KINETIC STUDIES OF ANALYTICAL INTEREST USING BOTH CONVENTIONAL AND STOPPED-FLOW MIXING

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Submitted to the Faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the Degree of
DOCTOR OF PHILOSOPHY
December, 1983

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STOPPED-FLOW MIXING

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ACKNOWLEDGEMENT

I appreciate the helpful advice and guidance from my advisor, Dr. Mottola, through the past several years. When I failed to recognize "what do I do now", Dr. Mottola provided thoughtful insights from his experience to goad me forward. I remain indebted to Dr. Mottola for providing me with my first "real research experience" in the summer of 1977. The research itself (a real pleasure working with Dr. Ch-Michel Wolff) was rewarding but an unexpected tangent resulted in meeting my "peanut butter and jelly" girl, now Mrs. Jim Thompsen. Special regards are given to Dr. Gorin for The Picnic.

My committee members have each contributed to the final product - Dr. Moore gave me a good start in his challenging Inorganic Chemistry class, Dr. McGown has introduced me to fluorescence immunoassay, and Dr. Phillips helped to give me the "computer bug" during Software for Microcomputers. I thank the Chemistry Department at OSU for the support necessary to provide all of the services a graduate student requires to finish. The day-to-day work environment affects the quality of work one produces and my association with Monte, Shekar, Kazi, Dennis, Sudha, Ricky, Al I and II, and Matt has been a pleasure and provided useful criticism of my work.

I sincerely appreciate a father and mother who have encouraged me to be my best and have not allowed me to waste any God-given talents. Most of all I thank them for their love. My wife (a very dedicated and intelligent scientist herself) has been a vital part of this effort.

She has unreservedly and without complaint dedicated five years of her life for this moment. I am honored and deeply appreciative of her love.

Finally, I wish to dedicate this thesis to the Glory of God. The parents of a six year old boy were told that their son was a discipline problem and could not do well in school. I wonder where that boy would be now, 20 years later. My story changed when in 1963 I accepted Jesus Christ as my Savior and Lord. The accomplishments of my life are rightfully His - He paid the price and to Him I owe my life. Glory be to God forever.

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INTRODUCTION

Analytical chemistry is often introduced in beginning chemistry texts as the branch of chemistry dealing with "the identification of the composition, both qualitative and quantitative, of substances" (1). This definition is somewhat narrow and most closely describes the applied science. Research in analytical chemistry draws heavily from all branches of science because every analysis deals with organic and inorganic compounds and their physical and chemical properties. The underlying theme throughout this work is the application of kinetics to study reactions of analytical interest. Kinetics has not traditionally been emphasized in analytical chemistry but is becoming an essential tool to solve or understand complex problems plaguing the analyst. A careful kinetic study of a reaction may reveal useful chemical information (reaction orders, side reactions, possible mechanisms) that can be exploited to overcome limitations or to develop new analytical schemes. Also it is much easier to optimize reaction conditions based on a fundamental knowledge of a reaction mechanism than to use a purely empirical fit based on variation of all the reaction parameters.

On the other hand, kinetic methods offer, in some cases, unique advantages over equilibrium methods for determinations in chemical analysis. These advantages will in many cases make up for the necessity of measuring both an experimental parameter (absorbance, conductivity,

etc.) and time simultaneously. Kinetic methods, however, generally require stricter control of ionic strength and temperature. The first advantage is the reduced time necessary for analysis. It is not necessary to wait until the reaction reaches equilibrium to obtain a measurement of the analyte's concentration. A dramatic illustration of the time reduction is the determination of total protein in which the time per analysis was reduced from 30 minutes to 10 seconds (2). The second advantage is overcoming side reactions with the reagent which is not specific for the analyte. An equilibrium measurement will give the concentration of product formed from both the analyte and any interferring species present in the sample. If the interferring species reacts slower with the reagent than the analyte it is possible to extract the concentration of the analyte alone from a reaction profile. The concentration may be determined then without the need to physically separate the analyte from interferring substances. A third advantage is the possibility of simultaneous determination of two or more analytes in a single sample. If the analytes react at different rates with a common reagent it is possible to determine their concentrations, again without physical separation. Finally, a fourth advantage is the use of relative measurements compared to absolute measurements required in equilibrium methods. This is important especially in analyzing natural samples where the matrix can differ from sample to sample. If the background differs, an equilibrium technique requires the preparation of a separate blank for each sample. The kinetic method will not require this because only the change in signal is used and not its absolute value.

Kinetic methods of determination have in general been limited to reactions which are complete in more than about fifteen seconds. Faster

reactions would be almost complete by the time the reagent and sample were manually mixed and placed in the recording instrument. Therefore equilibrium methods of determination have been used with fast reactions. Recently the stopped-flow mixer (a method of fast mixing) has been exploited for kinetic determinations with indication reaction times of a few milliseconds. Rapid, simultaneous injection of a sample and reagent solution through a mixer and into an observation cell in less than 5 milliseconds is possible with stopped-flow mixing. After mixing the reaction is monitored by an appropriate method (spectrophotometric, electrochemical, etc.). The stopped-flow mixer can not only decrease mixing times dramatically but also has the added advantage of being readily adapted to automation. With a stopped-flow system samples can be aspirated and analyzed automatically by adding a sample turntable and a control device. This system retains the capability for studying the kinetics of fast reactions (the reason for its initial introduction) and can be used to develop methods of kinetic determination based on a knowledge of the chemistry of the reaction.

The output of the observation system for a stopped-flow mixer changes too quickly to be recorded on a conventional strip-chart recorder. Electronic means are required and originally only the oscilloscope was available to provide high speed signal acquisition. Permanent records were kept by photographing the oscilloscope screen, and further analysis was accomplished by hand digitizing the curve from the photograph. If a stopped-flow mixer is to be truly useful as a sample processor, as mentioned above, then the analysis of a reaction should be available on the same time scale as the reaction itself. The ideal stopped-flow system would include both fast data acquisition and

analysis. This is possible at a reasonable cost by employing a digital data acquisition system linked to a microcomputer for fast analysis of the data collected. The presence of a computer in the system could also be beneficial in controlling the functions of the separate parts of an automated system.

Our research is an application of kinetic techniques to the study of analytically important reactions. Two reactions of interest have been addressed as follows: 1) the complexation of Fe(II) by Ferrozine was studied and 2) the reported catalytic action of aldehydes on the oxidation of p-phenylenediamine was studied. A digital data acquisition system was also developed to implement the use of a stopped-flow mixer in the study of the Fe(II)-Ferrozine complexation.

Ferrozine was introduced in 1970 (3) as a reagent for the determination of iron in aqueous solutions. It is well suited for this application because its water solubility and molar absorptivity are higher than those of other popular iron reagents. Ferrozine, as a reagent for iron, has been used in a variety of samples including blood serum (4-6), lake and tap water (7), and plant nutrient solutions (8). Both batch and continuous-flow sample processing have been used in these applications. Equilibrium studies and some properties of the iron (II)-Ferrozine complex have been reported since the ligand's introduction (3,9). These studies have shown that the complex exists in a 3:1 ratio of Ferrozine to iron (3) and that it is stable in the pH range of 3 to 6 (9), although no reason has been advanced for this. A value of 27,900 $M^{-1}cm^{-1}$, at λ_{max} = 562 nm, has been reported for the molar absorptivity of the complex (3), which compares favorably in terms of sensitivity with other complexes commonly used for iron determination

e.g. 1,10-phenanthroline (11,100 $M^{-1}cm^{-1}$ at λ_{max} = 510 nm (3)) and 2,2'-bipyridine (8,650 $M^{-1}cm^{-1}$ at λ_{max} = 522 nm (10)) .

No report has been found describing the kinetics of this complexation reaction. A study of the complexation was undertaken because besides the basic interest in this, the rate information is of theoretical relevance in situations of continuous-flow sample processing such as flow injection analysis (11-13) and is an aid in the development of reaction rate determinations. With this relevance in mind, kinetic information was obtained with the help of a stopped-flow mixing device and a microcomputer-based data acquisition system for the Fe(II)-Ferrozine complexation reaction and is reported here. Briefly the complexation was found to be first order in Fe(II) and third order in Ferrozine. The pH dependence has been attributed to the reactivity of only the unprotonated form of the ligand. As such the pK_a was determined independently of the rate method using a potentiometric titration. The results of the study are compared with those found in the literature for 1,10-phenanthroline and 2,2'-bipyridine.

A flexible, low-cost and high-speed method was desired for both acquiring and analyzing data from the stopped-flow system. For this purpose a microcomputer-based data acquisition system was constructed (14). The actual hardware for an analog to digital converter and link to an Apple II Plus microcomputer were designed and implemented. The graphics capabilities and disk storage system of the computer were utilized in analyzing reaction profiles.

Our interest in studying the effect of glutaraldehyde on the oxidation of p-phenylenediamine by H_2O_2 was stimulated by a publication in which O. D. Shapilov (15) described a catalytic method for

determining aldehyde groups on molecules covalently bonded to a glass surface. An analytical technique of this type is of particular interest to chemists who desire to immobilize specific molecules onto a support surface. A commonly used immobilization process (16) begins by treatment of the glass surface with 3-amino-propyltriethoxysilane and then attaching glutaraldehyde to the amino group. The compound to be immobilized is then coupled through the aldehyde group now attached to the glass surface. Shapilov's method provides a means to determine the coverage obtained by the first two steps in this process. Since researchers in our laboratory were currently involved in such immobilizations (attaching enzymes to coils for continuous flow analysis) attempts were made to utilize Shapilov's method. A flow method was designed to pass hydrogen peroxide through a coil known to contain attached aldehyde groups and then inject a plug of p-phenylene-diamine which would subsequently be catalyzed by the aldehyde in the presence of H_2O_2 to form a brown compound. The situation was regarded as similar to passing a substrate through a coil of immobilized enzyme and then monitoring the reaction products to determine the extent of reaction. In this case the presence of more aldehyde should catalyze more p-phenylenediamine and less aldehyde would produce less reaction. This setup was made and it was found upon repeated injection of p-phenylenediamine that less reaction occurred after each injection using the same coil. This was not expected as the catalyst aldehyde should produce the same results each time. The study described in this work was motivated by the failure of Shapilov's method in the determination of aldehyde bound to glass reactors using continuous flow analysis. Our aim was to elucidate the mechanism of this reaction

enabling us to determine why the reported catalytic method did not apply to the situation of continuous flow analysis. Details of the "catalytic" effect in homogeneous solution were needed before consideration of the heterogeneous situation is attempted. Searching for those details constituted part of the work reported here.

Our studies involved conducting the oxidation at a constant ionic strength and varying each of the reactants to determine further kinetic information about the reaction. Of paramount interest is the discovery that the action of aldehydes is not catalytic but instead constitutes a case of promotion. A promoter (17,18) accelerates a reaction, similar to a catalyst, but is destroyed during the course of the reaction. The promoter is not regenerated as is a true catalyst. Other findings include the experimental orders of the reactants, and the effect of pH in changing the amount of unprotonated p-phenylenediamine present (the reactive species). The rate equation developed was tested by numerically fitting the rate equations for each of the reaction species in both the catalyzed and uncatalyzed reactions. A good fit is obtained for the curves and a mechanistic interpretation of the effect is offered.

CHAPTER II

A LITERATURE REVIEW OF ANALYTICAL APPLICATIONS OF STOPPED-FLOW MIXING

The technique of stopped-flow mixing evolved out of a need to characterize reactions occurring so rapidly that manual methods of study were inadequate. For several decades this technique was applied almost exclusively to the study of fast reactions of biological interest. Only recently several qualities of the technique have been exploited to the advantage of the analytical chemist. These qualities include low reagent consumption per reaction, fast and efficient mixing, and its suitability for automated techniques of sample handling. Because of these advantages, as well as the capability to handle fast kinetics, a review of the analytical applications of the stopped-flow mixing device and its development is of interest.

This review includes six basic instrumental developments, the determination of over thirty species by stopped-flow, and five kinetic studies of analytical importance, published since 1960.

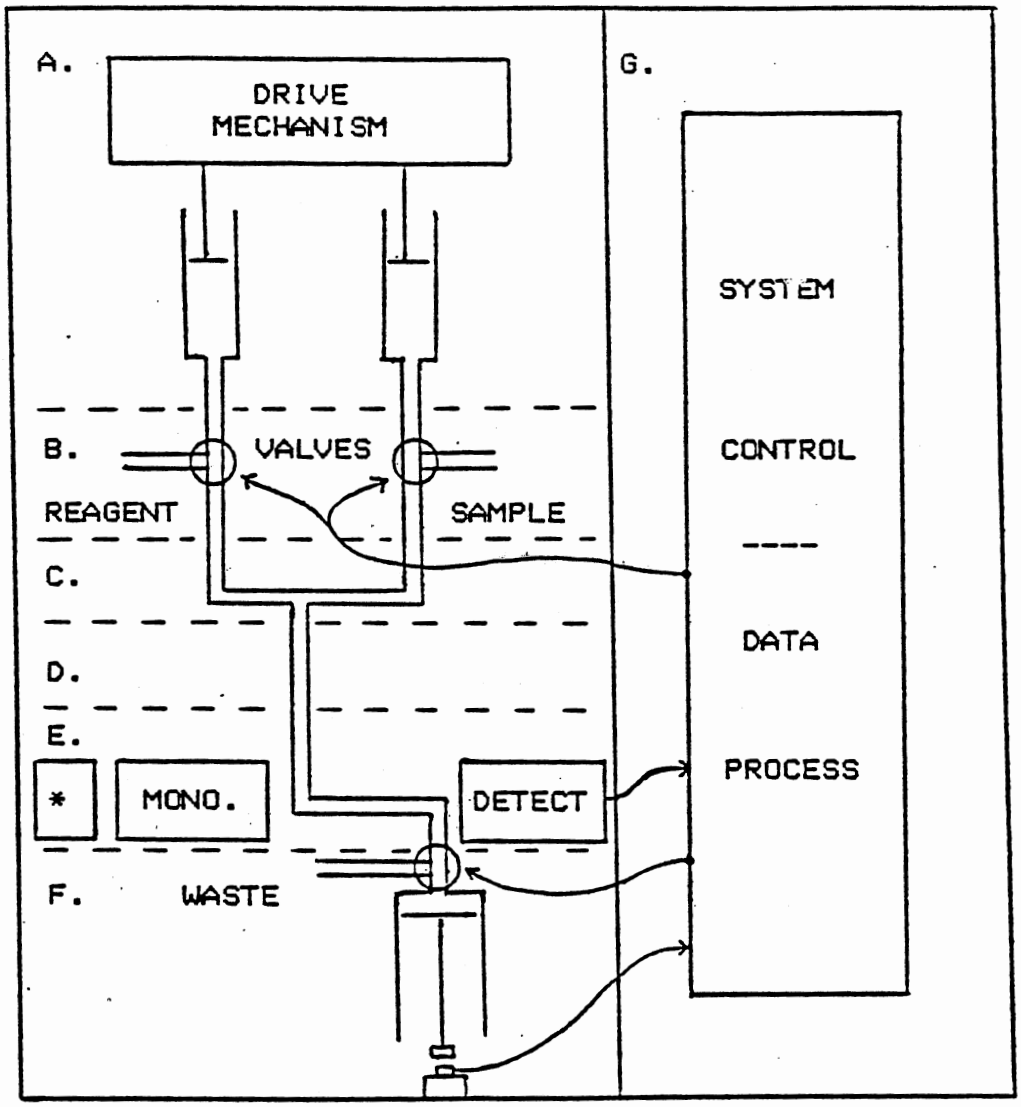
2.1 Development of the Stopped-Flow Instrument

The stopped-flow technique consists of rapidly mixing two solutions containing reacting species and then abruptly stopping their movement to allow the reaction to be monitored. The instrument which implements the stopped-flow technique consists of several parts. These are illustrated

in Figure 1. In this section the developments of the stopped-flow instrument will be traced, placing special emphasis on the importance of the improvements to the analytical chemist. Table I outlines the development of each part of the stopped-flow instrument designed for chemical analysis.

The first use of the stopped-flow technique in analytical chemistry was the kinetic study of the formation of 12-molybdophosphoric acid in 1968 by Malmstadt (19). The instrument which Malmstadt used in his work consisted of the basic stopped-flow components which include two drive syringes, a mixer, an observation cell, and a stopping syringe. The method of driving the syringes consisted of an offset cam on a motor. He used an oscilloscope to acquire the data and a camera to record the traces. This first system was a redesigned version of an automatic refill pipet which Malmstadt and Pardue had reported in 1962 (20). Malmstadt designed the stopped-flow unit (19) so that he could monitor the reaction between Mo(VI) and phosphate because it is too fast for conventional manual mixing techniques.

Malmstadt improved his original instrument in 1969 and reported one of the first analytical determinations performed by the stopped-flow technique (21). The new instrument incorporated many of the components necessary to decrease the processing time of samples with a stopped-flow instrument. The method of obtaining a signal on an oscilloscope, photographing it, and then hand digitizing the trace was too slow. In place of this Malmstadt employed a digital rate meter (22) to decrease the data processing time. The meter integrated the signal from the detector for a fixed period of time. The integrated value obtained was proportional to the concentration of the reacting species, assuming that the



BLOCK	FUNCTION
A	push syringes to force solutions together
B	deliver sample and reagent to S-F
C	mix reagent and sample
D	transport mixed solution to cell
E	optical system to monitor reaction progress
F	stop solution flow before monitoring reaction
G	control of S-F, acquire and process data

Figure 1. Block Diagram of the Stopped-Flow Instrument

TABLE I

DEVELOPMENT OF THE SEPARATE PARTS
IN THE STOPPED-FLOW INSTRUMENT

A. DRIVE MECHANISM
   1. Manual Push
   2. Offset Cam on a Motor
   3. Air Piston Drive

B. SAMPLE AND REAGENT INTRODUCTION
   1. Manual Sample Change and Valves
   2. Sample Turntable
   3. Automated Valves
   4. Automated Aliquoting of Solutions

C. MIXER
   1. T Mixer
   2. Tangential Mixer
   3. XY Mixer

D. TRANSPORT TO OBSERVATION CELL
   1. Fixed Distance, minimum length
   2. Variable Length Storage Coil

E. OPTICAL SYSTEM
   1. Single Beam
   2. Stabilized Optics
      a. Reference Feedback to Lamp Supply
      b. Monitor Reference PMT

F. SYSTEM CONTROL AND DATA ACQUISITION
   1. Oscilloscope and Photographs
   2. Digital Rate Meter
   3. Digital Sequencing of Valves
   4. Minicomputer Data System
   5. Whole System Control by Minicomputer
   6. Microcomputer Control and Data Handling

reaction is pseudo first-order in this component. Another innovation in this design is the sample turntable which shortens the time needed to analyze several samples. Even though many parts of the instrument were mechanized, most still required operator intervention to control them. For example, in one determination a valve must be opened to empty the stopping syringe, the sample turntable must be advanced, the rate-meter must be zeroed, and the syringe drive motor must be put back in its starting position. Malmstadt's instrument does, however, represent a dramatic improvement in stopped-flow instrumentation by combining fast signal processing and semi-automation.

The introduction of a computer for data acquisition and processing is credited to two biochemists, Desa and Gibson (23), in 1969. Pardue described a similar system (24) a year later which emphasized its application to quantitative kinetic analysis. His instrument did not use a cam to push the syringes, but instead used an air driven piston. A solenoid valve under computer control operated this air piston. The pneumatic drive became a standard feature in all subsequent instruments because of its speed, simplicity, and ease of automation. The computer used by Pardue was an HP2115A minicomputer. While the computer was utilized for automating the syringe drive, its primary function was to acquire and process data. Most of the other functions of the stopped-flow system were manually performed by the operator. Pardue used an optically stabilized spectrometer (25) in this instrument. The light from the source was split and ten percent went to a reference photomultiplier tube (PMT) while 90 percent passed through the cell of the stopped-flow to the sample PMT. The output of the reference PMT was fed back to a programmed power supply for the lamp. This kept the light

intensity stable for several hours and eliminated the need to readjust the 100 percent transmittance setting between every sample.

In 1972 Crouch (26) introduced a stopped-flow instrument which further automated its functions. Rather than use a minicomputer, the system used digital sequencing to control the pneumatic drive, the valve for introducing reagent and samples, and the waste valve. A digital sequencer uses a clock and digital flip-flops to turn valves on or off at preset times. This approach was less expensive than using a minicomputer, but the data processing ability was lost. Crouch used the digital rate-meter in his work to avoid the time consuming process of photographing oscilloscope traces.

The first instrument which could be described as fully automated was introduced in 1975 by Malmstadt (27). The instrument incorporated digital sequencing of all the valves and rotation of the sample turntable. This sequencer was in turn controlled by a PDP-8/f minicomputer which also collected and processed the data from the stopped-flow unit. The spectrometer employed in the system used a beam splitter to obtain a reference signal which was used with the sample signal to minimize the effects of light source drift.

The stopped-flow mixing module at this time was completely automated. In order to decrease the time required to analyze a sample further, it was necessary to automate the sample preparation steps. Malmstadt (28) introduced an instrument in 1977 which decreased the amount of time required for the sample preparation steps. His system employed a sample turntable with a weight sensor so that samples or standards could be delivered and then diluted accurately. With the instrument calibration curves could be programmed and performed

automatically, as well as analyzing samples and diluting them if necessary. The instrument also used a programmable monochromator so that the wavelength for different analyses could be changed under computer control. At this time the microprocessor was introduced (28,29,30), but only as a peripheral device for the minicomputer. The power of this inexpensive device was used to replace many of the functions of the digital sequencer.

In 1980 Malmstadt (31,32) introduced two stopped-flow systems which can be considered state of the art. The first instrument (31) was very similar to his 1977 model but incorporated only a low cost microcomputer and eliminated the minicomputer completely. The stopped-flow module, as well as the data acquisition system, was completely automated through its dedicated microcomputer. The main advantages of this instrument over its predecessors were its simplicity, low cost, and small size. It maintained the high accuracy, precision, and speed of the minicomputer system. The second instrument (32) which Malmstadt designed contained the only significant alteration to the basic design of the stopped-flow mixer. In all previous designs the path from the mixer to the observation cell was made as short as possible so that once a reaction began in the mixer it would not take much time to observe it in the observation cell. Several methods for determining species by the stopped-flow technique use reactions which are slow (several seconds). In this case the mixed solutions may sit in the observation cell for several seconds while no further sample processing occurs. In an effort to decrease the time per analysis while maintaining the flexibility to monitor slow or fast reactions, Malmstadt introduced a storage coil between the mixer and the observation cell (32). He named the instrument the stopped-flow

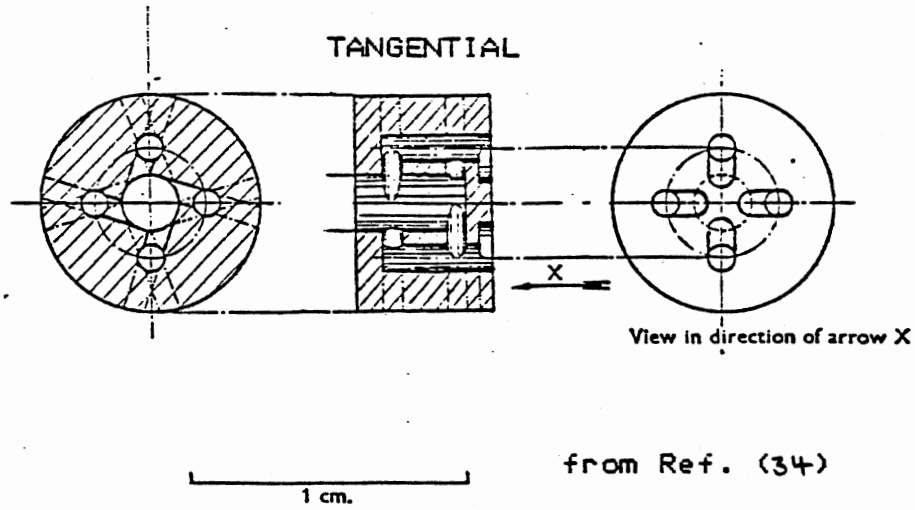
unsegmented solution storage analyzer (SF/USA). The sample and reagent can be mixed and pushed into the storage coil, allowing other samples to be mixed immediately. Later samples will push the first sample through the coil as it reacts until it reaches the observation cell where the absorbance at this fixed time after mixing is recorded. By varying the length of this storage coil the time between mixing and observation can be changed. The improved rate of analyzing samples is presented in Table II.

A review of stopped-flow instrumentation would not be complete without mentioning the mixing chamber design because the stopped-flow technique is, after all, simply a method of fast mixing. Hartridge and Roughton (33) in 1923 introduced the stopped-flow technique and included a study of mixing chamber designs. They found that the best one was the tangential mixer which is illustrated in Figure 2. To determine the efficiency of a stopped-flow mixer the mixing time is determined for the instrument. This is the time required for a nonreacting dye to fully mix with water once the solutions have entered the observation cell. The mixing time is affected by the configuration of the mixer and the flow velocity. The first four instruments reviewed (19,21,24,26) used tangential mixers as described by Gibson and Milnes (34). Malmstadt reported a mixing time of 20 milliseconds for his first two instruments which used an offset cam on a motor to drive the syringes (19,31). Crouch added a pneumatic drive to his system (26) and was able to achieve a mixing time of 5 milliseconds. This reduction in time was due to the higher velocities obtained using this drive system. Malmstadt also reported 5 millisecond mixing times in his instruments using pneumatic drives (27,28). It is interesting to note, however, that in

TABLE II

COMPARISON OF THE DETERMINATION RATE FOR THE STOPPED-FLOW AND THE SF/USA ANALYZERS

delay time (sec)	delay aliquots	changeover aliquots	throughput samples/hr	method used
0	-	4	486	SF
2	-	4	382	SF
5	-	4	290	SF
10	-	4	206	SF
20	-	4	131	SF
30	-	4	96	SF
5	3	7	260	SF/USA
10	6	7	260	SF/USA
20	12	8	233	SF/USA
30	18	8	211	SF/USA



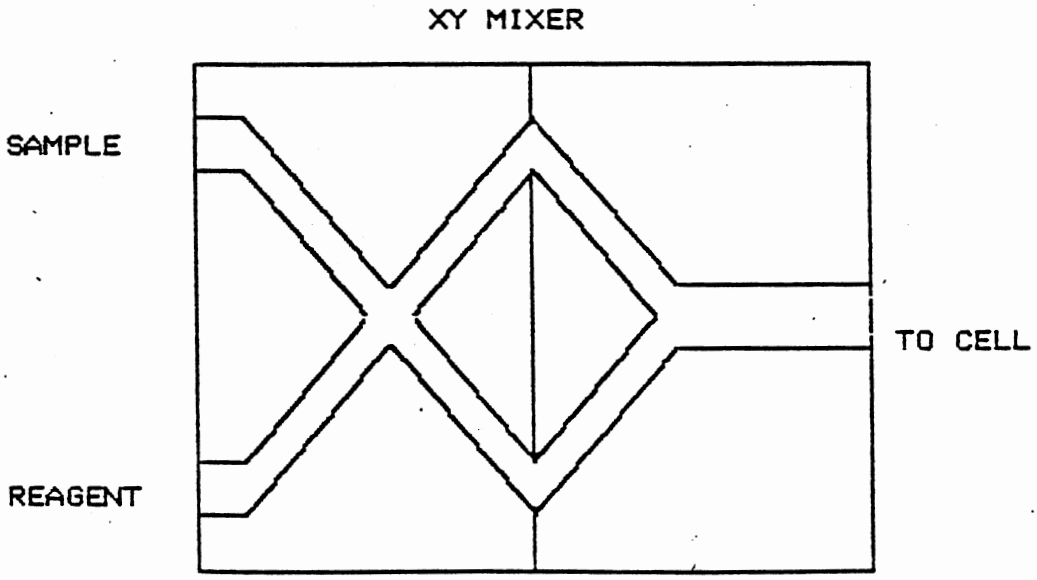


Figure 2. Diagrams of the Tangential and the XY Mixers

the last two instruments reviewed (31,32) the tangential mixer was replaced with an XY design, shown in Figure 2, because of its simplicity to construct. The mixing times reported were over 200 milliseconds for these designs. In this situation determinations based on fast reactions (0.5 seconds) use equilibrium measurements, while determinations based on slower reactions (1-10 seconds) may take advantage of kinetic methods. Here the faster mixers will not provide any advantages. For fast kinetic studies, however, the tangential mixer is a necessity.

2.2 Summary of Instrumental Developments

Table III traces the development of the stopped-flow instrument used for chemical analysis. Initially manual operation of the stopped-flow technique was used to exploit the study of fast reactions. Slowly automation was incorporated into each of the parts of the stopped-flow instrument to increase sample throughput. The introduction of digital data acquisition systems increased the precision and accuracy of this method. Several sample handling techniques, including sample turntables and automatic reagent dispensing, were introduced to shorten the analysis time for many samples. The incorporation of microcomputers as system controllers has reduced the cost of a complete stopped-flow instrument considerably. Finally, the storage coil has made even the determination of slower reacting species a faster process.

TABLE III

HISTORICAL VIEW OF STOPPED-FLOW INSTRUMENTATION
USED FOR CHEMICAL ANALYSIS

year (reference) Author : species determined ---- instrumentation used
1968 (19) Malmstadt : study of 12-molybdophosphoric acid ---- motor driven SF, photograph oscilloscope
1969 (21) Malmstadt : determination of phosphate ---- motor driven SF, sample turntable, digital sequencing, rate meter
1970 (24) Pardue : determination of Fe(III) and Sr ---- pneumatic drive, minicomputer data system, stabilized optics
1972 (26) Crouch : determination of Fe(III) and phosphate ---- pneumatic drive, digital sequencing, rate meter
1975 (27) Malmstadt : determination of glucose ---- pneumatic drive, sample turntable, dual beam, minicomputer control and data system
1977 (28) Malmstadt : determination of glucose ---- pneumatic drive, sample turntable, automatic aliquoting, dual beam, programmed monochromator, mini and microcomputer control and data system
1980 (31) Malmstadt : determination of Fe, ascorbic acid, P, creatinine, and glucose ---- same as 1977, but total microcomputer control
(32) Malmstadt : determination of Fe, ascorbic acid, P, and protein nitrogen ---- same as (11), but includes storage coil (SF/USA)

2.3 Determination of Chemical Species Using the Stopped-Flow Technique

Since 1969 the number of applications of the stopped-flow technique for the determination of chemical species has increased. Much of the interest has been a result of the improved designs introduced by analytical chemists since its introduction. The species which have been determined by stopped-flow are broadly classified in three categories for this review. The categories are: a) general biological interest, b) metal ions, and c) miscellaneous species. Table IV contains a complete listing of all the species determined.

2.3.(a) Species of General Biological Interest

The compounds included in this category are those which might be analyzed in a clinical or hospital laboratory. This section contains the bulk of the papers reviewed which may be attributed to the stopped-flow mixer originating as a tool used for the study of fast reactions of biological interest. Also, although the stopped-flow technique is not widely used in clinics or hospitals, its sample handling capabilities make it ideal for such settings where many samples are analyzed daily for specific components. The approximate order of introducing these species is chronological. Table V contains pertinent reactions for determining each of the species. Table VI compares the precision, accuracy, and speed of the techniques covered in this section.

2.3.(a).1 Phosphate. The determination of phosphate is based on the reaction of phosphate with Mo(VI) in acidic solution to form 12-molybdophosphoric acid (12-MPA). In three references (21,26,35) the

TABLE IV

SPECIES DETERMINED BY THE STOPPED-FLOW TECHNIQUE

GENERAL BIOLOGICAL INTEREST

Phosphate	Thiocyanate
Cysteine	Albumin
Thiolactic Acid	Total Protein
Glucose	Protein Nitrogen
Ascorbic Acid	Creatinine
Cholesterol	Alkaline Phosphatase
Lactate Dehydrogenase	

METAL IONS

Cd(II)	Mg(II)	Ca(II)
Ce(III)	Nd(III)	Mg(II)
Co(II)	Pb(II)	Sr(II)
Cu(II)	Pr(III)	Fe(III)
Eu(III)	Sm(III)	Mn(II)
Gd(III)	Tb(III)	
La(III)	Ba(II)	

MISCELLANEOUS

Aminopolycarboxylic Acids	NO and NO_2
Oxalic Acid	Water

TABLE V

REACTIONS USED IN THE DETERMINATION OF SPECIES OF GENERAL BIOLOGICAL INTEREST

PHOSPHATE (21,26,35)

$H_3PO_4 + 6\ Mo(VI)_t \rightleftharpoons 12\text{-MPA} + 9\ H^+$

PHOSPHATE (36,32)

$H_3PO_4 + 6\ Mo(VI)_t \rightleftharpoons 12\text{-MPA} + 9\ H^+$

12-MPA + Ascorbic Acid → Phosphomolybdenum Blue

CYSTEINE AND THIOLACTIC ACID (37)

Ni(II) + TA → Intermediate → NiTA

GLUCOSE (27,38,28,31)

$\text{Glucose} + O_2 + H_2O \xrightarrow{GOD} \text{Gluconic Acid} + H_2O_2$

H_2O_2 + p-diphenylamine sulfonate → Chromogen

ASCORBIC ACID (32,31,39)

Ascorbic Acid + 2,6 dichlorophenol-indophenol → Chromogen

CHOLESTEROL (38)

$\text{Cholesterol Ester} \xrightarrow{\text{Cholesterol Ester Hydrolase}} \text{free Cholesterol}$

$\text{free Cholesterol} \xrightarrow{\text{Cholesterol Oxidase}} \text{Cholest-4-en-3-one} + H_2O_2$

$2H_2O_2 + \text{4-aminoantipyrine} + \text{Phenol} \xrightarrow{\text{Peroxidase}} \text{Chromogen}$

LACTATE DEHYDROGENASE (38)

$\text{Lactate} + \text{DPN} \xrightarrow{LDH} \text{Pyruvate} + \text{DPNH}$

TABLE V (Continued)

THIOCYANATE (38)

$2\ N_3^- + I_3^- \xrightarrow{SCN^-} 3\ I^- + 3\ N_2$

THIOCYANATE (29)

$SCN^- + 4\ Br_2 + 4\ H_2O \rightarrow CNBr + 7\ Br^- + SO_4^{-2} + 8\ H^+$

$CNBr + Pyridine \rightarrow Chromogen$

ALBUMIN (40)

Albumin + Bromcresol Green → Chromogen

TOTAL PROTEIN (40,2)

Cu(II) + Peptide Bonds → Chromogen

PROTEIN NITROGEN (32,41)

$Protein + H_2SO_4 \xrightarrow{heat} Products + NH_3$

$NH_3 + HOCl \rightarrow H_2NCl + H_2O$

$H_2NCl + Phenol \rightarrow Indophenol$

CREATININE (31)

Creatinine + Alkaline Picrate → Chromogen

ALKALINE PHOSPHATASE ACTIVITY (35)

p-nitrophenyl phosphate $\xrightarrow[\text{phosphate}]{\text{alkaline}}$ p-nitrophenylate ion

TABLE VI

COMPARISON OF THE PRECISION, ACCURACY, AND SPEED OF THE STOPPED-FLOW METHODS FOR DETERMINING SPECIES OF BIOLOGICAL INTEREST

PHOSPHATE

ref.	year	concentration	method[1]	sec.	%RSD	#rep	%err
21	69	0.1-5 ppm	FT	0.7	0.7	10	1.4
26	72	0.5-4 ppm	FT	0.6	<1	10	1.4
36	79	8-80 ppm	IR	7.0	0.3	5	8.5
35	80	1-15 ppm	FT	0.6	2-23	7	-
32	80	4.8-48 ppm	FT	22.2	0.5	4	0.7

CYSTEINE AND THIOLACTIC ACID

ref.	year	concentration	method	sec.	%RSD	#rep	%err
37	72	5-50 E-5 M	RG	0.25	1.2	-	5.5

GLUCOSE

ref.	year	concentration	method	sec.	%RSD	#rep	%err
27	75	0.5-3.5 g/L	IR	15	1.8	4	2.2
38	77	0.6-2 g/L	IR	-	0.2	-	0.9
28	77	0.2-4 g/L	IR	20	2.0	5	3.2
31	80	0.02-0.32 g/L	IR	33	0.43	5	±0.1

ASCORBIC ACID

ref.	year	concentration	method	sec.	%RSD	#rep	%err
39	75	0.01-1.8 g/L	VT	0.5	-	-	2.3
31	80	5-40 g/L	EQ	33	0.38	5	-
32	80	5-40 g/L	EQ	31.8	0.24	4	-

CHOLESTEROL

ref.	year	concentration	method	sec.	%RSD	#rep	%err
38	77	1-3 g/L	EQ	-	-	-	-

LACTATE DEHYDROGENASE

ref.	year	concentration	method	sec.	%RSD	#rep	%err
38	77	50-200 I.U.	IR	200	-	-	-

TABLE VI (Continued)

THIOCYANATE

ref.	year	concentration	method	.sec	%RSD	#rep	%err
29	77	20-120 uM	RG	20	0.5	-	0.6
38	77	20-60 uM	IR	20	-	-	0.4

ALBUMIN

ref.	year	concentration	method	sec.	%RSD	#rep	%err
40	79	31-62 ppm	EQ	7	0.6	3	2.5

TOTAL PROTEIN

ref.	year	concentration	method	sec.	%RSD	#rep	%err
40	79	50-100 ppm	IR	3	1.3	3	-
2	80	20-80 ppm	FT	10	0.8	6	-

PROTEIN NITROGEN

ref.	year	concentration	method	sec.	%RSD	#rep	%err
41	79	56-300 ppm	IR	25	0.5	4	-
32	80	32-300 ppm	FT	20.8	0.44	4	0.38

CREATININE

ref.	year	concentration	method	sec.	%RSD	#rep	%err
31	80	10-50 ppm	IR	30	0.43	5	-

ALKALINE PHOSPHATASE ACTIVITY

ref.	year	concentration	method	sec.	%RSD	#rep	%err
35	80	19-138 U/L	IR	60	1.2	4	-

1. FT = fixed time, IR = initial rate, VT = variable time
   EQ = equilibrium, RG = regression

formation of 12-MPA was monitored at 400 nanometers while in two others (32,19) ascorbic acid was added to reduce the 12-MPA to the well known phosphomolybdenum blue compound. In all cases an excess of Mo(VI) was added so that the reaction would be pseudo first-order in phosphate.

These methods exemplify the advantage which can be obtained by using a kinetic rather than an equilibrium method for determining species. Two interfering species commonly encountered in this determination are arsenate and silicate. They do not interfere, using this method, in levels up to a 1,000 fold excess of arsenate and an 80 fold excess of silicate because they react slowly with Mo(VI). Therefore, their contribution to the absorbance, during the few seconds in which the reaction with phosphate occurs, is insignificant. Javier (21) used the method to analyze blood serum and found his results agreed well with those obtained with the Auto-Analyzer. Another advantage of the method for phosphate determinations is the reduction of matrix blank effects. Since a change in absorbance is measured and not an absolute value, the absorbance of the blank cancels and will not affect the results. This is of particular importance in determinations for agricultural products such as those performed by McCracken (36) where the matrix differred between the samples. It seems that Malmstadt (32) and McCracken (36) would have benefited from the faster method of Javier (21) where the ascorbic acid step was eliminated entirely. Javier's method is about 10 times as fast. Of course the faster method is less precise and accurate, so these factors may be a compelling consideration where speed is not the only factor. It is interesting to note that Bonnell's dual-microcomputer system has the worst precision. Simply adding more hardware does not necessarily improve a method.

2.3.(a).2 Cysteine and Thiolactic Acid. The method for determining cysteine and thiolactic acid is based on the exchange reaction between Ni(II)-citrate and these thiol acids. Sanderson et al. (37) used a simultaneous kinetic determination for these two acids in a mixture. The concentrations were computed using a nonlinear regression of the data. Both thiol acids reacted under first order conditions. An important advantage of this method is that previous methods required separation prior to the measurement step while this one does not. Elimintation of the separation step is possible because the differences in the rates of the two reactions allow them to be determined simultaneously, whereas an equilibrium measurement of absorbance would not distinguish between the two components.

2.3.(a).3 Glucose. The determination of glucose is based on its oxidation by dissolved oxygen in the presence of the catalyst glucose oxidase. The products of the reaction are gluconic acid and hydrogen peroxide. In three of the four methods presented, the hydrogen peroxide formed reacts with p-diphenylamine sulphonate forming a chromogen detected at 470 nanometers. An alternate reaction senses the production of hydrogen peroxide via the molybdate catalyzed oxidation of iodide to iodine at 355 nanometers. In all cases the reaction is followed for a few seconds and the initial rate is proportional to the concentration of glucose in the sample. This is an interesting application of the stopped-flow technique because these methods allow between 10 seconds and 10 minutes for the reaction to occur. With these times conventional mixing techniques could work equally as well. An advantage which may be realized with the stopped-flow system is its sample processing

capabilities which eliminate time consuming manual procedures. Pardue (38) attempted to increase the throughput of the method by premixing the reagents with the samples and then using the stopped-flow as an automated spectrophotometer to measure their equilibrium absorbance. This method does give the largest throughput of the four. As mentioned in the phosphate section the most hardware does not always produce the best results. Even though Koupparis (31) used an inexpensive microcomputer, his accuracy and precision are almost an order of magnitude better than those of Krottinger (28) who used a minicomputer in his system. Koupparis includes five applications of his low cost system that demonstrate its power as a good sample processor.

2.3.(a).4 Ascorbic Acid. The three stopped-flow methods to determine ascorbic acid monitor the decrease in absorbance of 2,6-dichlorophenolindophenol (DCPI) at 522 nanometers as it is oxidized by ascorbic acid.

Karayannis (39) developed the kinetic determination of ascorbic acid by stopped-flow as a result of a kinetic study which he conducted (42). His method is faster than the conventional titration technique even though the slopes of the reaction are measured manually from photographs. Koupparis (31) used an equilibrium method which required 30 seconds for the reaction to reach completion. Malmstadt (32) applied his SF/USA for this determination and increased the number of samples analyzed per hour from 90 to 211. The increase shows the utility of his new design in reducing the time per analysis while maintaining good precision.

2.3.(a).5 Cholesterol. The determination of cholesterol is based on a standard A-Gent Cholesterol enzymatic kit involving cholesterol ester hydrolase and cholesterol oxidase in an equilibrium method. Pardue's application of the stopped-flow technique to the determination of cholesterol (38) is questionable because this reaction requires 15 minutes. Some time reduction per analysis may be experienced if all of the samples are premixed with reagent and then the stopped-flow is used to automatically measure the equilibrium absorbance. Since fast mixing is unnecessary, other automated techniques may be equally suited for the job and much less expensive. For example one might use an automated peristaltic pump to deliver the samples to a flow cell in a conventional spectrophotometer.

2.3.(a).6 Lactate Dehydrogenase. Pardue et al. (38) also determined lactate dehydrogenase (LDH) as well as glucose and cholesterol. The determination was based on the reaction of Brooks et al. (43) with Versatol E where the enzymatic activity was computed from a least squares fit of the absorbance versus time data. This initial rate method requires approximately seven minutes. The time saved by the method is due to the stopped-flow's use as an automated sample handler. A correlation coefficient of 0.996 was found between the stopped-flow method and a method using a stabilized spectrophotometer in the range of 50 to 200 units of LDH. Again, other methods might have been as suitable for this determination and also less expensive.

2.3.(a).7 Thiocyanate. Landis et al. (29) based their method of determining thiocyanate on the reaction of thiocyanate with bromine to

form cyanogen bromide. The cyanogen bromide formed reacts with pyridine to form a yellow product monitored at 400 nanometers. The method measures both thiocyanate and cyanide in a sample. This is not a problem in blood because cyanide concentrations are very low, but sample pretreatment may be required for other types of samples. This method does correlate well with the Pettigrew-Fell equilibrium method yeilding a correlation coefficient of 0.983.

A different reaction is the basis for the determination by Pardue et al. (38). In their method, thiocyanate is used as a catalyst for the oxidation of azide to nitrogen by triiodide. The initial rate method of calculating concentration from the rate data was used. A correlation with the Pettigrew-Fell method gave a coefficient of 0.98. An advantage of this method is the freedom from errors due to turbidity which plague the equilibrium methods. The absorbance due to turbidity will be constant and will not affect the change in absorbance which is the basis for determining concentration from the data.

2.3.(a).8 Albumin. The determination of albumin in serum is based on the binding of albumin with Bromcresol Green in acidic solution. The method was developed by Krottinger et al. (40) to eliminate as much of the sample pretreatment as possible. A study over a period of six days in which the working curve was calculated for the first, third, fifth and sixth days showed good reproducibility with a relative standard deviation of 2.3%. The reagent was remade after day three. This method is fast, accurate, and precise. The authors include the determination of two other species, calcium and total protein, each taking less than ten seconds. These times are less than can be obtained

by other methods where the mixing itself is rarely complete in ten seconds.

2.3.(a).9 Total Protein. Both of the references (40,2) use the biuret reaction in determining the total protein concentration in serum. In this reaction Cu(II) ions bind with peptide bonds in proteins to form a complex which is monitored at 520 nanometers. Krottinger (40) found that the reagent was stable for five days and suggested that only one calibration curve per week was necessary. Law et al. (2) analyzed samples for total protein using fixed times of 0.1 seconds and 10 seconds and found that the relative standard deviation for six replicates decreased from 3.1% for 0.1 second to 0.8% for 10 seconds. This is one of the best examples which demonstrates the shortened analysis time made possible with stopped-flow methods. The widely used procedure for total serum protein requires thirty minutes while these procedures take 10 seconds or less. The method is also better because the standard procedure is susceptible to errors resulting from turbidity in serum samples. In the longer determination, the effects of interfering side reactions will also be increased because more time is allowed for them to occur.

2.3.(a).10 Protein Nitrogen. The determination of protein nitrogen is based on the Berthelot reaction in which ammonia, formed by digesting the sample, reacts with hypochlorite to form chloramine. The chloramine then reacts with phenol under alkaline conditions to form indophenol which is the species spectrophotometrically monitored at 635 nanometers. In the stopped-flow procedures the reaction does not come to completion, which would require more than thirty seconds.

McCracken et al. (41) used an initial rate method to determine protein nitrogen. The slope for the initial rate was found by least squares analysis. The authors note that many of these Berthelot procedures use a catalyst to speed the reaction to decrease analysis time. They found that use of a catalyst decreased the precision and so they chose not to use one.

Malmstadt et al. (32) used the same reaction in their SF/USA but used only a single point at a fixed time of 20.8 seconds. The previous stopped-flow method (41) processes 114 samples per hour, a flow injection analysis technique (44) processes 110 to 180 samples per hour, while this procedure processes 233 samples per hour. Not only is the processing speed high, but this method exhibits excellent precision and accuracy. This system represents state of the art stopped-flow sample processing.

2.3.(a).11 Creatinine. Koupparis et al. (31) determined creatinine by measuring the rate at which alkaline picrate reacts with creatinine to form a complex. This is referred to as the Jaffe reaction. They reported an analysis rate of 109 samples per hour using their reaction rate method. In many references these processing times are quoted leaving out some steps so that they are not acheivable. This analysis rate does include considerations for flushing the system between samples, incrementing the sample turntable, and computer calculation time.

2.3.(a).12 Alkaline Phosphatase Activity. The stopped-flow system of Bonnell et al. (35) was mentioned under the heading of phosphate determinations. They also used their dual microcomputer system to

determine alkaline phosphatase activity. The method is based on the alkaline phosphatase catalyzed hydrolysis of p-nitrophenyl phosphate to p-nitrophenolate ion in pH 10.3 buffer. The initial rate is found by least squares. The authors report that measurement times exceed 60 seconds due to the slow reaction. They do not indicate if this slow reaction makes their method slow or if this is something overcome by their rate method.

2.3.(b) Metal Ions

The metal ions in this category are presented in two ways. The first two references reviewed cover over twenty ions so these papers will be reviewed by author rather than having twenty separate categories. The other sections will include particular metal ions and may include several references per ion. Many references involve determinations of Fe(III) because this has become the "benchmark" for evaluating the performance of a stopped-flow instrument. The order of introducing the metal ions is chronological. Table VII contains the reactions which were involved in these determinations. Table VIII compares the precision, accuracy, and speed of these methods.

2.3.(b).1 Cd(II), Ce(III), Co(II), Cu(II), Eu(III), Gd(III), La(III), Mg(II), Nd(III), Pb(II), Pr(III), Sm(III), Tb(III). Margerum et al. (45) may be considered with Javier (21) as true pioneers in the field of analytical stopped-flow applications. Margerum's work is very broad, covering data for thirty metals, and demonstrates the usefulness of the stopped-flow technique for simultaneous determination of two or more metal ions in one solution. The reaction used in these studies is

TABLE VII

REACTIONS USED IN THE DETERMINATION OF METAL IONS

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30 METALS (45)

$$\text{Metal-CyDTA} + H^{+} \rightarrow HCyDTA^{-3} + \text{Metal ion}$$

$$HCyDTA^{-3} + (Cu^{+2} \text{ or } Pb^{+2}) \rightarrow CuCyDTA^{-1}$$

Ba(II), Ca(II), Mg(II), Sr(II) (26)

(same as above)

Ca(II) (40)

$$Ca^{+2} + \text{o-cresolphthalein complexone} \rightarrow \text{chromogen}$$

Fe(III) (26,32,31,24,30)

$$Fe^{+3} + SCN^{-} \rightarrow FeSCN^{+2}$$

Mn(II) (46)

$$2\ MnO_4^{-} + 5\ C_2O_4^{-2} + 16\ H^{+} \xrightarrow{Mn^{+2}} 2\ Mn^{+2} + 10\ CO_2 + 8\ H_2O$$

TABLE VIII

COMPARISON OF THE PRECISION, ACCURACY, AND SPEED OF THE STOPPED-FLOW METHODS FOR DETERMINING METAL IONS

13 METALS

ref.	year	concentration	method	sec.	%RSD	#rep	%err
45	69	10^{-5}-10^{-4} M	RG	-	10	-	-

BARIUM, CALCIUM, MAGNESIUM, AND STRONTIUM

ref.	year	concentration	method	sec.	%RSD	#rep	%err
47	69	-	RG	-	10	3-9	5

CALCIUM

ref.	year	concentration	method	sec.	%RSD	#rep	%err
40	79	50-150 ppm	EQ	5	1	3	2.3

IRON(III)

ref.	year	concentration	method	sec.	%RSD	#rep	%err
24	70	6-28 ppm	IR	1	0.29	3	0.39
26	72	6-28 ppm	IR	-	4.2	5	1.6
30	78	28-100 ppm	RG EQ IR	-	-	-	-
31	80	2-20 ppm	EQ	16	0.4	5	-
32	80	2-20 ppm	EQ	12.2	0.2	5	0.75

MANGANESE(II)

ref.	year	concentration	method	sec.	%RSD	#rep	%err
46	82	10^{-4}-10^{-2} M	IR	0.5	1	5	2.2

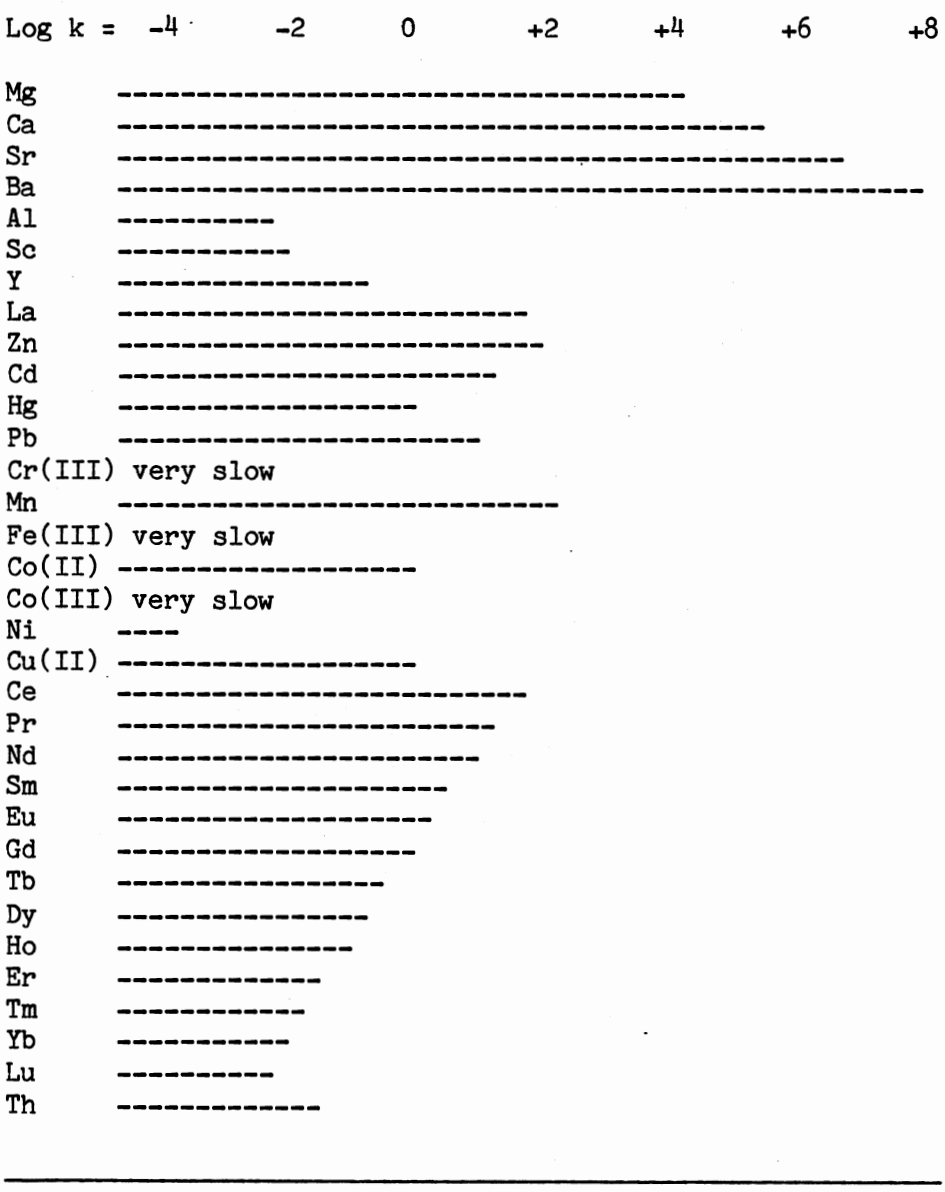
the exchange reaction of the metal-CyDTA (trans-1,2-diaminocyclohexane-N,N,N',N'-tetraacetate) complexes with a single exchanging metal ion. There is no direct attack of one metal on another metal-CyDTA complex so that each complex has a characteristic dissociation rate. This is due to the CyDTA's structure. The cyclohexane ring restricts the ligand's motion so that it can accommodate only one ion in its cage. This becomes important in the assumptions which are used to interpret the rate data to find concentrations. These rates are acid dependent which allows the pH to be adjusted to give any desired reaction time for the metals to be determined. As seen in Table IX, this is an important experimental parameter because the rates at one pH vary over twelve orders of magnitude. As a test of the approach to multicomponent analyses, Margerum et al. prepared a sample containing Fe(III), Ni(II), Mg(II), Ca(II), Zn(II), Mn(II), Cd(II), Hg(II), and Co(II). The sample was analyzed at four different pHs. Seven of the nine metals were determined within about 10% error.

This work is very comprehensive considering that all the data was taken from photographs of oscilloscope traces. The digitized points were then used to solve the equation for concentrations using a linear regression analysis program on an IBM 7094 computer. Although the accuracy and precision are not exceptional, these techniques could be optimized in methods to determine subsets of these metals.

2.3.(b).2 Ba(II), Ca(II), Mg(II), Sr(II). Pausch et al. (47) used the same reaction scheme as Margerum (45) to determine Ba(II), Ca(II), Mg(II), and Sr(II). The large differences in the rate constants of the four ions are exploited in the method. The ratio of rate constants for Mg:Ca:Sr:Ba is 1:6.5:96:1660. The errors for these

TABLE IX

RATE CONSTANTS FOR THE REACTION OF HYDROGEN ION WITH METAL-CyDTA COMPLEXES



determinations range from about 5% for metals determined separately to about 10% for the determination of ternary mixtures of these metals. They note that the limit of detection of these methods is good and that as little as 10^{-6} M metal ions can be determined. The major factors for errors were determining the rate constants, reading data from a photograph, and determining the molar absorptivity of Pb-CyDTA. They suggest possible remedies including the implementation of a better data acquisition system to eliminate oscilloscope errors and hand digitizing. They also recommend that the CyDTA be purified before its use. As with Margerum's paper, the impact of this work lies more in its introduction of the scope of kinetic methods utilizing the stopped-flow mixer rather than the actual refinement of a single determination for a particular metal.

Krottinger et al. (40) have determined Ca(II) in blood serum. Their method is based on calcium complexing with o-cresolphthalein complexone in basic solution. The standard deviation of this method in the range of 50 to 150 ppm was less than 1% for three replicates and had an error of 2.3%. This is a good method because of its precision, accuracy, and speed.

2.3.(b).3 Fe(III). Many chemists use the reaction of Fe(III) with thiocyanate to test the performance of their stopped-flow systems. Only those references which have reported viable determinations of Fe(III) and not just measurements of the dead time of their stopped-flow instruments have been reviewed here. In each case the product, Fe(III)-thiocyanate, is monitored at about 450 nanometers.

Willis et al. (24) compared the determination of Fe(III) on two instruments; one was their own stopped-flow mixer with an optically

stabilized spectrometer, and the other was a commercial stopped-flow instrument. While the commercial stopped-flow mixer had a precision of 0.95% for three replicates and an error of 0.79%, the stabilized stopped-flow mixer gave a precision of 0.29% for three replicates and an error of 0.39%. Since stopped-flow mixing systems in general are single beam instruments, this comparison illustrates the importance of stabilizing the spectrometer to obtain superior quality results.

Beckwith et al. (26) designed an instrument to automate the stopped-flow operation and minimize dead time. This was used in the determination of Fe(III) to indicate the high degree of precision which can be achieved. From their results, an error of 1.6% and a standard deviation of 4.2% for 5 replicates, it is apparent that the system does not stand up to their claims.

Mieling et al. (30) used the Fe(III) determination to compare three methods of analyzing kinetic data for analytical content. They used the initial rate, equilibrium, and regression curve methods. They also tested the sensitivity of each method to reaction variables such as pH, temperature, and ionic strength. They found that the regression method, which computes initial and final concentrations along with the rate constant to give a best fit of the data, was the best for accuracy, precision, and insensitivity to changing reaction variables. The second best technique was the equilibrium method and the worst case was the initial rate method. The precision of the regression and equilibrium methods are similar while that of the initial rate method was up to 10% less under some conditions. This study should be considered when choosing the means of extracting concentrations from kinetic data.

A versatile microprocessor-based stopped-flow analyzer developed by Koupparis et al. (31) has been used to determine Fe(III). With their system they report the capability of analyzing 183 samples per hour. Malmstadt et al. (32) improved the method by using the SF/USA to determine Fe(III). They could process 260 samples per hour. This again demonstrates the superior performance of the SF/USA analyzer.

2.3.(b).4 Mn(II). The determination of Mn(II) is based on the reduction of permanganate by oxalate in the presence of Mn(II) in acidic media. The rate of disappearance of permanganate is followed at 525 nanometers by Koupparis et al. (46). The reaction is pseudo first-order in Mn(II) with an excess of permanganate and oxalate present. The authors admit that the limit of detection for this method is not as good as that of other methods, but is rapid and selective in its range.

2.3.(c) Miscellaneous Species

The compounds in this category did not fit into either of the first two categories so they are reviewed separately here. The order of reviewing these compounds is chronological. Table X contains the reactions which are involved in the determinations. Table XI compares the precision, accuracy and speed of these methods.

2.3.(c).1 Aminopolycarboxylic Acids. Coombs et al. (48) analyzed solutions for the following aminopolycarboxylic acids, either alone or in mixtures: NTA (nitrilotriacetic acid), EDTA (ethylenediaminetetraacetic acid), EDDA (ethylenediamine-N,N'-diacetic acid), EGTA (ethylene glycol bis(aminoethyl)tetraacetic acid), HPDTA (1,3-diamino-2-hydroxypropanetetraacetic acid), and HEEDTA

TABLE X

REACTIONS USED IN THE DETERMINATION OF MISCELLANEOUS SPECIES

AMINOPOLYCARBOXYLIC ACIDS (48)

$NiA^{2-n} + CN^- \rightleftharpoons NiA(CN)^{1-n}$

$NiA(CN)^{1-n} + CN^- \rightleftharpoons NiA(CN)_2^{n-}$

$NiA(CN)_2^{n-} + CN^- \rightarrow NiA(CN)_3^{(n+1)-}$

$NiA(CN)_3^{(n+1)-} + CN^- \rightarrow Ni(CN)_4^{-2} + A^{n-}$

NO AND NO_2 (49)

$Fe^{+2} + NO \rightleftharpoons Fe(NO)^{+2}$

$3\ Fe^{+2} + NO_2 + 2\ H^+ \rightleftharpoons Fe(NO)^{+2} + 2\ Fe^{+3}$

OXALIC ACID (46)

$2\ MnO_4^- + 5\ C_2O_4^{-2} + 16\ H^+ \rightarrow 2\ Mn^{+2} + 10\ CO_2 + 8\ H_2O$

WATER (50)

$SO_2 + Ac^- + CH_3OH \rightleftharpoons HAC + CH_3SO_3^-$

$CH_3SO_3^- + I_2 \rightleftharpoons CH_3SO_3I_2^-$

$CH_3SO_3I_2^- + H_2O \rightarrow CH_3SO_4^- + 2\ I^- + 2\ H^+$

TABLE XI

COMPARISON OF THE PRECISION, ACCURACY, AND SPEED OF THE STOPPED-FLOW METHODS FOR DETERMINING MISCELLANEOUS SPECIES

AMINOPOLYCARBOXYLIC ACIDS

ref.	year	concentration	method	sec.	%RSD	#rep	%err
48	72	-	RG EX	-	2.2	3-4	10

NO AND NO_2

ref.	year	concentration	method	sec.	%RSD	#rep	%err
49	73	10^{-4}-10^{-3} M	FT EX	120	-	-	5

OXALIC ACID

ref.	year	concentration	method	sec.	%RSD	#rep	%err
46	82	5-20 ug/ml	IR	18	2.3	5	-

WATER

ref.	year	concentration	method	sec.	%RSD	#rep	%err
50	82	0.02-0.2 mg/ml	EQ	15	0.7	5	0.8

(N-hydroxyethylethylenediaminetriacetic acid). These acids were determined by following the reaction of cyanide ion with the acid-Ni(II) complexes in basic solution. The percent error increased from about 7% for binary mixtures to 10% for ternary mixtures and to very poor values, up to 50%, for quarternary mixtures. These results were obtained from a regression of the data points. Coombs did however determine NTA in the parts per billion range in natural waters. NTA was determined within 10 ppb for samples containing from 19 to 90 ppb NTA. This is an excellent lower limit compared to other techniques of ion-exchange chromatography (1 ppm) and polarographic procedures (10 ppb to 10 ppm), which have much higher or equivalent limits of detection. A study of interferences indicated that triphosphate was not a problem at concentrations up to 10^{-3} M. They recommended that most metals could be eliminated from possibly interfering by passing the sample through a cation exchange column before analysis.

2.3.(c).2 NO and NO_2. The determination of NO and NO_2 is based on the formation of the Fe(II)-nitrosyl complex from Fe(II) and NO or NO_2. The ratio of the rates for NO to NO_2 was 270 to 1. Coetzee et al. (49) analyzed the data using two different methods. In the first the concentration of NO was calculated from the absorbance at two seconds and the NO_2 was found from the differences in absorbance at four minutes and two seconds. After four minutes the reaction has reached equilibrium. Errors here arise from the assumption that NO has finished reacting and little NO_2 has begun to react at two seconds. But if there is a large ratio of NO_2 to NO this will not be true. The second method involves a logarithmic extrapolation of the data from 20 to 120 seconds from the $-\ln(A_{inf} - A_t)$ versus time curve back to time zero. The

concentration of NO is obtained from the difference between the total concentration of NO and NO_2 and the concentration of NO_2 from the logarithmic extrapolation. It is difficult to evaluate the actual errors in determining these species from this reference (49) because only the ratios of NO to NO_2 are reported. It is possible for the ratios to be correct but both concentrations determined may be higher or lower than the true values. The authors admit that the limit of detection (10^{-5} M) is only moderate.

2.3.(c).3 Oxalic Acid. The reaction rate method for determining oxalic acid is based on the reduction of permanganate by oxalate in the presence of Mn(II) in acidic media. Koupparis et al. (46) followed the disappearance of permanganate at 525 nanometers and then calculated the initial rate by least squares from data obtained with a PDP 8/f mini-computer. The thrust of this paper is the kinetic study of the reaction and the Mn(II) determination. As such the consideration for the oxalic acid determination is minimal and warrants further improvement.

2.3.(c).4 Water. Koupparis et al. (50) have used a pyridine free Karl Fischer reaction to determine water. The solvent in which the sample was mixed was methanolic sodium acetate-sulfur dioxide. This was mixed in the stopped-flow with the reagent, a methanolic iodine solution. The system has several advantages over the standard Karl Fischer titration. The absence of toxic pyridine, the stability of the iodine reagent, and the absence of SO_2I^-, which interferes spectrophotometrically in the Karl Fischer titration, are some of the advantages. The short analysis time provided by the method does not allow interfering reactions to occur to a significant extent.

Interfering reactions include carbonyl compounds reacting with methanol to form water, and mercaptans and amines reacting with iodine. The analysis rate was reported as 130 samples per hour including time for the sample turntable rotation, flushing between samples, and calculations. The reagents were found to be stable over a 15-day test period. This is an outstanding new stopped-flow method and enjoys the decreased interferences and increased sample throughput that is demonstrated by many of these analyses. The authors do comment that the limit of detection (0.5 ng/ml) is not as good as the conventional method.

2.4 Kinetic Studies of Chemical Species Using the Stopped-Flow Technique

Although many kinetic studies have been performed using the stopped-flow mixer, most of these involved reactions of biological interest. Our interest lies with those studies which provide insight into reactions which are important to analytical chemists. A good understanding of how a reaction occurs, and what influences it, can be used in method development to provide maximum speed, accuracy, precision, and a minimum of interferences. This section includes five such studies which involve the stopped-flow mixer and give the analytical chemist a better working knowledge of the chemistry he uses. These studies are introduced in chronological order.

2.4.(a) Reaction for Phosphate Determination

The reaction studied by Javier et al. (51) involved the formation of 12-molybdophosphoric acid (12-MPA) from Mo(VI) and phosphate in nitric acid solutions. The reaction forms the basis of a rapid

determination of phosphate (21), therefore the mechanism of this reaction is of interest. Their first experiments involved measuring the initial rate while changing the nitric acid concentration. These results show that at low acid concentration (<0.5 M) the rate is acid independent and at higher acid concentration (>0.8 M) the rate varies with the inverse fourth order of acid concentration. The reaction orders of phosphate and Mo(VI) were determined in the acid independent region and the acid dependent region. This was accomplished by plotting the log of the rate constant versus the log of the concentration where the slope is indicative of the reaction order. In 0.5 M acid the orders of both species, Mo(VI) and phosphate, were one. At higher acidity, 1 M, the reaction order of Mo(VI) changes to second. The mechanism from the preceeding data was postulated as:

$$HMo(VI)^{+} + H_3PO_4 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} PO_4\text{-}Mo(VI)^{-3} + 4\ H^{+} \qquad (2.1)$$

$$PO_4\text{-}Mo(VI)^{-3} + HMo(VI)^{+} \xrightarrow{k_2} PO_4\text{-}Mo(VI)_2^{-3} + H^{+} \qquad (2.2)$$

$$PO_4\text{-}Mo(VI)_2^{-3} + 4\ HMo(VI)^{+} \rightarrow 12\text{-}MPA + 4\ H^{+}. \qquad (2.3)$$

The second step is assumed to be rate determining. The rate equations do not indicate what species of Mo(VI) reacts, but from the stoichiometry of 12-MPA ($H_3PMo_{12}O_{40}\cdot 3\ H_2O$) it is assumed to be dimeric. The rate constant k_1 was evaluated to be 22.03 ± 0.42 L-mole^{-1} sec^{-1} and k_2/k_{-1} was calculated as 15.38 ± 1.09 mole^3 L^{-3}. The rate data was then recalculated on the basis of these values and found to be within approximately 5% of the experimental values. The authors mention that the ionic strength was not controlled due to the large excess of acid present. Some of the descrepancy between the calculated and

experimental data may be due to ionic strength effects. Their work exemplifies the advantage of knowing detailed reaction mechanisms when setting up an analysis. Since the mechanism of this reaction was understood, a new faster method of determining phosphate resulted because the slow ascorbic acid reduction of 12-MPA was eliminated.

2.4(b) Reaction for Thiol Acid Determinations

The reaction used in the thiol acid determinations is the exchange reaction between the Ni(II)-citrate complex and thiol acids resulting in a Ni(II)-thiol acid complex. Sanderson et al. (37) studied the effects of pH, ratio of citrate to Ni(II), and the concentrations of Ni(II) and the thiol acids upon the reaction. They used two acids in particular, cysteine and thiolactic acid. They observed a maximum in the rate constant versus pH curve near pH 9.0 and used this pH in all subsequent experiments. Since Ni(II) forms insoluble hydroxides at pH 9, citrate was added to keep the Ni(II) in solution. They studied the effect of the citrate to Ni(II) ratio and found that the rate was fastest at low citrate to Ni(II) ratios. They first chose a ratio of 0.6 because the rate did not increase even at ratios lower than this. Since this did not stabilize the solution enough, a compromise was made to use a 0.65 ratio of citrate to Ni(II). In studying the effect of Ni(II) on the reaction (at a fixed 0.65 ratio) it was found that at about 1.25×10^{-2}M Ni(II) the reaction was independent of Ni(II). This was the optimum value selected for the determination of thiol acids since larger concentrations of the Ni(II)-citrate contributed slightly to the absorbance at 275 nanometers, which is the absorbance maximum for the Ni(II)-thiol acid complexes. The thiol acids obey pseudo first-order

kinetics between 15 and 200 milliseconds. The computed data agrees with the assumption that the acids are first order and do not interfere with each other. This kinetic study deals primarily with establishing optimum experimental conditions for conducting an analysis for cysteine and thiolactic acid and does not establish the mechanism or a kinetic rate expression. They do, however, draw a few conclusions about the kinetics of the reaction from their data.

Sanderson et al. mention that an induction period occurs during the first few milliseconds and suggest that this is due to the formation of an intermediate. Since the reaction is independent of citrate at low citrate to Ni(II) ratios, they suggest that two parallel reactions may be taking place between the thiol acid and either an aqueous complex of Ni(II) or the citrate complex of Ni(II). The reaction scheme could be:

$$\text{Ni(II)} + \text{TA} \xrightarrow{k_1} \text{Intermediate} \xrightarrow{k_2} \text{Ni(II)-TA}. \qquad (2.4)$$

The calculated values of k_1 and k_2 are 783 sec^{-1} and 21.5 sec^{-1} respectively for cysteine and 156 sec^{-1} and 11.0 sec^{-1} respectively for thiolactic acid. The determination of the thiol acids using the above information is described in the determination section of this review.

2.4.(c) Reaction for Ascorbic Acid Determination

The reaction under investigation by Karayannis (42) was the oxidation of ascorbic acid by 2,6-dichlorophenolindophenol (DCPI). Oxalic acid was present in the reaction mixture because it is commonly used in the solvents to extract ascorbic acid from biological samples. The reaction is assumed to be first-order in both reactants. The purpose of his paper was to find the second-order rate constant. The author

measures this with four methods. The first two methods use pseudo first-order conditions where ascorbic acid is in excess. The first method uses the equation RATE = kA_0B_t, where A_0 is the initial concentration of ascorbic acid and B_t is the concentration of DCPI at time t. The rate is measured at a particular time and the absorbance is used to find B_t. The rate divided by A_0 and B_t gives the second-order rate constant. This same data is analyzed a different way for the second method. This method is based on the equation RATE = $k_{obs}B_t$, where $k_{obs} = kA_0$. Several plots, using a different A_0 in each, are used to find the rate at the same value of B_t on each one. Then k_{obs} in each case is found by dividing the rate by this B_t. A graph of k_{obs} versus A_0 will have a slope of the second-order rate constant. The other two methods use second-order experimental conditions where RATE = kA_tB_t holds. The third method finds the rate at various times and divides it by the product A_tB_t for that time. A plot of the rate versus A_tB_t gives a slope equal to the second-order rate constant. The final method uses the integrated form of the rate equation:

$$[1/(B_0 - A_0)] \ln[(A_0B_t)/(B_0A_t)] = kt. \qquad (2.5)$$

This equation was solved for k by substituting in values of A_t and B_t at various times. The second-order rate constants obtained from methods 1, 2, 3, and 4 were 55.9×10^3, 56.8×10^3, 56.8×10^3, and 56.2×10^3 M^{-1} sec^{-1}, respectively. The average obtained was 56.5×10^3 $M^{-1}sec^{-1}$ with a standard deviation of 0.6×10^3 $M^{-1}sec^{-1}$.

In a study of the effect of pH, an S-shaped curve which decreased as pH increased in the region of 1 to 7 pH units was found. The conclusion was made that hydronium ions do not participate directly but

that they affect the concentration of the undissociated ascorbic acid in the solution. When the curve was calculated by multiplying the rate constant by the ratio of the acid form to the analytical concentration of ascorbic acid at each pH, it agreed very closely to the experimental points. Because this reference shows how the reaction varies with pH and provides a good value of the second order rate constant, it can provide some insight to improve the analysis of ascorbic acid.

2.4.(d) Reaction for Fe(III) Determination

Mieling et al. (52) adopt the reaction mechanism which accounts for previous studies of Fe(III) and thiocyanate. The reaction involves two parallel pathways; one is the direct reaction of Fe(III) and thiocyanate, while the other involves Fe(III) hydroxide reacting with thiocyanate and subsequently losing the hydroxide from the complex. These can be represented by the following reactions:

$$Fe^{+3} + SCN^{-} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} FeSCN^{+2} \qquad (2.6)$$

$$Fe^{+3} + OH^{-} \overset{K_{h1}}{\rightleftharpoons} FeOH^{+2} + SCN^{-} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} Fe(OH)SCN^{+1} \overset{K_{h2}}{\rightleftharpoons} FeSCN^{+2}. \qquad (2.7)$$

The equilibrium processes represented by K_{h1} and K_{h2} are fast in comparison with the others. The authors developed the following rate equation from the preceeding reactions:

$$RATE = (k_1 + k_2K_{h1}/[H^+])[Fe(III)][SCN^-] - (k_{-1} + k_{-2}K_{h2}/[H^+])[FeSCN^{+2}]. \qquad (2.8)$$

With an excess of thiocyanate the reactions will appear to be two competing first order reactions and Mieling arrived at the following

integrated rate equation:

$$\ln\{[FeSCN^{+2}]_{inf} / ([FeSCN^{+2}]_{inf} - [FeSCN^{+2}]_t)\} = [(k_1 + k_2K_{h1}/[H^+])[Fe(III)] + (k_{-1}+k_{-2}K_{h2}/[H^+])]t. \quad (2.9)$$

This equation is simplified to:

$$\ln(A_{inf} - A_t) = -(k_f[Fe] + k_r)t + \ln(A_{inf} - A_0), \quad (2.10)$$

where k_f and k_r are just combined rate and equilibrium constants from the integrated rate equation. From these equations k_f and k_r can be calculated by plotting $\ln(A_{inf} - A_t)$ versus time for several Fe(III) concentrations. The slope of each curve will be equal to $k_f[Fe(III)] + k_r$. If these slopes are plotted versus [Fe(III)] then k_f and k_r can be determined from the slope and intercept respectively. Since $k_f = k_1 + k_2K_{h1}/[H^+]$ and $k_r = k_{-1} + k_{-2}K_{h2}/[H^+]$, a plot of k_f versus $1/[H^+]$ will have a slope of k_2K_{h1} and intercept of k_{-1}, while k_r versus $1/[H^+]$ will have a slope of $k_{-2}K_{h2}$ and an intercept of k_{-1}. Previously reported values of K_{h1} and K_{h2} were used to compute k_2 and k_{-2}. One hundred and sixty-eight experiments were run to evaluate the rate constants. The authors used their system to demonstrate the speed and accuracy with which detailed kinetic studies can be performed using an automated stopped-flow system with automatic reagent handling equipment and a computer. The values are within the range of other works which this reference quotes, as seen in Table XII. It is unfortunate that Mieling chose an ionic strength of 1 M because the other chemists used ionic strengths near or below 0.5 M. Since he does not include an ionic strength study one cannot directly compare the values. The error bounds

TABLE XII

COMPARISON OF RATE CONSTANTS FOR THE REACTION OF Fe(III) AND THIOCYANATE

Method (ionic strength)	(ref)	k_1, $M^{-1}s^{-1}$	k_2, $M^{-1}s^{-1}$ $\times 10^{-3}$	k_{-1}, s^{-1}	k_{-2}, s^{-1} $\times 10^{-3}$
stopped-flow (u = 1)	(52)	97±3	9.6±5	0.75±0.03	2.2±0.1
stopped-flow (u = 0.4)	(53)	127±10	10±1		
press.-jump (u = 0.5)	(54)	150±50	26±2		
stopped-flow (u = 0.2)	(55)	90±5	5.1±0.5	1.6±0.2	1.0±0.1
press.-jump (u = 0.2)	(56)	132±50	42±5		
temp.-jump (u = 0.5)	(57)	130±40	13±2		

of his values are some of the lowest although k_2 is less accurate than the other reported values.

2.4.(e) Reaction for Mn(II) and Oxalic Acid Determination

Koupparis et al. (46) studied the reduction of permanganate by oxalate in the presence of Mn(II) in acidic media. Previous studies indicated that Mn(II) accelerates the reaction between permanganate and oxalate. Their studies involved determining the reaction orders of all the species participating in the reaction; Mn(II), permanganate, oxalate, and hydronium ions. This was done by using low concentrations of permanganate relative to the other three species. The reaction under these conditions was found to be first order. Then the concentrations of the other three species were changed, but kept in excess over permanganate, while the change in the apparent rate constant was observed. The slope of the log of the rate constant versus the log of the concentration gives the reaction order of the species. The rate equation resulting from this study was:

$$-d[MnO_4^-]/dt = k[Mn^{+2}][C_2O_4^{-2}]^2[MnO_4^-][H^+]^{-2}. \qquad (2.11)$$

The value of k was 5.4×10^4 $M^{-1}s^{-1}$ with a relative standard deviation of 11%. The rate equation was not used to hypothesize a plausibe mechanism. The authors did, however, use the information to set up conditions to determine Mn(II) and oxalic acid. The determinations have been discussed previously in this review.

2.5 Conclusion

This review describes how the stopped-flow mixer has been incorporated into a new analytical instrument. The drive syringes provide rapid and reproducible aliquoting of the sample and reagent to the mixer and the observation cell. In the past this rapid mixer was exploited primarily for the study of fast reactions. Another of its capabilities, which is more important to the analytical chemist, is the possibility of using the same system to process samples very quickly. All the elements required for this are present; the injection of a reproducible volume of sample and the transportation of the mixed sample and reagent both to and from the cell. Since each step is automated, samples can be processed very rapidly. For example, the determinations of phosphate and total protein have been improved dramatically by the technique. The time for determining phosphate has been reduced from several minutes to one second and for total protein from 30 minutes to less than 10 seconds. The problem of processing samples requiring long reaction times and therefore tieing up the observation cell, has been remedied by the SF/USA. Kinetic methods performed with the stopped-flow instrument benefit from the advantages inherent in all kinetic determinations. These include reduction of the interferences from slow side reactions and elimination of the effects of a background absorbance. The ability of the stopped-flow instrument to study fast reactions should not be ignored. By gaining detailed information about the kinetics of a reaction it enables the analyst to choose the best conditions for a determination. Because the stopped-flow technique has been demonstrated to be ideal for both characterizing reactions and then using them in rapid, selective, and precise measurements, its future does not depend

on further physical improvements but rather it depends on changing the way the instrument is perceived by the analytical community. This tool is no longer just a means for biochemists to study fast reactions, it is now a powerful sample processor.

CHAPTER III

EXPERIMENTAL METHODS AND PROCEDURES FOR A KINETIC STUDY OF THE COMPLEXATION OF FE(II) BY FERROZINE

3.1 Apparatus

The stopped-flow spectrophotometer used in the study of Fe(II) and Ferrozine was constructed from a number of components and is illustrated in Figure 3. The following is a list of the separate units used to construct the system:

(1) A 24-volt, 5-amp APS24-5 power supply (Adtech Power, Anaheim, CA);

(2) A 24-volt, 100-watt tungsten lamp, Osram 64460 (Berlin, Germany) in an AH10 housing (Instruments SA, Inc., Meutuchen, NJ);

(3) An H-10 1200VIS Jobin-Yvon concave holographic grating monochromator (Instruments SA, Inc., Meutuchen, NJ);

(4) A GCA/McPherson EU-730-11 stopped-flow mixing module (Acton, MA) with a five millisecond dead time;

(5) An EG&G UV-040 BG photodiode detector;

(6) A Lauda/Brinkman K-2/R water circulator (Westbury, NY) with $\pm 0.05\ ^{\circ}C$ temperature control;

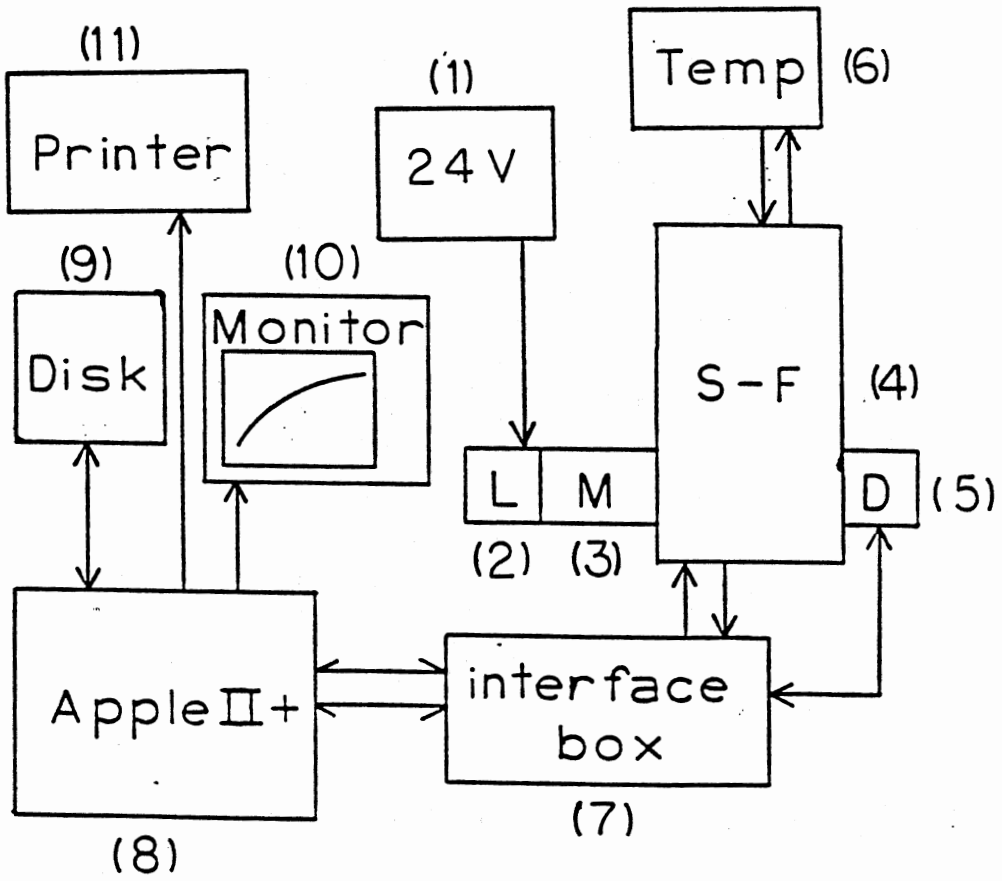


Figure 3. Block Diagram of the Stopped-Flow Spectrophotometer

(7) An in-house analog signal acquisition unit (described in Chapter IV) with ± 10 volt offset, $>10^{12}$ ohms impedence,and 12 bit resolution;

(8) An Apple II+ microcomputer (Cupertino, CA) upgraded to 64K of read/write memory;

(9) An Apple II disk drive with a controller card;

(10) A Zenith ZVM-121 12-inch green phosphor monitor;

(11) A C-ITOH 8510P Prowriter 80 column dot-matrix printer.

The Apple-based data acquisition system, items 7-11, was designed to replace the use of a Tektronix power supply (for signal offset), a Tektronix differential amplifier (for signal amplification), and a Nicolet digital oscilloscope with an interface to an HP9825 microcomputer. The HP-based system was comparable in most respects although the present system gives the operator greater software control of the data acquisition system.

The stopped-flow module was set to deliver 150μL of the reacting solutions for each reaction performed. The required pressure for pneumatic activation of the push syringes was obtained from a tank of nitrogen regulated at 35 psi. Nitrogen was chosen because it is not corrosive to the internal surface of the drive cylinder or driving piston. Figure 4 illustrates the mechanical workings of the stopped-flow module (58). The stopped-flow module consisted of several operational sections including: 1) the pneumatic drive system (N,R,V1,P1,P2,P3); 2) the solution flow system (S,R,V2,V3,V4,M,OC,SS); and 3) the waste release system (SS,VR). The stopped-flow unit contains electronic digital sequencing to operate solenoid valves V1 and V5. Only one control pulse is necessary to cycle the unit, which can be

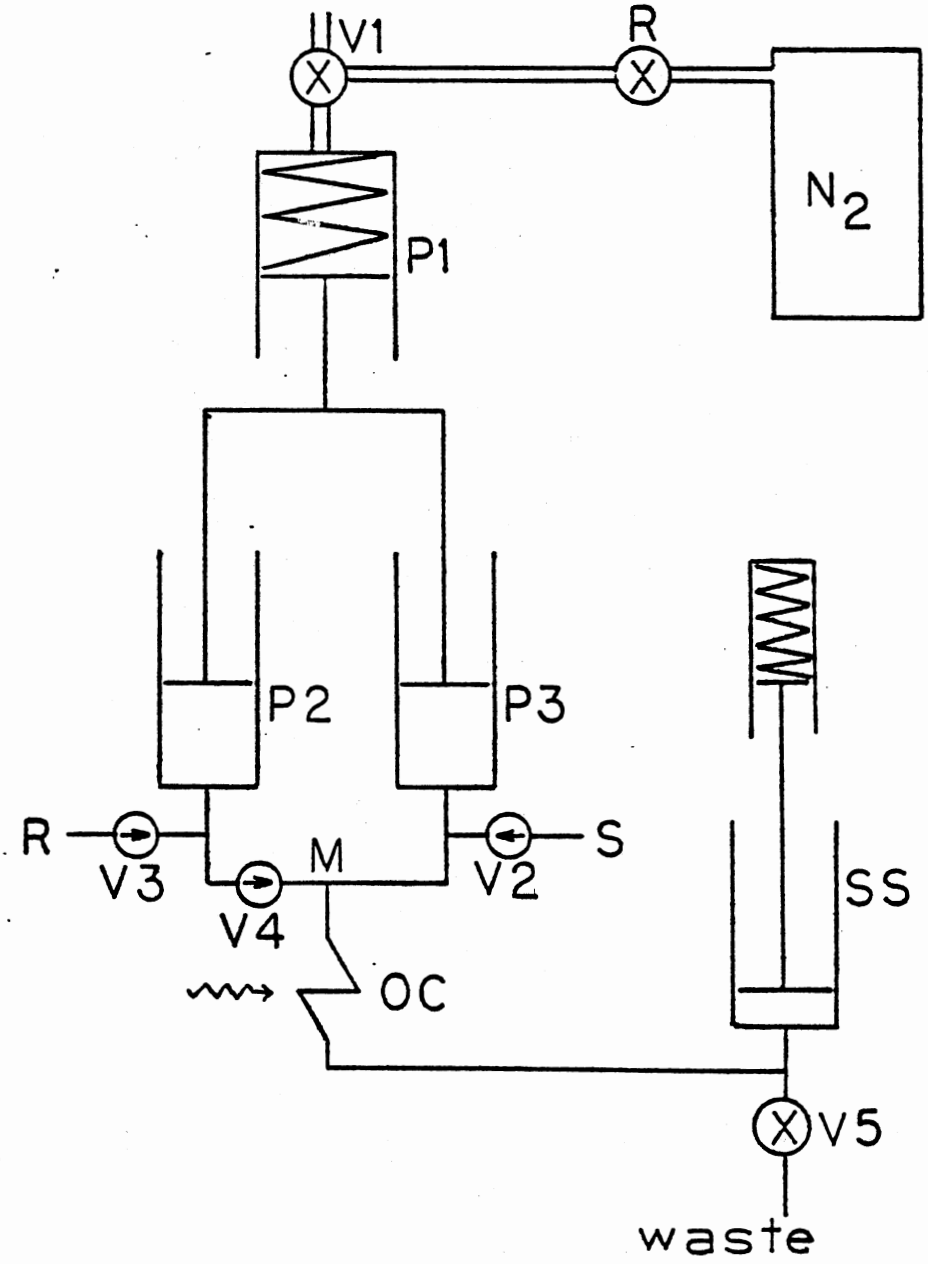


Figure 4. Mechanical Diagram of the Stopped-Flow Module

accessed from a front panel push button or can be activated from a microcomputer control line. The line is TTL compatible. Upon activation of the cycle control, solenoid valve V5 is opened allowing the solution in the stopping syringe SS to be released. After a delay V5 closes and the two position solenoid valve V1 releases the pressure in cylinder P1 which draws up pistons P2 and P3, aspirating fresh sample and reagent into the syringes. The unit now activates V1 to the position where the nitrogen gas enters P1 which drives P2 and P3 forcing the solution out of the drive syringes. One way valves V2 and V3 prevent any solution from returning to the containers from which they were drawn. One way valve V4 opens only under high pressure to prevent the reagent from being aspirated into drive syringe P3 but it allows the reagent to be forced by the drive syringe, along with the sample from the other syringe, into the tangential mixer M, through the observation cell OC and into the stopping syringe SS. When the stopping syringe is full the flow stops abruptly and the absorbance of the mixed reactants in the observation cell is monitored. The reaction can be monitored as long as necessary before another cycle command is given and the sequence begins again with the waste valve V5 opening.

The consumption of the nitrogen drive gas was low and a tank could be expected to last more than six months if it was shut off at night and when the stopped-flow unit was not being used. Several procedures were followed prior to the collection of quality data from the stopped-flow spectrophotometer. First, the lamp was allowed to warm up for approximately 30 minutes or until a stable baseline was obtained. Second, the constant temperature water circulator was used to circulate water at the desired temperature both through the copper coil in the stopped-flow

module itself and through a jacketed beaker where the samples would be thermally equilibrated before injection. For reactions which are not performed at room temperature, at least 15 minutes should be allowed for the samples to equilibrate with the water bath.

A Beckman 25 UV/VIS spectrophotometer was employed to monitor the reverse reaction (dissociation) of the complex in acid solution. Reagent solutions were added to the cuvette using syringes. The time necessary for manual mixing (approximately ten seconds) was insignificant in comparison to the time required for the reverse reaction (approximately eight hours). The Beckman 25 was equipped with a recorder/controller unit which allowed reactions to be recorded unattended overnight. Up to four cells could be sequentially switched in and out of the light path so that several reactions could be monitored at the same time.

The adjustment of pH in the buffer solutions was performed using an Orion Research (Cambridge, MA) Model 601A digital pH meter with a resolution of ±0.01 pH units. The potentiometric titration for the calculation of the pK_a of Ferrozine was also carried out using this instrument.

3.2 Reagents and Solutions

All of the chemicals used for the complexation study were of AR grade. The water used for solution preparation was deionized and further purified by distilling in an all-borosilicate glass still with a quartz immersion heater. Each solution was made 0.10 M in ionic strength by adding appropriate amounts of $NaClO_4$ to them. A phthalate buffer system was used in the pH study by adding volumes of either

0.010 M NaOH or 0.010 M $HClO_4$ to 0.010 M potassium acid phthalate until the desired pH was attained. Iron(II) solutions were prepared daily from $FeSO_4 \cdot 7H_2O$. Fresh solutions of Fe(II) are required because about 20% of the iron in such a solution is oxidized to Fe(III) in a 24-hour period. Ferrozine, in the monosodium dihydrate form, was found to be stable in solution for several weeks.

3.3 Procedure

The stopped-flow spectrophotometer was standardized daily by setting the zero absorbance with water in the cell and the maximum absorbance with a standard solution of iron(II)-Ferrozine complex of the same concentration as that expected at the completion of the reaction. The zero was set using the voltage offset adjustment on the interface unit and the maximum was set by adjusting the gain. The output of the detector was a voltage linear with absorbance so that a logarithmic function was not required. These adjustments were repeated when different iron concentrations were used (iron is the limiting reagent and only its concentration affects the final absorbance obtained). At least fifteen 250-μL injections were made before starting the collection of data to ensure a reliable run (after about ten injections all reaction profiles, for a given set of conditions, exhibited excellent replication). The graphical display of each run was inspected on the monitor for any anomalies that might be caused by disturbances such as air bubbles passing through the observation cell. If any extraneous artifacts were present, the run was discarded and another injection was made to replace it. After collecting a series of reaction profiles and

saving them to disk, the data was recalled and analyzed for a first order fit.

Because the reverse reaction required several hours, a double beam instrument (the Beckman 25) was used to avoid the long term drift present in the single beam system utilized by the stopped-flow unit. The reverse reaction was initiated by injecting 1.5 mL of 0.20 M HCl into 1.5 mL of a 9.00×10^{-6} M complex solution in a 1-cm cuvette in the Beckman 25 spectrophotometer.

Potentiometric titrations of Ferrozine solutions were performed by placing a 0.010 M Ferrozine solution (0.10 M in $NaClO_4$) into a thermostated titration vessel (25 °C) and titrating with a 0.115 M NaOH solution standardized against potassium acid phthalate.

CHAPTER IV

A MICROCOMPUTER-BASED DATA ACQUISITION SYSTEM

The design and operation of a microcomputer-based data acquisition system, which includes input signal conditioning, a 12-bit analog-to-digital converter, and a programmable clock, are described. The unit is suitable for acquiring signals with amplitudes from 50 mV to 3 V at rates of 5,000 points per second to 10 seconds per point. The system was designed to interface an Apple II+ microcomputer to a stopped-flow mixing unit. The rapid signal changes present when observing fast reactions (in the millisecond time scale) require high-speed recording units such as oscilloscopes. Since laboratory computers are frequently used today to analyze kinetic data, direct analog-to-digital conversion and storage within the computer itself eliminates the slow step of manually enterring data into the computer. The Apple II-based system has several advantages including its low cost and versatility. The unit could easily be adapted for use in applications such as chromatography and continuous-flow sample processing [e.g. flow injection analysis].

The design of the data acquisition system described here resulted from several constraints placed upon it by the stopped-flow mixing unit. Three basic criteria needed to be met: 1) high-speed data acquisition (since a reaction may be complete in 100 ms, a speed of at least 1 KHz is required for data collection), 2) provision for triggering the data acquisition system from the stopped-flow unit (average human reaction

times are too slow for hand triggering), and 3) impedance matching considerations (using high impedance sensors requires very high input impedance for the data acquisition system). The input amplifier should also provide convenient gain and offset adjustments. Each of these features is included in the data acquisition system described here.

4.1 Instrumentation

Three major functional blocks constitute the unit reported in this chapter. They are as follows: 1) the input amplifier, 2) the analog-to-digital section, and 3) the interface between the data acquisition components and the microcomputer bus. A description of each of these sections follows.

The input amplifier was constructed around three FET input operational amplifiers arranged so as to form a differential input instrumentation amplifier. The wiring block diagram is illustrated in Figure 5. Many low-cost amplifiers boast impedances of less than 100 K ohms which eliminates the possibility of using them directly with such high impedance signal sources such as pH probes or photodiodes. One side of our amplifier provides a high impedance (more than 10^{12} ohms) input for the photodiode detector. The other input provides an offset of ±10 V using a voltage divider between the ±15 V power supplies. The gain is adjusted by varying the resistance between the inverting sides of the input operational amplifiers. The gain is defined by the equation:

$$E_o = [(20{,}000 / R_g) + 1]\ E_i \qquad (3.1)$$

As with all high impedance sources, precautions such as using shielded

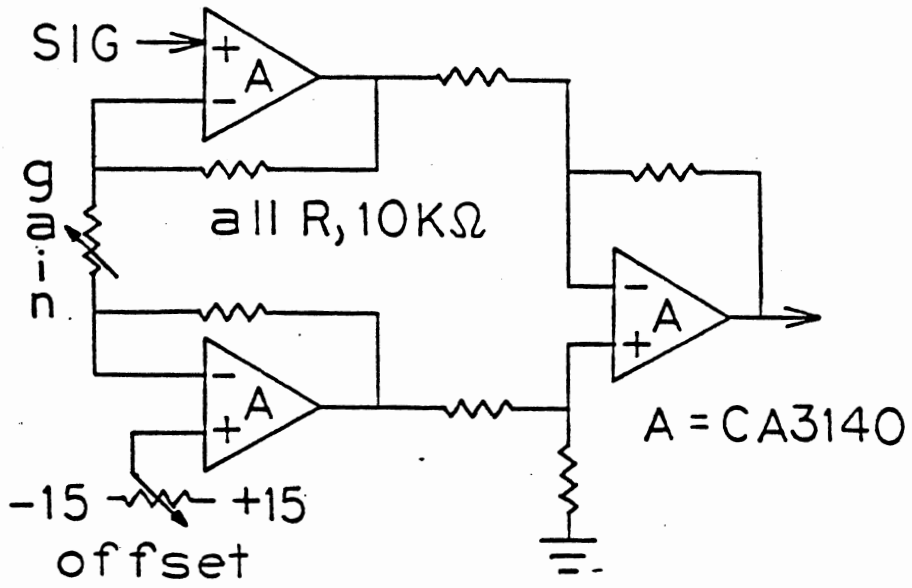


Figure 5. Wiring Diagram of the Input Amplifier

cable are required to keep noise levels to a minimum.

In order to achieve both the high speed and the resolution desired the Analog Devices AD574 analog-to-digital converter (59) was selected (Figure 6). In Figure 6 the values of the various discrete components are as follows: R1 = 50K, R2 = 10K, R3 = 2K, R4 = 18K, R5 = 5K (in ohms), C1 = C2 = 0.05μF, C3 = 1000pF, and C4 = C5 = 10pF. The A/D chip performs a 12-bit conversion in 25 microseconds. Pin 5 of the AD574 must be pulsed low to begin a conversion, otherwise it is held at 5 V to permit the data to appear on the output pins. The accuracy of the conversion is improved by holding the input voltage to the converter constant while a conversion is taking place. This is accomplished with the AD582 sample/hold amplifier (60) illustrated in Figure 6 in a unity gain configuration. The hold capacitor, C3, is a polystyrene unit to minimize the charge leakage while in the hold mode. The hold mode is initiated from the A/D at the beginning of a conversion with a 5 V signal going from pin 28 of the AD574 to pin 1 of the AD582. When the conversion is done, this line returns to 0 V and the sample/hold returns to sampling the signal.

The link from the data acquisition components to the microcomputer bus is provided by the Rockwell 6522 Versatile Interface Adapter (VIA) (61). The VIA (illustrated in Figure 6) performs the following four necessary functions: 1) latching the A/D data to the computer, 2) sending the begin conversion signal to the A/D , 3) providing a flag register for the trigger input, and 4) providing continuous interrupts with its built-in timer. Table XIII summarizes the addresses and functions of the VIA which are used in the data acquisition system. The timer consists of a 16-bit counter and two 8-bit registers. The counter

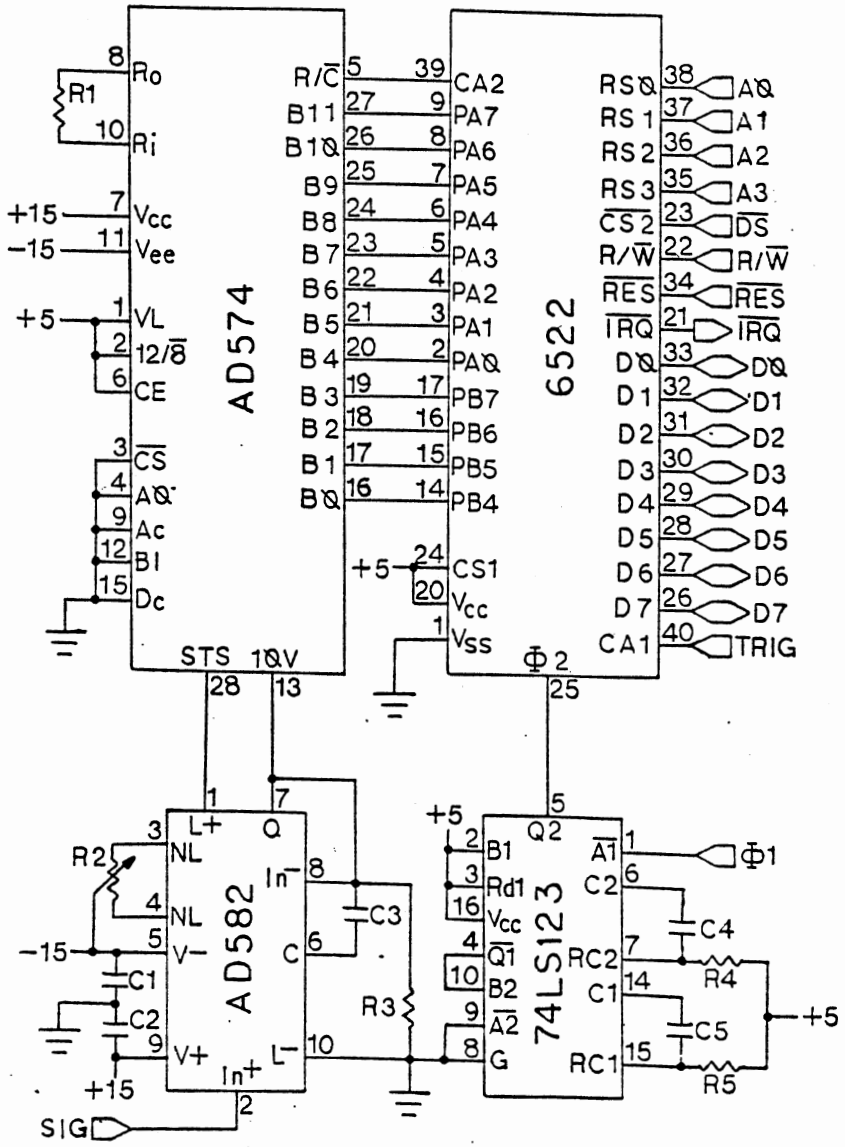


Figure 6. Wiring Diagram for the A/D and Interface Sections

TABLE XIII

ADDRESSES AND FUNCTIONS OF THE 6522 VIA

ADDRESS : NAME	FUNCTION
0 : Port B Latch	input of the four least significant bits of the A/D value
1 : CA2 Control	read to send start conversion low pulse to the A/D
4 : Timer 1 Low 5 : Timer 1 High	Timer 1 decrements to zero, interrupts the computer and counts down again
11 : Auxiliary Control	contains "64" for "free run" mode
12 : Peripheral Control	contains 10 (CA & CB operation mode)
13 : Interrupt Flags	bit 6 = 1 at time out of Timer 1 bit 1 = 1 at trigger in CA1
14 : Interrupt Enable	if bit 6 = 1 interrupt at Timer 1 out
15 : Port A Latch	input the eight most significant bits of the A/D value

decrements at the 1.023 MHz computer clock rate (62) until it reaches zero. The VIA then sends an interrupt request to the microprocessor, reloads the counter from the two registers, and counts down again. These interrupts provide the equally spaced intervals at which data is collected. The 74LS123 in Figure 6 is required to convert the phase 1 clock to phase 2 since phase 2 is not available at the Apple II+ expansion ports. The 74LS123 delays phase 1 about 400 nanoseconds. The other unused lines in the VIA could be used to expand the capabilities of the system, for example control lines could multiplex several channels for signal input.

4.2 Software

User interaction with the data acquisition system is provided by a control program (63) written in BASIC. Initially the operator may change any parameter such as the time per point, scale expansion, or trigger source. The allowable options are listed in Table XIV. When all the parameters are correct a call is made to an assembly language subroutine "GETDATA12" that stores the data during the timed interrupts after the trigger has occurred and until all the data has been collected. At this point the interrupts are turned off and a call is made to another assembly language program "PLOTDATA12". This program prepares the data for disk storage and plots it on the monitor for visual inspection. The data may then be saved on disk for later display or analysis. The self-documented listings of these programs are included in Appendix A.

TABLE XIV

SOFTWARE OPTIONS FROM THE BASIC CONTROL PROGRAM

KEY TO PRESS : FUNCTION
A : store data to disk
H : list settings
L : set trigger level and polarity
I : set calibration of the timer
M : sweep singly, continuously, or automatically
O : plot points or lines
P : view pretrigger data
S : select scale expansion
T : set time per point
V : display a data file
X : trigger external to signal
Y : display value of any point
R : collect data

4.3 Construction

Because of the digital and radio-frequency noise present within the Apple II's case, the data acquisition system was built in its own enclosure (a WA2-1A Cono-Case, Vector, Sylmar, CA). For flexibility in design and to provide enough amps for future expansions, a separate power supply (a PSB 203, ±15 V, 5 V power supply board and PT0006 transformer, Albia, New Haven, CT) was installed in the case. All the integrated circuits were mounted on a Vector (4112-5) breadboard and soldered in place. A 44-pin circuit board connector (Vector R644) holds the board and is attached by a ribbon cable to an RS232 jack in the case. Another ribbon cable connects the interface case to the Apple case RS232 connector. The RS232 jack on the Apple case is connected to the appropriate pins of an Apple prototyping card (A2B0001) in Slot 2. Mounted to the interface case are an on/off switch, a BNC connector for signal input, a fuse, and two Bourns counting dials (H-494-3, Riverside, CA) for the offset and gain adjustments.

4.4 Experimental

The data acquisition system was used to interface an Apple II+ microcomputer to a GCA/McPherson stopped-flow module, model EU730-11, for a study of the complexation of Fe(II) with Ferrozine (for the complete study refer to Chapters III and V). Figure 7 shows a typical plot of the results collected from a reaction of 8.00×10^{-6} M iron(II) and 8.00×10^{-4} M Ferrozine in 0.10 M $NaClO_4$ at a pH of 3.00. The temperature was 25 °C and the reaction was monitored at 562 nm. The data from each run was processed by a first order fit to find the initial and final concentrations of iron(II) as well as the

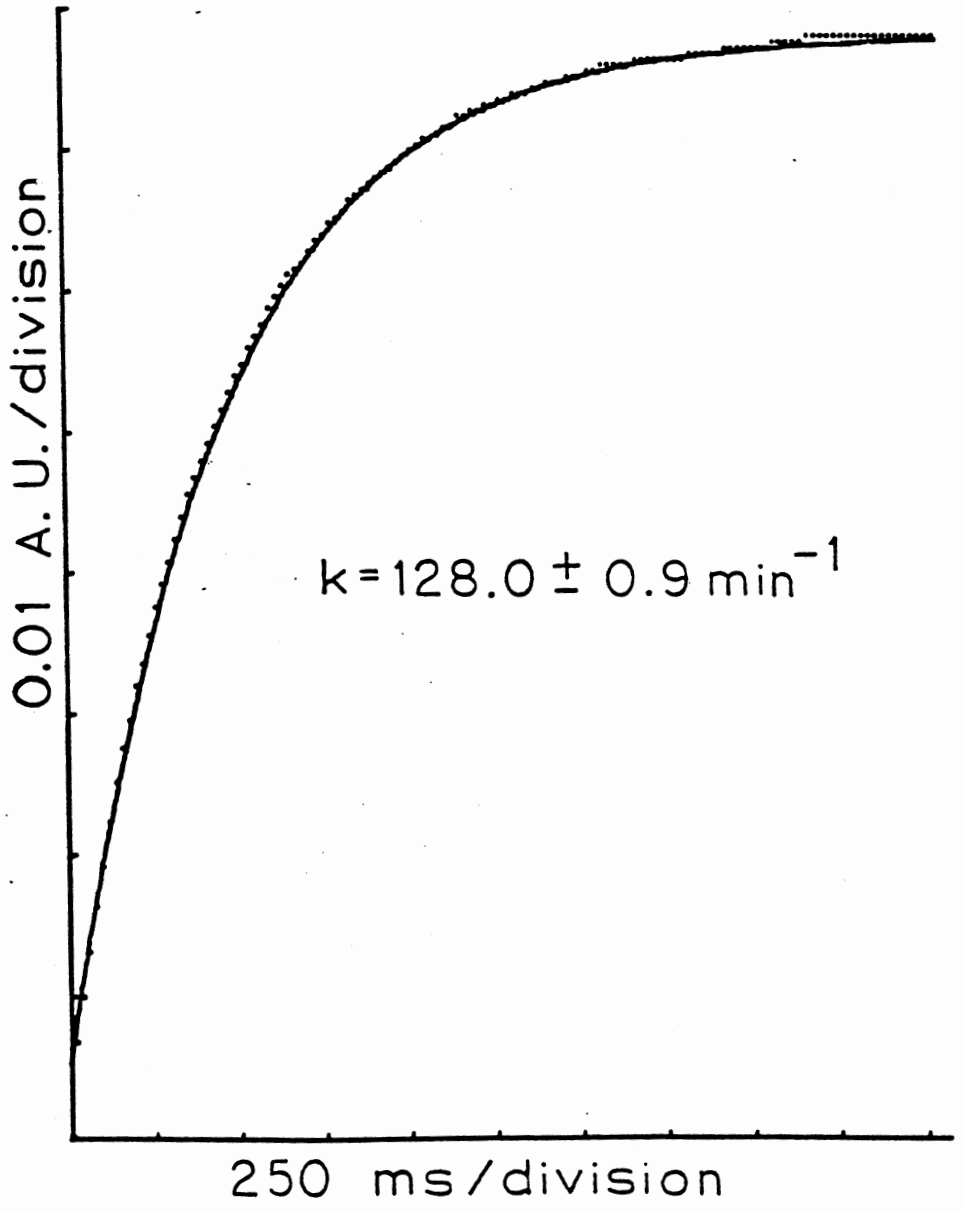


Figure 7. Typical Plot of Acquired Rate Data

corresponding rate coefficient. The reproducibility of the data from run to run was in most cases better than 99%.

4.5 Conclusion

Although the data acquisition system described here was designed for stopped-flow studies, its versatility and performance make it ideal for many other applications. The high input impedance is compatible with many probes or detection systems such as pH or ion-selective electrodes. The low cost of the unit (less than $300) has not been achieved at the expense of either speed or accuracy. The data acquisition system is well suited as a low-cost high-performance interface from a laboratory instrument to a computer.

CHAPTER V

EXPERIMENTAL RESULTS AND DISCUSSION FOR A KINETIC STUDY OF THE COMPLEXATION OF FE(II) BY FERROZINE

The complexation of Fe(II) by the reagent Ferrozine, benzenesulfonic acid,4,4'-[3-(2-pyridinyl)-1,2,4-triazine-5,6-diyl]bis-,monosodium salt dihydrate, was studied to determine the rate equation and the mechanism for the reaction. The reverse reaction, acid-base properties of Ferrozine, and the temperature dependence of the forward reaction were also studied. A comparison with two other common reagents for the spectrophotometric determination of iron in aqueous samples, 1,10-phenanthroline and 2,2'-bipyridine, is included. The following reaction is consistent with the known 3:1 ligand to metal stoichiometry of the complex (3):

$$Fe(II) + 3Fz \rightarrow Fe(II)Fz_3 \qquad (5.1)$$

A general rate equation was initially proposed as follows:

$$d[FeL_3] / dt = k_{obs} [Fe(III)]^x [Fz]^y \qquad (5.2)$$

5.1 Order with Respect to Fe(II)

Our initial goal was to determine the reaction order of the Fe(II) and Ferrozine. The Ferrozine concentration was held at 100 times the Fe(II) concentration with the thought that the resulting curve might be

first order since only one iron was involved in forming the complex. Numerical analysis of the curve to determine the order verified first order behavior for Fe(II). All subsequent reactions were run with an excess of the ligand. The first order curves were then fit by a nonlinear least square method proposed by Zuberbuhler and Kaden (64) to compute the rate coefficients and their standard deviations. Their method does not require either an initial or final concentration to be known. This is an advantage when analyzing stopped-flow data, where the first value is collected a few milliseconds after the reaction has started and there is no true time zero value.

5.2 Order with Respect to Ferrozine

The order with respect to Ferrozine was determined, again with the Ferrozine concentration about 100 times that of iron. The excess of Ferrozine insures a pseudo first order rate. The first order rate coefficients obtained under these conditions were used to compute the experimental order with respect to Ferrozine according to:

$$d[FeL_3] / dt = k_{obs} [Fe(II)] \qquad (5.3)$$

with $k_{obs} = k [Ferrozine]_o^y$. A log k_{obs} vs. log $[Ferrozine]_o$ plot allows one to obtain from the slope the value of y, the order with respect to the ligand. Values of y found under different conditions are tabulated in Table XV and indicate that the experimental order with respect to Ferrozine is three.

5.3 Rate Expression and Proposed Mechanism

Because of the low probability of a four-body collision, the

TABLE XV

EXPERIMENTAL ORDER WITH RESPECT TO FERROZINE

Temperature: 25.0 °C ; Ionic Strength: 0.10 M

Experimental Conditions	Order[a]
Phthalate buffer, pH = 5.00	2.98 2.94
Phthalate buffer, pH = 2.80	2.84
0.185 M H_2SO_4	2.80
The overall average order (9 replicas) was found to be 2.95 ± 0.14	

[a]See text for calculation.

proposed mechanism (in accordance with what has been reported for similar iron ligands (65,66)) contemplates the following steps:

$$Fe(II) + L \overset{K_1}{\rightleftharpoons} FeL \tag{5.4}$$

$$FeL + L \overset{K_2}{\rightleftharpoons} FeL_2 \tag{5.5}$$

$$FeL_2 + L \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} FeL_3 \tag{5.6}$$

where L is the Ferrozine ligand, K_1 and K_2 are equilibrium constants, and k_3 and k_{-3} are rate coefficients. Steps 5.4 and 5.5 are rapid pre-equilibrium ones and step 5.6 is the rate-determining step. These three steps lead to the following rate expression which is in accordance with the experimental orders reported above:

$$d[FeL_3] / dt = k_3 K_1 K_2 [Fe(II)] [L]^3 \tag{5.7}$$

Equation 5.7 is a result of considering the change in concentration of FeL_3 in step 6 as:

$$d[FeL_3] / dt = k_3[Fe(II)L_2][L] - k_{-3}[FeL_3] \tag{5.8}$$

and since $k_{-3} << k_3$ (as shown later), this leaves:

$$d[FeL_3] / dt = k_3 [FeL_2] [L] \tag{5.9}$$

Substituting the expressions for $[FeL_2]$ and the [FeL], derived from the equilibrium constant expressions, in Equation 5.9 one obtains Equation 5.7.

5.4 Rate Dependence on pH

In early experiments changes in pH were found to change the rate coefficient obtained using Equation 5.3. The reaction was noted as still following first order kinetics but with a change in k_{obs}. Therefore the effect of pH on the reaction rate was studied in the pH range of 2.80 to 5.50 fixed by phthalate buffers. For each reaction the concentration of iron(II) and Ferrozine were 8.00×10^{-6} M and 8.00×10^{-4} M respectively, and the ionic strength was adjusted to 0.10 M with $NaClO_4$. Figure 8 illustrates the effect of the hydrogen ion concentration on the observed rate coefficient, k_{obs}. This effect can be explained by recognizing the basic characteristics of the two nitrogens responsible for coordination to the iron (II) central ion and the proton competition for those basic sites. Such consideration leads to a postulation that the rate is directly proportional to the concentration of the unprotonated form of the ligand, which, from equilibrium considerations, is given by:

$$[L] = (K_a\ [HL^+]) \ / \ ([H^+]) \qquad (5.10)$$

By taking into consideration the mass balance equation for the ligand, $[L]_T = [HL^+] + [L]$, Equation 5.10 can be rewritten as:

$$[L] = ([L]_T\ K_a) \ / \ (K_a + [H^+]) \qquad (5.11)$$

and substituting in Equation 5.7 results in:

$$d[FeL_3] \ / \ dt = k_f\ [Fe(II)]\ \{\ ([L]_T\ K_a) \ / \ (K_a + [H^+])\ \}^3 \qquad (5.12)$$

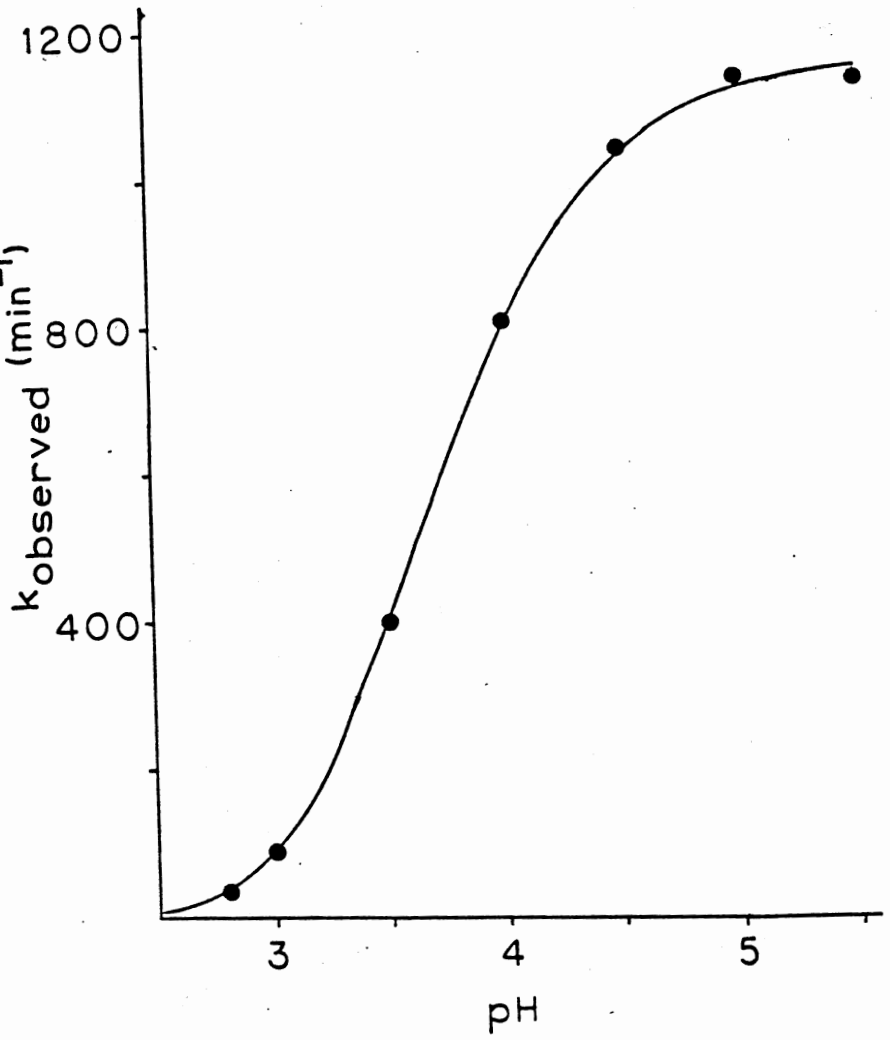


Figure 8. Effect of pH on the Observed Forward Rate Coefficient

To establish the validity of Equation 5.12 it was used to fit the k_{obs} vs. pH data in Figure 8 by assuming:

$$k_{obs} = k_f \{ ([L]_T K_a) / (K_a + [H^+]) \} \quad (5.13)$$

and finding the best k_f and K_a. The solid line in Figure 8 represents the best fit with a pK_a of 3.13 ± 0.02 and a forward rate coefficient of $1.85 \pm 0.02 \times 10^{13}$ $min^{-1}M^{-3}$. As a second test of the assumption that the rate is affected by the ratio of the unprotonated to the protonated form of the ligand, the pK_a of the ligand was independently determined by potentiometric titration and a value of 3.27 ± 0.05 was obtained, which agrees closely with the value extracted from the kinetic interpretation.

5.5 Reverse Reaction

The reverse reaction was studied in 0.10 M hydrochloric acid and allowed to react overnight. The dissociation was found to be first order in complex with a reverse rate coefficient of $2.55 \pm 0.3 \times 10^{-3}$ min^{-1}. This value in conjunction with the value of the forward rate coefficient reported earlier yields a formation constant for FeL_3 equal to $7.2 \pm 0.3 \times 10^{15}$ M^{-3}, which is of the same order of magnitude as the value of $3.6 \pm 1.6 \times 10^{15}$ M^{-3} obtained by a study under equilibrium conditions (9).

5.6 Temperature Dependence of the Forward Reaction Rate

Rate coefficients were experimentally determined from temperatures of 18.0 °C to 40.0 °C. Figure 9 illustrates the change in the

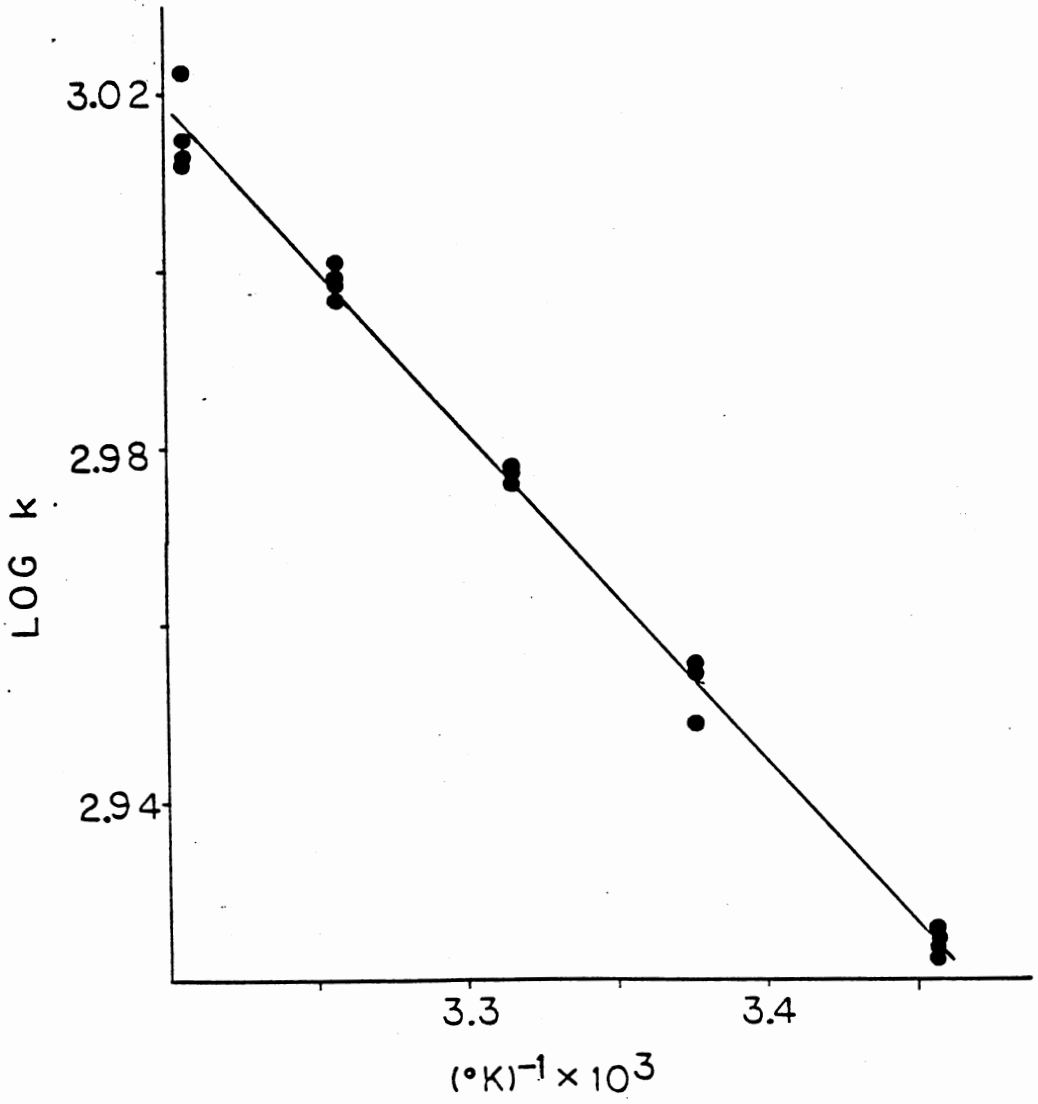


Figure 9. Temperature Dependence of the Forward Rate Coefficient

observed forward reaction rate coefficient with T^{-1}. The Arrhenius plot for the values yielded an activation energy of 2.36 ± 0.04 kcal/mole for the forward reaction. Low values for the activation energy seem to be characteristic of this type of ligand since for the iron(II) complexation by 2,2'-bipyridine a value of zero activation energy has been reported (65).

5.7 Comparison with 1,10-Phenanthroline and 2,2'-Bipyridine

Some information is available regarding ligands which have a similar structure to Ferrozine and are common reagents for determining iron(II). The most popular of these are 1,10-phenanthroline and 2,2'-bipyridine. Table XVI contains a summary of the characteristics of these ligands and their complexes with iron(II). The Ferrozine complex has the lowest values for both the forward rate coefficient and the equilibrium constant. These values, however, are relatively large and the complex formation may be classified as favored and fast. Also, because Ferrozine has the lowest pK_a of the three ligands, the rate of formation can be made faster than the others by lowering the pH. At a low solution pH the other ligands are more highly protonated than Ferrozine. Because the free ligand is the reactive form, less of these other ligands are available to react and the reaction proceeds at a slower rate.

TABLE XVI

COMPARISON OF FERROZINE WITH 1,10-PHENANTHROLINE AND 2,2'-BIPYRIDINE

	1,10-PHENANTHROLINE	2,2'-BIPYRIDINE	FERROZINE
Molar absorptivity, $M^{-1}cm^{-1}$	11,100 (1)	8,650 (3)	27,900 (1)
Solubility in water, g/100 mL,25 °C	0.3 (16)	0.5 (16)	7 (2)
pK_a	4.96 (17)	4.33 (18)	3.13 ± 0.02[a] (c) 3.27 ± 0.05[b] (c)
Forward rate coefficient, k_f, $min^{-1}M^{-3}$	$1.35 \pm 0.13 \times 10^{19}$ I = 0.50 25 °C (15)	$8.4 \pm 1.3 \times 10^{14}$ I = 0.025 17 °C (14)	$1.85 \pm 0.02 \times 10^{13}$ I = 0.10 25 °C (c)
Reverse rate coefficient, k_d, min^{-1}	4.5×10^{-3} I=0.50 25 °C (15)	7.3×10^{-3} I= 0.025 25 °C (14)	$2.55 \pm 0.03 \times 10^{-3}$ I = 0.10 25 °C (c)
Formation (k_f/k_d) constant, M^{-3},25 °C (direct)	3×10^{21} (15) 2×10^{21} (17)	1.21×10^{17} (14) 1.17×10^{17} (14)	$7.2 \pm 0.3 \times 10^{15}$ (c) $3.6 \pm 1.6 \times 10^{15}$ (2)
Activation energy of formation, kcal/mole	-	0 (14)	2.36 ± 0.04 (c)

[a] by kinetic interpretation.
[b] by potentiometric titration.
[c] This work.

CHAPTER VI

EXPERIMENTAL METHODS AND PROCEDURES FOR A KINETIC STUDY OF THE EFFECT OF GLUTARALDEHYDE ON THE OXIDATION OF p-PHENYLENEDIAMINE BY H_2O_2

6.1 Apparatus

A Beckman 25 UV/VIS spectrophotometer was used to monitor the progress of the reaction at 485 nm and 25 °C. The Beckman 25 is equipped with a chart recorder and an automatic cell changer. The cell changer allowed four reactions to be monitored at the same time by switching individual cells in and out of the light path. Manual mixing was employed and allowed adequate time to observe the reaction. Approximately the first thirty minutes were of the greatest interest so the 90 seconds used to mix the four solutions did not cause any significant loss of information. The individual reaction profiles were subsequently entered into an Apple II Plus microcomputer for numerical analysis.

The adjustment of pH in the buffer solutions was performed using an Orion Research (Cambridge, MA) Model 601A digital pH meter with a resolution of ±0.01 pH units.

6.2 Reagents and Solutions

All of the chemicals used were of AR grade. The water used for solution preparation was deionized and further purified by distilling in

an all-borosilicate glass still with a quartz immersion heater. All the solutions were maintained at 1.0 M ionic strength by the addition of appropriate amounts of $NaClO_4$. A phosphate buffer system was used and 0.1 M NaH_2PO_4 solution was added to 0.1 M Na_2HPO_4 solution (both in 1.0 M $NaClO_4$) until the desired pH was attained. Solutions of p-phenylenediamine were prepared about every three days. Fresh solutions were required because discoloration occured after several days. Hydrogen peroxide solutions were prepared from stock 30% H_2O_2 and were stored in the refrigerator. The glutaraldehyde was prepared from a 25% stock solution. Some controversy exists over the stabilization of these solutions for work with electron microscopy so two grades are available (70). Grade I is shipped in dry ice and under nitrogen while Grade II is delivered without any temperature precautions. Neither grade seemed to differ in terms of the effect on the oxidation of p-phenylenediamine so Grade II would be preferred as it is much less expensive.

6.3 Procedure

The reagents were added to the cuvettes in the same order and quantity to maintain reproducible experimental conditions. One milliliter of buffer was added first followed by 1 mL of hydrogen peroxide. One hundred and fifty μL of glutaraldehyde were then added. If a reaction without glutaraldehyde was to be performed then 150 μL of 1.0 M $NaClO_4$ was added instead to maintain the same overall volume in each experiment. This mixture was then put in the Beckman 25 and each cell was zeroed in absorbance at this time. The next step was the addition of 1 mL of p-phenylenediamine solution to each cell. Since the

addition of all reagents was performed with an automatic pipette little mixing occured during the introduction of the solutions. After the p-phenylenediamine was added, the cells were Teflon capped and inverted several times to insure complete mixing at the beginning of the reaction. Approximately 90 seconds elapsed between the addition of the final reactant and the recording of the first data point. The Beckman 25 was set to plot one point every 2 minutes for each cell and leave 20 seconds to cycle from one cell to the next. The scale expansion for absorbance depended upon the concentration of the reagents added and could be varied from 0.1 A to 2 A full scale.

CHAPTER VII

EXPERIMENTAL RESULTS AND DISCUSSION FOR A KINETIC STUDY OF THE EFFECT OF GLUTARALDEHYDE ON THE OXIDATION OF p-PHENYLENEDIAMINE BY H_2O_2

The product from the oxidation of p-phenylenediamine (PD) by hydrogen peroxide was first described by Bandrowski in 1894 (71). He proposed the following stoichiometry for this reaction:

$$3\ H_2O_2 + 3\ C_6H_8N_2 \rightarrow C_{18}H_{18}N_6 + 6\ H_2O. \qquad (7.1)$$

A structure for the product was also proposed at that time and is included in Figure 10a. Some controversy over the exact structure of this compound, referred to as Bandrowski's Base (BB), has arisen (72,73,74) and even now no consensus seems to have been reached as several current reference works use different structures to refer to the same species (Figure 10). Our interest has not been directed toward the structural determination of Bandrowski's Base but rather is concerned with reports about the catalytic activity of aldehydes upon the oxidation. Feigl (75) reported the use of the acceleration of the oxidation of p-phenylenediamine and visual detection of the formation of the brown Bandrowski's Base in the determination of the presence of aldehydes in solution. His work draws upon Woker's 1914 paper (76) describing the action of aldehydes in the acceleration of oxidations by hydrogen peroxide. Woker proposes a complex between the aldehyde and

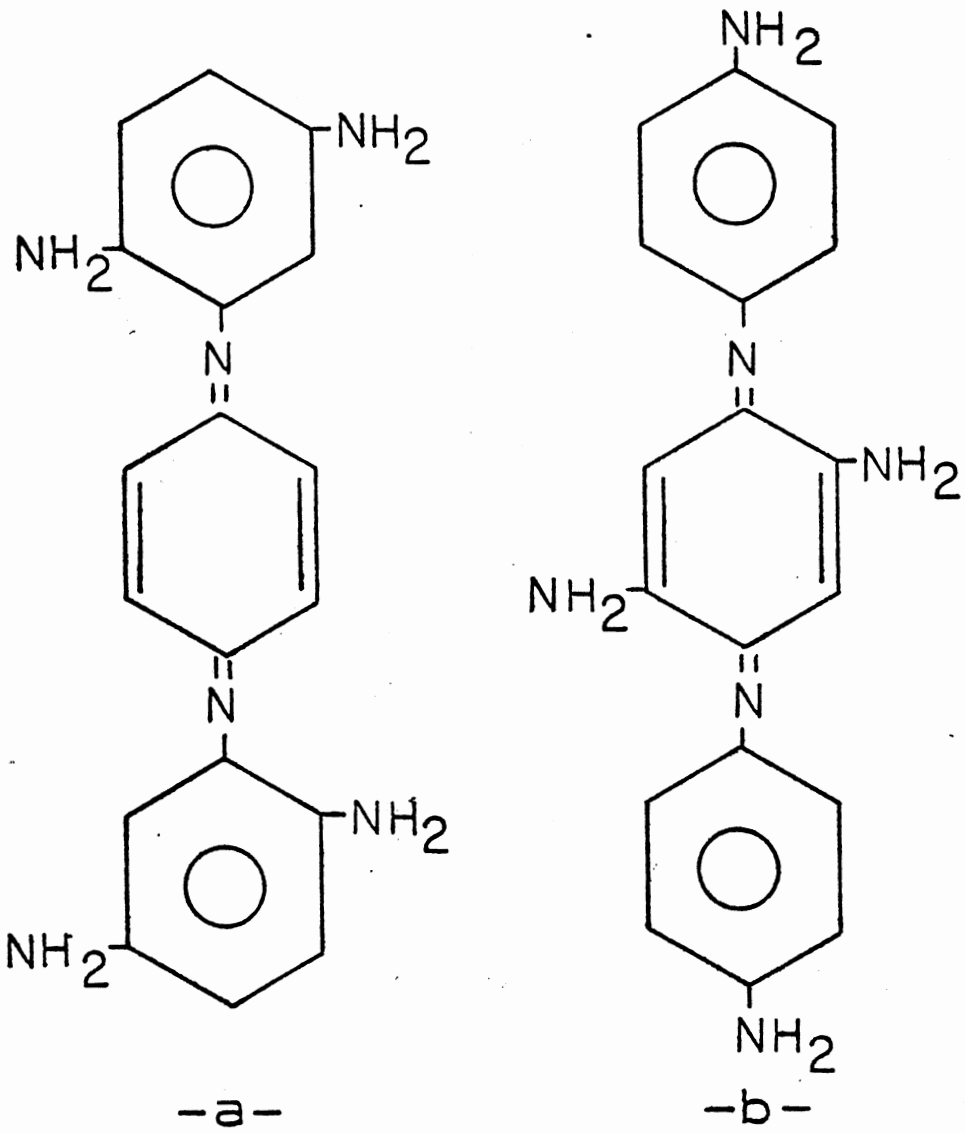


Figure 10. Proposed Structures of Bandrowski's Base

hydrogen peroxide that more easily releases oxygen for an oxidation than the action by the peroxide alone. Very few, if any, applications have been reported for using this reaction outside of Feigl's spot tests until recently. In 1980 Shapilov reported a kinetic determination of aldehyde groups on porous glass based on the acceleration of the oxidation of p-phenylenediamine to Bandrowski's Base in the presence of aldehyde groups (15). The attachment of aldehyde groups to glass is one of the steps in the most commonly used synthetic approach for immobilizing compounds on a glass surface. This is important in such areas as chromatography (with biospecific sorbents attached to glass) or continuous flow analysis (with enzymes attached to mixing coil walls). Unsuccessful attempts to utilize Shapilov's "catalytic" method in a continuous flow system prompted the following studies of the action of aldehyde on the oxidation of p-phenylenediamine by hydrogen peroxide. The continuous flow system consisted of a flowing stream of H_2O_2 into which p-phenylenediamine was intercalated and passed through a coil which was prepared to contain aldehyde groups covalently bonded to the glass. The absorbance of the p-phenylenediamine plug, after passage through the coil, indicates the amount of aldehyde in the coil. Tests of the reproducibility of the results were performed but it was noted that each subsequent injection of p-phenylenediamine gave lower results. Our study of the "catalytic" action in the homogeneous reaction was performed with the hope of providing an understanding of the problems observed with the heterogeneous system described above.

The spectrum of Bandrowski's Base (Figure 11) was taken and the maximum was found at approximately 485 nm and this wavelength was used for monitoring all the reactions. Initial experiments were conducted to

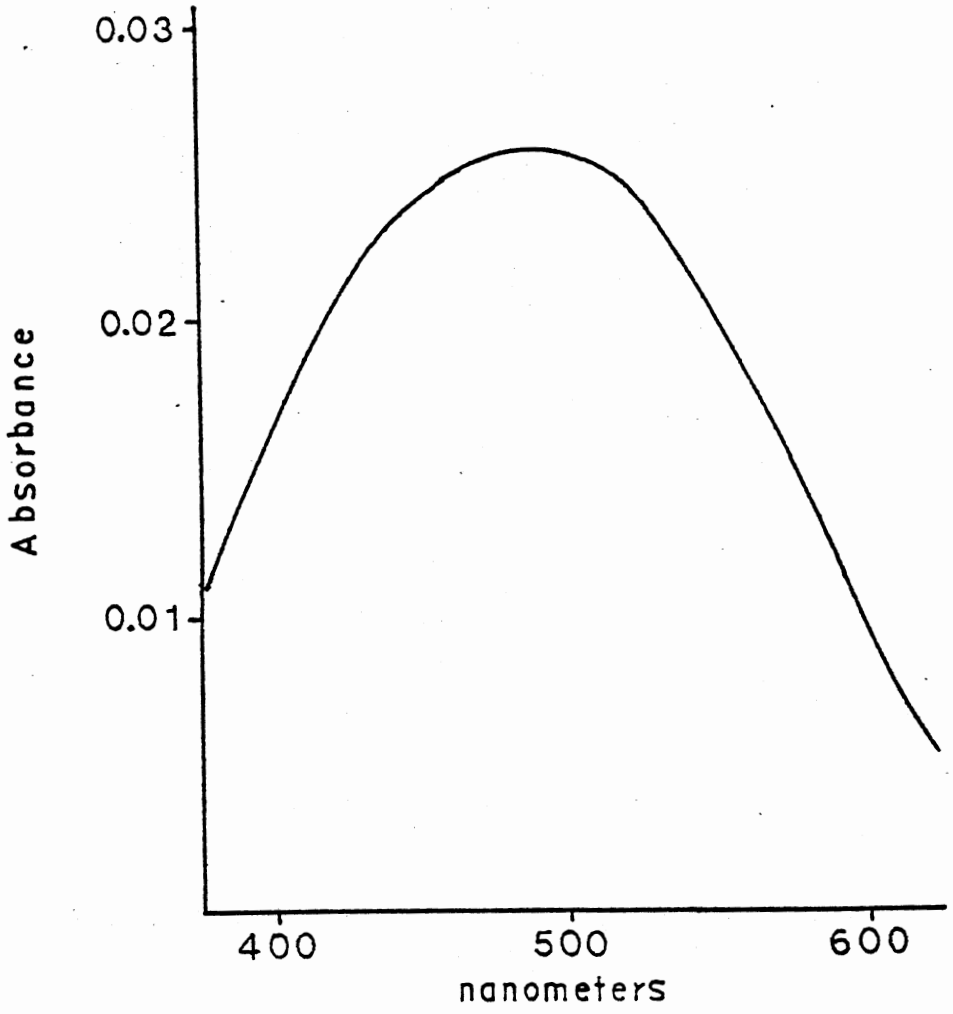


Figure 11. Visible Spectrum of Bandrowski's Base

give some indication of the reaction times involved. A problem was noticed immediately because Bandrowski's Base precipitated when an 8.75×10^{-3} M p-phenylenediamine solution was used. The absorbance at precipitation was 0.85 and all subsequent reactions were run at concentrations to insure that precipitation did not occur. Using the information from the literature the rate equation was believed to be of the form:

$$d[BB] / dt = k_u [PD]^x [H_2O_2]^y + k_c [PD]^x [\text{catalyst}]^z \qquad (7.2)$$

where k_u represents the uncatalyzed rate coefficient and k_c represents the catalyzed rate coefficient.

7.1 Order with Respect to p-Phenylenediamine and pH Effect

Reactions of p-phenylenediamine at concentrations of 7.0×10^{-5} M, 2.1×10^{-4} M, 3.5×10^{-4} M, 7.0×10^{-4} M with 0.34 M H_2O_2 at a pH of 5.00 and ionic strength of 1.0 M were conducted in the presence of 9.51×10^{-6} M glutaraldehyde. Assuming that the uncatalyzed reaction is very slow (very little change in absorbance was seen at these concentrations with no catalyst) the initial rates were used to determine the reaction order of p-phenylenediamine. The slope of the log of the initial rates versus the log of the initial concentrations yields the reaction order of p-phenylenediamine according to the equation:

$$\log (d[BB] / dt) = \log k_c + \log [\text{catalyst}] + x \log [PD]_o \qquad (7.3)$$

where x is the reaction order of p-phenylenediamine. This analysis resulted in an order of one for p-phenylenediamine.

Because the rate is dependent upon pH (15,75) the reactions both with and without glutaraldehyde were performed at pHs of 4.00, 5.00, 6.00, and 7.00. The reaction proceeded much faster at high pH. The reported value of the pK_a of p-phenylenediamine was used to calculate the concentration of just the unprotonated form of the ligand according to the following equation:

$$[PD] = ([PD]_T K_a) / (K_a + [H^+]) \qquad (7.4)$$

where [PD] is the concentration of the unprotonated form of p-phenylenediamine and $[PD]_T$ is the analytical concentration of p-phenylenediamine. Using this equation it was found that the initial rates were proportional to the initial concentration of the unprotonated form of the reactant p-phenylenediamine indicating that only the unprotonated form is reactive. At this point nothing unexpected was found regarding the catalyzed or uncatalyzed reaction. The concentration of unprotonated p-phenylenediamine was used in subsequent rate equations according to the findings above.

7.2 Effect of Glutaraldehyde

The effect of glutaraldehyde was studied by preparing solutions of 0 to 5.7×10^{-6} M glutaraldehyde used with 8.4×10^{-4} M (unprotonated concentration equaled 5.57×10^{-5} M) p-phenylenediamine and 0.34 M H_2O_2 at pH 5.00 and ionic strength of 1.0 M (Figure 12). The relationship of the initial rates and initial glutaraldehyde concentration were used to determine the reaction order of glutaraldehyde. In this case an

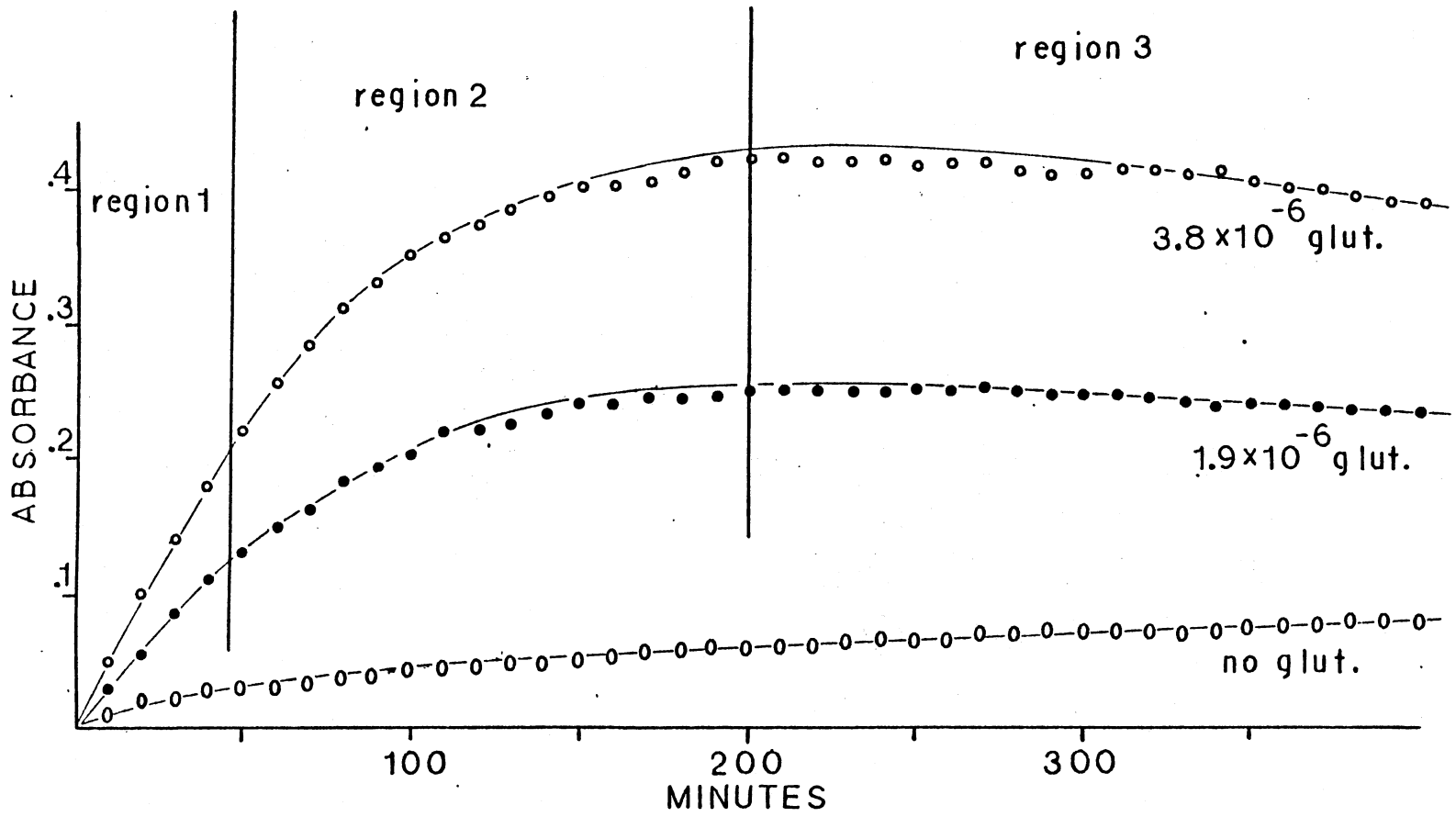


Figure 12. Reaction Curves for the Oxidation of p-Phenylenediamine with and Without Glutaraldehyde Present

experimental order of 2/3 was found for glutaraldehyde. However this study provided the first evidence that the effect of glutaraldehyde might not be catalytic. If glutaraldehyde were a catalyst and p-phenylenediamine were reacting by an order of one then the curves should have been a series of first order curves with different rates of ascent based on the concentration of the "catalyst" glutaraldehyde. The following equation should describe such curves:

$$d[BB] / dt = k_c [catalyst]_o [PD] = k' [PD] \qquad (7.5)$$

where the first-order rate coefficient, k' increases with increasing catalyst. Our findings did not correlate with first order curves of increasing rate coefficients and it was found that the maximum absorbance was less for each smaller amount of glutaraldehyde used. Also it was noted that after several hours the absorbance began to decrease. At this point we began to consider alternatives to a formation of product via a catalyzed and an uncatalyzed pathway as described by Equation (7.2). Subsequent experiments with the reaction in the presence of glutaraldehyde but with various concentrations of H_2O_2 revealed no changes in the initial portion of the curve but showed deviations closer to the maximum absorbance. This indicates that indeed some promotion in rate is experienced as a result of the glutaraldehyde alone and not H_2O_2. A promoter is destroyed in the process of accelerating a reaction whereas a true catalyst is regenerated (17,18). According to the type of aldehyde-hydrogen peroxide complex described in Woker's paper (76) we proposed a fast preequilibrium step between glutaraldehyde and H_2O_2 as follows:

$$H_2O_2 + \text{glutaraldehyde} \rightleftharpoons H_2O_2\text{-glutaraldehyde} \qquad (7.6)$$

Because H_2O_2 is in an approximately 10^5 molar excess over glutaraldehyde we can assume that almost all of the glutaraldehyde in solution is in the complexed form and promotes the oxidation of p-phenylenediamine. This complex explains why the catalyzed reaction is initially unaffected by the H_2O_2 concentration. The change at later reaction times was proposed to be an effect of the H_2O_2 on the loss of product through a subsequent reaction. A general rate equation was proposed at this point to describe all the experimental details:

$$d[BB] / dt = k_1 [PD]^1 [H_2O_2]^1 + k_2 [PD]^1 [\text{glutaraldehyde-}H_2O_2]^{2/3} - k_3[?] \qquad (7.7)$$

with a term k_3 to describe some reaction to account for the loss of Bandrowski's Base with time.

7.3 Loss of Bandrowski's Base

Since the previous set of reactions varying the glutaraldehyde had shown that glutaraldehyde may be destroyed during the reaction its concentration could not necessarily be considered constant during these reactions. To determine the effect of glutaraldehyde under conditions where glutaraldehyde did not change concentration, reactions were performed with a large excess of glutaraldehyde over p-phenylenediamine. In this manner the glutaraldehyde concentration could be considered constant. We varied the concentration of p-phenylenediamine from 1.16×10^{-6} M to 4.64×10^{-6} M (concentrations of the unprotonated species) in 0.34 M H_2O_2 and 4.76×10^{-4} M glutaraldehyde at pH 5.00 (Figure 13). These curves were first order in p-phenylenediamine as expected and the maximum absorbance of each was proportional to the

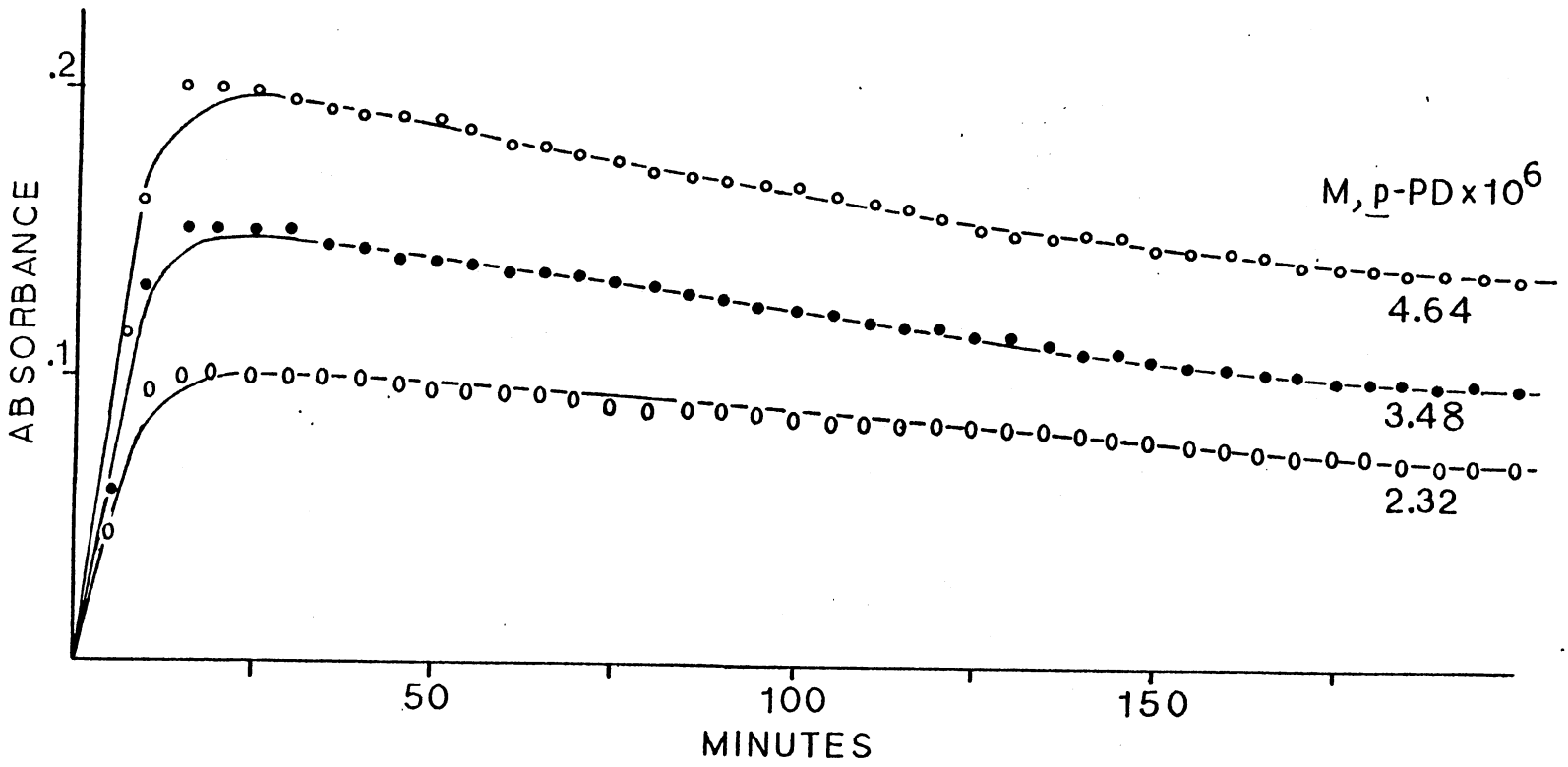


Figure 13. Reaction Curves for the Oxidation of p-Phenylenediamine with Excess Glutaraldehyde Present

concentration of p-phenylenediamine at the start of reaction. The reaction reached completion in a few minutes due to the excess of glutaraldehyde. Since the molar absorptivity of Bandrowski's Base is not reported, equilibrium absorbances gave us a means to estimate the molar absorptivity of this species. The value we determined from these four curves was $1.35 \pm 0.03 \times 10^5$ at the λ_{max} of 485 nm. This value filled an important gap because all previous absorbances could not be directly related to the concentration of the product. Another important consequence of conducting this reaction at constant glutaraldehyde concentration was further observation of the destruction of Bandrowski's Base after the absorbance reached the maximum. The rate of decrease was similar to the case with much lower glutaraldehyde concentration and was not affected by the glutaraldehyde to any appreciable extent as would be expected for over a hundred fold change in concentration. The experimental order of the "destruction reaction" was determined to be 2/3 with respect to the concentration of Bandrowski's Base. This was determined by measuring the initial rates of the decrease in absorbance after four different concentrations of the product had been formed. The rate equation proposed after these experiments (in accordance with all previous experimental results) was:

$$d[BB] / dt = k_1 [PD]^1 [H_2O_2]^1 + k_2 [PD]^1 [\text{glutaraldehyde-}H_2O_2]^{2/3} - k_3 [BB]^{2/3} [H_2O_2] \qquad (7.8)$$

7.4 Numerical Fit of Experimental Curves

The experimental curves using a constant concentration of p-phenylenediamine and H_2O_2 and varying the concentration of glutaraldehyde were subjected to a more rigorous numerical analysis to

determine the validity of the proposed rate equation. Because of the complexity of the reaction, a simple relation between the rate equation and the experimental curve does not exist. To help rationalize what is happening at each stage of the reaction the following reaction steps should be kept in mind:

$$3\ PD + 3\ H_2O_2 \xrightarrow{k_1} BB + 6\ H_2O \tag{7.9}$$

$$H_2O_2 + \text{glutaraldehyde} \overset{K_{eq}}{\rightleftharpoons} \text{glutaraldehyde-}H_2O_2 \tag{7.10}$$

$$3\ PD + \text{glutaraldehyde-}H_2O_2 \xrightarrow{k_2} BB + 3\ H_2O + \text{glutaraldehyde}' \tag{7.11}$$

$$BB + H_2O_2 \xrightarrow{k_3} P \tag{7.12}$$

Each of these reactions predominates at different times during the reaction. Initially Equation 7.11 predominates depleting p-phenylenediamine, H_2O_2, and glutaraldehyde. As glutaraldehyde is diminished the uncatalyzed reaction (7.9) will also be a significant contributor to the formation of Bandrowski's Base. As Bandrowski's Base increases, the reaction in Equation 7.12 predominates when glutaraldehyde has been almost entirely exhausted and the concentration of p-phenylenediamine is lowered significantly.

As mentioned previously there is no direct relationship between the curve obtained from the experiment and Equation 7.8, the experimental reaction rate equation. This arises from the fact that all the concentrations in the rate equation are changing, with the exception of H_2O_2 which is in great excess. To perform a numerical fit the change in concentration of each species must be calculated and substituted into

Equation 7.8 to determine how Bandrowski's Base has changed. The rate equations for each species can be proposed based on the series of equations (7.9-7.12) as follows:

$$d[PD] / dt = -3\ k_1\ [A]\ [B] - 3\ k_2\ [A]\ [C]^{2/3} \quad (7.13)$$

$$d[H_2O_2] / dt = -3\ k_1\ [A]\ [B] - 3\ k_2\ [A]\ [C]^{2/3} - k_3\ [D]^{2/3}\ [B] \quad (7.14)$$

$$d[\text{glutaraldehyde}] / dt = -\ k_2\ [A]\ [C]^{2/3} \quad (7.15)$$

$$d[BB] / dt = k_1\ [A]\ [B] + k_2\ [A]\ [C]^{2/3} - k_3\ [D]^{2/3}\ [B] \quad (7.16)$$

where A is p-phenylenediamine, B is H_2O_2, C is the glutaraldehyde-H_2O_2 complex, and D is Bandrowski's Base. To reconstruct the reaction curve, the change in Bandrowski's Base is computed for a given time increment and then added to the present concentration of Bandrowski's Base to give the concentration at time t + dt later. A Basic program was developed in house (Appendix B) to generate the change in concentration for each species. The new concentrations of these species at this time were used to compute the change in Bandrowski's Base. The change in Bandrowski's Base was added to the previous concentration and plotted at the time dt later. The fitted curve and the experimental curve are visually inspected for differences to find good initial values for k_1, k_2, and k_3. The sum square difference in the generated and experimental curves were used to refine the k values for the best fit via a successive approximations procedure. Table XVII lists the best fit of k_1 through k_3 values for a number of experimental curves. Figures 12 and 13 show the best fit line through the data points. The test of a rate equation lies in the consistency of the k values and those found indicate a good fit. The large standard deviation for k_2 may be a result of the error

TABLE XVII

BEST FIT RATE COEFFICIENTS

CONDITIONS	k_1	k_2	k_3
p-PD = 5.57×10^{-5} M			
H_2O_2 = 0.34 M			
Glut = 0	1.7×10^{-4}		1.0×10^{-4}
Glut = 1.9×10^{-6} M	1.7×10^{-4}	2.7	6.6×10^{-5}
Glut = 3.8×10^{-6} M	1.9×10^{-4}	2.9	8.0×10^{-5}
Glut = 4.76×10^{-3} M			
H_2O_2 = 0.34 M			
p-PD = 4.64×10^{-6} M	2.0×10^{-4}	7.6	6.4×10^{-5}
p-PD = 3.48×10^{-6} M	1.7×10^{-4}	8.1	6.4×10^{-5}
p-PD = 2.32×10^{-6} M	1.8×10^{-4}	8.7	5.6×10^{-5}
p-PD = 1.16×10^{-6} M	1.7×10^{-4}	8.9	6.4×10^{-5}
AVERAGE VALUES	$1.8 \pm 0.1 \times 10^{-4}$	6.5 ± 2.6	$7.0 \pm 1.4 \times 10^{-5}$

in determining the time of the initial data point because of the time required for mixing the reactants. The value of k_2 will be the most affected by errors in the initial time. Also there is less certainty in the value of the glutaraldehyde concentration because more dilutions are required to obtain the low concentrations used.

It should be noted that over twenty different combinations of reaction rate equations for these species were subjected to the same fit and not found satisfactory. We found that every mechanism based on the assumption that glutaraldehyde was a true catalyst did not provide even a marginal fit. The empirical reaction model (77) and the rate equations describe the experiments considered and also provide an explanation for the unsatisfactory performance of this reaction for the determination of bound aldehyde groups using continuous-flow analysis. Since the glutaraldehyde is destroyed during the reaction, in a continuous-flow system each pass of p-phenylenediamine will destroy some of the aldehyde groups and result in lower amounts of reaction each time.

CHAPTER VIII

CONCLUSIONS

The kinetic study of the Fe(II)-Ferrozine complexation described in this thesis provides a complete mechanism for the reaction and explains previous observations concerning the effect of solution pH on the complex. Solution pH contributes largely to the rate of complex formation because it affects the amount of reactive Ferrozine (the unprotonated form) present in solution. Since the effect of Ferrozine is third order in the rate equation, small changes in pH will have large effects on the observed rate of reaction. This provides a convenient way to tailor the reaction rate of a kinetic method when a determination of Fe(II) is contemplated. The pK_a was determined and is important when comparing the forward rate coefficients of other Fe(II) ligands to that for Ferrozine. Because the pK_a of Ferrozine is lower than for 1,10-phenanthroline or 2,2'-bipyridine (two other popular Fe(II) reagents) the reaction of Ferrozine and Fe(II) will still be very fast at low pH even though its k_f is smaller than for the other two reagents. The high molar absorptivity of the Fe(II)-Ferrozine complex coupled with the favorable kinetic information indicated that the reaction is both favored and fast and justifies why Ferrozine should be a reagent of choice for the determination of Fe(II). The information obtained as a result of the kinetic study is also useful for future Fe(II) determinations and for studies of the effect of kinetics in

continuous-flow analysis where a kinetically well characterized reaction system is necessary.

A microcomputer-based data acquisition system was developed to aid in the previous study. It has proven to be extremely useful for rapidly acquiring signals with high resolution and permitting them to be analyzed immediately after they are collected. The acquisition system is many times faster and more accurate than using a conventional oscilloscope and photographed reaction traces. The system has also proven to be lower in cost and of greater flexibility than a digital oscilloscope and a parallel interface to a microcomputer. The general design of the input section with its range of offsets and gains and the high input impedance make this data system readily suited for other systems such as chromatography or continuous-flow analysis.

The study of the oxidation of p-phenylenediamine by H_2O_2 and the effect of glutaraldehyde on this reaction has led to an empirical reaction model and rate equations consistent with experimental details. The study has also provided a value for the molar absorptivity of Bandrowski's Base (the reaction product). The general reaction scheme has been elucidated by considering the reaction profile in the presence of glutaraldehyde to contain three main regions of interest (Figure 12). The first involves the rate promoting effect of glutaraldehyde on the oxidation by considering a previous fast complex formation between glutaraldehyde and H_2O_2. This region is the one reported in the two published works where a spot test indicates the presence of aldehydes by their "catalytic" effect and bound aldehyde groups on glass "catalyze" the oxidation of p-phenylenediamine. By consideration of this region alone one may easily conclude that the effect of glutaraldehyde is

indeed catalytic. However study of the reaction for several hours indicates the presence of two other regions which by their presence demonstrate that glutaraldehyde is not catalytic (regenerated) but instead only promotes the reaction (destroyed after reaction). The second region is an area where the glutaraldehyde is almost gone and the formation of Bandrowski's Base is contributed to by both the promoted and unpromoted reaction. Finally the third region is described by using a subsequent reaction of Bandrowski's Base with H_2O_2 to form another compound causing a decrease in absorbance. The rate of this last reaction is not affected by glutaraldehyde. The effect of glutaraldehyde then is that of a promoter for the formation of Bandrowski's Base from p-phenylenediamine and H_2O_2. This effect describes the inadequacy of a continuous-flow analysis system to measure the amount of glutaraldehyde immobilized on glass with repetitive injections of p-phenylenediamine into a reagent stream of H_2O_2. Considering glutaraldehyde as a promoter as our results indicate, instead of a catalyst, will cause less glutaraldehyde to be available after each reaction with p-phenylenediamine. This is what has occured in attempts to use this method with continuous-flow analysis where less product was formed in each subsequent determination. The promotion of the reaction of p-phenylenediamine and H_2O_2 is still a useful means to determine aldehyde groups if the limitations discovered in this study are considered and the initial portion of reaction profiles are used.

BIBLIOGRAPHY

(1) Mortimer, C. E., Introduction to Chemistry, D. Van Nostrand Co., New York, New York, 1977, p. 6.

(2) Law, W. T., Crouch, S. R., Anal. Letters, 13, 1115-1128 (1980).

(3) Stookey, L. L., Anal. Chem., 42, 779-781 (1970).

(4) Carter, P., Anal. Biochem., 40, 450-458 (1971).

(5) White, J. M., Flashka, H. A., Clin. Chem., 19, 526-528 (1973).

(6) Yee, H. Y., Goodwin, J. F., Clin. Chem., 20, 188-191 (1974).

(7) Eswara Dutt, V. V. S., Hanna, A., Mottola, H. A., Anal. Chem., 48, 1207-1211 (1976).

(8) Brown, J. C., Agron. J., 64, 240-243 (1972).

(9) Gibbs, C. R., Anal. Chem., 48, 1197-1201 (1976).

(10) Schilt, A. A., Analytical Applications of 1,10-Phenanthroline and Related Compounds, Pergamon Press, New York, New York, 1969.

(11) Painton, C. C., Mottola, H. A., Anal. Chem., 51, 1713-1715 (1981).

(12) deAndrade, J. C., Rocha, J. C., Pasquini, C., Baccan, N., Analyst (London), 108, 621-625 (1983).

(13) Betteridge, D., Sly, T. J., Wade, A. P., Tillman, J. E. W., Anal. Chem., 55, 1292-1299 (1983).

(14) Thompsen, J. C., Mottola, H. A., Chem. Biol. Environ. Instrumentation (in press).

(15) Shapilov, O. D., J. Anal. Chem. USSR, 35, 1429-1431 (1980).

(16) Iob, A., Mottola, H. A., Clin. Chem., 27, 195-196 (1981).

(17) Mottola, H. A., CRC Crit. Rev. Anal. Chem., 4, 254 (1975).

(18) Eswara Dutt, V. V. S., Mottola, H. A., Anal. Chem., 46, 1090-1094 (1974).

(19) Javier, A. C., Crouch, S. R., Malmstadt, H. V., Anal. Chem., 40, 1922-1925 (1968).

(20) Malmstadt, H. V., Pardue, H. L., Anal. Chem., 34, 299-301 (1962).

(21) Javier, A. C., Crouch, S. R., Malmstadt, H. V., Anal. Chem., 41, 239-243 (1969).

(22) Cordos, E., Crouch, S. R., Malmstadt, H. V., Anal. Chem., 40, 1812 (1968).

(23) Desa, R. J., Gibson, Q. H., Comput. Biomed. Res., 2, 494 (1969).

(24) Willis, B. G., Bittikofer, J. A., Pardue, H. L., Margerum, D. W., Anal. Chem., 42, 1340-1349 (1970).

(25) Pardue, H. L., Rodriguez, P. A., Anal. Chem., 39, 901 (1967).

(26) Beckwith, P. M., Crouch, S. R., Anal. Chem., 44, 221-227 (1972).

(27) O'Keefe, K. R., Malmstadt, H. V., Anal. Chem., 47, 707-714 (1975).

(28) Krottinger, D. L., McCracken, M. S., Malmstadt, H. V., Amer. Lab., 3, 51-59 (1977).

(29) Landis, J. B., Rebec, M., Pardue, H. L., Anal. Chem., 49, 785-788 (1977).

(30) Meiling, G. E., Pardue, H. L., Anal. Chem., 50, 1611-1618 (1978).

(31) Koupparis, M. A., Walczak, K. M., Malmstadt, H. V., J. of Auto. Chem., 2, 66-75 (1980).

(32) Malmstadt, H. V., Walczak, K. M., Koupparis, M. A., Amer. Lab., 12(9), 27-40 (1980).

(33) Hartridge, H., Roughton, F. J. W., Roy. Soc. London A, 104A, 376-394 (1923).

(34) Gibson, Q. H., Milnes, L., Biochem. J., 91, 161-171 (1964).

(35) Bonnell, I. R., Defreese, J. D., Anal. Chem., 52, 139-142 (1980).

(36) McCracken, M. S., Malmstadt, H. V., Talanta, 26, 467-471 (1979).

(37) Sanderson, D., Bittikofer, J. A., Pardue, H. L., Anal. Chem., 44, 1934-1939 (1972).

(38) Pardue, H. L., Gaw, H. T., Mieling, G. E., Mathews, V. L., Fast, D. M., Rubesch, M. A., Clin. Chem., 23, 1230-1237 (1977).

(39) Karayannis, M. I., Anal. Chim. Acta, 76, 121-130 (1975).

(40) Krottinger, D. L., McCracken, M. S., Malmstadt, H. V., Talanta, 26, 549-561 (1979).

(41) McCracken, M. S., Malmstadt, H. V., J. Assoc. Off. Anal. Chem., 62, 23-28 (1979).

(42) Karayannis, M. I., Talanta, 23, 27-30 (1976).

(43) Brooks, L., Olken, H. G., Clin. Chem., 11, 748 (1965).

(44) Stewart, J. W. B., Ruzicka, J., Bergamin Filho, H., Zagatto, E. A., Anal. Chim. Acta, 81, 371 (1976).

(45) Margerum, D. W., Pausch, J. B., Nyssen, G. A., Smith, G. F., Anal. Chem., 41, 233-238 (1969).

(46) Koupparis, M. A., Karayannis, M. I., Anal. Chim. Acta, 138, 303-310 (1982).

(47) Pausch, J. B., Margerum, D. W., Anal. Chem., 41, 226-232 (1969).

(48) Coombs, L. C., Vasiliades, J., Margerum, D. W., Anal. Chem., 44, 2325-2331 (1972).

(49) Coetzee, J. F., Balya, D. R., Chattopadhyay, P. K., Anal. Chem., 45, 2266-2268 (1973).

(50) Koupparis, M. A., Malmstadt, H. V., Anal. Chem., 54, 1914-1917 (1982).

(51) Javier, A. C., Crouch, S. R., Malmstadt, H. V., Anal. Chem., 40, 1922-1925 (1968).

(52) Mieling, G. E., Pardue, H. L., Anal. Chem., 50, 1333-1337 (1978).

(53) Below, J. F., Connick, R. E., Coppel, C. P., J. Am. Chem. Soc., 80, 2961 (1965).

(54) Wendt, H., Streklow, H., Z. Elektrochem., 66, 228 (1962).

(55) Funakaski, S., Adachi, S., Tanoka, M., Bull. Chem. Soc. Jpn., 46, 479 (1973).

(56) Cavasino, F. P., Eigen, M., Ric. Sci., Part2, Sez. A, 4, 509 (1964).

(57) Goodall, D. M., Harrison, P. W., Hardy, M. J., Kirk, C. J., J. Chem. Educ., 49, 675 (1972).

(58) GCA/McPherson Instrument, Instruction Manual for Model 730-11 Stopped-Flow Module, GCA Corporation, Acton, MA, 1978.

(59) Analog Devices, Data Acquisition Components and Subsystems, Norwood, MA, 1980, pp. 10-35 to 10-42.

(60) Analog Devices, Data Acquisition Components and Subsystems, Norwood, MA, 1980, pp. 13-7 to 13-10.

(61) Rockwell International, R6500 Hardware Manual, Anaheim, CA, 1978, pp. 6-1 to 6-33.

(62) Apple Computer Inc., Apple II Reference Manual, Cupertino, CA, 1981, p. 90.

(63) Korba, L. L., Byte, 9, 520 (1982).

(64) Zuberbuhler, A. D., Kaden, T. A., Chimia, 31, 442-444 (1977).

(65) Baxendale, J. M., George, P., Trans. Faraday Soc., 46, 736-744 (1950).

(66) Lee, T. S., Kolthoff, I. M., Leussing, D. L., J. Am. Chem. Soc., 70, 3596-3600 (1948).

(67) Windholz, M., The Merck Index, Merck and Co., Inc., Rahway, NJ, 1976.

(68) Lee, T. S., Kolthoff, I. M., Leussing, D. L., J. Am. Chem. Soc., 70, 2348-2352 (1948).

(69) Baxendale, J. M., George, P., Trans. Faraday Soc., 46, 55-63 (1950).

(70) Gillett, R., Gull, K., Histochemie, 30, 162 (1972).

(71) Bandrowski, E., Ber., 27, 480-486 (1894).

(72) Green, J., J. Chem. Soc., 1913, 933 (1913).

(73) Ritter, J. J., Schmitz, G. H., J. Am. Chem. Soc., 51, 1587-1589 (1929).

(74) Lauer, W. M., Sunde, C. J., J. Org. Chem., 3, 261-264 (1938).

(75) Feigl, F., Spot Tests in Organic Analysis, Elsevier, New York, New York 7th Ed., 1966, p. 198-199.

(76) Woker, G., Ber., 47, 1024-1029 (1914).

(77) Come, G. M., Modern Methods in Kinetics, Vol. 24, C. H. Bamford and C. F. H. Tipper (Eds.), Elsevier, New York, New York, 1983, p. 252.

APPENDIXES

APPENDIX A

PROGRAM LISTING OF ROUTINES USED BY THE MICROCOMPUTER-BASED DATA ACQUISITION SYSTEM

```
100  REM -------  ACQUIRE12  -------
120  GOTO 920: REM JUMP TO START
140  REM **R**R**  RUN ACQUIRE  **R**R**
160  HGR
180  GOSUB 800
200  GOSUB 660
220  IF  PEEK (BG + 15) = 0 GOTO 280: REM  READY=0(RUN DONE)
240  IF  PEEK ( - 16384) ) 128 GOTO 360: REM KEY PRESSED
260  GOTO 220
280  GOSUB 420
300  IF ML$ = "S" THEN  GOTO 360
320  GOSUB 660
340  GOTO 240
360  CALL BG + 3: REM TURN OFF INTERRUPT
380  RETURN
400  REM *** PLOT DATA ***
420  CALL BP
440 LP% =  PEEK (BP + 280) * 16 +  INT ( PEEK (BP + 536) / 16): REM VAL LST PT
460  VTAB (22): HTAB (27): PRINT  SPC( 14)
480  VTAB (22): PRINT "VIEWING, MS/PT= ";T;"  LST PT= ";LP%
500  IF PRTR% = 0 GOTO 560
520  HCOLOR= 7
540  HPLOT PRTR%,0 TO PRTR%,159: REM  DRAW PRETRIGGER
560  IF XS = 1 THEN  GOTO 600
580 LS =  INT (159 - 8 * L% / 13): REM  DRAW LEVEL
600  HPLOT 1,LS TO 256,LS
620  RETURN
640  REM *** ACQUIRE DATA ***
660  POKE BG + 35,0: REM  RESET COUNTER
680  CALL BG: REM INIT INTERRUPT
700  IF ML$ (  ) "C" THEN  VTAB (21): PRINT "WAITING FOR TRIGGER ": GOTO 740
720  POKE BG + 27,0: POKE BG + 20,128:PRTR% = 0: REM SET PRETRIG=0,TRIG ON
740  POKE BG + 15,128: REM  SET READY,START ACQUIRING DATA
760  RETURN
780  REM *** DRAW AXES ***
800  HCOLOR= 7: FOR I = 0 TO 250 STEP 25: HPLOT I,159 TO I,156: NEXT I
840  FOR I = 0 TO 159 STEP 16: HPLOT 0,I TO 3,I: NEXT I
860  HPLOT 0,80 TO 7,81
880  RETURN
900  REM * * *PROGRAM START* * *
920 D$ =  CHR$ (4): REM  CNTRL-D
940  PRINT D$;"BLOAD GETDATA12.OBJ0"
960  PRINT D$;"BLOAD PLOTDATA12.OBJ0"
980 BG = 16384:BP = 20480: REM   BASE ADDRESS,GETDATA PLOTDATA
1000 T = 1:XS = 1:CH% = 0:PR% = 0:L% = 128:D% = 6:S = 0
1020 ML$ = "A":P$ = "+":TM = 1031:TB = 0
1040 M$(1) = "AUTOMATIC":M$(2) = "CONTINUOUS":M$(3) = "SINGLE"
1060 XS$(0) = "SET ON":XS$(1) = "EXTERNAL TO"
1080  GOSUB 1900: REM DISPLAY SETTINGS
1120  VTAB (21): HTAB (1): PRINT  SPC( 40)
1140  VTAB (21): PRINT "ENTER COMMAND KEY   (H=HELP)"
1160  VTAB (21): HTAB (29): GET K$: PRINT K$
```

```
1180 G =  FRE (0): REM  COLLECT GARBAGE
1200  IF K$ = "A" THEN  GOSUB 1560: REM  ADD TO DISK
1220  IF K$ = "C" THEN  GOSUB 1660: REM  CHANNEL
1240  IF K$ = "D" THEN  GOSUB 1740: REM  DELAY
1260  IF K$ = "E" GOTO 1860: REM         END
1280  IF K$ = "H" THEN  GOSUB 1900: REM  HELP
1300  IF K$ = "I" THEN  GOSUB 2320: REM  TIME CALIBRATE
1320  IF K$ = "L" THEN  GOSUB 2380: REM  LEVEL
1340  IF K$ = "M" THEN  GOSUB 2600: REM  MODE
1360  IF K$ = "O" THEN  GOSUB 2680: REM  ONLY POINTS
1380  IF K$ = "P" THEN  GOSUB 2780: REM  PRETRIGGER
1400  IF K$ = "R" THEN  GOSUB 160: REM   RUN
1420  IF K$ = "S" THEN  GOSUB 2920: REM  SCALE
1440  IF K$ = "T" THEN  GOSUB 3020: REM  TIME
1460  IF K$ = "V" THEN  GOSUB 3200: REM  VIEW DATA FILE
1480  IF K$ = "X" THEN  GOSUB 3340: REM  TRIGGER SOURCE
1500  IF K$ = "Y" THEN  GOSUB 3460: REM  Y VALUE
1520  GOTO 1120
1540  REM **A**A**  DATA TO DISK  **A**A**
1560  VTAB (23): INPUT "FILENAME TO DISK= ";F$
1580  PRINT D$;"BSAVE";F$;",A";BP + 25;",L512": REM SAVDAT
1600  VTAB (23): PRINT  SPC( 40)
1620  RETURN
1640  REM **C**C**  SET CHANNEL  **C**C**
1660  VTAB (23): PRINT "RECORDING CHANNEL = 0"
1680  POKE BG + 25,0: VTAB (23): PRINT  SPC( 40)
1700  RETURN
1720  REM **D**D**  A/D DELAY  **D**D**
1740  VTAB (23): INPUT "DELAY, START TO READ OF A/D= ";D%
1760  IF D% ) 255 GOTO 1740
1780  IF D% ( 0 GOTO 1740
1800  POKE BG + 26,D%: VTAB (23): PRINT  SPC( 40)
1820  RETURN
1840  REM **E**E**  PROGRAM END **E**E**
1860  TEXT : END
1880  REM **H**H**  HELP LISTING  **H**H**
1900  TEXT : HOME
1920 MN = 1: IF ML$ = "C" THEN MN = 2
1940  IF ML$ = "S" THEN MN = 3
1960  HTAB (10): PRINT " * * * * * * * * * *"
1980  HTAB (9): PRINT "DIGITAL SCOPE SETTINGS"
2000  PRINT : PRINT "## TO CHANGE VALUE PRESS (KEY) ##"
2020  PRINT : PRINT "(KEY)(PARAMETER LIMIT)*CURRENT STATUS"
2040  PRINT "-----------------------------------"
```

```
2060  PRINT "(M)(A,S,C)*";M$(MN);" SWEEP MODE"
2080  PRINT "(T)(.2-45)*";T;" MS/POINT"
2100  PRINT "(P)(0-255)*";PRTR%;" PRETRIGGER POINTS"
2120  PRINT "(L)(1-254 +/-)*TRIGGER LEVEL IS ";L%;" (";P$;")"
2140  PRINT "(X)*TRIGGER IS ";XS$(XS);" SIGNAL IN"
2160  PRINT "(D)(0-255)*A/D INTERNAL DELAY = ";D%
2180  PRINT "(C)(0)*A/D CHANNEL = ";CH%
2200  PRINT "(I)*CALIBRATION:M = ";TM
2220  PRINT "                   B = ";TB
2240  PRINT "(A)ADD TO DISK,(E)END,(R)RUN,(V)VIEW": PRINT "(Y)POINT VALUE"
2260  HTAB (10): PRINT " * * * * * * * * * *"
2280  RETURN
2300  REM **I**I**  CALIBRATE TIMER  **I**I**
2320  VTAB (23): PRINT "M,B=";TM;",";TB: VTAB (24): INPUT "M,B=";TM,TB: VTAB (23): PRINT  SPC(
      40): VTAB (24): PRINT  SPC( 40)
2340  GOTO 3100
2360  REM **L**L**  TRIGGER LEVEL  **L**L**
2380  VTAB (23): INPUT "TRIGGER LEVEL = ";L%
2400  IF L% < 1 GOTO 2380
2420  IF L% > 254 GOTO 2380
2440  VTAB (23): INPUT "POLARITY( +/- )= ";P$
2460  IF P$ = "+" THEN PN = 0: GOTO 2520
2480  IF P$ = "-" THEN PN = 128: GOTO 2520
2500  GOTO 2440
2520  POKE BG + 30,L%
2540  POKE BG + 16,PN: VTAB (23): PRINT  SPC( 40)
2560  RETURN
2580  REM **M**M**  SET MODE  **M**M**
2600  VTAB (23): PRINT "SINGLE S,CONTINUOUS C, AUTO A "
2620  VTAB (23): HTAB (35): GET ML$: PRINT ML$: VTAB (23): PRINT  SPC( 40)
2640  RETURN
2660  REM **O**O**  PLOT ONLY POINTS  **O**O**
2680  VTAB (23): INPUT "0=LINE, 1=POINTS";O
2700  IF O > 1 OR O < 0 GOTO 2680
2720  POKE BP + 20,O: VTAB (23): PRINT  SPC( 40)
2740  RETURN
2760  REM **P**P**  PRETRIGGER  **P**P**
2780  VTAB (23): INPUT "PRETRIGGER = ";PRTR%
2800  IF PRTR% < 0 GOTO 2780
2820  IF PRTR% > 255 GOTO 2780
2840  POKE BG + 27,PRTR%
2860  VTAB (23): PRINT  SPC( 40)
2880  RETURN
2900  REM **S**S**  SCALE OF DISPLAY **S**S**
2920  VTAB (23): INPUT "SCALE= (0-5)  11-5(0);10-4(1);...";S
2940  IF S > 5 OR S < 0 GOTO 2920
2960  POKE BP + 19,S: CALL BP + 3: VTAB (23): PRINT  SPC( 40)
2980  RETURN
```

```
3000  REM **T**T**  TIME PER POINT  **T**T**
3020  VTAB (23): INPUT "MS / POINT = ";T:TS = T:TE = 1
3040  IF T < .2 GOTO 3020
3060  IF T > 11430 GOTO 3020
3070  IF T > 45 THEN TE =  INT (T / 45) + 1:TS = T / TE
3080 TS = (TM * TS) + TB
3100  POKE BG + 29, INT ((TS / 256 -  INT (TS / 256)) * 256 + .05) *  SGN (TS / 256)
3120  POKE BG + 28, INT (TS / 256): POKE BG + 31,TE
3140  VTAB (23): PRINT  SPC( 40)
3160  RETURN
3180  REM **V**V**  VIEWING FILE  **V**V**
3200  VTAB (23): INPUT "FILENAME TO VIEW= ";F$
3220  PRINT D$;"BLOAD";F$;",A";BP + 25: REM SAVDAT
3240  POKE BG + 35,00: REM POINT
3260  CALL BP + 3
3280  VTAB (23): PRINT  SPC( 40)
3300  RETURN
3320  REM **X**X**  TRIGGER SOURCE  **X**X**
3340  VTAB (23): INPUT "TRIGGER SOURCE;A/D=0 EXTERNAL=1";XS
3360  IF XS < 0 GOTO 3340
3380  IF XS > 1 GOTO 3340
3400  POKE BG + 17,XS: VTAB (23): PRINT  SPC( 40)
3420  RETURN
3440  REM **Y**Y**  Y VALUE  **Y**Y**
3460 FL =  - 1
3480  HTAB (1): VTAB (23): INPUT "(ENTER 999 TO QUIT) POINT # = ";VX
3500  IF VX > 256 THEN 3640
3520  IF FL >  - 1 THEN  HCOLOR= 0: HPLOT FL,0 TO FL,159: HCOLOR= 7: HPLOT FL,159 -  INT ((8
      / 13) * VY%)
3540 VY% =  PEEK (BP + 281 + VX)
3560  HTAB (1): VTAB (24): PRINT  SPC( 20): HTAB (1): PRINT "Y = ";VY%;
3580 FL = VX - 1
3600  HPLOT FL,0 TO FL,159
3620  GOTO 3480
3640  HTAB (1): VTAB (23): PRINT  SPC( 40): PRINT  SPC( 40)
3660  RETURN
```

```
SOURCE FILE: GETDATA12
0000:           1 *SERVICE 6522 TIMER 1 INTERRUPTS
0000:           2 *
0000:           3 *----UPON INTERRUPT REQUEST----
0000:           4 *--(1) EXIT IF NOT TIMER 1
0000:           5 *--(2) PUT A/D & TRIGGER VALUES IN "SMPLHI" & "TRGSIG"
0000:           6 *--(3) EXIT IF NOT "READY"
0000:           7 *--(4) IF NOT ENOUGH PRETRG DATA,(13)
0000:           8 *--(5) ENOUGH PRETRG DATA SET "PREMET"
0000:           9 *--(6) IF "TRGSIG" NOT OPPOSITE OF "POL",(13)
0000:          10 *--(7) OPPOSITE SO SET "OPPMET"
0000:          11 *--(8) "TRGSIG" NOT CROSSED "TRLVL",(13)
0000:          12 *--(9) CROSSED SET "TRGMET" BEGIN POST TRG COUNT,(13)
0000:          13 *-(10) POST TRIG COUNT DONE - - FINISHED - -
0000:          14 *-(11) "POINT" SHOWS "RAWDTH" POSITION 1
0000:          15 *-(12) RESET "READY,PREMET,OPPMET,TRGMET & PSTCNT"
0000:          16 *-(13) PUT (2) VALUE IN "RAWDTH", INC "POINT"
0000:          17 *
0000:          18 *DEFINE ADDRESSES
03FF:          19 IRQAH   EQU  $03FF
03FE:          20 IRQAL   EQU  $03FE
C0A0:          21 ORB     EQU  $C0A0     49312
C0A1:          22 ORA     EQU  $C0A1
C0A4:          23 T1CL    EQU  $C0A4
C0A5:          24 T1CH    EQU  $C0A5
C0AB:          25 ACR     EQU  $C0AB
C0AC:          26 PCR     EQU  $C0AC
C0AD:          27 IFR     EQU  $C0AD
C0AE:          28 IER     EQU  $C0AE
C0AF:          29 ORAN    EQU  $C0AF     NO EFFECT ON PORTS
00C0:          30 INTEN   EQU  $C0
0040:          31 INTDIS  EQU  $40
0000:          32 *
----- NEXT OBJECT FILE NAME IS GETDATA12.OBJ0
4000:          33         ORG  $4000
4000:          34 *
4000:          35 *ENTRANCE POINTS
4000:4C 26 43  36         JMP  SETUP     16384 SET UP ROUTINE
4003:4C 66 43  37         JMP  INTOFF    SHUT OFF INTERRUPTS
4006:4C 76 43  38         JMP  FUT1      FUTURE EXPANSION
4009:4C 79 43  39         JMP  FUT2      -
400C:4C 7A 43  40         JMP  FUT3      -
400F:          41 *
400F:          42 *--FLAGS--MAY BE 0 OR 128
400F:00        43 READY   DFB  $00       16399 0DONE,128GO
4010:00        44 POL     DFB  $00       0+,128- TRIGGER POLARITY
4011:01        45 EXTRNL  DFB  $01       0A/D,1EXTERNAL
4012:00        46 PREMET  DFB  $00       128 ENOUGH PRETRIG DATA IN
4013:00        47 OPPMET  DFB  $00       128 TRGSIG OPOSIT SMPL FROM POL
4014:00        48 TRGMET  DFB  $00       128 TRIGGER MET
```

```
4015:           49 SA      DS   1         SAVE FOR FUTURE EXPANSION
4016:           50 SB      DS   1         -
4017:           51 SC      DS   1         -
4018:           52 SD      DS   1         -
4019:           53 *
4019:           54 *VARIABLES FROM BASIC
4019:00         55 CHNL    DFB  $00       16409 CHANNEL #
401A:06         56 DELAY   DFB  $06       A/D DELAY
401B:00         57 PRETRG  DFB  $00       PRETRIGGER AMOUNT
401C:04         58 T1CHV   DFB  $04       TIMER 1 HI VALUE
401D:00         59 T1CLV   DFB  $00       TIMER 1 LO VALUE
401E:80         60 TRLVL   DFB  $80       TRIGGER LEVEL
401F:01         61 EXTEND  DFB  $01       NUMBER OF INTERRUPTS TO SKIP
4020:01         62 SKIP    DFB  $01       INTERRUPTS LEFT TO SKIP
4021:           63 SG      DS   1         -
4022:           64 SH      DS   1         -
4023:           65 *
4023:           66 *VARIABLES FOR INTERNAL USE
4023:00         67 POINT   DFB  $00       16419 ARRAY POINTER
4024:           68 POSTRG  DS   1         INVERTED PRETRG
4025:           69 PSTCNT  DS   1         COUNT TO END OF DATA
4026:           70 SMPLHI  DS   1         CURRENT A/D VALUE HI
4027:           71 TRGSIG  DS   1         TRIGGER SIGNAL VALUE
4028:           72 SMPLLO  DS   1         CURRENT A/D VALUE LO
4029:           73 SJ      DS   1         -
402A:           74 SK      DS   1         -
402B:           75 SL      DS   1         -
402C:           76 SM      DS   1         -
402D:           77 *
402D:           78 *DATA ARRAY STORAGE
402D:           79 RAWDTH  DS   256       16429 DATA ARRAY HI ,1110987654
412D:           80 RAWDTL  DS   256       DATA ARRAY LO ,3210XXXX
422D:           81 *
422D:           82 *INTERRUPT SERVICE
422D:           83 *
422D:8A         84 START   TXA             X,Y TO STACK,ACC IN $45
422E:48         85         PHA             -
422F:98         86         TYA             -
4230:48         87         PHA             -
4231:AD AD C0   88         LDA  IFR        EXIT IF NOT TIMER1 INTERRUPT
4234:10 03      89         BPL  EXIT1      -
4236:0A         90         ASL  A          -
4237:30 03      91         BMI  CONT       -
4239:4C 1F 43   92 EXIT1   JMP  EXIT       -
423C:AD A4 C0   93 CONT    LDA  T1CL       RESET TIMER1 FLAG
423F:AD AD C0   94         LDA  IFR        SAVE EXTERNAL TRIGGER
4242:8D 27 40   95         STA  TRGSIG     -
4245:           96 *
4245:           97 *FOR EXTENDED ACQUIRE TIMES SKIP "EXTEND" INTERRUPTS
4245:CE 20 40   98         DEC  SKIP
```

```
4248:F0 03      99          BEQ  ATOD
424A:4C 1F 43  100          JMP  EXIT
424D:AD 1F 40  101 ATOD     LDA  EXTEND    START NEW COUNT
4250:8D 20 40  102          STA  SKIP
4253:          103 *
4253:          104 * STORE SMPLHI
4253:AD A1 C0  105          LDA  ORA       START A/D, CLEAR TRG CA1
4256:AE 1A 40  106          LDX  DELAY     DELAY TIL A/D DONE
4259:CA        107 WAIT     DEX             -
425A:D0 FD     108          BNE  WAIT      -
425C:AD AF C0  109          LDA  ORAN      GET DATA
425F:8D 26 40  110          STA  SMPLHI    -
4262:AD A0 C0  111          LDA  ORB       -
4265:8D 28 40  112          STA  SMPLLO    -
4268:          113 *
4268:          114 *CHECK FOR EXTERNAL SIGNAL
4268:AD 11 40  115          LDA  EXTRNL    IF = 1 EXTERNAL
426B:D0 09     116          BNE  EXTRG     -
426D:          117 *
426D:AD 26 40  118          LDA  SMPLHI    OR OTHER CHANNEL(LATER)
4270:8D 27 40  119          STA  TRGSIG    -
4273:4C 82 42  120          JMP  RDY       -
4276:          121 *
4276:AD 27 40  122 EXTRG    LDA  TRGSIG    SEE IF BIT1 = 1
4279:4A        123          LSR  A         TRIG FROM 6522 CA1 (5T08V)
427A:4A        124          LSR  A         -
427B:90 05     125          BCC  RDY       -
427D:A9 80     126          LDA  #$80      SET TRGMET
427F:8D 14 40  127          STA  TRGMET    -
4282:          128 *
4282:          129 * SEE IF READY FOR DATA COLLECTION
4282:AD 0F 40  130 RDY      LDA  READY     IF 0 EXIT ELSE GO
4285:D0 03     131          BNE  GO        -
4287:4C 1F 43  132          JMP  EXIT      -
428A:          133 *
428A:          134 * CHECK FOR ENOUGH PRETRIG DATA
428A:AD 12 40  135 GO       LDA  PREMET    ENOUGH PRETRIG DATA = $80
428D:F0 73     136          BEQ  BEFORE
428F:          137 *
428F:          138 * CHECK IF SIGNAL TRIGGERED
428F:AD 14 40  139          LDA  TRGMET    TRIGGERED = $80
4292:D0 4A     140          BNE  AFTER     STORE DATA IF TRIG
4294:          141 *
4294:          142 *CHECK FOR EXTERNAL TRIGGER
4294:AD 11 40  143          LDA  EXTRNL    SKIP LEVEL CHECK IF EXTERNAL
4297:D0 69     144          BNE  BEFORE    -
4299:          145 *
4299:          146 *WAS TRGSIG OPPOSITE SIDE OF SMPLHI FROM POL
4299:AD 13 40  147          LDA  OPPMET    OPPOSITE=80 SAME=0
429C:30 20     148          BMI  NOWOPP    JUMP IF READY
```

```
429E:           149 *
429E:           150 *IS IT OPPOSITE NOW
429E:AD 10 40   151         LDA  POL       CHECK POLARITY
42A1:F0 10      152         BEQ  PLUS1     -
42A3:           153 *
42A3:           154 *POLARITY(-) SO IS TRGSIG>TRLVL YET
42A3:AD 27 40   155         LDA  TRGSIG    CHECK FOR INPT>TRIG
42A6:CD 1E 40   156         CMP  TRLVL     -
42A9:90 57      157         BCC  BEFORE    -
42AB:           158 *
42AB:A9 80      159 SETOM   LDA  #$80      TRGSIG OPPOSITE TRLVL
42AD:8D 13 40   160         STA  OPPMET    SET UP INDICATOR
42B0:4C 02 43   161         JMP  BEFORE    -
42B3:           162 *
42B3:           163 *POLARITY(+) SO IS TRGSIG<TRLVL YET
42B3:AD 27 40   164 PLUS1   LDA  TRGSIG
42B6:CD 1E 40   165         CMP  TRLVL
42B9:90 F0      166         BCC  SETOM     CHECK FOR INPT<TRIG
42BB:4C 02 43   167         JMP  BEFORE    OTHERWISE CONTINUE
42BE:           168 *
42BE:           169 *
42BE:           170 *TRGSIG WAS OPPOSITE SO
42BE:           171 * HAS TRGSIG CROSSED TRLVL
42BE:AD 10 40   172 NOWOPP  LDA  POL       CHECK POLARITY
42C1:10 0B      173         BPL  PLUS2     -
42C3:           174 *
42C3:           175 * POLARITY(-) SO IS TRGSIG<TRLVL
42C3:AD 27 40   176         LDA  TRGSIG    SETTM IF<TRGSIG<TRLVL)
42C6:CD 1E 40   177         CMP  TRLVL     -
42C9:90 0B      178         BCC  SETTM     -
42CB:4C 02 43   179         JMP  BEFORE    -
42CE:           180 *
42CE:           181 * POLARITY(+) SO IS TRGSIG>TRLVL
42CE:AD 27 40   182 PLUS2   LDA  TRGSIG    TM NOT SET IF<TRGSIG<TRLVL)
42D1:CD 1E 40   183         CMP  TRLVL     -
42D4:90 2C      184         BCC  BEFORE    -
42D6:           185 *
42D6:A9 80      186 SETTM   LDA  #$80      INDICATE TRIGGERED
42D8:8D 14 40   187         STA  TRGMET    -
42DB:4C 02 43   188         JMP  BEFORE    -
42DE:           189 *
42DE:           190 *
42DE:           191 *JUMP HERE AFTER PRETIGGER DONE
42DE:AD 25 40   192 AFTER   LDA  PSTCNT    DATA LEFT BEFORE END
42E1:CD 24 40   193         CMP  POSTRG    POINTS AFTER PRETRIGGER
42E4:90 19      194         BCC  NOTEND    JUMP IF NOT DONE(PSTCNT<POSTRG)
42E6:           195 *
42E6:A9 00      196         LDA  #00
42E8:8D 14 40   197         STA  TRGMET    ZERO BUFFERS
42EB:8D 0F 40   198         STA  READY     -
```

```
42EE:8D 25 40  199         STA  PSTCNT    -
42F1:8D 13 40  200         STA  OPPMET    -
42F4:8D 12 40  201         STA  PREMET    -
42F7:A9 40     202         LDA  #INTDIS   -
42F9:8D AE C0  203         STA  IER       DISABLE INTERRUPTS
42FC:4C 1F 43  204         JMP  EXIT
42FF:EE 25 40  205 NOTEND  INC  PSTCNT    POINT TO NEXT STORE LOCATION
4302:          206 *
4302:          207 *JUMP HERE BEFORE PRETRIGGER
4302:AD 23 40  208 BEFORE  LDA  POINT     CURRENT DATA POSITION
4305:A8        209         TAY
4306:CD 1B 40  210         CMP  PRETRG    JUMP IF NOT ENOUGH(POINT(PRETRG)
4309:90 05     211         BCC  STORE     -
430B:A9 80     212         LDA  #$80      SET PREMET WHEN ENOUGH
430D:8D 12 40  213         STA  PREMET    -
4310:          214 *
4310:AD 26 40  215 STORE   LDA  SMPLHI    PUT SAMPLES IN ARRAY
4313:99 2D 40  216         STA  RAWDTH,Y  -
4316:AD 28 40  217         LDA  SMPLLO    -
4319:99 2D 41  218         STA  RAWDTL,Y  -
431C:EE 23 40  219         INC  POINT     INC STORE POINTER
431F:          220 *
431F:68        221 EXIT    PLA            RESTORE REGISTERS
4320:A8        222         TAY            -
4321:68        223         PLA            -
4322:AA        224         TAX            -
4323:A5 45     225         LDA  $45       RESTORE ACC
4325:40        226         RTI            RETURN FROM INTERRUPT
4326:          227 *
4326:          228 *END INTERRUPT ROUTINE
4326:          229 *
4326:          230 *INITIALIZE
4326:          231 *
4326:78        232 SETUP   SEI            DISABLE INTERRUPT
4327:A9 42     233         LDA  #(START   LOW ADDR OF START
4329:8D FF 03  234         STA  IRQAH     SET UP INTERRUPT VECTOR
432C:A9 2D     235         LDA  #)START
432E:8D FE 03  236         STA  IRQAL
4331:A9 0A     237         LDA  #10       LO PULSE ON CA2 ON R/W ORA
4333:8D AC C0  238         STA  PCR       -000/0/101/0
4336:A9 00     239         LDA  #00       SET UP VARIABLES
4338:8D 0F 40  240         STA  READY     FROM BASIC REDY=$80
433B:8D 23 40  241         STA  POINT     DATA POINTER
433E:8D 25 40  242         STA  PSTCNT    TO END OF DATA
4341:8D 14 40  243         STA  TRGMET    128=TRIGGERED
4344:A9 00     244         LDA  #00
4346:18        245         CLC            CLEAR CARRY
4347:ED 1B 40  246         SBC  PRETRG    SUB FROM ACC
434A:8D 24 40  247         STA  POSTRG    SAVE INVERTED PRETRIG
434D:A9 40     248         LDA  #$40      TIMER1"FREE RUN",PB7 DISABLED
```

```
434F:8D AB C0  249          STA  ACR       01/0/000/0/0
4352:AD 1D 40  250          LDA  T1CLV     SET UP TIMER 1
4355:8D A4 C0  251          STA  T1CL
4358:AD 1C 40  252          LDA  T1CHV
435B:8D A5 C0  253          STA  T1CH
435E:A9 C0     254          LDA  #INTEN    ENABLE INTERRUPTS
4360:8D AE C0  255          STA  IER
4363:D8        256          CLD             CLEAR DECIMAL
4364:58        257          CLI             ENABLE INTERRUPTS
4365:60        258          RTS
4366:          259 *
4366:          260 * TURN OFF INTERRUPT, CALL FROM BASIC
4366:A9 00     261 INTOFF   LDA  #00
4368:8D 14 40  262          STA  TRGMET
436B:8D 25 40  263          STA  PSTCNT
436E:8D 13 40  264          STA  OPPMET
4371:8D 12 40  265          STA  PREMET
4374:A9 40     266          LDA  #INTDIS
4376:8D AE C0  267 FUT1     STA  IER
4379:78        268 FUT2     SEI
437A:60        269 FUT3     RTS

*** SUCCESSFUL ASSEMBLY: NO ERRORS
```

```
 C0AB ACR        42DE AFTER      424D ATOD       4302 BEFORE
?4019 CHNL       423C CONT       401A DELAY      431F EXIT
 4239 EXIT1      401F EXTEND     4276 EXTRG      4011 EXTRNL
 4376 FUT1       4379 FUT2       437A FUT3       428A GO
 C0AE IER        C0AD IFR          40 INTDIS       C0 INTEN
 4366 INTOFF     03FF IRQAH      03FE IRQAL      42FF NOTEND
 42BE NOWOPP     4013 OPPMET     C0AF ORAN       C0A1 ORA
 C0A0 ORB        C0AC PCR        42B3 PLUS1      42CE PLUS2
 4023 POINT      4010 POL        4024 POSTRG     4012 PREMET
 401B PRETRG     4025 PSTCNT     402D RAWDTH     412D RAWDTL
 4282 RDY        400F READY     ?4015 SA        ?4016 SB
?4017 SC        ?4018 SD        42AB SETOM      42D6 SETTM
 4326 SETUP     ?4021 SG       ?4022 SH        ?4029 SJ
?402A SK         4020 SKIP      ?402B SL        4026 SMPLHI
?402C SM         4028 SMPLLO     422D START      4310 STORE
 401C T1CHV      C0A5 T1CH       C0A4 T1CL       401D T1CLV
 4014 TRGMET     4027 TRGSIG     401E TRLVL      4259 WAIT
```

```
   40 INTDIS        C0 INTEN        03FE IRQAL       03FF IRQAH
 400F READY       4010 POL         4011 EXTRNL      4012 PREMET
 4013 OPPMET      4014 TRGMET     ?4015 SA         ?4016 SB
?4017 SC         ?4018 SD         ?4019 CHNL       401A DELAY
 401B PRETRG      401C T1CHV       401D T1CLV       401E TRLVL
 401F EXTEND      4020 SKIP       ?4021 SG         ?4022 SH
 4023 POINT       4024 POSTRG      4025 PSTCNT      4026 SMPLHI
 4027 TRGSIG      4028 SMPLLO     ?4029 SJ         ?402A SK
?402B SL         ?402C SM         402D RAWDTH      412D RAWDTL
 422D START       4239 EXIT1       423C CONT        424D ATOD
 4259 WAIT        4276 EXTRG       42B2 RDY         428A GO
 42AB SETOM       42B3 PLUS1       42BE NOWOPP      42CE PLUS2
 42D6 SETTM       42DE AFTER       42FF NOTEND      4302 BEFORE
 4310 STORE       431F EXIT        4326 SETUP       4366 INTOFF
 4376 FUT1        4379 FUT2        437A FUT3        C0A0 ORB
 C0A1 ORA         C0A4 T1CL        C0A5 T1CH        C0AB ACR
 C0AC PCR         C0AD IFR         C0AE IER         C0AF ORAN
```

```
SOURCE FILE: PLOTDATA12
0000:           1 *PLOT DISPLAY PROGRAM FOR ACQUIRE
0000:           2 *
0000:           3 *--(1) REORDER "RAWDT" FOR DISK STORAGE-TO "SAVDT"
0000:           4 *--(2) 7 BITS OF "SAVDT" INTO "RAWDTH"
0000:           5 *--(3) OFFSET "RAWDTH"
0000:           6 *--(4) FIRST TIME, CLEAR "EDAT" (ERASE ARRAY)
0000:           7 *--(5) ERASE LINE WITH "EDAT"
0000:           8 *--(6) DRAW LINE WITH "RAWDTH"
0000:           9 *--(7) PUT "RAWDTH" INTO "EDAT" FOR NEXT ERASE
0000:          10 *--- A/D   11 10 9 8 7 6 5 4 3 2 1 0
0000:          11 *---RAWDTH  7  6 5 4 3 2 1 0
0000:          12 *---RAWDTL                  7 6 5 4 X X X X
0000:          13 *
0000:          14 *DEFINE ADDRESSES
F53A:          15 HLIN    EQU  $F53A     LINE WRITE
F457:          16 HPLOT   EQU  $F457     POSITION DOT
00E4:          17 SETHCOL EQU  $E4       COLOR BYTE LOCATION
402D:          18 RAWDTH  EQU  $402D     DATA ARRAY FROM GETDATA
412D:          19 RAWDTL  EQU  $412D     LO DATA ARRAY
4023:          20 POINT   EQU  $4023     FIRST POINT OF RAWDTH
0000:          21 *
----- NEXT OBJECT FILE NAME IS PLOTDATA12.OBJ0
5000:          22         ORG  $5000
5000:          23 *
5000:          24 *ENTRANCE POINTS
5000:4C 19 53  25         JMP  START     20480
5003:4C 34 53  26         JMP  UPDATE    VIEW DISK DATA OR RESCALE
5006:4C DE 53  27         JMP  FUT2      FUTURE EXPANSION
5009:4C DA 53  28         JMP  FUT3      -
500C:4C DC 53  29         JMP  FUT4      -
500F:          30 *
500F:          31 *PROGRAM VARIABLES
500F:00        32 XPOS    DFB  $00       20495 X POSITION
5010:00        33 GETPT   DFB  $00       POINT FROM GETDATA
5011:          34 HIBYT   DS   1         HIGH BYTE
5012:          35 LOBYT   DS   1         LOW BYTE
5013:00        36 SHIFT   DFB  $00       SHIFT LOW TO HIGH
5014:00        37 PTS     DFB  $00       POINTS=1, LINE=0
5015:FF        38 FIRST   DFB  $FF       FIRST TIME THRU
5016:          39 SD      DS   1         FUTURE EXPANSION
5017:          40 SE      DS   1         -
5018:          41 SF      DS   1         -
5019:          42 *
5019:          43 *DATA ARRAY STORAGE
5019:          44 SAVDTH  DS   256       20505 SAVE DATA FOR DISK
5119:          45 SAVDTL  DS   256       20761 SAVE DATA LO
5219:          46 EDAT    DS   256       ERASE ARRAY
5319:          47 *
5319:          48 *START OF PROGRAM
```

```
5319:           49 *
5319:AD 23 40   50 START   LDA  POINT     GET DATA INDEX
531C:8D 10 50   51         STA  GETPT     SAVE FOR INDEXING DATA
531F:           52 *
531F:           53 *REORDER RAWDATA TO SAVE
531F:AE 10 50   54         LDX  GETPT     OFFSET TO FIRST POINT
5322:A0 00      55         LDY  #00       POINTER TO SAVDAT
5324:BD 2D 40   56 DISK    LDA  RAWDTH,X  STORE IN ORDER
5327:99 19 50   57         STA  SAVDTH,Y  INTO SAVDATA HI
532A:BD 2D 41   58         LDA  RAWDTL,X  -
532D:99 19 51   59         STA  SAVDTL,Y  -
5330:E8         60         INX             INCREMENT RAWDATA POINTER
5331:C8         61         INY             INCREMENT SAVEDATA POINTER
5332:D0 F0      62         BNE  DISK      LOOP 256 TIMES
5334:           63 *
5334:           64 *PUT 7 BITS OF SAVDAT INTO RAWDTH
5334:           65 *(SHIFT):(0)11-5,(1)10-4,(2)9-3,(3)8-2,(4)7-1,(5)6-0
5334:A2 00      66 UPDATE  LDX  #00       SCALE 256 POINTS
5336:           67 *
5336:BD 19 51   68 FIX     LDA  SAVDTL,X  NO CHANGE TO SAVDAT ITSELF
5339:8D 12 50   69         STA  LOBYT
533C:BD 19 50   70         LDA  SAVDTH,X
533F:8D 11 50   71         STA  HIBYT
5342:AC 13 50   72         LDY  SHIFT
5345:F0 13      73         BEQ  HIGH      WANT 11-5
5347:           74 *
5347:AD 12 50   75 LOTOHI  LDA  LOBYT     BIT 7 LOBYT TO CARRY
534A:0A         76         ASL  A         -
534B:8D 12 50   77         STA  LOBYT     -
534E:AD 11 50   78         LDA  HIBYT     CARRY TO BIT 0 HIBYT
5351:2A         79         ROL  A         -
5352:B0 10      80         BCS  MAX       IF TOO BIG SET TO MAX
5354:8D 11 50   81         STA  HIBYT     -
5357:88         82         DEY             SHIFT Y TIMES
5358:D0 ED      83         BNE  LOTOHI    -
535A:           84 *
535A:4A         85 HIGH    LSR  A         0 TO BIT 7 HIBYT
535B:9D 2D 40   86 STORE   STA  RAWDTH,X  SAVE SCALED DATA
535E:E8         87         INX             -
535F:D0 D5      88         BNE  FIX       -
5361:4C 69 53   89         JMP  INVERT    -
5364:           90 *
5364:A9 7F      91 MAX     LDA  #127      SET TO MAX IF TOO BIG
5366:4C 5B 53   92         JMP  STORE     -
5369:           93 *
5369:           94 *COMPUTE 135-(RAWDTH)
5369:A2 00      95 INVERT  LDX  #00       INVERT FOR CORRECT DISPLAY
536B:38         96 SUB     SEC             -
536C:A9 91      97         LDA  #145      -
536E:FD 2D 40   98         SBC  RAWDTH,X  -
```

```
5371:9D 2D 40   99         STA  RAWDTH,X  -
5374:E8        100         INX             -
5375:D0 F4     101         BNE  SUB        -
5377:          102 *
5377:          103 *FIRST TIME, CLEAR "EDAT" FOR ERASE
5377:AD 15 50  104 CLEAR   LDA  FIRST      FIRST TIME = $FF
537A:F0 0D     105         BEQ  LINE       -
537C:A2 00     106         LDX  #00        -
537E:A9 00     107         LDA  #00        -
5380:9D 19 52  108 CLRALL  STA  EDAT,X     -
5383:E8        109         INX             -
5384:D0 FA     110         BNE  CLRALL     -
5386:8D 15 50  111         STA  FIRST      SET FIRST TO 0
5389:          112 *
5389:A9 00     113 LINE    LDA  #00        ZERO COUNTER
538B:8D 0F 50  114         STA  XPOS       -
538E:          115 *
538E:          116 *ERASE OLD LINE
538E:A9 00     117 ERASE   LDA  #00        BLACK COLOR
5390:85 E4     118         STA  SETHCOL    -
5392:AE 0F 50  119         LDX  XPOS       LO HORIZONTAL
5395:BD 19 52  120         LDA  EDAT,X     VERTICAL
5398:A0 00     121         LDY#00          HI HOR
539A:20 57 F4  122         JSR  HPLOT      -
539D:AD 14 50  123         LDA  PTS        0LINE 1POINTS
53A0:D0 0D     124         BNE  DRAW       -
53A2:AE 0F 50  125         LDX  XPOS       -
53A5:E8        126         INX              NEXT VALUE
53A6:BC 19 52  127         LDY  EDAT,X     VERT
53A9:8A        128         TXA              LO HORZ
53AA:A2 00     129         LDX  #00        HI HORZ
53AC:20 3A F5  130         JSR  HLIN       -
53AF:          131 *
53AF:          132 *DRAW NEW LINE
53AF:A9 7F     133 DRAW    LDA  #127       SET WHITE
53B1:85 E4     134         STA  SETHCOL    -
53B3:AE 0F 50  135         LDX  XPOS       LO HORZ
53B6:BD 2D 40  136         LDA  RAWDTH,X   VERT
53B9:9D 19 52  137         STA  EDAT,X     SAVE TO ERASE
53BC:A0 00     138         LDY  #00        HI HORZ
53BE:20 57 F4  139         JSR  HPLOT      -
53C1:EE 0F 50  140         INC  XPOS       POINT TO NEXT VALUE
53C4:AD 14 50  141         LDA  PTS        0LINE, 1POINTS
53C7:D0 0E     142         BNE  END        -
53C9:AE 0F 50  143         LDX  XPOS       -
53CC:BC 2D 40  144         LDY  RAWDTH,X   VERT
53CF:AD 0F 50  145         LDA  XPOS       LO HORZ
53D2:A2 00     146         LDX  #00        HI HORZ
53D4:20 3A F5  147         JSR  HLIN       -
53D7:          148 *
```

```
53D7:AD 0F 50  149 END     LDA  XPOS      DO ALL 256
53DA:C9 FF     150 FUT3    CMP  #255      -
53DC:90 80     151 FUT4    BCC  ERASE     -
53DE:AA        152 FUT2    TAX            SAVE LAST POINT
53DF:BD 2D 40  153         LDA  RAWDTH,X  -
53E2:9D 19 52  154         STA  EDAT,X    -
53E5:60        155         RTS

*** SUCCESSFUL ASSEMBLY: NO ERRORS
```

?5377 CLEAR	5380 CLRALL	5324 DISK	53AF DRAW
5219 EDAT	53D7 END	538E ERASE	5015 FIRST
5336 FIX	53DE FUT2	53DA FUT3	53DC FUT4
5010 GETPT	5011 HIBYT	535A HIGH	F53A HLIN
F457 HPLOT	5369 INVERT	5389 LINE	5012 LOBYT
5347 LOTOHI	5364 MAX	4023 POINT	5014 PTS
402D RAWDTH	412D RAWDTL	5019 SAVDTH	5119 SAVDTL
?5016 SD	E4 SETHCOL	?5017 SE	?5018 SF
5013 SHIFT	5319 START	535B STORE	536B SUB
5334 UPDATE	500F XPOS		

```
   E4 SETHCOL      4023 POINT      402D RAWDTH      412D RAWDTL
 500F XPOS         5010 GETPT      5011 HIBYT       5012 LOBYT
 5013 SHIFT        5014 PTS        5015 FIRST      ?5016 SD
?5017 SE          ?5018 SF         5019 SAVDTH      5119 SAVDTL
 5219 EDAT         5319 START      5324 DISK        5334 UPDATE
 5336 FIX          5347 LOTOHI     535A HIGH        535B STORE
 5364 MAX          5369 INVERT     536B SUB        ?5377 CLEAR
 5380 CLRALL       5389 LINE       538E ERASE       53AF DRAW
 53D7 END          53DA FUT3       53DC FUT4        53DE FUT2
 F457 HPLOT        F53A HLIN
```

APPENDIX B

PROGRAM LISTINGS OF THE FITTING ROUTINE FOR THE *p*-PHENYLENEDIAMINE OXIDATION

```
10  GOTO 110
20  REM RUN30 TO PRINT PROGRAM
30  PR# 1: PRINT  CHR$ (9);"100N"; CHR$ (27);"Q"; CHR$ (27);"L022": LIST 0 - : PR# 0: END
40  REM
50  REM   SAVE EQUALLY SPACED POINTS TO DISK
60  REM ** DATA USED IN KINETIC FIT PROGRAM **
70  REM
80  REM FORMAT OF DATA FILE IS
90  REM 1:NP,# OF POINTS 2:MP,MULTIPLIER PER POINT 3:UX$,UNIT OF X AXIS 4:UY$,UNIT OF Y AXIS
     5 - 4+NP:VALUES
100  REM
110  HOME : REM SAVDAT
120  REM
130  INPUT "FILE NAME =";F$
140  INPUT "# OF POINTS =";NP: DIM Y(NP)
150 D$ =  CHR$ (4): REM CNTRL-D
160  INPUT "MULTIPLIER PER POINT = ";MP
170  INPUT "UNIT OF X AXIS = ";UX$
180  INPUT "UNIT OF Y AXIS = ";UY$
190  INPUT "Y=Y(A/B)+C:ENTER:A,B,C ";A,B,C
200  REM
210  FOR I = 1 TO NP: PRINT I;: INPUT " VALUE= ";Y(I):Y(I) = (A * Y(I) / B) + C: NEXT
220  REM
230  PRINT D$;"OPEN";F$: PRINT D$;"WRITE";F$
240  PRINT NP
250  PRINT MP: PRINT UX$: PRINT UY$
260  FOR I = 1 TO NP: PRINT Y(I): NEXT
270  PRINT D$;"CLOSE";F$
280  END
```

```
40  REM
50  REM   FIT KINETICS MADE 10/23/83 BY JIM THOMPSEN
60  REM
70  HIMEM: 16383: HGR2 : TEXT
80  REM PLOTTING IS DONE BY "AMPERGRAPH", MADWEST SOFTWARE, BOX9822, MADISON, WI 53715
90  REM  --10-23-83--(--PLOT CON. D--)
100  REM MECHANISM
110  REM 3A + 3B -) D (K1)
120  REM B + G (=) C OR B-G, FAST
130  REM 3A + C -) D + G' (K2)
140  REM D + B -) E (K3)
150  REM  A=P-PD, B=H2O2, G=GLUT, D=BB, C=B-G, E
160  REM
170  INPUT "DATA FILENAME";F$:D$ =  CHR$ (4)
180  PRINT D$;"OPEN";F$: PRINT D$;"READ";F$
190  INPUT NP: DIM DR(NP): INPUT MP,UX$,UY$
200  REM
210  FOR I = 1 TO NP: INPUT DR(I): NEXT
220  PRINT D$;"CLOSE";F$
230  PRINT NP;" = # OF POINTS"
240  INPUT "WHAT FRACTION (1/X) TO USE, X = ";FP
250  REM
260  FOR I = 1 TO NP STEP FP
270 DR(I) = 7.733E - 6 * DR(I)
280  IF DR(I) ) MX THEN MX = DR(I)
290  NEXT
300  DIM D(NP * 10)
310  READ A(0),B(0),C(0),D(0),E(0): FOR I = 1 TO 6: READ K(I): NEXT
320 DT = MP * FP
330  PRINT "1  2  3  4  5  6  7   8  9   10  11 12"
340  PRINT "A0,B0,C0,D0,E0,K1,***,K2,***,K3,K4 ,DT"
350  PRINT "1 :";A(0): PRINT "2 :";B(0): PRINT "3 :";C(0)
360  PRINT "4 :";D(0): PRINT "5 :";E(0)
370  FOR I = 6 TO 9: PRINT I;" :";K(I - 5): NEXT
380  FOR I = 10 TO 11: PRINT I;":";K(I - 5): NEXT
390  PRINT "12:";DT
400  PRINT "MIN DT = ";MP / 5
410  INPUT "ENTER # TO CHANGE OR 99 TO BEGIN --) ";N
420  IF N = 1 THEN  INPUT "A0=";A(0): GOTO 410
430  IF N = 2 THEN  INPUT "B0=";B(0): GOTO 410
440  IF N = 3 THEN  INPUT "C0= ";C(0): GOTO 410
450  IF N = 4 THEN  INPUT "D0= ";D(0): GOTO 410
460  IF N = 5 THEN  INPUT "E0= ";E(0): GOTO 410
470  IF N = 6 THEN  INPUT "K1= ";K(1): GOTO 410
480  IF N = 7 THEN  INPUT "K-1= ";K(2): GOTO 410
490  IF N = 8 THEN  INPUT "K2=  ";K(3): GOTO 410
500  IF N = 9 THEN  INPUT "K-2= ";K(4): GOTO 410
510  IF N = 10 THEN  INPUT "K3=  ";K(5): GOTO 410
520  IF N = 11 THEN  INPUT "K4 = ";K(6): GOTO 410
530  IF N = 12 THEN  INPUT "DT=";DT: GOTO 410
```

```
540  REM
550 A(1) = A(0):B(1) = B(0):C(1) = C(0):E(1) = E(0)
560  FOR I = 1 TO NP * MP / DT
570 Q1 =  - K(1) * A(1) * B(1)
580 Q2 =  - K(3) * A(1) * (C(1) ^ .6667)
590 Q3 =  - K(5) * B(1) * (D(I - 1) ^ .6667)
600  REM
610 DA = DT * (3 * Q1 + 3 * Q2)
620 DB = DT * (3 * Q1 + 3 * Q2 + Q3)
630 DC = DT * Q2
640 DD = DT * ( - Q1 - Q2 + Q3)
650  REM
660 A(1) = DA + A(1): IF A(1) < 0 THEN A(1) = 0
670 B(1) = DB + B(1): IF B(1) < 0 THEN B(1) = 0
680 C(1) = DC + C(1): IF C(1) < 0 THEN C(1) = 0
690 D(I) = DD + D(I - 1): IF D(I) < 0 THEN D(I) = 0
700  NEXT I
710 Z =  FRE (0)
720  REM
730  REM COMPUTE SUM SQUARE ERROR
740 SS = 0: FOR I = 1 TO NP STEP FP
750 SQ = (D( INT (MP * I / DT)) - DR(I)) ^ 2
760 SS = SS + SQ
770  NEXT : PRINT "ERROR = ";SS
780  REM
790  PRINT "TO PLOT PRESS P": GET A$: IF A$ <  > "P" THEN 330
800  PRINT "GO ON ANY KEY, ERASE -E-": GET A$: IF A$ = "E" THEN DN = 0
810  GOSUB 1060
820  IF DN = 1 THEN 930
830  REM
840  REM PLOT ROUTINES REQUIRE "AMPERGRAPH"
850  HGR2
860  & SCALE,0,NP * MP,0,5 * MX / 4
870  & AXES,0,0,NP * MP / 10,MX / 4
880  FOR I = 1 TO NP STEP FP
890  &  DRAW ,I * MP,DR(I)
900  NEXT
910  & PENUP
920 DN = 1
930  FOR I = 1 TO NP STEP FP
940  &  DRAW ,I * MP,D( INT (MP * I / DT))
950  NEXT I
960  & PENUP
970  FOR I = 1 TO 25:A =  PEEK ( - 16336): NEXT
980  GET A$: TEXT
990  PRINT "HIT Q TO QUIT ": GET A$: IF A$ <  > "Q" THEN  GOTO 330
1000  END
1010  REM STARTING VALUES
1020  DATA 5.568E-5,.336,7.000E-6,0,0
1030  DATA  0,0,0,0,0,0
1040  REM
1050  REM BUZZ AFTER PLOT
1060 A =  PEEK (49237):A =  PEEK (49234):A =  PEEK (49239):A =  PEEK (49232): RETURN
```

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