavelengths. Linearity indicates a single equilibrium, and construction of ADQ-diagrams is unnecessary. Linear A-diagrams are easily analyzed to obtain the single K value, as discussed in reference 9. On the other hand, curvature of A-diagrams indicates ≥ 2 successive equilibria, and it is then necessary to construct a number of ADQ-diagrams. Linearity indicates 2 successive equilibria. In this case, the A-diagrams can be analyzed with relatively little effort to obtain 1) the extinction coefficients of the intermediate species at the plotted wavelengths, which are usually not directly measurable; and 2) the equilibrium constants for the two equilibria involved, as discussed below. If the ADQ-diagrams are not linear, 3 or more successive equilibria are indicated. This situation will not be further discussed here, since our focus is on the 2-step situation. The reader is referred to reference 9 for derivations of relevant equations and detailed explanations of the uses of A-diagrams for multistep processes.

The FeL/MeIm System. Spectra obtained during a typical titration of FeL with MeIm in An at 25°C are shown in Figures 2a and 2b. Figure 2a shows the evolution of spectra during the first half of the titration, in which eqn 1 dominates. Similarly, Figure 2b shows spectral changes due primarily to eqn 2. Isosbestic points are observed at 567 and 624 nm. The point at 567 nm is observed early in the titration and corresponds to crossover of the spectra of FeL and FeL(MeIm). It persists until the concentration of FeL(MeIm)₂ becomes significant, then blurs and disappears. It is replaced by the point at 624 nm,

the spectral crossover for $\text{FeL}(\text{MeIm})$ and $\text{FeL}(\text{MeIm})_2$. As the titration proceeds, the initial pink color of the solution (due to FeL) changes to purple, blue, and finally cyan (due to $\text{FeL}(\text{MeIm})_2$). Concentration and absorbance data are collected in Table 1.

Figure 3 shows one of the three possible A-diagrams for the data in Table 1. The plot clearly consists of two segments separated by a region in which the plot changes direction, suggesting at a glance that the FeL/MeIm system is governed by 2 successive overlapping equilibria. (It should be noted that although for this system the number of equilibria is readily perceived from the progression of titration spectra in Figure 2, there are many systems for which the spectra alone do not clearly reveal the number of equilibria involved. For such systems, A-diagrams often quickly disclose this.) The linear ADQ-diagram shown in Figure 4 confirms two successive equilibria. The A-diagrams can now be analyzed to obtain the extinction coefficients for $\text{FeL}(\text{MeIm})$ at 551, 604, and 663 nm, and the values of K_1 and K_2 . This is accomplished using the so-called *absorbance triangle*.

Figure 5 reproduces the A_{604} - A_{551} A-diagram of Figure 3. The point corresponding to the beginning of the titration is labelled B, and that corresponding to the end of the titration, E. The absorbance triangle is obtained by constructing tangents to the A-diagram at points B and E, and extending them until they intersect at the point labelled I, for intermediate. (The construction of tangents may be carried out by a number of methods. Those in Figure 5 were done using the mirror technique¹. More rigorous methods based on differentiation of the equation describing the best polynomial fit of the A-diagram can be used if desired.) The triangle is completed by connecting points B and E. This procedure effectively resolves the observed A-diagram into three linear A-diagrams, one corresponding to each of the equilibria in eqs 1 and 2, and the third corresponding to direct conversion of FeL to $\text{FeL}(\text{MeIm})_2$ in one step, without involvement of the intermediate complex. Thus, the line connecting B and I, BI, is the A-diagram that *would be* observed if eq 1 were the only process occurring in the system. A similar correspondence exists between eq 2 and line IE, while line BE is the A-diagram that would be observed if the sum of eqs 1 and 2 were to occur in a single step. The point I therefore corresponds to the intermediate complex, $\text{FeL}(\text{MeIm})$, which is the terminal species for eq 1 and the initial species for eq 2. Point I reveals the absorbances that would be observed at 551 and 604 nm if the intermediate complex were the only Fe-containing species present in solution. From these absorbance values and the known concentration of Fe, extinction coefficients for the intermediate at 551 and 604 nm are found to be 4.4×10^3 and 9.0×10^3 , respectively. These values and those obtained from similar analysis of the remaining A diagrams (A_{663} vs. A_{604} and A_{663} vs. A_{551}), which are not shown, are presented in Table 2. By this method, extinction coefficients for FeL may be obtained even though they cannot be directly measured experimentally.

The absorbance triangle may also be used to evaluate the equilibrium constants for eqns 1 and 2, using the *bisection of sides method* (9). A line, or ray, is drawn connecting each vertex of the triangle with the midpoint of the opposite side. These rays are shown in Figure 5. The ray BM connects the point representing the beginning of the titration with the midpoint of the linear absorbance diagram for the second equilibrium. The intersection of BM with the experimental A-diagram gives the half-equivalence absorbances at both plotted wavelengths for the second equilibrium, eq 2. The half-equivalence absorbance is the absorbance at the half-equivalence point of the equilibrium step concerned. Since at the half-equivalence point of the second equilibrium $\text{p}K_2 = -\log[\text{MeIm}]$, $\text{p}K_2$ is found by determining the concentration of MeIm corresponding to the half-equivalence absorbance. This is done using an absorbance-concentration plot of the type shown in Figure 6. In Figure 5, BM intersects the experimental A-diagram at $A_{551} = 0.36$. From Figure 6, this absorbance corresponds to $-\log[\text{MeIm}] = \text{p}K_2 = 2.76$. Similarly, intersection of the ray EN (which connects the end of the titration with the midpoint of the linear A-diagram for the first equilibrium) with the experimental A-diagram gives the half-equivalence absorbance for the first equilibrium, eq 1. $\text{p}K_1$ may be obtained by the same procedure used to obtain $\text{p}K_2$. Finally, intersection of the ray IP with the diagram gives absorbance values corresponding to $-\log[\text{MeIm}] = (\text{p}K_1 + \text{p}K_2)/2$. (This results because at the half-equivalence point for conversion of FeL directly to $\text{FeL}(\text{MeIm})_2$,

according to the sum of eqs 1 and 2, $[\text{MeIm}]^2 = K_1K_2$.) In Figure 5, IP intersects the curve at $A_{551} = 0.55$, which translates to $-\log[\text{MeIm}] = (\text{p}K_1 + \text{p}K_2)/2 = 3.34$. Substitution of $\text{p}K_2$ from above gives $\text{p}K_1 = 3.92$. Application of the bisection of sides method to all three A-diagrams for the FeL/MeIm system gives the results collected in Table 2. Values of $\text{p}K_1$ have been determined indirectly from $\text{p}K_2$, rather than directly using ray EN. Direct determination would require knowledge of the concentration of *unbound* MeIm at the point of the titration represented by the intersection of EN with the A-diagram. At this early point in the titration, a significant and unknown fraction of the total concentration of MeIm is bound, so that the total and unbound concentrations differ. At later points in the titration, where the concentration of bound MeIm is insignificant with respect to the total, use of the total MeIm concentration to determine $\text{p}K$ values introduces negligible error. The values of $\text{p}K_1$ and $\text{p}K_2$ obtained spectrometrically compare favorably with earlier estimates obtained using NMR spectroscopy: $\text{p}K_1 - \text{p}K_2 = 0.85$ (12); and $\text{p}K_1 > 2.70$, $\text{p}K_2 > 1.85$ (15).

The graphical procedures of reference 9, though somewhat awkward to describe narratively, are simple to apply. After some initial assistance in constructing A-diagrams, ADQ-diagrams, and absorbance triangles, students will readily accomplish the analysis of the two-step FeL/MeIm system. Hopefully they will then realize that quite complex equilibrium systems can be analyzed by simple methods which do not require the use of a computer for data analysis.

In conclusion, the experimental procedures, including the preparation of iron working solution and the titration, can readily be accomplished in a 3-hour laboratory period. A-diagrams, ADQ-diagrams, and absorbance-concentration plots may be generated using any of a number of plotting routines now available. Figures 3-6 in this article were generated using ProPLOT Scientific Graphics, Version 1.0, from Cogent Software.

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Footnotes

1. A mirror is held perpendicular to the curve at the point of interest and aligned until the curve and its reflection in the mirror appear to join smoothly. A straight line perpendicular to the curve is then drawn using the edge of the mirror. Finally, the tangent is constructed perpendicular to the first straight line and touching the curve at the point of interest.

Table 1: Concentration and Absorbance Data for the Titration of FeL with MeIm in An

Scan	μL 0.1 M MeIm	μL 1.0 M MeIm	μL neat MeIm	$[\text{MeIm}]_{\text{total}}, *10^3$	$\log [\text{MeIm}]$	A at 551	A at 604	A at 663
1	0			0		1.038	0.038	0.010
2	1			0.0333	-4.477	0.947	0.240	0.017
3	1			0.0666	-4.176	0.874	0.403	0.027
4	1			0.0999	-4	0.814	0.533	0.040
5	2			0.166	-3.779	0.724	0.703	0.072
6	2			0.233	-3.633	0.666	0.798	0.107
7	4			0.365	-3.437	0.590	0.886	0.179
8	4			0.498	-3.303	0.543	0.914	0.246
9	8			0.761	-3.119	0.478	0.913	0.357
10	10			1.088	-2.963	0.426	0.887	0.468
11		1		1.42	-2.849	0.390	0.860	0.554
12		1		1.75	-2.758	0.360	0.831	0.627
13		2		2.40	-2.619	0.321	0.787	0.732
14		2		3.06	-2.514	0.296	0.755	0.809
15		4		4.37	-2.359	0.260	0.709	0.903
16		4		5.68	-2.246	0.239	0.679	0.963
17			1	9.80	-2.009	0.204	0.632	1.061
18			1	13.9	-1.856	0.186	0.606	1.114
19			2	22.2	-1.655	0.171	0.585	1.158
20			5	42.7	-1.370	0.157	0.565	1.194
21			10	83.5	-1.078	0.151	0.554	1.213
22			10	124	-0.906	0.146	0.547	1.215

Spectrophotometer cell initially contained 3.00 mL of 1.26×10^{-4} M $\text{FeL}(\text{An})_2(\text{PF}_6)_2$. T = 25°C.

Table 2: Results of Analysis of Absorbance Triangles Constructed from A Diagrams

A Diagram Used	λ, nm	A	ϵ ($M^{-1}cm^{-1}$) for FeL(MeIm) * 10^3
604 vs 551	551	0.55	4.4
	604	1.14	9.0
604 vs 663	604	1.13	9.0
	663	0.044	0.35
663 vs 551	551	0.57	4.5
	663	0.039	0.31

Mean ϵ Values for FeL(MeIm): 4.4×10^3 at 551 nm; 9.0×10^3 at 604 nm; 0.33×10^3 at 663 nm

A Diagram Used	pK₂	(pK₁+pK₂)/2	pK₁
604 vs 551	2.76	3.34	3.92
604 vs 663	2.77	3.40	4.03
663 vs 551	2.76	3.42	4.08
Mean:	2.76 ± 0.01		4.01 ± 0.08

Figure Captions

Figure 1. The complex $\text{Fe}(\text{Me}_4[14]\text{tetraeneN}_4)^{2+}$, abbreviated FeL.

Figure 2. Titration spectra obtained during the spectrometric titration of FeL with MeIm. Inset a shows scans 1-8, proceeding in the direction of the arrow. Inset b shows scans 8-22. See Table 1 for corresponding absorbance and concentration data.

Figure 3. A at 604 nm versus A at 551 nm for the FeL/MeIm System.

Figure 4. ADQ-diagram for the FeL/MeIm System.

Figure 5. Absorbance triangle for the A-diagram of Figure 3.

Figure 6. Absorbance at 551 nm versus total concentration of MeIm.