

than the phenanthroline ligand and so is more easily distorted. If the distortion is large enough, it is no longer sufficient to use an averaged crystal field splitting in the formulas for the low temperature  $g$ -values. Assuming that  $\lambda$  remains constant, this reasoning leads to a splitting of about  $20000\text{ cm}^{-1}$  of the degenerate electronic state in the distorted configuration. This figure is reasonable when compared to other studies in which the ligands around the  $\text{Cu}^{+2}$  are tetragonally distorted.

The spectrum does not show a sharp transition point with temperature but rather goes over slowly from one limiting spectrum to the other. Figure 4 shows a series of spectra run at various temperatures. It is not possible to account for these spectra quantitatively at the intermediate temperatures, but they seem to be superpositions of the two limiting spectra.

### Conclusion

The line width of the high temperature spectrum is equal to the line width of the two components observed at low temperature. This observation would seem to lend weight to the interpretation given here. One

would expect that the hyperfine structure would be washed out at the high temperature owing to the breadth of the energy levels involved in the transitions.

This work on polycrystalline samples poses some questions to be answered. Single crystals of the diluted compounds are being grown to help answer these questions. The optical spectrum will be investigated at low temperature to see if the Jahn-Teller splitting of the electronic state can be observed. Similarly, since the Liehr-Ballhausen treatment assumes that the distortion arises from a vibronic coupling to the E-modes of vibration, the infrared spectrum should be investigated as the vibrational states should also be perturbed. Observation of these effects would eliminate any doubts that may exist concerning the origin of the observed effects. X-Ray diffraction investigations should be done on the crystals at various temperatures to see if any information can be deduced as to the extent of the distortion.

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## Machine Computation of Association Constants from Spectrophotometric Data: An Analysis of Errors

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Fortran language programs have been written which successfully calculate equilibrium constants,  $K$ , and molar absorptivities,  $\epsilon$ , from experimental data for self-association and heteroassociation. The calculated parameters are, however, inordinately sensitive to variations in the input data. Experiments using the programs with synthetic data reveal the results to be most sensitive to concentration errors. The errors are discussed in terms of the Rose-Drago treatment of this kind of data. Suggested approaches for the improvement of this situation are given.

The simplicity and accuracy of spectrophotometric measurements have led to their use in the determination of a great variety of association constants, especially where the association occurs between two different species. A wide variety of methods has been devised for the treatment of the experimental data, but owing to the complexity of the problem it has usually been necessary to limit the approach to special cases (such as special concentration relationships) or to make simplifying approximations.<sup>2</sup> The best known methods are graphical and are comparatively tedious and often imprecise. One digital method has been proposed for the determination of self-association constants<sup>3</sup> but it has been shown to fail.<sup>4</sup> A digital method has been presented by Liptay for the calculation of association constants of electron donor-acceptor complexes which avoids many of the restrictions implicit in the earlier methods but which, too, necessarily makes simplifying assumptions in order to keep the labor of the calculation within bounds.<sup>5</sup>

In the current work we sought to write a program for a digital computer which would accept spectrophotometric data and fit to it, without simplifying approximations, equilibrium constants and molar absorptivi-

ties according to the least-squares criterion. Activity coefficient effects were assumed to be negligible; Beer's law was assumed to hold for every species involved. The kind of approach used in developing the computer program is akin to that used by Coburn and Grunwald<sup>6</sup> and more recently by Sillén.<sup>7</sup>

The problem may be stated as follows: The three independent variables which have an effect on the value of the optical density (at a given temperature in a given solvent) are the initial concentration of substrate(s), the values of the equilibrium constants for the equilibria involved, and the values of the molar absorptivities for the species involved. Of these three independent variables, only the concentration is experimentally controlled.

The task which confronts one is to deduce values of the equilibrium constants and molar absorptivities which predict the experimentally determined values of the optical density ( $D_m$ ) at various wave lengths and experimental values of the concentration of substrate(s). The variety of possible ways this might be done is summarized with the following diagram. To illustrate the interpretation of this diagram consider the upper left leg. If one knows the values of  $K$  and the initial concentrations ( $C_0$ ) one can calculate the equilibrium concentrations ( $C_e$ ) of the species. Conversely, if one knows the equilibrium concentrations and the initial concentrations one can derive the equilibrium constant.

(1) National Science Foundation Research Participation for College Teachers Fellow.

(2) F. J. C. Rossotti and H. Rossotti, "The Determination of Stability Constants," McGraw-Hill Book Co., Inc., New York, N. Y., 1961.

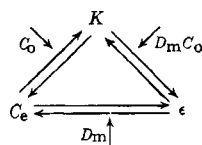
(3) P. A. D. de Maine and A. G. Goble, *Trans. Faraday Soc.*, **53**, 427 (1957); P. A. D. de Maine, M. M. de Maine, A. A. Briggs, and G. E. McAlonie, *J. Mol. Spectry.*, **4**, 398 (1960).

(4) B. Musulin, W. Lee, and R. L. Foley, *ibid.*, **9**, 254 (1962).

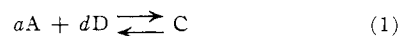
(5) W. Liptay, *Z. Elektrochem.*, **65**, 375 (1961).

(6) W. C. Coburn and E. Grunwald, *J. Am. Chem. Soc.*, **80**, 1318, 1322 (1958).

(7) D. Dyrssen, N. Ingre, and L. G. Sillén, *Acta Chem. Scand.*, **15**, 694 (1961); L. G. Sillén, *ibid.*, **16**, 159, 191 (1962).



We may illustrate with the heteronuclear case. Given the stoichiometry



the relationships between initial concentration and equilibrium concentration are

$$A_e = A_0 - aC; D_e = D_0 - dC \quad (2)$$

Substituting into the chemical equilibrium expression, we obtain

$$K = \frac{C}{(A_0 - aC)^a (D_0 - dC)^d} \quad (3)$$

or

$$C = K(A_0 - aC)^a (D_0 - dC)^d$$

According to Beer's law, the total absorbance will be the sum of the absorbances due to each species present

$$D_M = \epsilon_a A_e + \epsilon_d D_e + \epsilon_c C \quad (4)$$

Equation 3 is used in the upper left leg of the diagram, eq. 4 is used in the lower leg of the diagram, and a combined equation obtained by suitable elimination between eq. 3 and 4 is used in the upper right leg of the diagram.

In an iterative procedure one would take an initial guess for one of the independent variables and proceed in either sense around the diagram adjusting each variable in turn until convergence to a self-consistent set of values was obtained. The diagram points out clearly that no new information is injected into the cycle in the third leg; this suggests that the procedure may fail from lack of information (*cf.* ref. 4). In numerous attempts we have not been able to devise an iterative procedure which will converge reproducibly (different initial guesses give different final answers). In several of our attempts, equations which had to be solved simultaneously proved to be dependent (not linearly) so that a unique solution was impossible. On the other hand, an iterative procedure based on algebra given by de Maine appears to converge reproducibly to approximately correct answers, though it is difficult to say what criterion that procedure adopts in coming to a "best" fit.<sup>8</sup>

In developing the programs used in this work, a search procedure has been adopted as in the work of Coburn and Grunwald<sup>6</sup> and Sillén.<sup>7</sup> To keep the algebra simple, the upper left and lower legs of the diagram (*i.e.*, eq. 3 and 4) were used. The program was written for any integral stoichiometry relationship (*i.e.*, integral  $a$  and  $d$ ). The program takes an arbitrary initial value for  $K$  and the corresponding values of the equilibrium concentrations are calculated from 3, using Newton's<sup>9</sup> method with the initial concentrations. Then, using 4 and the usual least-squares procedure, it finds the values of  $\epsilon$  at each wave length that best fit the experimental optical density data at that wave length. The fit obtained in this way with the

arbitrary value of  $K$  is then measured by calculating the optical density at each point and accumulating the sum of the squares of the deviations of the calculated from the experimental values. An arbitrary change in  $K$  is then made and the whole process repeated. The fit obtained is compared with that obtained previously. When the fit is improved the new values are remembered and the same arbitrary change in  $K$  is made until no further improvement results. When a fit is obtained that is poorer than a previous one, the change in  $K$  is made in the opposite direction. When the change in  $K$  has been tried unsuccessfully in both directions, the size of the change is decreased. When the size of the change has become arbitrarily small, the calculation is terminated and then the best values of  $K$  and  $\epsilon$  are taken as the best fit. Since each  $\epsilon$  is chosen explicitly to give best fit for each  $K$ , and  $K$  is adjusted by a search procedure to give the best fit, it follows that the programs give the best values of both  $K$  and  $\epsilon$  according to the least-squares criterion.

TABLE I

Concentration group	Concentration $A_0$ , mole l. <sup>-1</sup>	Concentration $D_0$ , mole l. <sup>-1</sup>
A	0.0050	0.0100
	.0050	.0600
	.0050	.1000
B	.0050	.0150
	.0100	.0050
	.0075	.0075
C	.0015	.0500
	.0075	.0500
	.0150	.0500

TABLE II

Wave length	$\epsilon_c$	$\epsilon_a$
1	10	...
2	25	0.0005
3	75	.005
4	120	.1
5	850	7.5
6	1000	12.0
7	1050	18.0
8	1150	26.0
9	1310	115.0
10	1300	140.0
11	1290	160.0
12	1300	185.0

The programs have been extensively checked with synthetic data developed for the purpose, with data published in the literature, and with new data from this laboratory. The programs converge reliably to a value which is independent of the starting point. In favorable cases, they reproduce the parameters used to generate synthetic data quite accurately. Thus, we concluded that the programs operate satisfactorily.

However, in unfavorable cases there were remarkably large deviations in  $K$  and  $\epsilon$  from those used to generate the synthetic data. Since in preliminary work not described here optical density data were truncated to four digits in order to imitate the maximum number of digits available experimentally, this deviation seemed most likely to be due to accumulation of errors resulting from truncation of  $D_m$ . Data from the literature sometimes gave results rather different from that reported and rather more different than the uncertainty quoted by the authors would lead one to expect. Data obtained for a given equilibrium at different temperatures in this laboratory showed an impossibly large

(8) P. A. D. de Maine, "Digital Computer Programs for Physical Chemistry," Macmillan and Co., New York, N. Y., 1963, in press.

(9) F. A. Willers, "Practical Analysis," Dover Publications, New York, N. Y., 1948, p. 218.

TABLE III

Concn. group	Input K	Determined K (sharpness)			
		No errors	$D_m$ and $\epsilon_a$ errors	$A_0$ and $D_0$ errors	All errors
A	10.00	9.975 (174)	9.984 (73.8)	8.863 (48.5)	8.878 (32.3)
B	10.00	10.05 (193)	10.12 (8.20)	15.05 (0.257)	13.11 (0.240)
C	10.00	9.205 (21.3)	8.358 (0.156)	3.990 (0.87)	3.622 (0.141)
A	30.00	29.95 (4782)	...	...	27.99 (36.8)
B	30.00	29.95 (2566)	...	...	35.36 (1.14)
C	30.00	26.86 (1.16)	...	...	82.95 (1.034)

and erratic temperature dependence. All these results indicate an unusual and unexpected sensitivity of the results of the calculation to small changes in the input data. A series of experiments using the computer were then initiated to discover the source of this unexpected result. For this purpose synthetic data were prepared using values of  $K$  and  $\epsilon_c$  approximately appropriate for the 2-methylnaphthalene-1,3,5-trinitrobenzene complex.

### Experimental

The digital computer programs were written in IBM Fortran I (with Format) language and were run on an IBM 1620 computer with 20K memory. The standard accuracy of 8 significant digits was used throughout. Copies of the programs will be made available to interested parties.

The synthetic data consisted of nine concentrations of acceptor and donor (Table I), 12 values of  $\epsilon_c$  and  $\epsilon_a$  (the donor was assumed not to absorb in this region) corresponding to 12 wave lengths (Table II), two values of the equilibrium constant (10.0 and 30.0), and the corresponding calculated absorbances. The absorbances were rounded off to give the same number of significant figures as could be obtained experimentally.

The synthetic data were processed in groups of three concentrations and 12 wave lengths using (1) the calculated data and (2) data with random errors introduced. The data (containing errors in  $A_0$ ,  $D_0$ ,  $\epsilon_a$ , and  $D_m$ ) were introduced in different combinations so that the effects of various types of errors (singly and in combination) on the equilibrium constant could be evaluated. The results are summarized in Table III.

The errors introduced into the absorbances ranged from 0 to 0.003 absorbance unit. The errors in concentration were based on weighing errors of 0 to 0.3 mg. in samples ranging from 20 to 500 mg. Errors in the molar absorptivity of the acceptor were within 1.5 standard deviations (based on a least-squares determination of a typical acceptor as carried out in this laboratory). Table IV summarizes the values of the slopes, the errors in the

TABLE IV

Concn. group	Wave length	$m \times 10^3$	$\Delta m \times 10^3$			$\frac{\Delta m(A_0, D_0)}{\Delta m(D_m)}$
			Due to $\Delta A_0$	Due to $\Delta D_0$	Due to $\Delta D_m$	
A	3	1.5185				
		2.1510	0.018	0.021	0.015	2.6
		2.6683				
B	10	0.0984				
		.1391	.0012	.0040	.0010	5.2
		.1727				
B	3	1.5086				
		1.4661	.024	.017	.014	2.9
		1.4588				
C	10	0.1023				
		.0984	.0016	.0040	.0006	9.3
		.0984				
C	3	2.0107				
		2.0680	.032	.020	.012	4.3
		2.1369				
C	10	0.1302				
		.1339	.0022	.0055	.0011	6.5
		.1341				

slopes, and the contribution to these errors due to errors in  $A_0$ ,  $D_0$ , and  $D_m$ .

Wherever there could be found in the literature tabulated concentration and absorbance data for association equilibria, these data were processed using the present programs. The results are summarized in Table V.

### Discussion

For the purpose of discussion of the sources of error in this type of work, a more specialized equation will be used. In the case of a 1:1 complex ( $a = 1$ ,  $d = 1$ ), elimination of  $C$ ,  $A_e$ , and  $D_e$  from eq. 3 and 4 gives an equation in two unknowns ( $K$  and  $\epsilon_c$ )

$$\frac{1}{K} = \frac{D_0 A_0 (\epsilon_c - \epsilon_a - \epsilon_d)}{D_m - \epsilon_a A_0 - \epsilon_d D_0} - (D_0 + A_0) + \frac{D_m - \epsilon_a A_0 - \epsilon_d D_0}{\epsilon_c - \epsilon_a - \epsilon_d} \quad (5)$$

This equation is the same as the Rose-Drago equation<sup>10</sup> except that the contribution of the donor molecules to the total observed absorbance has been included.

Consider the results in Table III. Errors of less than 1% introduced into the concentrations  $A_0$  and  $D_0$  in concentration group B caused the calculated value of the equilibrium constant to be in error by more than 50%. Solving the equations directly or graphically gave equally erroneous results.

The reason for this behavior can best be seen by plotting  $K^{-1}$  vs.  $\epsilon_c$  as in the Rose-Drago method.<sup>10</sup> The errors can be considered in two groups: errors affecting the slopes and errors causing displacement in the curves. Differentiation of eq. 5 with respect to  $\epsilon_c$  gives the slope of the curves in the Rose-Drago plot

$$m = \frac{A_0 D_0}{D_m - \epsilon_a A_0 - \epsilon_d D_0} - \frac{D_m - \epsilon_a A_0 - \epsilon_d D_0}{(\epsilon_c - \epsilon_a - \epsilon_d)^2} \quad (6)$$

Since the slopes are so nearly identical (less than 3% separates the slopes of any of the curves resulting from concentration group B), slight errors in the slope or in the displacement of the curves will give widely varying intersections or, in the case of parallel lines, none at all.

In general if a particular set of data gives curves with slopes differing by as much as 10-15% of each other, extremely small errors in  $A_0$ ,  $D_0$ , and  $D_m$  are sufficient to shift the points of intersection of the curves by large amounts.

These qualitative observations are corroborated by a more formal error analysis.<sup>11</sup> Since in our case  $\epsilon_d$  is zero and  $\epsilon_a$  can always be made very small (by choice of wave length and least-squares treatment of data for pure acceptor), an approximate expression (omitting terms with  $(\epsilon_c - \epsilon_a)^2$  in the denominator) for the error in the slope,  $\Delta m$ , as a function of errors in  $A_0$ ,  $D_0$ , and  $D_m$  can be written

$$\Delta m = \frac{A_0}{D_m - \epsilon_a A_0} \Delta D_0 + \frac{D_m D_0}{(D_m - \epsilon_a A_0)^2} \Delta A_0 + \frac{A_0 D_0}{(D_m - \epsilon_a A_0)^2} \Delta D_m \quad (7)$$

This analysis emphasizes that the conditions necessary to minimize the effects of errors are dependent on

(10) N. J. Rose and R. S. Drago, *J. Am. Chem. Soc.*, **81**, 6138 (1959).

(11) H. D. Young, "Statistical Treatment of Experimental Data," McGraw-Hill Book Co., Inc., New York, N. Y., 1962.

TABLE V

TABLE OF LITERATURE RESULTS FOR HETEROASSOCIATION AND COMPARISON WITH RESULTS OF THE PRESENT WORK

	Literature results				Present results			
	Ref.	$K$	$\epsilon$	$\Sigma(\text{dev.})^2$	$K$	$\epsilon$	$\Sigma(\text{dev.})^2$	Sharpness
Thiacyclobutane, iodine	<i>a</i>	$87 \pm 4$	1690	$2.771 \times 10^{-3}$	99.57	1459	$1.950 \times 10^{-4}$	1.02
			1855			1656		
			1920			1701		
			1680			1608		
	<i>4</i>	$91 \pm 0.2$	1572	$2.795 \times 10^{-4}$				
			1785					
			1830					
	<i>b</i>	$93 \pm 33$	$1800 \pm 440$	<i>g</i>				
	<i>b</i>	$82 \pm 8$	$2010 \pm 200$	<i>g</i>				
	<i>b</i>	87.7	1870	<i>g</i>				
Diphenylamine, trinitrobenzene	<i>c</i>	0.51			0.5008	1391	$1.778 \times 10^{-6}$	51.35
		.49			0.5051	1652	$2.962 \times 10^{-6}$	16.85
	<i>4</i>	$.49 \pm 0.1$	1410	$2.563 \times 10^{-6}$				
		$.51 \pm 0.1$	1630	$2.349 \times 10^{-6}$				
Naphthalene, picric acid	<i>d</i>	2.29	377	$3.576 \times 10^{-2}$	6.941	157.7	$5.850 \times 10^{-3}$	0.34
	<i>4</i>	$3.6 \pm 0.3$	250	$1.293 \times 10^{-2}$				
Dimethyl sulfide, iodine	<i>e</i>	$71.4 \pm 2$	1140	$1.217 \times 10^{-1}$	70.45	1157	$4.979 \times 10^{-2}$	52.2
			1490			1505		
			1830			1842		
			2020			2043		
			2040			2044		
			1860			1865		
Dimethyl selenide, iodine	<i>e</i>	$471.4 \pm 4.4$	2030	$1.119 \times 10^{-1}$	481.4	2066	$2.705 \times 10^{-2}$	20.78
			2310			2356		
			2660			2714		
			2850			2915		
			2790			2857		
			2500			2563		
Dimethyl selenide, bromine	<i>e</i>	$2.5 \times 10^6$	30	$8.757 \times 10^{-1}$	$10^{11}$	31.30	$1.476 \times 10^{-4}$	<i>ca.</i> 0
			15			16.31		
			8			8.40		
			5			5.13		
			3			3.52		
			2			2.02		
Naphthalene, iodine	<i>f</i>	0.62	2395	$1.194 \times 10^{-2}$	0.76	90294	$1.090 \times 10^{-4}$	<0.009
Biphenyl, iodine	<i>f</i>	0.37	4000	$3.457 \times 10^{-5}$	.127	11480	$2.876 \times 10^{-6}$	0.054
	10	$0.39 \pm 0.15$	3925	$1.989 \times 10^{-5}$				
Stilbene, iodine	<i>f</i>	0.34	8000	$1.640 \times 10^{-2}$	$4.198 \times 10^{-5}$	$3.02 \times 10^8$	$3.080 \times 10^{-5}$	<i>ca.</i> 0
Phenanthrene, iodine	<i>f</i>	1.06	1492	$1.926 \times 10^{-2}$	1.82	4469	$8.520 \times 10^{-6}$	0.008
Pyrene, iodine	<i>f</i>	36.49	161	$5.563 \times 10^{-2}$	42.29	703.5	$3.068 \times 10^{-6}$	1.54
	10	$43 \pm 7$	1396	$8.998 \times 10^{-2}$				
	<i>b</i>	$43.6 \pm 5.6$	$142 \pm 15$	$5.760 \times 10^{-2}$				
Anthracene, iodine	<i>f</i>	52.35	112	$2.311 \times 10^{-2}$	8.27	1305	$1.650 \times 10^{-5}$	0.109

<sup>a</sup> J. D. McCullough and D. Mulvey, *J. Am. Chem. Soc.*, **81**, 1291 (1959). <sup>b</sup> M. Tamres, *J. Phys. Chem.*, **65**, 654 (1961). <sup>c</sup> R. Foster, D. L. Hammick, and A. A. Wardley, *J. Chem. Soc.*, 3817 (1953). <sup>d</sup> R. Foster, *ibid.*, 5098 (1957). <sup>e</sup> N. W. Tideswell and J. D. McCullough, *J. Am. Chem. Soc.*, **79**, 1031 (1957). The concentrations for the fourth concentration set of the dimethyl sulfide, iodine data were taken as  $S \times 10^3 = 99.79$ ,  $B \times 10^3 = 5.438$ ; J. D. McCullough, private communication. <sup>f</sup> R. Bhattacharya and S. Basu, *Trans. Faraday Soc.*, **24**, 1286 (1958). <sup>g</sup> The  $\Sigma(\text{dev.})^2$  are not comparable because the values given in ref. *b* are based on calculations at a single wave length (the third of those above).

the particular system being studied; *i.e.*, the particular values of  $K$ ,  $\epsilon_c$ ,  $\epsilon_a$ ,  $\epsilon_d$ ,  $A_0$ , and  $D_0$ . Note that synthetic data without errors deliberately introduced (thus corresponding to ideal experimental technique) sometimes fail to reproduce the value of  $K$  within 10% (Table III, group C). No degree of sophistication in the treatment of the data can overcome this inherent indeterminism resulting from the poor choice of experimental conditions. The value of  $K$  cannot be improved by selective rejection of data in this case because there are no erroneous points to reject.

A study of Table IV indicates that the total error in

the slopes in concentration groups B and C are often larger than the deviation of the various slopes within the groups. In general, the errors in  $A_0$  and  $D_0$  have the most pronounced effects on the slopes. The slopes will differ the most (and hence give the best results) when the concentration of the absorbing species is held fairly constant and the concentration of non-absorbing species is varied over a wide range (as in concentration group A and the diphenylamine-trinitrobenzene data from the literature). This is borne out by considering the slopes given in Table IV for the various concentration groups.

Concentration group A gives the most favorable case (and the best results). In this concentration group the concentration of the donor (nonabsorbing) was varied over a 10:1 range. In concentration group C, the concentration of the acceptor (absorbing) was varied over the same range, but the slopes of this group differ by only small amounts. This effect is due to the contribution of the acceptor molecule to the observed absorbance.

Compared to errors in  $A_0$  and  $D_0$ , the errors in  $D_m$  are less important by a considerable factor (*cf.* Table IV). Reduction of the concentration errors by an order of magnitude would greatly enhance the accuracy of the determination of the equilibrium constants and make the measured absorbances the limiting factor in the accuracy of the determination. This is a desirable condition since (1) absorbances can be measured at a large number of wave lengths (whereas obtaining a large number of concentrations with slopes differing by sufficient amounts is difficult) and (2) computer techniques minimizing the sum of the squares of the deviations of the observed absorbances from the calculated absorbances would then be optimally applicable.

Errors in displacement of the curves can be considered by qualitative inspection of eq. 5. To minimize errors in displacement due to  $D_m$ , the terms  $D_m - \epsilon_a A_0 - \epsilon_d D_0$  and  $\epsilon_c - \epsilon_d - \epsilon_a$  should be large. Both these conditions can be satisfied if  $\epsilon_c C$  (the absorbance due to the complex) is large compared to  $\epsilon_a A_0 + \epsilon_d D_0$  (the combined absorbances of donor and acceptor). This involves carefully selecting the wave lengths at which data will be obtained.

In an attempt to obtain some measure of the reliability of the equilibrium constants calculated, we have adopted a suggestion of Coburn and Grunwald's<sup>6</sup> involving the sharpness of the fit as a function of the value of  $K$ . To this end, the fit was calculated for values of  $K$  10% on each side of the best value (10% was arbitrarily chosen as a reasonable goal to strive for in determining  $K$ ). The ratio of the percentage change in fit to 10 (the percentage change in  $K$ ) is presented in Tables III and V as the sharpness. A large sharpness corresponds to a reliable value of  $K$ ; note that in these cases agreement of the present results with the literature is satisfactory. A low sharpness value corresponds to a poorly determined  $K$ . The same relationship is evident in the synthetic data, although in some cases better fits were obtained by chance than the sharpness value would indicate.

In terms of the Rose-Drago treatment, when the various intersections occur in a region small compared to a 10% change in  $K$ , the sharpness will be large because a change in  $K$  of this size causes a large change in fit. On the other hand, when the various intersections occur over a region large compared to 10% of  $K$ , a change in  $K$  of this size causes only a small change in fit.<sup>12</sup>

(12) In an alternate approach to a criterion for a satisfactorily determined value of  $K$ , the ratio of the fit at  $K \pm 10\%$  to that at the best value of  $K$  may be compared to the  $F$ -value found in statistical tables, a higher value indicating within a given confidence limit that the determination is accurate within 10%. Although not strictly a correct application of the  $F$  tables, this method offers a way to account for the effect of the number

The synthetic data suggest that the sharpness should be 20 or more for a reliably determined value of  $K$ .<sup>13</sup> We withhold data obtained in this laboratory because it does not yet meet this criterion. Systematic errors whose effect is a systematic displacement of all the Rose-Drago curves will have little effect of the sharpness; only errors having a random effect on the curves will be estimated by the sharpness criterion. Non-absorbing impurities in acceptor and donor samples might constitute such a systematic error.

To determine equilibrium constants accurately, several conditions must be met: (1) The slopes of the plots of  $K^{-1}$  and  $\epsilon_c$  within a concentration group must differ from each other by as much as possible. (Any attempt to employ many concentrations within a group will only decrease the variation between the slopes. Although more intersections will result, they will be scattered over a large area and the resulting  $K$  may be of greatly reduced accuracy. Processing data from several concentrations in well-chosen groups and checking that the values of  $K$  and  $\epsilon_c$  resulting from each group agree with those resulting from the other groups offer a preferable alternative.)

(2) The errors in the concentrations must be reduced to the point that their contributions to the errors in the slopes will be small compared to the variations between the slopes. (Ideally the concentration errors should contribute less to the error in the slopes than do the errors in the absorbance.)

(3) In order to minimize errors in the displacement of the curves a region of the spectrum should be chosen in which the contribution of the complex to the observed absorbance is large (as in the dimethyl selenide-iodine data from the literature).

In order to meet these conditions it will usually be necessary to make a preliminary run and a preliminary calculation to get rough values for  $K$  and  $\epsilon_c$  on the basis of which the exact experimental conditions for the final run can be chosen intelligently. If these conditions cannot be met, presently available experimental techniques are insufficient to determine  $K$  accurately.

The foregoing discussion has been based entirely on the heteronuclear equilibrium case, but the same considerations will obviously apply in the self-association case except that in the case where both monomer and polymer absorb at the same wave length, the incursion of errors is even more serious, because this is equivalent to *both acceptor and donor* absorbing at the critical wave lengths. In other words, it is less possible in the self-association case to make an intelligent choice of experimental conditions. Preliminary synthetic data experiments with the appropriate program confirm this.

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of data points on the sharpness of the fit. We thank Drs. N. A. Coward and G. S. Krause for discussions of this point.

(13) A sharpness of 20 corresponds to an  $F$ -value of 3.0.