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Kinetic Analysis Applied to Aluminum Citrate Complexing

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Standard nonlinear regression algorithms were adapted for the treatment of simultaneous multicomponent kinetic data arising in kinetic analysis. It was observed that good initial estimates of the regression parameters must be available before successful implementation of a standard library nonlinear regression algorithm can be assured. The Guggenheim method adapted to multicomponent kinetics was incorporated into the scheme of analysis for preliminary treatment of the data to produce the necessary initial approximations. The integrated approach was applied in the treatment of kinetic analysis data from an Al^{3+}*/Al–citrate mixture, the stability constant for the aluminum citrate complex obtained from such a kinetic analysis is* 5.55×10^{-4} *at 19.5 °C. Kinetic analysis parameters on the mixtures with various ratios of* Al^{3+} *and Al–citrate were both internally and externally consistent with results established in separate studies. Thus we present a correction of this stability constant.*

Multicomponent Kinetics – A Non-linear Model

Kinetic analyses [1] may be based on the following reaction scheme:

$$\begin{array}{l} A + R \xrightarrow{k_A} P \\ B + R \xrightarrow{k_B} P \\ \vdots \qquad\qquad\quad \vdots \\ N + R \xrightarrow{k_N} P \end{array}$$

where A, B, ..., and N are the analytes which react with a large excess of reagent R, *i.e.*, $[R]_o \gg [A]_o + [B]_o + \ldots\ldots + [N]_o$, to yield a common product P which is monitored by a physical method. The time dependent concentration profile of the product P is described by the following expression,

$$[P]_t = [A]_o(1 - e^{-k_A t}) + [B]_o(1 - e^{-k_B t}) + \\ + \ldots. + [N]_o(1 - e^{-k_N t}) + \text{constant} \qquad (1)$$

where brackets denote concentrations, k's are rate constants, t is time, and subscript zeros denote initial values. Mathematically, equation (1) represents a nonlinear model. Numerical data generated by such a system can be analysed via linear regression when linearization of the mathematical model can be accomplished either by a transformation of the original model or if *a priori* knowledge of the values of the rate constants is assumed [2, 3]. A serious limitation on the scope of kinetic methods of analysis has been that the concept of using the rate constant as a 'qualitative' analysis parameter is rarely achieveable. It is usually necessary to know these in advance to get good fits. Alternatively, nonlinear data must be treated directly with an iterative method such as curve fitting (non-linear regression) when linearization of the model is proven unfeasible. A prevalent criticism of the latter approach is that the model is ill-conditioned [4]. The present work is an attempt to demonstrate that *provided reliable initial estimates of the values of the parameters to be fitted are ascertainable*, the kinetic data of a well resolved 2 or 3 component system where the respective rates observed are separated by nearly an order of magnitude can be treated satisfactorily by *any one* of the common non-linear regression methods. (It must be made clear at the outset that we make no claim to be able to resolve the simultaneous rates of a closely overlapping multicomponent system of analytes). This work is preliminary to application of kinetic methods to the study of Al in natural water models. The novel feature is the use of the Guggenheim method to achieve an *objective* method of *obtaining preliminary parameter* estimates so that the nonlinear regression package is less likely to converge

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TABLE I. Fitted Values of a Poorly Resolved 2-Component Test System.

Trial	Parameters	Initial Approximation	Minimization Algorithm	Fitted* Parameter
I	A1	0.100	Marquardt's	0.392
	A2	0.100		0.109
	A3	0.100		6.76×10^{-2}
	A4	1.00×10^{-3}		7.30×10^{-3}
II	A1	0.200	Marquardt's	0.170
	A2	0.150		0.129
	A3	5.00×10^{-3}		0.240
	A4	2.00×10^{-3}		8.92×10^{-2}
III	A1	5.00×10^{-2}	Marquardt's	0.392
	A2	7.00×10^{-2}		0.109
	A3	4.00×10^{-2}		7.11×10^{-2}
	A4	5.00×10^{-4}		6.85×10^{-3}
IV	A1	9.00×10^{-2}	Marquardt's	0.171
	A2	0.107		0.129
	A3	0.150		0.239
	A4	0.100		8.91×10^{-2}
V	A1	6.50×10^{-2}	Marquardt's	0.190
	A2	0.239		0.126
	A3	0.133		0.220
	A4	0.300		8.75×10^{-2}
VI	A1	0.500	Marquardt's	0.395
	A2	8.00×10^{-2}		0.102
	A3	1.00×10^{-2}		1.36×10^{-2}
	A4	1.70		0.229
VII	A1	0.500	Gauss's	0.398
	A2	8.00×10^{-2}		0.102
	A3	1.00×10^{-2}		1.04×10^{-2}
	A4	1.70		0.330

*The data were generated with the values A1 = 0.16, A2 = 0.13, A3 = 0.25, A4 = 9.00×10^{-2}.

on a physically false (even if statistically best) solution. That is, we have some assurance that the *input parameters* to the nonlinear regression are *physically meaningful* even though we have *no advance information on the kinetic properties* of the analyte solution. This is a distinct advantage in kinetic analysis. It allows us to approach raw data without advance knowledge of any rate constants.

Nonlinear Parameters Estimates

Non-linear parameter estimate methods are well documented in applied mathematics literature [5–9] and applications in chemical problems [10–12].

In the present NONLINEAR REGRESSION package [13] (an on-line interactive routine available in the Carleton University computing library and in many libraries) two different popular gradient methods, those of Gauss (1809) and Marquardt (1963) are combined. The program is representative of the tool readily available to most chemists interested in routine application of nonlinear regression.

Testing of the Nonlinear Regression

To illustrate the problems with the algorithm *even with favourable chemical kinetic data,* and to

TABLE II Nonlinear Fitted Parameters of a Badly Overlapping 3-Component Test System.

Trial	Number of Data Points	Number of Iterations	Parameter	Initial Approximation	Minimization Algorithm	Fitted* Parameter
I	250	200	A1	0.407	Marquardt's	0.247
			A2	0.365		0.458
			A3	0.300		0.196
			A4	0.200		0.149
			A5	0.218		0.163
			A6	5.29×10^{-2}		4.04×10^{-2}
II	250	200	A1	0.247	Marquardt's	0.247
			A2	0.459		0.459
			A3	0.195		0.195
			A4	0.149		0.150
			A5	0.163		0.164
			A6	4.05×10^{-2}		4.05×10^{-2}
III	250	500	A1	0.247	Gauss's	0.237
			A2	0.459		0.466
			A3	0.195		0.184
			A4	0.149		0.165
			A5	0.200		0.181
			A6	5.00×10^{-2}		4.5×10^{-2}
IV	1000	1000	A1	0.247	Marquardt's	0.247
			A2	0.459		0.459
			A3	0.195		0.195
			A4	0.149		0.149
			A5	0.163		0.164
			A6	4.05×10^{-2}		4.06×10^{-2}
V	1000	1000	A1	0.247	Marquardt's	0.217
			A2	0.459		0.483
			A3	0.195		0.191
			A4	0.149		0.184
			A5	0.200		0.193
			A6	5.00×10^{-2}		4.83×10^{-2}

*Data were generatated using the parameters: A1 = 0.20, A2 = 0.50, A3 = 0.20, A4 = 0.20, A5 = 0.20, A6 = 5.00×10^{-2}.

underline the advantage of some supplemental procedure to simplify the fitting processes the Nonlinear Regression routine was tested in feasability studies with simulated noise-free data. An artificial file of 700 records was generated using the function,

$$Y = [A]_o(1 - e^{-k_A t}) + [B]_o(1 - e^{-k_B t}) \qquad (2)$$

simulating a 2-component kinetic system without a baseline signal. The first case studied was where the reaction rates between the first and second component are well resolved, *e.g.*, $t_{1/2}(A)/t_{1/2}(B) \gg 10$. Various sets of initial estimates of the parameters were subjected to a thousand iterations. In all cases, correct parameters were recovered and the nonlinear regression engendered no problems. In a separate study, the rates of the two components are programmed to be very close to each other; results of the fittings after a thousand iterations are tabulated in Table I.

It is obvious that provided the reaction rates between the two components are only marginally overlapping, the Nonlinear Regression is dependable even if the initial approximations are poor. *As the rates for the two components approach each other, however, the influence of the initial approximation values is crucial* as demonstrated in Table I. Satisfactory routine fitting results only *when reasonable initial guesses are available.* It should be emphasized that the present exercise does not prove that closely overlapping reaction rates of a 2-component system can not be successfully resolved by Nonlinear Regression, but rather that *adequate procedures must* *be developed to procure adequate initial approximations of the parameter values* if library routines are to be applied routinely. When this stipulation is satisfied, the proposed numerical analysis is applicable in spite of the response function being classically ill-conditioned.

In a 3-component kinetic system, the probability of determining accurately the unknown parameter values is not as good, particularly when the three reaction rates overlap closely. However, a hypothetical situation will demonstrate some of the potential limitations of the Nonlinear Regression. The fitting results are tabulated in Table II. Two crucial features emerged from this study. (i) A small quantity of data is often sufficient to achieve the same final regression result as that which was obtained from a comparatively large volume of information, as long as the behaviour of the response function is fully represented. Typically, the number of data pairs in Trial II in Table II is only a quarter of that of Trial IV, but when both describe the same interval of the response function, the fitted parameter values are identical; (ii) it is apparent from the preceding example as well as in the case of Trial I and Trial II that 200 iterations are sufficient to produce the 3 significant figures best-fit set of parameter values. These features are particularly attractive in that they economize both the workspace and execution time required for routine implementation of the Nonlinear Regression programme. Allowing for the closeness of the parameters values used in simulating the 3-component system (a situation which we cannot hope to resolve satisfactorily when encountered in experiment), the final fitting results in Trials I and V are acceptable.

In summary, rates that do not overlap extensively, can be analyzed accurately in a 2-component system, and in all likelihood a 3-component system as well by the chosen algorithms provided that reasonable initial estimates are available. The same numerical method was successfully applied by Moore [10] to analyze the kinetic data of a first-order reaction with unknown initial and final (infinity) readings plus the rate constant in question, Alcock *et al.* in either two separate studies reported the determination of equilibrium constants [9] and rate constants [12] through the application of Marquardt's algorithm. The nonlinear treatment of kinetic data was found to be particularly effective in mechanistic studies involving either multiple reaction networks or resolving the overall rate of reaction into the rates of stepwise components. A perfect example of the potential benefit from using the nonlinear routine was demonstrated in the study of the substitution reaction of chloride and bromide in the $[Pt(NH_3)_3X]^+$ complex by iodide [12]. Assuming a second-order path only, nonlinear analysis consistently failed to produce good fits to the data until a first-order reaction step (k_1) leading to the formation of an intermediate $[Pt(NH_3)_3(OH_2)]^{2+}$ was incorporated into the reaction model. The magnitude of k_1 was very small compared to k_{obs}, thus plots of k_{obs} *vs.* the concentration of entering ligand L passed virtually through the origin. The presence of k_1 could have been easily overlooked, and when detected, its value could only be approximated with linear treatments. The nonlinear data treatment on the contrary produced reliable values for k_1 while simultaneously serving as a sensitive detector of deviations from the original set of conditions (*e.g.* a constant rate constant or blank) and/or physical model assumed. From this study, Alcock *et al.* concluded that in a situation where one reaction step is very much slower (factor of 10^3) than another (an ideal case of the condition depicted in Table I), nonlinear analysis is more likely to resolve the observed data than linear treatments. In the section to follow, it will be shown that this method is far superior in comparison to the relatively primitive graphical method in the extraction of kinetic parameter values from a simultaneous 2-component system. Thus, the routine nonlinear packages available in the libraries of most computing centres can be an important aid to kinetic analysis of genuine 'unknowns' if a method for initial parameter estimation is available.

Kinetic Analysis of a Mixture of Hexaaquo Aluminum Ion and Aluminum Citrate Complex. The Guggenheim Method for Initial Approximations

The objective of this work is to implement the algorithm described above in the treatment of an experimental system using a new approach to generate reasonable first approximations. The system being examined is

$$Al^{3+} + \text{Citrate} \overset{K_s}{\rightleftharpoons} \text{Al-Citrate} \qquad (3)$$

where K_s is the stability constant of the aluminum citrate complex generally expressed as,

$$K_s = \frac{[ML^{(n-1)^+}][H^+]}{[M^{n+}][HL]},$$

for any degree of metal–ligand complex formation. The reaction in equation (3) is obviously pH dependent, therefore, by manipulating the pH, mixtures with different ratios of Al^{3+}/Al–Citrate distribution can result. Provided that the ratios $[ML^{(n-1)^+}]/[M^{n+}]$ and $[H^+]/[HL]$ can be determined, the stability constant K_s can be evaluated directly. The present strategy is to react the free metal ion/metal complex mixture with a common reagent which will displace the citrate ligand from the parent aluminum complex. Such a reagent is found in a metalfluorochromic reagent commonly known as Calcein Blue (abbreviated as CB from here on). The properties and conditions for CB as a reagent for kinetic analysis of aluminum have been carefully characterized [16]. The reaction scheme of the analysis is then,

$$\begin{aligned} &Al^{3+} + CB \xrightarrow{k_x} Al\text{–}CB \\ &Al\text{–}Citrate + CB \xrightarrow{k_y} Al\text{–}CB + Citrate \end{aligned} \qquad (5)$$

If the reaction pH is controlled and the successive protonation constants [17] of the ligand are known, the stability constant K_s of aluminum citrate can be determined with the information ascertained from a kinetic analysis of the Al^{3+}/Al–Citrate mixture. In a study reported by Langford and Khan [18], the concentration of free and bound aquo Fe(III) in equilibrium mixtures of aquo Fe(III) and a soluble soil ligand, fulvic acid, were determined directly by kinetic analyses. It was found that free aquo Fe(III) reacted rapidly with a large excess of the kinetic analytical reagent 5-sulfosalicylic acid prior to significant perturbation of the iron–fulvic acid complex, consequently, both the concentrations of free and bound aquo Fe(III) were determined. From the analytical data, the conditional formation constant K_1 which was defined as [Fe–fulvic acid]/[Fe(III)-free] [fulvic acid] was determined at three pH values.

The Guggenheim Method

In order that the Nonlinear Regression analysis may be successfully implemented, suitable initial estimates of the unknown parameters must be provided. A logical approach would be to subject the composite kinetic data to a graphical type analysis with the resultant information subsequently employed as initial 'guesses', in the nonlinear analysis. Such a precursor treatment of data is best implemented by the Guggenheim method [19] which involves, essentially, converting the originally observed data into a logarithmic form and considering Δlog(Measured parameters) over successive fixed time intervals. If the components are kinetically well separated, *i.e.* reaction rates for each component are at least one order of magnitude apart, the Guggenheim plot would resemble a curve that is not smooth but rather comprised of distinguishable linear segments with different gradients (since it was invented to linearize first order kinetics where infinity values are unknown). The segments reflect the number of reacting components that can be kinetically resolved. Thus, a routine Guggenheim analysis of the data from an unknown mixture would: (i) aid assessment of the number of resolvable reacting species present in the mixture, and (ii) enable one to obtain, through *linear* least-squares regression analysis, fairly reasonable initial estimates of the parameter values to be entered in the nonlinear analysis. In the case of a simple 2-component system, a parameter estimate for the faster component is often obtained using exclusively Guggenheim data points early in the plot while the reverse is true for the estimation of the parameter value of the slower components. This is by no means a golden rule for exceptions are found in cases where the Guggenheim plots are noisy. In such cases, most of the data points in the available plots have to be used to obtain reasonable linear regression. Success still depends on the judgement of the analyst, and partly on the nature and/or condition of the observed data.

Experimental

Reagents

Aluminum chloride hexahydrate (reagent grade) was obtained from BDH Chemicals and twice recrystallized from doubly distilled water. The hydration number of the recrystallized aluminum chloride salt was determined gravimetrically [20] by precipitation with 8-hydroxyquinoline. The average of three determinations was 6.00 ± 0.0024. Analytical reagent grade tri-sodium citrate dihydrate ($Na_3C_6H_5O_7 \cdot 2H_2O$, M.W. = 294.10 gm) obtained from BDH Chemicals and the dye Calcein Blue (CB) from G. Frederick Smith Co. (F.W. = 321.29 gm) were used without further purification. Reagent grade anhydrous sodium acetate and acetic acid were used in the preparation of pH 5.0 buffer. Reagent grade perchloric acid used in the present work was standardized [21] against mercuric oxide using methyl purple as the indicator. Water doubly distilled from glass was used in all the solution preparations.

Kinetics

A stock solution of 100 ml of 1.00×10^{-3} *M* tri-sodium citrate dihydrate was prepared and used in the subsequent preparation of 200 ml of 1.00×10^{-4} *M* Al^{3+} + 1.00×10^{-4} *M* Na_3-Citrate stock solution. Sample solutions were prepared from the stock Al^{3+}/Na_3–Citrate solution by successive dilutions. The hydrogen ion concentrations of the sample solutions were adjusted with $HClO_4$ to the desired level as monitored by an Orion Research (Model 701A) 3-decimal digital pH meter. All the prepared sample solutions were allowed to stand for twenty-four hours to assure equilibrium. Reagent solutions of Calcein Blue were prepared directly from the crystals supplied by G. Frederick Smith Co., and always at concentrations 100-fold in excess of those of the Al^{3+}/Na_3-Citrate samples being analyzed. The CB reagent solutions were made up to contain 0.2 *M* acetate buffer at pH 5.0 such that after mixing, the final concentration of buffer was set at 0.1 *M*. Aluminum recovery experiments with the analytical reagent CB were performed on a Perkin-Elmer

TABLE III. Equilibrium (24 Hours) Recoveries of Aluminum by the Reagent CB from Mixtures of Ionic Aluminum and Tri-Sodium Citrate at Various Acidities.*

$[Al]_{Total}$ x 10^5 *M*	$[Citrate]_{Total}$ x 10^5 *M*	$[CB]_{Total}$ x 10^3 *M*	pH	Recovery %
2.0	1.0	2.0	4.00	97.03
2.0	2.0	2.0	2.70	99.49
2.0	2.0	2.0	3.00	98.98
0.20	0.10	0.20	4.00	96.06
0.20	0.20	0.20	3.00	98.53
0.20	0.20	0.20	3.55	97.17
0.20	0.20	0.20	4.00	98.75

*The aluminum recoveries at various acidities and mixture compositions were calculated using a theoretical 100% aluminum recovery derived by substituting the reaction mixture in question with a reference solution of ionic aluminum at pH 3.0 identical in concentration to the total aluminum concentration of the mixture, the final emission intensity obtained from equilibrating this reference with the reagent CB for 24 hours was used as the value of total possible recovery.

TABLE IV. Kinetic and Thermodynamic Data of the Aluminum–Citrate Complex as Determined by Nonlinear Regression Kinetic Analyses.

$[Al]_{Tot}$ x 10^5 *M*	$[Cit]_{Tot}$ x 10^5 *M*	pH	$[CB]_{Tot}$ x 10^3 *M*	$[Al]_{Free}/[Al]_{Tot}$	$k_{(Al)}$[a] M^{-1} sec^{-1} x 10^{-2}	$k_{(Al-Cit)}$[a] sec^{-1} (obs) x 10^3	Equil.[b] Infinity	Kinetic[b] Infinity	% Recovery	Stability Constant (K) x 10^4
2.0	2.0	2.70	2.0	0.61	3.37	4.70	25.23	24.98	99.01	7.13
2.0	2.0	3.00	2.0	0.40	2.92	4.88	18.77	18.88	100.6	4.49
0.20	0.20	2.80	0.20	0.88	2.82	5.01	17.53	16.65	94.98	6.05
0.20	0.20	3.00	0.20	0.77	2.66	4.41	19.74	20.03	101.5	4.74
0.20	0.20	3.20	0.20	0.57	3.16	4.68	19.74	20.27	102.7	5.34
0.20	0.20	3.55	0.20	0.30	2.29	4.90	22.50	22.92	101.9	5.56

[a] Since the rate law governing the pseudo first order process observed is presumably rate = k[Al] [CB] for the free ion, a 2nd order constant is reported. We cannot be confident of the rate law for the reaction with the complex without a very extensive study, so only the phenomenological pseudo first order constant is given. [b] Both the equilibrium and kinetic infinities are expressed in arbitrary units. The equilibrium infinity is obtained after equilibrating the reaction mixture with the reagent CB for 24 hours, the subsequent recovery of aluminum is used as the theoretical 100% aluminum recovery.

spectrofluorimeter (Model 204S). λ_{ex} was at 340 nm with the fluorescence monitored at λ_{em} = 410 nm. All the kinetic runs were performed on an automated Aminco-Bowman stopped-flow spectrofluorimeter [16] at 19.5 ± 0.1 °C. The resulting data stored on disk in the minicomputer can be transferred into the mainframe computer for the Nonlinear Regression analysis via paper tape. Both linear regression and Guggenheim analysis routines can be performed on either one of the computers. Theoretical equilibria or reaction infinities (24 hours) were determined by reacting the reagent with aluminum solutions that were identical in hydrogen ion concentrations and Al^{3+} concentrations both without the presence of any citrate. A minimum of ten kinetic runs for each point were recorded for the purpose of data averaging. An experimental emission blank was recorded with each run and subsequently entered into the nonlinear analysis using a 2-component model with a constant described in equation (1).

Some of the noisier kinetic spectra were subjected to data smoothing by the digital filter technique [22] based on Fourier Transformation [23] of the signal. The smooth data, when analyzed by the nonlinear regression, did not demonstrate any significant improvement over the untreated data, an observation in agreement with those previously reported by McKinnon *et al.* [24]. There are, however, several aspects of data analyses which should benefit from the implementation of noise reduction.

TABLE V. Part A. Analyses of a Well-Resolved 2-Component Experimental System.[a] Part B. Analyses of an Intermediate 2-Component Experimental System.[b]

Parameter	Experimental Blank	Guggenheim Estimates	Raw Data Fit	Filtered Data Fit
(A)				
A1		13.45	10.28	10.11
A2		0.2122	0.2896	0.2903
A3		11.84	10.84	10.99
A4		4.250×10^{-3}	4.964×10^{-3}	4.862×10^{-3}
A5	7.500		6.901	7.101
(B)				
A1		16.67	14.99	14.99
A2		2.338×10^{-2}	2.448×10^{-2}	2.450×10^{-2}
A3		14.17	5.396	5.394
A4		8.990×10^{-2}	4.929×10^{-3}	4.932×10^{-3}
A5	4.000		4.294	4.333

[a] Fitting results obtained after 300 iterations floating all five variables. [b] Fitting results obtained from 600 iterations. Initial estimates of A2, A4, and A5 were held constant in the first 300 iterations, while in the last 300 iterations all five variables were floated.

(i) Due to the pair of point nature of the Guggenheim algorithm, excessively noisy data can bring about a failure of the Guggenheim plot.

(ii) 'Cleaner' data would produce a 'cleaner' Guggenheim spectrum which in turn would facilitate assessment of the number of components present in the analyte. Finally, (iii) a large residual sum of squares could only mean either that the data were noisy or that the physical model (response function) supplied does not describe the observed data adequately. With relatively noise-free data, a large residual sum of squares can be attributed to the failure of the proposed model. Thus, filtering is useful for purposes other than improvement of iterated parameter values.

Results

To ensure that, under the present experimental conditions, the reagent CB would displace the citrate ligand completely from its aluminum complex, equilibrium recovery experiments were performed and the results tabulated in Table III. It was found that the infinity emissions after 24 hours of equilibration with CB for the pure Al^{3+} samples and the Al^{3+} + Al–Citrate mixtures were identical within experimental error. This qualified the reagent CB in the analysis of this chosen model system.

The results from a study of six different mixtures of Al^{3+} and its citrate complex are tabulated in Table IV. The experimental reaction rate constant of the aluminum citrate complex k, was found to be independent of the reagent concentration in all the situations. In such a case, the two component composite kinetics observed should be better resolved at higher reagent (CB) concentrations since the pseudo first-order rate constant for the free-Al^{3+} ions would then be much larger than k_{obs} of the citrate complex. Indeed, Guggenheim plots for the first and second mixtures in Table IV demonstrated two linear segments of drastically different gradients while data of the rest of the four mixtures displayed two slopes which were still easily differentiable but noticeably closer in magnitude. Typical Guggenheim plots of the two groups of data are shown in Fig. 1(a) and (b) and 2(a) and (b). Examples of nonlinear regression analysis on the data corresponding to the depicted kinetic runs are compiled in Table V. Fittings performed with the filtered data did not substantially improve the parameter values determined, but is clearly easier to recognize the number of components.

Discussion

The second-order rate constants of the free-Al^{3+} ion are in good agreement with those established in an independent single component Al-CB complex formation kinetic study [16]. For instance, the second-order rate constant for reactions between 10^{-6} M of free-Al^{3+} and 2×10^{-4} M CB was 2.62×10^{2} M^{-1} sec^{-1}, very similar to what is determined in the 2-component analysis, an average of ~2.70×10^{2} M^{-1} sec^{-1}. In the six experiments reported in

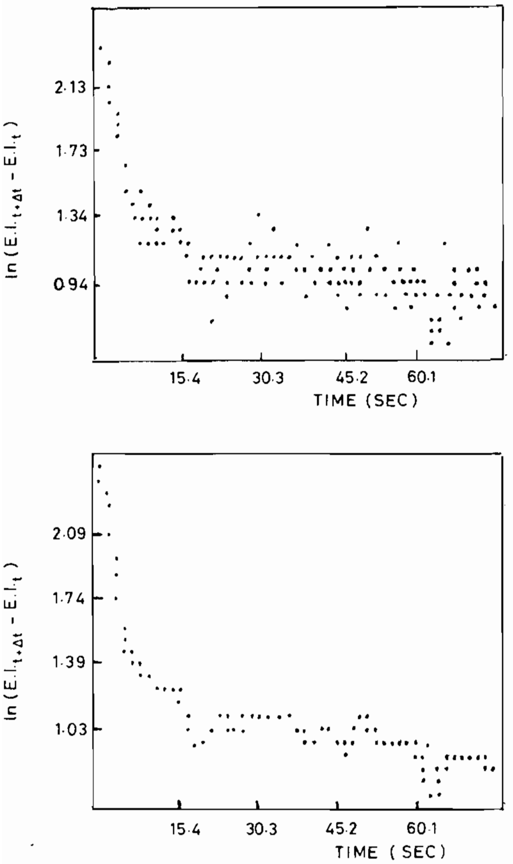

Fig. 1. (a) Guggenheim Plot of Data (Unfiltered) of a Typical Well-Resolved 2-Component System. System under observation is: $\{10^{-5}\ M\ [Al^{3+}]/10^{-5}\ M\ [Na_3\text{-Citrate}]\}$ mixture at pH = 3.0 + 10^{-3} *M* CB, in 0.1 *M* acetate buffer at pH = 5.0. (b) Guggenheim Plot of Data (Filtered) of a Typical Well-Resolved 2-Component System. System under observation is as in Fig. 1(a).

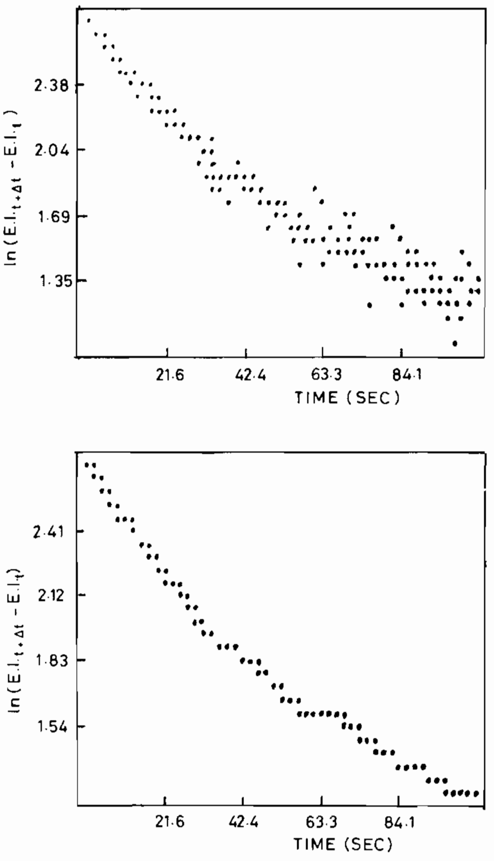

Fig. 2. (a) Guggenheim Plot of Data (Unfiltered) of a Typical Interdiately-Resolved 2-Component System. System under observation is: $\{10^{-6}\ M\ [Al^{3+}]/10^{-6}\ M\ [Na_3\text{-Citrate}]\}$ mixture at pH = 3.0 + 10^{-4} *M* CB, in 0.1 *M* acetate buffer at pH = 5.0. (b) Guggenheim Plot of Data (Filtered) of a Typical Intermediately-Resolved 2-Component System. System under consideration is as in Fig. 2(a).

Table IV, the metal (Al^{3+}) to reagent concentration ratio and the initial pH of the Al^{3+}/Al-Citrate mixtures were all different, this has been proven in reference 16 to produce slight discrepancies in the second-order rate constants. Taking into consideration these various facotrs, it is not unreasonable to anticipate some slight fluctuations in the second-order rate constants in Table IV.

The observed independence of the rate constant (k_{obs}) between Al–Citrate and CB of the reagent concentration seems to suggest the *dissociation* of citrate as the rate determining step in the ligand exchange. It is likely that such a dissociation occurs stepwise with the initial step, the breaking of an Al–O bond being rate determining. However, the present results are not definitive. Secco and Venturini [25] established the dissociation rate constant for the complex aluminum salicylate, yielding $AlOH^{2+}$ and HL^-, as 7.90 × 10^{-3} sec^{-1} at 25 °C. The average 'dissociation' rate constant k_{obs} determined in this work for the complex Al–Citrate is 4.76 × 10^{-3} sec^{-1} at 19.5 °C, and comparable to that of the Al–Salicylate complex. Buck and Moore [26] suggested that complexes of comparable stabilities should have similar rates of dissociation (consequence of the dissociative mechanism). If this is relevant, the overall intrinsic stability of the citrate complex of aluminum is comparable to that of the salicylate complex and the pentaaquo hydroxy aluminum ion is involved in the kinetics of formation of the aluminum citrate complex at pH 5.0.

The stability constants K_c of the aluminum citrate complex reported in Table IV were calculated with the following expression,

$$K_c = \frac{[\text{Al–Citrate}]\,[H^+]^3}{[Al^{3+}]\,[H_3\text{Citrate}]} \tag{6}$$

The concentration of Al–Citrate and Al^{3+} were the experimentally determined quantities. Using the value of $K_{OH} = 3.55 \times 10^{-6}$ *M* for the hydrolysis constant between $Al(H_2O)_6^{3+}$ and $Al(H_2O)_5OH$ adopted by Perlmutter-Hayman and Tapuhi [27], the percentage of hydrolysis at the highest pH level employed in the preparations of the complex, *i.e.* 3.55, is calculated to be 1.24%, therefore, the experimentally determined concentration of the free-Al^{3+} component is assumed to be $Al(H_2O)_6^{3+}$ in all instances. The hydrogen ion concentration is taken to be that at which the complex was prepared and equilibrated. Finally, the concentration of the undissociated citric acid can be calculated from the literature values of the protonation constants [17], *i.e.*, $K_1 = 4.79 \times 10^5$, $K_2 = 2.24 \times 10^4$, and $K_3 = 7.42 \times 10^2$. The average stability constant established in the present work is 5.55×10^{-4} at 19.5 ± 0.1 °C, a value which is substantially higher than a previously reported value of 1.95×10^{-5} at 33 °C in 0.25 *M* $NaClO_4$ determined by Pattnaik and Pani [30] using the pH titration method. It seems unlikely that only temperature causes the difference. If a value of 1.95×10^{-5} is assumed in a theoretical calculation, it can be shown that at pH 3.0, 12.41% of the aluminum ions exist as the citrate complex in a mixture of 2×10^{-5} *M* Al^{3+} + 2×10^{-5} *M* Na_3-Citrate, the percentage drops even lower to 1.57% in the case of a mixture of 2×10^{-6} *M* Al^{3+} + 2×10^{-6} *M* Na_3-Citrate. This is obviouly contrary to the present observation where the percentages were determined to be 59.93% and 23.22% in the respective mixtures. Any error of the kinetic method would *underestimate* the complex concentration. The experimental evidence in this work supports the trustworthiness of our stability constant for the following reasons. The rate constants of free-Al^{3+} and Al–Citrate determined from 2-component nonlinear regression analyses are in good agreement with what was observed in an independent study of the free-Al^{3+} [12] and the literature value [25] for the dissociation rate constant of the Al–Salicylate complex which is not altogether unlike the citrate complex. Secondly, the stability constants established in six different experimental conditions are within experimental uncertainties 'internally' consistent. Finally, the substantial presence of the aluminum citrate complex in the reaction mixtures at pH 3.0 are indisputable in the Guggenheim plots in Figs. 1(a) and (b), and 2(a) and (b). In fact, in the majority of cases, the concentration of the citrate complex was significant enough that it was possible to diagnose its presence from just the raw kinetic data. The plausibility of a rapid reversion of the citrate complex to free-Al^{3+} by shifting the complex equilibrium with the depletion of free-Al^{3+} (by the reagent CB) can be eliminated by virtue of the large difference separating the second-order formation rate constant of the Al–CB complex (2.87×10^2 M^{-1} sec^{-1}) and the first order dissociation rate constant of the Al–Citrate complex (4.76×10^{-3} sec^{-1}). Provided that the reagent concentration is kept artificially high, the initial formation rate of Al–CB is always faster than the initial Al–Citrate complex dissociation rate. In addition, under the present experimental design, the final pH immediately after mixing was 5.0, a condition which favoured the formation of the citrate complex rather than dissociation.

As a consequence of this study, it is apparent that both the nonlinear regression algorithm in the treatment of 2-component kinetics and the analytical reagent Calcein Blue (CB) in the fluorimetric determination of aluminum species fare very well in their respective applications. The average stability constant of aluminum citrate at 19.5 °C was determined to be 5.55×10^{-4}. From two separate experiments which the results were not included in Table IV, the regression was found to be less reliable in extreme cases where the presence of one component is predominant. In the case where free-Al^{3+} was prevalent, *e.g.* 96.2%, the experimental stability constant obtained was 4.90×10^{-3}, the respective rate constants for the free-Al^{3+} and the citrate complex were 2.84×10^2 M^{-1} sec^{-1} and 5.07×10^{-3} sec^{-1}. When the situation is reversed, *e.g.* the presence of free-Al^{3+} at 7.70%, the stability constant was determined to be 1.61×10^{-3}, the corresponding rate constants were 2.69×10^2 M^{-1} sec^{-1} and 4.72×10^{-3} sec^{-1} for the free-Al^{3+} and the bound. It is apparent from these results that in the event of poor conditioning of the experimental data, the concentration parameters are more susceptible to nonlinear regression uncertainties in comparison with their counterparts, the rate constants and the blank. From the results tabulated in Table V, which included kinetic parameters determined by the Guggenheim method (equivalent to a graphical analysis) and nonlinear regression, the shortcomings of the graphical method *alone* are clearly demonstrated.

The following are a number of helpful constraints which can assure better success a majority of the time when used to appraise the reliability of input parameters from the Guggenheim plots. First, a useful constraint to remember when evaluating the values of the concentration parameters derived from the Guggenheim plot is that the sum of these values should sum together to the experimental infinity of the reaction mixture. Whenever this condition is not met, the initially estimated concentration values (but not the experimental blank) should be scaled proportionally. Secondly, a high level of confidence should be associated with the value of the time independent blank determined experimentally, therefore, its value may be not allowed to float in the initial fitting but permitted to do so only later on.

Finally, it is well known in linear regression that the slope can often be determined with much better accuracy than the intercept. Consequently, under most circumstances, the Guggenheim estimated rate constants should weigh more than the estimated concentration values. In any attempt to analyze ill-conditioned data, it is advisable to initially constrain the rate constants and the blank floating only the scaled concentration estimates. The resultant values from these preliminary fittings are then refined in further iterations floating all parameters. A note of caution pertaining to the effectiveness of constraining certain parameters: it was observed throughout this work that reducing the number of degrees of freedom in the regression can often result in adverse effects leading to erroneous analyses. A similar observation was made by Alcock *et al.* [12].

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