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KINETIC ANALYSIS OF ALUMINIUM IN ENVIRONMENTALLY AND
GEOCHEMICALLY RELEVANT PROBLEMS

by

Mark K.S. Mak

A thesis submitted to the Faculty of
Graduate Studies in partial fulfilment
of the requirements for the degree of
Doctor of Philosophy

Department of Chemistry

Carleton University

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ABSTRACT

The application of kinetic analysis to multicomponent systems was developed for several areas. These included:

- (a) instrumentation development in the form of a digital computer controlled stopped-flow fluorescence spectrometer;
- (b) nonlinear regression data analysis of multicomponents kinetic data;
- (c) the development and characterization of a suitable fluorescence reagent for low concentration aluminium analysis;
- (d) the application of developments in (a), (b), and (c) to a model 2-component mixture;
- and (e) the study of aluminium speciations in a simulated natural water system by the kinetic technique developed.

Instrumentally, an Aminco-Bowman* spectrofluorimeter was adapted to accomodate a Canterbury type stopped-flow apparatus with a dead time of 10 msec. The stopped-flow spectrofluorimeter was interfaced to a digital minicomputer for rapid data collection. Data analysis was handled by the university mainframe Sigma-Honeywell computer using a library available nonlinear regression programme. Signal to noise improvements were handled both by electronic hardwares such as active and RC filters, and software filtering using Fast Fourier Transform digital filtering techniques.

A metalfluorechromic reagent Calcein Blue (CB) was found to be highly sensitive (2.7 ppb or less), for the detection of aluminium ions as well as having characterizable kinetic behaviour. An average rate

constant of $\sim 2 \times 10^2 \text{ M}^{-1} \text{ Sec}^{-1}$ at 19.5°C was determined for the reaction between $\text{Al(III)}_{\text{aq}}$ and Calcein Blue.

The reagent CB was subsequently applied in a study of the aluminium-citrate complex prepared in solutions of various acidities. Direct identifications of two separate components, i.e., free aluminium ions and aluminium ions bound to citrate ligands, were achieved. The average dissociation rate constant of the aluminium-citrate complex was determined to be $\sim 4.76 \times 10^{-3} \text{ Sec}^{-1}$ at 19.5°C . From the kinetic analyses, an average stability constant of the aluminium-citrate complex according to the reaction,

was determined to be 5.55×10^{-4} at 19.5°C .

Finally, kinetic analyses of aluminium-fulvic acid (Al-FA) mixtures showed that the resulting complexes are not homogeneous in nature but rather comprised of a range of complexes different in their stabilities. The distribution ratios of these complexes were mostly governed by the polyelectrolyte properties of fulvic acid. At least three fractions of these complexes were directly identified and quantified by the present kinetic analyses. The findings are demonstrative of the potential of kinetic analysis in the treatment of speciation in natural water systems.

* Aminco-Bowman Spectrophotofluorometer is manufactured by American Instrument Co., Inc. (8030 Georgia Avenue, Silver Spring, Maryland 20910).

(I) INTRODUCTION

In recent years, there is an increasing necessity to perform chemical analysis that would yield information pertaining to the chemical state or speciation of the metal ions in environmentally and geochemically relevant problems. Electrochemical methods such as complexometric titration followed with anodic stripping voltammetry (ASV) or pulse polarography have enjoyed enormous popularity with analytical chemists.^{1,2,3} Under most circumstances, these methods provide a qualitative identification of the species as well as a quantitative determination of the concentrations. An attractive alternative would be the kinetic analytical methods,^{4,5} a form of kinetic spectrometry in which the rate constants of a mixture of species reacting with a common reagent are usefully regarded as the reciprocals of frequencies serving to differentiate the components. The concentrations of the various species are determined from the differing rates of reaction. In the present work, the author explores a model study of environmental or geochemical interest which includes,

- (A) the modification of an Aminco-Bowman spectrofluorimeter into a stopped-flow kinetic spectrometer for fast reaction rate studies,
- (B) numerical analysis of multicomponent kinetic data,
- (C) the characterization of a fluorescence reagent for aluminium analysis,
- (D) the verification of the validity of multicomponent kinetic analysis, and

(E) a kinetic analysis of aluminium interactions with fulvic acid (FA) at natural water pH levels.

The choice of fluorimetry as the monitoring mode in kinetic analysis has two significant advantages. First, the intrinsically high sensitivity of fluorimetry, and second, for study of reactions that are generally too fast for stopped-flow, the high sensitivity of fluorimetry would allow the use of much lower initial reactant concentrations and hence bring the observed rate down to the stopped-flow range.

Since the topics of the study are inherently diverse, the interrelations will not be apparent until the parts have been described. Without further introduction, the first topic is treated.

(A) MODIFICATION OF AN AMINCO-BOWMAN SPECTROFLUORIMETER FOR STOPPED-FLOW KINETICS

The basic design of the present stopped-flow apparatus has incorporated features that are drawn from reported designs as well as novel elements. Special attention is focused on two priorities, (a) that the unit can be economically produced; and (b) that the modular unit (photos-1a & b) can be easily inserted and removed from a popular model fluorimeter such as the Aminco-Bowman. In the present design, whenever it was possible, the construction employed commercially available components which reduced the production cost substantially. After the removal of the original observation compartment, the stopped-flow unit with its own observation chamber can be attached to the fluorimeter easily via four bolts fastened to the top of the excitation monochromator housing. There is no alteration made to the original mechanical and optical configurations of the fluorimeter. The original electronics of the Aminco-Bowman spectrofluorimeter were modified in order to handle the fast rise time of the kinetic signal. In the rest of this chapter, the mechanical design of the stopped-flow unit and the modifications to the electronics will be documented in separate sections, namely (i) mechanical, and (ii) electronic designs.

(i) STOPPED-FLOW MECHANISMS

The conventional architecture of most first generation stopped-flow apparatus is depicted in figure (1). The three areas of interest for design are (a) the driving mechanism, (b) the mixer, and finally, (c) the stopping mechanism. Traditionally, stopped-flow units are

The Modular Stopped-Flow Unit

PHOTO 1(a)

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The Aminco-Bowman Stopped-Flow Spectrofluorimeter.

PHOTO 1(b)

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FIGURE 1 - CONVENTIONAL ARCHITECTURE OF A STOPPED-FLOW APPARATUS

- (1) Pneumatic or Manual Drive Unit
- (2) Driving Syringes
- (3) Reactant Reservoirs
- (4) 3-Way T-Taps
- (5) Mixer (2 jets T-mixer or multi-jets tangential mixer)
- (6) Outlet for Circulating Thermostating fluid
- (7) Transmitted Radiation (monitoring mode - absorption spectrometry)
- (8) Photomultiplier placed inline with Excitation and Transmitted Radiation (absorption mode)
- (9) Photomultiplier placed 90° to Excitation Radiation (fluorescence mode)
- (10) Emission Radiation (90° to Excitation)
- (11) Thermostating Fluid Circulation Tubing
- (12) Inlet for Circulating Thermostating Fluid from an External Bath
- (13) Incident Excitation Radiation
- (14) Excitation Source
- (15) Effluent Exhaust
- (16) Stopping Syringe
- (17) Oscilloscope Display
- (18) Microswitch Trigger (initiation of data acquisition coinciding with the stoppage of flow)
- (19) Micrometer (adjustment of the position of the microswitch)

FIGURE 1

designed around the particular spectrometer to which they are adapted. With the advent of light guides, a different design philosophy emerged, that is, the Canterbury type second generation stopped-flow apparatus.⁶

In this radically different approach, the stopped-flow apparatus is a totally separated unit detached from the spectrometer which is then used as the monitor. Excitation radiation and the subsequent optical change (absorption or emission) are conducted to and from the spectrometer via flexible light guides. One great advantage of this design is major improvement of temperature control of the reaction under investigation since the mixing unit of the stopped-flow apparatus can be wholly immersed in a thermostated bath. In previous designs, overall thermostating was achieved by a combination of immersion of the reactant reservoirs into a thermostated bath and simultaneously circulating the thermostated liquid in tubing around the mixer. This method was found much less than desirable due to the long period of time required before temperature equilibration can be achieved particularly in cases of high or low temperature studies. At the time of construction of the present stopped-flow apparatus, UV light guides were generally very ineffective. The average percentage of transmittance was 35 - 40%. Today, improved efficiency leads to reported averages 80% of transmittance. In order to retain the high sensitivity intrinsic to fluorimetry and maximum versatility, the original design of the present apparatus has abandoned the use of light guides while retaining a good part of the thermostating feature of the

Canterbury instrument. Consequently, one can describe this apparatus as a hybrid of the first generation and the second generation design. The features are detailed in figure (2).

A new and distinguishing feature in figure (2) is the conspicuous absence of the stopping syringe. Instead, the stopping mechanism is incorporated into the driving mechanism. An ordinary pneumatic drive unit (borrowed from a Durrum stopped-flow drive assembly/part 16199) operated under 60 psi of pressure was used to provide the driving action. Stoppage of the forward motion of the piston was effected by the mechanism seen in photo (2). The rear section of the piston shaft was threaded and a stainless steel cylinder was mounted on it which can be rotated clockwise for forward motion and counter-clockwise for reverse motion. With every new experimental run, the cylinder was rotated backwards to produce the desired travelling distance. An activation of the pneumatic drive would propel both piston and the cylinder forward until stopped by the posterior of the casing against the cylinder for the pre-set distance. A micro switch mounted on the posterior of the casing was triggered simultaneously when the flow was stopped. This initialized the data collection sequence of the computer.

The thermostating system which was described earlier as a cross between the first generation and second generation designs is schematically represented in figure (2). More details of the assembly are presented in photos (3)(a) and (b). Reactant from each driving syringe was delivered to the mixer via a suitable length of PVC tubing. This tubing is heat shrinkable, and therefore, can be easily mated to

FIGURE 2 - STOPPED-FLOW AMINCO-BOWMAN SPECTROFLUORIMETER

(i) Stopped-flow apparatus:

- (A) Threaded Piston Shaft
- (B) Stainless Steel Cylinder (mounted on (A))
- (C) Microswitch Trigger (mounted on the back side of the pneumatic drive casing)
- (D) Compressed Air Inlet from the Actuation Unit
- (E) Pneumatic Drive Unit (borrowed from a Durrum stopped-flow drive assembly/Part 16199)
- (F) Compressed Air Outlet to the Actuation Unit
- (G) Driving Syringes (2 ml B-D)
- (H) 3-Way T-Taps
- (I) Reactant Reservoirs
- (J) Thermostating Fluid Reservoir
- (K) Extra Length of Heat Shrunken PVC Tubing Serving as Thermostated Reactant Reservoirs
- (L) Outlet for Thermostating Fluid from an External Bath Pumped into the Thermostating fluid Reservoir thorough (M)
- (M) Inlet for Thermostating Fluid Pumped in from an External Bath
- (N) Brass Tubings Encasing the Heat Shrunken PVC Reactant Delivery Tubes/vertical positioning of the cell holder (Q) can be achieved by sliding these brass tubings up and down (P)
- (O) Overflow Outlet for the Thermostating Fluid Reservoir/back to the external bath via a peristaltic pump

- (P) A Rotating Disc Mounted on the Bottom of the Cell Holder (Q)
- (Q) T-Mixer Cell Holder with a Cavity at the Top Allowing Thermostating Fluid Coming in from (M) to Circulate around the Top Part of the T-Mixer and Exit via (L)
- (R) Photomultiplier Mounted 90° to the Excitation Radiation
- (S) Emission Radiation (fluorescence mode)
- (T) Excitation Radiation
- (U) T-Mixer (manufactured by Hellma of W..Germany/refer to Figure 3)
- (V) Excitation Source (150W Xenon lamp)
- (W) A Length of Tapered Heat Shrunken PVC Tubing Acting as the Exhaust for the Reacted Mixture
- (X) Thermometer Placed in the Neighbourhood of (L) for an Accurate Temperature Display of the Thermostating Fluid Reservoir
- (Y) Excitation Monochromator Housing on which the Stopped-Flow Apparatus is Mounted

(ii) Electronics

- (1) Preamplifier (refer to Figure 5)
- (2) Main Amplifier
- (3) Differential Voltmeter (DVM)
- (4) Analog-Digital-converter (ADC) (manufactured by Computer Data Conversion System, model Analogic, AN5800 Series)
- (5) Minicomputer Manufactured by Data General (Nova 1220)
- (6) Teletype Terminal
- (7) Paper Tape Puncher
- (8) Oscilloscope/Display
- (9) Analog Control Unit which Includes an Actuation Unit for the Pneumatic Drive, a Digital Counter, and the Triggering Circuit for the ADC and Oscilloscope

SIDE
VIEW

FIGURE 2

TOP
VIEW

ELECTRONICS

BLOCK DIAGRAM

The Front-Mount Stopping Mechanism

PHOTO 2

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The Reactant(s) Delivery and Thermostating Assembly

PHOTO 3(a)

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The Reactant(s) Thermostating Reservoir

PHOTO 3(b)

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the three way T-tap and the inlet of the mixer. Additional lengths of tubing between the driving syringe and the mixer were submerged in a thermostated water bath. This was a direct adaptation of the Canterbury design such that large volumes of reactants can be equilibrated to the desired temperature prior to mixing and reacting.

The mixer currently being used in this instrument is a simple T-mixer fabricated in quartz by Hellma of W. Germany. The measurements and configuration are illustrated in figure (3) and photo (4). Two jets, 1.1 mm each in diameter, are arranged staggered to each other. The two reactants coming from opposite directions, flow together and merge perpendicularly down a square channel 2x2 mm in cross section. The mixer cell is accommodated in a jacket shown in figure 4 and photos 5a & b. The top part of the cell jacket is hollow where thermostating fluid can enter and exit via the brass tubing affixed to the sides. The bottom part of the jacket is solid except for a 2x2 mm cross section channel bored out to accommodate the stalk of the stopped-flow cell. On two adjacent sides of the jacket, two relatively large holes were bored centering 7.5 mm down from the point of mixing in the cell. These two perpendicular holes were not bored out completely such that they intersected the central channel but stopped with approximately 1.5 mm of material left. A smaller concentric hole, 1.5 mm in diameter, was subsequently drilled all the way through to intersect the central channel at each site. These pin holes function as slits for the excitation and emission radiations. The peripheral larger cavities permit easier positioning of the cell holder in relation to monochromator systems in

FIGURE 3 - DIMENSIONS OF THE T-MIXER

External Dimensions:

Length x Width = $F \times F = 5.5 \text{ mm} \times 5.5 \text{ mm}$

Height = $G = 25 \text{ mm}$

External Diameter of the Inlet and Exhaust Ducts = $D = 3.5 \text{ mm}$

Length of the Ducts = $E = 10 \text{ mm}$

Internal Dimensions:

Internal Diameter of the Inlet and Exhaust Ducts = $C = 2.2 \text{ mm}$

Internal Diameter of the Inlet Jets = $A = 1.1 \text{ mm}$

Length x Width of the Internal Column = $B \times B = 2 \text{ mm} \times 2 \text{ mm}$

Distance between the top of the internal column from the top of the cell

= $H = 2.5 \text{ mm}$

Height of the internal column = $I = 22.5 \text{ mm}$

TOP VIEW

SIDE VIEW

FIGURE 3

The Quartz Stopped-Flow T-Mixer

PHOTO 4

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FIGURE 4

A CROSS-SECTION AND TOP VIEW OF THE STOPPED-FLOW CELL JACKET.

- (A) Reactant(s) Delivery Flow Entrance
- (B) Entrance of the Thermostating Fluid from an External Bath
- (C) Exit of the Thermostating Fluid returning to the External Bath
- (D) Removable Lid of the Stopped-Flow Cell Jacket
- (E) An O-Ring placed between the Lid(D) and the Cell Jacket
- (F) Brass Tubing for the Conveyance of the Thermostating fluid
- (G) PVC Tubing for the Conveyance of the Reactant(s)
- (H) Brass fitting affixing the Brass Tubing(F) to the Cell Jacket
- (I) An O-Ring placed between the Brass Fitting(H) and the Cell Jacket
- (J) Distance between Point of Mixing and Point of Observation (7.5 mm)
- (K) Quartz Stopped-Flow Mixer Cell
- (L) Reaction Mixture Exit
- (M) Tapered PVC Tubing for the Conveyance of the Reacted Mixture
- (N) Hollow Cavity for the Circulation of Thermostating Fluid from an External Bath
- (O) Four Screws affixing the Lid(D) to the Cell Jacket

CROSS SECTION

FIGURE 4

TOP VIEW

The Stopped-Flow Cell Jacket Assembly Mounted at the Bottom of the Thermostating Reservoir

PHOTO 5(a)

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The Cavity in the Top Part of Cell Jacket

PHOTO 5(b)

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order to achieve optional sensitivity.

With the present cell jacket, direct temperature control of the reactants can be effected up to the point of mixing. The heat shrinkable PVC tubing delivering the reactants to their respective jets are encased in brass tubing. One of the two brass tubes serves as an entry for the thermostating fluid which flows through the upper cavity of the jacket and exits via the other tube. Since the top part of the mixing cell is in direct contact with the thermostating fluid, it is not unreasonable to suggest that indirect but very efficient temperature control of the reaction at the point of observation can be achieved if a minimum period of time is allowed for temperature equilibration to occur between the top and the rest of the cell.

Finally, a section of taper PVC tubing was heat shrunk onto the exhaust of the mixer cell where the stopping syringe was attached in earlier designs. Displaced reaction mixture can exhaust freely into a refuse beaker. This design configuration has eliminated much of the drudgery and accidents previously associated with the operation of the stopping syringe. It was discovered experimentally that a slight degree of taperness built into the exhaust tubing can assist to dampen the momentum of the flow after it has been stopped.

(ii) ELECTRONICS OF THE KINETIC SPECTROFLUORIMETER

There are a number of reasons for the adaptation of the electronics built for the present apparatus. The original electronics of the Aminco-Bowman spectrofluorimeter were intended for emission measurements of samples at equilibrium using a 1x1 cm cross section cuvet. This

permits the liberal application of RC filters to obtain a clean signal but often suffers from response time with the system. The original response time of $\frac{1}{2}$ second is obviously inadequate for the handling of the faster kinetic signal change in the region of milliseconds. The 2x2 mm cross section stopped-flow cell while drastically reducing self-absorption by the sample, at the same time produces a comparatively much weaker signal than a standard cuvet. The immediate objective was to alleviate this problem by increasing the gain of the system such that the same sensitivity can be restored and improving the response time for monitoring fast reactions without degrading the signal-to-noise ratio unduly. Due to the nature of kinetic experiments, it is desirable to supply two features which were originally missing, i.e., a voltage suppression circuit to offset systems with a large baseline emission and a time clock to enable delayed triggering in cases where one wishes to observe the reaction at a time later than immediately after mixing.

The modification of electronics began with the photomultiplier.

The housing of the photomultiplier was altered in such a way that a pre-amplifier can readily be hooked up to it directly. This was to minimize the inductance effect of a lead when the current travelling through it varied. A separate input source was installed for the high voltage supplied to operate the tube. The pre-amplifier was a parallel-parallel (PP) negative feedback amplifier providing a very low noise and low distortion current-to-voltage converter. Three levels of output voltage gain were

obtained by using feedback resistors of the magnitude 10^5 , 10^6 , and 10^7 ohms in the resistive network. A series of RC filters with various time constants were included in the circuit trailing the feedback amplifier. These RC filters were designed to be optional and therefore can be switched in at will. A typical voltage output from the pre-amplifier is in the range of 20 - 30 millivolts. This is insufficient for the purpose of interfacing the analog voltage signal with an analog-to-digital converter (ADC) (Analogic AN5800 Series, manufactured by Computer Data Conversion System) operated in conjunction with a digital minicomputer (Nova 1220, manufactured by Data-General) for rapid, binary data collection. In the case of a unipolar signal, most ADC's operate in an amplitude range between 0 volt and a maximum of 10 volts. The analog and digital zeros correspond to each other, and the full-scale signal of 10V corresponds to 2^N , where N-bit coding is used. According to binary arithmetic, the largest number that can be encoded with N-bits is $2^N - 1$, therefore, the largest voltage that can be encoded is $10V \times (2^N - 1) / 2^N$ and there is no digital code for 10V. To achieve maximum resolution of the analog signal in terms of binary coding, output from the pre-amplifier in the millivolt range must be further amplified by a factor of $10^3 \times$. A main amplifier which combined an active filter with a gain of $10 \times$ placed before two operational amplifiers each with a gain of $10 \times$ provided the desired total amplification of the pre-amplifier signal. The choice of a fourth-order fast rolloff low pass filter was adopted after extensive Fast Fourier Transform (FFT) spectrum analyses were performed on the chemical system under investigation. The present

filter has a cutoff frequency at 25 Hz and a rolloff at 12 db/octave. Provisions were made for ready and easy interchange for filters with higher cutoff frequencies (e.g. 100 Hz). Also incorporated in the main amplifier was a voltage suppression circuit which was coupled fore of the amplifying circuit. For an incoming pre-amp signal which has a large component of baseline voltage, the suppression circuit was activated and consequently enabled the amplification of the net signal only. Details of the amplifiers' circuits are shown in figures (5)(a) and (b). After the final stage of amplification, independent voltages are output to the ADC and an oscilloscope.

The sequence of events beginning with the actuation of solution flow and leading up to data collection by the ADC were coordinated by a relatively simple control assembly. It consisted of the original commercial actuation unit (Durrum/part 16199) for the pneumatic drive, a digital counter that counts from milliseconds to minutes, and an integrated circuit that can generate both rising and dropping voltage pulses. The circuit is activated when the microswitch is triggered by stoppage of the drive piston (as described earlier). A rising voltage pulse triggers the oscilloscope and a dropping (5V) voltage pulse triggers the computer. The simultaneous generation of pulses occurs only if the circuit is decoupled from the digital counter; in other words, the circuit is being operated in an immediate (or normal) mode. In a time delayed mode, the pulses generation circuit is coupled to and subsequently controlled by the digital counter. A desired time-delay between the moment of stoppage of flow and the initiation of data

FIGURE 5(a)

BLOCK DIAGRAM OF THE MAIN AMPLIFIER

FIGURE 5A

FIGURE 5(b)

DETAILS OF THE LOW-PASS FILTER AND THE OFFSET CIRCUIT OF THE MAIN AMPLIFIER

LAYOUT OF FILTER-AMPLIFIER

BOARD. POSITIONS OF OP-AMPS 1-B.

FIGURE 5B

collection and display can be easily programmed by presetting the digital counter. This is a useful feature particularly when it becomes necessary to observe exclusively the slower component of a 2-component system.

By implementing the voltage suppression and time delay facilities judiciously, it is possible to achieve maximum resolution of any segment of a multicomponent kinetic spectrum.

(iii) GENERAL INFORMATION ON THE AUTOMATED STOPPED-FLOW FLUORIMETER SYSTEM

The choice of driving syringes was two 2 ml B-D glass syringes. Three way T-taps with 2 mm capillary were modified and fitted with reservoirs. Connections between the syringes and the three way taps and the mixer were heat shrinkable tubing 5.5 mm in internal diameter before and 2.0 mm I.D. after shrinking. Temperature control up to $\pm 0.1^\circ\text{C}$ was achieved in conjunction with an external thermostated bath. Dead volume of the mixer cell was calculated to be 0.03 ml. Dead time of the T-mixer was established to be 10 milliseconds (figure (6)) by observing the time necessary to restore to equilibrium fluorescence level the reaction between water soluble fluorescein in acid and sodium hydroxide.

Fluorescein in an acidic medium does not fluoresce, therefore, in an acid-base reaction (with a reaction rate constant that is in the order of $10^{10} - 10^{11} \text{ M}^{-1}\text{Sec}^{-1}$) as described, the emission level should be at its equilibrium value instantaneously after mixing. The duration elapsed before equilibrium fluorescence level can be restored is attributed to the dead time of the mixer.

FIGURE 6

EXPERIMENTAL DEAD TIME OF THE STOPPED-FLOW T-MIXER CELL

System under observation was:

Acidic Fluorescein in Ethanol + NaOH in Ethanol

Vertical Scale: Emission Intensity - 1Volt/Div.

Horizontal Scale: Scan time - 10msec/Div.

FIGURE 6

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All the operational amplifiers used in the circuit were 10 KHz in bandwidth. According to the relationship,⁷ $f_b = 0.35/T_R$, where f_b is the break frequency beyond which the voltage gain drops off at 20 dB per decade, and T_R is the amount of time or risetime it takes the voltage to go from the 10% point to the 90% point; the risetime of the present amplifiers with f_b at 10 KHz is 3.5×10^{-5} Sec and therefore, can be considered to be negligible. Experimentally, the risetime of the active filter was estimated to be 50 msec. For reactions in the high concentration range where the S/N ratio is favourable but the rate is fast, no filter of any kind was deployed. In the medium range where S/N ratio is average and rates of 0.5 - 10 seconds are typical, the active filter was applied. Finally, in low concentration reactions, the S/N ratio is often poor but the rates are slow, an RC filter in conjunction with the active filter was used.

Voltage output from the main amplifier was hardwired to an input port of the ADC. Initiation of the ADC was controlled by computer software. A FORTRAN and ASSEMBLER language (Data General Nova) package was written to handle the data collection and file transfer via paper tape (Appendix A). The dropping voltage pulse (5V) transmitted from the control assembly was recognized by the software as a signal for the commencement of data collection. The ADC in use was a 14 bit coding machine which digitized an analog signal by a successive approximation method. Each conversion cycle time or sample and hold time was 66 μ s. The Nyquist sampling theory⁸ states that in order to prevent distortions (aliasing) which are introduced by sampling the waveform at regular time

intervals, t , t must be equal to $\frac{1}{2f_c}$ where f_c is the critical frequency or the maximum frequency component in the waveform, therefore, the maximum frequency that the present ADC can handle is 7600 Hz without aliasing. An average kinetic experiment followed ten half-lives of a reaction. Assuming that a thousand data points were collected, then 66 ms would be required in order to observe ten half-lives of a reaction. The time interval for sampling by the ADC was user controlled by software. At the selected time intervals, one of the pulses generated at a constant rate by the internal time clock of the computer would be recognized by the software and subsequently used to activate a series of subroutines which controlled the start-of-conversion (SOC) and end-of-conversion (EOC) sequences of a single analog-digital-conversion cycle. The real-time clock for the Nova computer is crystal-controlled and supplies an operating frequency of 1 KHz. The clock cycle time which is equal to one period of the clock is 1 ms. Accuracy of the crystal-controlled oscillator is rated at better than a few parts in 10^9 per day, and therefore, cannot be considered as a source of error in data acquisitions. The two principle sources of error in an ADC are (i) "quantization error" in digitizing due to the least significant bit (LSB) and is commonly ± 1 bits, and (ii) an analog error caused by the comparator in the approximation circuit. In the present ADC, such A/D conversion is estimated to be less than 0.2%.

Finally, the finished instrument was tested by reinvestigating the kinetics of a reported reaction. In this case, the system chosen was the oxidation of Diethylenetriaminepentaacetic Acid (DTPA) by

Cerium (IV) reported by Hanna et. al.⁹ A second-order rate constant of $23.1 \text{ M}^{-1}\text{Sec}^{-1}$ in a $[\text{H}^+] = 1.3 \text{ M}$ medium was reported by these authors using a Durrum-Gibson stopped-flow absorption apparatus. In the present study, the same reaction at concentration levels two orders of magnitude down was monitored by observing the emission of Ce(III) at $\lambda_{\text{excitation}} = 260 \text{ nm}$ and $\lambda_{\text{emission}} = 350 \text{ nm}$. The rate constant obtained was $23.08 \text{ M}^{-1}\text{Sec}^{-1}$, in excellent agreement with the literature.

(B) MULTICOMPONENT KINETIC DATA ANALYSIS

(i) MULTICOMPONENT KINETICS

In our multicomponent system of interest,⁵

where $[R]_0 \gg [A]_0 + [B]_0 + \dots + [N]_0$, i.e. pseudo zero-order in $[R]_0$, the concentration of product P at any time t is:

$$[P]_t = [A]_0(1 - e^{-k_A t}) + [B]_0(1 - e^{-k_B t}) + \dots + [N]_0(1 - e^{-k_N t}) + X \quad (1)$$

where $[A]_0$, $[B]_0$, ..., $[N]_0$ are initial concentrations and X is a blank.

In the application of kinetic spectrometry as an analytical method, it is necessary to determine quantitatively $[A]_0$, $[B]_0$, ..., $[N]_0$ as the concentrations of the corresponding species and k_A , k_B , ..., k_N for their qualitative identification. Under these stipulations, it would be extremely optimistic to suggest that the kinetic spectrum of any system with more than three components can be adequately resolved. It is then more realistic if we restrict ourselves to systems that contain no more than three similarly reactive components which have relatively

well separated rate constants. A number of data reduction procedures had been reported in the literature, both graphical and numerical.⁵

A very common graphical method for the treatment of pseudo-first-order reactions described in the above scheme is with the conventional semilogarithmic first-order plot. In a 2-component system, such a plot would produce a curvature in the early stages of reaction and gradually linearize as the faster component has completely reacted. An extrapolation to zero time yields the concentration of the slower component. Another interesting graphical interpretation of pseudo-first-order multicomponent kinetics was reported by K.A. Connors,^{10,11} an approach which is very elegant and capable of minimizing simultaneous kinetic effects such as catalysis, inhibition due to complexation, general medium effects and side-reactions. The method is based upon what the author called "matching strategies", in which the kinetic concentration-time curve of a sample mixture (the analyte) is matched completely by a reference mixture formulated with this matching condition as the end point. Where this condition is met, the initial sample concentrations are then equal to the initial reference concentrations. Graphic interpolation methods for both 2-component and 3-component mixtures were presented by the author. It can be best viewed as a modified graphic adaptation of the calibration method first introduced by Lee and Kolthoff¹² who prepared a calibration curve of extent of reaction at a fixed time against mixture composition. The only drawback of this method is that it cannot be implemented unless the exact identities of the components in the mixture are known, (i.e. the k 's).

An example of a well known numerical analysis method for the treatment of multicomponent kinetics is the method of proportional equations reported by Garmon and Reilley.¹³ The method is applicable to all first- and pseudo-first-order reactions. Consider equation (1), it can be rewritten in the following form,

$$[P]_t = K_{A,t}[A]_0 + K_{B,t}[B]_0 + \dots + K_{N,t}[N]_0 + X \quad (2)$$

where $K_{A,t}$, $K_{B,t}$, ..., $K_{N,t}$ are proportionality constants which are linearly related to each other. If the reaction kinetics are observed at N different times, determination of the contents of the mixture is possible by solving simultaneously N equations. Once again, the technique assumes an a priori knowledge of the identities of the species in the mixture. Another numerical method is the much discussed weighted least square regression analysis reported by Margerum et. al.^{14,15} According to the principle of the method, the deviation, u_t , between the measured parameter $[P]_t$, and the theoretical value $[P]$, is

$$u_t = K_{A,t}[A]_0 + K_{B,t}[B]_0 + \dots + K_{N,t}[N]_0 + X - [P]_t \quad (3)$$

The best fit line that can be drawn through the series of data points must be one in which the sum of the squared deviations is a minimum with respect to each coefficient $[A]_0$, $[B]_0$, ..., $[N]_0$, and X . From equation (3), the sum of the squared deviations is,

$$\sum_{t=1}^n (u_t)^2 = \sum_{t=1}^n W_t (K_{A,t}[A]_0 + K_{B,t}[B]_0 + \dots + K_{N,t}[N]_0 + X - [P]_t)^2 \quad (4)$$

where W_t is the weighting factor chosen as:

$$W_t = \frac{e^{-k_A t} + e^{-k_B t} + \dots + e^{-k_N t}}{[P]_t} \quad (5)$$

Partial differentiation of the right hand side expression of equation (4) with respect to each coefficient produces (N+1) equations with (N+1) unknowns which are resolved by matrix algebra. This method was found to be very satisfactory for the treatment of three-component kinetics and partially valid in four-component systems. Ridder and Margerun¹⁵ later reported a modified version of the same algorithm which enabled them to investigate system where each reactant and each product were different and each had a measurable absorbance. There are two constraints, one being that each of the rate constants $k_A, k_B, \dots, k_N$ have to be determined separately in an identical matrix of the sample; and secondly, sample components have to be converted chemically to favourable forms prior to the analysis, thus effectively eliminating information on speciation of the components.

(ii) NONLINEAR REGRESSION ANALYSIS

Mathematically, numerical analysis of a function that is the sum of a linear combination of exponential terms such as that of equation (1) is often very difficult and sometimes impossible. From the outset, it was never the intention or claim of the present work to present any numerical resolution of the pseudo first-order kinetics of a multi-component mixture which has closely overlapping rate processes. Instead, we will restrict our endeavours to systems with no more than three

components which are well separated by rate constants that differ from each other by approximately one order of magnitude. These conditions are frequently found in natural water systems where the monomeric metal ions react much faster than the small to medium size polymeric materials which in turn react faster than the large polymers or colloidal materials. By imposing the proper constraints, it is not unreasonable to expect the development of numerical methods which will differentiate the combination of rate processes in the mixture.

Instead of the linear least square regression analysis adopted by Margerum et. al.^{14,15}, the choice of algorithm in the current work is a least-squares estimation of nonlinear parameters developed by Marquardt and his coworkers.^{16,17,18} This routine allows the experimental data to be analysed according to the function originally described in equation (1) without reducing it to a linear relationship such as equation (2). The linearity or nonlinearity of an expression such as equation (2) describes the manner in which the unknown parameters enter into the algebraic function. In this case, the concentration terms $[A]_0$, $[B]_0$,, $[N]_0$ and the blank X are linear because these parameters enter in the first degree.

For a general function of the following,

$$y = f(x_1, x_2, \dots, x_m; \beta_1, \beta_2, \dots, \beta_k) \quad (6)$$

where $x_1, x_2, \dots, x_m$ are independent variables and $\beta_1, \beta_2, \dots, \beta_k$ are the population values of k parameters; the experimental data points

are denoted as,

$$(Y_i, X_{1i}, X_{2i}, \dots, X_{mi}); \quad i = 1, 2, \dots, n. \quad (7)$$

Let $\hat{Y}_i$ represents the theoretical value that is predicted by the function, i.e.,

$$\hat{Y}_i = f(X_{1i}, X_{2i}, \dots, X_{mi}; b_1, b_2, \dots, b_k), \quad (8)$$

then the sum of the squared deviations is,

$$\phi = \sum_{i=1}^n [Y_i - \hat{Y}_i]^2 \quad (9)$$

In equation (8), $b_1, b_2, \dots, b_k$ are the sample values of $\beta_1, \beta_2, \dots, \beta_k$, the population values of the parameters. A particular set of data which is called a sample is a subset of a hypothetically infinitely large population of values. According to the least-square principle, the values of the unknown parameters, β 's, are to be adjusted until ϕ is a minimum. Therefore, the values of β 's can be elucidated by setting the partial derivatives of ϕ to zero, i.e.,

$$\frac{\partial \phi}{\partial b_1}, \frac{\partial \phi}{\partial b_2}, \dots, \frac{\partial \phi}{\partial b_k} = 0 \quad (10)$$

and solving the equations simultaneously. If f is linear in the β 's, the partial derivatives in equation (10) are linear equations and can be solved readily. However, if f is nonlinear in the parameters, the simultaneous equations are nonlinear as well and the solution is not straightforward.

Two popular methods of estimating the nonlinear parameters in the

least-squares context are practised. The first technique is to linearize the mathematics while retaining the nonlinear physical model. Unfortunately, this is applicable to only a small minority of the natural occurring systems. The second approach is an iterative technique where determination of the least-squares is estimated by a direct trial-and-error fitting.

In the first iterative method, a straightforward Taylor Series expansion; the nonlinear function in question is expanded into a Taylor Series about a set of current trial values for the parameters ($b_1, b_2, \dots, b_k$) and their corresponding correction terms ($\Delta b_1, \Delta b_2, \dots, \Delta b_k$). The expansion is restricted to first order so that the resultant expression is linear in the correction terms, Δb 's, e.g.,

$$\hat{y}_i = f_i^{(r)} + \Delta b_1 \left(\frac{\delta f_i}{\delta b_1} \right)^{(r)} + \Delta b_2 \left(\frac{\delta f_i}{\delta b_2} \right)^{(r)} + \dots + \Delta b_k \left(\frac{\delta f_i}{\delta b_k} \right)^{(r)} \quad (11)$$

where the superscript r denotes numerical evaluation using the values of the parameters at the r^{th} iteration trial, and the subscript i denotes numerical evaluation using the values of the independent variables for the i^{th} data point. A linear least square analysis of the following equations can be set up and the values of Δb 's solved.

$$\left(\frac{\delta \phi}{\delta (\Delta b_1)} \right)^{(r)} = 0, \left(\frac{\delta \phi}{\delta (\Delta b_2)} \right)^{(r)} = 0, \dots, \left(\frac{\delta \phi}{\delta (\Delta b_k)} \right)^{(r)} = 0 \quad (12)$$

The new estimates of the parameters for the $(r+1)^{\text{st}}$ iteration trial are:

$$b_1^{(r+1)} = b_1^{(r)} + \Delta b_1^{(r)}; b_2^{(r+1)} = b_2^{(r)} + \Delta b_2^{(r)}; \dots \text{etc.} \quad (13)$$

The iteration is repeated until the magnitude of Δb 's are negligible, that is, until convergence of the iteration to the least-squares estimates. In practice, this method proves to converge very rapidly, but only when the initial trial values supplied for the parameters are quite good; otherwise, the algorithm diverges.

An alternative algorithm to minimize ϕ is the steepest-descent or gradient method. Mathematically, ϕ decreases most rapidly in the direction that is defined by the negative gradients at the r^{th} iteration,

$$-\left(\frac{\delta\phi}{\delta b_1}\right)^{(r)}, -\left(\frac{\delta\phi}{\delta b_2}\right)^{(r)}, \dots, -\left(\frac{\delta\phi}{\delta b_k}\right)^{(r)} \quad (14)$$

The steepest-descent procedure determines the corrections terms, Δb 's, which are in the steepest downhill direction from the current set of trial values. Therefore, the estimate parameter values for the $(r+1)^{\text{th}}$ iteration are,

$$b_1^{(r+1)} = b_1^{(r)} - t \left(\frac{\delta\phi}{\delta b_1}\right)^{(r)},$$

$$b_2^{(r+1)} = b_2^{(r)} - t \left(\frac{\delta\phi}{\delta b_2}\right)^{(r)},$$

$$b_k^{(r+1)} = b_k^{(r)} - t \left(\frac{\delta\phi}{\delta b_k}\right)^{(r)}, \quad (15)$$

where t is a proportionality factor chosen to minimize ϕ . The gradient method was found to work well on the earlier iterations where it converged rapidly from almost any reasonable initial guess; however, it became exceedingly slow as it approached the minimum. This is attributed to the fact that in the vicinity of the minimum, the steepest-descent vector of any trial value is nearly at right angles to the direction from the current point to the minimum of ϕ . Obviously, a modified version of the gradient method is necessary so that the numerical analysis may be expedited. There have been various modified steepest-descent methods reported in the literature.^{18,19} The discussion here is restricted to the Marquardt^{18,19,20,21} variation which was chosen exclusively for all the data reductions in the present work.

The modification proposed by D.W. Marquardt can best be described as an interpolation between the straightforward Taylor Series method and the gradient method. In a qualitative analysis of the problem, Marquardt suggested that in most cases, the correction vector δ_t in the Taylor Series expansion algorithm is usually almost 90° away from δ_g of the gradient method. Therefore, any proper method must result in a correction vector with a direction somewhere within 90° of the negative gradient of ϕ . An implicit feature common to both of the minimization procedure is the choice of direction of the correction vector prior to the determination of an appropriate step size for the correction terms. In the modified algorithm by Marquardt, the vector direction and step size are determined

simultaneously.

At the r^{th} iteration, the linear equations denoted in equation (12) are in detail

$$\sum_{i=1}^n (Y_i - f_i^{(r)}) \left(\frac{\delta f_i}{\delta b_j} \right)^{(r)} = \sum_{j'=1}^k \Delta b_{j'} \sum_{i=1}^n \left(\frac{\delta f_i}{\delta b_j} \right)^{(r)} \left(\frac{\delta f_{j'}}{\delta b_{j'}} \right)^{(r)}, \quad j=1,2,\dots,k \quad (16)$$

In terms of linear algebra, the above can be conveniently rewritten as

$$A \vec{\delta}_t = \vec{g} \quad (17)$$

where

$$A^{[k \times k]} = P^T P \quad (\text{superscript } T \text{ denotes a matrix transpose}) \quad (18)$$

$$P^{[n \times k]} = \begin{pmatrix} \delta f_i \\ \delta b_j \end{pmatrix}, \quad \begin{matrix} i=1,2,\dots,n; \\ j=1,2,\dots,k \end{matrix} \quad (19)$$

and

$$\vec{g}^{[k \times 1]} = \begin{pmatrix} \sum_{i=1}^n (Y_i - f_i) \frac{\delta f_i}{\delta b_j} \end{pmatrix}, \quad j=1,2,\dots,k \quad (20)$$

The main thrust of Marquardt's algorithm was based upon the following theorems (proofs of the theorems can be found in reference 18).

There exists a correction vector

$$\vec{\delta}_0 = (A + \lambda I) \vec{g} \quad (21)$$

Where λ is a non-negative and I is an identity matrix, such that:

(i) The solution $\vec{\delta}_0$ of equation (22) minimizes $\langle \phi \rangle$ on the boundary of a sphere centered at $\vec{b}^{(0)}$ (initial guess) whose radius $\|\vec{\delta}\|$ satisfies

$$\|\vec{\delta}\|^2 = \|\vec{\delta}_0\|^2 \quad (22)$$

(ii) $\|\delta_0\|$ is a continuous decreasing function of λ such that

$$\|\delta_0\| \rightarrow 0 \text{ as } \lambda \rightarrow \infty$$

(iii) If γ is the angle between δ_0 and δ_g , then $\gamma \rightarrow 0$ monotonically as $\lambda \rightarrow \infty$, i.e., δ_0 rotates toward δ_g as $\lambda \rightarrow \infty$.

The correction vector δ_t is scale invariant under linear transformations of the $\vec{b}$ -space, however, δ_g is not. Since δ_0 is effectively an interpolation between δ_t and δ_g , it is necessary, then, to scale the $\vec{b}$ -space in some convenient manner. The choice of Marquardt was to scale in units of the standard deviations of the $(\delta f_i / \delta b_j)$ that make up the matrix P . This choice of scale causes the matrix A to be transformed into the matrix of simple correlation coefficients among the $(\delta f_i / \delta b_j)$.

The equivalent scaled quantities in equation (17) are:

$$A^* = (a_{jj} / \sqrt{a_{jj}} \sqrt{a_{jj}}) = (a_{jj}^*) \quad (23)$$

$$\vec{g}^* = (g_j / \sqrt{a_{jj}}) = (g_j^*) \quad (24)$$

At the r^{th} iteration, expression (21) is rewritten as,

$$(A^{*(r)} + \lambda^{(r)} I) \delta_0^{*(r)} = \vec{g}^{*(r)} \quad (25)$$

and

$$\delta_j = \delta_j^* / \sqrt{a_{jj}} \quad (26)$$

This equation is then solved for $\delta_0^{*(r)}$ and a new trial vector

$$\vec{b}^{(r+1)} = \vec{b}^{(r)} + \delta_0^{*(r)} \quad (27)$$

used to produce a new sum of squares $\phi^{(r+1)}$.

From equation (21), it is obvious that when $\lambda \rightarrow \infty$ (where simultaneously $\|\delta_0\| \rightarrow 0$), convergence to the minimum is very slow; an undesirable feature which prevails the latter iterations of the steepest descent method. On the contrary, when $\lambda \rightarrow 0$, the situation approaches that described in equation (21); a straightforward Taylor Series method which often gives trouble on the initial iterations. It is therefore necessary to choose judiciously an initial value of λ in order that it will converge rapidly as well as satisfy the condition, $\phi^{(r+1)} < \phi^{(r)}$. A common practice is to start initially with a relatively large value of λ which is decreased steadily as the iterations progress. The general strategy is described in the following scheme.

Let $v > 1$, (in practice, $b=10$ has been established to be a good choice) $\lambda^{(0)} = 10^{-2}$, and $\lambda^{(r-1)}$ be the value of λ from the previous iteration, then compute $\phi(\lambda^{(r-1)})$ and $\phi(\lambda^{(r-1)}/v)$. If $\lambda^{(r-1)}$ is already negligible compared to 1, say $\lambda^{(r-1)} < 10^{-15}$, proceed to

(ii) directly; otherwise start at (i).

(i) If $\phi(\lambda^{(r-1)}/v) < \phi(\lambda^{(r-1)})$, then $\lambda^{(r)} = \lambda^{(r-1)}/v$.

(ii) If $\phi(\lambda^{(r-1)}/v) > \phi(\lambda^{(r-1)})$, and $\phi(\lambda^{(r-1)}) < \phi(\lambda^{(r-1)})$ then

$$\lambda^{(r)} = \lambda^{(r-1)}.$$

(iii) If $\phi(\lambda^{(r-1)}/v) > \phi(\lambda^{(r-1)})$, and $\phi(\lambda^{(r-1)}) > \phi(\lambda^{(r-1)})$, λ is

increased by successive multiplication with v until for

for some smallest value of w , $\phi(\lambda^{(r-1)} v^w) < \phi(\lambda^{(r-1)})$; then

$$\lambda^{(r)} = \lambda^{(r-1)}_w.$$

Realistically, condition (iii) is encountered very rarely. It is implicit in conditions (i)-(iii) that in most cases, it is required that equation (25) has to be solved for two values of $\lambda^{(r)}$ at each iteration. In a standard Taylor Series method, only one such solution is required. This algorithm described above functions as a self-regulating mechanism in which the iterations begin by sharing the characteristics of the gradient method the ability to converge from a region far from the minimum when the λ values are relatively large; and gradually switching over to a straightforward Taylor Series method which should converge rapidly once the vicinity of the minimum is reached where the λ values are much decreased. In the present Nonlinear Regression Package*, the set of linear equations in expression (25) can be solved with two optional minimization algorithms which are user controlled. If the user supplied function or model is twice differentiable with respect to the sample values, $b_1, \dots, b_k$; a solution for the linear equations can be elucidated by Newton's iterative method.²² An alternative routine would be the Gauss²³ interpolation formulae (backward and forward) for convergent procedures. In practice, it was noted that the Newton's method converged more rapidly than the Gauss's method; however, it was also more unpredictable if the initial guesses were poor or the function supplied ill-conditioned. The present on-line Nonlinear Regression Package is fully interactive, therefore, it is often useful to explore both algorithms with each application. An example of running the package both on-line and batch

appears in Appendix B.

(iii) EVALUATION OF THE NONLINEAR REGRESSION

An expression such as the one represented in equation (1) is generally considered to be mathematically ill-conditioned. A number of attempts had been made to improve the mathematics while retaining the physical model. The most desirable situation is that if one can linearize the nonlinear model. Failing that, the differential form of equation (1) was considered together with two transform techniques, i.e. a Laplace Transform²⁴ or Fourier Transform²⁵ of the expression. The findings from these studies were in accordance with the conclusion from a deconvolution of photoluminescence data study reported by McKinnon et. al.²⁶ favouring the iterative method using the original function describing the physical model.

To establish the reliability of the algorithm, the Nonlinear Regression routine was tested in feasibility studies with simulated noise-free data. It will be demonstrated in the next section that the iterative algorithm is relatively immune from the influence of noise in the elucidation of parameter values. An artificial data file of 700 records was generated using the function,

$$Y = [A]_0 (1 - e^{-k_a t}) + [B]_0 (1 - e^{-k_b t}) \quad (28)$$

{ In FORTRAN, $Y = (A1 - A1/DEXP(A2 * X)) + (A3 - A3/DEXP(A4 * X))$ }

where Y is the concentration (emission intensity) of the common product,

A1 is the initial concentration of the first species $[A]_0$,

A2 is the rate constant of A, k_a ,

A3 is the initial concentration of the second species $[B]_0$,

A4 is the rate constant of B, k_b ,

and X is the time.

The first case studied was where the reaction rates between the first and second component are well resolved, e.g., $t_{1/2}(A)/t_{1/2}(B) \geq 10$. After a thousand iterations, the regression results from using various sets of initial approximation values are as tabulated in the following Table 1.

The parameter values used originally to generate the data file were:

$A1=0.16$, $A2=0.13$, $A3=9.00 \times 10^{-2}$, and $A4=5.65 \times 10^{-3}$. In a separate study, the rates of the two components are programmed to be very close to each other; results of the fittings are tabulated in Table 2. The original parameter values were: $A1=0.16$, $A2=0.13$, $A3=0.25$, and $A4=9.00 \times 10^{-2}$, and the number of iterations was a thousand.

It is obvious in Table 1 that provided the reaction rates between the two components are only marginally overlapping, the Nonlinear Regression is dependable even if the initial approximations may be inadequate. As the rates of the two components approach each other, the influence of the initial approximation values is significant as demonstrated in Table 2. Satisfactory fitting results are observed only when reasonable initial guesses are available, e.g., trials II and IV.

In the case of trial V, the initial approximations are less than ideal, nevertheless, the fitted parameter values can still be considered to be acceptable. It should be emphasized that it is not the pretension of the present exercise to prove that closely overlapping reaction rates of a 2-components system can be successfully resolved by the Nonlinear

TABLE 1

NONLINEAR REGRESSION FITTED PARAMETER VALUES OF A WELL RESOLVED
2-COMPONENT TEST SYSTEM

The original parameter values were:

$$A1 = 0.16$$

$$A2 = 0.13$$

$$A3 = 9.00 \times 10^{-2}$$

$$A4 = 5.65 \times 10^{-3}$$

TABLE 1

TRIAL	PARAMETER	INITIAL APPROXIMATION	MINIMIZATION ALGORITHM	FITTED PARAMETER
I	A1	0.100	NEWTON'S	0.160
	A2	0.100		0.130
	A3	0.100		9.00×10^{-2}
	A4	1.00×10^{-3}		5.65×10^{-3}
II	A1	0.200	NEWTON'S	0.160
	A2	0.150		0.130
	A3	5.00×10^{-3}		9.00×10^{-2}
	A4	2.00×10^{-3}		5.65×10^{-3}
III	A1	5.00×10^{-2}	NEWTON'S	0.160
	A2	7.00×10^{-2}		0.130
	A3	4.00×10^{-2}		9.00×10^{-2}
	A4	5.00×10^{-4}		5.65×10^{-3}

TABLE 2

NONLINEAR REGRESSION FITTED PARAMETER VALUES OF A POORLY RESOLVED
2-COMPONENT TEST SYSTEM

The original parameter values were:

$$A1 = 0.16$$

$$A2 = 0.13$$

$$A3 = 0.25$$

$$A4 = 9.00 \times 10^{-2}$$

TABLE 2

TRIAL	PARAMETER	INITIAL APPROXIMATION	MINIMIZATION ALGORITHM	FITTED PARAMETER
I	A1	0.100	NEWTON'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	NEWTON'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}	NEWTON'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}	NEWTON'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}	NEWTON'S	0.190
	A2	0.239		0.126
	A3	0.133		0.220
	A4	0.300		8.75×10^{-2}

TABLE 2 - CONTINUED

TRIAL	PARAMETER	INITIAL APPROXIMATION	MINIMIZATION ALGORITHM	FITTED PARAMETER
VI	A1	0.500	NEWTON'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	9.00×10^{-2}	GAUSS'S	0.171
	A2	0.107		0.110
	A3	0.150		0.235
	A4	0.100		0.106
VIII	A1	6.50×10^{-2}	GAUSS'S	0.111
	A2	0.239		0.201
	A3	0.133		0.176
	A4	0.300		0.202
IX	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

Regression; but rather that adequate procedures must be developed in order to procure sufficiently adequate initial approximations of the parameter values. When this stipulation is satisfied, the proposed numerical analysis is applicable in spite of the mathematical function being classically ill-conditioned. This conclusion is supported by a study on the deconvolution of photoluminescence data reported by McKinnon et. al.²⁶ particularly in its resilience to noisy data and its ability to resolve exponentials. This same numerical method was successfully applied by Moore²⁷ to analyze the kinetic data of a first-order reaction with unknown initial and final (infinity) readings plus an unknown rate constant. Finally, Alcock et. al.^{19,28} in two separate studies reported the determination of equilibrium constants¹⁹ and rate constants²⁸ through the use of Marquardt's algorithm. These authors concluded that the method was very versatile in that it can elucidate an equilibrium constant from either spectrophotometric or potential data, or a combination of both types of data. In the kinetic study, the algorithm was first tested in the treatment of synthetic data for five types of reaction networks, and later applied in the study of halide-substitution reactions of the (3-azapentane-1, 5-diamine)-halogenoplatinum (II) or $[\text{Pt}(\text{3NH-pd})\text{X}]^+$ complex. It was found that the method offered a very satisfactory and more rigorous method of testing a variety of kinetic models on large volumes of data than previous linear approaches. A particular merit of the method was its ability to resolve the overall rate of reaction into the rates of the component steps. This was achieved with much better precision by the

nonlinear approach compared to previous linear treatments in the situation in which one step is very much slower (factor of 10^3) than another. This last observation is in excellent agreement with our present conclusion from Table 1. It will be demonstrated in the next section that this method is far superior in comparison with the relatively primitive graphical method in the extraction of kinetic parameter values from a 2-components system.

A perfect example of the potential benefit from using the nonlinear routine was typified in the study of the substitution reaction of chloride and bromide in the $[\text{Pt}(\text{3NH-pd})\text{X}]^+$ complex by iodide.²⁸ 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 $[\text{Pt}(\text{3NH-pd})(\text{OH}_2)]^{2+}$ was incorporated into the reaction model. The magnitude of k_1 was negligible 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 estimated in the case 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. In this context, the present approach in handling multicomponent kinetics is potentially more informative than the method adopted by Margerum et al.^{14,15} By floating the values of the rate constants and the

blank, any mutual kinetic effects, general medium effects, and side-reactions as suggested by K.A. Connors^{10,11} would be reflected accordingly in the corresponding parameter values elucidated. Such information is inevitably lost with numerical methods that dictate that the values of the rate constants have to be predetermined independently in a chemical environment simulating that of the reaction mixture; and subsequently held constant during linear regression analysis.

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. Such a hypothetical situation however provides the necessary acid test for the potential limitation of the Nonlinear Regression. In a simulation study, synthetic data were generated using an expression similar to that of equation (28) with the linear addition of a third exponential term, i.e., unknown in six parameters in the absence of a blank or constant term. The original parameter values were : $A_1=0.20$, $A_2=0.50$, $A_3=0.20$, $A_4=0.20$, $A_5=0.20$, and $A_6=5.00 \times 10^{-2}$ where the identities of A_1 , A_2 , A_3 and A_4 were as defined in equation (28), A_5 is the initial concentration $[C]_0$ and A_6 it's corresponding rate constant. Regression results are tabulated in Table 3. In such an exercise, the feasibility and limitation of a 3-component (six unknown parameters) fitting attempt are tested. The final fitting results in trial V are rather satisfactory considering that the original parameter

TABLE 3

NONLINEAR REGRESSION FITTED PARAMETER VALUES OF AN OVERLAPPING
3-COMPONENT TEST SYSTEM

The original parameter values were:

$$A1 = 0.20$$

$$A2 = 0.50$$

$$A3 = 0.20$$

$$A4 = 0.20$$

$$A5 = 0.20$$

$$A6 = 5.00 \times 10^{-2}$$

TABLE 3

TRIAL	NUMBER OF DATA POINTS	NUMBER OF ITERATIONS	PARAMETER	INITIAL APPROXIMATION	MINIMIZATION ALGORITHM	FITTED PARAMETER
I	250	200	A1	0.407	NEWTON'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	NEWTON'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

TABLE 3 - CONTINUED

TRIAL	NUMBER OF DATA POINTS	NUMBER OF ITERATIONS	PARAMETER	INITIAL APPROXIMATION	MINIMIZATION ALGORITHM	FITTED PARAMETER
III cont.	250	500	A4	0.149	GAUSS'S	0.165
			A5	0.200		0.181
			A6	5.00×10^{-2}		4.5×10^{-2}
IV	1000	1000	A1	0.247	NEWTON'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	NEWTON'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}

values are extremely ill-conditioned. At the same time, the initial approximations supplied to trial I which eventually provided the approximations for trial V are extraordinarily close to the neighbourhood of the real parameter values. It is not unreasonable to suggest that well separated 3-component system can be attempted with the present method with a good likelihood of success; assuming of course that quality initial approximations equivalent to or better than those suggested in the case of a 2-component system can be procured. Two other distinctive features emerging from this study are, (i) that satisfactory fittings can be obtained with as few as 250 points (rather than 700 or 1000 points), and (ii) that 200-300 iterations are often enough to produce the true "best-fit" set of parameter values. These features are particularly attractive because it permits most of the data files to be processed on-line instead of batch, simultaneously limiting the collection of data points to under 500. In the current Nonlinear Regression Package, due to a workspace restriction, any file which exceeds 300 pairs of data points cannot be processed on-line but must be run batch.

(IV) EXPERIMENTAL DATA TREATMENT

(a) DATA FILES CREATION

Raw experimental data from stopped-flow runs were stored as files on cartridge disk in a 2-column format with the observations or emission intensities (Y) in the first and the corresponding variable or time (X) in the adjacent column. These files were later transcribed from disk onto paper tapes which were subsequently read into the Xerox-Honeywell

mainframe computer via a teletype coupled to a modem. In the cases of very slow kinetics, the runs were recorded on chart paper by a strip-chart recorder. These were analysed manually and the resultant data files entered manually via an interactive terminal. Since all the kinetic emission spectra were digitized at regular time intervals, it is therefore unnecessary to enter the corresponding time intervals when recreating these data files in the main computer. Simple programmes had been written to alleviate the tedium associated with data files entered manually. In this way, the time intervals can also be entered in the unit preferred by the user.

(b) DIGITAL SMOOTHING OF THE EXPERIMENTAL KINETIC SPECTRUM

There are a number of reasons why supplementary digital filtering of the kinetic data is occasionally implemented in addition to the hardware analog filters described earlier in section (A). The active low pass filter in use currently has a cutoff frequency set at 25 Hz. Admittedly, this is a fairly low value; however, from our own spectra analyses, it was shown that many of the frequency components of the kinetic signal lie far below 25 Hz particularly if the reaction was a relatively slow one. It is also true that the active filter has a rapid rolloff; but the fact remains that the cutoff is not vertical in nature. Consequently, although much reduced in amplitudes, there are still high frequency noises present in the signal. The digital filter approach provides a convenient mean for complete control of the cutoff frequency as well as eliminating the problem of response times. These advantages are often difficult to duplicate with analog filters.

The digital filtering technique in this work is based on the Fourier Transformation²⁹ of the signal. In an extensive study reported by Hayes et. al.,³⁰ digital smoothing of exponential decay type data was investigated in detail. Separation of the signal from the noise is achieved by transforming a function of time into frequency space, multiplied by an appropriate filter function and then transformed back into time space. Such a filter is the commonly used rectangular filter which has the characteristics of retaining fully the frequency amplitudes below the cutoff and reducing everything to zeros above the cutoff. Mathematically, the common exponential decay represents a discontinuous transient,⁸ and like all sudden transients, a continuous broadband Fourier spectrum is required for accurate representation. This is the consequence of an effect termed "leakage" which is a discrepancy between the discrete and continuous Fourier Transform because of the required time domain truncation. The effect of an abrupt truncation of any continuous broadband Fourier spectrum will produce ringing (i.e. small, spurious side lobes appearing in the original signal spectrum) in place of the sudden "transient". To minimize this effect, Hayes et. al. proposed to rotate the original spectrum and translate it so that the initial and terminal points are zero in value. The rotated waveform is time-limited,⁸ thus it's discrete Fourier Transform will agree (within a constant) reasonably well with the continuous Fourier Transform, drastically reducing "leakage".

In practice, the strategy involved the following procedures executed in sequence.

(i) The kinetic curve was rotated by using the expression,

$$R_n = A_n - \Delta_n \quad (29)$$

where R_n was the magnitude of the n^{th} point in the rotated-translated array, A_n was the magnitude of the n^{th} point in the original array; and

$$\Delta_n = A_1 + \frac{(A_k - A_1)(n-1)}{k-1} \quad (30)$$

where $n=1,2,3, \dots, k$.

(ii) The rotated spectrum was transformed into its corresponding Fourier spectrum via an IMSL FFT³¹ (Fast Fourier Transform algorithm⁸) library routine.

(iii) The frequency components were multiplied by a rectangular filter with an adjustable cutoff limit and then inverse Fourier transformed back to a time domain spectrum.

(iv) Finally, the smoothed spectrum was inversely rotated and translated back to the original form with non-zero data initiation and termination.

A listing of the necessary computer programmes for these routines appears in Appendix C.

The purpose of the smoothing routine was to improve the signal-to-noise ratio of the data such that well-resolved plots resulted when the data were subjected to Guggenheim³² type linear analyses. This will be discussed in the next section. The smoothed data, when analysed by the nonlinear regression, did not demonstrate any significant improvement

over the untreated data. This is in good congruence with the observation reported by McKinnon et. al.²⁶ where the nonlinear method under test responded exactly the same to the smoothed data as it had to the raw data. A typical example illustrating the resilience of the nonlinear method towards noisy data is included in Table 4.

(c) DEDUCTION OF INITIAL APPROXIMATION PARAMETER VALUES FROM THE RAW DATA

It was emphasized in section (iii) that in order to extract proper and maximum performance from the Nonlinear Regression Package, it was necessary to procure quality initial guesses for the outset of the iterations. A convenient method is the well established Guggenheim³² logarithmic linear treatment. The method is basically a graphical method developed to analyze straightforward first-order kinetics. The fundamental principle of the technique is described in the following mathematical expressions. Let P_t and $P_{t+\Delta}$ be the values of the observable (in this case, the emission intensity) at time t and $t+\Delta$ where Δ is an arbitrary but constant time increment chosen to be two or three time the half-life of the reaction. The arithmetic difference between $P_{t+\Delta}$ and P_t is (refer to equation (1)):

$$P_{t+\Delta} - P_t = P_0 e^{-kt} (1 - e^{-k\Delta}) \quad (31)$$

where P_0 is the value of the observable at time zero, t_0 , and k , the rate constant of the 1st order reaction under observation. The natural logarithmic form of equation (31) is,

TABLE 4

ANALYSES OF A WELL-RESOLVED 2-COMPONENT EXPERIMENTAL SYSTEM*

* Fitting results obtained after 300 iterations floating all five variables.

TABLE 4

PARAMETER	EXPERIMENTAL	GUGGENHEIM ESTIMATES	RAW DATA	FILTERED DATA
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

$$\ln(P_{t+\Delta} - P_t) = C - kt \quad (32)$$

where the constant C is,

$$C = \ln P_0 (1 - e^{-k\Delta}) \quad (33)$$

A plot of $\ln(P_{t+\Delta} - P_t)$ vs t yields a straight line with slope equal to $-k$ and an intercept equal to C . A distinctive advantage with the Guggenheim analysis is that a knowledge of the value of P at time infinity is not required. In the case of a system with two components which are kinetically well separated, a Guggenheim plot should provide an accurate picture which consists of two straight lines displaying distinctively different slopes. An example is shown in Figures 7(a) and (b).

A routine Guggenheim analysis of the smoothed data of an identified kinetic mixture has two outstanding advantages. First of all, the graphic display provides a ready means of assessing the number of reacting species present in the mixture. Secondly, with the aid of any of the many linear least-square regressions available, one can actually obtain fairly reasonable estimations of the parameter values which will serve as the initial approximations entering the nonlinear analysis. Parameter estimates for the faster component are often obtained using exclusively the Guggenheim points in the proximity of the beginning of the plot; while the reverse is true for the estimation of the parameter values of the slower component. 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 would have

FIGURE 7(a)

GUGGENHEIM PLOT OF DATA (UNFILTERED) OF A TYPICAL WELL-RESOLVED
2-COMPONENT SYSTEM. SYSTEM UNDER OBSERVATION IS:

$(10^{-5} \text{M } [\text{Al}^{3+}] / 10^{-5} \text{M } [\text{Na}_3\text{-CITRATE}])$ mixture at $\text{pH}=3.0 + 10^{-3} \text{M CB.}$;
in 0.1M acetate buffer at $\text{pH}=5.0$.

$$\ln (E.I._{t+\Delta t} - E.I._t)$$

E.I. - EMISSION INTENSITY

FIGURE 7(b)

GUGGENHEIM PLOT OF DATA (FILTERED) OF A TYPICAL WELL-RESOLVED
2-COMPONENT SYSTEM. SYSTEM UNDER OBSERVATION IS:

$\{10^{-5} \text{M } [\text{Al}^{3+}] / 10^{-5} \text{M } [\text{Na}_3\text{-CITRATE}]\}$ mixture at $\text{pH}=3.0 + 10^{-3} \text{M CB}$;

in 0.1M acetate buffer at $\text{pH}=5.0$.

FIGURE 7(b)

to be used to obtain reasonable linear regressions. It is evident that the Guggenheim analysis can be implemented with better consequences if relatively noise-free or smoothed data are available. Two possible undesirable effects can occur with noisy data, (i) the failure of equation (32) if the quantity $(P_{t+\Delta} - P_t)$ is negative, and (ii) a poor Guggenheim plot can often cause difficulties in visually differentiating the number of components present. The computer programmes for the Guggenheim conversion and the subsequent linear regression are listed in Appendix D. These estimated parameter values are finally entered into the Nonlinear Regression Package and fitted to a 2-component model with an unconstrained blank. The regression model used throughout this work for a 2-component mixtures was,

$$Y = (A1 - A1/\text{DEXP}(A2 * X)) + (A3 - A3/\text{DEXP}(A4 * X)) + A5 \quad (34)$$

where $A1, A2, A3, A4, X$ were as defined in equation (28) and $A5$ was the value of an experimental blank to be fitted. There is not any standard rule to best implement the Nonlinear Regression in conjunction with the Guggenheim estimation analysis. Much of the success is dependent upon the imagination and discretion of the user. A number of useful constraints however can assure better success with the above combination of mathematical analyses.

First of all, 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 identical reaction mixture. Whenever this

condition is not met, the 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 blank determined experimentally, therefore, it's value should not be allowed to float in the initial fittings 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-condition 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. Several examples are included in the following demonstrating the various approaches to the Nonlinear Regression. The experimental data involved in these examples represent kinetic conditions which are quite different from each other.

In figures 7(a) and (b), the Guggenheim plots of the raw data and the digital filtered data clearly display a case of two distinctive kinetic components. The gradients of the two segments are drastically different from each other indicating that the fast component is much faster than the other component present. The situation is analogous to the simulated data used in the study reported in Table 1. The poor resolution of the slow component is attributable to a very minimal signal change during the sampling time interval selected. The

Guggenheim estimated parameter values for both components are listed in Table 4 together with the nonlinearly fitted parameter values of the raw and filtered data. The Guggenheim parameters were entered into the Nonlinear Regression directly without any scaling, with all five parameters floating simultaneously right from the start. Very satisfactory fittings were produced readily after 300 iterations using 300 data points. The system under observation was a mixture of equal molar Al^{3+} and Tri-Sodium Citrate reacting with a 100-fold excess of a ligand CB (Calcein Blue). Extensive studies of the reactions of the Al^{3+} ion and the mixture systems with the ligand CB will be discussed independently in the next two chapters of this work. The fitted parameter values were found to be in good agreement with these other studies and hence good confidence can be placed upon the results after 300 iterations. A comparison of the results from the analyses of the raw and filtered data does not demonstrate any significant discrepancy, a characteristic of the algorithm's resilience towards noisy data. The success of this exercise supports the earlier conclusion that for two well resolved components, convergence can be effected even with poorly estimated initial approximations.

An intermediate case where the two components are not as well separated as the one just illustrated but still not totally unresolvable is depicted in figures 8(a) and (b). This condition can easily be distinguished in graphical analysis as the gradients of the two components approach each other. Initial guesses of the parameters from least square analysis of the Guggenheim plot of the smoothed data,

FIGURE 8(a)

GUGGENHEIM PLOT OF DATA (UNFILTERED) OF A TYPICAL INTERMEDIATELY-
RESOLVED 2-COMPONENT SYSTEM. SYSTEM UNDER OBSERVATION IS:

$\{10^{-6} \text{M } [\text{Al}^{3+}] / 10^{-6} \text{M } [\text{Na}_3\text{-CITRATE}]\}$ mixture at $\text{pH}=3.0 \pm 10^{-4} \text{M CB}$;

in 0.1M acetate buffer at $\text{pH}=5.0$.

FIGURE 8(a)

E.I. - EMISSION INTENSITY

FIGURE 8(b)

GUGGENHEIM PLOT OF DATA (FILTERED) OF A TYPICAL INTERMEDIATELY-
RESOLVED 2-COMPONENT SYSTEM. SYSTEM UNDER OBSERVATION IS:

$(10^{-6} \text{M } [\text{Al}^{3+}] / 10^{-6} \text{M } [\text{Na}_3\text{-CITRATE}])$ mixture at $\text{pH}=3.0 + 10^{-4} \text{M CB}$;

in 0.1M acetate buffer at $\text{pH}=5.0$.

FIGURE 8(b)

E.I. - EMISSION INTENSITY

together with the fitted parameters are reported in Table 5. The strategy for the Nonlinear Regression involved holding the two estimated rate constants and the experimental blank constant in the initial 300 iterations and subsequently floating all five parameter in another 300 iterations. After 600 iterations, the resultant parameter values were found to be very satisfactory. Results from both the raw and smoothed data again confirm the effectiveness of the algorithm in treating noisy data.

Finally, in an additional example where conditions are very similar to the last one excepting that the two components overlap more extensively, crude estimates from the Guggenheim plots figures 9(a) and (b) are proven to be inadequate for the Nonlinear Regression purpose. The only successful approach involved a sequence where the first 300 iterations were executed with constraints placed upon the values of the rate constants established from previous experiments and the experimental blank, fitting only the concentration parameters which were properly scaled before being entered into the regression; proper parameter values were produced in the ensuing 300 iteration floating all the variables. Results of the analyses are compiled in Table 6. The present example represents perhaps a limiting situation where useful informations can be extracted reliably by the Nonlinear Regression method. A necessary prerequisite to modest success with very ill-condition data includes some a priori knowledge of either the values of the rate constant and/or the concentrations besides the experimental blank.

Experimental data throughout this work can all be treated by

TABLE 5

ANALYSES OF AN INTERMEDIATE 2-COMPONENT EXPERIMENTAL SYSTEM*

* Fitting results obtained from 600 iterations.

Initial estimates of A2, A4, A5 were held constant in the first 300 iterations. In the final 300 iterations, all five variables were fitted.

TABLE 5

PARAMETER	EXPERIMENTAL	GUGGENHEIM ESTIMATES	RAW DATA	FILTERED DATA
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^{-3}	4.929×10^{-3}	4.932×10^{-3}
A5	4.000		4.294	4.333

is adopted for kinetic analysis, the behaviour of the real rate constants with various reagent concentrations will have to be carefully characterized. This practice to a large extent resembles the fingerprint technique in most classical analytical approaches.

It is evident that there is seldom a reagent which can fulfil all of the above criteria, therefore, when deciding for or against the choice of a reagent, the priorities of these criteria should be evaluated judiciously. When the detection mode is spectrophotometry, appropriate reagents are often more readily available. Generally speaking, the absorption characteristics of a complex are less susceptible to the influences of external parameters such as pH and medium composition. An extreme variation in one of these parameters will likely result in a minor perturbation of the original spectral features. When the detection mode becomes fluorescence, the magnitude of such a perturbation is amplified sufficiently enough to produce adverse consequences. For instance, the intensity of fluorescence is a function of both the concentrations of quenchers and absorbers present; consequently, any change in the concentrations of the medium components can represent a potential experimental failure. In the present work, the following priorities govern the final choice of a reagent.

- (i) The reagent should be highly sensitive and specific towards aluminium species, i.e. the resulting complex should have a relatively high quantum yield.
- (ii) There should also be a sufficiently large Stokes shift between the

FIGURE 9(a)

GUGGENHEIM PLOT OF DATA (UNFILTERED) OF A TYPICAL CLOSELY-OVERLAPPING
2-COMPONENT SYSTEM. SYSTEM UNDER OBSERVATION IS:

$\{10^{-6} \text{M } [\text{Al}^{3+}] / 10^{-6} \text{M } [\text{Na}_3\text{-CITRATE}]\}$ mixture at $\text{pH}=3.55 + 10^{-4} \text{M CB}$;
in 0.1M acetate buffer at $\text{pH}=5.0$.

[illegible]

E.I. - EMISSION INTENSITY

FIGURE 9(b)

GUGGENHEIM PLOT OF DATA (FILTERED) OF A TYPICAL CLOSELY-OVERLAPPING
2-COMPONENT SYSTEM. SYSTEM UNDER OBSERVATION IS:

$(10^{-6} \text{M } [\text{Al}^{3+}] / 10^{-6} \text{M } [\text{Na}_3\text{-CITRATE}])$ mixture at $\text{pH}=3.55 + 10^{-4} \text{M CB}$;
in 0.1M acetate buffer at $\text{pH}=5.0$.

$$\ln(E.I._{t+\Delta t} - E.I._t)$$

E.I. - EMISSION INTENSITY

TABLE 6

ANALYSES OF A CLOSELY OVERLAPPING 2-COMPONENT SYSTEM*

* Fitting results obtained from 600 iterations.

A1, A3 approximations were scaled Guggenheim* estimates, while

A2, A4 were supplied from other established experimental determin-

ations. In the first 300 iterations, A2, A4, A5 were held constants,

but permit to vary together in the last 300 iterations with A1 and A3.

TABLE 6

PARAMETER	EXPERIMENTAL	INITIAL APPROXIMATIONS	RAW DATA	FILTERED DATA
A1		10.01	11.43	11.42
A2		3.000×10^{-2}	3.041×10^{-2}	3.042×10^{-2}
A3		9.790	8.865	8.864
A4		5.000×10^{-3}	5.331×10^{-3}	5.329×10^{-3}
A5	5.200		5.643	5.751

implementing either one or a combination of the above approaches. The described numerical analysis scheme was strongly adhered to in all data reduction procedures. In most cases, raw data can be treated without any refinement.

(C) CHARACTERIZATION OF A FLUORESCENCE REAGENT FOR THE DETERMINATION
OF Al^{3+} BY KINETIC ANALYSIS

(i) INTRODUCTION

In complexometric analysis, there are a number of features which characterized all the potential reagent systems. When one of these reagents is adopted for the specific purpose of kinetic analysis, additional parameters such as the kinetic behaviours and dependences of the system under investigation have to be considered. An adequate analytical reagent in kinetic analysis will generally meet the following four specifications.

(a) The chosen reagent must demonstrate reasonably high sensitivity and specificity towards the analyte in question. This implies not only a low detection limit, but the ability of the reagent to remain resilient towards potential interferences present in the matrix which contains the analyte. These criteria are fundamental to all complexometric analyses.

(b) The chemistry associated with the complex formation should be thermodynamically favourable and well defined. For complexometric analyses at equilibrium, some of these criteria that are traditionally assumed to be essential may in fact turn out to be a matter of convenience rather than absolute necessity. These same criteria are however very pertinent and significant in the practice of kinetic analyses. For instance, both the data interpretation and experimental design aspects of kinetic analysis can be drastically improved if the complexometric system selected produces a stoichiometrically well defined, preferably 1:1,

complex which is highly favoured thermodynamically. The reagent of choice should also be compatible in a medium in which the analyte of interest is found. For example, an analyte in an aqueous system may at best be marginally analysed by a reagent which is only soluble in ethanol; the reason being that when ethanol is mixed with water, a large temperature change occurs coupled with an opalescence phenomenon, both obscuring the real kinetic information. In some instances, the signal being monitored is influenced by the nature of the solvent, this is particularly prevalent among fluorescence species where the quantum yield depends strongly on the solvent. The advantage of choosing a thermodynamically favourable reagent is that it can effectively eliminate any masking problem yielding the true concentration of the analyte. A well known reagent which displays most of the above qualities is the EDTA system.

(c) Independent of the detection mode, the product of a complexometric analysis is expected to have radically different properties from the original analyte and the reagent. When the detection mode is spectroscopic, an ideal reagent should effect a well defined and substantial spectral shift on complexation. This feature is particularly relevant in kinetic analysis since the kinetics can be followed in the presence of excess reagent. In this manner, resolution of the kinetic signal is immensely improved.

(d) The treatment of kinetic data has been the subject of many

discussions, certainly indicative of the difficulty of the subject. Most of the simple chemical reactions are second-order, a situation which is generally treated as pseudo first-order by using an excess of either one of the reactants. Consequently, complex second-order kinetics are reduced to relatively simple first-order kinetics. In a multicomponent system, the composite rate can become extremely complex. It is therefore essential, when selecting an analytical reagent in kinetic analysis, not to complicate the kinetics any further. A simple complexation reaction is one which is zero-order in the reagent and first-order in the analyte where the observed reaction rate is only dependent upon the concentrations and natures of the species present. This situation is sometimes found in ligand substitution reactions where the dissociation rate of the departing ligand is rate limiting. Experimentally, the most commonly encountered arrangement is still that one of a combination of simple, one-step second-order processes which are conveniently converted into a series of pseudo first-order events by using an excess of reagent. In cases described so far, reagent concentration dependence of the reaction rate is either zero or simple and straightforward. Barring any mutual kinetic effect, real rate constants converted from pseudo rate constants³³ in all of the above systems are constant. When the final product in a complexometric analysis is not formed by a simple, one-step bimolecular process, but rather a multi-order or mixed-order rate law,³⁴ the real rate constant for the formation of the complex will often display complex dependence on the concentration of the reagent. In the event that such a reaction system

is adopted for kinetic analysis, the behaviour of the real rate constants with various reagent concentrations will have to be carefully characterized. This practice to a large extent resembles the fingerprint technique in most classical analytical approaches.

It is evident that there is seldom a reagent which can fulfil all of the above criteria, therefore, when deciding for or against the choice of a reagent, the priorities of these criteria should be evaluated judiciously. When the detection mode is spectrophotometry, appropriate reagents are often more readily available. Generally speaking, the absorption characteristics of a complex are less susceptible to the influences of external parameters such as pH and medium composition. An extreme variation in one of these parameters will likely result in a minor perturbation of the original spectral features. When the detection mode becomes fluorescence, the magnitude of such a perturbation is amplified sufficiently enough to produce adverse consequences. For instance, the intensity of fluorescence is a function of both the concentrations of quenchers and absorbers present; consequently, any change in the concentrations of the medium components can represent a potential experimental failure. In the present work, the following priorities govern the final choice of a reagent.

- (i) The reagent should be highly sensitive and specific towards aluminium species, i.e. the resulting complex should have a relatively high quantum yield.
- (ii) There should also be a sufficiently large Stokes shift between the

excitation spectrum and emission spectrum of the complex. An extensive overlap will reduce the region where the quantum yield is constant.

- (iii) When the reagent luminesces independently, it is necessary that the emission spectrum of the free reagent and that of the complex do not overlap extensively.
- (iv) The reagent must be capable of forming a reasonably strong complex in an all aqueous medium, preferably at a low pH level as well.
- (v) The complex formation kinetics, when it is not simple and straightforward, is at least manageable and most of all, can be characterized.

Assays of Al^{3+} using fluorescent metal chelates have been well studied and documented in the literature.^{35,36} In majority of the cases, the reagents are either derivatives of the 2,2'-dihydroxyazo dye (A)³⁶

(A)

or derivatives of the salicylidene aromatic Schiff base (B).³⁷

(B)

The other very popular reagents include lumogallion,³⁸ morin,³⁹ and hydroxyquinoline.^{40a,40b} A comparative study⁴¹ of these reagents in terms of the product (ϵQ) of the quantum yield of luminescence (Q) and the molar extinction coefficient (ϵ) of the respective Al-reagent

complex suggested that salicylal-o-aminophenol⁴² and morin³⁹ were the most sensitive. The diazodihydroxy dyes and lumogallion were readily dismissed from consideration due to their poor sensitivities. In the case of lumogallion, inspite of the literature's claim of a detection limit of approximately 1 ppb, an exploratory study in the present work with 0.27 ppm of Al^{3+} seemed to indicate that the signal was very weak. This was in good agreement with the work reported by Babko et. al.⁴¹ where the poor sensitivity of lumogallion was attributed to the strong absorption of the free reagent in the region where the complex was absorbing. The sensitivity could be improved slightly if 8-hydroxyquinoline was chosen as the designated reagent. A severe drawback eliminating this reagent as a serious contender was that the reaction had to be performed in a nonaqueous medium. An otherwise excellent reagent morin was rejected for more or less the same reason. In a feasibility study, the formation rate of the Al-morin complex was investigated with a morin reagent solution which was prepared by dissolving the reagent in a 10% ethanol solution. It was concluded from several studies with the Al^{3+} concentration varying from $10^{-5}M$ - $10^{-7}M$, that if ten half lives of the reaction were followed, only the last three half lives were analyzable. The most informative part of the kinetic spectrum was rendered useless due to the side effects from mixing water with a 10% ethanol solution. Finally, an obvious choice is the remaining reagent, salicylal-o-aminophenol, one which is most often mentioned as the most sensitive reagent for the luminescent determination of aluminium. Dagnall et. al.⁴² in their study reported a

detection level as low as 0.27 ppb. The optimal conditions were established to be at pH 5.6, with the reagent in excess a 1000-fold, and the reaction accomplished in a basically aqueous medium. Although it may have been an excellent reagent for the determination of Al^{3+} in the conventional sense, its application cannot be extended to kinetic analysis for the following reasons. According to Dagnall et. al., the resultant aluminium chelate was found to be stable 15 mins. after mixing in an aqueous solution, however, simultaneous thermal hydrolysis of the reagent was also noted. Experimentally, the reagent was prepared in acetone at a high concentration and only a small volume was used to react with the aluminium ion in an aqueous solution. Immediately after mixing, a yellow colour was observed which was eventually assigned to a thermal hydrolysis of the reagent to salicylaldehyde. The requirement of a relatively large excess of reagent was attributed partially to the depletion of the reagent due to hydrolysis. A further complication arising from this phenomenon was that aluminium was found to react with salicylaldehyde forming a complex which gave a green fluorescence, in a region where the emission maxima (520 nm) of the aluminium-salicylidene-o-aminophenol complex was located. The described sequence of events is likely to produce very complex rate dependences which may not be manageable, hence rendering this reagent inadequate for kinetic analysis purpose. A second serious drawback is that it is doubtful whether the stability of the fluorescent chelate is adequately favourable thermodynamically. The authors reported that the presence of anions such as citrate, tartrate, oxalate and fluoride interfered seriously by diminishing the

fluorescence due to aluminium. It was not clear whether the quenching was physical (dynamic) or chemical (competitive complexation by the interfering anions) in nature. It is very probable that both mechanisms were active. However, the question remains whether the potential tetradentate structure (B) depicted on P. 79 indeed has more complexing power compared to basically dicarboxylate structures such as tartrates and oxalates, and citrates which are tri-carboxylate. In the present scheme of analysis, the chosen reagent must be able to displace a ligand such as citrate from its aluminium complex, the reason being that the aluminium citrate complex is a good model representing aluminium complexes potentially present in natural water systems.

The reagent of choice in the present work is Calcein Blue (4-methylumbelliferone-methyleneiminodiacetic acid), a metalfluorechromic reagent which is a condensation product of 4-methylumbelliferone, formaldehyde, and iminodiacetic acid.⁴³ The structure of the reagent is as described below. There are four possible coordination sites, the original three sites of the iminodiacetic acid and the phenolic site of

the 7-hydroxy-4-methyl coumarin moiety.⁴⁴ The dye was most often used as an indicator for the titrimetric determination of alkaline earth and

transition metal ions.^{43,45,46} Other luminescent complexometric analyses include the determination of zirconium,⁴⁷ and a wide range of metal ions.⁴⁴ The free dye, CB, and its corresponding aluminium complex, Al-CB, were reported⁴⁸ to be extremely quantum efficient, having a quantum yield near unity in water in each case, and are used as organic dyes in a tunable liquid laser.⁴⁹

The spectral properties and ionization characteristics of CB, and the laser action of Al-CB with various pH were investigated extensively by the authors Imasaka et. al.⁴⁹ The acid dissociation constants for the second carboxyl proton and the phenolic proton were (i) $pK_2=7.2$, and (ii) $pK_3=12.2$. Therefore, below pH 7.2, CB is singly negatively charged; between pH 7.2 - 12.2, the dye is doubly negatively charged; and finally, above pH 12.2, the molecule is triply negatively charged. In an absorption study of the free dye with pH that varied from 3.3 to 12.8, two isobestic points at 335 nm and 354 nm along with a shift of the absorption maximum from 325 nm to 370 nm were observed by these authors. The coordination compound Al-CB which was formed at around pH 5.0 was found to "lase" at 410 nm when excited at 340 nm. A gradual red shift to 450 nm was observed when the pH of the aqueous medium was increased from 5.0 to 12.0. In the case of the free dye at pH 5.0, when excited at 337 nm "lase" at 450 nm. The fluorescence intensity was reduced drastically with a shift to higher pH. This was attributed to the non-fluorescent characteristic of the triply charged species prevalent at $pH > 12.2$. The absence of fluorescence was subsequently explained in terms of the breakage of the hydrogen bond between the

phenolic proton and the imino nitrogen, enabling the methyleneiminodiacetic acid side chain to become flexible and thus served as a non-radiative decay mechanism. It was estimated that, for both the free dye and its aluminium complex, the superimposition of the fluorescence and absorption spectra was below 1%. In an attempt by these authors to improve the quantum efficiency of the Al-CB complex, ethanol-water mixtures were introduced to replace a strictly aqueous medium; the result was that there was not any significant improvement since the quantum efficiency was already very high and near unity in water. The stoichiometry of the complex was explored in two independent studies. A mole ratio plot⁵⁰ of a fluorometric study and a continuous variation plot⁵⁰ of an absorption study both indicated a 1:1 binary complex at pH 5.0.

A careful evaluation of the chemical and spectral properties of Calcein Blue supports the application of this reagent in the kinetic analysis of aluminium. It is not unreasonable to suspect that the methyleneiminodiacetic acid moiety is more powerful in complexing ability than an average dicarboxylate structure. In a study reported by Aguila⁵¹ where an 'O,Q'-dihydroxyazo group was in the place of the methyleneiminodiacetic acid, the substituted coumarin was found to react with aluminium very slowly at room temperature. It will be shown in this work that CB reacts with Al^{3+} at a rate which is characteristic of aluminium substitution rates reported in the literature;^{52,53} and can indeed replace a ligand such as citrate from its aluminium complex. The excitation and emission maxima of both the free

dye (CB: $\lambda_{ex}=337$ nm, $\lambda_{em}=450$ nm) and the aluminium complex (Al-CB: $\lambda_{ex}=337$ nm, $\lambda_{em}=410$ nm) are well separated with minimal superimpositions, this feature has virtually eliminated all the problems attributable to self reabsorptions. The difference between the Stokes shifts of the complex and the free dye is 40 nm, a situation where it is possible to resolve the luminescent signal of Al-CB even in the presence of an excess amount of CB. An undesirable feature of the reagent is that both the free dye and the complex absorb strongly at 337 nm. In kinetic analysis, a condition of an excess of reagent is almost inevitable. Under such a condition, the fraction of incident radiation which can be consumed in the excitation of the luminescent complex is reduced. It is assumed that this adverse effect, if not completely offset by the extraordinary quantum efficiency of the luminescent complex, is at least largely compensated for.

(ii) EXPERIMENTAL

(a) REAGENTS

The dye Calcein Blue (CB) was obtained from G. Frederick Smith Co. (F.W.=321.29 gm) and used without further purification. Aluminium chloride hexahydrate (reagent grade) was obtained from British Drug House Chemicals and twice recrystallized from doubly distilled water. Hydration number of the recrystallized aluminium chloride salt was determined gravimetrically⁵⁴ by precipitation with 8-hydroxyquinoline. The average of three determinations was 6.00 ± 0.0024 . Reagent grade anhydrous sodium acetate and acetic acid were used in the preparation of pH 5.0 buffer. Reagent grade perchloric acid in the present work was

standardized⁵⁵ against mercuric oxide using methyl purple as as indicator. Doubly distilled water was used in all the solution preparations.

(b) REACTANT PREPARATIONS

A 0.2 M stock solution of $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ was prepared by dissolving the purified crystals in a 0.1 M HClO_4 solution. The stock was stored in a polycarbonate bottle previously leached with 1:1 HNO_3 . These procedures were adopted to minimize the hydrolysis of the Al^{3+} ion and the loss of Al^{3+} due to surface adsorption on glass.⁵⁶ Solutions of Al^{3+} at various concentrations and pH values were obtained by gradually diluting the stock and simultaneously adjusting the pH with HClO_4 . The pH change was monitored by a 3-decimals digital pH meter manufactured by Orion Research (model 701A). Aluminium solutions of 10^{-4} M - 10^{-7} M with pH values ranging from 2.0 - 4.0 were obtained according to this procedure. All the prepared solutions were permitted to stand and equilibrate for 24 hours before being used in kinetic experiments.

In all of the experiments in this work, the final pH of the reactants after mixing was set to be 5.0. This was achieved by preparing the reagent solutions of CB in the presence of a large excess of pH 5.0 acetic acid-sodium acetate buffer. This final pH value at 5.0 was chosen after several factors pertaining to the potential effectiveness of the present reagent in the kinetic analysis of aluminium species were considered. The priorities were, at the chosen pH, the complexation reaction should be largely favoured to go to completion; and that the resultant complex would luminesce very strong. It was reported by

Imasaka et. al.⁴⁹ that a stable complex which produced the most intense fluorescence was formed at around pH 5.0. It is conceivable that by shifting to pH 6.0, the stability of the complex is further increased; a feature that is very much desired if the reagent is to displace a ligand such as citrate from its aluminium complex directly. There are two negative aspects chemically associated with such a change. First of all, Al^{3+} ions at pH 6.0 hydrolyse very extensively to form large and relatively unreactive polymers.^{56,57,58} Although the hydrolysis is kinetically very slow at room temperature,⁵⁸ and therefore should not be able to compete on the same time scale with the rate of complexation after mixing; it nevertheless is undesirable to introduce another potentially competing rate process into the reacting system particularly when the Al^{3+} ion is present only at a very low concentration. At pH 5.0, hydrolysis is drastically reduced in aluminium solutions of low concentrations,⁵⁶ specifically $<10^{-5}$ M. Secondly, by shifting the medium pH to 6.0, the fluorescence intensity of the luminescent complex is attenuated. An alternative approach was to perform the kinetic analysis in a medium with the final pH set at 4.0. A lower pH condition will favour the acid hydrolysis of aluminium complexes present as analytes but simultaneously destabilizing the formation of the luminescent complex of interest. The emission intensity of Al-CB at pH 4.0 according to Imasaka et. al. was reduced slightly. It is apparent then at pH 5.0, both the emission intensity and stability of the luminescent complex are optimized without undue complications.

A 1 M stock acetic acid-sodium acetate buffer solution was

prepared by dissolving 57.75 gm of anhydrous sodium acetate in 296.00 ml of 1 M acetic acid and then diluted to 1 litre with doubly distilled water.

Stock solutions of Calcein Blue were found to be unstable on standing, therefore, fresh solutions were prepared each time 24 hours prior to the experiments. The desired amount of dye was weighed out and dissolved in a small amount of water. A calculated volume of stock buffer was pipetted into the dissolved dye solution such that when made up to the final volume, the concentration of the acetate buffer was 0.2 M.

A typical blank was prepared by mixing equal volumes of the dye reagent with a HClO_4 solution at a pH identical to the aluminium solution being analyzed.

(c) APPARATUS

The faster kinetic runs were observed with stopped-flow kinetic spectrofluorimeter described in Section (A). In the case of low concentration studies of Al^{3+} , i.e. $<10^{-6}$ M, the rates were much slower and were observed with a Perkin-Elmer (model 204S) spectrofluorimeter coupled to a Hewlett-Packard strip-chart recorder (model hp Moseley 7100B). Fused quartz cuvettes of 10 nm pathlength were employed. The fluorescence intensity of the Al-CB was extremely high and therefore was attenuated with a 10% transmittance neutral density filter. The slit widths for both the excitation and emission radiations were 10 nm.

(d) PROCEDURE

There were a number of procedures which were standardized from the outset and practised throughout this work whenever and wherever it was permitted by the experimental conditions. The concentration of the reagent was kept at a hundred times in excess of the aluminium concentration which was being analyzed. After some studies on the time dependence of the complex formation, it was established that for all intent and purpose, an experimental infinity was achieved after the reaction mixture was allowed to equilibrate for 24 hours. In each set of stopped-flow experiment, a minimum of at least ten runs were recorded and processed for the purpose of data averaging. For the slower experiments performed with the Perkin-Elmer spectrometer, an average of a minimum of five runs were used in producing the final data. All the experiments were observed at a temperature of $19.5 \pm 0.1^\circ \text{C}$ with the stopped-flow spectrometer and $19.5 \pm 0.5^\circ \text{C}$ when the Perkin-Elmer spectrofluorimeter was employed.

In characterization studies such as calibration curve, Job's-plots and molar ratio plots,⁵⁰ the Al-CB complex was invariably generated by reacting the calculated amounts of Al^{3+} and CB in a buffer medium which after being diluted to the final volume yielded the desired ratio of $[\text{Al}^{3+}]$ to $[\text{CB}]$ in 0.1 M acetate buffer at pH 5.0. This mixture was subsequently equilibrated for 24 hours before being examined. The concentrations reported from these studies are the equivalents of the effective concentrations (after mixing) in a kinetic study.

The following procedures were followed for all the kinetic experiments observed with the stopped-flow spectrometer. An experimentally prepared blank was first loaded into the observation area and the baseline emission was displayed on the oscilloscope and the differential voltmeter (DVM). The voltage suppression circuit in the main amplifier was adjusted to offset the voltage due to the blank. The blank was subsequently displaced by an experimental infinity of the Al-CB system under investigation. A combination of the gain circuits of the pre-amplifier and main amplifier were adjusted to just below 10 volts. The cycle was repeated until the electronics and the oscilloscope were conditioned so that the magnitude of the corrected signal was approximately 9.5 volts, i.e. ~0 volt for the blank and ~9.5 volts for the experimental infinity. An expansion to exactly 10 volts is not advisable for fear of saturating the ADC with the slightest drift of the electronics, e.g. thermal drift. The exact final magnitudes of the blank and infinity were recorded and stored as separate files in the computer. This information was later retrieved and served as the value of an equilibrium recovery of the aluminium species in a comparison with the recovery value elucidated from the kinetic data. It is important to note that whenever fresh reactants are loaded into the storage coils (i.e. the extra lengths of tubing between the three way T-taps and the inlets of the mixer) which are immersed in the thermostating fluid, it is necessary to allow the system a minimum of five minutes to reach a temperature equilibrium. Besides the two amplifiers, it is also possible to modulate the signal by varying the high voltage supplied to

the photomultiplier (~750 volts) and the slit widths of the excitation and/or emission windows.

For the slower reactions which were followed by the Perkin-Elmer spectrofluorimeter, manual mixing of the reactants was required. As was noted before, due to the high intensity of the emission from Al-CB, it was necessary to impose a 10% transmittance neutral density filter at the emission window in order to attenuate it. The electronics of the spectrometer were set up similarly to those of the stopped-flow spectrometer. The conditions were optimized when a maximum resolution of the corrected signal was achieved. The signals of the suppressed blank and the experimental infinity were recorded on the chart-recorder and later served as references. At the start of an experiment, 1.5 ml of the reagent CB was pipetted into the quartz cuvet positioned in the cell holder; the shutter of the spectrometer was closed. With a hypodermic syringe, 1.5 ml of an aluminium containing analyte was delivered rapidly into the cuvet, achieving efficient mixing in the course of the turbulent delivery. The scanning motor of the strip-chart recorder was switched on simultaneously with the initiation of the syringe delivery. Immediately after the delivery was completed, the observation compartment of the spectrometer was closed and shutter opened for data acquisition. With some practice, it is possible to execute the manual mixing procedure in a matter of 2 - 3 seconds. From the outset of the experiments, results obtained from the manual runs were verified against the same experiments performed by the stopped-flow spectrometer and found to be identical within experimental

uncertainties. The resultant data charts were digitized and entered into the computer manually.

In all the experiments, a minimum of 10 half-lives of the reaction were followed. An average of 500 data pairs are collected in each stopped-flow experiment and 150 - 200 data pairs in a manual run.

(iii) RESULTS

The spectral characteristics of the reagent and the complex were briefly reinvestigated for verification purposes and found to concur with the observations reported by Imasaka et. al.⁴⁹. At pH 5.0, maximum fluorescence of the Al-CB complex was observed. The excitation and emission maxima of the reagent CB were found to be $\lambda_{ex} = 340$ nm and $\lambda_{em} = 450$ nm respectively. For the complex Al-CB, the maxima were $\lambda_{ex} = 340$ nm and $\lambda_{em} = 410$ nm.

The dependence of the emission intensity of Al-CB upon the aluminium concentration was investigated in two separate calibration studies. Various concentrations of the complex were prepared in reactions which were pseudo zero-order in the reagent CB. The calibration study was carried out on the Perkin-Elmer spectrometer with a 10 mm pathlength cuvet, and the result is depicted in figure 10. The other study was observed in the 2 mm pathlength stopped-flow cell, the result is shown in figures 11(a) and 11(b).

FIGURE 10

CALIBRATION CURVE OF THE DEPENDENCE OF THE EMISSION INTENSITY OF THE ALUMINIUM-CALCEIN BLUE COMPLEX ON THE CONCENTRATION OF ALUMINIUM. (10 mm cuvet)

CONDITIONS UNDER WHICH THE CALIBRATION CURVE WAS DERIVED:

$$[\text{CB}]_{\text{EFF}*} = 5 \times 10^{-5} \text{M}$$

$$[\text{ACETATE BUFFER}]_{\text{EFF}*} = 0.1 \text{M at pH} = 5.0.$$

* Denotes the effective concentration of the species after mixing.

Emission readings were developed at room temperature for 24 hours and observed with a 10 mm pathlength normal cuvet.

FIGURE 10

FIGURES 11(a) & (b).

CALIBRATION CURVE OF THE DEPENDENCE OF THE EMISSION INTENSITY OF THE
ALUMINIUM-CALCEIN BLUE COMPLEX ON THE CONCENTRATION OF ALUMINIUM. (2 mm cuvet)

CONDITIONS UNDER WHICH THE CALIBRATION CURVE WAS DERIVED:

$$[CB]_{\text{EFF}*} = 2 \times 10^{-3} \text{ M}$$

$$[\text{ACETATE BUFFER}]_{\text{EFF}*} = 0.1 \text{ M at pH} = 5.0$$

* Denotes the effective concentration of the species after mixing.

Emission readings were developed at room temperature for 24 hours and
observed with a 2 mm pathlength stopped-flow cuvet.

EMISSION INTENSITY OF ALUMINIUM-CALCEIN BLUE COMPLEX (ARBITRARY UNITS)

The structure of the complex was investigated by Imasaka et. al. first in a Yoe and Jones⁵⁰ mole-ratio study monitored by the fluorescence of the complex; followed by a Job's⁵⁰ continuous variation plot obtained by monitoring the absorbance of the complex at $\lambda_{\text{abs}} = 350 \text{ nm}$. The results from a routine reinvestigation of the Job's plot at various total concentrations of $([\text{Al}^{3+}] + [\text{CB}])$ are shown in figures 12(a), (b), and (c). The observations from the complementary mole-ratio studies are illustrated in figures 13(a), (b), and (c).

The kinetics of the analytical system were characterized in the aluminium concentration range from $10^{-4} \text{ M} - 10^{-7} \text{ M}$. The advantage of the high sensitivity of fluorescence detection is readily reflected not only in the calibration studies, but also in the easily analyzable kinetics at such low aluminium concentration, representing the potential capability in the present system to achieve aluminium speciation at ppb levels. The aluminium ion at each concentration level was investigated at three different pH conditions, the results are collected and tabulated in Table 7. The percentage of recovery is a ratio between the concentration of metal ion as determined from a theoretical infinity which was experimentally prepared by equilibrating the reaction mixture for a minimum of 24 hours. The kinetic study was completed with an investigation of the ligand concentration dependence of the complex formation rate, the results are compiled in Table 8. Tabulated in Table 9 are results obtained in low metal to reagent concentration ratio (1:20) studies clearly indicated the incompatibility of the present data with a simple one component model but instead supported a

FIGURE 12(a)

CONTINUOUS VARIATION PLOT OF EMISSION INTENSITY OF THE ALUMINIUM-

CALCEIN BLUE COMPLEX; CONCENTRATION OF THE MIXTURE WAS KEPT AT,

$[Al^{3+}]_{EFF*} + [CB]_{EFF*} = 2 \times 10^{-6} M$ IN 0.1M ACETATE BUFFER AT pH=5.0.

* Denotes the concentrations of aluminium and Calcein Blue after mixing.

Emission readings were developed at room temperature for 48 hours and observed with a regular 10 mm pathlength cuvet.

FIGURE 12(a)

FIGURE 12(b)

CONTINUOUS VARIATION PLOT OF EMISSION INTENSITY OF THE ALUMINIUM-

CALCEIN BLUE COMPLEX; CONCENTRATION OF THE MIXTURE WAS KEPT AT,

$[Al^{3+}]_{EFF*} + [CB]_{EFF*} = 2 \times 10^{-5} M$ IN 0.1M ACETATE BUFFER AT pH=5.0.

* Denotes the concentrations of aluminium and Calcein Blue after mixing.

Emission readings were developed at 40°C for 24 hours and observed with a regular 10 mm pathlength cuvet.

FIGURE 12(b)

FIGURE 12(c)

CONTINUOUS VARIATION PLOT OF EMISSION INTENSITY OF THE ALUMINIUM-
CALCEIN BLUE COMPLEX; CONCENTRATION OF THE MIXTURE WAS KEPT AT,

$[Al^{3+}]_{EFF*} + [CB]_{EFF*} = 2 \times 10^{-4} M$ IN 0.1M ACETATE BUFFER AT pH=5.0.

(A) - ● Emission readings were developed at room temperature for
24 hours and observed with a regular 10 mm pathlength cuvet.

(B) - ● Emission readings were developed at 40°C for 8 hours and
observed with a 2 mm pathlength cuvet.

* Denotes the concentrations of aluminium and Calcein Blue after mixing.

FIGURE 12 (C)

FIGURE 13(a)

MOLE-RATIO PLOT OF THE EMISSION INTENSITY OF THE ALUMINIUM-CALCEIN BLUE
COMPLEX WITH $[Al^{3+}]_{EFF*} = 10^{-6}M$, IN A 0.1M ACETATE BUFFER AT pH=5.0.

* Denotes the effective concentration of aluminium after mixing.

Emission readings were developed at room temperature for 24 hours and
observed ~~with~~ a regular 10 mm pathlength cuvet.

FIGURE 13 (a)

FIGURE 13(b)

MOLE-RATIO PLOT OF THE EMISSION INTENSITY OF THE ALUMINIUM-CALCEIN BLUE
COMPLEX WITH $[Al^{3+}]_{EFF*} = 10^{-5}M$, IN 0.1M ACETATE BUFFER AT pH=5.0.

* Denotes the effective concentration of aluminium after mixing.

Emission readings were developed at room temperature for 24 hours and
observed with a 2 mm pathlength stopped-flow cuvet.

EMISSION INTENSITY OF ALUMINIUM-CALCEIN BLUE COMPLEX (ARBITRARY UNITS)

FIGURE 13(c)

MOLE-RATIO PLOT OF THE EMISSION INTENSITY OF THE ALUMINIUM-CALCEIN BLUE

* COMPLEX WITH $[Al^{3+}]_{EFF*} = 10^{-4} M$, IN 0.1M ACETATE BUFFER AT pH=5.0.

* Denotes the effective concentration of aluminium after mixing.

Emission readings were developed at room temperature for 24 hours and
observed with a 2 mm pathlength stopped-flow cuvet.

EMISSION INTENSITY OF ALUMINIUM-CALCEIN BLUE COMPLEX (ARBITRARY UNITS)

TABLE 7

KINETIC CHARACTERIZATION OF THE COMPLEX FORMATION BETWEEN IONIC
ALUMINIUM AND CALCEIN BLUE (CB)

* Equilibrium infinity is the emission intensity of the reaction
mixture 24 hours after mixing.

+ % ALUMINIUM RECOVERY = $\text{KINETIC INFINITY} / \text{EQUILIBRIUM INFINITY}$.

TABLE 7

$[Al^{3+}]_{TOT}$	pH of Al^{3+}	$[CB]_{TOT}$	$k(Al^{3+})$ $M^{-1}Sec^{-1}$ $\times 10^{-2}$	EQUILIBRIUM* INFINITY (ARBITRARY UNIT)	KINETIC INFINITY	% ALUMINIUM+ RECOVERY
$1.0 \times 10^{-4} M$	2.0	$4 \times 10^{-3} M$	1.35	28.70	30.15	105.1
"	3.0	"	1.46	28.19	29.72	105.4
"	4.0	"	1.50	29.96	29.26	97.66
$3.0 \times 10^{-5} M$	2.0	$4 \times 10^{-3} M$	1.20	23.58	25.76	109.3
"	3.0	"	1.46	23.02	24.75	107.5
"	4.0	"	1.59	24.55	25.11	102.3
$2.0 \times 10^{-6} M$	2.0	$2 \times 10^{-4} M$	2.20	63.00	64.00	101.6
"	3.0	"	3.11	"	61.90	98.25
"	4.0	"	3.26	"	63.51	100.8
$1.0 \times 10^{-6} M$	2.0	$2 \times 10^{-4} M$	1.98	27.00	26.07	96.56
"	3.0	"	2.62	"	27.76	102.8
"	4.0	"	2.98	"	27.13	100.5
$5.0 \times 10^{-7} M$	2.0	$10^{-4} M$	1.14	26.00	26.23	100.9
"	3.0	"	1.44	"	26.02	100.1
"	4.0	"	1.62	"	26.27	101.0
$1.0 \times 10^{-7} M$	2.0	$10^{-5} M$	0.457	75.50	64.97	86.05
"	3.0	"	0.441	37.50	28.53	76.08
"	4.0	"	0.428	39.40	31.01	78.71

TABLE 8

LIGAND CONCENTRATION DEPENDENCE STUDIES OF THE Al-CB COMPLEX
FORMATIONS AT pH = 3.0

@ $[Al^{3+}]I$ and $[Al^{3+}]II$ are concentration fractions of $[Al^{3+}]_{TOTAL}$ which will react with the reagent CB at two different rates $k_{(Al-I)}$ and $k_{(Al-II)}$ respectively forming presumably complexes with relatively different metal to ligand ratios.

+ The kinetic aluminium recovery is expressed as a percentage of the theoretical 100% equilibrium aluminium recovery which was the emission intensity of the reaction mixture of interest 24 hours after mixing.

TABLE 8

$[Al^{3+}]_{TOTAL}$ $\times 10^5 M$	$[CB]_{TOTAL}$ $\times 10^3 M$	$k_{(Al^{3+})}$ $M^{-1} Sec^{-1}$ $\times 10^{-2}$	KINETIC ⁺ ALUMINIUM RECOVERY %
3.0	6.0	1.55	95.14
3.0	4.0	1.46	107.5
3.0	2.4	2.32	100.4
3.0	1.2	2.89	92.50*
3.0	0.60	2.64	82.05*
0.10	0.40	2.43	104.4
0.10	0.20	2.62	102.8
0.10	0.16	2.50	105.8
0.10	0.080	2.50	100.0
0.10	0.040	2.59	101.7

* If the kinetic data were refitted with a 2-component model, the following results were obtained.

$[Al^{3+}]_{TOT}$ $\times 10^5 M$	$[CB]_{TOT}$ $\times 10^3 M$	$[Al^{3+}]_{I/II}$ $[Al^{3+}]_{II}$	$k_{(Al-I)}$ $M^{-1} Sec^{-1}$ $\times 10^{-2}$	$k_{(Al-II)}$ $M^{-1} Sec^{-1}$ $\times 10^{-2}$	KINETIC ⁺ ALUMINIUM RECOVERY %
3.0	1.2	1.59	3.99	9.82	105.6
3.0	0.60	0.605	6.25	14.9	93.85

TABLE 9

KINETIC ANALYSES OF Al-CB COMPLEX FORMATIONS AT LOW Al:CB
CONCENTRATION RATIO (1:20)

* $[Al^{3+}]I$ and $[Al^{3+}]II$ are concentration fractions of $[Al^{3+}]_{total}$ which will react with the reagent CB at two different rates $k_{(Al-I)}$ and $k_{(Al-II)}$ respectively forming presumably complexes with relatively different metal to ligand ratios.

+ The kinetic aluminium recovery is expressed as percentage of the theoretical 100% equilibrium aluminium recovery which was the emission intensity of the reaction mixture of interest 24 hours after mixing.

TABLE 9

$[Al^{3+}]_{TOT}$ $\times 10^5 M$	pH of Al^{3+}	$[CB]_{TOT}$ $\times 10^4 M$	$[Al^{3+}]I^* /$ $[Al^{3+}]II^*$	$k_{(Al-I)}$ $M^{-1}Sec^{-1}$ $\times 10^{-2}$	$k_{(Al-II)}$ $M^{-1}Sec^{-1}$ $\times 10^{-1}$	KINETIC ALUMINIUM ⁺ RECOVERY %
20	3.0	40	1.39	9.72	8.98	98.51
20	4.5	40	3.32	7.93	9.82	101.4
2.0	2.5	4.0	0.888	9.80	21.6	97.75
2.0	3.0	4.0	1.27	9.60	21.3	99.53
2.0	4.0	4.0	1.42	10.7	22.6	103.4
1.0	2.5	2.0	0.721	10.8	22.2	101.1
1.0	3.0	2.0	0.841	11.6	21.1	97.88
1.0	4.0	2.0	0.891	11.3	18.3	100.6

two components regression model. Finally, typical results from an experiment demonstrating the consistently constant equilibrium recovery of the Al^{3+} ion independent of its pH history are tabulated in Table 10. The final pH of a solution formed from mixing an Al^{3+} sample at pH 2.0 with the reagent CB in 0.2 M of pH 5.0 acetate buffer was found to be 4.978. The present concentration of buffer was proven to be adequate in effectively controlling the final pH in all of the experimental situations.

(iv) DISCUSSION

A linear relationship was observed in all the calibration curves within the range of working concentration of Al^{3+} of interest. In figure 10, the nonlinearity which occurred at the highest concentration of Al^{3+} is attributed mostly to the effect of self reabsorption of the luminescence radiation by the complex; and to a much lesser extent, secondary effects such as the background emission of the reagent blank in this particular case can no longer be considered constant, and the possibility of a luminescent chelate of different structure but with very similar spectral characteristics. The nature of the latter will become more apparent as this discussion progresses. In figures 11(a) and (b), nonlinearity occurred much later in spite of the overall higher working concentrations of Al^{3+} . This supports the conclusion that the deviations from linearity of the calibration curves at high concentrations of Al^{3+} are attributable primarily to the self-reabsorption of the complex. The 2 mm pathlength of the stopped-flow cell is obviously more effective in reducing the distortion comparing to the 10 mm

TABLE 10

EXPERIMENTAL INFINITIES OF Al-CB COMPLEXES DERIVED FROM ALUMINIUM
SOLUTIONS WITH DIFFERENT pH HISTORIES

TABLE 10

$[Al^{3+}]$ $\times 10^6 M$	pH of Al^{3+}	$[CB]$ $\times 10^4 M$	EMISSION INTENSITY IN % (ARBITRARY UNIT)
0.50	2.0	1.0	26.00
0.50	3.0	1.0	27.00
0.50	4.0	1.0	25.00
1.0	2.0	2.0	38.25
1.0	3.0	2.0	38.25
1.0	4.0	2.0	38.00
2.0	2.0	2.0	44.50
2.0	3.0	2.0	44.50
2.0	4.0	2.0	44.50

pathlength of a regular cuvet. Figure 11(b) is just another plot of figure 11(a) except that the data at high aluminium concentrations were excluded. The upper limits of the two calibration curves are $[\text{Al}^{3+}] = 7.50 \times 10^{-6} \text{ M}$ for the regular cuvet and $[\text{Al}^{3+}] = 2.5 \times 10^{-4} \text{ M}$ for the stopped-flow cell. It is important to stress that the lower limit represented in the working curves are not indicative of the absolute detection limit of the analytical system. As it was stated earlier, the complex was prepared pseudo-zero order in CB, therefore, the lower limits in the present calibration curves represent a point where the background emission of the reagent becomes comparable to the fluorescence of the complex Al-CB. Indeed, when this occurs in the case with a normal cuvet, the concentration of CB would have been five hundred times that of Al^{3+} ; and with the narrower stopped-flow cell, this happens where the concentration of CB is equal to two thousand times that of Al^{3+} . It is felt that the working concentration range in aluminium analysis with the present reagent can easily be extended another order of magnitude down from the present 10^{-7} M or 2.7 ppb. In this respect, Calcein Blue appears to be a comparable metalfluorechromic complex reagent to the most sensitive reagent in the literature thus far, salicylidene-o-aminophenol.⁴²

The structure of the Al-CB complex was reinvestigated in three separate studies using the continuous variation method. A wide range of $([\text{Al}^{3+}] + [\text{CB}])$ spanning from $2 \times 10^{-6} \text{ M}$ to $2 \times 10^{-4} \text{ M}$ was examined. The mode of detection was the emission of the complex as opposed to the absorption at 350 nm employed by Imasaka et. al.. In contrast to the

1:1 stoichiometry that was postulated by these authors, it was observed consistently in all the three present studies that the ratio of metal to ligand was near 1:n where $4 \leq n \leq 6$. Under such a circumstance, three follow-up studies with the mole-ratio method were conducted in the hope that it would provide the additional information necessary for the identification of a definitive structure for the complex. However, this was not to be so since collectively the mole-ratio plots were inconclusive. The plot in figure 13(a) was an attempt to verify the existence of either a 1:1 or a 1:4 complex, as it can be seen, there is neither a break nor a change in the gradient of the plot. In the two subsequent mole-ratio studies at higher Al^{3+} concentrations observed with a 2 mm pathlength cell, an apparent metal:ligand ratio of 1:6 was established. Both stability constant methods are subjected to similar conditions limiting their applicabilities, these conditions are that the stability constant must not be excessively large or exceedingly small, and in a 1:n complex, n must not be greater than 3.0. Although the information ascertained from the studies with the two different methods do not concur, nevertheless, it can be agreed upon that Al-CB is not a simple 1:1 complex but rather a higher order aggregate type complex. From the data in figure 12(c), a conditional stability constant of the Al-CB complex is estimated⁵⁹ to be 5.74×10^{16} by assuming a 1:4 metal to ligand ratio. If the protonated form of the ligand is being taken into consideration, the stability

constant is re-estimated to be 3.70×10^{25} .

The existence of a discrete inner sphere 1:4 complex is doubtful for a number of reasons. The Al^{3+} ion is known to be a very small ion with an ionic radius of $0.50 \text{ \AA}$.⁶⁰ The ligand Calcein Blue on the contrary is a rather bulky structure. Its ionic radius can be assumed to approximate $10 \text{ \AA}$ or more. It is difficult to envisage packing four molecules of CB around the small metal ion. An alternative interpretation is to conjecture the formation of an aggregate type complex. The Al^{3+} ion is pictured as being encapsulated in a network of the ligand molecules. A plausible mechanistic explanation of such a phenomenon is the formation of an inner sphere type complex in the first step, followed by further ligand associations in the outer sphere leading to the formation of the aggregate. It is not unreasonable to suspect that the experimental information obtained thus far are average of the true structures of the complex. The validity of such a mechanism is largely based upon the assumption that the coordination symmetry of the Al^{3+} ion is retained in the course of the reaction. Unfortunately, it is impossible with the present information to establish the nature of the Al^{3+} ion in its corresponding Al-CB complex, and therefore, no assignment was made regarding the true structure of the complex. Henceforth, the luminescent complex in the present analytical system will be described as $\text{Al}(\text{CB})_n^m$; where $n \geq 4$ and the magnitude of the charge of the complex is undetermined. It is conceivable that the spectral characteristics of the luminescent species are primarily governed by the structure of the inner sphere complex, additional outer sphere

associations will only effect a modulation of the intensity of the luminescence, the pathway in which the final complex is arrived at may vary as a function of the concentrations of Al^{3+} and CB, and therefore will subsequently be reflected in the corresponding complex formation kinetics. It is on this basis that a detailed characterization of the complex formation kinetics was performed, providing the necessary standard references in the future for the "fingerprinting" of kinetic data from actual analyses. The structure of the complex at present is not assumed to be polynuclear although such species have often been suggested in the literature.^{40(a),(b), 42} Systems where Al^{3+} was found to form complexes of higher order, i.e., 1:n where $n > 1$, include a 1:3 complex with 2-hydroxy-3-naphthoic acid⁶¹ and 1:2 complex with morin.⁶²

The rate constants in Table 7 were determined in a series of reactions with a wide range of Al^{3+} concentrations adjusted to pH levels extending from 2.0 to 4.0; the average magnitude of the second order rate constants is approximately $10^2 \text{ M}^{-1} \text{ Sec}^{-1}$. These rate constants were derived by assuming that the forward formation rate constant, k_f , is much larger than the reverse dissociation rate constant, k_d , i.e. $k_f \gg k_d$. Very few complex formation kinetic data are available in the literature other than a group of relaxation studies on weak aluminium complexes such as AlSO_4^+ ,⁶³⁻⁶⁶ $\text{AlFe}(\text{CN})_6^{67}$, $\text{AlCo}(\text{CN})_6^{68}$ and $\text{Al}(\text{CH}_3\text{COO})_2^+$;⁶⁹ and the two independent studies on the formations of aluminium salicylate⁵² and aluminium sulfosalicylate⁵³ by the stopped-flow method. In light of the nature of the present complex, it will be more relevant to interpret the significance of the rate constants in Table 7 in terms

of the reaction mechanisms established in the salicylate and sulfo-salicylate studies. Secco and Venturini⁵² concluded that below pH 3.0, the salicylate ion (HL^-) reacted with Al^{3+} via the following pathways,

where it was established that $k_1 = 9.1 \times 10^{-1} \text{ M}^{-1} \text{ Sec}^{-1}$, $k_{-1} = 7.8 \times 10^{-1} \text{ M}^{-1} \text{ Sec}^{-1}$, $k_2 = 1.02 \times 10^3 \text{ M}^{-1} \text{ Sec}^{-1}$, and $k_{-2} = 7.9 \times 10^{-3} \text{ Sec}^{-1}$ at 25°C and in a 0.1 M $NaClO_4$ medium. Perlmutter-Hayman and Tapuhi⁵³ in their study with 5-sulfosalicylic acid came to a very similar conclusion. Between the pH range 1.3 and 4.1 at 25°C and at ionic strength of 0.1 M, the rate constants in the following reaction scheme were,

$k_1' = 1.5 \text{ M}^{-1}\text{Sec}^{-1}$, $k_2' = 2.00 \times 10^3 \text{ M}^{-1}\text{Sec}^{-1}$, $k_3' = 0.02 \text{ M}^{-1}\text{Sec}^{-1}$, and $k_4' = (2.50 \pm 0.04) \times 10^3 \text{ M}^{-1}\text{Sec}^{-1}$. The forward paths in both studies were consistent with the observations reported in the relaxation studies.

In the scheme of an Eigen multistep formation mechanism, e.g.,
 a bidentate complex,

the overall forward and reverse rate constants, k_f and k_r , obtained by imposing a steady state condition on the intermediate, i.e.

$d[\text{MS}_5\text{-L-L}]^{2+}/dt = 0$, are;

$$k_f = K_o k_{11} k_{22} / (k_{22} + k_{-11}) \quad (\text{A10})$$

$$k_r = k_{-11} k_{-22} / (k_{22} + k_{-11}) \quad (\text{A11})$$

If it is assumed that chelate ring enclosure is much faster than the breaking of the first metal-ligand bond; i.e. $k_{22} \gg k_{-11}$, k_f can then be re-expressed as,

$$k_f = (1/S) K_o k_{ex} \quad (\text{A12})$$

where $(1/S)$ is a statistical factor first proposed by Langford and Stengle^{71(a),(b)} to account for the discrepancy between first-order rate constants of solvent substitution and exchange; K_o is the outer-

sphere association constant of the ion-pair, and k_{ex} is the solvent or water exchange rate constant of the metal ion. The value of K_o was calculated from the Fuoss relation.⁷² In the aluminium-salicylate study,⁵² K_o for the $\text{Al}^{3+}/\text{HL}^-$ ion-pair was reported to be 5.3 M^{-1} at 25°C in a medium 0.1 M in ionic strength. Using a value of $0.13 - 0.22 \text{ Sec}^{-1}$ for the water exchange rate constant, $k_{\text{H}_2\text{O}}$, of the Al^{3+} ion;⁷⁰ the k_f value calculated according to equation (A12) agreed very well with k_1 and k_1' in expressions (A1) and (A4). This suggests that the rate limiting step is the conversion of the outer-sphere complex into the inner-sphere complex which in turn is governed by the inner water exchange rate of the $\text{Al}(\text{H}_2\text{O})_6^{3+}$ ion. The reactions appear to proceed via a straightforward dissociative, d, mechanism. The much faster rate constants k_2 , k_2' and k_4' of reactions (A3)', (A5) and (A7) respectively were attributed to the more rapid rate of water exchange of the hydrolysed $\text{Al}(\text{H}_2\text{O})_6^{3+}$ ion. No direct measurement (e.g. NMR or O^{17}MR data) of the water exchange of the $\text{Al}(\text{H}_2\text{O})_5\text{OH}^{2+}$ ion has been reported, however, from their complexation formation kinetic data, Perlmutter-Hayman and Tapuhi⁵³ estimated the rate constant, k^* , for the exchange between ligand and water in the inner sphere to be $1.22 \times 10^3 \text{ Sec}^{-1}$. At pH 4.09, the overall formation rate constant for aluminium-5-sulphosalicylate was $1.04 \times 10^3 \text{ M}^{-1} \text{ Sec}^{-1}$ when the dissociation rate constant of the complex is negligible by comparison. This is roughly an order of magnitude faster than what is established in the present system.

Due to the nature of this work, i.e. the kinetic study was more characterization rather than mechanism oriented; it is not possible to

assign exact reaction pathways for the formation of the luminescent complex $\text{Al}(\text{CB})_n^m$. It is however possible to partially interpret the present data in a manner similar to that just described. The arguments to follow support a predominant reaction pathway which involves the $\text{Al}(\text{H}_2\text{O})_5\text{OH}^{2+}$ ion and the H_2CB^- ion. In their calculation of the K_0 value for the $\text{Al}^{3+}/\text{HL}^-$ ion pair, Secco and Venturini⁵² adopted a value of $5\text{\AA}$ to be the distance of closest approach between the two ions. The present ligand CB is considerably larger than the salicylate ion. For an ion pair such as $\text{Al}^{3+}/\text{H}_2\text{CB}^-$, at 19.5°C and in 0.1 M ionic medium, the calculated values of K_0 using $10\text{\AA}$ and $15\text{\AA}$ as the distances of closest approach are 7.36 M^{-1} and 15.03 M^{-1} respectively; corresponding to maximum k_f values of $1.62\text{ M}^{-1}\text{Sec}^{-1}$ and $3.31\text{ M}^{-1}\text{Sec}^{-1}$ according to equation (A12). This has practically eliminated any possible contribution to the final complex formation by the reaction between $\text{Al}(\text{H}_2\text{O})_6^{3+}$ and H_2CB^- via a dissociative, d, mechanism (analogous to that described in equation (A1)). An alternative is to suggest that complex formations proceed via reaction pathways which are associative, a, in nature. This proposed interpretation is also abandoned in light of the following reasons. From a comparison of the kinetic data obtained from Al^{3+} and the singly charged salicylate ion (HL^-) with those of the reaction between Al^{3+} and SO_4^{2-} (66); Secco and Venturini⁵² concluded that the basicity of the entering ligand played no role in the reactions of aluminium ion. In addition, formation rate constants of aluminium complexes studied with a variety of entering ligands^{52-53,63-69} did not demonstrate any significant discrepancy; contrary to what is normally

expected with an associative pathway where the rate is very sensitive to the nature of the entering group. A reaction between the $\text{Al}(\text{H}_2\text{O})_5\text{OH}^{2+}$ ion and the H_2CB^- ion is seen as favourable in light of the reaction pH being buffered at 5.0. Using the thermodynamic data reported by Frink and Peech,⁵⁷ Perlmutter-Hayman and Tapuhi⁵³ established the hydrolysis constant of the reaction depicted in equation (A2) at 0.1 M ionic medium to be $K_{\text{OH}} = 3.55 \times 10^{-6}$ M. Quick calculations reveal that at pH=5.0, 26.20% of a solution of Al^{3+} of any concentration is present as AlOH^{2+} . At the same time, the ligand CB at pH 5.0 is a singly negatively charged species. Therefore, provided the water exchange rate constant of the $\text{Al}(\text{H}_2\text{O})_5\text{OH}^{2+}$ ion is 2-3 orders of magnitude faster than the unhydrolysed $\text{Al}(\text{H}_2\text{O})_6^{3+}$ ion, complex formation via the ion pair $\text{Al}(\text{H}_2\text{O})_5\text{OH}^{2+}/\text{H}_2\text{CB}^-$ would prevail. The rate-limiting step in such a process is governed by the rate constant for the exchange between ligand and water in the inner coordination sphere of the $\text{Al}(\text{H}_2\text{O})_5\text{OH}^{2+}$ ion. If the value of $1.22 \times 10^3 \text{ Sec}^{-1}$ reported by Perlmutter-Hayman and Tapuhi⁵³ is adopted as the exchange rate constant; then the formation rate constant presently observed ($\sim 10^2 \text{ M}^{-1} \text{ Sec}^{-1}$) is approximately an order of magnitude slower than expected. In terms of the Eigen multistep mechanism in equation (A9), this is best explained as a partial failure of the condition $k_{22} \gg k_{-11}$; i.e. not every successful ligand attack resulting in the formation of the metal ligand monodentate complex would convert automatically into the final complex. It can be seen in equation (A10) that when k_{-11} is not negligible comparing to k_{22} , the overall complex formation rate constant is substantially reduced.

It is noted in Table 7 that the second-order rate constants elucidated are not constant in the strictest sense but vary somewhat between the range $1 \times 10^2 \text{ M}^{-1} \text{Sec}^{-1}$ to $3 \times 10^2 \text{ M}^{-1} \text{Sec}^{-1}$. The discrepancy is far too large to be attributed solely to experimental errors. From the data in Table 8 which is a study of the ligand concentration dependence of the second-order rate constant, it can be shown that depending on the ratio of the concentration of metal to ligand present in the reaction system; there is a very complex ligand concentration dependence of the second-order rate constant. It is this very complex ligand concentration dependence behaviour that renders the careful characterization of the kinetics of the system absolutely necessary. An analytical system involving the present reagent in the identification of aluminium species kinetically is best implemented via an operation that is analogous to the conventional "fingerprint" technique. In the study of the reactions between $3 \times 10^{-5} \text{ M}$ of Al^{3+} at pH 3.0 with various concentrations of CB, it is noted that when the ligand concentrations were $1.2 \times 10^{-3} \text{ M}$ and $6 \times 10^{-4} \text{ M}$, the metal recovery percentages dropped off poorly if the two reactions were continually being treated as a one component system. Nonlinearity of the data is illustrated in a typical example in figure 14. A refitting of the experimental data using a two component mathematical model resulted in much improved recoveries. There is additional evidence tabulated in Table 9 demonstrating that with a comparatively closer ratio of $[\text{Al}^{3+}]:[\text{CB}]$, e.g., 1:20, as opposed to the normal ratio of 1:100 adopted in the present work; the experimental data seem to support a physical model with two reacting

FIGURE 14

AN EXAMPLE OF NONLINEARITY IN THE GUGGENHEIM PLOT INDICATING DEVIATION FROM A STRAIGHTFORWARD ONE COMPONENT SYSTEM THAT IS CAUSED BY A LOW [CB] TO $[Al^{3+}]$ RATIO. SYSTEM UNDER OBSERVATION IS:

$[Al^{3+}]_{TOTAL}^* = 3 \times 10^{-5} M + [CB]_{TOTAL}^* = 6 \times 10^{-4} M$; in 0.1M acetate buffer at pH=5.0.

* Denotes the total concentration of the species before mixing.

TIME $\longrightarrow$ (Sec)

E.I. - EMISSION INTENSITY

components rather than one. Guggenheim treatment (refer to text in Section (B)) of the data in Table 9 uniformly yield plots with two distinct segments of different gradients. A ready interpretation of such an observation would be that there are possibly two kinetically distinguishable species of ionic aluminium present in the experimental system; a plausible combination being the monomeric $\text{Al}(\text{H}_2\text{O})_6^{3+}$ ion and the dimeric $\text{Al}_2(\text{H}_2\text{O})_{10}^{4+}$ ion.^{73(a),(b)} Such a postulation is dubious in light of the validity of the straightforward one component fit across the data reported in Table 7. An alternative interpretation is to attribute the phenomenon to the effect of only partial aggregate formation. It can be argued that both the kinetic and thermodynamic aspects of the aggregate formation are ligand concentration dependent. A lower metal to ligand concentration ratio, such as 1:20, may prove not to be conducive to uniform formation of a homogeneous aggregate complex. Consequently, a mixture of higher order aggregates (identified as the ones formed in a one component system) and simpler ones are formed in the same reaction. If such an interpretation is correct, then it is natural to associate the faster rate constant, $\sim 10^3 \text{ M}^{-1} \text{ Sec}^{-1}$, with the formation of the simpler complexes while the slower rate constant, $\sim 10^2 \text{ M}^{-1} \text{ Sec}^{-1}$, is likely to be associated with processes which lead to the formation of a similar higher order aggregate type complex as discussed earlier. The complex ligand concentration dependence of the reaction rate displayed in the present system is characteristic of that of a multistep or stepwise complex formation scheme.³⁴ The observed reaction rate of such a system is very often composite, in the form of

a quotient where both the numerator and the denominator contain linear combinations of concentration terms of the reactants. Prevalent among these terms are higher order ligand concentration terms of various degrees appearing in both the numerator and the denominator. The evidence here is consistent with the proposed complex formation mechanism in which the aggregation process leading to the final Al-CB complex is perceived to be stepwise. In order to avoid unnecessary complications in the treatment of the kinetic data, it is recommended that a large metal:ligand concentration ratio such as 1:100 or more be used in all applications of the reagent Calcein Blue. The only exception here being in the case of 10^{-4} M Al^{3+} , the presence of 10^{-2} M CB was found to obscure the fluorescence signal of the luminescent complex; therefore, for the benefit of better signal resolution, the ligand concentration was reduced to forty times that of the aluminium ion concentration.

A consistent trend present in the data reported in Table 7 is that as one progress from pH 2.0 down to pH 4.0, the value of the second-order rate constant increases. This can be attribute to the effect of the concentration of free acetate ions present in the reaction medium after mixing. Although it was determined experimentally that the presence of 0.1 M of acetic acid-sodium acetate buffer in the final reaction medium is more than capable of maintaining the overall solution pH value at 5.0 regardless of the original pH value of the aluminium solution which may range from 2.0 to 4.0; it remains true that the concentration of free acetate ions is lower in a final solution which

is the result of a reaction between a solution of Al^{3+} set at pH 2.0 and the ligand than a similar reaction medium where the original Al^{3+} solution was set at pH 4.0. The presently observed dependence of the second-order rate constant on the concentration of the free acetate ion is contrary to an earlier report by Turner and Sulaiman⁷⁴ where these authors observed that the reaction rate constant of the polynuclear hydroxyaluminium cations with 8-quinolinol was dependent on the concentration of the acetic acid rather than the acetate ion. The acetate ion concentration influence on the rate constant is probably very complex in nature, much like the ligand concentration influence, only that the former effect is less in comparison with the latter. A very similar situation was reported by Goto and Yotsuyanagi⁷⁵ in their study of the reaction of hydroxyaluminium polymers with 8-quinolinol and acetate.

In Table 7, the lowest concentration of Al^{3+} analyzed was 10^{-7} M or 5×10^{-8} M after mixing. The corresponding rate constants and percentages of recovery were generally on the low side. The Guggenheim plots of the rate data did not indicate any deviation from the adopted regression model of a single exponential term plus a constant (blank). The observed decreases in the second-order rate constants must then be attributed to the metal to ligand concentration dependence of the complex formation rate constant. It must be cautioned that the net signal (i.e. blank suppressed) of the luminescent complex at very low levels of Al^{3+} is small, therefore, the effect of noise or drift in the electronic circuits would be drastically amplified and manifested in the

final quality of the corresponding rate data. It was observed during this work that the baseline suppression circuit of the Perkin-Elmer spectrometer experienced a serious drift problem (presently assigned to be thermal in nature) over an average period of 10 minutes. The reaction times (ten half-lives) of low concentrations of Al^{3+} are expected to be long and indeed experimentally observed to span anywhere from five to fifteen minutes. In practice, the thermal drift in the baseline voltage suppression circuit was principally responsible for the net signal to drift to a lower value than what it should have been. In light of this, the smaller rate constants must partially be the consequence of the overall rate reduction observed due to the voltage drift that is opposite to the growth of the signal voltage; in addition to the metal to ligand concentration effect cited above. The poor recoveries at low concentrations of Al^{3+} are directly and totally attributable to the adverse effect of such a thermal drift. It should be clarified that the present inadequacies in the analyses of low concentrations of Al^{3+} , i.e., $[\text{Al}^{3+}] < 10^{-7} \text{ M}$, is not due to the limitations of the chemical system; but rather, the poor performance of the electronics. It is necessary to have the voltage suppression circuit in the Perkin-Elmer spectrometer modified substantially before any work at $[\text{Al}^{3+}] < 10^{-7} \text{ M}$ levels can become reliable.

(v) CONCLUSION

The kinetics of the present analytical system are neither simple nor uniform in their behaviour, however, the situation is far from being unmanageable. It is shown in the present results that the system can be

implemented readily when accompanied by a careful characterization study of its responses to various influencing parameters. Advantages such as the high intensity of the luminescent complex and strong complex formation serve as a compensation for some of the tedium associated with the present reagent. In the presence of a large excess of reagent, the second-order reaction rate constants were found to be a function of both the concentrations of the ligand and the acetate ion. Within experimental error, the recoveries of the metal ion determined from the kinetic analyses agree well with the values of theoretical recoveries at equilibrium. In the current study, there is not any evidence suggesting the presence of a dimeric aluminium species; alternatively, if the dimers do exist then the reaction rate of the species with the chosen reagent must be kinetically undifferentiable. A definite structure of the complex cannot be derived from the available data, but it is conjectured to be a structure with a high number of ligands aggregating around the aluminium ion. Independent of the complexity of the structure of the luminescent complex and its exact formation kinetics, the analytical system was found to be applicable for a wide range of aluminium concentrations.

(D) KINETIC ANALYSIS OF A MIXTURE OF HEXAAQUO ALUMINIUM ION AND
ALUMINIUM CITRATE COMPLEX

(i) INTRODUCTION

In the two preceding chapters, a nonlinear regression numerical analysis method for the treatment of 2-component kinetic data was presented along with the characterization of an analytical reagent for the kinetic determination of Al^{3+} . The objective of this work is to implement these methods in the treatment of a model system in order that their respective applicabilities to a real experimental situation can be carefully scrutinized. Consider the following mononuclear complex formation reaction,

depending upon the hydrogen ion concentration prevailing, the extent of complex formation can vary drastically. Consequently, at various pH levels, there are a number of possible combinations of free metal ion concentration to metal complex concentration distributed in the system. Provided that the reaction rates of the free ion and the complex are sufficiently well separated, and suitably fast with respect to the function of k_r and k_f governing relaxation of equilibrium (B1), the equilibrium mixture can be analyzed kinetically and the corresponding concentrations of each component determined along with their characteristic rate constants. In a kinetic analysis as such, both

the equilibrium and some kinetic properties of the free ion/complex system are investigated simultaneously.

The stability constant, K , of the complex system depicted in equation (B1) is generally expressed as,

$$K = \frac{[ML^{(n-1)+}][H^+]}{[M^{n+}][HL]} \quad ; \quad (B2)$$

classical methods⁵⁹ available for the determination of K are mainly based upon the preparation of a series of solutions containing known amounts of the complex forming components where the concentration of one component is gradually varied and the concentration of one of the reactants or products is observed directly or indirectly by a convenient analytical means. Usually the concentration of the metal ion is held constant and that of the ligand varies within wide limits. The most frequently employed "direct" methods are potentiometric and pH titrations. In a potentiometric titration, the activity of the uncomplexed metal ion is measured directly by potentiometry during the course of the titration with the ligand; the stability constant is calculated from the related free metal and free ligand concentration data. Analogously, the activity of the hydrogen ion concentration is monitored directly in a pH titration. Some of the popular "indirect" methods include the continuous variation method (Job's plot), mole-ratio method, and the slope-ratio method discussed briefly in the last chapter.

From the complex formation rate of equation (B1), an overall

stability constant of the complex is given as

$$K' = \frac{k_f}{k_r} \quad (B3)$$

where k_f and k_r are the formation and dissociation rate constants of the complex respectively. When complex formation via the pre-equilibrium step involving "ion-pairing" of an aquo metal ion and the ligand is considered.

K' above is rewritten as

$$K' = \frac{k_a k_c}{k_b k_d} = K_o \frac{k_c}{k_d} \quad (B5)$$

For a straightforward 1:1 complex, the value of K' can be evaluated quite readily from the complex formation kinetic data with an a priori knowledge of K_o . The value of k_d can be determined directly⁵² in a separate set of experiments or extracted indirectly⁵³ from the formation rates. In terms of a kinetic method of complex stability constant determination, the approach described can be considered as the equivalent of an "indirect" method in the classical methods of determining stability constants.

In a study reported by Langford and Khan,⁷⁶ the concentrations of free and bound aquo Fe(III) in equilibrium mixtures of aquo Fe(III) and a soluble 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-sulphosalicylic 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 this information, the conditional formation constant K_1 which was defined as $[\text{Fe-fulvic acid}]/[\text{Fe(III)-free}][\text{fulvic acid}]$ was determined at three pH values. The derivation of conditional formation constant by kinetic method in such a manner is analogous to a direct classical method such as potentiometric titration where the activity of the free metal ion is monitored directly.

The equilibrium system under investigation in this work is:

where the monitoring or analysis system is,

The reaction depicted in equation (B6) is pH dependent. This gives rise to different ratios of $\text{Al}^{3+}/\text{Al-CITRATE}$ distribution at various pH. The successive protonation constants at 20°C and in 0.1 M NaClO_4 are:⁷⁷

$$K_1 = \frac{[\text{HCITRATE}^{2-}]}{[\text{H}^+][\text{CITRATE}^{3-}]} = 4.79 \times 10^5 \quad (\text{B8})$$

$$K_2 = \frac{[H_2CITRATE^-]}{[H^+][HCITRATE^{2-}]} = 2.24 \times 10^4 \quad (B9)$$

$$K_3 = \frac{[H_3CITRATE]}{[H^+][H_2CITRATE^-]} = 7.42 \times 10^2 \quad (B10)$$

The strategy in this work is to apply the previously characterized reagent Calcein Blue (CB) in the analyses of both the free hexaquo Al^{3+} ion and aluminium citrate complex where the reagent ligand CB is expected to displace the citrate ligand directly from the parent aluminium complex. The composite kinetic data of the 2-component system is analyzed by the Nonlinear Regression Package developed. The resultant concentrations of both components determined directly are then used in conjunction with the citrate protonation data to calculate a stability constant for the aluminium citrate complex. The rate constants k_x and k_y serve as the qualitative parameter identifying the nature of the two species present.

(ii) EXPERIMENTAL

The preparations of stock solutions of Al^{3+} from purified $AlCl_3 \cdot 6H_2O$ crystals were described in the last chapter. Analytical reagent grade tri-sodium citrate dihydrate ($Na_3C_6H_5O_7 \cdot 2H_2O$, M.W.=294.10 gm) obtained from British Drug House Chemicals was used without purification. A stock solution of 100 ml of 10^{-3} M tri-sodium citrate dihydrate was prepared and used in the subsequent preparation of 200 ml of 10^{-4} M Al^{3+} + 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 and monitored by an Orion Research (model 701A) 3-decimals digital pH meter. All the prepared sample solutions were left to stand for twenty-four hours to attain 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 $\text{Al}^{3+}/\text{Na}_3\text{-Citrate}$ 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. All the kinetic runs were performed by the Aminco-Bowman stopped-flow spectrofluorimeter at $19.5 \pm 0.1^\circ \text{C}$ and the data resulting analyzed via the Nonlinear Regression Package as described earlier. Theoretical infinities (24 hours) of the reactions were prepared by reacting the reagent with aluminium solutions that were identical in hydrogen ion concentrations and Al^{3+} concentrations but without the presence of any citrate ion. A minimum of ten kinetic runs were recorded for the purpose of data averaging.

(iii) RESULTS

The results from a study of six different mixtures of Al^{3+} and its citrate complex are tabulated in Table 11. The experimental reaction rate constant of the aluminium citrate complex k_{obs} was found to be independent of the reagent concentration in all the situations. The fact that k_{obs} is constant throughout various compositions of free Al^{3+} and its citrate complex is reflected in the Guggenheim plots of

TABLE 11

KINETIC AND THERMODYNAMIC DATA OF THE ALUMINIUM-CITRATE COMPLEX AS DETERMINED BY NONLINEAR REGRESSION KINETIC ANALYSES

* 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 aluminium is used as the theoretical 100% aluminium recovery.

TABLE 11

[Al] _{TOT} x10 ⁵ M	[CIT] _{TOT} x10 ⁵ M	pH	[CB] _{TOT} x10 ³ M	[Al] _{FREE} / [Al] _{TOT}	k _(Al) M ⁻¹ Sec ⁻¹ x10 ⁻²	k _(Al-CIT) Sec ⁻¹ (obs) x10 ³	EQUIL. * INFINITY	KINETIC * INFINITY	% RECOVERY	STABILITY CONSTANT(K) x10 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

the experimental data. In figures 7(a) and (b), the raw and FFT filtered kinetic data for the reaction rate between a mixture of 10^{-5} M Al^{3+} + 10^{-5} M $\text{Na}_3\text{-Citrate}$ at pH 3.0 and 10^{-3} M Calcein Blue are demonstrated to have two distinct segments with very different gradients. The observed rate constants for the free- Al^{3+} and its citrate complex are separated by almost two orders of magnitude. Similar Guggenheim plots of a mixture of 10^{-6} M Al^{3+} + 10^{-6} M $\text{Na}_3\text{-Citrate}$ at pH 3.0 reacting with 10^{-4} M CB are illustrated in figures 8(a) and (b). Although the two components characteristics are still very obvious in these plots, the gradients of the slopes had become much closer to each other. In this case, the observed rate constants are approximately one order of magnitude apart. When k_{obs} of the aluminium citrate complex is reagent concentration independent, the two component kinetics are better resolved at higher reagent concentrations since the pseudo first-order rate constant for the free- Al^{3+} ions will then be much larger than k_{obs} of the citrate complex.

(iv) DISCUSSION

One of the criteria for the success of kinetic analysis of a multi-component system is that the reagent must form very strong complex with all the components present in the analyte mixture. Therefore, it must be established that the reagent Calcein Blue employed in the present work is capable of displacing the citrate ligand totally from its parent aluminium complex. By virtue of the stipulation under which the kinetics must be observed, i.e. in the presence of a large excess (100 fold) of the reagent CB, the formation of Al-CB is favoured

even if the stabilities of both complexes, Al-CB and Al-Citrate, are comparable. This is assuming that the concentration of free citrate ion present is small. It is shown in Table 12 that the infinity readings after 24 hours of equilibration for the pure Al^{3+} samples and the Al^{3+} -Al-Citrate mixtures are identical within experimental error. This qualifies the reagent CB in the analysis of this chosen model system.

The second-order rate constants of the free- Al^{3+} ion are in good agreement with those established earlier. The first two second-order rate constants, k_1 , in Table 11 are characteristic of reactions between free- Al^{3+} concentrations of approximately 10^{-5} M (assuming 50% of complexation by the citrate ions) and reagent concentrations of 2×10^{-3} M. The average magnitude of the determined rate constants $\sim 3.00 \times 10^2 \text{ M}^{-1} \text{Sec}^{-1}$ is comparable to the second-order rate constant of a reaction between 3.00×10^{-5} M of free- Al^{3+} and 2.40×10^{-3} M of the reagent, $2.32 \times 10^2 \text{ M}^{-1} \text{Sec}^{-1}$, reported in Table 8. The other four second-order rate constants in Table 11 are associated with reactions between an average of $\sim 10^{-6}$ M free- Al^{3+} and 2×10^{-4} M CB. It can be seen in Table 7 that for a reaction between 10^{-6} M of free- Al^{3+} and 2×10^{-4} M CB, the second-order rate constant was $2.62 \times 10^2 \text{ M}^{-1} \text{Sec}^{-1}$; very similar to what is determined presently in a 2-component analysis, an average of $\sim 2.70 \times 10^2 \text{ M}^{-1} \text{Sec}^{-1}$. It is to be expected that the rate constants elucidated from a 2-component system will not have the same accuracy as those in a simple single component model. In addition, the metal to reagent concentration ratios in all six experiments are different. This has been proven to produce differences in the rate constants due to the dependence of the

TABLE 12

EQUILIBRIUM (24 HOURS) RECOVERIES OF ALUMINIUM BY THE REAGENT CB FROM MIXTURES OF IONIC ALUMINIUM AND TRI-SODIUM CITRATE AT VARIOUS ACIDITIES.*

- * The aluminium recoveries at various acidities and mixture compositions were calculated using a theoretical 100% aluminium recovery derived by substituting the reaction mixture in question with a reference solution of ionic aluminium at pH 3.0 identical in concentration to the total aluminium 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.
- 2

TABLE 12

$[Al]_{TOTAL}$ $\times 10^5 M$	$[CITRATE]_{TOTAL}$ $\times 10^5 M$	$[CB]_{TOTAL}$ $\times 10^3 M$	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

reaction kinetics on the ligand concentration. Taking these influences into consideration, it is not unreasonable to anticipate some slight variations in the second-order rate constants in Table 11.

The reaction between the aluminium citrate complex and the reagent CB is essentially a ligand exchange reaction;

where there are two possible reaction pathways. The first mechanism is one in which the dissociation of the chelate is the rate controlling process, i.e., the observed reaction rate is first-order in the concentration of the complex ML only. The second mechanism can be classified as ligand-assisted dechelation⁷⁸ in which the observed forward rate is first-order in both the concentrations of ML and L'. Reactions where both mechanisms are active are documented in the literature.^{79,80(a),(b)} The observed rate constant of the reaction described in expression (B11) can be written as,

$$k_{obs} = k_1 + k_2[L'] \quad (B12)$$

where k_1 is the dissociation rate constant of the ligand or chelate L, and k_2 is the second-order rate constant of the reaction between ML and L'. In the present ligand substitution reaction, the observed rate constant, k_{obs} , in Table 11 is consistently reagent concentration independent. The evidence seems to support the dissociation of citrate as the rate determining step in the ligand exchange. It is likely that such a dissociation occurs stepwise with the first step, the breaking

of an Al-O bond, being rate determining. In a kinetic study of the dissociations of some Ni(II) chelate complexes, Buck and Moore⁸¹ concluded that complexes of comparable stabilities should have similar rates of dissociation. Secco and Venturini⁵² established in their study the dissociation rate constant k_{-2} for the complex aluminium salicylate, AlL^+ , yielding $AlOH^{2+}$ and HL^- , to be $7.90 \times 10^{-3} \text{ Sec}^{-1}$ at 25°C . The average dissociation rate constant k_{obs} determined in this work for the complex Al-Citrate is, under the assumption that k_1 dominates, $4.76 \times 10^{-3} \text{ Sec}^{-1}$ at 19.5°C , very similar to that of the Al-Salicylate complex. There are two implications with such an observation, (i) that the overall intrinsic stability of the citrate complex of aluminium is comparable to that of the salicylate complex; and (ii) that the pentaquo hydroxy aluminium ion is involved in the formation of the aluminium citrate complex at pH 5.0.

The stability constants K_C of the aluminium citrate complex reported in Table 11 were calculated on the basis of the following reactions,

where K_C is expressed as

$$K_C = \frac{[Al-CITRATE][H^+]^3}{[Al^{3+}][H_3CITRATE]} \quad (B14)$$

The concentrations of Al-Citrate and Al^{3+} in equation (B14) are determined experimentally. Using a value of $K_{OH} = 3.55 \times 10^{-6} \text{ M}$ for the

hydrolysis constant between $\text{Al}(\text{H}_2\text{O})_6^{3+}$ and $\text{Al}(\text{H}_2\text{O})_5\text{OH}$ adopted by Perlmutter-Hayman and Tapuhi,⁵³ 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 totally $\text{Al}(\text{H}_2\text{O})_6^{3+}$ in all of the instances. The hydrogen ion concentration is taken to be that in which the complex was prepared and equilibrated at. Finally, the concentration of the undissociated citric acid can be calculated from the protonation constants described in expressions (B8)-(B10). Succinctly, the concentration of any unbound or free citrate ions present in the complex system is,

$$[\text{Citrate}]_{\text{free}} = [\text{Citrate}]_{\text{total}} - [\text{Al-Citrate}] \quad (\text{B15})$$

where $[\text{Citrate}]_{\text{free}}$ is simultaneously a mixture of the protonated acids of the ion; the distribution ratio of these species are pH dependent, i.e.,

$$[\text{Citrate}]_{\text{free}} = [\text{H}_3\text{Citrate}] + [\text{H}_2\text{Citrate}^-] + [\text{HCitrate}^{2-}] + [\text{Citrate}^{3-}]. \quad (\text{B16})$$

In terms of the protonation constants K_1 , K_2 , and K_3 in expressions (B8)-(B10), the concentration of total free citrate ions in expression (B16) can be re-expressed as,

$$[\text{Citrate}]_{\text{free}} = \frac{[\text{H}_3\text{Citrate}]}{[\text{H}^+]^3} \left([\text{H}^+]^3 + \frac{[\text{H}^+]^2}{K_3} + \frac{[\text{H}^+]}{K_2 K_3} + \frac{1}{K_1 K_2 K_3} \right) \quad (\text{B17})$$

If the ratio of the concentration of the undissociated acid to the concentration of total free citrate ions is defined as α_0 , i.e.

$$\alpha_0 = [\text{H}_3\text{Citrate}]/[\text{Citrate}]_{\text{free}} \quad (\text{B18})$$

then the corresponding α_0 values for the five different pH settings in Table 11 can be calculated from equation (B17) and tabulated in Table 13 below.

The average stability constant established in the present work is 5.55×10^{-4} at $19.5 \pm 0.1^\circ \text{C}$, 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 by the authors Pattnaik and Pani.⁸² The stability constant was determined by the method of pH titrations. If the value 1.95×10^{-5} is assumed in a theoretical calculation, it can be shown that at pH 3.0, 12.41% of the aluminium ions exist as the citrate complex in a mixture of $2 \times 10^{-5} \text{ M Al}^{3+} + 2 \times 10^{-5} \text{ M Na}_3\text{-Citrate}$; the percentage drops even lower to 1.57% in the case of a mixture of $2 \times 10^{-6} \text{ M Al}^{3+} + 2 \times 10^{-6} \text{ M Na}_3\text{-Citrate}$. This is obviously contrary to the present observation where the percentages were determined to be 59.93% and 23.22% in the respective mixtures. 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+} (Section C) and the literature value⁵² 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 aluminium citrate

TABLE 13

FRACTIONS OF UNIONIZED CITRIC ACID AT VARIOUS ACIDITIES

$$\alpha_0 = [\text{H}_3\text{CITRATE}] / [\text{CITRATE}]_{\text{free}}$$

TABLE 13

pH	
2.00	0.881
2.70	0.592
2.80	0.533
3.00	0.415
3.20	0.304
3.55	0.153
4.00	4.85×10^{-2}

complex in the reaction mixtures at pH 3.0 are indisputable in the Guggenheim plots in figures 7(a) and (b), and 8(a) and (b). In fact, in majority of the cases, the concentrations of the citrate complex were 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 ($2.87 \times 10^2 \text{ M}^{-1} \text{Sec}^{-1}$) and the first-order dissociation rate constant of the Al-Citrate complex ($4.76 \times 10^{-3} \text{ Sec}^{-1}$). The two rate constants differ by an average factor of 6.03×10^4 . Rough calculations indicate that in order for the initial dissociation rate of the citrate complex to be competitive with the initial formation rate of Al-CB in a reaction between ($2 \times 10^{-6} \text{ M Al}^{3+} + 2 \times 10^{-6} \text{ M Na}_3\text{-Citrate}$) and $2 \times 10^{-4} \text{ M CB}$; 86% of the total concentration of Al^{3+} will have to exist as the citrate complex. The highest percentage of citrate complex present in this study is 69.70% at pH 3.55, where the initial formation rate of Al-CB is approximately 2.70 times faster than the initial Al-Citrate complex dissociation rate. Reactions involving higher concentrations of the mixture are even less likely to be susceptible to the reversion phenomenon described above. For example, in a reaction between ($2 \times 10^{-5} \text{ M Al}^{3+} + 2 \times 10^{-5} \text{ M Na}_3\text{-Citrate}$) and $2 \times 10^{-3} \text{ M CB}$, if 90% of the free- Al^{3+} is assumed to be complexed, the formation rate of Al-CB is still approximately one order of magnitude faster than the dissociation rate of Al-Citrate.

(v) CONCLUSION

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 CB in the fluorimetric determination of aluminium species fare very well in their respective applications. The rate constants determined by the nonlinear regression method besides being internally consistent are in good agreement with those established in independent studies. In spite of the slight discrepancy between the stability constants of this work and that reported in the literature⁸²; the observed stability constants were internally consistent and there are good evidences favouring the average value of 5.55×10^{-4} derived here. To compensate for the slower complex formation rate with the present reagent CB (second-order rate constant is $\sim 10^2 \text{ M}^{-1} \text{ Sec}^{-1}$ as oppose to $\sim 10^3 \text{ M}^{-1} \text{ Sec}^{-1}$ in the case of salicylate ions^{52,53} which is assumed to be homologous to the citrate ion), a large excess of reagent is used in all the analyses. A recommended arrangement would be to have the concentration of CB a hundred fold in excess of either the total aluminium species concentration or the total free complexing ligands concentration, whichever may be larger. In this manner, the observed rate of the reaction being monitored is guaranteed to be at least an order of magnitude faster than the other simultaneous competing processes. The dissociation of the aluminium complex under investigation will not hamper the analysis (assuming an average dissociation rate

constant of $\sim 10^{-3} \text{ Sec}^{-1}$), unless the unknown aluminium complex was originally present in a large quantity in the neighbourhood of approximately 90% of the total aluminium concentration at very low levels, e.g., $\sim 10^{-6} \text{ M}$. From two separate experiments which the results were not included in Table 11 for reasons which will become obvious, the regression was found to be less reliable in extreme cases where the presence of one component is predominant. In a reaction between a mixture of ($2 \times 10^{-5} \text{ M Al}^{3+} + 10^{-5} \text{ M Na}_3\text{-Citrate}$) at pH=2.0 and $2 \times 10^{-3} \text{ M CB}$, the ratio of the free- Al^{3+} concentration to the total Al^{3+} concentration was determined to be 0.9617, the corresponding stability constant 4.90×10^{-3} . The respective rate constants for the free- Al^{3+} and the citrate complex were $2.84 \times 10^2 \text{ M}^{-1} \text{ Sec}^{-1}$ and $5.07 \times 10^{-3} \text{ Sec}^{-1}$. In another reaction between a mixture of ($2 \times 10^{-6} \text{ M Al}^{3+} + 2 \times 10^{-6} \text{ M Na}_3\text{-Citrate}$) at pH 4.0 and $2 \times 10^{-4} \text{ M CB}$, the ratio $[\text{Al}^{3+}]_{\text{free}}/[\text{Al}^{3+}]_{\text{total}}$ was found to be 7.70×10^{-2} and the corresponding stability constant 1.61×10^{-3} . The rate constants were $2.69 \times 10^2 \text{ M}^{-1} \text{ Sec}^{-1}$ and $4.72 \times 10^{-3} \text{ Sec}^{-1}$ for the free- Al^{3+} and bound- Al^{3+} respectively. 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. This observation is very similar to that established in data analysis with the graphical method. From the results tabulated in Tables 4 and 5, which are kinetic parameters determined by the Guggenheim method (a graphical method) and nonlinear regression analysis; the short comings of the graphical method are clearly

demonstrated. Finally, a technical note on the implementation of the nonlinear regression. It was observed throughout this work that by reducing the number of degrees of freedom in the regression; i.e., constraining a certain number of parameters; adverse effects can result in the consequent regression analysis. A similar observation was reported by Alcock et. al.²⁸ in their nonlinear least square evaluation of reaction rate constants study.

(E) KINETIC STUDY OF INTERACTIONS BETWEEN IONIC ALUMINIUM AND FULVIC
ACID AT NEAR NEUTRAL pH VALUES

(i) INTRODUCTION

The pH dependence of the aluminium ion behaviour in an aqueous medium is well documented in the literature.^{56,57,83-86} At $\text{pH} \leq \text{pH}_p$ (~ 4.5), where pH_p is the critical hydrogen ion concentration at which condensed (colloidal) phase just begins to form, there is a general consensus that the predominant species is the $\text{Al}(\text{H}_2\text{O})_6^{3+}$ ion. At higher total aluminium concentrations, e.g. $[\text{Al}^{3+}]_{\text{total}} \geq 0.01 \text{ M}$, the dimeric species $\text{Al}_2\text{OH}_2^{4+}$ is also present. In the region, pH_p (~ 4.5) $\leq \text{pH} \leq \text{pH}_c$ (~ 7.0), where pH_c is the boundary pH between the stable and settleable precipitate (coagulation), a condensed phase containing various forms of aluminium polycations exist in the presence of $\text{Al}(\text{H}_2\text{O})_5\text{OH}^{2+}$. These polycations are generally divided into two categories according to their reacting rates with a complexing reagent.^{74,87} The existence of a polycationic species⁸⁸ and the subsequent differentiation⁸⁹ of a faster and slower reacting species are confirmed spectroscopically. From pH_c (~ 7.0) to pH_d (~ 9.0), where pH_d is the critical hydrogen ion concentration at which the precipitate is just completely dissolved, precipitation of the crystalline complex $\text{Al}(\text{OH})_3$ or gibbsite occurs. At $\text{pH} > \text{pH}_d$ (~ 9.0), aluminium species becomes soluble again in the form of the aluminate ion, $\text{Al}(\text{OH})_4^-$. A zero point of charge (ZPC) occurs generally around pH 8.0 where the aluminium hydroxo complexes are approximately neutral in charge. It must be emphasized that the designated pH intervals indicated in the above corresponding to the respective phenomena are

by no means constant but are total aluminium concentration dependent. From a study reported by Rubin and Hayden,⁵⁶ it was demonstrated that as the total aluminium concentration is varied from 3.0×10^{-2} M to 6.7×10^{-4} M, pH_p was shifted from 4.08 to 4.63, and correspondingly pH_c from 6.02 to 7.15, and finally pH_d from 10.66 to 9.13.

In a natural water system, the acidity often varies between pH 4.0 - 9.0 depending upon the geological composition of the surrounding environment. In view of the very sensitive pH dependent solubility of aluminium, the dissolution and transportation of aluminium in a natural water system must then involve additional species other than the simple ionic aluminium hydroxides. The current understanding^{90,92a} suggests that the solubility of aluminium can be greatly enhanced by the formation of soluble organoaluminium complexes. This can certainly be related to the phenomena of organic chelation and leaching of aluminium from soil materials. Both iron and aluminium are assumed to be transported from the upper reaches of a water system to the lower reaches via such widely soluble organometallic complexes. At a site of deposition, the metal is gradually precipitated by either one or both of the two mechanisms proposed by Cronan and Schofield.⁹⁰ The metals can ultimately precipitate from solution as hydroxides and sesquioxides when the organic moieties of the complexes are degraded by microbes; or the organic ligands during transportation can complex with an increasing numbers of metal cations, eventually reaching metal complexation saturation and precipitate as organomineral colloids. In recent times, much attention^{90,91} has been focussed on the subject of

"acid rain" and its detrimental effects on the environment. Being one of the most abundant metals in the earth crust, there is a significant increase in the concentrations of dissolved aluminium in the surface and ground waters in areas which are recipients of the meteoric H_2SO_4 . The phenomenon is attributed to consequences arising from the current contemporary increases in soil aluminium leaching by the "acid precipitation". The subsequent transportation of this aluminium to the acidified lakes led to a drastic increase in the rate of fish mortality. The mortality is believed to have been caused from exposure to acutely toxic concentrations of aluminium. Therefore, the present interest in the interactions between ionic aluminium species and soil organics is no longer strictly for the benefit of mapping the deposition of the mineral but an attempt to formulate an overall picture of an environmental problem.

A major organic component in the constitution of soils and sediments is fulvic acid, a low molecular weight, poorly condensed aromatic polymer that has resulted from the chemical and biological degradation of plant and animal residues, and from synthetic activities of microorganisms.^{92a} Fulvic acid is separated from the rest of the humic materials by its solubility in both dilute alkali and acid. The aromatic polymer is suggested to be composed of monomeric molecular units which are benzene rings bearing various functional groups including carboxylate, phenolic OH, alcoholic OH, ketonic $C=O$, and quinoid $C=O$. The distribution ratio of these groups in units of equivalents of functional group/gm of fulvic acid is approximately

7.71:3.3:3.6:2.5:0.6 respectively in a well characterized sample used in our work.^{93,99} Hydrogen bonding is established to be the primary bonding force linking these individual molecular units together.^{92b}

A number-average molecular weight, $\bar{M}_n$ of 951 gm was reported by Hansen and Schnitzer⁹⁴ using the vapour pressure osmometry method and corrected for the dissociation of the acidic functional groups. This widely accepted value was later revised by Wilson and Weber⁹⁵ to be averages 644 gm and 626 gm for podzol soil fulvic acid and river water fulvic acid respectively. It is commonly acknowledged that fulvic acid in an aqueous solution will exhibit some polyelectrolytic behaviour, e.g., the ionization of the weakly acidic functional groups are strongly influenced by the ionic strength of the medium and that the acid strength decreases with increasing degree of ionization. In addition, the molecular conformation of the polymer varies as a function of its own concentration,⁹⁶ a phenomenon attributed to the electrostatic effects on the polyvalent molecule. Gamble^{97,98} in two separate potentiometric titration studies attempted a systematic characterization of the rather complex ionization behaviour of the acidic functional groups of fulvic acid. From these studies and supporting literature evidence, that author concluded that although it is likely that no two acidic functional groups (mostly carboxyl groups) are inherently chemically identical in fulvic acid, it nevertheless is possible to categorize them into two general classes according to the two equivalence points which were derived from the titration data. The Type A functional groups include all the more readily ionizable functional groups up to

the first equivalence point, 4.99 ± 0.25 , and Type B are those that fall between the two equivalence points, i.e. $4.99 \pm 0.25 < \text{pH} < 7.63 \pm 0.28$. Amongst Type A acidic functional groups are Type I carboxyl groups which are ortho to the phenolic OH groups on the aromatic rings constituting salicylic acid type structures which are the most likely chelating sites for metal ions. The ratio of Type A to Type B acid groups has been established⁹⁸ to be 4.99 mequiv. acid group/gm FA:2.64 mequiv. acid group/gm FA. In the poly-electrolyte chemistry of fulvic acid, it is more appropriate to describe the acid ionization equilibrium as a continuous function of the concentration of the ionized acidic functional groups as oppose to the conventional concept of an acid dissociation constant developed for monomeric acids.

Salicylic acid type chelation sites are most readily available in fulvic acid. The distributions of carboxyl and phenolic OH groups in fulvic acid are 7.63 mequiv./gm FA and 3.30 mequiv./gm FA respectively.^{98,93} The current feeling is that in the event of a saturation of all the salicylic acid type binding sites, i.e.

3.30 mequiv./gm FA; additional binding sites are available in the form of dicarboxylic structures. Therefore, for each gram of fulvic acid, the total quantity of binding sites available is $(7.63 + 3.30)/2 = 5.47$ mequivalents if the incorporations of metal ions are assumed to proceed via straightforward chelation mechanisms. This is however a very simple minded assumption in light of the rather complex molecular conformation of fulvic acid. At low pH, the hydrogen bonded molecular units which constituted fulvic acid are flexible enough to permit the units to aggregate; thus forming elongated fibers and bundles of fibers.^{92b}

It is not unreasonable to suspect that these aggregates may possess tertiary structures. As the pH increases, the hydrogen bonds rupture and dispersion occurs. The nature of the interaction between metals ions and fulvic acid is not exactly known at the present, however, it is quite certain that it cannot be treated as a pure classical metal complexation or chelation process. It is possible that the conformations of metal-fulvates are effected via a combination of processes such as ion-exchange, surface adsorption, chelation, coagulation/peptization, and inclusion/occlusion. The likelihood is that one of these processes will prevail depending upon the chemical conditions. In view of the polyelectrolytic nature of fulvic acid, any metal fulvate complex equilibrium⁹⁶ should really be described in terms of a metal binding function of the concentration of fulvic acid.

In the present model study, relatively acidic solutions of aluminium-fulvate are titrated gradually to near neutral pH levels simulating the course which an organoaluminium complex will experience during its transportation in a natural water system. Constituents in the subsequent sample solutions are analysed kinetically by the nonlinear method reported in Sections (B) and (D) of this thesis. The results will be compared to those of the control standards which are aluminium solutions of identical total aluminium concentrations and pH to the samples in the absence of fulvic acid. It is hoped that studies like the present one will produce more information regarding, (i) the role of fulvic acid as a typical organic substance in the leaching and transportation of elemental aluminium in a natural water system, (ii)

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the equilibrium distribution of aluminium species in near neutral pH typical of a natural water system, and finally (iii) the nature of metal-fulvic acid interactions under the experimental conditions set in this work.

(ii) EXPERIMENTAL

The preparations of standard aluminium stock solutions from purified $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ crystals was as described in Section (C) earlier. Purified fulvic acid extracted from the Bh horizon of an imperfectly drained podzol soil in Prince Edward Island was graciously supplied by Dr. D.S. Gamble, Soil Research Institute, Agriculture Canada. The number average molecular weight was assumed to be 900 gm, similar to that used in two previous studies.^{76,99} The choice of molecular weight in the present work is unimportant and only arbitrary since the molar concentrations of FA can always be re-expressed in the base unit of mequivalents of functional groups/gm of fulvic acid. Stock solutions of FA were prepared freshly for every new set of experiments by dissolving known weights of FA crystals. The solutions were filtered with Whatman #42 filter papers to eliminate any residual sediment particles. Fulvic acid solutions were found to be unstable on standing (approximately 2 weeks) and under normal laboratory lighting conditions. Analytical reagent grade sodium bicarbonate used in the titrations was supplied by the Nichols Chemical Co. Ltd.. As reported previously, the reagent Calcein Blue was obtained from G. Frederick Smith Co. and used without further purification.

Stock solutions of aluminium fulvate at relatively high acidities

(pH 3.0) were prepared by reacting calculated volumes (that are necessary to establish the desired $[Al^{3+}]:[FA]$ ratios) of stock aluminium and fulvic acid solutions together and set to equilibrate for 24 hours. Sample solutions of aluminium fulvate of controlled $[Al^{3+}]:[FA]$ ratios at the desired pH levels were prepared by very slowly titrating the corresponding stock aluminium fulvate solutions with sodium bicarbonate. This procedure was to simulate the carbonate/bicarbonate buffering capacity present in a natural water system. The pH change was monitored by an Orion Research (model 701A) 3-decimals digital pH meter. After the titrations, the sample solutions were allowed 24 hours to reach an experimental pseudo equilibrium. The final pH of the sample solutions were rechecked after equilibration and found to be generally within ± 0.15 pH unit of the desired levels. The reagent solutions of Calcein Blue in 0.2 M of sodium acetate buffer at pH 5.0 were prepared as described in Section (C) earlier.

The total aluminium concentrations being analyzed were generally very low, therefore, it was possible to achieve both the reactant mixing and kinetic data acquisition via manual techniques. The method was described in details in Section (C) under the heading of PROCEDURE. Briefly, after the electronics were properly adjusted, the mixing was achieved by delivering, with a hypodermic needle, one of the reactants rapidly into a cuvet which contained the other reactant. Simultaneously the Hewlett-Packard, strip-chart recorder (model hp Moseley 7100B) which was coupled to the Perkin-Elmer spectrofluorimeter (model 2045) was switched on initiating data recording. The resulting kinetic spectra

were manually digitized and fed into the mainframe computer for nonlinear regression analysis. The number of data pairs in various runs ranged from 150 - 250. All the reported values in the RESULTS section are averages of at least five runs.

(iii) RESULTS

To establish the time duration required for the reaction systems of interest to reach experimental equilibria, (not generally 100% recovery of Al) the emission intensities were monitored at various time intervals after mixing, the results are tabulated in Table 14. Also included in Table 14 are emission intensities of reaction mixtures where the concentrations of fulvic acid were comparatively higher; the increases in recoverable aluminium are significant. The results of detailed kinetic analyses of various reaction mixtures of Al^{3+} /FA at three different pH levels are reported in Table 15. Graphical analyses or Guggenheim plots of an example of an experimental 2-component system (mixture of 2×10^{-5} M Al^{3+} / 2×10^{-5} M FA at pH 6.0/ 2×10^{-3} M CB) and an experimental 1-component system (mixture of 2×10^{-6} M Al^{3+} / 5×10^{-5} M FA at pH 5.0/ 2×10^{-4} M CB) are illustrated in figures 15 and 16 respectively.

(iv) DISCUSSION

It is evident from the results in Table 14 that the reaction systems will reach their experimental equilibria in 24 hours or less. Based on this observation, all the experimental equilibrium infinities of the reaction systems were prepared by equilibrating the mixtures over a period of 24 hours at room temperature. There is a general decrease

TABLE 14

TIME DEPENDENT EQUILIBRIUM RECOVERIES OF ALUMINIUM FROM ALUMINIUM/
FULVIC ACID MIXTURES.*

* The aluminium recoveries at various time intervals were calculated using a theoretical 100% aluminium recovery derived by substituting the reaction mixture in question with a reference solution of ionic aluminium at pH 3.0 identical in concentration to the total aluminium 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 14

[Al] _{TOT} x10 ⁵ M	[FA] _{TOT} x10 ⁶ M	pH	[CB] _{TOT} x10 ³ M	ALUMINIUM RECOVERIES IN			
				13 HOURS %	24 HOURS %	61 HOURS %	2 WEEKS %
2.0	-	5.0	2.0	87.25	86.28	80.89	
2.0	-	6.0	2.0	17.57	20.43	15.79	
2.0	6.67	5.0	2.0	87.17	90.43	87.80	
2.0	6.67	6.0	2.0	64.07	66.82	65.29	
0.20	-	5.0	0.20	33.75	35.69	34.27	
0.20	-	6.0	0.20	0.000	5.502	3.265	
0.20	0.667	5.0	0.20	12.50	14.83	14.57	
0.20	0.667	6.0	0.20	8.053	10.84	10.20	
2.0	20.0	5.0	2.0		65.42		60.10
2.0	20.0	6.0	2.0		56.52		49.74
0.20	2.00	5.0	0.20		38.95		36.77
0.20	2.00	6.0	0.20		40.00		36.45
0.20	-	8.0	0.20		0.000		

TABLE 15

KINETIC ANALYSES OF ALUMINIUM-FULVIC ACID MIXTURES WITH REAGENT CALCEIN BLUE AT pH=5.0 IN 0.1M ACETATE BUFFER.*

* A theoretical 100% aluminium recovery for each type of mixture was derived by substituting the reaction mixture in question with a reference solution of ionic aluminium at pH 3.0 identical in concentration to the total aluminium 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.

These are observed rate constants.

+ It includes all species that are observed kinetically in the present experimental time scale, i.e. Al-FA(I), Al-FA(II), and Al-FA(C).

TABLE 15

MIXTURE	[A1] _{TOTAL} x10 ⁶ M	[FA] _{TOTAL} x10 ⁵ M	pH OF MIXTURE	[CB] _{TOTAL} x10 ⁴ M	k _(A1) M ⁻¹ Sec ⁻¹
a	20.0	-	5.0	20.0	2.81x10 ²
b	20.0	2.0	5.0	20.0	-
c	20.0	5.0	5.0	20.0	-
d	20.0	2.0	6.0	20.0	-
e	20.0	5.0	6.0	20.0	-
f	2.00	-	5.0	2.00	6.24x10 ¹
g	2.00	0.10	6.0	2.00	-
h	2.00	0.20	6.0	2.00	-
i	2.00	0.50	6.0	2.00	-
j	2.00	2.0	6.0	2.00	-
k	2.00	5.0	6.0	2.00	-
l	2.00	5.0	5.0	2.00	-
m	2.00	0.20	8.0	2.00	-
n	2.00	2.0	8.0	2.00	-

$k_{(A1-FA(I))}^{\#}$ $\times 10^2 \text{Sec}^{-1}(\text{obs})$	$k_{(A1-FA(II))}^{\#}$ $\times 10^3 \text{Sec}^{-1}(\text{obs})$	$k_{(A1-FA(C))}$ $\text{Sec}^{-1}(\text{obs})$	EQUILIBRIUM (24 hours) RECOVERY %	TOTAL KINETIC ⁺ RECOVERY %
-	-	-	86.28	78.51
2.71	3.36	-	65.22	61.44
2.62	3.30	-	33.33	35.13
2.35	1.98	-	56.52	48.02
2.64	3.54	-	33.33	31.31
-	-	-	51.11	40.96
2.34	3.57	-	17.41	15.91
1.85	1.92	-	40.00	26.16
1.66	1.76	-	51.85	39.39
1.35	1.53	-	75.93	56.95
-	-	5.51×10^{-4}	37.06	35.07
-	-	7.77×10^{-4}	38.46	42.04
116.6	30.9	-	35.09	31.69
-	-	1.87×10^{-3}	29.83	20.29

FIGURE 15

GUGGENHEIM PLOT OF A TYPICAL 2-COMPONENT SYSTEM IN AN
ALUMINIUM/FULVIC ACID MIXTURE.

SYSTEM UNDER OBSERVATION IS:

$\{2 \times 10^{-5} \text{M } [\text{Al}^{3+}] / 2 \times 10^{-5} \text{M } [\text{FA}]\}$ mixture at $\text{pH}=6.0 + 2 \times 10^{-3} \text{M } [\text{CB}]$;

in 0.1M acetate buffer at $\text{pH}=5.0$.

FIGURE 15

E.I. - EMISSION INTENSITY

FIGURE 16

GUGGENHEIM PLOT OF A TYPICAL 1-COMPONENT SYSTEM IN AN
ALUMINIUM/FULVIC ACID MIXTURE.

SYSTEM UNDER OBSERVATION IS:

$\{2 \times 10^{-6} \text{ M } [\text{Al}^{3+}] / 5 \times 10^{-5} \text{ M } [\text{FA}^-]\}$ mixture at $\text{pH}=5.0 + 2 \times 10^{-4} \text{ M } [\text{CB}]$;

in 0.1M acetate buffer at $\text{pH}=5.0$.

FIGURE 16

E.I.- EMISSION INTENSITY

in the emission intensities after prolonged periods of equilibration; this is probably due to the instability of the aluminium-Calcein Blue complex on standing. As described earlier, hydrolysis of the aluminium ion beyond pH 4.5 is significant. This is reflected in the poor equilibrium recoveries of aluminium at pH 5.0 and 6.0 for both aluminium samples at 2×10^{-5} M and 2×10^{-6} M in the absence of fulvic acid. The percentage of aluminium recovery at equilibrium is the ratio between the equilibrium emission intensity of a sample and that of a reference identical in aluminium concentration to that of the sample but with the pH set at 3.0 in the absence of fulvic acid. It is evident that some of the hydrolysis products are relatively unreactive towards complexation by the reagent Calcein Blue. For a constant concentration of aluminium, i.e. either 2×10^{-5} M or 2×10^{-6} M, an increase in pH will increase the extent of hydrolysis as is reflected in the lower aluminium recoveries at pH 6.0. From the turbidity studies reported by Rubin and Hayden,⁵⁶ pH_p (the critical hydrogen ion concentration at which condensed phase just begins to appear) for 5.0×10^{-5} M of $Al(NO_3)_3$ was determined to be 4.95; therefore, there is good reason to believe that in the present circumstance, 2×10^{-5} M of $AlCl_3$ at pH 5.0 will experience minimal hydrolysis. Experimentally, this is demonstrated in the 86.28% of equilibrium aluminium recovery. With the concentration of $AlCl_3$ still at 2×10^{-5} M, the recovery drops drastically to 35.29% when the pH is raised to 6.0. When the molar concentration of $AlCl_3$ is reduced by one order of magnitude to 2×10^{-6} M, the recovery at pH 5.0 is 51.11%. This is a substantial reduction in comparison to 86.28% particularly in

light of what was observed as a general trend by Rubin and Hayden⁵⁶ in their studies that as the concentration of total aluminium decreases, pH_p increases proportionally; therefore, the hydrolysis of 2×10^{-6} M of total aluminium at pH 5.0 should then have been negligible. This apparent contradiction can be resolved if it is assumed that the hydrolysis products of 2×10^{-5} M and 2×10^{-6} M of total aluminium are different. Matijević et. al.^{100,101} in a series of studies on aluminium ion hydrolysis by the coagulation of hydrophobic sols (e.g. AgI/I^-) reported that the critical coagulation concentration (CCC) of the aluminium salt solution which is the minimum concentration above which coagulation of the sol is produced was 7.2×10^{-6} M of $Al(NO_3)_3$ at $4.0 < pH < 7.0$. A typical turbidity curve¹⁰⁰ reported by these authors showed a maximum at 10^{-5} M $Al(NO_3)_3$ where restabilization of the colloid accompanied by a reversal of the charge of the colloidal particles occurred. In the region to the right of the maximum, the sol stability was attributed to the adsorbed iodide ions; while in the region to the left of the maximum, the sol stability was thought to be due to the adsorption of counter-ions such as the polyhydroxo cation of aluminium. The polycation was identified as $Al_8(OH)_{20}^{4+}$ by these authors; a species which is more strongly adsorbed by the colloid than Al^{3+} because of the increases in both the ionic charge and the hydroxylation. In the present study, 2×10^{-5} M of total aluminium at $5.0 < pH < 6.0$ falls in the region to the left of the maximum indicating the presence of polycations such as $Al_8(OH)_{20}^{4+}$. On the contrary, 2×10^{-6} M of total aluminium at $5.0 < pH < 6.0$ falls in the region to the right of the maximum. Although Matijević

et. al. had not proposed a structure for the aluminium species in this region; it remains implicit in their findings that different aluminium species prevail on different sides of the turbidity maximum. The reason for the stronger resistance to complexation of this undetermined but definitely polymeric aluminium species present in low aluminium concentration solutions, $\sim 2 \times 10^{-6}$ M, is not known at the present.

In the presence of fulvic acid, the recoveries of aluminium varied. A simple calculation shows that a 6.67×10^{-6} M FA solution will have 2×10^{-5} equivalents/litre of salicylic acid binding sites and 3.32×10^{-5} equivalents/litre of total sites, providing a minimum aluminium binding capacity of 1:1 for 2×10^{-5} M of total aluminium. The subsequent aluminium recoveries with the introduction of equal equivalents of FA were substantially improved particularly when the pH was equal to 6.0. This observation suggests that fulvic acid does participate in the solubilization of aluminium that is previously unavailable for complexation; and when bound to aluminium in roughly stoichiometric equivalents, can be readily displaced by the reagent Calcein-Blue present in large excess. When the concentration of FA was tripled to 2×10^{-5} M, the aluminium recoveries actually decreased. This can best be ascribed to some retention of the metal by the FA ligands rendering them "nonlabile"⁷⁶. The evidence here seems to indicate that FA in moderate excess can form highly stable complexes that are not homogeneous in their bonding natures and that a fraction of these

complexes is inert to substitution by the present reagent CB, such species may be hydroxo-organo-aggregates. The contrary is observed for a total aluminium concentration of 2×10^{-6} M, an increase in the concentration of fulvic acid generally resulted in an increase in the equilibrium aluminium recovery. The nature of this reaction system is likely to be different from that of the higher aluminium concentration case discussed earlier. There are two possible factors that can give rise to the difference, (i) that the aluminium species involved are different, and (ii) that the conformations of FA at the two concentrations are different. The nature of the aluminium fulvates formed from various fulvic acid concentrations were studied kinetically and the results reported in Table 15. The concentrations of fulvic acid chosen spanned from 0.9 mg/litre to 45 mg/litre covering all reasonable fulvic acid concentrations in natural systems (~10 mg/litre).

The reaction rates of the aluminium ion at concentrations of 2×10^{-5} M and 2×10^{-6} M were investigated at both pH levels 5.0 and 6.0. From the equilibrium data, it was determined that the metal ion hydrolysed extensively at pH 6.0 such that only a small fraction of it was reactive and recoverable with the reagent CB. In the rate studies, the reactive fraction was found to be extremely slow reacting, on time scales of tens of minutes and hours. At pH 5.0, the aluminium ion reacts with CB at two different rates depending on the total aluminium concentration. For a 2×10^{-5} M aluminium solution, the second-order rate constant was $2.81 \times 10^2 \text{ M}^{-1} \text{ Sec}^{-1}$, not altogether unlike the

average of the second-order rate constants, $\sim 2.5 \times 10^2 \text{ M}^{-1} \text{Sec}^{-1}$, reported in Table 8. This reflects the fact that the prevailing species is very similar to that in the high acidity regions (pH 2.0 - 4.0), i.e. $\text{Al}(\text{H}_2\text{O})_6^{3+}$. It was suggested earlier in this discussion that based upon the results of Rubin and Hayden,⁵⁶ hydrolysis of the aluminium ion at pH 5.0 at low concentrations should be minimal. The present kinetic result seems to bear out this suggestion. The kinetic percentage of aluminium recovery is calculated in a very similar fashion as the equilibrium percentage of recovery. The total kinetic recovery was found to be 7.77% lower than the equilibrium recovery and without placing undue weight on this, it could be accounted for by a fraction of the aluminium ions which would react only very slowly. Experimentally, the second-order rate constant associated with the reaction between $2 \times 10^{-6} \text{ M}$ of total aluminium at pH 5.0 and $2 \times 10^{-4} \text{ M}$ of CB was determined to be $6.24 \times 10^1 \text{ M}^{-1} \text{Sec}^{-1}$; 4 - 5 times slower than its counterparts at higher acidities (Table 7). The finding here supports what was proposed earlier in the discussion that at $2 \times 10^{-6} \text{ M}$ of total aluminium, there are different aluminium species prevailing. From the kinetic data, it is apparent that the unidentified aluminium species is relatively homogeneous. The kinetic recovery is 10.15% lower than that at equilibrium, indicating the presence of an even slower reacting aluminium species than the one already mentioned.

In a mixture of aluminium and fulvic acid, there are generally two resolvable rate processes within an acceptable experimental time scale, excepting the cases when the molar concentration of EA is greater

than ten times that of aluminium. The discrepancy between any kinetic recovery and equilibrium recovery is attributed to a much slower reacting component, not observable within the experimental time scale presently chosen. It was concluded that since the fraction of the slow reacting aluminium component was generally small, it would have been impractical to monitor the small signal change over a long period of time in light of the limitation imposed by the electronics. The observed rate constants were independent of the reagent concentrations, therefore, it is concluded that the reactions between aluminium-fulvates and Calcein Blue are first-order, dependent upon the concentration of aluminium-fulvate only. Average rate constants of the two processes are $2.19 \times 10^{-2} \text{ Sec}^{-1}$ and $2.62 \times 10^{-3} \text{ Sec}^{-1}$ at 19.5°C respectively, separated by one order of magnitude.

In the discussion to follow, complex formation between ionic aluminium and fulvic acid is associated with certain types of reaction sites and bonding interactions. It is necessary to stress that these are only arbitrary assignments, not to be taken as a complete representation of the real picture. There is growing evidence (Weber and Saar,⁹⁶ Langford, Gamble, and Underdown¹⁰²) supporting the theory that interactions between metal ions and fulvic acid are very complex in nature and are best described in terms of their respective stability functions rather than the classical approach with stability constants. The assignments of kinetic components in the following discussion are strictly for the purpose of facilitating the identification of groups of reaction sites or bonding interactions which are kinetically

differentiable from each other. Within each assigned group, the aluminium-fulvic acid complexes are found to exhibit kinetic behaviour which collectively can best be reflected by the reaction site and/or bonding interaction assigned to describe them. The assigned reaction site and bonding interaction are chosen in such a way that maximum clarity and consistency is achieved. In the following proposed interpretation of the experimental results, much of the behaviour observed in the interactions between ionic aluminium and fulvic acid are explained in terms of the complex behaviour of fulvic acid in solution. Three features are particularly prominent in affecting the thermodynamic and kinetic characteristics of the aluminium-fulvate complexes, these are, (i) the diverse variety of possible binding sites in the fulvic acid molecules, (ii) a wide range of changing electrostatic effects, and (iii) the dynamic conformational changes of the fulvic acid molecules. For example, the degree of aggregation (potentially ternary in structure) of the FA molecules is influenced extensively by a great number of parameters such as the hydrogen ion concentration, both the metal and fulvic acid concentrations, and ionic strength of the medium. The properties cited above reflect strongly the polyelectrolytic and macromolecular characteristics of the fulvic acid molecules. It would be foolhardy to suggest that any one of these factors can singularly account for all that was observed experimentally, but instead, it must be emphasized that all these factors operate concurrently and very often in competing processes. To avoid being repetitive and laborious, there are instances during the course of this discussion where

only one of the characteristic properties or behaviour of FA is being singled out and used to describe the observation in question. Generally, the factor chosen to reflect the event being examined is one which is felt to best represent the prevalent thermodynamic and kinetic properties of the particular aluminium-fulvate complexes involved; and should not be confused as the sole element responsible for the observed phenomenon.

Consider the magnitude of the slower rate constant, $2.62 \times 10^{-3} \text{ Sec}^{-1}$ at 19.5°C , this is similar to the dissociation rate constant of the aluminium citrate complex that was determined earlier in this work, $4.76 \times 10^{-3} \text{ Sec}^{-1}$ at 19.5°C ; and that of aluminium salicylate reported by Secco and Venturini,⁵² $7.90 \times 10^{-3} \text{ Sec}^{-1}$ at 25°C . The rate limiting step was perceived to be the initial breaking of an Al-O bond which is likely to be the aluminium carboxyl oxygen in light of the two following points; (i) that an oxygen next to a carboxyl carbon centre is more acidic than a phenolic oxygen, and (ii) that if in any of the three instances an aluminium phenolic oxygen bond was broken, it would have been reflected in the dissociation rate constants; but instead, there is a remarkable agreement between the three cases. In a study of Fe^{3+} binding by fulvic acid, Langford and Khan⁷⁶ reported a formation rate constant of $1.1 \times 10^2 \text{ M}^{-1} \text{ Sec}^{-1}$ and estimated the dissociation rate constant to be $7.0 \times 10^{-3} \text{ Sec}^{-1}$ for the iron-fulvic acid complex at 25°C in a pH=1.65 medium. It was suggested that Fe^{3+} ions were incorporated into the salicylic acid type sites of the fulvic acid with the rate limiting step being the substitution of an inner sphere water molecule of the Fe^{3+} ion by the chelate donor group. On

the basis of the similarities between the dissociation rate constants cited above, it would not be overly presumptuous to associate the present aluminium incorporations with the simple salicylic acid type sites of the fulvic acid, presently designated as monosalicylate sites type II.

The faster dissociation process characterized by an average rate constant of $2.19 \times 10^{32} \text{ Sec}^{-1}$ was originally thought to be complex dissociations involving phthalic acid type sites on the fulvic acid. Available circumstantial evidence tends to favour such an assumption. It was shown earlier that carboxyl groups are prevalent in each gram of fulvic acid, 7.71 mequiv./gm FA. Amongst that, 3.30 mequiv./gm FA are used in the furnishing of the salicylic acid sites leaving 4.41 mequiv./gm FA for the formation of 2.21 mequiv./gm FA of phthalic acid type sites. The stability constant of an aluminium-phthalic acid complex is not available in the literature; however, generally speaking the stabilities of metal-phthalates are 2 - 3 orders of magnitude lower than their monooxalate analogues. The stability constant⁷⁷ of a monooxalate aluminium complex was 1.82×10^7 (at -18°C), approximately six orders of magnitude lower than the 1.29×10^{14} (at -26°C) reported for a monosalicylate aluminium complex. If the formation rate constants of the salicylate, oxalate, and phthalate complexes of aluminium are assumed to be comparable; then the dissociation rate constant of an aluminium phthalate complex is expected to be faster than that of the oxalate complex which in turn will be much faster than the salicylate complex. Presently, the dissociation rate constant in question is only one order, faster than that alleged to be associated with the salicylic acid type sites. In light of this, an alternative to the notion of phthalic acid type site may be considered. In the present work, the aluminium-fulvic

acid complexes were prepared in such a way that the ratio of milliequivalents of aluminium ion to theoretically available salicylic acid sites (3.30 mequiv./gm FA) from fulvic acid is never less than 1:1. Therefore, there is no reason why the faster dissociation rate constant here cannot be associated with a type of salicylic acid site which is just slightly different from the Type II ones described. A comparison of the stability constants⁷⁷ between a monosalicylato aluminium complex (1.29×10^{14} at -26°C), and a monosulphosalicylato aluminium complex (8.13×10^{12} or 1.58×10^{13} at 25°C) demonstrates that depending upon the neighbouring environments of the salicylic acid sites, metal-complex thermodynamic stabilities can differ by one to two orders of magnitude. Electron withdrawing functional groups in the immediate vicinity of the binding site can destabilize the chelate enough to produce a faster complex dissociation rate. A significant factor which can influence or regulate the binding power and subsequently the stability of the resulting complex is the intramolecular hydrogen bonding prevalent in FA. The effect of hydrogen bonding is invariably electron withdrawing from the phenolic and carboxyl functional groups; consequently destabilizing the salicylic acid type site. This could be the principle cause responsible for these faster dissociation rate constants. The implications here are that these less strongly binding salicylic sites are either very close to the hydrogen bonding centres or in direct competition with hydrogen bonding (thus sharing the phenolic and/or carboxyl functional group), and the Type II salicylic acid sites described earlier must be relatively free of the hydrogen bonding effects.

From here on, these faster salicylic acid type sites will be addressed as monosalicylato sites Type I.

Independent of the nature of the binding sites in question, the general rule which suggests that if the forward rate constants are assumed to be comparable, the complex with the slower dissociation rate constant is the more stable should still apply. Therefore, the complex Al-FA(II) in Table 15 is expected to be more stable than that designated as Al-FA(I). According to Gamble,⁹⁸ at the present experimental pH levels (5.0 and 6.0), all the Type I carboxyl groups are essentially ionized along with 80% of the Type A functional groups; a condition that is most conducive for metal ion chelations.

The kinetic analysis method determines not only the presence of two distinctive classes of aluminium-fulvates, but their respective concentrations as well. The sums of the concentrations of Al-FA(I) and Al-FA(II) from the kinetic analyses were expressed as fractions of the theoretical total recoveries of ionic aluminium at low pH; the percentages are reported under the heading "total kinetic recovery" in Table 15. In aluminium/fulvic acid mixtures at pH 6.0 (mixtures g - k) employing a constant concentration of total aluminium, i.e. 2×10^{-6} M, and varying concentrations of FA, from 10^{-6} M to 2×10^{-5} M; the overall kinetic recoveries of aluminium demonstrate systematic increases, 15.91 - 56.95%, with the corresponding fulvic acid concentration increments. When the FA concentration was increased to 5×10^{-5} M, the kinetic recovery of aluminium dropped off severely to 35.07%. This observation seems to indicate that the composite or resultant stability of the aluminium-

fulvates decreases with decreasing values of $[Al]_{total}/[FA]_{total}$; passes a minimum and then increases with further decreases of $[Al]_{total}/[FA]_{total}$. A similar observation was reported by Saar and Weber⁹⁶ in their complexation study of Cadmium (II) with water- and soil- derived fulvic acids. These authors reported that, at pH 6.0 (in the absence of any hydrolysis of Cd^{2+}), the behaviour of the cadmium fulvate stability constant as a function of $[Cd-FA]/[FA]_{total}$ displayed a minimum which is very similar to the one that is being observed in the present work. It was concluded that when $[FA]_{total} \leq 10^{-4}$ M, the stability constant of cadmium-fulvate exhibit a fulvic acid concentration dependence. Fulvic acid binding to cadmium was found to be strongest when FA was most dilute. As the concentration of fulvic acid rose, the authors surmised that there were accompanying conformational changes forceful enough to block off some potential cadmium ion binding sites and consequently reducing the stability of the cadmium-fulvate complex. The decrease in stability constant would continue until the increasing FA concentration reached a critical value (reflected as the minimum in the stability constant function) where from that point on, an ever increasing supply of sites were available on the fulvic acid for binding, thus masking the effects of intramolecular attractions that prevail before the critical FA concentration. The characteristics of such a stability constant function were attributed to the polyelectrolyte properties of the fulvic acid ligands.

There is a strong temptation to interpret the present experimental results in terms of the scheme proposed by Saar and Weber⁹⁶ due to the

resemblance between the data behaviour patterns of the two independent studies. The kinetic results of mixtures g - k in Table 15 can be understood as a variety of bonding processes active in the incorporation of aluminium by fulvic acid. According to their respective reaction rates with the reagent CB, the resultant aluminium-fulvate complexes in this work can be collectively classified into five major kinetically differentiable categories reflecting the nature of the prevalent binding sites. In conjunction with the results in Table 16 which shows the equilibrium distribution of aluminium species in the presence of fulvic acid as determined by kinetic and equilibrium analyses; possible bonding interactions for each of the five categories of binding sites will be explored.

From the percentage of equilibrium aluminium recoveries, it is quite obvious that there is persistently a fraction of the theoretically totally recoverable aluminium which is not recovered by the present reagent CB. According to the results in Table 14, at pH 6.0, the recovery of aluminium in the absence of fulvic acid by the reagent CB after 24 hours of equilibration was 5.50%. In the presence of fulvic acid (mixtures g - k), equilibrium aluminium recoveries increase systematically from 17.41 - 75.93% corresponding to [FA] increases from 10^{-6} M to 2×10^{-5} M. This demonstrates that the addition of FA will increase the solubilization of aluminium. In any aluminium-fulvic acid mixture above pH 4.50, there are two competitive processes occurring simultaneously. The first is the solubilization of aluminium by complexation with fulvic acid forming both labile and "nonlabile"

TABLE 16

EQUILIBRIUM DISTRIBUTION OF ALUMINIUM-FULVIC ACID COMPLEXES DETERMINED
BY KINETIC AND EQUILIBRIUM ANALYSES*

* A theoretical 100% aluminium recovery for each type of mixture was derived by substituting the reaction mixture in question with a reference solution of ionic aluminium at pH 3.0 identical in concentration to the total aluminium 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 16

MIXTURE	EQUILIBRIUM		KINETIC		
	UNRECOVERABLE	RECOVERABLE	RECOVERABLE	RECOVERABLE	RECOVERABLE
	NONLABILE	KINETICALLY	Al-FA(I)	Al-FA(II)	Al-FA(C)
	Al-FA(A) %	VERY SLOW Al-FA(B) %	%	%	%
b	34.78	3.780	29.50	31.94	
c	66.67	-1.800	14.46	20.67	
d	43.48	8.500	18.21	29.81	
e	66.67	2.020	12.70	18.61	
g	82.59	1.500	10.67	5.240	
h	60.00	13.84	12.51	13.65	
i	48.15	12.46	16.80	22.59	
j	24.07	18.98	16.10	40.85	
k	62.94	1.990	-	-	35.07
l	61.54	-3.580	-	-	42.04
m	64.91	3.400	11.79	19.90	
n	70.17	9.540	-	-	20.29

complexes. In the case of the latter, aluminium is retained by fulvic acid rendering them unavailable for complexation by the reagent CB. The other concurrent process is the hydrolysis of the aluminium ions which is driven very strongly thermodynamically, yielding unreactive aluminium hydroxides. This would also reduce the availability of aluminium to the reagent CB accordingly. In the present work, it is impossible to estimate the relative contribution from each of the two processes. Consequently, the exact nature of aluminium retention in majority of the Al/CB mixtures cannot be identified except to speculate that in mixtures where the ratio of concentration of aluminium to fulvic acid is high, the inert products of hydrolysis may prevail; and as the concentration of FA rises, dissolution of aluminium increases accordingly and so will the "nonlabile" fraction of the soluble aluminium-fulvate complexes as more and more binding sites become available. From here on, the "nonlabile" aluminium-fulvic acid complex and the unreactive aluminium hydroxides will collectively be labelled as Al-FA(A). In view of the inertness of the Al-FA(A) species towards substitution, very strong bonding involving substantial chelation by carboxyl groups in addition to the salicylic acid type chelation must be involved. Taking into account the flexibility of the fulvic acid molecule, it is not difficult to envisage the folding and twisting of the molecule into a conformation which will provide multiple chelating sites comprised of one salicylic acid type site plus n carboxyl sites resulting in chelating power comparable to that of the powerful EDTA ligand. In addition, after the incorporation of the aluminium ions, the molecule

can acquire ternary structures which can further entrap the aluminium ion. In such an environment, the competition between any bond rupture and re-formation in the aluminium-fulvate complex would be lop-sided, favouring the latter. Therefore, this strongest type aluminium-fulvate complex is not only thermodynamically stable, but kinetically nonlabile as well.

The second type of aluminium species in the presence of fulvic acid reported in Table 16 as Al-FA(B), is the fraction that makes up the balance between the kinetically recoverable aluminium and the equilibrium recoverable aluminium. This discrepancy is attributed to a very slow reacting (i.e. extremely small dissociation rate constant) aluminium-fulvate species that is not observable on the present experimental time scale. A likely bonding arrangement is one which is similar to that described for the species Al-FA(A) except that the number (m) of carboxyl groups participating is substantially reduced, i.e. $n > m$, plus that the present species may be situated on parts of the fulvic acid where they are more kinetically accessible and/or less kinetically stable.

The third and fourth classes of aluminium-fulvic acid complexes are those described respectively as Al-FA(I) and Al-FA(II) in Tables 15 and 16. The bonding arrangements are as described earlier, i.e. Al-FA(II) are formed primarily at sites described as monosalicylato Type II and Al-FA(I) at sites described as monosalicylato Type I.

A fifth type of aluminium-fulvate, Al-FA(C), is typified by those present in mixture k where $[Al]_{total}/[FA]_{total}$ is small. The complex is characterized by an average first-order dissociation rate

constant of $5.51 \times 10^{-4} \text{ Sec}^{-1}$. Since the dissociation rate constant is only approximately seven times slower than that of the aluminium-fulvates involving monosalicylato Type II sites, the suggestion of any bonding arrangement which includes possible contribution(s) from carboxyl group(s) may not be warranted. Instead, the higher stability/slower dissociation property of the present complex is more adequately described or reflected by introducing a third type of monosalicylato site labelled as Type III. It is perceived that, for reasons that may be electrostatic and/or conformational, these Type III monosalicylato sites are stronger binding than the regular Type II sites. The disappearance of the Type I sites, or rather, the deactivation of the Type I sites in an abundance of stronger binding sites is somewhat expected in view of its already minor role in mixtures g - j (Table 16).

The binding sites that appropriately characterize the five categories of complexes in Table 16 are, (i) Al-FA(A)/monosalicylato site + n carboxyl sites or "nonlabile" sites ($n \geq 2$), (ii) Al-FA(B)/monosalicylato site + m carboxyl sites or "intermediate" sites ($m < n$), (iii) Al-FA(I)/monosalicylato Type I site, (iv) Al-FA(II)/monosalicylato Type II site, and (v) Al-FA(C)/monosalicylato Type III site. Depending upon the fulvic acid concentration, the relative contribution from each of these five categories of binding sites are reflected in the equilibrium distribution of their corresponding aluminium-fulvic acid complexes. It must be emphasized that the classification scheme is a relatively straightforward interpretation of the results and that the five categories of binding sites introduced are probably not at all

homogeneous, it by no means excludes the possibility of other combinations and arrangements.

An inspection of Table 16 shows that as the concentration of FA increases (mixtures g - j), the concentrations of Al-FA(B), Al-FA(I), and Al-FA(II) increase accordingly while the concentration of Al-FA(A) decreases. The reader is reminded at this juncture that Al-FA(A) is a fraction of the total aluminium that is rendered "nonlabile" or inert by a combination of two processes; (i) hydrolysis of the aluminium ions leading to the formation of unreactive aluminium hydroxides, and (ii) strong retention of aluminium by fulvic acid in the form of a nonlabile complex. In terms of this proposed argument, the decrease in [Al-FA(A)] from 82.59% - 24.07% is not surprising since an increase in the solubilization of aluminium is expected with the corresponding increase in the fulvic acid concentration. Hydrolysis of the aluminium ion is anticipated to be particularly pronounced in mixtures such as g and h where the number of theoretical salicylic acid type sites do not exceed the number of aluminium ions by a large amount. The present feeling is that concurrent to the ever present retention of aluminium by fulvic acid (in the form of a nonlabile complex) which is dependent upon the complex behaviour of fulvic acid in solution, at low ratios of theoretical binding sites to the number of aluminium ions (typified in mixtures g and h), hydrolysis of the Al^{3+} ions is significant, therefore, a substantial portion of the nonlabile fraction Al-FA(A) is actually constituted of inert hydroxo polymers; and as the FA concentration increases, hydrolysis of Al^{3+} decreases, but simultaneously retention of aluminium by the presence of

excess fulvic acid binding sites also increases, this is typified in mixture k. The observed aluminium recovery pattern with various FA concentrations is thought to be the consequence of two concurrent processes. Maximum aluminium recovery by the reagent CB occurs at a point where the ratio between the quantity of aluminium solubilized by FA and that retained by the same concentration of FA is the greatest. It is with this in mind that we proceed on to interpret the results in Table 16.

The rapid increase in solubilization of aluminium (decrease in $[Al-FA(A)]$) going from mixture g to j is seen to result in increased participation of aluminium incorporation by the other three types of binding sites. In the first transition from mixture g to h, $[Al-FA(B)]$ jumped from 1.5% (effectively 0% within experimental error) to 13.84% while $[Al-FA(II)]$ increased moderately by comparison from 5.24% to 13.65%. This is contrary to what was observed in going from mixture h to j where there was relatively little change in $[Al-FA(B)]$ from 13.84% to 18.98%, but $[Al-FA(II)]$ rose steadily from 13.65% to 40.85%. This seems to suggest that when $[FA]$ is increased from 10^{-6} M to 2×10^{-6} M in the presence of 2×10^{-6} M of aluminium at pH 6.0 (mixture g+h), there is an accompanying molecular process which alters the electrostatic and/or conformational effect(s) of the FA molecules such that aluminium binding at the "intermediate" sites are initially favoured over the Type II monosalicylate sites. According to the results in Table 16, somewhere in the transition from mixture h to i where $[FA]$ is increased from 2×10^{-6} M to 5×10^{-6} M in the presence of 2×10^{-6} M of aluminium at pH 6.0;

there is a change in the nature of the molecular process which effects another change in the electrostatic and/or conformational influence(s) of the FA molecules in aluminium incorporation, the result is manifested in a shift which then favours aluminium binding via the Type II monosalicylato sites. There is relatively little change until the transition from mixture j to k where presumably, a very significant change occurs in the prevalent electrostatic/conformational effect(s) of the FA molecules resulting in virtually exclusive aluminium binding via the Type III monosalicylato sites. This is accompanied by an actual decrease in equilibrium aluminium recovery from 75.93% to 37.06% rather than continuing the trend of increased aluminium recovery with an increasing FA concentration which can be partially attributed to the increase in aluminium retention by a large surplus of binding sites in the fulvic acid molecules. In terms of classical treatment, the distribution of the various aluminium-fulvates is governed by two thermodynamic equilibria; the first is an internal equilibrium established between the different types of binding sites, and the second involves an equilibrium between the aluminium ions and the various types of binding sites. Both equilibria are expected to be strongly and constantly influenced by the specific electrostatic and/or conformational effect(s) of the FA molecules prevalent in each of the mixtures g - k.

In the present work, the kinetic behaviour of the labile fractions of each mixture of Al/FA were characterized according to their respective substitution rates by the reagent Calcein Blue.

Unfortunately, the fractions of Al-FA(B) were generally small and their corresponding reaction rate constants too small, making their determinations too uncertain. However, with the information determined for the other fractions, Al-FA(I), Al-FA(II), and Al-FA(C), a general trend emerges which is consistent with the present picture of aluminium-fulvic acid complexations established from the equilibrium species distribution data in Table 16. The significant decreases in both $k_{\text{Al-FA(I)}}$ and $k_{\text{Al-FA(II)}}$ observed in the first [FA] transition from 10^{-6}M to $2 \times 10^{-6}\text{M}$ coincide with the suspected change in the electrostatic/conformational effect(s) of the FA molecules initially proposed to account for the major surge in [Al-FA(B)] from 1.50% to 13.84%. Therefore, both the data from species distribution and their corresponding substitution rate constants reflect a substantial change in the electrostatic/conformational environment of the binding sites in the FA molecules and subsequently their corresponding aluminium complexes during the transition from mixture g to h. From Table 15, it can be seen that the magnitude of the changes in the rate constants $k_{\text{Al-FA(I)}}$ and $k_{\text{Al-FA(II)}}$ in going from mixture h to j is relatively small. Therefore, any suggestion of a departure from the prevalent conditions in mixture h during these [FA] transitions may not be valid. Instead, the more moderate decreases in the rate constants in going from mixture h to j can be interpreted as the consequence of minor variations in the prevalent electrostatic/conformational effect(s) of the FA molecules upon aluminium binding as [FA] rises. This observation is in good agreement with what was concluded earlier from the species distribution

data that there is no change in the prevalent factors in the transition from mixture h to j favouring aluminium binding via the Type II monosalicylato sites. Finally, in the transition from mixture j to k, the rate constants $k_{\text{Al-FA(I)}}$ and $k_{\text{Al-FA(II)}}$ disappear; instead, a single rate constant $k_{\text{Al-FA(C)}}$ that is considerably smaller than either $k_{\text{Al-FA(I)}}$ or $k_{\text{Al-FA(II)}}$ emerges. The significance of the behaviour exhibited here parallels the drastic reduction in equilibrium aluminium recovery that marked the transition from mixture j to k discussed earlier. In other words, all the available evidence supports the theory that there corresponds a molecular process that significantly alters the electrostatic/conformational environment of the binding sites in the fulvic acid molecules during a transition from mixture j to k. The species distribution in mixtures g - k and the substitution rate constants for the species Al-FA(I) and Al-FA(II) as a function of the fulvic acid concentrations are plotted and illustrated in figures 17(a) & (b), and 18.

Brief studies of Al/FA mixtures with the total aluminium concentrations set at $2 \times 10^{-5} \text{ M}$ were conducted at both pH 5.0 and pH 6.0; the results of mixtures b - e are as listed in Tables 15 and 16. There are insufficient information from these studies to establish anything conclusive, however, what is immediately apparent is that there is no clear difference between the dissociation rate constants determined at the two pH levels. A similar conclusion can be derived if the results of mixtures k and l in Table 15 are compared. It can be seen in Table 15

FIGURE 17(a)

EQUILIBRIUM PERCENTAGE CONCENTRATION OF THE SPECIES "Al-FA(A)" IN
MIXTURES WITH DIFFERENT [Al]/[FA] RATIOS.

MIXTURES UNDER OBSERVATION:

<u>MIXTURE</u>	<u>[Al]/[FA]</u>
g	$2 \times 10^{-6} \text{M} / 10^{-6} \text{M}$
h	$2 \times 10^{-6} \text{M} / 2 \times 10^{-6} \text{M}$
i	$2 \times 10^{-6} \text{M} / 5 \times 10^{-6} \text{M}$
j	$2 \times 10^{-6} \text{M} / 2 \times 10^{-5} \text{M}$
k	$2 \times 10^{-6} \text{M} / 5 \times 10^{-5} \text{M}$

Percentage Concentration of "Al-FA(A)" is calculated as:

$$\frac{[\text{Al-FA(A)}]}{[\text{Al}]_{\text{TOTAL}}} \times 100\%$$

FIGURE 17(a)

FIGURE 17(b)

EQUILIBRIUM PERCENTAGE CONCENTRATIONS OF THE SPECIES "Al-FA(B)",
 "Al-FA(I)", AND "Al-FA(II)" IN MIXTURES WITH DIFFERENT [Al]/[FA] RATIOS.

MIXTURES UNDER OBSERVATION:

<u>MIXTURE</u>	<u>[Al]/[FA]</u>
g	$2 \times 10^{-6} \text{M} / 10^{-6} \text{M}$
h	$2 \times 10^{-6} \text{M} / 2 \times 10^{-6} \text{M}$
i	$2 \times 10^{-6} \text{M} / 5 \times 10^{-6} \text{M}$
j	$2 \times 10^{-6} \text{M} / 2 \times 10^{-5} \text{M}$

Percentage Concentrations of "Al-FA(B)", "Al-FA(I)", and "Al-FA(II)"
 are calculated as:

$$\frac{[\text{Al-FA(B)}]}{[\text{Al}]_{\text{TOTAL}}} \times 100\% ; \frac{[\text{Al-FA(I)}]}{[\text{Al}]_{\text{TOTAL}}} \times 100\% ; \frac{[\text{Al-FA(II)}]}{[\text{Al}]_{\text{TOTAL}}} \times 100\%$$

- ——— Al-FA(B)
- - - - Al-FA(I)
- ★ Al-FA(II)

FIGURE 17 (b)

FIGURE 18

DISSOCIATION RATE CONSTANTS OF THE "A1-FA(I)" AND "A1-FA(II)" COMPLEXES
IN MIXTURES OF DIFFERENT [A1]/[FA] RATIOS.

MIXTURES UNDER OBSERVATION:

MIXTURE	[A1]/[FA]
g	$2 \times 10^{-6} \text{M} / 10^{-6} \text{M}$
h	$2 \times 10^{-6} \text{M} / 2 \times 10^{-6} \text{M}$
i	$2 \times 10^{-6} \text{M} / 5 \times 10^{-6} \text{M}$
j	$2 \times 10^{-6} \text{M} / 2 \times 10^{-5} \text{M}$

Ⓐ ● ————— $k_{\text{A1-FA(I)}}$

Ⓑ ● - - - - - $k_{\text{A1-FA(II)}}$

FIGURE 18

that in the absence of fulvic acid, the aluminium recovery by the present reagent CB for a $2 \times 10^{-5} \text{ M Al}^{3+}$ solution at pH=5.0 is very high at 86.27%. In the presence of $2 \times 10^{-5} \text{ M}$ of FA (mixture b), the percentage of aluminium recovery was 65.22%; and when the concentration of FA was increased to $5 \times 10^{-5} \text{ M}$ (mixture c), the recovery was reduced to 33.33%. In view of the fact that the extent of hydrolysis of $2 \times 10^{-5} \text{ M}$ of Al^{3+} at pH=5.0 is relatively small, the decrease in aluminium recovery in the presence of FA must be attributed almost totally to strong retention of aluminium by the fulvic acid molecules rendering the complex nonlabile to substitution by the present reagent CB. Experimentally, a solution of $2 \times 10^{-5} \text{ M Al}^{3+}$ at pH 6.0 was found to hydrolyse extensively forming inert hydroxo polymeric species, the equilibrium recovery by the reagent CB after 24 hours was 20.43% (Table 14). Therefore, the increase in aluminium recovery in the presence of fulvic acid (mixtures d - e) are attributed to increased solubilization of aluminium by fulvic acid.

From these results, it can be concluded that increased solubility of aluminium ions at near neutral acidities are effected by the presence of fulvic acid in the medium solubilizing the aluminium ions by forming intermediate to very strong complexes, some of which are considered to be substitution inert to the present reagent Calcein Blue. The data on aluminium-fulvates species distribution for the mixtures b - e reported in Table 16 are insufficient for the purpose of anything else but to demonstrate that the thermodynamically more stable Type II monosalicylato sites are favoured over the Type I monosalicylato sites.

Kinetically, the lack of variation between the dissociation rate constants $k_{\text{Al-FA(I)}}$ and $k_{\text{Al-FA(II)}}$ when $[\text{FA}]$ was increased from $2 \times 10^{-5} \text{M}$ to $5 \times 10^{-5} \text{M}$ is quite unlike what was observed between the mixtures j and k; this reflects the lack of electrostatic/conformational change(s) in the environment of the binding sites in the fulvic acid molecules in this series of mixtures. Since it has been established that a pH change from 5.0 to 6.0 has literally no effect upon the magnitudes of the dissociation rate constants $k_{\text{Al-FA(I)}}$ and $k_{\text{Al-FA(II)}}$, therefore, the lack of electrostatic/conformational change in fulvic acid for the mixtures b and c observed at pH 5.0 and the mixtures d and e observed at pH 6.0 must solely be attributed to the increased presence of aluminium from $2 \times 10^{-6} \text{M}$ in mixtures j and k to the present $2 \times 10^{-5} \text{M}$ in mixtures b - e. It may be conjectured that the conformation of the FA molecules is partially dependent upon the concentration of aluminium ions present in the mixture.

Finally, in mixtures m and n, the interactions between $2 \times 10^{-6} \text{M}$ of total aluminium and $2 \times 10^{-5} \text{M}$ of FA at pH 8.0 were investigated. A $2 \times 10^{-6} \text{M}$ solution of ionic aluminium titrated to pH 8.0 was found to be inert when reacted with the reagent CB. In the presence of fulvic acid, the pattern of the kinetic results is by and large consistent with that observed in mixtures h and k. The amalgamation of the two species Al-FA(I) and Al-FA(II) is visible corresponding to the $[\text{FA}]$ increase from $2 \times 10^{-6} \text{M}$ to $2 \times 10^{-5} \text{M}$. This affirms the presence of a significant electrostatic/conformational change of the FA molecules

analogous to that discussed earlier. Equilibrium recoveries (Table 15) for the mixtures m and n are 35.09% and 29.83% which compare favourably to those of mixtures h and k, 40.00% and 37.06%. The species distributions of aluminium fulvates determined (Table 16) for the mixtures m and n when compared to that for the mixtures h and k demonstrate some minor differences. A plausible explanation of the observed will become more apparent as this discussion proceeds further. The outstanding discrepancy is the overall labilization of the complexes Al-FA(I), Al-FA(II), and Al-FA(C) as reflected in the increases in their respective dissociation rate constants, $k_{\text{Al-FA(I)}}$, $k_{\text{Al-FA(II)}}$, and $k_{\text{Al-FA(C)}}$, corresponding to 8.97, 16.09, and 3.39 times that of the same mixtures determined at pH 6.0. The phenomena associated with FA at elevated pH levels such as 8.0 are that the degree of aggregations between FA molecules are substantially reduced along with the size(s) of the FA molecules due to the ruptures of the hydrogen bonds responsible for the cohesions of these structures. In addition, the two equivalence points established by Gamble⁹⁸ in the titrations of fulvic acid are ~5.0 and ~7.63, i.e., at pH 5.0, all the Type A acidic functional groups are ionized while Type B groups will not be all ionized until the pH is 7.63. It can be assumed that at pH 8.0, there would be additional proton ionizations from functional groups other than those already involved in complexations. Consequently, the state of ionization of the FA molecules in an Al/FA mixture at pH 8.0 cannot really be compared to that at pH 5.0. In the present experimental design, upon mixing with

the analytical reagent CB, the final pH is immediately re-established to 5.0. Such an increase in acidity will spontaneously reprotonate majority of the Type B functional groups and others leading to substantial rapid molecular rearrangements in view of the polyelectrolytic properties of the FA molecules. Although nothing conclusive can be derived from such a consideration, it is felt that the molecular rearrangements subsequent to the rapid reprotonations are principally responsible for the observed increases in the dissociation rate constants $k_{\text{Al-FA(I)}}$, $k_{\text{Al-FA(II)}}$, and $k_{\text{Al-FA(C)}}$; and the slightly different equilibrium aluminium-fulvate species distributions in the present mixtures m and n as compared to those in mixtures h and k.

(v) CONCLUSION

The present findings are consistent with the current theory that the stability of metal-fulvate can be accurately described only by the use of a stability function instead of the classical treatments. The advantages of kinetic analysis are best illustrated in the present study where the aluminium-fulvic acid complex was identified as being inhomogeneous and subsequently some of the components determined semi-directly. In mixtures simulating those occurring in nature, i.e., where the number of equivalents of binding sites on FA is present in excess of that of total aluminium, the overall conditional stability of the combined complexes is largely governed by the polyelectrolytic properties of the FA molecules

through various conformational interactions. It is perceived that the presence of different concentrations of total aluminium giving rise to various ratios of aluminium ion to theoretically available binding site(s) will affect the electrostatic effect/conformation of the FA molecules and subsequently the values of the stability constant function. Electrostatically and/or conformationally modified salicylic acid type sites provide the backbone for most or all of the bonding interactions, both inter- and intramolecular ones. The carboxyl groups are seen in an auxiliary role acting in conjunction with the principle salicylic acid type sites. In the solubilization and transportation of aluminium by fulvic acid, the labile fraction according to the present scheme, is determined by the concentration of fulvic acid present in the equilibrium mixture. A maximum aluminium recovery of 75.93% occurred when 18 mg/litre of fulvic acid was present in the equilibrium mixture. Comparing to the binding capacity of the present reagent Calcein Blue, the fulvic acid molecules are capable of forming very strong complexes which can be classified as substitution "nonlabile". Maximum recovery of aluminium occurs presumably at a point where the concentration of fulvic acid is such that solubilization or prevention of hydrolysis of aluminium ions is greatest, and simultaneously, retention of the metal ions in the form of a "nonlabile" fraction is minimum. Drastic pH changes will alter the lability of the reactive fractions. The binding of aluminium ions by fulvic acid is extremely complex, the assumption of one equivalent of available site to one equivalent of aluminium ion cannot be maintained since a substantial

fraction of the possible sites could be engaged in intramolecular activities. For sites which are available, a spectrum of sites with different characteristics are possible; equilibrium distribution of the various sites for a particular FA molecular conformation is probably thermodynamically (electrostatically) controlled.

BIBLIOGRAPHY

- (1) L.C. Michael, G.P. Woodward, and M. Shulman, Environ. Sci. Eng. Notes, 11, 2, 2 (1974)
- (2) K.W. Hanck, and J.W. Dillard, Anal. Chim. Acta, 89, 329 (1977)
- (3) T.A. O'Shea, and K.H. Mancy, Anal. Chem., 48, 1603 (1976)
- (4) C.H. Langford, Water Quality Parameters, ASTM STP 573, American Society For Testing and Materials, 414 (1977)
- (5) M.K.S. Mak, C.H. Langford, and I.R. Khan, J. Indian Chem. Soc., LIV, Jan., Feb., Mar., 51 (1977)
- (6) E.F. Caddin, J.E. Crooks, and A. Queen, J. of Physics E: Scientific Instruments, 6, 930 (1973)
- (7) A.P. Malvino, "Electronic Principles", 2nd. Ed., McGraw-Hill, (1979)
- (8) E.O. Brigham, "The Fast Fourier Transform", Prentice-Hall, Inc., (1974)
- (9) S.B. Hanna, and R.K. Hessley, Inorg. Nucl. Chem. Lett., 7, 83 (1971)
- (10) K.A. Conners, Anal. Chem., 48, 97 (1976)
- (11) K.A. Conners, Anal. Chem., 49, 1651 (1977)
- (12) T.S. Lee, and I.M. Kolthoff, Ann. N.Y. Acad. Sci., 53, 1093 (1951)
- (13) R.G. Garmon, and C.N. Reilley, Anal. Chem., 34, 600 (1962)
- (14) B.G. Willis, W.H. Woodruff, J.R. Frysinger, D.W. Margerum, and H.L. Pardue, Anal. Chem., 42, 1350 (1970)
- (15) G.M. Ridder, and D.W. Margerum, Anal. Chem., 49, 2090 (1977)
- (16) D.W. Marquardt, Chem. Eng. Prog., 55, 66 (1959)
- (17) D.W. Marquardt, R.G. Bennett, and E.J. Burnell, J. of Mol. Spect., 7, 269 (1961)

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;
; RDSTRNG < STRING ARRAY>
; ROUTINE READS A STRING FROM THE TELETYPE
; AND STORES IN MEMORY FOLLOWED BY A NULL
; AFTER THE STRING
;

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;
; .TITL RDSTR
; .ENT RDSTR
; .EXTD .CIN .STBT
; .EXTU
; .NREL
;

```

```

RDSTR: 1
JSR 6.CPYL ; GET STRING ADDRESS
LDA 2..STNG,3 ; CREATE A BYTE POINTER
MOVZL 2,2

;
; .NXT: JSR 6.CIN ; READ A CHARACTER INTO R0
; LDA 1,CR ; CARRIAGE RETURN
; SUB 0,1,SZR ; IS IT A CR
; JMP .OK ; NO!

;
; SUB 1,1 ; PREPARE A NULL
; MOVZ 2,0 ; WHERE IT GOES
; JSR 6.STBT ; SAVE IT AWAY
; FRET ; AND RETURN
;

```

```

; .OK: MOVZ 0,1 ; GET CHARACTER READY
; MOVZ 2,0 ; GET BYTE POINTER READY
; JSR 6.STBT ; AND STORE IT
; INC 0,2 ; INDEX TO NEXT CHARACTER
; JMP .NXT ; AND GO BACK AGAIN
;

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; .STNG= -167
;

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; .CR: 15
;

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; .END
;

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- (32) E.A. Guggenheim, *Phil. Mag.*, 2, 538 (1926)
- (33) R.G. Wilkins, "The Study of Kinetics and Mechanism of Reactions of Transition Metal Complexes", P.14, Allyn and Bacon, Inc., Boston, (1974)
- (34) *ibid.*, P.30, P.65
- (35) R.A. Passwater, "Guide to Fluorescence Literature", Vol. 1-3, IFI/Plenum, N.Y., (1967-1974)
- (36) G.G. Guilbault, "Fluorescence and Phosphorescence Spectrometry", An ACS Audio Course, (1973)
- (37) K. Morisige, *J. Inorg. Nucl. Chem.*, 40, 5, 843 (1978)
- (38) D.J. Hydes, and P.S. Liss, *Analyst*, 101, 1209, 922 (1976)
- (39) F. Will III, *Anal. Chem.*, 33, 1361 (1961)
- (40a) W.E. Ohnesorge, and A. Capotosto Jr., *J. Inorg. Nucl. Chem.*, 24, 829 (1962)
- (40b) W.E. Ohnesorge, *J. Inorg. Nucl. Chem.*, 29, 485 (1967)
- (41) A.K. Babko, A.I. Volkova, and T.E. Get'man, *Zh. Anal. Khim.*, 22, 7, 1004 (1967)
- (42) R.M. Dagnall, R. Smith, and T.S. West, *Talanta*, 13, 609 (1966)
- (43) D.H. Wilkins, *Talanta*, 4, 182 (1960)
- (44) J.H. Eggers, *Talanta*, 4, 38 (1960)
- (45) H.N. Elheimer, *Talanta*, 14, 97 (1967)
- (46) G.F. Kirkbright, and W.I. Stephen, *Anal. Chim. Acta.*, 27, 294 (1962)
- (47) R.V. Hems, G.F. Kirkbright, and T.S. West, *Anal. Chem.*, 42, 784 (1970)
- (48) N. Ishibashi, T. Imasaka, T. Ogawa, M. Maeda, and Y. Miyazoe, *Chem. Lett.*, 1315 (1974)

- (49) T. Imasaka, T. Ogawa, and N. Ishibashi, Bull. Chem. Soc. Japan, 49, 10, 2687 (1976)
- (50) D.A. Skoog, and D.M. West, "Fundamentals of Analytical Chemistry", 3rd. Ed., Holt Rinehart Winston, Inc., N.Y. (1976)
- (51) J.F. Aguila, Talanta, 14, 1195 (1967)
- (52) F. Secco, and M. Ventruini, Inorg. Chem., 14, 8, 1978 (1975)
- (53) B. Perlmutter-Hayman, and E. Tapuhi, Inorg. Chem., 16, 11, 2742 (1977)
- (54) A.I. Vogel, "Quantitative Inorganic Analysis", Longmans, Green and Co., London, P.516 (1971)
- (55) I.M. Kolthoff and E.B. Sandell, "Textbook of Quantitative Inorganic Analysis", 3rd. Ed., McMillan, P.525 (1953)
- (56) A.J. Rubin and P.L. Haden, "Studies on the Hydrolysis and Precipitation of Aluminium (III)", NTIS PB-241, (April, 1973)
- (57) C.R. Frink, and M. Peech, Inorg. Chem., 2, 473 (1963)
- (58) R.E. Mesmer, and C.F. Baes, Jr., Inorg. Chem., 10, 2290 (1971)
- (59) J. Inczedy, "Analytical Applications of Complex Equilibria", translation editor J. Tyson - Chechester : Ellis Horwood (1976)
- (60) J.E. Huheey, "Inorganic Chemistry, Principles of Structure and Reactivity", P. 73, Harper and Row, N.Y., (1972)
- (61) A.I. Cherkasov, Dokl. Akad. Nauk. SSSR, 118, 309 (1958)
C.A. 52, 8840c (1958)
- (62) T. Sawada, T. Shikamoto, and H. Kamada, Bull. Chem. Soc., Japan, 51, 6, 1736 (1978)
- (63) B. Behr, and H. Wend, Ber. Bunsenges. Phys. Chem., 66, 223 (1962)

- (64) C. Kalidas, W. Knoche, and D. Paradoulas, Ber. Bunsenges. Phys. Chem., 75, 106 (1971)
- (65) A. Bonsen, W. Knoche, W. Berger, K. Giese, and S. Petrucci, Ber. Bunsenges. Phys. Chem., 82, 678 (1978)
- (66) J. Miceli, and J. Stuhler, J. Am. Chem. Soc., 90, 6976 (1968)
- (67) M. Matusék, and H. Strehlow, Ber. Bunsenges. Phys. Chem., 73, 427 (1969)
- (68) C. Kuehn, and W. Knoche, Trans. Faraday Soc., 67, 2101 (1971)
- (69) M. Hiraishi, S. Harada, and T. Yasunaga, Bull. Chem. Soc., Japan, 51, 8, 2317 (1978)
- (70) D. Fiat, and R.E. Connick, J. Am. Chem. Soc., 90, 608 (1968)
- (71a) C.H. Langford, and T.R. Stengle, Annu. Rev. Phys. Chem., 19, 193 (1968)
- (71b) C.H. Langford, and T.R. Stengle, Coord. Chem. Rev., 2, 349 (1967)
- (72) R.M. Fuoss, J. Am. Chem. Soc., 80, 5059 (1958)
- (73a) E. Grunwald, and D-W Fong, J. Phys. Chem., 73, 3, 650 (1969)
- (73b) J.W. Akitt, N.N. Greenwood, and G.D. Lester, Chem. Comm., 988 (1969)
- (74) R.C. Turner, and W. Sulaiman, Can. J. Chem., 49, 1683 (1971)
- (75) K. Goto, and T. Yotsuyanagi, Nippon Kagaku Zasshi, 89, 49 (1968)
- (76) C.H. Langford, and T.R. Khan, Can. J. Chem., 53, 20, 2979 (1975)
- (77) L.G. Sillén, and A.E. Martell, "Stability Constants of Metal-Ion Complexes", The Chemical Society Special Publication, 17 (1964)
- (78) R.G. Wilkins, "The Study of Kinetics and Mechanism of Reactions of Transition Metal Complexes", P.217-218, Allyn and Bacon, Inc., Boston (1974), and references therein.

- (79) D.W. Rogers, D.A. Aikens, and C.N. Reilley, J. Phys. Chem., 66, 1582 (1962)
- (80a) J. Burgess, J.C.S. Dalton, 1061 (1972)
- (80b) J. Burgess, and R.H. Prince, J. Chem. Soc., 6061 (1965)
- (81) D.M.W. Buck, and P. Moore, J.C.S. Dalton, 409 (1975)
- (82) R.K. Pattnaik, and S. Pani, J. Indian Chem. Soc., 38, 7, 379 (1961)
- (83) R.C. Turner, Can. J. Chem., 47, 2521 (1969)
- (84) R.W. Smith, "Relations among Equilibrium and Non-Equilibrium Aqueous Species of Aluminium Hydroxy Complexes", Am. Chem. Soc., Advances in Chemistry Series, 106, P.250-279 (1971)
- (85) R.W. Smith, and J.D. Hem, "Effect of Aging on Aluminium Hydroxide Complexes in Dilute Aqueous Solutions", U.S. Geological Survey Water Supply Paper 1827-D (1972)
- (86) C.F. Baes, Jr., and R.E. Mesmer, "The Hydrolysis of Cations", Ch. 6, Wiley, N.Y., (1976)
- (87) R.C. Turner, Can. J. Chem., 12, 1910 (1976)
- (88) D.W. Waters, and M.S. Henty, J.C.S. Dalton, 243 (1977)
- (89) D.A. Riesgraf, and M.L. May, Applied Spectroscopy, 32, 4, 362 (1978)
- (90) C.S. Cronan, and C.L. Schofield, Science, 204, 304 (1979), and references therein.
- (91) N.M. Johnson, Science, 204, 497 (1979)
- (92a) M. Schnitzer, and S.U. Khan, editors, "Soil Organic Matter", Developements in Soil Science 8, Ch. 1, Elsevier, N.Y., (1978)

- (92b) ibid., P.47-53
- (93) C.H. Langford, and D.S. Gamble, Proceedings of the International Conference on Transport of Persistent Chemicals in Aquatic Ecosystems. II (37-39), Ottawa, Canada. (1974)
- (94) E.H. Hansen, and M. Schnitzer, Anal. Chim. Acta., 46, 247 (1969)
- (95) S.A. Wilson, and J.H. Weber, Chem. Geol., 19, 285 (1977)
- (96) R.A. Saar, and J.H. Weber, Can. J. Chem., 57, 1263 (1979), and references therein.
- (97) D.S. Gamble, Can. J. Chem., 48, 2662 (1970)
- (98) D.S. Gamble, Can. J. Chem., 50, 2680 (1972)
- (99) C.H. Langford, G-W Quance, R. Kay, and T.R. Khan, Anal. Letters, 10, 14, 1249 (1977)
- (100) E. Matijevic, K.G. Mathai, R.H. Ottewill, and M. Kerker, J. Phys. Chem., 65, 826 (1961)
- (101) E. Matijevic, E.F. Janauer, and M. Kerker, J. Colloid Science, 19, 333 (1964)
- (102) C.H. Langford, D.S. Gamble, and A. Underdown, (to be published)

Load Module can be initiated by typing "SFRUN" followed with a carriage return.

```

?SFLIST?
@PARM.SF
@COMMON.SF
      IRUN=0
      TYPE"FULL PROGRAM DESCRIPTION DESIRED?(Y OR N)"
      CALL RDSTRNG(IFLG3)
      IF(IFLG3.EQ."N")GO TO 3
      TYPE"      THIS PROGRAM INPUTS DATA FROM THE A/D CONVERTER"
      TYPE"AND PUTS IT INTO A FILE. THE USER IS GIVEN "
      TYPE"THE CHOICE OF TAKING THE READINGS AT REGULAR INTERVALS"
      TYPE"OR INCREASING THE LENGTH OF THE INTERVALS AS THE "
      TYPE"HALF-LIFE OF THE REACTION INCREASES.(CHECKING FOR A "
      TYPE"SECOND REACTION.) IF THE USER CHOOSES TO PROCESS"
      TYPE"A FILE, HE MAY CONVERT THE FILE TO THE GUGGENHEIM FORM,"
      TYPE"CALCULATES THE FIRST AND SECOND DERIVATIVES, OR PERFORM"
      TYPE"A LEAST SQUARES FIT ANALYSIS ON THE FILE.IN THE"
      TYPE"FIRST TWO CASES THE USER MAY OBTAIN A COPY OF THE"
      TYPE"OUTPUT FILE ON PAPER TAPE. THE USER MAY ALSO"
      TYPE"PRINT A COPY OF ANY DATA FILE ON PAPER TAPE."
      TYPE"      WHEN THE USER CHOOSES TO RECORD A RUN, HE"
      TYPE"WILL BE ASKED FOR THE TIME INTERVAL AND THE UNITS."
      TYPE"THE DESIRED UNITS SHOULD BE INPUT AS FOLLOWS:"
      TYPE"MILLISECONDS=1"
      TYPE"SECONDS=2"
      TYPE"MINUTES=3"
3     TYPE"INPUT OPTION:"
      TYPE"1)RECORD A RUN"
      TYPE"2)PROCESS A FILE"
      TYPE"3)RECORD A DATA FILE ON TAPE"
      TYPE"4)EXIT FROM PROGRAM"
14    ACCEPT"?",IOPI
      IF(IOPI.EQ.1)GO TO 1
      IF(IOPI.EQ.2)GO TO 4
      IF(IOPI.EQ.3)GO TO 13
      IF(IOPI.EQ.4)STOP
      GO TO-14
13    TYPE"INPUT FILENAME AND TURN ON PAPER TAPE PUNCH"
      CALL RDSTRNG(INAME(1))
      CALL OPEN(1,INAME,1,IER,123)
      READ BINARY(1) (ICOM(1),I=1,11)
      WRITE(12,100) (ICOM(1),I=1,11)
120   FORMAT(11I5)
      IPTS=ICOM(10)
      DO 12 I=1,IPTS
      READ BINARY(1) IX,IX
12    WRITE(12,101) IX,IX
131   FORMAT(2I6)
      TYPE"
      CALL CLOSE(1,IER)
      GO TO 3
1     IRUN=IRUN+1

```

- Load Module continues on next page.

```

CALL SAMINIT
CALL FILINIT
CALL COMMENT
ACCEPT"START VALUE ", ISTER
TYPE"INITIALIZATION COMPLETE. "
TYPE"PRESS START WHEN READY."
CALL STATE(ITER)
GO TO 31
CALL SFSTATE(CTEL)
CONTINUE
CALL LGHTSW(ON)
DO 2 I=1, IPTS
ITIM(I)=0
ICHAN=6
A LDA 0, ICHAN-200, 3
A INTDS
A DOAS 0, 53
A SKPEN 53
A JMP --1
A DICC 1, 53
A INTEN
A MOVL 1, 1
A MOVL 1, 1
A LDA 0, ISK
A AND 0, 1
A STA 1, IAD-200, 3
AMSK: 177774
ISF(I)=IAD
IF(IFLGR.EQ."Y")CALL TCHECK(IAD, IDEL)
IF(I.EQ.1)GO TO 7
ITIM(I)=ITIM(I-1)+IDEL
7 CALL WAIT(IDEL, IUNITS, IER)
IF(IER.NE.1)CALL MSGWR(10, "ERROR ON WAIT CAL", IER)
2 CONTINUE
DO 6 I=1, IPTS
6 WRITE BINARY(2) ISF(I), ITIM(I)
CALL CLOSE(2, IER)
CALL LGHTSW(OFF)
GO TO 3
4 TYPE"INPUT OPTION:"
TYPE"1) CONVERT TO GUGENHEIM FORM"
TYPE"2) CALCULATE DERIVATIVES"
TYPE"3) PERFORM LEAST SQUARE FIT"
11 ACCEPT"?", IOP2
IF(IOP2.EQ.1)GO TO 8
IF(IOP2.EQ.2)GO TO 9
IF(IOP2.EQ.3)GO TO 10
GO TO 11
8 CALL GUGG
GO TO 3
9 CALL BETTER
GO TO 3
12 CALL LSQFIT
GO TO 3
END

```

The running of the Load Module is self-explanatory step-by-step during execution.

```

; RDSTRNG < STRING ARRAY>
; ROUTINE READS A STRING FROM THE TELETYPE
; AND STORES IN MEMORY FOLLOWED BY A NULL
; AFTER THE STRING
;

```

```

.TITL RDSTR
.ENT RDSTR
.EXTD .CIN .STBT
.EXTU
.NREL

```

```

RDSTR: 1
JSR 6.CPYL ;GET STRING ADDRESS
LDA 2..STNG,3 ;CREATE A BYTE POINTER
MOVZL 2,2

.NXT: JSR 6.CIN ;READ A CHARACTER INTO R0
LDA 1,CR ;CARRIAGE RETURN
SUB 0,1,SZR ;IS IT A CR
JMP .OK ;NO!

SUB 1,1 ;PREPARE A NULL
MOVZ 2,0 ;WHERE IT GOES
JSR 6.STBT ;SAVE IT AWAY
FRET ;AND RETURN

.OK: MOVZ 0,1 ;GET CHARACTER READY
MOVZ 2,0 ;GET BYTE POINTER READY
JSR 6.STBT ;AND STORE IT
INC 0,2 ;INDEX TO NEXT CHARACTER
JMP .NXT ;AND GO BACK AGAIN

```

```

.STNG= -167

```

```

.CR: 15

```

```

.END

```

*PARAM.SF

SUBROUTINE TCHECK(IAD, IDEL1)

*COMMON.SF

RK = 0.269

IF(I.EQ.1)RETURN

TIM = FLOAT(ITIM(I))

SLOTH = IFIN*RK/EXP(RK*TIM)

SLOBS = FLOAT(ISF(I)-ISF(I-1))/FLOAT(ITIM(I)-ITIM(I-1))

IDEL1 = IDEL*SLOTH/SLOBS

RETURN

END

SUBROUTINE SAMINIT

*COMMON.SF

DIMENSION JDAY(3),JTIME(3)

CALL DATE(JDAY,IER)

CALL TIME(JTIME,IER)

DO 3 K=1,3

IDAY(K)=JDAY(K)

3 ITIME(K)=JTIME(K)

ACCEPT"INPUT TIME INTERVAL AND UNITS ",IDEL,IUNITS

ACCEPT"INPUT NO. OF DATA POINTS (.LT.500) ",IPTS

CALL MSGWR(10,"DO YOU WANT A CHECK FOR A SECOND

* REACTION?(Y OR N)",IER)

CALL RDSTRNG(IFLG1)

IFIN=3

IF(IFLG1.EQ."N")RETURN

ACCEPT"FINAL READING=",IFIN

RETURN

END

```
*PAR1.SF
SUBROUTINE FILINIT
COMMON,SF
CALL MSGWR(16,"FILENAME=",1ER)
CALL RDSRNG(IFNAM(1))
CALL CPILW(IFNAM(1),2,1EP)
CALL OPEN(2,IFNAM,3,1EP,128)
RETURN
END
```

```
*PARM.SF
SUBROUTINE COMMENT
COMMON,SF
WRITE BINARY(2) IDAY(1),IDAY(2),IDAY(3)
WRITE BINARY(2) ITIME(1),ITIME(2),ITIME(3)
WRITE BINARY(2) IRUN
WRITE BINARY(2) IDEL
WRITE BINARY(2) IUNITS
WRITE BINARY(2) IPTS
WRITE BINARY(2) IFIN
RETURN
END
```


C
C
COMMON FILE FOR STOP-FLOW DATA

INTEGER ITEMP(16), IFNAM(20), INAME(20), I2NAME(20)
COMMON ICON(12)
COMMON IDAY(3), ITIME(3)
COMMON I, IRUN, IDEL, IUNITS, IPTS, IFIN,
& ITIM(1000), ISF(1000), IFLG1, A

:
: <INTEGER> SFSTATE <DUMMY>
: RETURNS THE STATE OF DEVICE 42(BIT 12)
: STOP-FLOW START COMMAND
:

.NREL
.TITL SFSTATE
.ENT SFSTA
.EXTU
.NREL

SFSTA: JSR @.CPLY
SUB 0,0
DIA 0,42
LDA @1,SFMSK
AND 0,1,SZR
JMP -3
STA 0,0-167,3
JSR @.FRET
SFMSK: @00010
.END

```

; MSGWR <CHANNEL> <STRING>
;
; OUTPUTS A STRING.
;
; COMPUTEK LTD. (1976)

.NREL
.TITL MSGWR
.ENT MSGWR
.EXTD .LDBT
.EXTU
.NREL

.C4: 4

MSGWR: JSR 6.CPYL
LDA 0,.STNG,3 ; ADDRESS OF STRING
MOVZL 0,0 ; BYTE POINTER
SUBZ 1,1
STA 1,.CNT,3 ; ZERO COUNT
STA 1,.FLG,3 ; ZERO FLAG
;

.NXT: JSR 6.LDBT ; GET A CHARACTER
MOVZ# 1,1,SNR ; NULL?
JMP .DONE

ISZ .CNT,3 ; NO AND INCREMENT COUNT
LDA 2,.C15 ; CARRIAGE RETURN?
SUBZ# 2,1,SNR
JMP .DI
LNC 0,0 ; NO AND INCREMENT TO NEXT BYTE
JMP .NXT

.DI: STA 2,.FLG,3 ; SET CARRIAGE RETURN FLAG
.DONE: LDA 0,.STNG,3 ; GET ADDRESS
MOVZL 0,0 ; BYTE ADDRESS
LDA 2,.IOCA ; GET SYSTEM CHANNEL
LDA 1,0.CHAN,3
MOVZL 1,1
ADDZ 1,2
LDA 2,0,2
LDA 1,.CNT,3 ; GET COUNT

```

ASSEMBLER programme "MSGWR" continues on next page.

```

      .SYSTEM
      .WRS      77

      JMP      .+1
      LDA      0,.FLG,3           ; GET FLAG
      MOVZ     0,0,SNR
      JMP      .EXIT             ; NOT SET
      LDA      0,.LFA             ; PUT OUT LINE FEED
      MOVOL    0,0
      SUBZL    1,1

      .SYSTEM
      .WRS      77

      FRET
.EXIT: FRET

      .LFA:     .LF
      .LF:      12

      .CIS:     15
               .CHAN= -167
               .STNG= -166
               .CNT=  -165
               .FLG=  -164

      .END

```

R

The three subroutines, "GUGG", "BETTER", and "LSQFIT" are simple FORTRAN routines written to convert the experimental data into the Guggenheim form, the first and second differentials, and to perform least square analysis respectively; they are included in the Load Module "SFRUN" as data processing options. The source programmes would not be included here.

C<LSQFIT

(D2)

```
REAL X(300),Y(300)
REAL DX(300),DY(300)
REAL PLUS/'+',MINUS/'-',
READ (3,999) N,H
999 FORMAT (2G.0)
DO 10 I=1,N
  READ(105,3) Y(I),X(I)
  3 FORMAT (2F18.12)
10 CONTINUE
  SX=0
  SY=0
  SXX=0
  SXY=0
  SYY=0
  DO 20 I=1,N
    SX=SX+X(I)
    SY=SY+Y(I)
    SXX=SXX+X(I)*X(I)
    SXY=SXY+X(I)*Y(I)
    SYY=SYY+Y(I)*Y(I)
  20 CONTINUE
  SLOPE=(N*SXY-SX*SY)/(N*SXX-SX*SX)
  B=(SXX*SY-SX*SXY)/(N*SXX-SX*SX)
  D=SYY-2*SLOPE*SXY-2*B*SY+SLOPE**2*SXX+B*B*N+
  12*SLOPE*B*SX
  U=N-2
  STADEV=SQRT(ABS(D/U))
  XBAR=SX/N;YBAR=SY/N
  XX=0;XY=0;YY=0
  DO 30 I=1,N
    DX(I)=X(I)-XBAR
    DY(I)=Y(I)-YBAR
    XX=XX+DX(I)*DX(I)
    YY=YY+DY(I)*DY(I)
    XY=XY+DX(I)*DY(I)
  30 CONTINUE
  R=XY/SQRT(XX*YY)
  OUTPUT
  OUTPUT      ****PROGRAM OUTPUT****
  OUTPUT
  OUTPUT THE LINEAR EQUATION FOR THIS CURVE IS:
  OUTPUT
  SIGN=PLUS
  IF(B.LT.0) SIGN=MINUS;B1=ABS(B)
  PRINT 43,SLOPE,SIGN,B1
  43 FORMAT(10X,'Y=',1PG10.4,'X',X,A1,1PG10.4)
  OUTPUT
  OUTPUT
  PRINT 44,R
  44 FORMAT('THE CORRELATION COEFFICIENT IS',X,F9.4)
  OUTPUT
  PRINT 45,STADEV
  45 FORMAT('STANDARD DEVIATION IS:',X,1PG10.4)
  RC=-SLOPE
  PRINT 100,RC
  100 FORMAT('THE GUGGENHEIM RATE CONSTANT IS:',2X,F10.5)
  A=EXP(B)
  GOOD=SLOPE*X
  CONC=A/(1-EXP(GOOD))
  PRINT 200,CONC
  200 FORMAT('THE CONC. OF REACTANT IS:',2X,F10.5)
  STOP
  END
```

Remote "batched" NONLINEAR REGRESSION analysis

```
1.000 !JOB D1007650,MAK,7.
2.000 !LIMIT (CORE,40),(TIME,2)
3.000 !RUN (LMN,NONLINEAR,TSL)
4.000 !DATA
5.000 )D FROM TEST1 BY 0
6.000 #NOBS=700
7.000 )F=(26.0)
8.000 )R INTO Y,X1
9.000 A1=0.1
10.000 A2=0.1
11.000 A3=0.1
12.000 A4=0.1
13.000 #NUMBER OF ITERATIONS=1000
14.000 #SIGNIFICANCE LEVEL=0.999
15.000 )MODEL=(A1-A1/DEXP(A2*X1))+(A3-A3/DEXP(A4*X1))
16.000 )GAUSHAUS Y
17.000 A1=0.05
18.000 A2=0.25
19.000 A3=0.02
20.000 A4=0.3
21.000 )GAUSHAUS Y
22.000 A1=0.5
23.000 A2=0.08
24.000 A3=0.01
25.000 A4=1.7
26.000 )GAUSHAUS Y
27.000 )STOP
--EOF HIT AFTER 27.
*END
```

!BATCH FOOL

ID=1A4A SUBMITTED 10:00 JUL 12, '77
WAITING: 3 TO RUN

APPENDIX (C)

(C1)

```

C ROTATION
IMPLICIT DOUBLE PRECISION (A-H,O-Z)
DIMENSION X(1050),Y(1050),R(1050),DELTA(1050)
WRITE (4,2)
2 FORMAT ('FOR FORWARD ROTATION,TYPE IN "1" IMMEDIATELY',X
1'AFTER PROMPT;TYPE IN "0" FOR INVERSE ROTATION.')
READ (3,999) N1,MAX
999 FORMAT (2G.0)
IF (N1.EQ.0) GOTO 77
READ (105,100), (Y(I),X(I),I=1,MAX)
100 FORMAT (2G.0)
DO 10 J=1,MAX
DELTA(J)=Y(1)+(Y(MAX)-Y(1))*(J-1)/(MAX-1)
R(J)=Y(J)-DELTA(J)
WRITE (108,200) R(J),X(J)
200 FORMAT (2F18.12)
WRITE (6,300) DELTA(J)
300 FORMAT (F18.12)
10 CONTINUE
GOTO 78
77 READ (105,400), (Y(I1),X(I1),I1=1,MAX)
400 FORMAT (F18.12,36X,F18.12)
READ (5,500), (DELTA(J1),J1=1,MAX)
500 FORMAT (F18.12)
DO 20 K=1,MAX
R(K)=Y(K)+DELTA(K)
20 CONTINUE
WRITE (108,600), (R(K1),X(K1),K1=1,MAX)
600 FORMAT (2F18.12)
78 STOP
END

```

APPENDIX (C) includes all the programmes that are required for the implementation of the FFT digital filter.

"ROTATION" - The kinetic spectrum is rotated such that the first and last emission readings are both reduced to zeros. (FORWARD ROTATION)

"PREINFFT" - A simple programme to convert the number of observations in the rotated kinetic spectrum to the nearest larger number that is a multiple of 2, e.g. 400 observations will be converted into 512 which is equal to 2^9 . This is a condition dictated by the algorithm of the Fast Fourier Transform.

"FFTRANS" - Forward Fourier Transform of the output from "PREINFFT". This load module was created from the source routines "FOURTRANS" and "FOURTRANS1".

"INFFTRANS" - Inverse Fourier Transform of the output from "FFTRANS". The load module was created from the source programmes "INVFFTR" AND "INVFFTR1".

Continue Next Page

continue from page (C1).....

"ROTATION" - The output from "INFFTRANS" is inversely rotated such that the original kinetic spectrum is restored with the high frequency noise filtered. (INVERSE ROTATION)

```

PREINFFT
1.000  DIMENSION Y(1050),T(1050),D(1050)
2.000  READ (3,999) N1
3.000  999 FORMAT (G.0)
4.000  IF (N1.EQ.1) GOTO 77
5.000  READ (3,888) N
6.000  888 FORMAT (G.0)
7.000  READ (105,100), (Y(I),T(I),I=1,N)
8.000  100 FORMAT (2G.0)
9.000  DO 10 I1=1,11
10.000 K=2**I1
11.000 IF (N.LT.K) GOTO 15
12.000 10 CONTINUE
13.000 15 L=T(N)-T(N-1)
14.000 K1=K-N
15.000 DO 30 I2=1,K1
16.000 Y(N+I2)=0.0
18.000 T(N+I2)=T(N)+L*I2
19.000 30 CONTINUE
20.000 OUTPUT K,I1
22.000 WRITE (6,101), (Y(I3),T(I3),I3=1,K)
23.000 101 FORMAT (2F18.12)
24.000 GOTO 777
25.000 77 READ (3,666) N
26.000 666 FORMAT (G.0)
27.000 READ (105,111), (D(J),J=1,N)
28.000 111 FORMAT (F18.12)
29.000 DO 40 J1=1,11
30.000 M=2**J1
31.000 IF (N.EQ.M) GOTO 78
32.000 IF (N.LT.M) GOTO 16
33.000 40 CONTINUE
34.000 16 M1=M-N
35.000 DO 41 J2=1,M1
36.000 D(N+J2)=0.0
37.000 41 CONTINUE
38.000 78 WRITE (6,112), (D(J3),J3=1,M)
39.000 112 FORMAT (F18.12)
40.000 777 STOP
41.000 END
--EOF HIT AFTER 41.
*END

```

IC FOURTRANS

```

    DIMENSION IWK(10000),A(1050),B2(1050),
    1B2(1050),C(1050),C1(1050),T(1050)
    COMPLEX C,C1,GAMN
    EQUIVALENCE (A,C)
    WRITE (106,2)
    2 FORMAT ('FOR "HANNING",TYPE IN "1" IMMEDIATELY AFTER "X
    1'PROMPT; OTHERWISE, "0" FOR NO "HANNING", THE SECOND "X
    1'INPUT IS THE MAX. # OF DATA POINTS')
    READ (3,999) N1,MAX
    999 FORMAT (2G.0)
    N=MAX
    READ (105,100) (A(I),T(I),I=1,N)
    100 FORMAT (2G.0)
    CALL FFTR(A,GAMN,N,IWK)
    C(N/2+1)=GAMN
    IF (N1.EQ.0) GOTO 10
    A(N+1)=REAL(C(N/2+1))
    A(N+2)=AIMAG(C(N/2+1))
    A2(1)=(A(1)-A(3))/2
    A2(N/2+1)=(A(N+1)-A(N-1))/2
    B2(1)=0.0
    B2(N/2+1)=0.0
    DO 15 I1=1,N/2-1
    I2=I1*2
    A2(I1+1)=(-A(I2-1)+2*A(I2+1)-A(I2+3))/4
    B2(I1+1)=(-A(I2)+2*A(I2+2)-A(I2+4))/4
    15 CONTINUE
    DO 20 I3=1,N/2+1
    C1(I3)=CMPLX(A2(I3),B2(I3))
    20 CONTINUE
    WRITE (10,200) (C1(J1),T(J1),J1=1,N/2+1)
    200 FORMAT (3F18.12)
    GO TO 17
    10 WRITE (108,300) (C(L),T(L),L=1,N/2+1)
    300 FORMAT (3F18.12)
    17 STOP
    END

```

BATCH FOURTRANS1

TO CREATE ROMME CALL B6AR

THAN:

! LINK B6AR OVER FFTRANS; IMSL

MC FOURTRANS1

```

!JOB D1007650,MAK,7
!LIMIT (CORE,40),(TIME,2),(FI,1)
!SETUP(FILES,FFRDR2,IMSL,FFTP,IMSL,FFT2,IMSL,ZZZZ,IMSL)
!ASSIGN F:105,(DEVICE,SI)
!ASSIGN F:3,(DEVICE,LO)
!ASSIGN F:105,(FILE,DATA2),(IN),(SAVE)
!ASSIGN F:108,(FILE,DATA3),(OUT),(SAVE)
!ASSIGN F:10,(FILE,DATA3A),(OUT),(SAVE)
!ASSIGN M:SI,(FILE,FOURTRANS),(IN),(SAVE)
!ASSIGN M:BO,(FILE,BEAR),(OUT),(SAVE)
!FORTRAN LS,BO
!LOAD (EF,(BEAR),(FFTR,IMSL),(FFRDR2,IMSL),(FFTP,IMSL),;
! (FFT2,IMSL),(AAAA,IMSL),(UNSAT,(IMSL),(LMN,FFTRANS),;
! (PERM),(MAP)
!RUN (LMN,FFTRANS)

```



```

IC INVFTR
  DIMENSION A(1050),B(1050),C(1050),D(1050),IWK(10000),
  IT(1050)
  COMPLEX C
  WRITE (104,2)
  2 FORMAT ('TOTAL OF 4 INPUTS, 1) THE MAX. # OF POINTS, MAX
  1/ 2) THE POWER OF 2, L.G. 128-2**7, THEREFORE, THE END, 3) X
  1/ INPUT IS 7, 3) THE POINT WHERE FILTERING IS TO X
  1/ BEGIN, AND FINALLY, 4) TYPE IN "1" FOR NO FILTERING AND "X
  1/" 0" FOR FILTERING.')
  READ (3,999) MAX,M,N1,N2
  999 FORMAT (4E.0)
  READ (105,100) (A(I),B(I),T(I),I=1,MAX/2+1)
  100 FORMAT (3F18.12)
  IF (N2.EQ.1) GOTO 77
  DO 5 J=N1,MAX/2+1
    A(J)=0.0
    B(J)=0.0
  5 CONTINUE
  77 C(MAX/2+1)=CMPLX(A(MAX/2+1),B(MAX/2+1))
  DO 10 K=1,MAX/2
    C(K)=CMPLX(A(K),B(K))
    IF (K.EQ.1) GOTO 10
    L=MAX+2-K
    C(L)=CONJG(C(K))
  10 CONTINUE
  DO 20 K1=1,MAX
    C(K1)=CONJG(C(K1))
  20 CONTINUE
  CALL FFT(C,M,IWK)
  DO 15 L1=1,MAX
    C(L1)=CONJG(C(L1))/MAX
  15 CONTINUE
  CALL FFRDR2(C,M,IWK)
  DO 25 I1=1,MAX
    D(I1)=CABS(C(I1))
  25 CONTINUE
  DELTA=T(MAX/2+1)-T(MAX/2)
  DO 30 L2=MAX/2+2,MAX
    T(L2)=T(L2-1)+DELTA
  30 CONTINUE
  WRITE (108,200) (C(I2),D(I2),T(I2),I2=1,MAX)
  200 FORMAT (4F18.12)
  STOP
  END

```

BATH INVFTR1 TO CREATE RANING

CALL MONSTER

THEN:

! LYNX MONSTER OVER INFFTRANS; IMSL

```

IC INVFTR1
!JOB D1007650,MAX,7
!LIMIT (CORE,40),(TIME,2),(FI,1)
!SETUP (FILES=FFRDR2,IMSL,ZZZZ,IMSL)
!ASSIGN F:105,(DEVICE,SI)
!ASSIGN F:3,(DEVICE,LO)
!ASSIGN F:105,(FILE,DATA3),(IN),(SAVE)
!ASSIGN F:108,(FILE,DATA4),(OUT),(SAVE)
!ASSIGN M:SI,(FILE,INVFTR),(IN),(SAVE)
!ASSIGN M:BO,(FILE,MONSTER),(OUT),(SAVE)
!FORTRAN LS,BO
!LOAD (EF,(MONSTER),(FFT2,IMSL),(FFRDR2,IMSL)),;
! (UNSAT,(IMSL)),(LMN,INFFTRANS),(PERM),(MAP)
!RUN (LMN,INFFTRANS)

```

APPENDIX (D) - Guggenheim estimation of rate parameter values /

(Initial inputs for the final NONLINEAR REGRESSION analysis)

```

C- GUGGENHEIM
  IMPLICIT DOUBLE PRECISION (A-H,O-Z)
  DIMENSION Y(1050),X(1050),P(1050)
  READ (3,999) MAX,N
  999 FORMAT (2G.0)
  READ (105,100), (Y(I),X(I),I=1,MAX)
  100 FORMAT (2F18.12)
  K=MAX-N
  DO 10 J=1,K
    DY=Y(J+N)-Y(J)
    P(J)=DLOG(DY)
    WRITE (108,20) P(J),X(J)
  20 FORMAT (2F18.12)
  10 CONTINUE
  STOP
  END

```

"GUGGENHEIM" - A simple programme to convert a set of filtered or unfiltered data into the Guggenheim form for Linear Regression Analysis.

"LSQFIT" - Linear Regression Analysis of the output from "GUGGENHEIM".

C-LSQFIT

(D2)

```

REAL X(300),Y(300)
REAL DX(300),DY(300)
REAL PLUS/'+',MINUS/'-',
READ (3,999) N,H
999 FORMAT (2G.0)
DO 10 I=1,N
  READ(105,3) Y(I),X(I)
  3 FORMAT (2F18.12)
10 CONTINUE
  SX=0
  SY=0
  SXX=0
  SXY=0
  SYY=0
  DO 20 I=1,N
    SX=SX+X(I)
    SY=SY+Y(I)
    SXX=SXX+X(I)*X(I)
    SXY=SXY+X(I)*Y(I)
    SYY=SYY+Y(I)*Y(I)
  20 CONTINUE
  SLOPE=(N*SXY-SX*SY)/(N*SXX-SX*SX)
  B=(SXX*SY-SX*SXY)/(N*SXX-SX*SX)
  D=SYY-2*SLOPE*SXY-2*B*SY+SLOPE**2*SXX+B*B*N+
  12*SLOPE*B*SX
  U=N-2
  STADEV=SQRT(ABS(D/U))
  XBAR=SX/N;YBAR=SY/N
  XX=0;XY=0;YY=0
  DO 30 I=1,N
    DX(I)=X(I)-XBAR
    DY(I)=Y(I)-YBAR
    XX=XX+DX(I)*DX(I)
    YY=YY+DY(I)*DY(I)
    XY=XY+DX(I)*DY(I)
  30 CONTINUE
  R=XY/SQRT(XX*YY)
  OUTPUT
  OUTPUT      ****PROGRAM OUTPUT****
  OUTPUT
  OUTPUT THE LINEAR EQUATION FOR THIS CURVE IS:
  OUTPUT
  SIGN=PLUS
  IF(B.LT.0) SIGN=MINUS;B1=ABS(B)
  PRINT 43,SLOPE,SIGN,B1
  43 FORMAT(10X,'Y=',1PG10.4,'X',X,A1,1PG10.4)
  OUTPUT
  OUTPUT
  PRINT 44,R
  44 FORMAT('THE CORRELATION COEFFICIENT IS',X,F9.4)
  OUTPUT
  PRINT 45,STADEV
  45 FORMAT('STANDARD DEVIATION IS:',X,1PG10.4)
  RC=-SLOPE
  PRINT 100,RC
  100 FORMAT('THE GUGGENHEIM RATE CONSTANT IS:',2X,F10.5)
  A=EXP(B)
  GOOD=SLOPE*XH
  CONC=A/(1-EXP(GOOD))
  PRINT 200,CONC
  200 FORMAT('THE CONC. OF REACTANT IS:',2X,F10.5)
  STOP
  END

```

END

1 8 1 2 8 1

FIN