ABSTRACT

AN AUTOMATED, COMPUTER-CONTROLLED STOPPED-FLOW SPECTROPHOTOMETER FOR FAST REACTION-RATE ANALYSIS

By

Phillip Kent Notz

A first generation stopped-flow spectrophotometer has been improved, automated and interfaced to a PDP 8/e minicomputer. The stopped-flow mixing system was completely thermostated and equipped to permit the measurement of absorbance, conductance and temperature on the millisecond time scale. Other design changes include a novel mixer, high throughput quartz optics and an optical trigger system. The operation of the stopped-flow mixing system and the collection, analysis, display and storage of data are all done under minicomputer control. On-line display of results via a high speed CRT terminal provides the experimenter with valuable feedback in seconds after the completion of a stopped-flow experiment.

The detection system can achieve a resolution of 1 part in 200,000, which allows the measurement of very small changes in absorbance. The overall accuracy of absorbance measurements is limited to about 0.5% due to drift in light source intensity. Temperature data, with a precision of

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0.01°C, can be acquired nearly simultaneously with the acquisition of absorbance data.

A complete characterization of the automated stopped-flow spectrophotometer is presented. The components of the mixing and spectrophotometric systems were characterized to determine their individual effects on the system accuracy and precision. As a check on the overall system performance, rate and equilibrium constants for the iron-thiocyanate reaction were determined. Equilibrium constants were determined at different concentrations, both by equilibrium absorbance measurements and by fitting both the forward and reverse rate constants to kinetics data. The equilibrium constants (for a given concentration of reactants), as determined by the two different methods, agreed by better than one percent in all cases.

A preliminary study of the proton consumption by Mo(VI) in strongly acid solution shows that the number of protons consumed per molybdenum atom approaches 2½ as the acid-to-molybdate ratio is increased to 10.

A study of the dependence of the rate of formation of 12-molybdophosphate (12-MPA) on nitric acid concentration is presented. Results indicate inverse first and inverse ninth order dependence on acid concentration and the formation of two different products at different acid concentrations.

Finally, the reaction-rate analysis of phosphate and the reaction rate analysis of silicate via their reactions with Mo(VI) are presented.

# AN AUTOMATED, COMPUTER-CONTROLLED STOPPED-FLOW SPECTROPHOTOMETER FOR FAST REACTION-RATE ANALYSIS

By

Phillip Kent Notz

A DISSERTATION

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Department of Chemistry

1977

Dedicated to Eleanor, Jennifer and Ryan,

and Mom and Dad

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# CHAPTER I

# INTRODUCTION

## A. Reaction-Rate Methods of Analysis

As implied by the name, reaction-rate methods utilize the rate, rather than the stoichiometry, of a reaction to perform a chemical analysis. Several books (1,2) and quite a few review articles (3-10) have been recently written on reaction-rate methods of chemical analysis.

Reaction-rate methods of analysis have been developed on a broad scale in recent years. This development is largely due to advances in instrumentation and modern electronics. Until the last decade, the growth of rate methods was inhibited because of the need for more complex analysis procedures and instrumentation than those required for equilibrium-based methods. However, with the advent of modern integrated circuit electronics and the consequent upsurge in small computer usage, the automation of reaction-rate instruments and reaction-rate data analysis has become routine and relatively inexpensive.

Another item which has given great impetus to the development of reaction-rate methods of analysis is the growing realization of the importance of enzymes in biological systems. In many diseases the enzyme level, or enzyme activity, is a critical parameter. Since enzymes

are biological catalysts, the determination of enzyme activity necessitates reaction-rate procedures.

For a general chemical reaction, the rate of disappearance of a reactant A with time may be expressed in terms of the concentrations of the chemical species involved in the reaction.

$$\frac{-dA}{dt} = k_1[A]^m[B]^n...$$

Equations relating the rate of disappearance of A or the concentration of A at any time t with the initial concentration of A, $[A]_o$ can be developed for specific cases. In rate methods of analysis conditions are usually chosen such that the reaction is first-order or pseudo-first-order in the species of interest. Thus the following equations would apply:

$$\frac{dA}{dt} = k[A] \qquad (1)$$

$$[A]_t = [A]_o \exp(-kt) \qquad (2)$$

$$-\left(\frac{d[A]}{dt}\right)_t = k[A]_o \exp(-kt) \qquad (3)$$

where k is the first-order or pseudo-first-order rate constant and the subscript t indicates the value of the variable at time t.

Equation (3) forms the basis for reaction-rate methods

of analysis. It indicates that the rate of a first-order reaction at a given time t is related to the initial concentration of the analyte by a constant, k exp(-kt). Thus the measurement of the reaction-rate at any fixed point in time can form the basis for analysis. In practice the rate must be measured over some finite time interval rather than at a point in time. If this time interval is during the initial portion of the reaction ($t << \frac{1}{k}$) the method is termed an initial rate method. In that case the exponential term in Equation (3) is approximately unity. The validity of this approximation has been discussed by Ingle and Crouch (11) and by Crouch (12). In addition, the reaction-rate is a maximum during the initial portion so that sensitivity is optimum there.

In comparing reaction-rate methods to equilibrium based methods, the most obvious advantage of the rate method is the shorter measurement time. This is particularly desirable for clinical or environmental applications where many routine analyses must be performed each day. Reaction-rate analysis may be performed in minutes even for reactions with half-lives on the order of an hour. This same analysis would take several hours using an equilibrium based method.

Reactions that are nonstoichiometric, produce unstable products or have interferring side reactions, which make them unsuitable for equilibrium-based methods, may be entirely suitable for reaction-rate analysis. Since the reaction-rate measurement can be made during the initial period of the

reaction, these situations often cause no interference. Also since reaction-rate analysis is a relative measurement, constant interferences such as dirty cell windows or slightly turbid solutions do not cause errors as they would in an equilibrium-based analysis.

The reaction-rate method is also advantageous in that it can be highly specific. It is possible to determine several different components in the same solution as long as their reaction rates are sufficiently different.

Reaction-rate methods of analysis also have certain disadvantages. Because only a part of the total reaction is measured, the precision of the chemical analysis is generally less than in equilibrium-based analyses. However, modern high quality measurement systems have lessened the seriousness of this disadvantage. Another disadvantage stems from the limitation in following very fast reactions. Reaction-rate methods utilizing mixing of reagents to initiate the reaction are limited to reactions with half-lives greater than a few milliseconds. However, relaxation techniques can follow reactions with half-lives in the submicrosecond range.

In addition all the parameters which affect the rate of a reaction such as temperature, ionic strength or pH must be controlled and/or monitored. These parameters are generally not as critical for equilibrium techniques.

## B. Techniques for Studying Fast Reactions

Techniques for studying fast reactions must be capable of initiating a reaction and making the appropriate measurements in less than a few seconds. A recent study (29) includes a review of techniques for studying fast reactions. To be of practical value, the technique should be able to approach the millisecond time scale. Techniques which meet this criteria can be divided into two categories: mixing methods and relaxation methods. The common mixing methods include continuous-flow, accelerated-flow and stopped flow. The common relaxation methods include temperature jump and pressure jump, although changes in electric field are also used.

### 1. Fast Mixing Methods

In the fast mixing methods the reaction is initiated by the rapid mixing of the solutions containing the reacting species. The solutions flow through separate channels into a mixer where they are combined and the reaction is initiated. The reacting solution then flows into an observation cell where the reaction is monitored. The basic difference between the three fast mixing methods discussed here is in the regulation of the flow velocity and its relationship to the measurement period. In the continuous flow method, the measurement is made while the solution is flowing at a constant rate. The measurement is made while the solution

velocity is being changed in the accelerated flow method. And, in the stopped-flow method, the measurement is made after the flow is stopped.

The continuous flow method (13,14) developed by Hartridge and Roughton in 1923 was the first technique for studying fast reactions in solution. The reacting solutions are pushed through a mixer and then through an observation tube at a constant rate. By moving a detector along the observation tube, the reaction can be observed at leisure at different points in time. Reaction times down to one millisecond are possible with this method. However, a disadvantage is the large volumes of solution consumed, which ranges from a few milliliters to several liters.

Unlike the continuous flow method, the accelerated flow method (15-19) maintains the detector at a fixed distance from the observation cell. The time profile of the reaction is obtained by varying the flow velocity. This method can be used to measure reactions on the millisecond time scale and solution consumption down to 0.1 milliliters can be attained. Its major disadvantages are a limited time scale and the added complexity of the fluid drive and flow velocity monitoring system.

Because of its major importance to this work the stopped-flow method will be discussed separately in Chapter II.

## 2. Relaxation Methods

Relaxation methods involve the perturbation of a chemical

system which is at equilibrium. The perturbation causes a shift in the chemical equilibrium and the system then adjusts or relaxes to the new equilibrium concentrations. The monitoring of this relaxation is then used to determine the reaction kinetics. The perturbation is caused by an abrupt change in one of the physical parameters of the system, such as temperature (20-22), pressure (20-22) or electric field (20,22,23). These methods can be used to follow reactions with half lives on the order of nanoseconds. This greatly extends the range of reaction rates accessible for fundamental kinetics. However, there is little utility in relaxation methods for analytical purposes. A chemical reaction at equilibrium is utilized and initial, analytical concentrations are not involved in the relaxation expressions.

# CHAPTER II

# BACKGROUND

## A. The Stopped-Flow Technique

The stopped-flow mixing technique was developed by Chance in 1940 (16-18). It has some basic advantages over the accelerated-flow technique which he had developed earlier. The main advantages are better reaction time accuracy and a wider time range available in one run. The accelerated-flow method can monitor a reaction from 1 millisecond to 10 milliseconds whereas the stopped-flow method can monitor a reaction from 1 millisecond to hours. Also, the accelerated-flow method relies on the accuracy of monitoring a varying flow rate to determine the reaction times at the observation cell. The stopped-flow method only relies on the reproducibility of the time between reaction initiation (mixing) and the stopping of the flow. The reaction time from that point on can be derived from any accurate electronic time base.

Since the stopped-flow method has the same solution volume requirements as the accelerated-flow method, there are no realistic disadvantages of stopped-flow compared to accelerated-flow. Thus, the stopped-flow technique has essentially obsoleted the accelerated-flow method. However, there are still applications where continuous-flow methods are advantageous over stopped-flow in spite

of the large solution volume requirements. This is because of the simplicity of design of the continuous-flow apparatus and because the use of slow responding detectors to follow fast reactions is possible.

## 1. Principles of Stopped-Flow Mixing

An ideal stopped-flow mixer would instantaneously mix reactant solutions and immediately deliver them into the observation cell where the reaction is to be monitored. In a real system these operations require a finite amount of time.

The most useful figure of merit for a stopped-flow mixing system is the dead time, $t_d$, which is the difference between the time of initial contact of the reactants and the time at which they are stopped in the observation cell (24-26). In a well designed system, mixing must be complete by the time the solution is stopped in the observation cell. Thus the dead time must be greater than or equal to the mixing time, $t_m$, which is the time between initial contact of the reactant solutions and "complete" mixing. A third time which is of significance because it affects $t_d$ and $t_m$ is the stopping time, $t_s$. This is the time required for flow to cease once the stopping device has begun to impede flow. For precise measurements $t_s$ should be much less than $t_d$.

a. Sequence of Operations - Figure 1 shows a pictorial diagram of a general stopped-flow mixing system with spectrophotometric detection.

A controller is shown which directs the sequence of events in the system. The controller can be a manual sequencer, an electronic hard wired sequencer, a minicomputer, or a microprocessor.

The sequence of operations necessary to obtain reaction-rate information by stopped-flow spectrophotometry begins with reagent preparation. Although this step is normally carried out manually, in principle all reagent preparation operations can be carried out under the supervision of the controller. After solutions are prepared, they must be introduced into the drive system, which normally consists of two drive syringes. Once solutions are introduced into the drive syringes, the drive system is actuated and the two solutions flow into a mixing chamber. The mixed solution flows through an observation cell into a stopping device which ceases the flow after a preset flow volume or time of flow. When the flow stops, the spectrophotometric detection system is activated and data acquisition (absorbance vs. time) begins. Data processing is often carried out in order to present the data in the desired format (initial rate, concentration of analyte, rate constants, etc.). Finally the desired information is presented via a readout device (recorder, print-out, plotter, etc.).

Figure 1. An Automated Stopped-Flow Mixing System with Spectrophotometric Detection.

STOPPED-FLOW
1P28
PMT

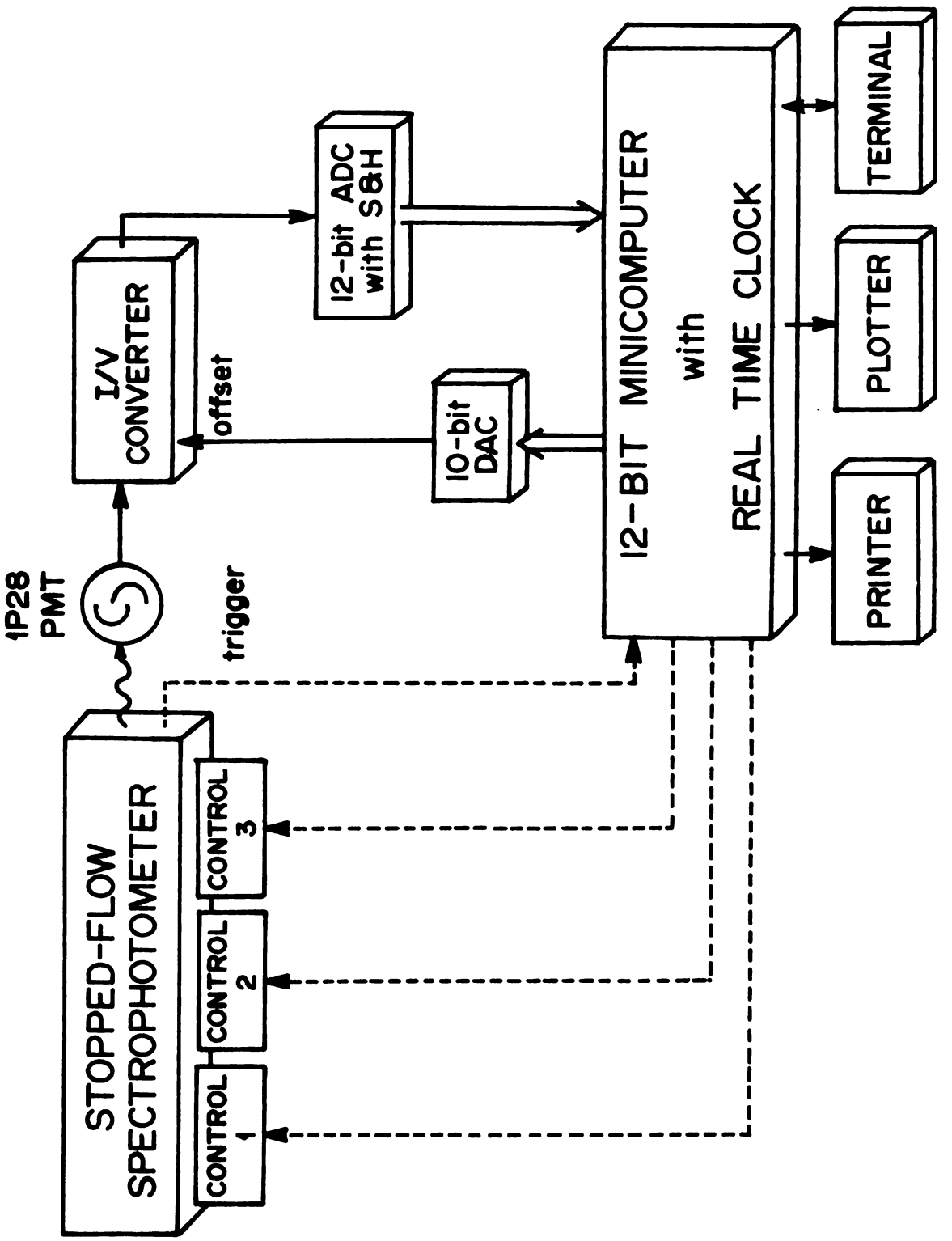

1P28
PMT
trigger
I/V
CONVERTER
offset
12-bit ADC
with S&H
10-bit
DAC
12-BIT MINICOMPUTER
with
REAL TIME CLOCK
TERMINAL
PLOTTER
PRINTER
STOPPED-FLOW
SPECTROPHOTOMETER
CONTROL
1
CONTROL
2
CONTROL
3

Figure 1.

b. Influence of Dead Time on Rate Measurements - Although the data acquisition system begins taking data the instant the flow stops, the reaction has already proceeded for a finite time $t_d$. If $t_d$ is very small compared to the reaction half-life, the reaction is essentially followed from its initiation, and the full reaction history can be recorded. In many cases (for analytical data and often in mechanistic studies), the initial reaction rate is the information of interest. This places special emphasis on the development of systems with short dead times so that the initial rates of rapid reactions may be measured. The importance of a short dead time can be deduced from a rearrangement of Eq. (3). Since the half-life, $\tau$, of a first- or pseudo-first-order reaction is given by $\tau = \frac{\ell n 2}{k}$, it can be seen that for a first-order or pseudo-first order reaction and $t/\tau << 1$,

$$\left(\frac{d[A]}{dt}\right)_t = \left(\frac{d[A]}{dt}\right)_o \exp\left(-0.693t/\tau\right) \approx \left(\frac{d[A]}{dt}\right)_o \left(1 - 0.693t/\tau\right) \quad (4)$$

where, $\tau = 0.693/k$ = half-life of the reaction, s

$t$ = reaction time, s

$\left(\frac{d[A]}{dt}\right)_t$ = reaction rate at time t, mole $\ell^{-1}s^{-1}$

$\left(\frac{d[A]}{dt}\right)_o$ = initial reaction rate, mole $\ell^{-1}s^{-1}$

For the measured rate to be within one percent of the initial rate, the rate measurement would have to be

made within $\tau/69.3$ seconds of the reaction initiation.

If the rate cannot be measured at $t << \tau$, the analysis becomes quite complicated. The reaction time t evaluated as a function of position in the observation cell then becomes important, since in most systems the observation cell volume is a large contributor to the total dead volume. Assuming (1) plug flow of solution from the exit of the mixer to the exit of the observation cell (this is approximately true for high flow velocities in straight tubes); (2) an observation cell with uniform cross-section; and (3) the stopping time is much less than the dead time, the reaction time would be uniform for any cross-section of the cell and would be a linear function of the distance from the entrance of the observation cell. Thus, the following would hold:

$$\left(\frac{d[A]}{dt}\right)_t = \frac{\int_{t_1}^{t_2} -k[A]_o \exp(-kt)dt}{\int_{t_1}^{t_2} dt} = \frac{[A]_o \exp(-kt_2 - \exp(-kt_1)}{t_2 - t_1} \tag{5}$$

So,

$$[A]_o = \left(\frac{d[A]}{dt}\right)_t \frac{t_2 - t_1}{\exp(-kt_2 - \exp(-kt_1)} \tag{6}$$

$\left(\frac{d[A]}{dt}\right)_t$ = average or measured reaction rate at time t where

$t = (t_2 + t_1)/2$.

$t_2$ = the total time that the solution at the exit end of the observation cell has been mixed.

$t_1$ = the total time that the solution at the entrance end of the observation cell has been mixed.

For solutions with approximately the same physical properties, $t_1$ and $t_2$ are characteristics of the stopped-flow system and can be determined by measuring the solution flow rate and the volume of the system. Then if k is known, $[A]_o$ can be determined from a measurement of $\frac{d[A]}{dt}$ at any time as long as the reaction remains first-order or pseudo-first order. The limiting factor in this type of analysis is the ability of the detection system to measure the reaction rate over a small time interval.

c. Character of Flow - An important aspect in the design of a fast mixing system is the character of flow. The parameter which is normally used to characterize flow is the Reynold's number, $R_e$ given by

$$R_e = \frac{\rho d}{\eta} v$$

where

$v$ = flow velocity, cm/s

$\eta$ = viscosity of the fluid, poises

$\rho$ = density of the fluid, g/ml

$d$ = diameter of the tube, cm

Flow can be classified into two categories, laminar and turbulent. The Reynold's number can be used to

differentiate between these two categories. Laminar flow is produced at Reynold's numbers less than 2100 whereas turbulent flow is produced at Reynold's numbers greater than 2100. This dividing line is not strictly quantitative but can be used as an approximate criterium in designing a flow system.

Laminar flow is characterized by a streamline flow pattern with no eddy currents or localized transverse flow. Laminar flow in circular tubes has a parabolic velocity profile with the maximum velocity at the center and zero velocity at the walls. The pertinent equations can be derived using Newton's law of viscosity. The result is

$$\frac{V}{V_{max}} = 1 - \left(\frac{r}{R}\right)^2$$

thus,

$$\frac{\langle V \rangle}{V_{max}} = \frac{1}{2}$$

where,

r = the radial distance from the center of the tube

R = the radius of the tube

V = the flow velocity at distance r

$V_{max}$ = the maximum flow velocity

$\langle V \rangle$ = the average flow velocity

In contrast to laminar flow, turbulent flow is characterized by the lack of a streamlined flow pattern and the presence of eddy currents and localized transverse flow. The velocity profile is also much flatter than the laminar flow velocity profile. Because of the random nature of turbulent flow it cannot be described mathematically by a straight forward application of Newton's Law of viscosity. Using a large collection of experimental data, the following empirical result was found to be reasonably accurate for fluids in circular tubes.

$$\frac{V}{V_{max}} = (1 - \frac{r}{R})^{\frac{1}{7}}$$

thus

$$\frac{<V>}{V_{max}} = \frac{4}{5}$$

The results on fluid flow were taken from a text on transport phenomena (27), although they can be found in any text on elementary fluid dynamics.

Turbulent flow has several important advantages over laminar flow in stopped-flow and other fast mixing systems. It is highly desirable to have uniform flow velocity throughout a cross-section (perpendicular to the flow) of the flow channel. This is necessary for good reaction-time resolution at the observation cell. Turbulent flow has a flatter velocity profile than laminar flow. Also

the transverse velocity components present in turbulent flow cause some exchange between the slower moving fluid near the wall and the faster moving fluid near the center of the tube.

Turbulent flow also improves the reagent mixing process. This was demonstrated by Chance (18). He observed the seemingly paradoxical phenomenon that the point of 98 percent complete mixing moves upstream toward the mixer as the flow velocity is increased.

Further advantages of turbulent flow can be seen by application of the Hagan-Poiseuille law to laminar flow and the Blasius formula to turbulent flow. The results indicate that with laminar flow in channels the flow velocity is proportional to the driving pressure and proportional to the inverse of the viscosity. However, with turbulent flow in channels, the velocity is proportional to the four-sevenths power of the driving pressure and proportional to the inverse of the one-seventh power of the viscosity. Therefore, the flow velocity would be effected less by changes in either pressure or viscosity in the case of turbulent flow.

One possible disadvantage of using high flow velocities is cavitation. Cavitation is the formation of tiny vapor bubbles when the momentum is suddenly changed so as to cause low pressure regions in the fluid. This occurs when the direction of the fast moving fluid is abruptly changed or when upstream stopping of the flow is used.

Cavitation is especially troublesome with optical methods of detection, although it interferes with all methods of detection to some extent.

Chance observed cavitation which originated in the mixer at high flow velocities (18). However, he was able to eliminate it up to flow velocities of 25 m/s by slightly modifying the mixer. A recent article (28) discusses cavitation in stopped-flow systems and methods to minimize or eliminate it.

## 2. The Components of a Stopped-Flow Mixing System

The goal of this section is to develop a perspective on the design of the components of a stopped-flow mixing system. The components include the reagent delivery and drive system, the mixing chamber, the observation cell, and the stopping device. No attempt will be made to cover every variation of each component. A recent thesis (29) has discussed the details of the components of various stopped-flow mixing systems reported in the literature.

It must be kept in mind that the designs of the individual components are not independent of each other. The components must be compatible with respect to flow volume, flow rate, fluid pressure and response time.

For a general purpose stopped-flow mixing system, all components which come in contact with the solution should be made from materials which are chemically inert.

Suitable materials of construction include glass, Kel F, Teflon and A.I.S.I. 316 stainless steel.

a. Reagent Delivery and Drive System - The only type of stopped-flow drive mechanism in common use utilizes plungers to force the solutions from the drive syringes through the mixing system. Therefore the discussion of reagent delivery and drive mechanisms will be limited to this type.

Usually the reagent delivery is accomplished by drawing back the drive plungers. A double 3-way stopcock or several valves can be used to connect the sample and reagent containers to the drive syringes during this operation and subsequently to connect the drive syringes to the mixing system. Alternatively, check valves can be used to switch connections automatically when the drive plungers are filled or discharged. The disadvantage to using check valves is that the solutions are subjected to a decrease in pressure (below atmospheric) when the drive syringes are being filled. This can cause degassing of the solutions or seepage of air around the plungers, which allows gas into the flow system.

The drive mechanism must be capable of forcing reproducible, and usually equal, amounts of both solutions through the flow system at high pressure. In order to accomplish this, there must be a good seal between the plunger and the syringe wall, and the driving mechanism

must be capable of exerting considerable force. In addition, the force should be reproducible and uniform throughout the drive so that accurate chemical rate measurements can be made. Suitable plungers have been constructed from stainless steel with neoprene o-rings or from Teflon with an embedded metal expansion ring. Specially constructed gas tight Teflon syringes are available commercially (30). The driving force has been derived from pneumatic cylinders, hydraulic cylinders, electric motors or the experimenters hand. Pneumatic cylinders are used most frequently because they are simple, reproducible, easily adjusted and trouble free.

The way in which the driving force created by a pneumatic cylinder is reported is often ambiguous. The quantity often reported is the air pressure of the pneumatic cylinder, whereas the critical item is the static fluid pressure created by the action of the pneumatic cylinder on the drive plungers. If mechanical losses are neglected the static fluid pressure can be calculated by the following equation.

$$P_f = P_c \times \frac{A_c}{A_f}$$

where,

$P_f$ = static fluid pressure

$P_c$ = air pressure in the pneumatic cylinder

$A_c$ = area of the plunger in the pneumatic cylinder

$A_f$ = sum of the areas of the plungers in the drive syringes.

As an example, the static fluid pressure was calculated for two similar stopped-flow systems. At an air pressure of 60 psi one system had a fluid pressure of 270 psi while the other system had a fluid pressure of 750 psi. Based on previous experience, both of these systems might be reported as having a drive pressure of 60 psi! It is most important that authors take the time to calculate and report the static fluid pressure of their stopped-flow systems.

b. Mixing Chamber - The purpose of the mixing chamber is to combine two solutions in a manner so as to produce rapid and thorough mixing with reasonable pressure drop and cavitation. Reasonable pressure drop is defined by the capabilities and limits of the rest of the flow system. Reasonable cavitation is that which subsides before the mixed solution reaches the observation cell. Rapid and thorough mixing is 99 percent mixing completed in 5 milliseconds, although somewhat less stringent mixing criteria may be acceptable.

There has been no lack of creativity in the design of stopped-flow mixers. Designs have included everything from the simple "Y" mixer to the "tangential offset jets" mixer of Gibson-Milnes (31) or the "turbulent wake of a sphere" mixer of Berger (32).

Jet mixers are the most commonly used. They are fairly easy to construct and can offer good mixing efficiency. There is a variety of designs including opposed jets, tangential jets and a compromise between the two. Chance found the compromise produced the least turbulence (18). Berger (33) found that the optimum offset between the pairs of tangential jets was one jet diameter. A general rule of thumb to avoid cavitation and excess pressure drop is the total area of the jets into one chamber should be equal to the area of the main flow channel. Increasing the number of jets will increase mixing efficiency if the flow rate is maintained. However the pressure drop will increase.

In designing a mixer, there is always a trade off between mixing efficiency on the one hand and increased pressure drop and cavitation on the other hand.

c. Observation Cell - The construction of the observation cell depends on the type of detection used. The only general criteria are that the volume should be small and that the cell should be constructed so that cavitation is avoided. Also, a cylindrical observation cell is best from a fluid dynamics point of view.

In spectrophotometric measurements, the path length will be important. It is desirable to have a long path length for good sensitivity, but a short path length gives better time resolution and a shorter dead time.

In molecular fluorescence, an observation cell with a square cross-section may be preferred. And, if conductometric measurements are to be made, the cell must be constructed of nonconducting materials except for the electrodes.

d. Stopping Device - The stopping device must be able to stop the flow rapidly without causing shock waves to propagate through the flow system. The time from when the device first begins to impede flow until it stops the flow completely should be much less than the dead time. It is difficult to find valves which close in a fraction of a millisecond. However, one was developed by Berger and coworkers (34). The most common type of mechanism utilizes a stopping syringe. The plunger of the syringe comes up against a fixed block thus causing an abrupt stop. The block and/or the plunger tip should be made of a relatively soft metal to prevent the formation of shock waves in the solution. Sturdevant (35) caused stopping upstream by the contact of tapered pins rather than a blunt stop block.

e. Detection and Readout System - Virtually any method of detection which has a response time on the millisecond time scale and can be used on small sample volumes can be applied to a stopped-flow mixing system. UV-visible spectrophotometry is the most common mode of detection.

The major improvements with this type of detection have been in the development of high intensity stabilized light sources (36-38). Several workers have utilized monitoring of the light source intensity in order to obtain highly accurate spectrophotometric data (37,39).

Scanning spectrophotometric systems have been developed in order to follow transient species. These systems utilize a rapid scanning monochromator (40-43) vidicon tubes, or diode arrays (44). In order to be useful, the system must be capable of scanning a spectra in a few milliseconds.

Photomultiplier tubes (PMT) or photodiodes are the two types of transducers used with spectrophotometric detection. The transducer which gives the best noise-drift characteristics has been the subject of considerable debate (45-50). The photodiode-high gain amplifier combination has superior characteristics at high light levels, but at lower levels the PMT-low gain amplifier combination gives better results.

Other means of detection include molecular fluorescence (51), light scattering (52-53), IR absorption (54), ESR (55) thermal methods (34) and electrochemical methods (56-58).

Readout devices for stopped-flow systems range from a storage oscilloscope equipped with a Polaroid camera to a minicomputer system including mass storage, CRT display and teletype. Numerous analog and digital hardware rate

meters have been developed for specific applications. The various types of readout systems have been recently reviewed (59,60).

## 3. Manual Stopped-Flow Systems

The development of stopped-flow systems up to 1972 has been covered in a recent thesis (29) and will not be repeated here. Also systems in which both the operation of the mixing system and the data acquisition and analysis have been automated are discussed in the next section.

A stopped-flow system with accurately controlled thermostating and a flow system entirely of glass or quartz has been described by Caldin and coworkers (61). The entire flow system except for the drive unit is emersed in a thermostating bath. Temperatures from -10 to +55°C have been used. It requires approximately 10 minutes for temperature equilibration.

Flexible fiber optics transfer light to and from the observation cell, which has a 2 mm path length. The authors report a 3-4 ms deadtime (determined by the extrapolation method) even though a simple 2-jet mixer is used. A storage oscilloscope is employed as the readout device.

Peterson and Mock (62) have described the use of a commercially available dual wavelength/split beam stopped-flow spectrophotometer for analysis of turbid samples. In the dual wavelength mode, accurate determinations

can be made on reactions with half-lives down to 50 ms. A PDP 11/05 minicomputer is used for data acquisition and analysis.

A variable temperature, rapid scanning stopped-flow spectrophotometer was reported by Dye and coworkers (40-42). The system is emersed in a thermostating bath for thorough temperature control. The entire flow and reagent delivery system is constructed of glass and Teflon and is vacuum tight for work with air sensitive solutions. The flow system requires a minimum of 2 ml of each reagent and 0.75 ml are used per run.

The detection system is double beam to cancel out source and PMT fluctuations. A high intensity, 1000 watt xenon arc lamp is used to obtain high light levels and high signal-to-noise ratio (S/N). Quartz flexible fiber optics lead to and from the reference and observation cells. The system contains two observation cells (0.199 cm and 1.85 cm) to allow measurements of a wide concentration range. Dead times of 2.7 and 6.7 ms were determined for the short path length cell and long path length cell, respectively.

The scanning system consists of a modified Perkin-Elmer model 108 rapid scan monochromator. The modifications involve optoelectronic transducers to encode the position and velocity of the scanning mechanism. A phase-locked loop frequency multiplication system allows the data sampling rate to be synchronized with the scanning

monochromator. The system can scan up to 150 spectra per second.

The detection system is interfaced to a PDP 8/I minicomputer for data acquisition and analysis. The interface is capable of sending parallel digital data over several hundred feet at rates up to 10 MHz.

The data points are averaged to provide a bandwidth which can be varied with time to optimize the S/N. Averaging is also desirable because it economizes the use of computer memory. The display of time dependent spectra via a high speed CRT display provides the experimentor with on-line feedback.

A second computer interfaced rapid scanning stopped-flow spectrophotometer was reported by Wightman and coworkers (43). The computer is used to control the scan and acquire and analyze data. The system employs a commercially available rapid scanning spectrometer (RSS) which was developed by Kuwana (63). The RSS accomplishes rapid scanning by the rotation of a mirror attached to a galvanometer armature. The instrument was modified so that the voltage to the galvanometer was supplied by a computer-controlled digital-to-analog converter. The system is capable of taking a 50 point, 250 nm spectrum in 1.2 ms with a repetition rate of 2 ms.

The stopped-flow mixing system is also a modified commercial unit. This system has an electric motor drive system. The flow system was modified from downstream

stopping to upstream stopping. To prevent cavitation caused by upstream stopping a pocket of air at the exit end is compressed during flow to create back pressure. About 1 ml of each reactant solution is required per run.

## 4. Automated Stopped-Flow Systems

Automation of rate measuring systems is an area of vigorous research (59,60). As will be demonstrated in this section, stopped-flow systems have certainly received their share of the attention. The operation of a complete stopped-flow system can be divided into three parts: (1) sample and reagent preparation; (2) mixing and flow stoppage and (3) data collection and analysis. Automation of the entire system not only reduces human labor tremendously, but for fast reactions it reduces the analysis time by several orders of magnitude. The major time efficiencies occur in the automation of steps (1) and (3), but automation significantly reduces human labor in all three steps. Another inherent advantage of automation is better reproducibility.

Automation can be accomplished by analog-digital hardware, but the use of a small computer to control the entire system greatly improves efficiency and flexibility. This discussion on automated stopped-flow systems will be restricted to those systems in which the automation of both the mixing system and the data analysis have

been accomplished.

One of the first systems to have automated sample handling as well as automated data handling and operation of the instrument was developed by Javier and coworkers (64). The automatic sampling system consists of a motor driven sample turntable and a sample introduction system based on a rapid injection and automatic refill pipet (65). The rapid injection pipet also serves as the drive mechanism for the stopped-flow mixing system. One cycle of the sampling system takes less than one second. The sampling system can be operated manually or programmed to operate in synchronization with the stopped-flow system.

The spectrophotometric observation and readout system utilizes a stable high intensity tungsten lamp as a light source and accomplishes automatic readout with a digital ratemeter. The average of ten results can be read out on the digital display in 10 seconds, which includes cell flushings between samples.

Another automated stopped-flow system utilizing a hardware rate meter was reported by Beckwith and Crouch (66). The basic flow system consists of two delivery syringes with a pneumatic drive, pneumatically operated delivery and waste release valves, a mixing chamber of the Gibson-Milnes design, an observation cell for spectrophotometric detection and a spring-loaded stopping assembly. Vertical flow was used to eliminate air bubble problems.

The entire operating cycle is controlled by a digital sequencing system.

Initial rates are measured automatically using a fixed-time digital readout system (67). The rate meter can measure both positive and negative slopes and has a dynamic range of over four orders of magnitude.

Approximately 1000 samples can be analyzed per hour, including changing solutions manually and several rinsing steps between samples of different concentrations.

The first reported use of a small digital computer for stopped-flow-data acquisition and evaluation was by DeSa and Gibson (68). They utilized a DEC PDP 8/I computer with a fast-analog-to-digital converter plus external control and timing circuits. A standard Durrum stopped-flow spectrophotometer was used except the hydraulic drive was replaced with a pneumatic cylinder.

The data acquisition involves taking 400 samples of the response curve at accurately known time intervals. The sampling rate can be varied from 1 Hz to $2 \times 10^4$ Hz and there is an option of changing the sampling rate during data acquisition. This allows some optimization of sampling rate with a changing response.

After data acquisition, the computer checks for overflow of the range of the analog-to-digital converter. If overflow did not occur, smoothing of the data to reduce noise effects can be carried out. Mean values and standard deviations are calculated from replicate runs and

the results outputed as printed copy and/or punched paper tape.

In cases where smoothing is not required, the computer can be instructed to take only 20 data points. This results in the capability of representing a response curve with points as close as 50 microseconds apart.

This system is capable of making absorption and fluorescence measurements and has been applied to problems involving multiple inputs as in polarization of fluorescence and dual wavelength measurements.

An automated computer-controlled stopped-flow system utilizing a novel mass-based solution preparation system was recently reported by O'Keefe and Malmstadt (39). The stopped-flow mixing unit fits into a modular spectrophtometric setup (69). A single TTL pulse from the computer causes the stopped-flow mixer to perform one complete cycle. Desirable features of the mixing system include small sample volumes (0.17 ml) and temperature monitoring of the reacting solution via a high speed thermistor.

Highly accurate spectrophotometric measurements are made with a high intensity unregulated light source. This is accomplished by monitoring the light intensity via a beam splitter and second photomultiplier tube.

The solution preparation system (70) is based on the weights of solutions rather than their volumes. An electronic weight sensor monitors the weight of the sample vials on a turntable as each solution is prepared. The

system is simple and accurate, but the dynamic range of the concentrations prepared is limited.

The computer controls the operation of the entire system from solution preparation to data analysis. The experimentor has a choice between a routine mode of operation for reaction-rate analysis and an investigative mode of operation for fundamental studies.

A highly automated stopped-flow system was developed by Sanderson and coworkers (71). The system consists of a sample preparation unit, a sampling unit, a Sturdevant-type stopped-flow mixer, an optically stabilized spectrophotometer, and a small computer (Hewlett-Packard 2115A). The computer is used to control the instrument and to analyze the reaction-rate data.

Instructions are given to the computer as to what samples are to be prepared and in what order they are to be prepared, the rate at which data is to be taken, the amount of data to be taken, a delay time, a noise increment and what mathematical operations are to be performed on the data.

The system delivers desired amounts of sample solutions and diluent to a receiving vial. This mixture is then transferred to the stopped-flow to be rapidly mixed with reagents and delivered to the observation cell. Spectrophotometric data are collected and processed by the computer. The results are displayed on an oscilloscope or printed out via a teletype.

The computer software is divided into two programs. First a "set-up program" is used to control the sample preparation and then an "operational program" is employed to perform data acquisition and analysis. In order to initiate sample preparation, stock concentrations of the individual constituents along with the volume of the receiving vial are entered as parameters via the teletype. The computer then asks for the concentrations to be used for each run.

The data acquisition and analysis procedure was described by Willis and coworkers (38). The analog-to-digital converter (ADC) can take data at rates from 0.1 Hz to $10^4$ Hz. The resolution of the 10-bit ADC was improved to between 14 and 15 bits by utilizing a real-time variable offset method described by Deming and Pardue (72).

The data processing method provides for operator interaction if desired. The operator has the option of displaying the unprocessed transmittance data on an oscilloscope for diagnostic purposes. Any given run can be rejected and the operating conditions changed if so desired. The program calculates absorbance information and can determine an apparent first-order rate constant at each of several successive points based on a 21 point least squares slope. Display of these slopes provides a check on the first-order assumption. At a later stage the program can determine a more accurate rate constant computed as the least squares slope over one half-life of the reaction.

Analysis of an unknown is performed by determining the ratio of the rate of change of absorbance of the unknown to that of a standard. This assumes first-order behavior.

The most sophisticated stopped-flow system reported to date (73) was developed in the same laboratory as the previous system (71). It utilizes the same stopped-flow spectrophotometer as its predecessor, but the solution preparation unit and the computer system are different.

The control system is a heirarchical arrangement in which a minicomputer directs a microcomputer to prepare reagents and operate the stopped-flow mixer. Thus, the minicomputer is freed of these time consuming tasks which do not utilize its computational power. In addition to sending directives to the microcomputer, the minicomputer acquires and analyzes data and designs new experiments based on simple criteria. The data acquisition routine uses an exponentially changing clock rate for optimum signal-to-noise ratio. The sample preparation unit uses a parastaltic pump to deliver the prescribed amounts of solutions to a sample turntable. The authors claim greater versatility for the new sample preparation unit, although the previous unit, which utilized micrometer driven syringes, had somewhat better accuracy.

## B. The Chemistry of 12-Molybdophosphate

The 12-molybdophosphate anion (12-MPA) is formed by the reaction of phosphate with cationic molybdenum(VI) species in strongly acid solutions. The study of the formation of 12-MPA is complicated by the lack of knowledge of the exact form of the molybdenyl species. Several recent studies (29,74) have reviewed the pertinent work on the chemistry of 12-molybdophosphate and molybdenum(VI). A brief summary of this work including any recent developments will be given here.

### 1. Molybdenum(VI) in Aqueous Solution

Molybdate dissolves in basic solution to form the $MoO_4^{2-}$ species. However, as the pH is decreased toward the isoelectric point, a variety of Mo(VI) species are formed. The techniques of spectrophotometric and pH titrations (75,76), Raman Spectroscopy and ultracentrifugation (76), enthalpy titrations (77), and dialysis and electromigration (78) have been used to identify these species. Sasaki and Sillen have reviewed the work in this area (75). At the isoelectric point insoluble molybdenum trioxide ($MoO_3$) forms. Upon further acidification, this precipitate redissolves as a cationic species. The exact form of the anionic or cationic molybdenum species depends on the bound acid-to-molybdate ratio (Z). However, investigations by numerous workers have resulted in conflicting

results.

Studies (79-82) have indicated that the first step in the protonation of tetrahedral $MoO_4^{2-}$ is a two proton addition which involves a change in coordination to octahedral $OMo(OH)_5^-$ or $Mo(OH)_6^-$. However, recent evidence suggests the protonated form exists as the less symmetric cis-dioxo $MoO_2(H_2O)\ (OH)_3^-$. At a bound acid-to-molybdate ratio (Z) of about 1.14 there is a rapid condensation of this monomer to the well known heptamer, $Mo_7O_{24}^{6-}$.

At about Z=1.5 various workers (76,81,84) have found evidence for the formation of an octameric species, $Mo_8O_{26}^{4-}$, which can be isolated in the solid state. However, the results of other workers (75,83,85) have not supported this contention.

As Z is increased above 1.5 further protonation occurs and eventually $MoO_3$ precipitates at the isoelectric point. If still more acid is added, $MoO_3$ dissolves as a cationic species (86). Both monomeric (78,87) and dimeric (75,88, 89) molybdenyl species have been suggested.

The work of Krummenacker and coworkers (90-94) indicates the presence of protonated and unprotonated monomeric and dimeric cationic species. Formation constants were determined as a function of solution acidity (93,94). Other workers (95) found evidence for a singly charged monomeric species and doubly charged dimeric species at perchloric acid concentrations greater than 3M. A recent study (87) determined equilibrium constants for the

protonation and dimerization of molybdenyl species in agreement with Krumenacker.

## 2. The Formation of 12-Molybdophosphate

Twelve-molybdophosphate is the most widely studied heteropolymolybdate compound. Both the solution chemistry and the solid crystal structure have been studied. The molecular formula for the solid has been determined (96,97) as $H_3Mo_{12}PO_{40}$ with 29-31 water molecules, six of which are structural. The $MoO_6$ octahedra surrounding the $PO_4$ tetrahedron have been shown to be somewhat distorted (97).

The formation of 12-MPA has been studied by numerous workers. Crouch and coworkers (89), who studied the reaction in nitric and perchloric acid solutions, found evidence in agreement with the overall stoichiometry suggested by Souchay (88), viz.,

$$H_3PO_4 + 6HMo_2O_6^+ \rightleftharpoons \text{12-MPA} + 9H^+$$

However, they found a slightly different stoichiometry in sulfuric acid. Other workers have postulated a different stoichiometry starting with a molybdate instead of a molybdenyl species (98).

Halasz and Pungor studied the formation and decomposition of 12-MPA (99). These authors postulated the pH dependent formation of two different forms of 12-MPA

similar to those found (100) for 12-molybdosilicate (12-MSA). However, no spontaneous transformation between the two forms was found as in the 12-MPA case.

Crouch and coworkers (89) concluded that the formation of 12-MPA involves an initial reaction of phosphate with the molybdenyl cation followed by several condensation steps. The reaction was always first order in phosphate, whereas it varied from first to sixth order in Mo(VI) depending on the acid-to-molybdate ratio. The rate varied from zero order in acid at low acid concentrations to inverse eighth order at high acid concentrations.

The formation of 12-MPA has been studied using proton and $^{31}P$ NMR (101,102). Also, numerous studies have been undertaken concerning the reduction of 12-MPA to the hetero-poly blues (89,103-107).

# CHAPTER III

## THE COMPUTER-CONTROLLED STOPPED-FLOW SPECTROPHOTOMETER

In this work a first-generation stopped-flow spectrophotometer (66) has been completely revamped and interfaced to a PDP 8/e minicomputer. The modifications include: installing quartz optics between the monochromator and the observation cell and between the observation cell and the photomultiplier tube (PMT); (2) thermostating the entire system including the drive syringes and the reagent containers; (3) inserting a manual valve between the mixer and the observation cell; (4) designing a more efficient, easily replaceable mixer; (5) inserting a fast thermistor probe in the flow stream just below the observation cell; and (6) installing an optoelectronic trigger module.

An extensive set of computer programs has been developed to operate the instrument and to acquire, analyze and display the spectrophotometric and temperature data.

A block diagram of the instrumental system is shown in Figure 1. The computer controls the operation of the stopped-flow mixing system via three TTL-switched optically isolated power relays (108). At the end of a push, a trigger signal is sent to the computer to initiate data acquisition. This signal is the sole synchronization link between the instrument and the computer.

The radiant power coming from the spectrophotometer (light source and monochromator not shown as separate units) is converted to a current via a 1P28 photomultiplier tube. The current is then converted to a voltage by a variable gain current-to-voltage (I/V) converter. Finally the voltage is converted to a binary number via the 12-bit analog-to-digital converter (ADC). The computer accepts this digital input and subsequently performs data analysis and outputs the results via a printer, a plotter or a fast CRT terminal. The data are also stored on a magnetic disk (not shown) for future use. The fast CRT display is particularly valuable in that it provides on-line feedback.

## A. The Stopped-Flow Mixing System

The stopped-flow mixing system consists of a reagent delivery and drive mechanism, a mixer, an observation cell and a stop syringe. Each of these parts will be discussed and then the operation of the stopped-flow mixing system as a whole will be explained. The system is shown in Figure 2. A description of the designated parts follows.

A, B, M: Air cylinders. These control, respectively, the drive plungers, valve D and valve J.

C: Drive syringes

D: Double 3-way stopcock valve

E: Reagent inlet tubes

Figure 2. A Stopped-Flow Spectrophotometer.

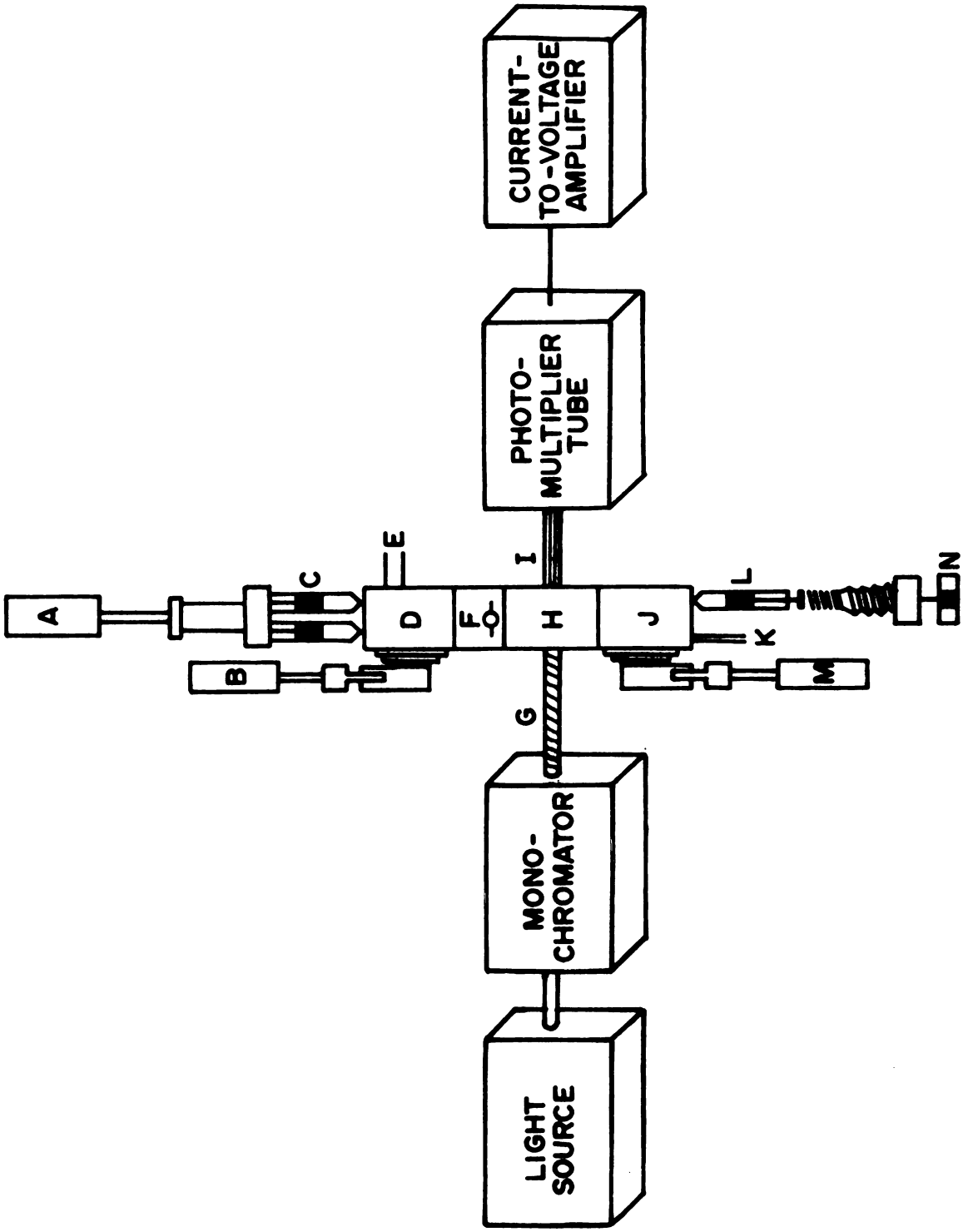

CURRENT-TO-VOLTAGE AMPLIFIER
PHOTO-MULTIPLIER TUBE
LIGHT SOURCE
MONO-CHROMATOR
A
B
C
D
E
F
G
H
I
J
K
L
M
N

Figure 2.

F: Mixer block. This contains the mixer and a manual valve after the mixer to prevent diffusion of unmixed reagents into the observation cell.

G: Flexible quartz fiber optic light guide.

H: Observation cell.

I: Quartz rod coated for internal reflection.

J: Waste release valve, single 3-way stopcock.

K: Solution exit tube.

L: Stop syringe.

M: See A.

N: Photo-interruptor module for trigger signal.

## 1. Reagent Delivery and Drive Mechanism

The purpose of the reagent delivery and drive mechanism is to deliver the reagents into the drive syringes and then to force them from the syringes through the flow system. The flow of reagents into and out of the drive syringes is caused by movement of the plungers via the pneumatic cylinder. The plungers are stainless steel with double neoprene O-rings. The direction of flow is controlled by the double 3-way stopcock. In one position of the stopcock the drive syringes are connected to the reagent tubes, whereas in the other position the syringes are connected to the flow system. When filled, the syringes contain enough solution for approximately six pushes (0.45 ml per push) with good purging of the solution from the previous push. The amount of solution used

per push is controlled by the stop syringe, which will be discussed later.

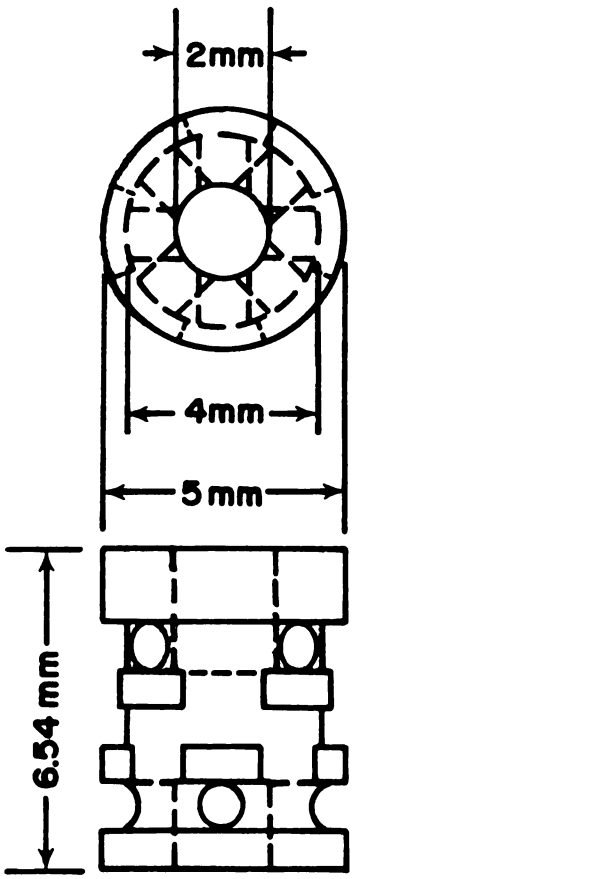
The important factor in determining the velocity of the fluid in the flow system is the static fluid pressure and not the air pressure in the pneumatic cylinder. However, as indicated in Chapter two, any back pressure at the exit end of the flow system must be subtracted from the static fluid pressure in order to obtain the true fluid driving pressure. This will be discussed further under "stopping syringe and trigger". The static fluid pressure is created by the force of the drive syringe plungers on the solutions. Since the 3/8" drive plungers are rigidly attached to the 1 1/8" pneumatic cylinder, the static fluid pressure is equal to 4.5 times the air pressure in the pneumatic cylinder. At a normal air pressure of 75 psi, the static fluid pressure is 338 psi.

## 2. Mixer

The first-generation stopped-flow originally had a tangential jet double mixer commonly used for stopped-flow applications (31). However, the mixing efficiency appeared to be poor. It was observed that no decrease in the dead time of 7.5 milliseconds was realized as the air pressure was increased from 60 psi to 90 psi. This pressure increase should result in an increase in flow velocity of more than 25 percent and a concomitant decrease

in the dead time. The dead time was determined by the extrapolation technique using the iron-thiocyanate reaction (24). At this point the alignment and other mechanical aspects were checked to make sure this observation was not due to some mechanical problems. Having determined that there were no mechanical problems, it was concluded that the mixer should be replaced. Two simple designs were tried, but they gave essentially the same results as the original mixer. The third mixer which was tried is shown in Figure 3. This mixer gave a dead time of 5.5 milliseconds at an air pressure of 70 psi and a dead time of just under 4 milliseconds at 90 psi.

The mixer was designed for easy replacement in the event that it proved unsatisfactory or for cleaning purposes. The mixer simply slips, very snuggly, into a cylindrical opening. Except for the entrance and exit, the flow is between the mixer body and the walls of the opening. The two solutions come together at the top of the mixer and then flow out of the entrance channel through the four upper holes. Mixing then continues around the perimeter as the solution is forced to flow around the lugs which seal against the containing walls. Finally, the solution flows into the four lower holes and out the exit channel toward the observation cell.




MIXER VOLUME = 0.0446cm$^3$

Figure 3. The Stopped-Flow Mixer.

3. C

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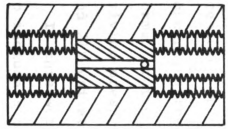
## 3. Observation Cell

The observation cell is designed to enable conductance as well as absorbance measurements. The cell was constructed by sandwiching four-3 mm platinum disks (with leads) between blocks of Kel F and then boring a 2 mm hole through the stock. Entrance and exit channels were bored at a right angle to the cell channel at either end. The Kel F sandwich is fitted into a stainless steel housing. The cell is shown in the upper left of Figure 4. The electrodes are not shown. Stainless steel inserts, which are threaded on the inside and outside, are used to compress the Kel F sections in order to seal the electrodes. The optics, also shown in Figure 4, are screwed into the inserts and seal the ends of the observation cell. The pathlength of the cell is approximately 2 cm.

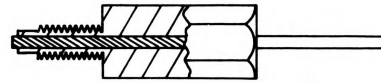
A threaded thermistor port intersects the flow channel just below the observation cell. A fast responding thermistor is epoxied into a stainless steel threaded plug. The plug is screwed into the port so that the thermister bead just protrudes into the flow channel.

## 4. Stopping Syringe and Trigger

The purpose of the stopping syringe is to suddenly stop the flow of solution. The flow of solution causes the plunger of the stopping syringe to be pushed back until it comes in contact with the stop block. At that



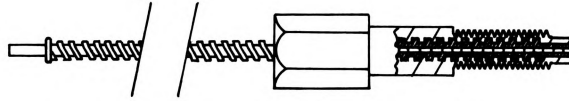
Observation Cell



Coated Quartz Rod and Delrin® Housing



Monochromator Fitting



Flexible Quartz Fiber Optics Bundle and S.S. Housing

Figure 4. The Stopped-Flow Observation Cell and Quartz Optics.

point the flow is suddenly stopped. Judging from the amount of "rounding" of the absorbance trace of a fast reaction, the time required to completely stop the flow is less than a tenth of a millisecond. The stop block consists of a threaded bolt so that the amount of flow before stopping is adjustable.

The plunger to the stopping syringe is spring loaded so that when the waste release valve is switched from the flow to the waste release position, the spent reagents are ejected automatically. An important consideration is the amount of back pressure caused by the loaded spring. The spring force was measured by suspending a weight from a rod which was attached to the syringe plunger on one end and rested on a pivot on the other end. The spring force is 5.7 pounds at the beginning of the push and 9.1 pounds at the end of the push. This translates to back pressures on the fluid of 74 and 119 psi. In the section concerning the drive mechanism it has been shown that the normal static fluid pressure is 338 psi. Thus the fluid driving pressure is 264 psi at the beginning of a push and 219 psi at the end.

In this work the stopping mechanism was modified slightly to include a triggering mechanism. The trigger signal must occur at a reproducible time prior to the end of a push so that the data collection can be synchronized to the time that the flow stops.

The triggering mechanism was constructed as follows.

A hole was bored through the center of the stop block (bolt). A rod was inserted through this hole and attached to the stopping syringe plunger. A photo-interruptor module (G. E., Model H13B1) was mounted so that the end of the rod interrupts the light path just before the plunger comes to rest against the stop block.

The time between the trigger signal and the actual stopping time (trigger time) can be varied by changing the position of the photo-interruptor module. This may be useful in characterizing the stopped-flow mixing system. However, under normal circumstances, the position would remain fixed. Of course the trigger time also depends on the driving pressure. Using an air pressure of 75 psig, the trigger time was 10.0 milliseconds. The trigger time was determined as the time (from the trigger signal) at which the absorbance trace of the iron-thiocyanate reaction begins to deviate from its level value during flow. This determination was performed several times on different days with a reproducibility of better than 0.2 millisecond.

## 5. Sequence of Operations

A description of the operation of the mixing system as a whole follows. See Figure 2 for locations of the components being referenced.

(1) Valve "D" is switched to allow the reagents to be drawn into the drive syringes.

(2) The drive syringe plungers "C" are drawn back via pneumatic cylinder "A". Then valve "D" is switched back to connect the drive syringes with the flow system.

(3) The pneumatic cylinder "A" is then pressurized in the downward direction to force the solutions through the flow system. However, no flow occurs because valve "J" is in the waste release position.

(4) Valve "J" is rapidly switched to the flow position at which point solution flows through the entire system pushing back the stop syringe plunger "L".

(5) Just before the stop syringe plunger hits the stop block, the rod which is attached to the plunger passes between the LED and the phototransistor of the photo-interruptor module. This causes the trigger signal to synchronize the data taking to the time at which the stop occurs. The flow stops shortly thereafter, typically 10 milliseconds.

(6) After the data are taken, valve "J" is switched to the waste release position whereupon the spent solution is expelled through tube "K".

(7) Operations (4) through (6) are repeated until the drive syringes are empty. normally six times.

## B. The Spectrophotometric Detection System

The components of the spectrophotometric detection system are illustrated in Figures 1 and 2. These

components are listed below.

(1) GCA/McPherson, EU-701-50 Light Source.

(2) GCA/McPherson, EU-700 Monochromator.

(3) Schott Optical, 25 cm, 2 mm diameter, quartz fiber optic bundle.

(4) Custom built Observation Cell (109).

(5) Schott Optical, QLG, 10 cm, 3 mm diameter quartz rod, coated for internal reflection.

(6) RCA, 1P28 Photomultiplier Tube and Heath, EU-42A High Voltage Power Supply.

(7) Keithley, 427 Current Amplifier.

(8) Heath, EU-800-GC, D/A Converter Card; utilizing an Analog Devices, MDA 10Z-25, 10-bit D/A converter.

(9) Datel, DAS-16-M12B, 12-bit, 8-channel A/D Converter.

The specifications for these components and other equipment used in this work are given in Appendix A. The non-standard items in this list are the observation cell, the quartz optics and the offset circuit. The observation cell has been discussed previously, and the other two items will be discussed here.

The optics were designed with two purposes in mind, viz., to maximize light throughput and to eliminate the disturbances caused by any vibrations of the stopped-flow mixing system. These vibrations are caused by the rapid switching of the waste release valve and by the stop syringe plunger striking the stop block. Since the

stopped-flow spectrophotometer was built as a modular system, these vibrations cause disturbances in the optical transmission between the monochromator and the observation cell. Since the PMT is bolted directly to the mixing unit, vibrations present no problem there.

The optics are shown in Figure 4. The 2 mm diameter flexible quartz fiber optic bundle which couples the monochromator to the observation cell has several advantages. Since it is rigidly fixed to both units and since its transmission is not affected by slight bending, the vibration problem is eliminated. The light throughput is quite good for several reasons. The fiber bundle diameter is the same as both the observation cell diameter and the usual slit width of the monochromator. The internally reflecting aspect of the fibers greatly reduces light losses. Light losses are further reduced since the end of the fiber bundle forms the window of the observation cell. This eliminates a quartz-air interface which would reduce transmission by about 10 percent due to Fresnel reflections.

Since vibrations are not a problem between the observation cell and the PMT, a 3 mm diameter, internally reflecting, quartz rod was utilized there. The rod must be slightly larger than the diameter of the observation cell because it forms the end seal. However, this causes no light loss since it is the (light) exit end of the observation cell. In the case of the fiber bundle, the

stainless steel sheath around the bundle forms the seal. Divergence losses are reduced in that the rod transmits the "trapped" light right up to the window of the PMT. The rod has the added advantage that the cross-sectional area is 100% transmitting as opposed to about 70% for the fiber bundle.

There was approximately a one hundred-fold increase in light intensity by adding these optics to the system. This was determined by the increase in the photocurrent at the same PMT voltage.

The other improvement in the detection system involves automatic, calibrated offset of the photocurrent to enable scale expansion measurements. The 12-bit ADC limits the resolution of signals to the computer to 1 part in 4000. However, with accurate offset and scale expansion, spectrophotometric resolution of 1 part in 200,000 with better than 1% accuracy has been achieved.

The Keithley 427 Current Amplifier was modified so that offset can be performed remotely. The normal method of offset is via a potentiometer, which is connected between the +15V and -15V sources within the current amplifier. The center tap of the potentiometer is connected to a resistor which leads to the summing point of the operational amplifier. Decade values for the summing point resistor are selectable via a switch on the front panel of the current amplifier. A single-pole-double-throw switch was inserted in the circuit so that the summing

point resistor can be connected to the potentiometer for manual offset or to a BNC connector for remote offset. The remote offset voltage is supplied by a Heath EU-800-GC, 10-bit digital-to-analog converter (DAC) followed by an Analog Devices AD518K operational amplifier used as a unity gain inverter. Since only negative currents are produced by the PMT, only positive offset voltages are required. The DAC is controlled by the computer.

## C. The Thermistor and Thermostating System

The entire flow system is thermostated by circulating water from a Neslab T.V. 45/250 thermostating bath. The temperature of the water in the 50 liter bath is controlled to 0.1°C. The temperatures from 10°C to 40°C have been used with no difficulties. The solution containers and the drive syringes have thermostating jackets. The rest of the flow system has numerous channels bored through the stainless steel blocks for thermostating purposes.

However, thermostating cannot control solution temperature changes on the millisecond time scale. Fast solution temperature changes can be produced by heats of reaction or dilution and by viscous heating of the flowing solutions. The temperature rise due to viscous heating of water flowing in the stopped-flow mixing system was measured by a fast thermistor and found to be a few tenths of a degree centigrade.

The rate of temperature equilibration in the 2 mm diameter observation cell can be calculated as follows. For a tube of circular cross-section and constant wall temperature (perfect thermostating), the heat flux through the wall is given by,

$$Q = hA\Delta T, \ cal \cdot sec^{-1}$$

where h = heat transfer coefficient, $cal \cdot cm^{-2} \cdot s^{-1} \cdot {}^{\circ}C^{-1}$

A = total wall area, $cm^2$

$\Delta T$ = temperature difference between the fluid and the wall, °C.

The value of h for free convection in water (still water) can be obtained from "Perry's Chemical Engineer's Handbook, 4th Edition, 10-20. It is 0.004 for a temperature difference of 0.1 to 0.5°C and goes up to 0.008 for a temperature difference of 5°C. The normal temperature difference would be less than 0.5°C, so the value of 0.004 for the heat transfer coefficient is used here. The rate of temperature equilibration, RTE, can be calculated as follows:

$$RTE = \frac{Q}{M \cdot C \cdot T}, \quad s^{-1}$$

$$= \frac{h \cdot A}{m \cdot C}$$

where, m = the mass of the fluid, g

c = the heat capacity of the fluid, $cal \cdot g^{-1} \cdot {}^{\circ}C^{-1}$.

The values for water in a 2 mm diameter by 2 cm long cell are:

$h = 0.004\ cal \cdot cm^{-2} \cdot s^{-1} \cdot {}^{\circ}C^{-1}$

$A = 1.26\ cm^2$

$m = 0.063\ g$

$c = 1.0\ cal \cdot g^{-1} \cdot {}^{\circ}C^{-1}$

Thus,

$RTE = 0.08\ s^{-1}$ or 8% per second.

Therefore the temperature of the solution would be approaching the wall temperature at a rate equal to 8% of the difference per second. Assuming that the temperature of the solution remains uniform through free convection, it would approach the wall temperature in a decaying exponential fashion. The rate is obviously too slow to assume temperature equilibration for fast reactions.

Because of the inability to control temperature precisely, a fast responding thermistor (7 ms time constant) was installed as described earlier. The thermistor and associated monitoring electronics were recently discussed (110).

## D. Computer Interface

All digital signals to and from the PDP 8/e computer are transferred through a Heath Computer Interface Buffer.

A Heath EU-801-21 I/O Patch Card is used to transfer data and timing pulses between the peripherals and the computer. The digital logic used to operate the stopped-flow system via signals from the computer consists of an octal decoder, NOR gates, NAND gates and flip-flops. The flip-flops hold the logic states for the optically isolated power relays which control the operation of the stopped-flow mixing system as explained earlier. The DAC, used for offset, is operated by signals from the I/O Patch Card. The logic and interface design for the ADC has been explained in a recent thesis (111). All the inter face cards except thosed for the ADC are housed in and powered by a Heath EU-801I Computer Interface ADD.

## E. Software

The software to operate the stopped-flow mixing system and acquire and analyze the spectrophotometric and temperature data was designed for maximum flexibility. The DEC OS/8 operating system allows chaining between programs with data and other parameters saved in a "common" area of the computer memory. Chaining involves the use of a file oriented mass storage device. The computer system used here has a DEC RK05 disk drive and also a SYKES dual Floppy Disk drive for mass storage. The program which is chained to is swapped into memory and its execution begun automatically. In this manner a closed loop

computational system involving many separate programs can be created.

In this work assembler language (PAL8) programs were created to operate the stopped-flow and acquire raw data related to absorbance and/or temperature. After the data are acquired, a FORTRAN program is swapped into memory. The FORTRAN program converts the raw data to absorbance and/or temperature, stores the data on the disk, analyzes the data and displays it if desired. The experimenter can then have the computer chain back to the PAL8 program to acquire more data or chain to some other program or exit. For maximum control all chaining is specified in real time by the experimenter. FORTRAN programs are also available for off-line analysis, display, etc. of the data files stored on the disk.

Appendix B lists the main programs used in this work along with a brief description of their capabilities.

Four different PAL8 programs were written to cover the options of taking temperature data along with the spectrophotometric data and using scale expansion for the spectrophotometric detection. The PAL8 program which includes scale expansion option is given in Appendix D and the dialog is given in Appendix C. The FORTRAN program which this PAL8 program would chain to is shown in Appendix E.

The calculation of absorbance values when using the scale expansion mode is somewhat complicated and will

be explained here. The offset is adjusted and calibrated and then the raw data (ADC readings) are acquired by the PAL8 program. The raw data and offset parameters are then passed to the FORTRAN program where absorbance values are calculated.

The offset calibration and data collection are performed as follows:

(1) The operator selects the amplification and offset range on the Keithley;

(2) With the light source shutter closed, the dark current level (ZRLVL) is recorded. Then the computer varies the offset and records the output levels in order to obtain the factor relating the change in the DAC setting. to the change in the ADC reading. This factor is stored as a binary number (FCTR) and a binary exponent (FEXP).

(3) The shutter is opened, and the offset is adjusted so that the blank level is just below the upper limit of the ADC (+5V). This ADC reading (BLNK) is saved along with the DAC setting (UDAS).

(4) The sample is run, and the ADC readings (PV) stored as raw data.

(5) The FORTRAN program is swapped into core with the offset parameters, and the blank value saved in "common" along with the raw data.

The transmittance range (TR) can be calculated in terms of the ADC readings as follows:

$$TR = (UDAS)(FCTR)(2)^{FEXP} + BLNK - ZRLVL$$

The transmittance of a data point (TP) can then be written as,

$$PT = 1 - \left(\frac{BLNK-PV}{TR}\right)$$

For computational purposes this can be broken down to,

$$PT = \left(1 - \frac{BLNK}{TR}\right) + \frac{PV}{TR}$$

Thus each raw data point needs only to be divided by a constant and added to another constant in order to obtain the transmittance. Absorbance is then calculated by taking the negative logarithm of the PT value.

# CHAPTER IV

# TESTING AND CALIBRATION OF THE AUTOMATED STOPPED-FLOW SPECTROPHOTOMETER

The testing and calibration of the automated stopped-flow spectrophotometer is a very important part of this work. It is imperative that the accuracy, reproducibility and limits of an instrument be known in order to use it in meaningful quantitative studies. The flow system and the detection system were both thoroughly tested and characterized. The specifications of all the instruments used to test the system as well as the specifications of the components of the stopped-flow system are given in Appendix A. Only the specifications which are pertinent to this work are given. Complete specifications can be obtained from the manufacturers.

## A. Testing and Calibration of the Flow System

When the stopped-flow drive syringes are full, they hold 2.35 ml each or a total of 4.7 ml. The amount of solution delivered per push can be adjusted by moving the stop block as explained earlier. The optimum amount of solution to be used per push is the minimum volume which will insure complete purging of the old solution from the observation cell. This can most easily be determined by using a relatively slow reaction which

results in a fairly large absorbance change. By starting with a small push volume and increasing it until the initial absorbance starts at zero, the volume for complete purging can be found. That point was found at approximately seven pushes per syringe filling. The instrument is normally run at six pushes per filling, allowing a safety margin. At this setting 0.39 ml of each solution or 0.78 ml total are used during each push. The volume from the mixer entrance to the exit of the observation cell is 0.16 ml. Thus, it takes approximately four times this volume for thorough purging. This factor will vary somewhat depending on the design of the flow system.

Two solutions were made up to calibrate the pathlength of the observation cell and to determine if equal volumes are delivered by the drive syringes. The solutions were:

(A) 0.0017 M $K_2Cr_2O_7$, 0.2 M$H_2SO_4$

(B) 0.2 M $H_2SO_4$

The absorbance of (A) and the absorbance of a 50-50 mixture of (A) and (B) were measured in a Cary 17 spectrophotometer with a standard quartz one centimeter cell, and in the stopped-flow. The pathlength of the stopped-flow was determined by comparing the absorbance values at 435 nm. The path length was calculated to be 1.94±0.01 cm, using either solution.

In order to ascertain that equal volumes are delivered by the drive syringes, solution (A) was loaded into one

drive syringe and solution (B) into the other. The resultant absorbance was compared to the absorbance obtained when the solutions were switched. The values agreed with each other to within 0.15%, and in either case the absorbance data had a relative standard deviation of less than 0.8%. This indicates that the volumes delivered by the syringes are equal, within a fraction of one percent.

The dead time of the stopped-flow mixing system was determined directly by measuring the flow velocity and indirectly by the reaction extrapolation technique (24) mentioned earlier. A flow velocity transducer utilizing an opto-interruptor module was used to measure the dead time. The result was 5.08±0.09 ms versus 5.4±0.4 ms when the reaction extrapolation technique was used (112). The slightly higher dead time measured by the extrapolation technique could indicate incomplete mixing.

The mixing efficiency was measured by monitoring a very fast reaction before and after stopping the flow. The reaction must be complete (assuming instantaneous mixing) in a time interval which is shorter than the dead time of the stopped-flow. A suitable reaction for this purpose is p-Nitrophenol plus sodium hydroxide, which goes to completion in less than 1 ms. Figure 5 shows the absorbance versus time curve. The beginning of the transition interval is the point where the flow stops. It can be seen that mixing is 98-99% complete by the time the solution reaches the observation cell. However,


Figure 5. Mixing Efficiency.

*The p-nitrophenol should be in slight excess to ensure proper indication of the degree of mixing.

the time for the absorbance to level off seems inordinately long, another 10-20 milliseconds. This did not cause any problems with the measurements on the reactions used in this work. However, this artifact should be investigated further and corrected if possible to enable precise measurements on faster reactions. This artifact is apparently a mixing problem. Nevertheless the transition period remained unchanged as the air pressure was changed from 70 psi to 90 psi.

## B. Testing and Calibration of the Detection System

The active components of the detection system are a light source, a photomultiplier tube (PMT) and its power supply, a current amplifier and the associated DAC offset circuit, and finally the analog-to-digital converter (ADC). These components are illustrated in Figures 1 and 2.

The light source was easily identified as the stability limiting component of the detection system. This is indicated by the manufacturer's specifications (see Appendix A) and was verified experimentally. The GCA/McPherson EU-701-50 light source module used in this work has a deuterium lamp for UV work and a stabilized tungsten lamp for the visible region. In addition, the tungsten lamp has two modes of regulation, a voltage control mode and an intensity control (optical feedback) mode. Although the deuterium lamp was not used in this work its stability was checked also. All intensity measurements

were made at 450 nm. A Heath SR-255B strip chart recorder was used for recording the spectrophotometer output.

The deuterium lamp produced drifts (over any 2 hour period) of 10% during the first 12 hours of warmup. After this warmup period maximum drifts of 1% over any 2 hour period were observed.

In the constant voltage mode the tungsten lamp produced drifts on the order of 5-10% during the first two hours of warmup. After this period the maximum drift in any 2 hour period was 2%. However, the drift could be as much as 1% in a 20 minute period.

The tungsten lamp gave better results when used in the optical feedback mode. Drifts were on the order of 5-10% during the first 4 hours of warmup. After 4 hours the maximum drift in any 2 hour period was down to 1%, and after 1 day of warmup it was 0.5%. The drifts after warmup are characterized by slow changes followed by abrupt feedback corrections. These feedback corrections are the most critical in that a change on the order of the maximum drift can occur in a few seconds.

The photocurrent measuring section of the detection system was thoroughly tested by determining the accuracy of the components and then determining the accuracy of the section as a unit. The section consists of the 10-bit digital-to-analog converter (DAC)-operational amplifier combination, the Keithley 427 current amplifier and the 12-bit analog-to-digital converter (ADC). The test

equipment consisted of a Power Designs 2005 precision power (voltage) source, an esi PVB 300 voltmeter bridge, a Fluke 8600A digital multimeter and a Keithley 261 precision current source.

The accuracy of absorbance measurements does not depend on the absolute accuracy of any of the components but only on their linearity. The linearity results reported here are the worst case values.

The offset circuit had a linearity of 0.2% for DAC settings as low as 20 (octal). This value improved to 0.1% for settings above 200 (octal). These values are valid for gain settings of $10^4$ to $10^{10}$ volts per ampere.

For settings of $10^6$ to $10^{10}$ volts per ampere, the linearity of the current amplifier was 0.02%. On the $10^5$ volts per ampere setting it was found to be 0.1%.

The linearity of the ADC was measured as 0.2% or ±1 LSB over its entire range.

The overall accuracy of the photocurrent measuring unit was determined by supplying currents from the precision current source. Simulated spectrophotometric measurements were made by using currents of $8.99 \times 10^{-8}$ to $8.99 \times 10^{-5}$ amperes as blank photocurrents and having the computer calculate absorbance values for currents which were a fraction of the blank value. The values computed from the simulated photocurrents were then compared to the theoretical absorbance values. For absorbance values from 0.0005 to 0.01, the absolute error was less than

0.0002. The relative error was less than 10% for absorbances as low as 0.0005. These error values apply to both the scale expansion mode and the manual offset mode. Above an absorbance of 0.01, the manual offset mode gave superior accuracy. For absorbance values between 0.01 and 2.25 errors were less than 0.3%.

Although no improvement in the absolute absorbance accuracy is gained by using the scale expansion mode, the accuracy of reaction rate measurements may be significantly improved. In the case of small absorbance changes, resolution becomes the limiting accuracy factor. By using the scale expansion mode, the resolution of the light intensity measurement can be improved from 1 part in 4000 to 1 part in 200,000 with better than 1% absolute accuracy.

## C. Accuracy of Monitoring a Chemical Reaction

A well behaved chemical reaction was utilized to examine the overall reproducibility of the automated stopped-flow system. The reaction chosen for this purpose was the iron-thiocyanate reaction (113,114). Under the conditions used, the appropriate equilibrium is

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

the rate law can be expressed as

$$\frac{d[Fe(SCN)^{2+}]}{dt} = k_1[Fe^{3+}][SCN^-] - k_{-1}[Fe(SCN)^{2+}]$$

All solutions used in this determination were prepared by diluting stock solutions. The stock solutions were made from reagent grade ferric nitrate, sodium thiocyanate and perchloric acid. The concentrations used in this work are given in Table 1. In all cases the ionic strength was adjusted to 0.40 with sodium perchlorate.

In this study kinetics data and equilibrium data (in separate runs) were taken at 450 nm. The molar absorptivity of the complex at 450 nm has been determined as 4600 $\ell \cdot mol^{-1} \cdot cm^{-1}$ (114). The kinetics data were analyzed in two different ways, and the results intercompared and compared to the equilibrium results. The agreement between the three results is a measure of the reproducibility of the automated stopped-flow system. The kinetics data were analyzed by the general purpose curve fitting program, KINFIT (115), on a CDC 6500 computer. The program determines the best fit rate constants. The differential equation (rate law) is integrated by a Runge-Kutta method.

In one kinetics analysis (Table 2) all 100 data points were fit. The final absorbance values were within 90% of the equilibrium values in all cases. In the other kinetics analysis (Table 3) only the first 20 points of each data set were used. The final absorbance values in those analyses ranged from 37% to 67% of the

Table 1. Iron-Thiocyanate Concentrations.

| Reaction Number | $[Fe^{3+}]$ | $[SCN^{-}]$ | $[H^{+}]$ |
|---|---|---|---|
| 1 | 0.004 | 0.00025 | 0.20 |
| 2 | 0.002 | 0.00100 | 0.20 |
| 3 | 0.002 | 0.00050 | 0.20 |
| 4 | 0.001 | 0.00025 | 0.20 |
| 5 | 0.001 | 0.00100 | 0.20 |
| 6 | 0.002 | 0.00050 | 0.10 |
| 7 | 0.001 | 0.00025 | 0.05 |

Table 2. Iron-Thiocyanate Results Using All 100 Data Points

| Reaction Number | $k_1$ | Std. Dev. | $k_{-1}$ | Std. Dev. | $K=k_1/k_{-1}$ |
|---|---|---|---|---|---|
| 1 | 202.7 | 0.10 | 1.330 | 0.0009 | 152.3 |
| 2 | 208.4 | 0.16 | 1.383 | 0.0013 | 150.7 |
| 3 | 206.4 | 0.16 | 1.323 | 0.0015 | 156.0 |
| 4 | 211.9 | 0.23 | 1.334 | 0.0020 | 158.8 |
| 5 | 211.1 | 0.09 | 1.301 | 0.0008 | 162.2 |
| 6 | 299.0 | 0.15 | 1.999 | 0.0012 | 149.6 |
| 7 | 493.4 | 0.85 | 3.291 | 0.0093 | 149.9 |

Table 3. Iron-Thiocyanate Results Using First 20 Data Points

| Reaction Number | $k_1$ | Std. Dev. | $k_{-1}$ | Std. Dev. | $K=k_1/k_{-1}$ |
|---|---|---|---|---|---|
| 1 | 203.1 | 9.59 | 1.334 | 0.2710 | 152.2 |
| 2 | 207.0 | 0.25 | 1.354 | 0.0071 | 152.9 |
| 3 | 207.0 | 0.33 | 1.335 | 0.0096 | 155.1 |
| 4 | 209.9 | 1.32 | 1.295 | 0.0359 | 162.1 |
| 5 | 212.2 | 0.49 | 1.331 | 0.0140 | 159.4 |
| 6 | 300.4 | 0.44 | 2.022 | 0.0090 | 148.6 |
| 7 | 484.6 | 5.93 | 3.040 | 0.2090 | 159.4 |

equilibrium values. The time between data points was 0.02 seconds for all concentrations except the last set, in which case it was 0.06 second.

Table 4 shows the values obtained from the equilibrium analysis. The equilibrium absorbance values are an average of from 20 to 50 data points. The standard deviations of these values illustrates the absolute precision obtainable at various absorbance levels.

There is good agreement between the rate constants obtained by using different subsets of the data points. The equilibrium constants obtained from the kinetics data (Table 2) agree with those obtained by measuring equilibrium absorbance values (Table 4) to better than 0.6% for all concentrations.

In the last two reactions, the acid concentration was varied to study the effect it would have on the rate constants. Below and coworkers (113) found that the forward rate, but not the reverse rate, had an inverse dependence on hydrogen ion concentration. The results here show that both the forward and reverse rates are affected by the same amount.

The equilibrium constants obtained here are in good agreement with those obtained by Lister and Rivington (114). They obtained values of 146 and 169 $\ell \cdot mol^{-1}$ for ionic strengths of 0.5 and 0.3, respectively. Although Below and coworkers postulated a hydrogen ion concentration effect on the forward rate law, a conditional forward

Table 4. Iron-Thiocyanate Equilibrium Results

| Reaction Number | Equilibrium Absorbance | Standard Deviation | K |
|---|---|---|---|
| 1 | 0.8393 | 0.00086 | 153.2 |
| 2 | 1.9045 | 0.00264 | 150.7 |
| 3 | 1.0220 | 0.00103 | 156.5 |
| 4 | 0.2983 | 0.00049 | 158.7 |
| 5 | 1.1166 | 0.00116 | 162.4 |
| 6 | 0.9880 | 0.00107 | 149.4 |
| 7 | 0.2825 | 0.00047 | 149.5 |

rate constant can be calculated from their results. The conditional constant would correspond to the forward rate constant obtained at a fixed acid concentration. At an acid concentration of 0.20, this conditional constant is 228 $\ell \cdot mol^{-1} \cdot s^{-1}$. This is in good agreement with the average value of 208 $\ell \cdot mol^{-1} \cdot s^{-1}$ obtained (Table 2) for that acid strength.

In summary, this chemical study has shown that accurate, reproducible absorbance data can be obtained with this automated stopped-flow system.

# CHAPTER V

## STUDY OF THE FORMATION OF 12-MOLYBDOPHOSPHATE

In order to study the formation of the 12-molybdophosphate anion (12-MPA), a preliminary study of molybdenum(VI) species in strongly acid solutions (molybdenyl species) was undertaken. The number of protons consumed in the transformation of the molybdate anion to the molybdenyl cation was determined. Also, the spectra of the molybdenyl species were recorded so that blank corrections could be made in the spectrophotometric study of 12-MPA. Finally, the effect of acid concentration on the formation of 12-MPA was studied with the automated stopped-flow spectrophotometer described earlier. A rather surprising conclusion is reached concerning the reaction at low acid concentrations.

### A. Proton Consumption by Molybdenum(VI)

#### 1. Characterization of the pH Instrument

The solutions used in this work were adjusted to pH values below 1.0 with nitric or sulfuric acid. Measuring pH values below 1.0 with a glass electrode is tenuous because as pH approaches zero, inaccuracies result due to saturation of the glass membrane. Therefore, it is

essential to calibrate the electrode and to make all measurements in a careful, reproducible manner.

Before each set of pH measurements was made, the pH instrument was standardized at pH - 4.00, with an NBS certified buffer, and at pH - 1.1, with 0.100 M HCl (116-118). All solutions which were measured with the pH instrument, except the two standards, were adjusted to an ionic strength of 1.00 with sodium nitrate. The stock sulfuric acid and nitric acid solutions (2.00 M), used in this work, were standardized with 1M sodium hydroxide which had been previously standardized with oven-dried potassium hydrogen phthalate (KHP).

This study involves the measurement of the change in hydrogen ion concentration when molybdate is added to an acid solution. An acid solution and an acid plus molybdate solution were made up for each acid concentration. The difference between the hydrogen ion concentrations of the two solutions is the acid bound to the molybdate. In order to determine the accuracy of such measurements, nitric acid solutions were made up to approximate the pH values of the pairs of solutions of interest. The pH values of these test solutions were read from the pH meter and converted to hydrogen ion "activities", $_{M}$. Since the analytical hydrogen ion concentrations of these test solutions are known, "activity coefficients", g, can be calculated to relate the measured hydrogen ion activities to the analytical concentrations as follows:

$$_{M} = g[H^{+}]$$

The subscript "M" denotes that the activity is derived from a pH meter reading and contains any inaccuracies associated with it. The g coefficients are calculated from the measurement of the higher hydrogen ion concentration of each pair. These values are then used to calculate the change in hydrogen ion concentration as follows:

$$[H^{+}]_2 - [H^{+}]_1 = \frac{(\ _M)_2 - (\ _M)_1}{g}$$

or

$$\Delta[H^{+}] = \frac{\Delta\ _M}{g}$$

The results of the pH instrument accuracy determination are shown in Table 5. For the average of five readings, errors of less than 5% can be expected in the measurement of the change in hydrogen ion concentration.

## 2. Protons Consumed by Molybdenum(VI)

The number of protons consumed per Mo(VI) molecule in strongly acid solution is termed the bound acid-to-molybdate ratio (Z). Acidification results in the conversion of molybdate anions to molybdenyl cations. It is important to know Z in order to determine the composition of the molybdenyl species which reacts with phosphate

Table 5. Accuracy of the pH Instrument.

| Concentrations, M | | | | | | |
|---|---|---|---|---|---|---|
| High $H^+$ | low $H^+$ | g* | $\Delta[H^+]^*$ (determined) | Std. Dev. | Error | % Error |
| 0.060 | 0.030 | 1.116 | 0.0294 | 0.0005 | -0.0006 | -2.0 |
| 0.200 | 0.150 | 1.033 | 0.0478 | 0.0024 | -0.0022 | -4.4 |
| 0.400 | 0.300 | 0.9805 | 0.0971 | 0.0048 | +0.0029 | +2.9 |

*Averages of 5 results.

to form 12-MPA. Also the 12-MPA reaction-rate is highly dependent on acid concentration. Therefore the consumption of protons by Mo(VI) may affect the reaction rate indirectly.

The bound acid-to-molybdate ratio was measured in nitric and sulfuric acids. Because of the limitations of the pH electrode, the maximum acid concentration used was about 0.4 molar. The results in nitric acid are shown in Table 6. The g coefficients were calculated, using the acid solutions with no molybdate. The results indicate that Z increases with the free acid-to-molybdate ratio and with the acid concentration. A maximum Z value of about 2.5 supports the existence of a protonated dimer, $HMo_2O_6^+$. It is unfortunate that higher acid concentrations could not be utilized in order to see if Z levels off at a value of 2.5. A Z value of 2.0 indicates the formation of molybdenum trioxide. Thus the Z values of 2.37 and 1.65 indicate the presence of molybdenum trioxide and even anionic molybdenum species. This contention is supported by the observation that a white precipitate (presumably molybdenum trioxide) forms over a period of time in solutions when the free acid-to-molybdate ratio is less than about 10.

Calculating Z values in sulfuric acid medium is not as simple as in the case of nitric acid because of the buffering effect of the bisulfate ion. Concentration dissociation constants, $K_c$, for the bisulfate ion, at

Table 6. Mo(VI) in Nitric Acid.

| Conc $HNO_3$ | Mo(VI) | g[a] | $\Delta[H^+]$[a] | Std. Dev. | Z[b] |
|---|---|---|---|---|---|
| 0.060 | 0.02 | 1.118 | 0.0330 | 0.0006 | 1.65 |
| 0.200 | 0.02 | 1.025 | 0.0464 | 0.0030 | 2.32 |
| 0.400 | 0.04 | 0.976 | 0.1012 | 0.0032 | 2.53 |

[a]Averages of 6 results.

[b]Bound acid to molybdate ratio, $\Delta[H^+]/[Mo(VI)]$

various sulfuric acid concentrations, are calculated by measuring the hydrogen ion concentrations. The results in sulfuric acid medium are shown in Table 7. The Z values are slightly lower than in the nitric acid case. However, they agree within experimental error.

In either acid only Z values measured after about two days were used. It was observed that Z increased to a maximum and then settled back down to a stable value within one to two days. The maximum value was as much as 15% greater than the equilibrium value. However, because of the limited precision of the pH instrument, the relationship between this phenomenon and concentrations was not determined. The stable period was observed to last at least two weeks. However, after one month the solutions (especially those with low Z values) showed signs of deterioration.

The pH measurements of the nitric acid solutions were made under approximately the same conditions as the determination of the instrument's accuracy which was shown to be better than 5%. However, in the case of sulfuric acid, g coefficients cannot be determined because of the uncertainty as to the exact hydrogen ion concentration. The coefficients from the nitric acid solutions of similar hydrogen ion concentrations were used.

In adjusting the ionic strength of the sulfuric acid solutions to 1.00, the approximation was made that sulfuric acid in solution exists entirely as hydrogen ions

Table 7. Mo(VI) in Sulfuric Acid.

| Concentration, M | | | Hydrogen ion concentration, M[b,c] | | | | | |
|---|---|---|---|---|---|---|---|---|
| $H_2SO_4$ | Mo(VI) | g[a] | $H_2SO_4$ | Std. Dev. | $H_2SO_4$ +Mo(VI) | Std. Dev. | $K_c$[d] | z[e] |
| 0.062 | 0.02 | 1.118 | 0.0901 | 0.0023 | 0.0605 | 0.0020 | 0.074 | 1.79 |
| 0.207 | 0.02 | 1.025 | 0.2553 | 0.0057 | 0.2188 | 0.0059 | 0.077 | 2.12 |
| 0.414 | 0.04 | 0.976 | 0.4647 | 0.0067 | 0.3824 | 0.0076 | 0.0648 | 2.29 |

[a]Taken from the nitric acid data (Table 6).

[b]Average of 6 results.

[c]Determined from the pH meter reading by using the g factor.

[d]$K_c$ = Bisulfate concentration dissociation constant.

[e]Calculated from the measured hydrogen ion concentration by using $K_c$.

and bisulfate ions. The results for the reference sulfuric acid solutions indicate ionic strengths of 1.04 to 1.08. The ionic strengths of the sulfuric acid solutions containing Mo(VI) were higher by an additional 1 to 2%.

As a check on the values obtained for the bisulfate concentration dissociation constants, a comparison can be made to the thermodynamic dissociation constant, K = 0.012 (119).

$$K = \frac{[H^+][SO_4^{2-}]}{HSO_4^-} \frac{\gamma^+\gamma^{2-}}{\gamma^-} = K_c \frac{\gamma^+\gamma^{2-}}{\gamma^-}$$

From the measurements made here,

$$\gamma^+ \approx g \approx 1$$

So,

$$K_c = \left(\frac{\gamma^-}{\gamma^{2-}}\right) K$$

The ratio of the activity coefficients is determined to be 4.2 by applying the Debye-Huckel equation with ion size parameters of 3 and 4 angstroms for the bisulfate and sulfate ions, respectively. This results in a value of 0.05 for the concentration dissociation constant which is in fair agreement with the values determined in this work (Table 7).

## B. Spectra and Properties of Molybdenyl and 12-MPA Solutions

Both molybdenyl and 12-MPA solutions have an appreciable absorbance in the ultraviolet region of the spectrum but absorb to a much lesser extent in the visible region. The absorbance of 12-MPA extends slightly farther into the visible region than the absorbance of the molybdenyl species does. It is in this region (above 400 nm) that 12-MPA abosrbance measurements are made when it is desirable to minimize the blank absorbance due to molybdenyl ions.

The spectra of molybdenyl solutions from 410 nm to 430 nm were recorded in order to determine blank corrections for the 12-MPA absorbance measurements. The Cary 17 spectrophotometer was used to record all spectra in this work.

The molar absorptivity of the molybdenyl ion was found to be a function of the free acid-to-molybdate ratio, R, rather than the acid concentration alone. Some approximate values were measured at a Mo(VI) concentration of 0.04 M. They are shown in Table 8. In all cases where 12-MPA absorbance values are reported, the appropriate molybdenyl blank absorbance has been subtracted.

Molybdenyl solutions were always allowed to stand for two days or longer before use. This is necessary in order to allow the solutions time to stabilize. This

Table 8. Molar Absorptivity of Mo(VI)[a]

| R[b] | Wavelength= 410 nm | Wavelength= 420 nm | Wavelength= 430 nm |
|---|---|---|---|
| 5-10 | 0.30 | 0.18 | 0.08 |
| 20-25 | 0.10 | 0.05 | 0.02 |

[a]0.04 M Mo(VI) at an ionic strength of 3.00 ($NaNO_3$)

[b]Free acid-to-molybdate ratio.

stabilization period has been recognized by other workers in its effect on the formation of 12-MPA (120). It has been observed directly in this work by the changing value of the bound acid-to-molybdate ratio as mentioned previously.

The overall equilibrium for the formation of 12-MPA can be represented as follows:

$$xMo(VI) + H_3PO_4 \rightleftarrows 12\text{-}MPA + yH^+$$

As of this writing the exact form of the Mo(VI) species has not been conclusively determined. However, evidence from this work and results from other workers (89) indicate that a protonated dimer ($HMo_2O_6^+$) is likely. The stoichiometric coefficient, x, would then be 6. A value of 9 has been suggested for y, the stoichiometric coefficient of the hydrogen ion (89). A high value is in agreement with the large inverse dependence of the reaction rate on hydrogen ion concentration. This will be discussed further in the next section. The concentration effects on the equilibrium must be considered in the determination of the molar absorptivity of 12-MPA.

The molar absorptivity of 12-MPA was determined by using an excess of molybdenyl ion. The amount of excess at a fixed acid concentration is limited because as the free acid-to-molybdate ratio is lowered to about 5, molybdenum trioxide begins to precipitate. And increasing

the acid concentration forces the equilibrium in the reverse direction. Using an excess of phosphate would seem to be an alternate method of forcing the reaction to completion. However, if the molybdenyl ion is not maintained in excess (perhaps as much as 40 times the stoichiometric amount), 12-MPA is not the sole product. Unsaturated heteropoly compounds are formed under these conditions. This was observed visually by the disappearance of the initially formed yellow color as additional phosphate was added to a phosphate plus molybdate solution. When the phosphate concentration had reached about one twentieth of the molybdenyl concentration, the yellow color had visually disappeared.

The change in absorbance of a 0.1 millimolar solution of phosphate as the Mo(VI) concentration is increased is illustrated in Figure 6. As the Mo(VI) concentration is increased, the absorbance should level off, corresponding to a 12-MPA concentration of 0.1 millimolar. Because of the precipitation of molybdenum trioxide, the Mo(VI) concentration was limited to about a 50-fold excess over the stoichiometric amount. However, the absorbance curve is fairly well leveled off at that point and the limit can be estimated. The estimated molar absorptivity at 420 nm is 770 $\ell \cdot mol^{-1} \cdot cm^{-1}$ with an estimated accuracy of ±70.

Spectra of 12-MPA solutions were recorded from 410 nm to 430 nm in order to determine the change in molar

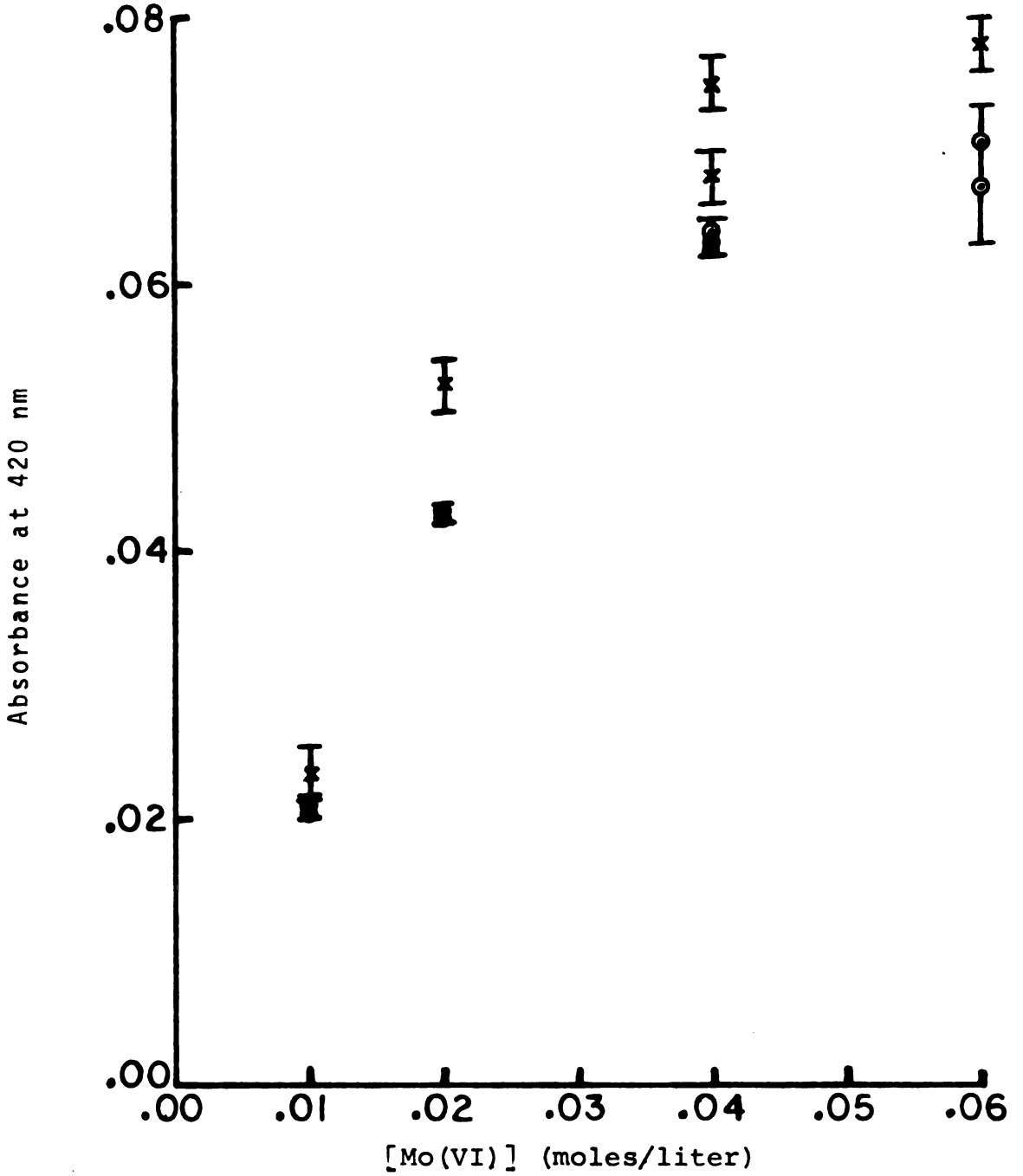



Figure 6. Molar Absorptivity of 12-MPA.

- stopped-flow data, normalized to 1 cm path length
- Cary 17 data, 1 cm cell

$[H_3PO_4] = 1.00 \times 10^{-4}$ M

$[H^+] = 0.0400$ M (nitric acid)

absorptivity with wavelength. The results are given in Table 9.

## C. Kinetics of the Formation of 12-MPA in Nitric Acid Solutions

The latest published study of the kinetics of formation of 12-MPA proposed a complicated rate law involving acid concentration to the inverse eighth, inverse fourth, inverse second and zeroth order for both nitric and perchloric acid (121). A slightly different rate law was determined for sulfuric acid solutions. In all cases acid was added only to the molybdenum(VI) solutions before mixing. Although this procedure maintains a constant free acid-to-molybdate ratio, R, it has the disadvantage that the acid concentration changes upon mixing. Since the form of the molybdate species depends on R, rather than the acid concentration per se, that procedure would eliminate effects on the rate of formation of 12-MPA due to transformation of the Mo(VI) reactant. However, the change in acid concentration can cause a temperature change due to the heat of dilution. This is significant for sulfuric acid at higher concentrations. For example, at a final acid concentration of 1 M, there would be 0.7°C increase in temperature upon mixing for sulfuric acid (122). The change for nitric acid or perchloric acid would be less than 0.01°C (123).

Table 9. Molar Absorptivity of 12-MPA Solutions[a]

| Wavelength, nm | 410 | 420 | 430 |
|---|---|---|---|
| Relative Absorbance[b] | 1.00 | 0.727±0.009 | 0.514±0.008 |
| Molar Absorptivity[c], $l \cdot mol^{-1} cm^{-1}$ | 1060±100 | 770±70 | 540±50 |

[a]Ionic strength is 3.00 ($NaNO_3$)

[b]Average of 6, the absorbance at 410 nm is the basis.

[c]Based on the measured value at 420 nm (Figure 6), with an estimated standard deviation of 70.

In this thesis work, the initial reaction rate was found to increase by a factor of 1.98 ± 0.10 for a change in temperature from 21.0 to 24.8°C. This was unchanged for nitric acid concentrations ranging from 0.2 to 0.4 M. This corresponds to an Arrhenius activation energy of 31.3 ±2.3 Kcal/mole. Beckwith and coworkers found the activation energy to be approximately the same (21 Kcal/mol) in all three acids (121). Although the determination in this present work involved only two temperature levels, the temperature measurements were quite precise (±0.02°C) and the thermistor was immersed in the reacting solution. In the other study, the temperature of the thermostating water was the measured quantity. Applying these results to the change in temperature by acid dilution (0.7°C) gives a change in reaction rate of 9% or 13% depending on which value for activation energy is used.

An experiment was carried out to compare the rates of reaction when all the acid was in the Mo(VI) solution before mixing and when the acid was evenly distributed between the Mo(VI) solution and the phosphate solution. The acid concentration after mixing was 0.4 M in either case. The average rates from the two different procedures were identical within experimental error.

For the acid dependence study, solutions were made up at constant phosphate and Mo(VI) concentrations, and the nitric acid concentration was varied to determine the dependence. In adjusting the acid concentration, it

was assumed that 2½ protons were consumed per Mo(VI) atom. The ionic strength was adjusted to a constant value with sodium nitrate. Reagent Grade or Analytical Reagent Grade chemicals were used in all cases. Concentrations within a series were varied by dilution of one stock solution.

In all determinations, the absorbance versus time curve used to calculate the initial reaction-rate was the point by point average of eight runs. The rate was determined as the linear least squares slope of a selected portion of the absorbance curve (124). The method of selecting this portion is illustrated in Figure 7. A Savitzky-Golay first derivative smoothing routine (125) was used to create a rate curve. Both this rate curve and the original absorbance curve are shown in the figure. The curves were displayed via the CRT terminal for visual discrimination. The plateau of maximum rate is the selected region which is then used to calculate the initial rate by the linear least squares fit of the absorbance data to a straight line. The standard deviations listed in this study are the calculated standard deviations of the slope of the best line through the selected points. This value may be different from the standard deviation obtained by determining slopes for individual runs and then calculating the standard deviation of the set of slopes.

The rate data are given in Tables 10-A, 10-B and 11. Table 10-B contains data for solutions with R values of

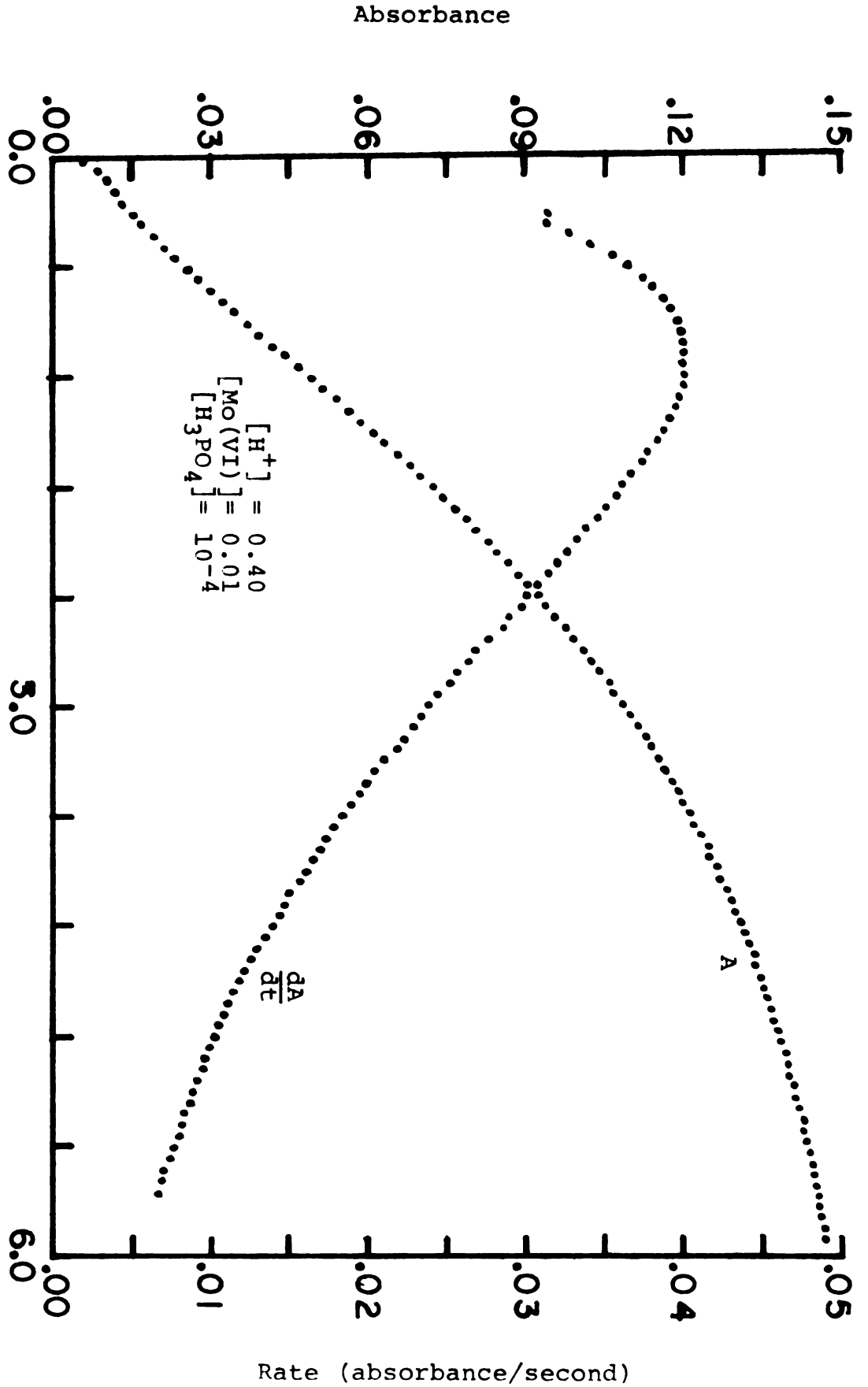



Figure 7. Absorbance and the First Derivative of Absorbance, 12-MPA Reaction.

Table 10-A. Initial Rate of Formation of 12-MPA, Conditions I

Conditions: 24.8±0.2°C
$[Mo(VI)] = 0.01$ M
$[H_3PO_4] = 5 \times 10^{-5}$ M
Ionic Strength ($NaNO_3$) = 2.0

| $[HNO_3]$, M | Time Interval, s[a] | Initial Reaction Rate,[b] A/s x $10^3$ | Std. Dev. x $10^4$ |
|---|---|---|---|
| 0.22 | 0.85-1.81 | 8.095 | 1.781 |
| 0.22 | 0.79-1.63 | 8.071 | 2.053 |
| 0.25 | 0.79-1.87 | 7.347 | 1.386 |
| 0.25 | 0.97-1.87 | 7.348 | 1.646 |
| 0.30 | 0.97-1.87 | 6.165 | 2.042 |
| 0.30 | 0.41-1.91 | 6.056 | 2.020 |
| 0.30 | 0.85-1.69 | 5.824 | 0.460 |
| 0.35 | 0.91-1.99 | 4.997 | 1.340 |
| 0.35 | 0.85-1.99 | 5.067 | 1.434 |
| 0.40 | 1.21-2.83 | 3.984 | 0.863 |
| 0.40 | 1.21-2.41 | 4.030 | 1.239 |
| 0.45 | 1.21-2.17 | 3.348 | 1.727 |
| 0.45 | 0.85-2.35 | 3.241 | 0.970 |
| 0.50 | 1.03-3.13 | 2.520 | 0.585 |
| 0.50 | 1.63-3.01 | 2.485 | 1.109 |
| 0.55 | 1.15-3.55 | 1.771 | 0.461 |
| 0.55 | 1.45-3.91 | 1.751 | 0.431 |
| 0.60 | 1.93-5.29 | 1.066 | 0.284 |
| 0.60 | 1.15-3.97 | 1.056 | 0.334 |
| 0.60 | 1.57-4.09 | 1.048 | 0.244 |
| 0.60 | 1.63-4.15 | 1.121 | 0.180 |
| 0.70 | 1.82-4.42 | 0.359 | 0.177 |

[a]Reaction time interval during which the rate was measured.

[b]The rate in $mol \cdot \ell^{-1} \cdot sec^{-1}$ can be calculated by using a path length of 1.94 cm and a molar absorptivity of $770 \pm 70 \ell \cdot mol^{-1} \cdot cm^{-1}$ at 420 nm.

Table 10-B. Initial Rate of Formation of 12-MPA, Conditions I.

Conditions: Same as Table 10-A

| $[HNO_3]$, M | Time Interval, s[a] | Initial Reaction Rate[b] A/s x $10^3$ | Std. Dev. x $10^4$ |
|---|---|---|---|
| 0.16 | 0.97-1.87 | 8.254 | 1.908 |
| 0.16 | 0.79-1.63 | 8.115 | 2.111 |
| 0.16 | 0.85-1.75 | 8.074 | 2.118 |
| 0.16 | 0.88-1.78 | 8.625 | 1.531 |
| 0.18 | 0.85-1.81 | 8.333 | 1.828 |
| 0.18 | 0.97-1.81 | 8.274 | 1.868 |
| 0.20 | 0.67-1.81 | 8.295 | 1.309 |
| 0.20 | 0.67-1.75 | 8.260 | 1.457 |

[a]Reaction time interval during which the rate was measured.

[b]The rate in $mol \cdot \ell^{-1} \cdot sec^{-1}$ can be calculated by using a path length of 1.94 cm and a molar absorptivity of $770 \pm 70 \ell \cdot mol^{-1} \cdot cm^{-1}$ at 420 nm.

20 or less. In Table 11 the acid concentration of 0.20 which results in an R value of 10 is not listed separately because of the shortness of the table. The reason for differentiating solutions with R values of less than about 20 is the apparent formation of unreactive Mo(VI) species. This formation results in the leveling off of the rate curve at low acid concentrations. Several observations support the contention that this "zero order" region is caused by unreactive Mo(VI) species rather than a real zero order dependence on acid concentration. First, the pH results discussed previously indicate the presence of $MoO_3$ or even anionic Mo(VI) species at low R values. Second, it was observed that those solutions form a precipitate of $MoO_3$ over a period of time. This precipitation was observed to be hastened when the solution was placed in a glass container (normally the solutions are kept in polyethylene bottles). And finally, it was observed that the best-fit rate equations containing zero order terms (in acid concentration) actually "ignored" the "zero order" region of the data and leveled off at much lower acid concentrations. A plot of the data in Tables 10-A and 10-B and the curve generated by the best-fit equation is shown in Figure 8. All data were analyzed using KINFIT, a general purpose curve-fitting and equation solving program (115). Since the data with acid concentrations of 0.20 M and below were not used in the curve fit, the best fit curve is terminated at an acid concentration of 0.22 M.

Table 11. Initial Rate of Formation of 12-MPA, Conditions II.

Conditions: $24.8 \pm 0.2^{o}C$
$[Mo(\bar{V}I)] = 0.02$ M
$[H_3PO_4] = 10^{-4}$ M
Ionic Strength ($NaNO_3$) = 3.0

| $[HNO_3]$, M | Time Interval, s[a] | Initial Reaction Rate[b] A/s x $10^2$ | Std. Dev. x $10^4$ |
|---|---|---|---|
| 0.20 | 0.73-1.51 | 2.370 | 1.926 |
| 0.20 | 0.73-1.51 | 2.358 | 2.228 |
| 0.40 | 0.73-1.57 | 2.257 | 2.607 |
| 0.40 | 0.73-1.57 | 2.245 | 1.763 |
| 0.80 | 1.45-3.31 | 0.611 | 0.592 |
| 0.80 | 1.39-2.59 | 0.615 | 1.359 |
| 1.00 | 1.87-5.47 | 0.148 | 0.228 |
| 1.00 | 2.21-4.41 | 0.149 | 0.253 |

[a] Reaction time interval during which the rate was measured.

[b] The rate in $mol \cdot \ell^{-1} \cdot sec^{-1}$ can be calculated by using a path length of 1.94 cm and a molar absorptivity of $770 \pm 70 \ell \cdot mol^{-1} \cdot cm^{-1}$ at 420 nm.

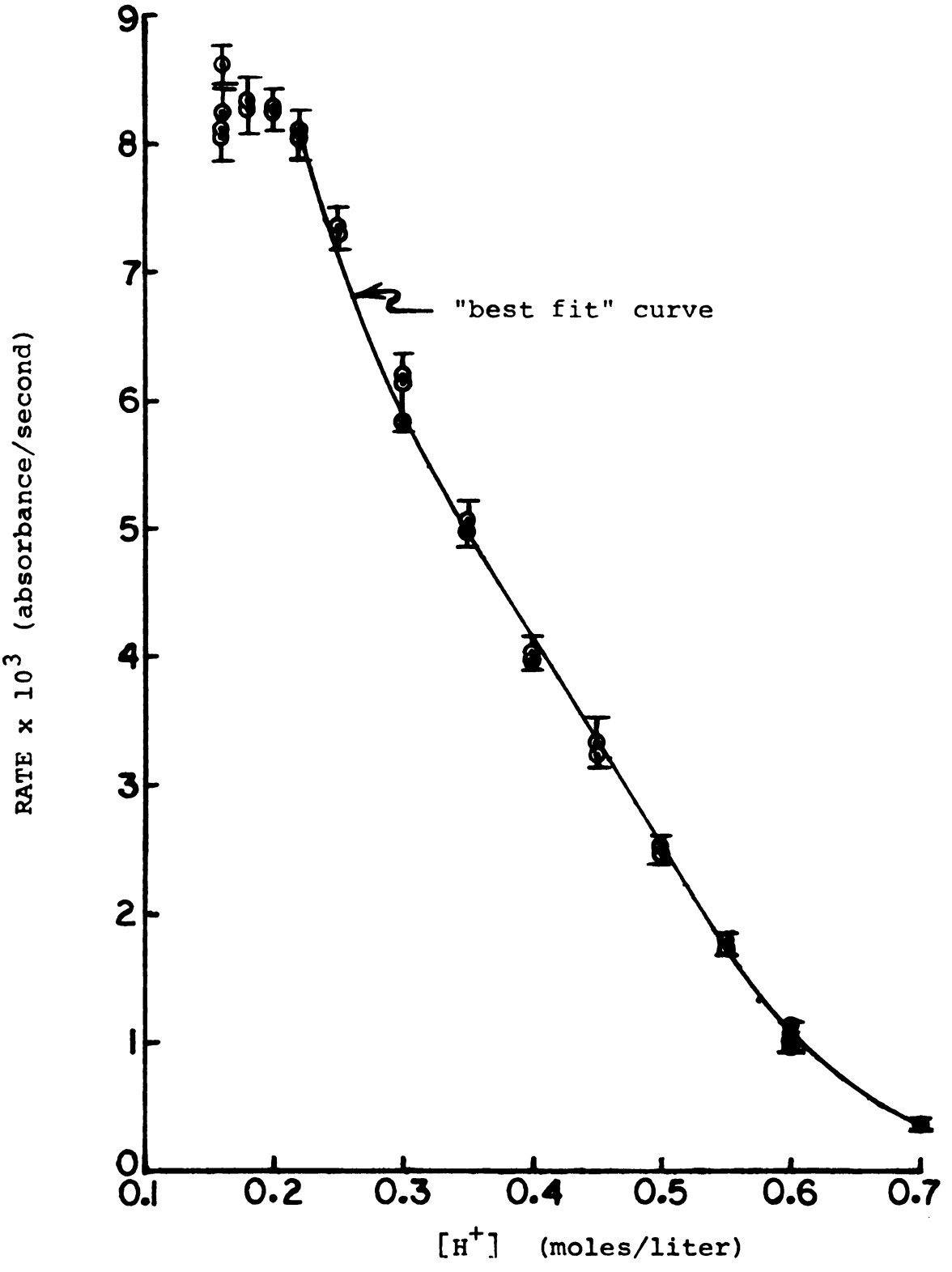



Figure 8. Dependence of the Rate of Formation of 12-MPA on $[HNO_3]$, Conditions I.

Based on the results from this data, it could not be said with certainty whether inverse eighth and inverse first order or inverse ninth and inverse first order gave the better fit. Other rate laws were tried but none gave a satisfactory fit. The results for the two cases are shown in Table 12. The inverse ninth order equation gave slightly better standard deviations for the conditional rate constants in all cases. However, when the data were split into a high acid set and a low acid set, the change in the value for the rate constant on the ninth order term was disturbing. Further analysis showed that at the low acid concentrations, which produced the inordinately high rate constant, there is very little dependence on the ninth order term. In fact, the large rate constant change from 56,036 to 104,963 shifts the curve an average of less than 0.8% in the low acid region. This is within the experimental error of the data.

The acid rate law is further clarified by analysis of the data in Table 11. These data are relatively more precise than the data in Tables 10-A and 10-B because of the higher concentrations of Mo(VI) and $H_3PO_4$. The results of fitting the two rate laws to this more precise data are given in Table 13. The best fit rate curve along with the data are plotted in Figure 9. The data at an acid concentration of 0.20 M were not included in the fit because of the low R value. There is little doubt that the better fit rate law involves acid concentration

Table 12. Determination of Rate Constants, Conditions I.

| Range of Concentration, M[a] | Rate Equation[b] | $K_1$ | RSD,% | $K_2$ | RSD,% |
|---|---|---|---|---|---|
| 0.22-0.70 | A | 34,440 | 2.3 | 554.6 | 0.82 |
| 0.22-0.70 | B | 57,539 | 2.1 | 562.8 | 0.68 |
| 0.22-0.40 | A | 42,284 | 27.2 | 553.9 | 1.1 |
| 0.40-0.70 | A | 35,274 | 3.1 | 536.6 | 2.8 |
| 0.22-0.40 | B | 104,963 | 21.7 | 555.7 | 0.79 |
| 0.40-0.70 | B | 56,036 | 2.6 | 579.2 | 2.0 |

[a]See Table 10-A for data

[b]Equation A: Rate = $1/K_1[H^+]^8 + K_2[H^+]$, absorbance/second

Equation B: Rate = $1/K_1[H^+]^9 + K_2[H^+]$, absorbance/second.

Table 13. Determination of Rate Constants, Conditions II.

| Range of Concentration, M[a] | Rate Equation[b] | $K_1$ | RSD,% | $K_2$ | RSD,% |
|---|---|---|---|---|---|
| 0.40-1.00 | A | 526.9 | 4.6 | 1.09.2 | 2.3 |
| 0.40-1.00 | B | 560.2 | 0.43 | 110.7 | 0.20 |

[a]See Table 11 for data

[b]Equation A: Rate = $1/K_1[H^+]^8 + K_2[H^+]$, absorbance/second.

Equation B: Rate = $1/K_1[H^+]^9 + K_2[H^+]$, absorbance/second.

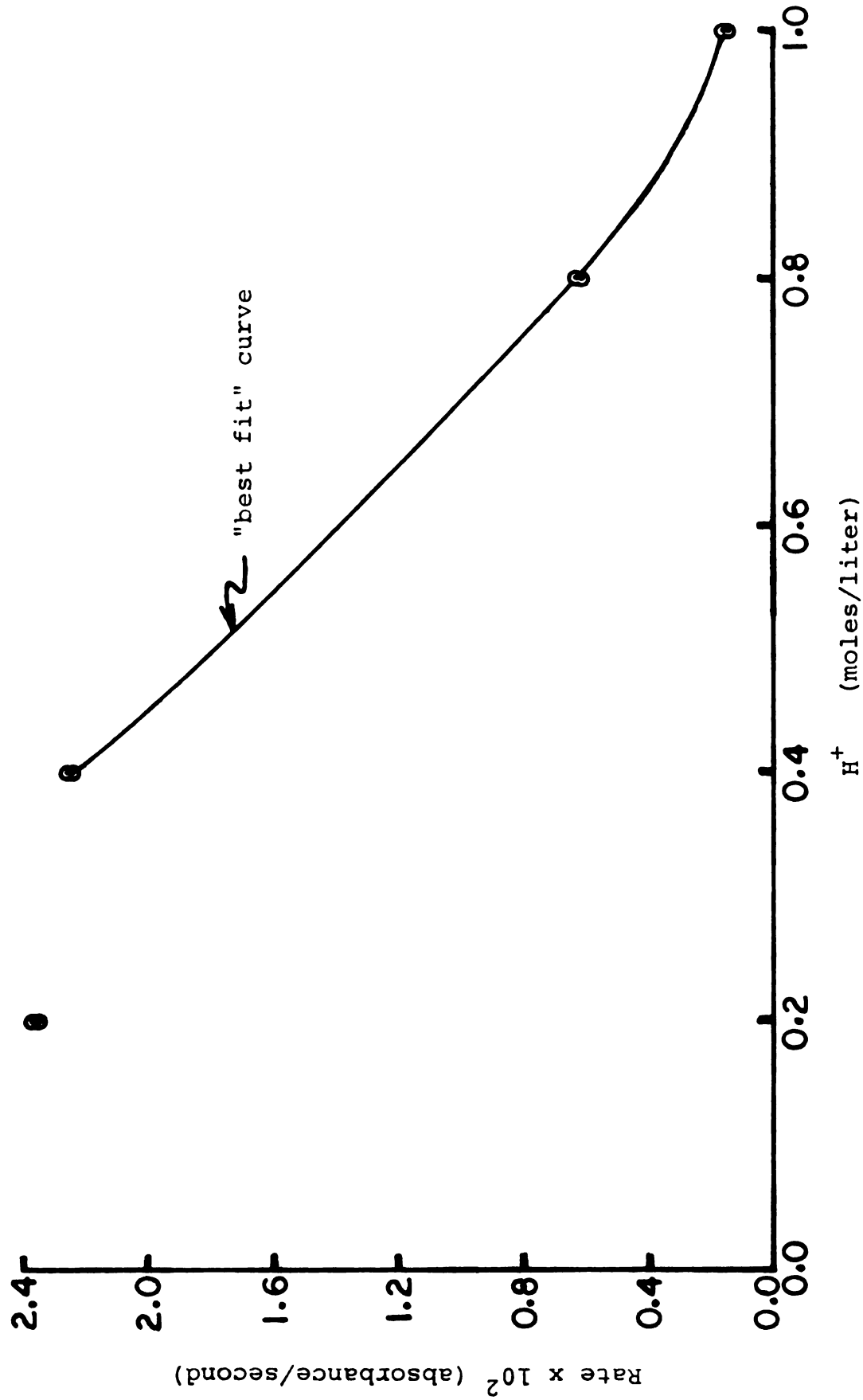



Figure 9. Dependence of the Rate of Formation of 12-MPA on $[HNO_3]$, Conditions II.

to the inverse ninth order. Thus this work indicates that the rate law in terms of acid concentration is

$$\text{Rate} = \frac{1}{K_1[H^+]^9 + K_2[H^+]} \text{ , absorbance/second}$$

where $K_1$ and $K_2$ are conditional rate constants which depend on [Mo(VI)] and $[H_3PO_4]$. Other workers have shown that the rate is dependent on $[H_3PO_4]$ to the first power but the dependence on [Mo(VI)] is more complicated (89). Although the dependence on Mo(VI) concentration was not determined in this work, some limiting conditions were. As mentioned previously in this work, concentration of Mo(VI) must be greater than about forty times the concentration of $H_3PO_4$ in order to avoid the formation of unsaturated heteropoly compounds. Evidence for this contention is also given in the next chapter. Another restraint on the Mo(VI) concentration is imposed by the requirement of keeping the free acid-to-molybdate ratio, R, above 20 to avoid the formation of unreactive Mo(VI) species. Thus for a given phosphate concentration and a given acid concentration, the first restraint will determine the upper limit for Mo(VI) concentration and the second restraint will determine the lower limit.

In analyzing rate data at low acid concentrations, an apparent transformation of 12-MPA was observed. This would be analogous to the transformations observed in similar heteropoly compounds of Mo(VI) (99,100,126-130). This

transformation of 12-MPA was observed to be dependent on the acid concentration per se and not on R. The transformation was observed for all acid concentrations between 0.16 and 0.25 M, but never for concentrations of 0.30 M and above. At an acid concentration of 0.25 M, the transformation was observed with R equal to 25 whereas at an acid concentration of 0.40 there was no measurable transformation for R values between 10 and 40. The rate of transformation was too slow in most cases to be measured quantitatively but there was no doubt about its presence at low acid concentrations.

The transformation was observed as a slow (several orders of magnitude slower than the initial rate) increase in absorbance which was detectable long after the formation of 12-MPA should have been complete. The point at which equilibrium should be attained can be estimated by observing a reaction of higher acid concentration in which the equilibration is attained without transformation. The primary equilibration is slower at higher acid concentrations so that the estimate of the equilibration point is conservative. The transformation has been measured for periods over five times as long as the estimated equilibration time and in that time (20 to 100 seconds) the absorbance was measured to increase up to 12.8±1.0%. It should be noted that acid concentrations below 0.30 M would generally not be used for analytical procedures and thus this transformation would not be observed.

# CHAPTER VI

## THE REACTION-RATE ANALYSIS OF PHOSPHATE AND SILICATE

The reaction-rate analysis of phosphate "unknowns" was performed under the control of the PDP 8/e minicomputer. The experimenter had only to specify the portion of the reaction curve to be used to determine the initial rate. A working curve was also developed for silicate analysis. The reaction of silicate with Mo(VI) at high acid concentrations is similar to the phosphate reaction, but proceeds at a much slower rate (99,100,126-130).

Reaction-rate data on standards and unknowns are processed by the computer and the analytical results printed out. The reaction-rates and concentrations of the standard solutions are fit to a linear equation (124). That equation is then used to determine the concentrations of the unknown solutions from their reaction rates. The computer programs are set up to handle up to 20 standards and 50 unknowns including averaging of multiple runs of each solution. This capability can be easily expanded to handle a greater number of solutions.

The experiments in this chapter were accomplished prior to the chemical studies discussed in Chapter V so that the limiting conditions determined in that work were not utilized here. In all experiments in the present chapter, the acid was contained only in the Mo(VI) solution before

mixing. However, since nitric acid was used, there was no problem with temperature effects due to heats of dilution. The acid concentrations given are the concentrations after mixing and no corrections were made for the amount of acid consumed by Mo(VI). Although optimum conditions were not used in these analyses, the results are indicative of what might be expected from the reaction-rate analysis of phosphate and silicate via the formation of their respective heteropolymolybdates.

Table 14 shows the computer printout of the results of the analysis of phosphate "unknowns". Only the column containing the actual concentrations of the "unknowns" was added to the original printout. The slope and intercept of the linear equation are printed out along with the data and results. The relative accuracy is 12% at 0.2 ppm P (as $H_3PO_4$) and improves to better than 2% for the concentrations from 0.5 to 3.5 ppm P. If only the data for the highest and the lowest standard concentrations are used to determine the working curve, the errors range from 4.5% at 0.2 ppm P to 1.9% at 3.5 ppm P. However bend off of the analytical curve occurred at concentrations of 7 ppm and above. This is illustrated in Figure 10. The solid line represents the best linear fit for all concentrations up to 4 ppm, and the dashed line represents the best linear fit for all concentrations up to 20 ppm. It can be seen that the latter case results in extreme relative errors at low concentrations. As

Table 14. Reaction-Rate Analysis of Phosphate.

| Std. Conc.[b] | Slope (Absorb/sec)[a] | Std. Dev. of Slope |
|---|---|---|
| 0.2000E+00 | 0.1059E-02 | 0.8213E-04 |
| 0.1000E+01 | 0.5233E-02 | 0.8126E-04 |
| 0.2000E+01 | 0.1033E-01 | 0.8437E-04 |
| 0.400E+01 | 0.2034E-01 | 0.9103E-04 |

Initial Rate = A * Concentration + B

A = 0.5066E-02

B = 0.1232E-03

| Unk. No. | Conc.[b] | Slope (Absorb/sec)[a] | Std. Dev. of Slope | Actual[b,c] Conc. |
|---|---|---|---|---|
| 1 | 0.1756E+00 | 0.1013E-02 | 0.7849E-04 | 0.200 |
| 2 | 0.4977E+00 | 0.2645E-02 | 0.8585E-04 | 0.500 |
| 3 | 0.2033E+01 | 0.1042E-01 | 0.8334E-04 | 2.00 |
| 4 | 0.3557E+01 | 0.1814E-01 | 0.8612E-04 | 3.50 |

[A]Rate of formation of 12-MPA; [Mo(VI)] = 0.080M,[$HNO_3$]=0.64M.

[b]Concentrations in ppm P, 1ppm P = $3.23 \times 10^{-5}$M $H_3PO_4$.

[c]This column not included in original computer printout.

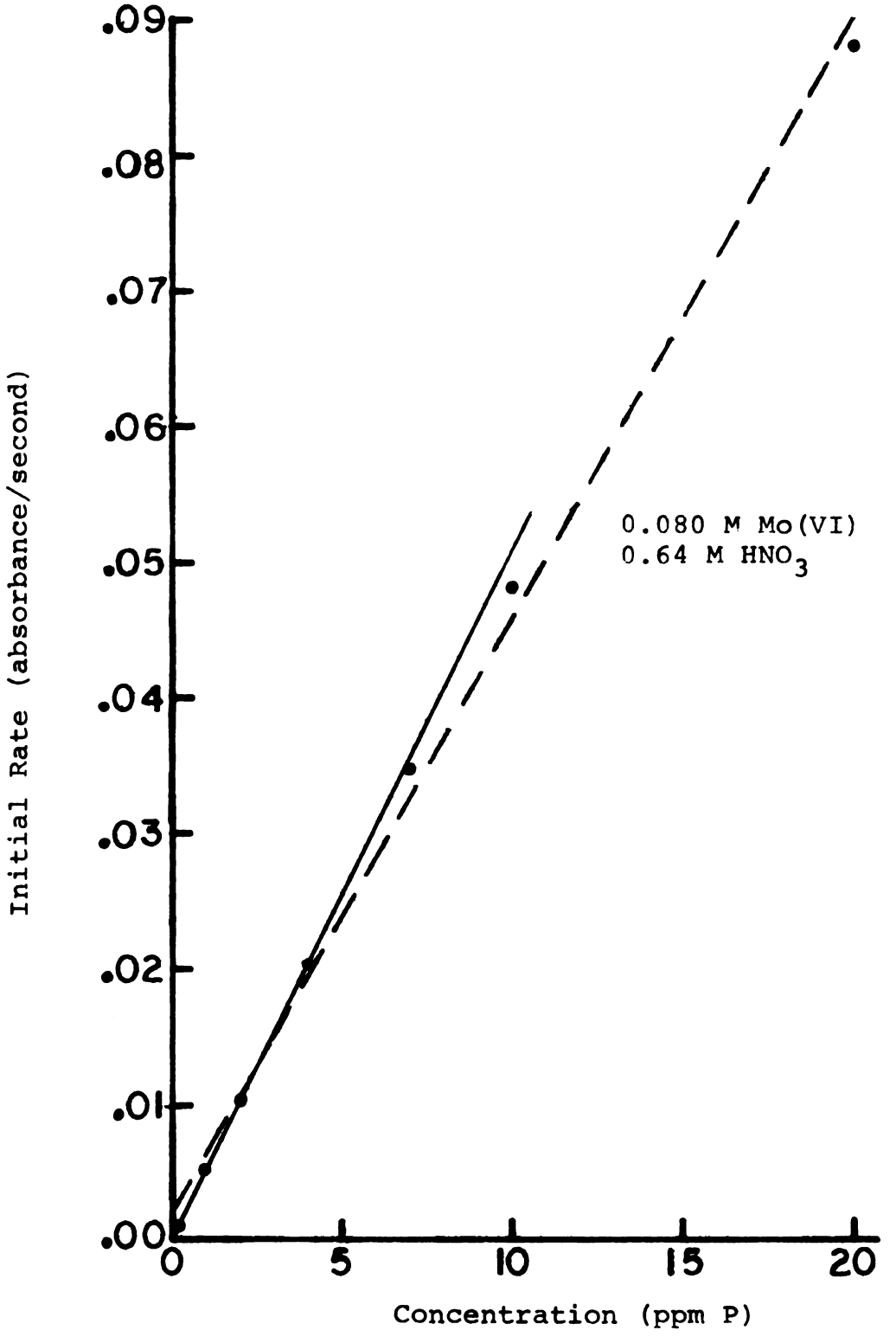



Figure 10. Reaction Rate Analysis of Phosphate, Low [Mo(IV)].

Initial rates measured from 0.6 to 1.4 seconds. Solid line represents best linear fit of points from 0.2 ppm to 4.0 ppm. Dashed line represents best linear fit of all points.

discussed previously in this thesis, the reason for the bend off of the curve is the formation of unsaturated heteropoly products at low Mo(VI) to phosphate ratios. From Figure 10, it can be estimated that the mole ratio of Mo(VI) to phosphate should be at least 350 (30 times the stoichiometric ratio) to prevent the formation of unsaturated products. The same range of phosphate concentrations was used with a higher Mo(VI) concentration to demonstrate this point. The reaction-rate results are listed in Table 15 and plotted in Figure 11. The improvement in linearity is marked. Fairly good linearity is obtained over two orders of magnitude.

Reaction-rate data were obtained for the formation of the 12-molybdosilicate anion (12-MSA) under the same conditions as used for the formation of 12-MPA. The results are given in Table 16 and plotted in Figure 12. The results are similar to those for 12-MPA, but relatively less precise because of the slower reaction-rate. The non-zero intercept indicates some bend off in the analytical curve. This is attributed to an insufficient excess of Mo(VI) and thus the formation of unsaturated heteropoly products at the higher silicate concentrations. All rates listed were the average of eight runs.

Table 15. Reaction-Rate Data: Phosphate Analysis[a]

| Conc.[b] | Time Interval (sec) | Slope (Absorb/sec)[c] | Std. Dev. of Slope |
|---|---|---|---|
| 0.20 | 0.5000E+00 to 0.8900E+00 | 0.1762E-02 | 0.1437E-03 |
| 0.20 | 0.5000E+00 to 0.8900E+00 | 0.1692E-02 | 0.1863E-03 |
| 1.00 | 0.5000E+00 to 0.8900E+00 | 0.7120E-02 | 0.2096E-03 |
| 4.00 | 0.5000E+00 to 0.8900E+00 | 0.2826E-01 | 0.1902E-03 |
| 10.0 | 0.5000E+00 to 0.8900E+00 | 0.6896E 01 | 0.2129E-03 |
| 20.0 | 0.5000E+00 to 0.8900E+00 | 0.1361E+00 | 0.2239E-03 |

[a] $[Mo(VI)] = 0.176$, $[HNO_3] = 0.64$ M

[b] Parts per million phosphorous.

[c] Rate of formation of 12-MPA.

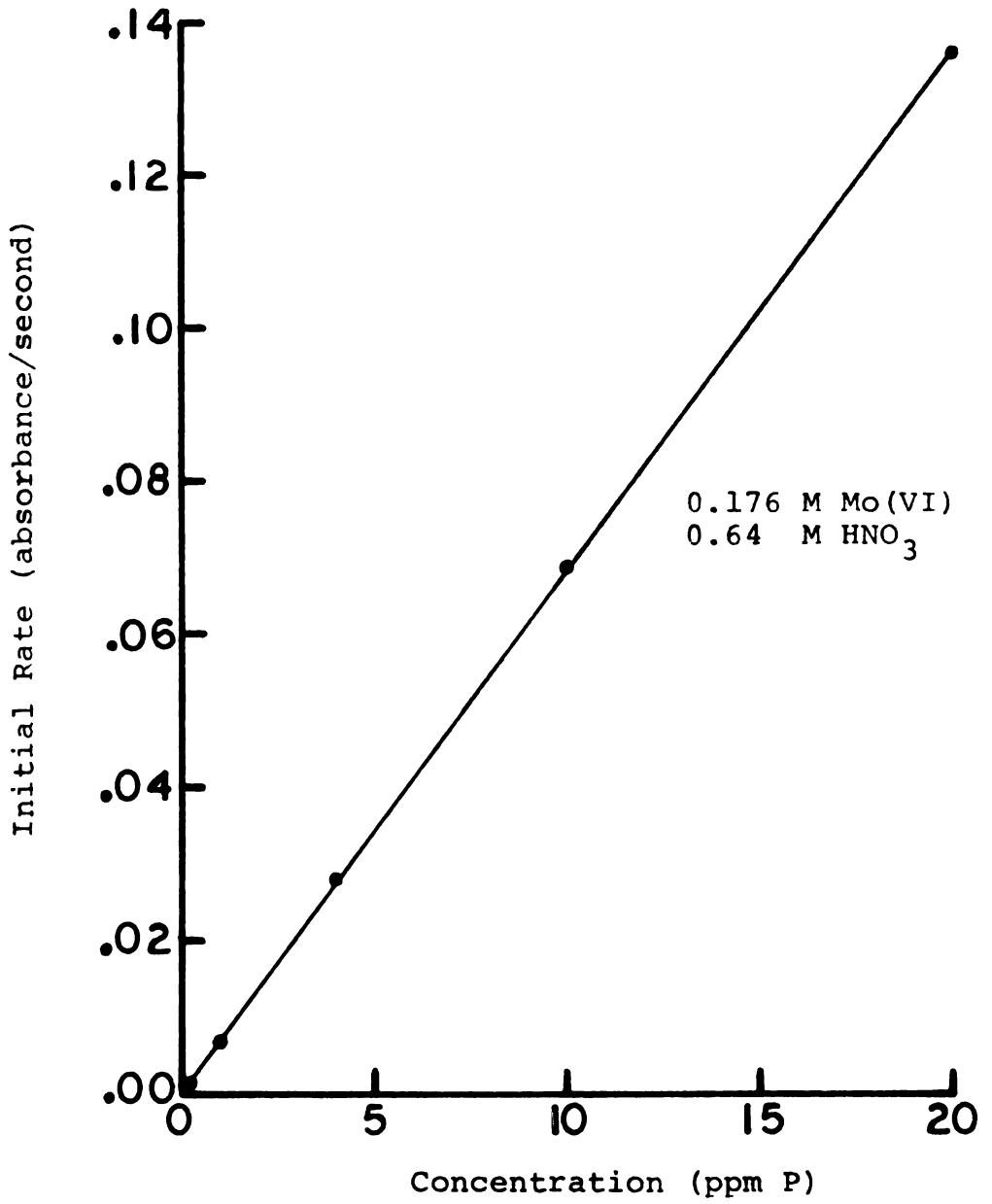



Figure 11. Analytical curve for Reaction-Rate Analysis of Phosphate.

Initial rates measured from 0.50 to 0.89 seconds.

Table 16. Reaction-Rate Data: Silicate Analysis

| Conc.[b] | Time Interval (sec) | Slope (Absorb/sec)[c] | Std. Dev. of Slope |
|---|---|---|---|
| 2.0 | 0.4100E+00 to 0.3410E+01 | 0.5403E-02 | 0.1661E-04 |
| 4.0 | 0.4100E+00 to 0.3410E+01 | 0.1175E-01 | 0.1982E-04 |
| 8.0 | 0.4100E+00 to 0.3410E+01 | 0.2123E-01 | 0.2210E-04 |
| 16.0 | 0.4100E+00 to 0.3410E+01 | 0.4149E-01 | 0.4318E-04 |

[a]Rate of formation of 12-MSA: [Mo(VI)] = 0.176 M, [$HNO_3$] = 0.64 M

[b]ppm Si, 1 ppm Si = $3.56 \times 10^{-5}$ M $H_2SiO_3$.

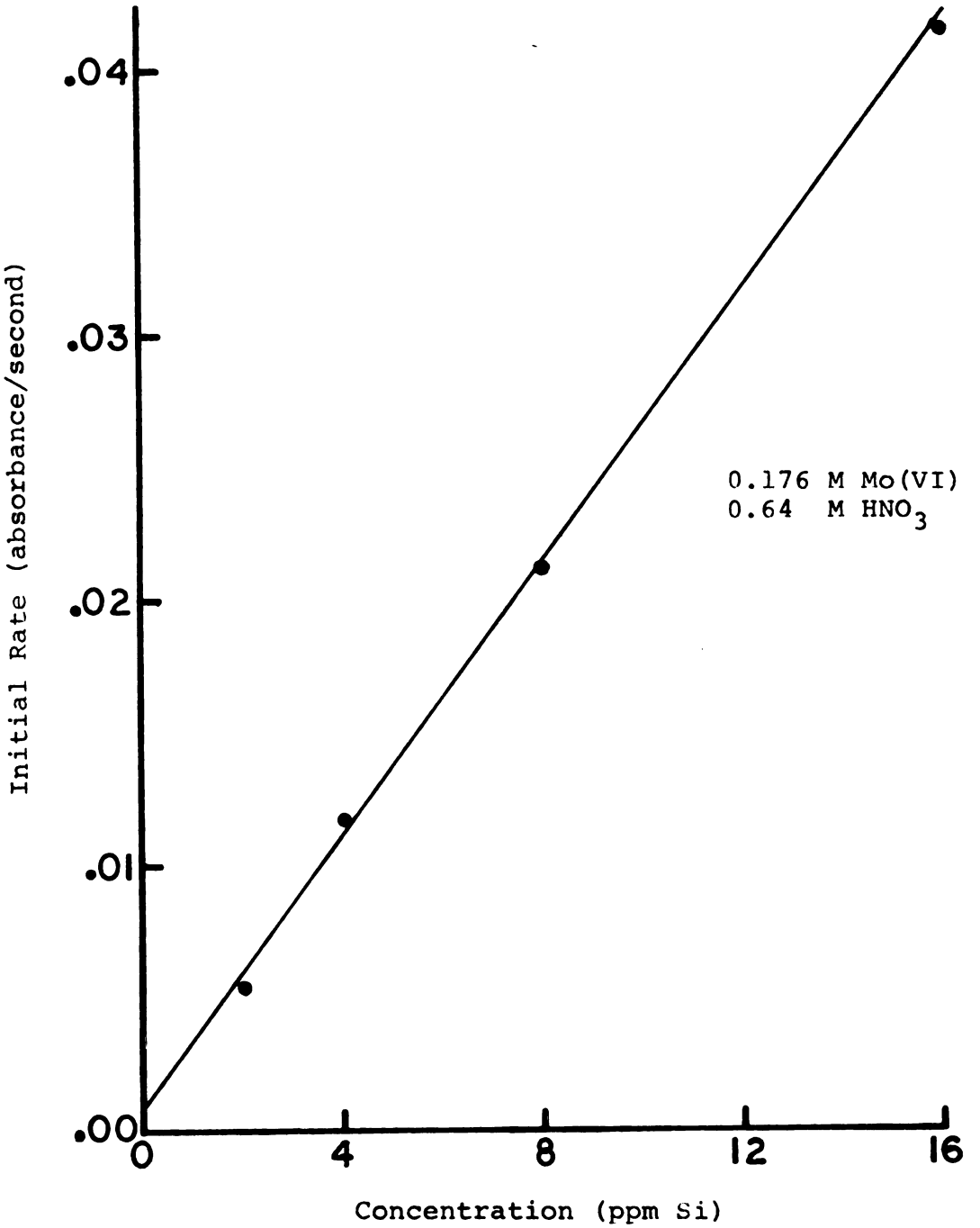



Figure 12. Analytical curve for the Reaction-Rate Analysis of Silicate.

Initial Rates measured from 4 to 34 seconds.

# CHAPTER VII

# FUTURE PROSPECTS

## A. The Automated Stopped-flow Instrument

Accurate analysis, high sample throughput and automation are major considerations in the design of analytical instruments and in the development of analytical techniques. The stopped-flow technique offers the potential for rapid accurate analysis and is readily amenable to automation. This present work was undertaken with those considerations in mind.

In this thesis work, the stopped-flow mixing system was completely thermostated and a thermistor was inserted into the flow channel for accurate solution temperature monitoring on the millisecond time scale. A new mixer was installed which improved the mixing efficiency. In addition, optics were designed to permit much greater radiation throughput, and the spectrophotometric detection system was redesigned to permit high precision measurements. The observation cell was also equipped with platinum electrodes for conductivity measurements, although they have not been used in this present work.

The stopped-flow instrument was interfaced to a PDP 8/e minicomputer so that the operation of the stopped-flow spectrophotometer and the collection and analysis of data could be done under computer control. Extensive

computer software has been developed to allow fundamental kinetics studies and routine reaction-rate analysis. The software is quite versatile and can be easily modified to accommodate different types of analyses or changes in the instrumentation.

The limiting factor affecting the accuracy of the spectrophotometric data is the fluctuation of the light source intensity. This can be eliminated as the limiting source of error by monitoring the light source intensity and correcting the spectrophotometric data for any fluctuations. This can be accomplished by inserting a beam splitter between the monochromator and the observation cell and using a separate PMT detector powered by the same supply as the main PMT. Several workers have achieved very accurate spectrophotometric results by monitoring the light source intensity (37,39). Light source intensity data can be collected by the computer along with the spectrophotometric data, and the latter corrected by software.

In this present work, errors in the spectrophotometric data were introduced when temperature data was being collected in the same run. The absorbance data for an entire run would occasionally be reduced by a factor of about two. This is presumably a problem with the interface buffer electronics or with the electronics of the temperature circuit. New connectors may have been installed in the buffer box and this may eliminate the problem.

Although the new mixer has improved the mixing

efficiency, there is still a need for further improvement. It is desirable to eliminate or reduce the 10-20 milliseconds it takes for the mixing to progress from 98-99% complete to 100% complete after the flow stops. This can be a problem for monitoring fast reactions. Dr. Dye and coworkers in this chemistry department have had very good results with a tangential jet double mixer (41,131). This design would probably be the most fruitful approach because of the close availability of the expertise. The mixer housing was purposely designed for easy replacement of the mixer so that different designs could be readily inserted and studied.

The one time limiting step remaining in the analysis procedure is the preparation of samples. An accurate (0.1%), wide ranging (6 orders of magnitude), versatile solution preparation system is presently being constructed in our laboratory (132). This system is to be controlled by a microcomputer and can be initialized by the PDP 8/e minicomputer to deliver specified solutions to the stopped-flow mixing system for analysis. The new software can be easily integrated with the existing software to enable hierachical control of the entire system for completely automated stopped-flow studies.

## B. Study of the Formation of 12-Molybdophosphate and Related Mo(VI) Compounds

Mo(VI) reacts with phosphate, silicate, arsenate and germanate at high acid concentrations to form yellow heteropolymolybdate compounds. This present study was concerned with the formation of 12-MPA and, to a much lesser extent, with the formation of 12-MSA. In either case, it is important to know the form of the Mo(VI) species in the highly acid solutions. This work demonstrated the feasibility of determining (via pH meter) the number of protons consumed by the Mo(VI) species upon acidification. The results showed that up to 2½ protons were consumed per Mo(VI) (R=2.5) as the free acid-to-molybdate ratio, Z, was increased to ten. This result indicates the formation of a protonated dimer, $HMo_2O_6^+$. This study should be carried out to higher Z values. The present results indicate that meaningful data would be expected up to acid concentrations of 0.6 M, with a Mo(VI) concentration of 0.02 M. This proposed study would indicate whether a plateau for the value of R has been reached and thus whether the protonated dimer would be the dominant species in the concentration range of interest.

The dependence of the rate of formation of 12-MPA on nitric acid concentration has been studied in this work. The results indicate inverse ninth and inverse first order dependence. These results are valid only for Z values above 10 because of the unreactivity and instability of

the Mo(VI) species below this value. Previous workers have determined that the rate dependence on phosphate concentration is a simple first order dependence (66,121). However, the dependence on Mo(VI) concentration was more complicated and terms in the rate law consisting of a ratio of acid concentration to Mo(VI) concentration were postulated. Thus it is necessary to perform further experiments in order to determine the rate dependence on Mo(VI). In this proposed study, the concentration of Mo(VI) should be maintained greater than that of phosphate by a factor of about 100 to avoid the formation of unsaturated heteropolymolybdates. Additionally the rate laws should be determined in perchloric and sulfuric acids.

The accidental discovery of the possible transformation of the 12-MPA species is in need of further corroboration. The occurrance of this transformation appears to be dependent on the acid concentration per se rather than on Z. It has only been observed for acid concentrations below 0.30 M. This type of transformation has been observed for other heteropolymolybdates (99,100,126-128). In those studies, differences in spectra and reduction potentials confirmed the presence of the two distinct species. Those techniques should prove useful in the identification of the two 12-MPA species. Also, the rate of transformation between the 12-MPA species and the molar absorptivities of the two species are in need of further investigation. The molar absorptivity of the

species which is formed at higher acid concentrations has been estimated in this present work to be 770 ± 70 $\ell \cdot mol^{-1} \cdot cm^{-1}$ at 420 nm.

The determination of the rate law and molar absorptivities would facilitate the determination of the equilibrium expression for the formation and dissociation of 12-MPA. The rate law and derived mechanism would suggest possible equilibrium expressions which could be tested with equilibrium and kinetics data.

The major goal of these fundamental studies is to specify conditions for the analysis of phosphate. The results of this present study indicate that for high stability of the Mo(VI) solutions the Z value should be above 20. However, faster reaction-rates are achieved for somewhat lower acid concentrations. This suggests having all the nitric acid contained in the Mo(VI) solution before mixing. This would not be appropriate for sulfuric acid because of the large heat of dilution. It is also desirable to have as high a Mo(VI) concentration as practical in order to increase the upper limit of phosphate concentration without forming unsaturated heteropolymolybdates. Increasing Mo(VI) concentration would also appear to increase the reaction rate. However, in order to maintain a high enough Z value, the acid concentration would also have to be increased. This leads to a decrease in the reaction rate as the concentrations are increased beyond a certain point. An optimum value would be a Mo(VI)

concentration of about 0.3 M. At this Mo(VI) concentration, the reaction-rate analysis of phosphate should be studied at acid concentrations from 0.3 M to 0.8 M. In addition to sensitivity and dynamic range, long term stability of the Mo(VI) reagent should be investigated.

The results on the formation of 12-MPA can then be used for a basis in studying the formations of 12-MSA and the arsenate and germanate analogs. A preliminary study of all four of these species has been done by Halaz and Pungor (99,126).

# APPENDICES

# APPENDIX A

## INSTRUMENT AND COMPONENT SPECIFICATIONS

This appendix contains manufacturers specifications of the instruments and components used in this work. Only the pertinent specifications are listed. More complete listings can be obtained from the manufacturers. The equipment is divided into three categories; stopped-flow components, stopped-flow test equipment and analytical instruments.

1. Stopped-flow Components

   a) Light source, Model EU-701-50 GCA/McPherson Instrument Company

   Deuterium Lamp

   Spectral range: 175-450 nm

   Stability: Less than 1% drift over 2 hours (after 30 minutes warm-up)

   Tungsten Lamp

   Spectral range: 350-3000 nm

   Stability-voltage Control: 1% over 2 hours

   Stability-intensity control: 0.1% for short periods, less than 1% drift over 2 hours (after 30 minutes warm-up)

   b) Monochromator, Model EU-700 GCA/McPherson Instrument Company

| | |
|---|---|
| Accuracy | 0.1 nm |
| Wavelength range: | 190-1000 nm |
| Aperature ratio: | f/6.8 @200 nm |
| Focal length: | 350 nm |
| Stray light: | less than 0.1% between 220 and 600 nm |
| Spectral bandwidth: | continuously adjustable between 0.05 and 8 nm |

c) Quartz flexible fiber optic bundle

Schott Optical Company

| | |
|---|---|
| Length: | 25 cm |
| Diameter: | 2 mm |
| Aperature angle, 2α* | 32±5° @ 254 nm |
| | 55±5° @ 546 nm |
| Transmission | 50% @ 250 nm |
| | 55% @ 300 nm |
| | 59% @ 500 nm |
| | 59% @ 700 nm |

*α is the angle for which the transmission is 50% of the transmission at an angle of 0°.

d) Quartz internally reflecting rod

Schott Optical Company

| | |
|---|---|
| Length: | 10 cm |
| Diameter: | 3 mm |
| Aperature angle, 2α: | 40±5° @ 254 nm |
| | 40±5° @ 546 nm |

Transmission: 85% @ 220-1100 nm

e) 1P28 Photomultiplier tube

RCA corporation

Luminous Sensitivity: 200 amperes per lumen

Dark current: $2 \times 10^{10}$ amperes maximum at 600 VDC

f) High voltage power supply for PMT, Model EU-42A

Heath Company

Range: 300-1500 VDC

Current: 1.5 mA, max

Voltage regulation with line voltage change of 105 to 125 VAC: 0.05% of full scale

With Load current change from zero to maximum: 0.1%

Ripple: Less than 5 mV peak, with 1000 VDC at 1 mA output

g) Current Amplifier, Model 427

Keithley Instruments, Inc.

Range: $10^4$ to $10^{11}$ volts per ampere in eight decade ranges

Output: -10 to +10 V at up to 3 mA

Output resistance: less than 10 ohms, dc to 30 KHz

Output accuracy: 2% or reading to $10^9$ V/A range

Rise time (10% to 90%) Nominally adjustable from 0.01 to 300 ms

| | |
|---|---|
| Voltage drift: | Less than 0.005%/°C |
| Effective input resistance: | Less than 15 ohms on the $10^4$ and $10^5$ V/A ranges, increasing to less than 4 megaohms on the $10^{11}$ V/A range. |

h) 10-bit digital-to-analog converter (DAC), Model EU-800-GC - Heath Company.

Voltage Mode Specifications

| | |
|---|---|
| Range: | 0 V to -10 V at 5 mA |
| Accuracy: | ±1 LSB (0.10%) |
| Linearity: | ±½ LSB (0.05%) |
| Settling time to 0.05% of full scale: | 25 microseconds for 10 V step |
| Temp. coef., zero: | 10 ppm of full scale/°C |
| gain: | 50 ppm of reading/°C |

i) Operational Amplifier, Model AD518K (used in the offset circuit)
Analog Devices Company

| | |
|---|---|
| Output voltage range: | -12 to +12 V |
| Output current: | -10 to +10 mA |
| Slew rate, unity gain: | 50 microseconds, min. |
| Settling time to 0.1%: | 800 ns |
| Temp. coef. of input offset voltage: | 15 microvolts/°C, max. |
| Differential input impedence: | 0.5 megaohm, min. |

j) 12-bit analog-to-digital converter (ADC), Model DAS-16-M12B

Datel Company

| | |
|---|---|
| Range: | -5 to +5 V |
| Input Impedence: | 100 megaohms. |
| Acquisition time: | 5 microseconds to 0.025% |
| Aperature time: | 50 ns |
| Accuracy: | 0.025% of full scale |
| Throughput rate: | 50 KHz |
| Temp. coef.: | 40 ppm/°C |

2. Stopped-flow Test Equipment

a) Potentiometric voltmeter bridge, Model 300A

Electro Scientific Industries (esi)

| | |
|---|---|
| Nominal accuracy: | 0.01% |

b) Digital multimeter, Model 8600A

Fluke Corporation

Voltmeter

| | |
|---|---|
| Ranges: | ±200 mV, ±2 V, ±20V, ±200V, ±1200V |
| Resolution: | 10 microvolts on 200 mV range |
| Accuracy: | 0.02% of input + 0.005% of range (for 2, 20, 200 V ranges) |
| 15°C to 35°C: | 0.02% of input + 0.008% of range for 1200V range<br>0.04% of input + 0.01% of range for 200 mV range |
| DC input resistance: | Greater than 1000 megaohms for |

| | |
|---|---|
| | 200 mV and 2V ranges, 10 mega-ohms for 20V, 200V and 1200V ranges |
| Common mode noise rejection: | 120 dB minimum |

c) Precision power source, Model 2005
Power Designs, Inc.

| | |
|---|---|
| Accuracy: | 0.1% $\pm$1 mV at outputs below 10V |
| Range: | 0-20 VDC |
| Ripple and noise: | less than 100 microvolts peak |
| Temp. coef.: | Less than 0.001%/°C. |

d) Precision current source, Model 261
Keithley Instruments, Inc.

| | |
|---|---|
| Worst case accuracy: | 0.25% for $10^{-7}$ to $10^{-4}$ A |
| | 0.5% for $10^{-8}$ to $10^{-7}$ A |
| | 0.8% for $10^{-9}$ to $10^{-8}$ A |
| Temp. coef. | 0.01%/°C for $10^{-7}$ to $10^{-5}$ A ranges |
| | 0.1%/°C for $10^{-12}$ to $10^{-8}$ A ranges |

e) Strip chart recorder, Model SR-255B
Heath Company

| | |
|---|---|
| DC input ranges: | 10 mV, 100 mV, 1 V, 10 V |
| Overall error: | Less than 1% of full scale |
| Standardization error: | Less than 0.005%/°C |
| Zero setting drift: | Less than 10 microvolts/°C, 10 mV range |
| Balancing time: | Less than 1 s full scale |

3. Analytical Instruments

a) Cary Spectrophotometer, Model 17

Varian Instrument Company

| | |
|---|---|
| Wavelength range: | 186-2650 nm |
| Wavelength accuracy: | 0.4 nm |
| Stray light: | Less than 0.0001% between 240 and 500 nm |
| Photometric accuracy: | 0.002 absorbance on 0-1 range<br>0.0005 absorbance on 0-0.1 range |
| Zero absorbance stability | Less than 0.0005 absorbance per hour drift with standard VIS-IR source. |

b) Servo-digital pH/volt meter, Model EU-302 A

Heath Company

| | |
|---|---|
| Range: | 0-14 pH |
| Resolution and precision | 0.02 pH |
| Accuracy: | 0.5% of full scale |
| Temperature compensation | 0-100°C, manual control |

c) Combination pH electrode, Model S30072-15

Sargent-Welch Scientific Company

| | |
|---|---|
| Range: | 0-14 pH |
| Temperature range: | 0-80°C |

# APPENDIX B

## A BRIEF DESCRIPTION OF THE CAPABILITIES OF THE COMPUTER PROGRAMS

The reader is referred to the "OS/8 Handbook", Digital Equipment Corporation, Maynard, MA, for clarification of the nomenclature used.

-----INFORMATION FOR USERS OF PN???? * PROGRAMS-----

I. PNSF1. PA--CHAINS WITH PNF?01.FT
PNSF3. PA--CHAINS WITH PNF?03.FT

PNSF1. PH: LO PNSF1

1) USES CHANNEL 0 of ADC
2) UTILIZES AUTOMATIC OFFSET-CAN OBSERVE 100% T TO, EG, 90% T FULL SCALE OF ADC
3) CALIBRATES OFFSET WITH LIGHT SOURCE SHUTTER CLOSED
4) LIGHT INTENSITY DATA IS TAKEN AS SPECIFIED BY THE USER AND STORED IN FIELD 1 (COMMON) ALONG WITH OFFSET AND AMPLIFICATION PARAMETERS
5) CHAINS TO SPECIFIED FORTRAN PROGRAM

PNSF3. PA: LO PNSF3

IV. 1) USES CHANNEL 0 OF ADC
2) MANUAL OFFSET AND AMPLIFICATION-0% T AND 100% T MUST BE WITHIN THE RANGE OF THE ADC (-5V to +5V)
3) COMPUTER RECORDS 0% T LEVEL WITH THE LIGHT SOURCE SHUTTER CLOSED
4) RELATIVE LIGHT INTENSITY DATA IS TAKEN AS SPECIFIED BY THE USER AND STORED IN FIELD 1 (COMMON) ALONG WITH BLANK AND 0% T LEVELS
5) CHAINS TO SPECIFIED FORTRAN PROGRAM

II. PNSFT1. PA--CHAINS WITH PNT?01. FT
PNSFT3. PA--CHAINS WITH PNT?03. FT

PNSFT1.PA: LO PNSFT1

1) SAME AS PNSF1.FT EXCEPT TAKES TEMPERATURE DATA ALONG WITH THE ABSORBANCE DATA

PNSFT3.PA: LO PNSFT3

1) SAME AS PNSF3.FT EXCEPT TAKES TEMPERATURE DATA ALONG WITH THE ABSORBANCE DATA

III. PNF?01.FT--CHAINS WITH PNSF1.PA
PNF?03.FT--CHAINS WITH PNSF3.PA

PNF101.FT,PNF103.FT: LO PNF10?,PNLLSQ(HO)$*ADDPLT$

1) UP TO 100 POINTS
2) STORES ABSORBANCE DATA ON RKB0 (3A6 FORMAT): TIME-ABSORBANCE-STD DEV OF ABSORBANCE
3) PLOT DATA ON ADDS AND/OR CALCULATE SLOPES OVER SELECTED TIME INTERVALS

PNF401.FT: LO PNF401,PNPR1(0)$*ADDPLT(L)$
PNF403.FT: LO PNF403,PNPR3(0)$*ADDPLT(L)$

1) 100 POINTS ONLY
2) STORES ABSORBANCE DATA ON RKB0-AS ABOVE
3) PLOTS (ADDS) ABSORBANCE AND/OR FIRST DERIVATIVE OF ABSORBANCE VS TIME. USES SAVITZKY-GOLAY 11-POINT SMOOTH TO CALCULATE THE FIRST DERIVATIVE

PNF801.FT,PNF803.FT: LO PNF80?,PNLLSQ(HOI)$*ADDPLT(L)$

1) UP TO 100 POINTS
2) STORES ABSORBANCE DATA ON RKB0 AS ABOVE
3) CALCULATES SLOPE OF ABSORBANCE FOR STANDARDS AND UNKNOWNS AND CALCULATES CONCENTRATIONS OF UNKNOWNS
4) PRINTS OUT RESULTS ON DECWRITER

IV. PNT?01.FT--CHAINS WITH PNSFT1.PA
PNT?03.FT--CHAINS WITH PNTSF3.PA

PNT101.FT: LO PNT101,PNTPL1(HOI)$*ADDPLT(L)$
PNT103.FT: LO PNT103,PNTPL3(HOI)$*ADDPLT(L)$

1) UP TO 100 POINTS
2) STORES ABSORBANCE AND TEMPERATURE DATA ON RKB0 (5A6 FORMAT): TIME--ABS--STD DEV ABS--TEMP--STD DEV TEMP
3) AVERAGE OR NOT AVERAGE THE RUNS
4) PLOT (ADDS) ABSORBANCE AND/OR TEMPERATURE DATA
5) LIST DATA ON DECWRITER

V. PNOD?.FT--OPERATE ON DATA FILES STORED (3A6 FORMAT) on RKB0 INDEPENDENT VARIABLE=COLUMN 1
DEPENDENT VARIABLE=COLUMN 2
STD DEV OF DEPENDENT VARIABLE=COLUMN 3

THE PROGRAMS WERE WRITTEN ASSUMING TIME HAS THE INDEPENDENT VARIABLE AND ABSORBANCE WAS THE DEPENDENT VARIABLE. FILES WITH OTHER VARIABLES CAN BE OPERATED ON CORRECTLY BY THESE PROGRAMS, AS LONG AS THEY ARE

IN 3E13.6 FORMAT. ONLY THE OUTPUT LISTINGS WILL BE LABELED INCORRECTLY.

PNOD1.FT: LO PNOD1(HOI)$*ADDPLT(L)$

1) PRINTS OUT DATA ON DECWRITER AND/OR PLOTS DATA ON ADDS TERMINAL

PNOD2.FT: LO PNOD2(IO)

1) COPIES DATA FILE FROM RKB0 TO FLP2 BUT WITH FORMAT: 'RD',3F15.8 FOR EASE IN TRANSFER TO 11/40 COMPUTER VIA TTR811

PNOD3.FT: LO PNOD3,PNLLSQ(HOI)

1) CALCULATES RATES (SLOPES) FROM THE DATA AND PRINTS OUT THE RESULTS ON THE DECWRITER.

PNOD4.FT: LO PNOD4,PNLLSQ(HOI)

1) DOES A REACTION RATE ANALYSIS OF UNKNOWNS BASED ON THE REACTION RATES OF KNOWNS (CONCENTRATIONS).
2) DATA FILES ON RKB0 ARE DESIGNATED AS KNOWNS OR UNKNOWNS BY THE USER

PNOD5.FT: LO PNOD5,AXIS,XYSYS(HOI)

1) PRINTS DATA ON DECWRITER AND/OR PLOTS DATA ON X-Y RECORDER

PNOD6.FT: LO PNOD6(HOI)

1) CALCULATES SLOPES OVER SELECTED TIME INTERVALS USING AN 11-POINT SAVITZKY-GOLAY QUADRATIC SMOOTH.
2) PRINTS OUT THE SLOPE AT EACH DATA POINT

PNOD7.FT: LO PNOD7(I)$*ADDPLT(L)$

1) PLOTS ABSORBANCE AND/OR FIRST DERIVATIVE OF ABSORBANCE (RATE) ON ADDS
2) USES SAVITZKY-GOLAY 11-POINT, QUADRATIC SMOOTH TECHNIQUE
3) CALCULATES AVERAGE OF THE RATE OVER SPECIFIED INTERVAL

VI. PNODT?.FT--OPERATE ON DATA FILES STORED (5E13.6 FORMAT) on RKB0
INDEPENDENT VARIABLE=COLUMN 1
DEPENDENT VARIABLE=COLUMN2
STD DEV OF DEPENDENT VARIABLE=COLUMN 3
DEPENDENT VARIABLE=COLUMN 4
STD DEV OF DEPENDENT VARIABLE=COLUMN 5

THESE PROGRAMS ARE WRITTEN ASSUMING TIME IS THE INDEPENDENT VARIABLE AND ABSORBANCE AND TEMPERATURE ARE THE DEPENDENT VARIABLES. FILES WITH OTHER VARIABLES CAN BE OPERATED ON CORRECTLY BY THESE PROGRAMS AS LONG AS THEY ARE IN 5A6 FORMAT. ONLY THE OUTPUT LISTINGS WILL BE LABELED WRONG.

PNODT1.FT: LO PNODT1,AXIS,XYSYS(HOI)$*ADDPLT$

1) PLOTS TEMPERATURE AND/OR ABSORBANCE VS TIME ON THE ADDS AND/OR AN X-Y RECORDER
2) PRINTS DATA ON THE DECWRITER.

# APPENDIX C

## DIALOG FOR PAL8 PROGRAM WHICH OPERATES THE STOPPED-FLOW AND ACQUIRES DATA

The printout halts at the appropriate times to accept responses from the experimentor. The experimentor's responses are not shown in the dialog.

```
  DO YOU WISH TO USE THE PREVIOUS 100 %T (BLANK) AND
0 %T LEVELS?  IF YOU DO, MAKE SURE THE LIGHT SOURCE
SHUTTER IS OPEN AND THE DRIVE SYRINGES ANE EMPTY AND
DO NOT CHANGE ANY OF THE PREVIOUS SETTINGS.  TYPE "1"
FOR YES OR TYPE "2" FOR NO:

                    ---SECTION I---

TYPE ANY CHARACTER AFTER YOU COMPLETE EACH INSTRUCTION
SET AND ARE READY FOR THE NEXT INSTRUCTION SET, UNLESS
A SPECIFIC RESPONSE IS REQUIRED.

(1) SET THE KEITHLEY OFFSET SWITCH TO "LOC" AND THE
STOPPED-FLOW TO MANUAL.
   SET THE KEITHLEY AMPLIFICATION SO THAT THE AMPLITUDE
OF CHANGE (INCLUDING NOISE) FOR THE MOST INTENSE REACTION
IS JUST UNDER 9V.  SET THE OFFSET RANGE SO THAT 100 %T
CAN BE OFFSET TO BELOW +5V WITH NO MORE THAN 6.5 TURNS
CLOCKWISE ON THE OFFSET "FINE" KNOB.
   CONSIDER THE NOISE AND DRIFT AND NOTE ITS MAXIMUM
AMPLITUDE (IN VOLTS).
   CLOSE THE LIGHT SOURCE SHUTTER AND SET THE KEITHLEY
OFFSET SWITCH TO "REM".

(2) WHAT IS THE MAXIMUM AMPLITUDE OF THE NOISE AND
DRIFT IN TENTHS OF A VOLT?  TYPE ONE OR TWO DIGITS,
THEN A SPACE.  NOISE=

(3) OPEN THE LIGHT SOURCE SHUTTER.  MAKE SURE THE
DRIVE SYRINGES ARE EMPTY, THEN SET THE STOPPED-FLOW
TO AUTOMATIC.
```

---SECTION II---

(1)TIME INTERVAL BETWEEN ANALOG POINTS:
A=0.2, B=1, C=5, D=10, E=100 MILLISECONDS

(2)NUMBER OF ANALOG POINTS AVERAGED FOR EACH DATA POINT:
A=1, B=3, C=10, D=30, E=100

(3)TIME INTERVAL BETWEEN THE CENTERS OF DATA POINTS IN UNITS OF THE TIME SPAN OF ONE DATA POINT: A=1, B=10, C=100, D=1000

(4)NUMBER OF DATA POINTS TO BE TAKEN:
A=10, B=50, C=100, D=500, E=1000

TYPE THE PROPER LETTER AFTER THE APPROPRIATE NUMBER.

(1)=

(2)=

(3)=

(4)=

(5) HOW MANY TENTH'S OF A MILLISECOND BETWEEN THE TRIGGER AND THE STARTING OF THE CLOCK FOR THE FIRST ANALOG POINT?
NOTE THAT IT WILL TAKE ONE ANALOG POINT TIME INTERVAL AFTER THE CLOCK STARTS BEFORE THE FIRST POINT IS TAKEN.
(HIGHEST 4-DIGIT NUMBER=4095, HIGHEST 5-DIGIT NUMBER=40950)
TYPE A WHOLE NUMBER THEN A SPACE (IF LESS THAN 5 DIGITS):
=

(6) NUMBER OF PUSHES PER SYRINGE FILLING=

(7) RINSE STOPPED-FLOW (THREE FLUSHINGS)? YES=1, NO=2
=

(8) BLANK OR SAMPLE RUN: BLANK=1, SAMPLE=2
=

(9) NUMBER OF SYRINGE FILLINGS PER RUN=

(10) NUMBER OF DATA PUSHES PER FILLING (MAX=5)=

CHECK STOPPED-FLOW SYSTEM, THEN HIT ANY KEY.

WHICH FORTRAN SAVE FILE DO YOU WISH TO CHAIN TO?
IF THE FILE NAME IS LESS THAN 6 CHARACTERS, FILL IT OUT WITH "CIRCLE A" TO MAKE 6 CHARACTERS. TYPE A "?" TO START THE NAME OVER IF YOU MAKE A MISTAKE. TYPE THE FILE NAME, THEN A PERIOD:

# APPENDIX D

## PAL8 PROGRAM, PNSF1.PA, WHICH OPERATES THE STOPPED-FLOW AND ACQUIRES SPECTROPHOTOMETRIC DATA

The Program is shown in the form of a CREF* listing with the cross-reference table at the end. The first column is the CREF reference number and the second column is the computer core location. Core locations from $4100_8$ and up contain 6-bit ASCII code for the dialog given in Appendix C and that section of the Program was not reproduced here.

*See "OS/8 Handbook", Digital Equipment Corporation, Maynard, MA.

```
                /       STOPPED-FLOW PROGRAM:  PNSF1.PA
                /PHILLIP KENT NOTZ - MICHIGAN STATE UNIVERSITY
          6133          CLAB=6133       /AC TO CLOCK BUFFER-PRESET.
          6132          CLOE=6132       /SET CLOCK ENABLE REGISTER PER AC.
          6135          CLSA=6135       /CLOCK STATUS TO AC & CLEAR FLAG.
          6131          CLSK=6131       /SKIP ON CLOCK INTERRUPT.
          6130          CLZE=6130       /CLEAR CLOCK ENABLE REGISTER PER AC.
          7407          DVI=7407
          7405          MUY=7405
          7417          LSR=7417
          7413          SHL=7413
          7411          NMI=7411
          7441          SCA=7441
          7447          SWBA=7447
          0017          *0017
00017     0000  DLOC,     0       /CURRENT LOCATION FOR DATA STORAGE.
00020     0205  SLD,      205     /STARTING LOCATION FOR DATA STORAGE -1.
00021     0003  FLS,      3       /# OF SYRINGE FILLINGS.
00022     0002  CLCT,     2       /CLOCK COUNTS PER ANALOG POINT.
00023     5400  TKHZ,     5400    /CLOCK RATE (10 KHZ).
00024     0012  APD,      12      /# ANALOG POINTS PER DATA POINT.
00025     0012  TID,      12      /TIME UNITS BETWEEN DATA POINTS.
00026     0144  NDP,      144     /# OF DATA POINTS PER PUSH.
00027     0000  SOB,      0       /SAMPLE=NONZERO, BLANK=ZERO.
00030     7777  BLNK,     7777    /BLANK LEVEL (DEFAULT LEVEL=7777).
00031     4000  XFM,      4000    /CONVERSION TO POSITIVE BINARY.
00032     7774  MNFSH,    7774    /MINUS # OF SYRINGE FLUSHINGS.
00033     7770  MNPSH,    7770    /MINUS # OF PUSHES PER FILLING.
00034     7774  MNC1,     7774    /MINUS # OF CLOCK
00035     7766  MNC2,     7766    /COUNTS FOR S.F. VALVES.
00036     7660  MTCC,     7660    /MINUS # OF CLOCK COUNTS AFTER TRIGGER.
00037     5400  EN,       5400    /CLOCK RATE FOR POST-TRIGGER DELAY.
00040     7777  MNGP,     7777    /MINUS # OF DATA PUSHES.
00041     0000  MNFL,     0       /STARTING VALUES IN COUNTERS.
00042     0000  MCC,      0
00043     0000  MNAP,     0
00044     0000  MNEP,     0
00045     0000  MNDP,     0
00046     0000  MFSH,     0       /COUNTERS-INCREMENTED EACH CYCLE.
00047     0000  MAP,      0
00050     0000  MEP,      0
00051     0000  MDP,      0
00052     0000  MFL,      0
00053     0000  MPSH,     0
00054     0000  MGP,      0
```

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                /       STOPPED-FLOW PROGRAM:  PNSF1.PA
                /PHILLIP KENT NOTZ - MICHIGAN STATE UNIVERSITY
          6133          CLAB=6133       /AC TO CLOCK BUFFER-PRESET.
          6132          CLOE=6132       /SET CLOCK ENABLE REGISTER PER AC.
          6135          CLSA=6135       /CLOCK STATUS TO AC & CLEAR FLAG.
          6131          CLSK=6131       /SKIP ON CLOCK INTERRUPT.
          6130          CLZE=6130       /CLEAR CLOCK ENABLE REGISTER PER AC.
          7407          DVI=7407
          7405          MUY=7405
          7417          LSR=7417
          7413          SHL=7413
          7411          NMI=7411
          7441          SCA=7441
          7447          SWBA=7447
          0017          *0017
00017     0000  DLOC,    0       /CURRENT LOCATION FOR DATA STORAGE.
00020     0205  SLD,     205     /STARTING LOCATION FOR DATA STORAGE -1.
00021     0003  FLS,     3       /# OF SYRINGE FILLINGS.
00022     0002  CLCT,    2       /CLOCK COUNTS PER ANALOG POINT.
00023     5400  TKHZ,    5400    /CLOCK RATE (10 KHZ).
00024     0012  APD,     12      /# ANALOG POINTS PER DATA POINT.
00025     0012  TID,     12      /TIME UNITS BETWEEN DATA POINTS.
00026     0144  NDP,     144     /# OF DATA POINTS PER PUSH.
00027     0000  SOB,     0       /SAMPLE=NONZERO, BLANK=ZERO.
00030     7777  BLNK,    7777    /BLANK LEVEL (DEFAULT LEVEL=7777).
00031     4000  XFM,     4000    /CONVERSION TO POSITIVE BINARY.
00032     7774  MNFSH,   7774    /MINUS # OF SYRINGE FLUSHINGS.
00033     7770  MNPSH,   7770    /MINUS # OF PUSHES PER FILLING.
00034     7774  MNC1,    7774    /MINUS # OF CLOCK
00035     7766  MNC2,    7766    /COUNTS FOR S.F. VALVES.
00036     7660  MTCC,    7660    /MINUS # OF CLOCK COUNTS AFTER TRIGGER.
00037     5400  EN,      5400    /CLOCK RATE FOR POST-TRIGGER DELAY.
00040     7777  MNGP,    7777    /MINUS # OF DATA PUSHES.
00041     0000  MNFL,    0       /STARTING VALUES IN COUNTERS.
00042     0000  MCC,     0
00043     0000  MNAP,    0
00044     0000  MNEP,    0
00045     0000  MNDP,    0
00046     0000  MFSH,    0       /COUNTERS-INCREMENTED EACH CYCLE.
00047     0000  MAP,     0
00050     0000  MEP,     0
00051     0000  MDP,     0
00052     0000  MFL,     0
00053     0000  MPSH,    0
00054     0000  MGP,     0
```

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46   00055  0000  APM,    0        /ANALOG DATA
47   00056  0000  APL,    0        /24-BIT WORD.
48   00057  0000  HPT,    0        /TEMPORARY HOLDING LOCATION.
49   00060  0000  NPTS,   0        /# OF DATA POINTS TAKEN.
50   00061  0000  MNPTS,  0        /MINUS # OF DATA POINTS TAKEN.
51   00062  0000  MPTS,   0        /MINUS # OF DATA POINTS.
52   00063  0000  MC1,    0        /CLOCK COUNTS
53   00064  0000  MC2,    0        /FOR S.F. VALVES.
54   00065  0000  BLKL,   0        /BLANK SUM VALUE
55   00066  0000  BLKM,   0        /24-BIT WORD.
56   00067  0000  OCTMG,  0        /UPPER MARGIN IN OCTAL.
57   00070  0000  ZRLVL,  0        /0 %T LEVEL.
58   00071  0000  MRG1,   0        /MARGIN-VOLTS.
59   00072  0000  MRG2,   0        /MARGIN-TENTHS OF A VOLT.
60   00073  0000  MPLFT,  0        /MINUS PUSHES LEFT AFTER SECTION I.
61   00074  0000  FCTR,   0        /OFFSET FACTOR.
62   00075  0000  FEXP,   0        /EXPONENT OF FACTOR.
63   00076  0000  KPL,    0        /KEEP PREVIOUS LEVELS INDICATOR.
64   00077  0000  TYPE,   0
65   00100  6041  TSFA,   TSF              /CHANGE FOR LPT.
66   00101  5100         JMP .-1
67   00102  6046  TLSA,   TLS              /CHANGE FOR LPT.
68   00103  7300         CLA CLL
69   00104  5477         JMP I TYPE
70   00105  0000  LISN,   0
71   00106  6031         KSF
72   00107  5106         JMP .-1
73   00110  6036         KRB
74   00111  6046         TLS
75   00112  6041         TSF
76   00113  5112         JMP .-1
77   00114  5505         JMP I LISN
78   00115  0000  OP1,    0
79   00116  0000  OP2,    0
80   00117  0000  OP3,    0
81   00120  0000  OP4,    0
82   00121  0000  TD1,    0
83   00122  0000  TD2,    0
84   00123  0000  TD3,    0
85   00124  0000  TD4,    0
86   00125  0000  DITD,   0
87   00126  0000  OP6,    0
88   00127  0000  OP9,    0
89   00130  0000  OP10,   0
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 90          0200          *200
 91   00200  7340  SFST,   CLA CLL CMA
 92   00201  6130          CLZE
 93   00202  7200          CLA
 94   00203  6066          6066            /CLEAR LPT FLAG.
 95   00204  6046          TLS
 96   00205  6032          KCC
 97   00206  6677          6677            /CLEAR VALVE FLIP-FLOPS.
 98   00207  6652          6652            /ACTIVATE FLIP-FLOPS.
 99   00210  7447          SWBA
100   00211  4777'         JMS STSF
101   00212  6451          6451            /LATCH D-A.
102   00213  1376          TAD (AS14
103   00214  4775'         JMS POOH
104   00215  4105          JMS LISN
105   00216  7300          CLA CLL
106   00217  1374          TAD (AS15
107   00220  4775'         JMS POOH
108   00221  4105  MRGN,   JMS LISN
109   00222  3071          DCA MRG1
110   00223  1071          TAD MRG1
111   00224  4241          JMS DOK         /FIRST DIGIT OK?
112   00225  4105          JMS LISN
113   00226  3072          DCA MRG2
114   00227  1072          TAD MRG2
115   00230  4241          JMS DOK         /SECOND DIGIT OK?
116   00231  4105          JMS LISN
117   00232  1373          TAD (-240
118   00233  7450          SNA
119   00234  5261          JMP WNM
120   00235  7300          CLA CLL
121   00236  1372          TAD (AS10
122   00237  4775'         JMS POOH
123   00240  5221          JMP MRGN
124   00241  0000  DOK,    0               /CHECK-LEGAL DIGIT?
125   00242  1373          TAD (-240
126   00243  7450          SNA             /IS IT A SPACE?
127   00244  5261          JMP WNM
128   00245  1371          TAD (-20
129   00246  7500          SMA
130   00247  5254          JMP CKPS
131   00250  7300  BDDG,   CLA CLL
132   00251  1372          TAD (AS10
133   00252  4775'         JMS POOH
134   00253  5221          JMP MRGN
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135   00254  1370  CKPS,   TAD (-12
136   00255  7500          SMA
137   00256  5250          JMP BDDG
138   00257  7300          CLA CLL
139   00260  5641          JMP I DOK
140   00261  1367  WNM,    TAD (215
141   00262  4077          JMS TYPE
142   00263  1366          TAD (212
143   00264  4077          JMS TYPE
144   00265  1071          TAD MRG1
145   00266  1373          TAD (-240
146   00267  7440          SZA
147   00270  5274          JMP .+4
148   00271  1372          TAD (AS10
149   00272  4775'         JMS POOH
150   00273  5221          JMP MRGN
151   00274  1371          TAD (-20
152   00275  3071          DCA MRG1
153   00276  1072          TAD MRG2
154   00277  1373          TAD (-240
155   00300  7440          SZA
156   00301  5306          JMP .+5
157   00302  1071          TAD MRG1
158   00303  3072          DCA MRG2
159   00304  3071          DCA MRG1
160   00305  5310          JMP .+3
161   00306  1371          TAD (-20
162   00307  3072          DCA MRG2
163   00310  3067          DCA OCTMG       /CLEAR OCTMG.
164   00311  1071          TAD MRG1
165   00312  7421          MQL             /LOAD MQ FROM AC, THEN CLEAR AC.
166   00313  7405          MUY
167   00314  0632          632             /A-D READING FOR 1 VOLT.
168   00315  7721          CLA SWP         /LOAD AC FROM MQ, THEN CLEAR MQ.
169   00316  3067          DCA OCTMG
170   00317  1072          TAD MRG2
171   00320  7521          SWP
172   00321  7405          MUY
173   00322  0051          51              /A-D READING FOR 0.1 VOLT.
174   00323  7721          CLA SWP
175   00324  1067          TAD OCTMG
176   00325  7010          RAR
177   00326  3067          DCA OCTMG       /OCTMG=MARGIN (A-D SCALE)
178   00327  7100          CLL
179   00330  6451          6451            /SET D-A PER AC
180   00331  4765'         JMS ADLVL
181   00332  3070          DCA ZRLVL
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182   00333  1364           TAD (40
183   00334  4763'          JMS LOLIM
184   00335  1762'          TAD LOLVL
185   00336  7041           CIA
186   00337  1070           TAD ZRLVL
187   00340  3761'          DCA DNDL
188   00341  3760'          DCA DNDM
189   00342  1757'          TAD LDAS
190   00343  3756'          DCA DVSR
191   00344  4755'          JMS DVDE
192   00345  1754'          TAD QT
193   00346  3074           DCA FCTR
194   00347  1753'          TAD BEXP
195   00350  3075           DCA FEXP
196   00351  5752'          JMP OHPT
197   00352  0707
198   00353  1145
199   00354  1142
200   00355  1056
201   00356  1126
202   00357  0552
203   00360  1140
204   00361  1141
205   00362  0554
206   00363  0454
207   00364  0040
208   00365  0400
209   00366  0212
210   00367  0215
211   00370  7766
212   00371  7760
213   00372  5151
214   00373  7540
215   00374  6021
216   00375  4000
217   00376  5276
218   00377  3000
219          0400' PAGE
220   00400  0000  ADLVL,  0
221   00401  7300           CLA CLL          /FIND AVERAGE OF
222   00402  3251           DCA LVL          /100 A-D POINTS
223   00403  3252           DCA LVM          /TAKEN AT 100 HZ.
224   00404  1377           TAD (-144
225   00405  3253           DCA CADL
226   00406  1377           TAD (-144
227   00407  6133           CLAB
228   00410  7240           CLA CMA
229   00411  6130           CLZE             /STOP CLOCK.
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230   00412  7300          CLA CLL
231   00413  1023          TAD TKHZ
232   00414  6132          CLOE
233   00415  6135          CLSA
234   00416  6131  LVFG,   CLSK
235   00417  5216          JMP .-1
236   00420  6135          CLSA
237   00421  7300          CLA CLL
238   00422  6531          6531
239   00423  6532          6532
240   00424  5223          JMP .-1
241   00425  6534          6534
242   00426  1031          TAD XFM
243   00427  7100          CLL
244   00430  1251          TAD LVL
245   00431  3251          DCA LVL
246   00432  7430          SZL
247   00433  2252          ISZ LVM
248   00434  7100          CLL
249   00435  2253          ISZ CADL
250   00436  5216          JMP LVFG
251   00437  7040          CMA
252   00440  6130          CLZE
253   00441  7200          CLA
254   00442  1251          TAD LVL
255   00443  7421          MQL
256   00444  1252          TAD LVM
257   00445  7407          DVI
258   00446  0144          144              /# POINTS IN THE AVERAGE.
259   00447  7721          CLA SWP
260   00450  5600          JMP I ADLVL      /GO BACK WITH AVG IN AC.
261   00451  0000  LVL,    0
262   00452  0000  LVM,    0
263   00453  0000  CADL,   0
264   00454  0000  LOLIM,  0                /SETS D-A TO OBTAIN
265   00455  3351          DCA LLM          /SPECIFIED LOWER LIMIT.
266   00456  3352          DCA LDAS
267   00457  7120          STL
268   00460  7012          RTR
269   00461  7010  LXDG,   RAR
270   00462  7450          SNA
271   00463  5330          JMP LDUN
272   00464  3353          DCA LDIG
273   00465  1353          TAD LDIG
274   00466  1352          TAD LDAS
275   00467  3352          DCA LDAS
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276   00470  1352          TAD LDAS
277   00471  6451          6451            /SET D-A PER AC.
278   00472  4200          JMS ADLVL
279   00473  3354          DCA LOLVL
280   00474  1351          TAD LLM
281   00475  7500          SMA
282   00476  5310          JMP LL2         /LLM IS POS.
283   00477  7200          CLA             /LLM IS NEG.
284   00500  1354          TAD LOLVL
285   00501  7500          SMA
286   00502  5315          JMP LDOF
287   00503  7041  LL1,     CIA             /LLM & ADLVL ARE BOTH
288   00504  1351          TAD LLM         /NEG OR BOTH POS.
289   00505  7500          SMA
290   00506  5315          JMP LDOF
291   00507  5325          JMP LIOF
292   00510  7200  LL2,     CLA
293   00511  1354          TAD LOLVL
294   00512  7510          SPA
295   00513  5325          JMP LIOF        /LLM IS POS & ADLVL IS NEG.
296   00514  5303          JMP LL1         /LLM IS POS & ADLVL IS POS.
297   00515  7300  LDOF,    CLA CLL         /DECREASE OFFSET.
298   00516  1353          TAD LDIG
299   00517  7041          CIA
300   00520  1352          TAD LDAS
301   00521  3352          DCA LDAS
302   00522  7100          CLL
303   00523  1353          TAD LDIG
304   00524  5261          JMP LXDG
305   00525  7300  LIOF,    CLA CLL         /INCREASE OFFSET.
306   00526  1353          TAD LDIG
307   00527  5261          JMP LXDG
308   00530  1352  LDUN,    TAD LDAS        /IS OFFSET SET OK?
309   00531  6451          6451
310   00532  4200          JMS ADLVL
311   00533  3354          DCA LOLVL
312   00534  1354          TAD LOLVL
313   00535  7510          SPA
314   00536  5776'         JMP NBOR
315   00537  7041          CIA
316   00540  1351          TAD LLM
317   00541  7500          SMA
318   00542  5345          JMP LLFU
319   00543  7300          CLA CLL
320   00544  5654          JMP I LOLIM
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321   00545  7240  LLFU,   CLA CMA
322   00546  1352          TAD LDAS
323   00547  3352          DCA LDAS
324   00550  5330          JMP LDUN
325   00551  0000  LLM,    0
326   00552  0000  LDAS,   0
327   00553  0000  LDIG,   0
328   00554  0000  LOLVL,  0
329   00576  0677
330   00577  7634
331          0600  PAGE
332   00600  0000  UPLIM,  0             /SETS D-A TO OBTAIN
333   00601  7041          CIA           /SPECIFIED UPPER LIMIT.
334   00602  3303          DCA MULM
335   00603  3304          DCA UDAS
336   00604  7120          STL
337   00605  7012          RTR
338   00606  7010  UXDG,   RAR
339   00607  7450          SNA
340   00610  5253          JMP UDUN
341   00611  3305          DCA UDIG
342   00612  1305          TAD UDIG
343   00613  1304          TAD UDAS
344   00614  3304          DCA UDAS
345   00615  1304          TAD UDAS
346   00616  6451          6451
347   00617  4777'         JMS ADLVL
348   00620  3306          DCA UPLVL
349   00621  1306          TAD UPLVL
350   00622  7500          SMA
351   00623  5243          JMP UL2
352   00624  7200          CLA
353   00625  1303          TAD MULM
354   00626  7510          SPA
355   00627  5250          JMP UIOF
356   00630  1306  UL1,    TAD UPLVL
357   00631  7500          SMA
358   00632  5250          JMP UIOF
359   00633  7300  UDOF,   CLA CLL
360   00634  1305          TAD UDIG
361   00635  7041          CIA
362   00636  1304          TAD UDAS
363   00637  3304          DCA UDAS
364   00640  7100          CLL
365   00641  1305          TAD UDIG
366   00642  5206          JMP UXDG
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367   00643  7200  UL2,    CLA
368   00644  1303          TAD MULM
369   00645  7500          SMA
370   00646  5233          JMP UDOF
371   00647  5230          JMP UL1
372   00650  7300  UIOF,    CLA CLL
373   00651  1305          TAD UDIG
374   00652  5206          JMP UXDG
375   00653  1304  UDUN,    TAD UDAS
376   00654  6451          6451
377   00655  4777'         JMS ADLVL
378   00656  3306          DCA UPLVL
379   00657  1306          TAD UPLVL
380   00660  1303          TAD MULM
381   00661  7510          SPA
382   00662  5273          JMP ULG
383   00663  7300  ULFU,    CLA CLL
384   00664  1304          TAD UDAS
385   00665  1376          TAD (-1777
386   00666  7500          SMA
387   00667  5277          JMP NBOR
388   00670  7200          CLA
389   00671  2304          ISZ UDAS
390   00672  5253          JMP UDUN
391   00673  7450  ULG,    SNA
392   00674  5263          JMP ULFU
393   00675  7300          CLA CLL
394   00676  5600          JMP I UPLIM
395   00677  7300  NBOR,    CLA CLL
396   00700  1375          TAD (AS17
397   00701  4774'         JMS POOH
398   00702  5773'         JMP SFST
399   00703  0000  MULM,    0
400   00704  0000  UDAS,    0
401   00705  0000  UDIG,    0
402   00706  0000  UPLVL,   0
403   00707  1372  OHPT,    TAD (-3
404   00710  3771'         DCA CHPT
405   00711  1370          TAD (AS16
406   00712  4774'         JMS POOH
407   00713  4105          JMS LISN
408   00714  7300          CLA CLL          /OPERATE STOPPED-FLOW.
409   00715  6661          6661             /SET FF1.
410   00716  4767'         JMS TM1
411   00717  6662          6662             /SET FF2.
412   00720  4766'         JMS TM2
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413   00721  6671           6671              /CLEAR FF1.
414   00722  4767'          JMS TM1
415   00723  6672           6672              /CLEAR FF2.
416   00724  4767'          JMS TM1
417   00725  6664           6664              /SET FF3.
418   00726  4767'          JMS TM1
419   00727  6674           6674              /CLEAR FF3.
420   00730  2771'          ISZ CHPT
421   00731  5324           JMP .-5
422   00732  4767'          JMS TM1
423   00733  1067           TAD OCTMG
424   00734  7041           CIA
425   00735  3765'          DCA HLMT
426   00736  1765'          TAD HLMT
427   00737  4200           JMS UPLIM
428   00740  5764'          JMP CHPL
429   00764  1000
430   00765  1023
431   00766  2306
432   00767  2265
433   00770  6125
434   00771  1022
435   00772  7775
436   00773  0200
437   00774  4000
438   00775  6225
439   00776  6001
440   00777  0400
441          1000' PAGE
442   01000  4777' CHPL,    JMS ADLVL
443   01001  3776'          DCA UPLVL
444   01002  1776'          TAD UPLVL
445   01003  7041           CIA
446   01004  1223           TAD HLMT
447   01005  7500           SMA
448   01006  5224           JMP POP
449   01007  7301           CLA CLL IAC
450   01010  1775'          TAD UDAS
451   01011  3775'          DCA UDAS
452   01012  1775'          TAD UDAS
453   01013  1374           TAD (-1777
454   01014  7500           SMA
455   01015  5773'          JMP NBOR
456   01016  7300           CLA CLL
457   01017  1775'          TAD UDAS
458   01020  6451           6451
459   01021  5200           JMP CHPL
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460   01022  0000  CHPT,   0
461   01023  0000  HLMT,   0
462   01024  7300  POP,    CLA CLL
463   01025  1372          TAD (0177
464   01026  3017          DCA DLOC
465   01027  1775'         TAD UDAS
466   01030  6211          CDF 10
467   01031  3417          DCA I DLOC
468   01032  6201          CDF 0
469   01033  1074          TAD FCTR
470   01034  6211          CDF 10
471   01035  3417          DCA I DLOC
472   01036  6201          CDF 0
473   01037  1075          TAD FEXP
474   01040  6211          CDF 10
475   01041  3417          DCA I DLOC
476   01042  6201          CDF 0
477   01043  1776'         TAD UPLVL
478   01044  6211          CDF 10
479   01045  3417          DCA I DLOC
480   01046  6201          CDF 0
481   01047  1070          TAD ZRLVL
482   01050  6211          CDF 10
483   01051  3417          DCA I DLOC
484   01052  6201          CDF 0
485   01053  1776'         TAD UPLVL
486   01054  3030          DCA BLNK
487   01055  5771'         JMP OPTNS
488   01056  0000  DVDE,   0       /DIVIDE SUBROUTINE
489   01057  7447          SWBA
490   01060  7621          CLA MQL
491   01061  7100          CLL
492   01062  1326          TAD DVSR        /SHIFT DVSR TO LEFT LIMIT.
493   01063  1346          TAD TFM
494   01064  7500          SMA
495   01065  5301          JMP NMV1
496   01066  1346          TAD TFM
497   01067  7411          NMI
498   01070  3326          DCA DVSR
499   01071  7441          SCA
500   01072  3343          DCA BPM1        /BPM1=# OF SPACES DVSR WAS MOVED.
501   01073  2343          ISZ BPM1
502   01074  1326          TAD DVSR
503   01075  7413          SHL
504   01076  0000          0
505   01077  3326          DCA DVSR
506   01100  5303          JMP DVND
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507   01101  7300  NMV1,   CLA CLL
508   01102  3343          DCA BPM1
509   01103  1341  DVND,   TAD DNDL
510   01104  7421          MQL             /LOAD MQ FROM AC, THEN CLEAR AC.
511   01105  1340          TAD DNDM        /SHIFT DND TO ONE LESS THAN
512   01106  1346          TAD TFM         /LEFT LIMIT.
513   01107  7500          SMA
514   01110  5320          JMP NMV2
515   01111  1346          TAD TFM
516   01112  7411          NMI
517   01113  3340          DCA DNDM        /BUT DNDL STILL IN MQ.
518   01114  7441          SCA
519   01115  3344          DCA BPM2        /BPM2=# OF SPACES DND WAS MOVED.
520   01116  1340          TAD  DNDM
521   01117  5325          JMP CQT
522   01120  7240  NMV2,   CLA CMA
523   01121  3344          DCA BPM2
524   01122  1340          TAD DNDM
525   01123  7417          LSR
526   01124  0000          0
527   01125  7407  CQT,    DVI
528   01126  0000  DVSR,   0
529   01127  7430          SZL
530   01130  7402          HLT             /DIVIDE OVERFLOW
531   01131  7721          CLA SWP         /LOAD AC FROM MQ, THEN CLEAR MQ.
532   01132  3342          DCA QT
533   01133  1344          TAD BPM2
534   01134  7041          CIA
535   01135  1343          TAD BPM1
536   01136  3345          DCA BEXP
537   01137  5656          JMP I DVDE
538   01140  0000  DNDM,   0
539   01141  0000  DNDL,   0
540   01142  0000  QT,     0
541   01143  0000  BPM1,   0
542   01144  0000  BPM2,   0
543   01145  0000  BEXP,   0
544   01146  4000  TFM,    4000
545   01171  1200
546   01172  0177
547   01173  0677
548   01174  6001
549   01175  0704
550   01176  0706
551   01177  0400
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                1200  PAGE
01200  7300  OPTNS,  CLA CLL          /INPUT OPTIONS FROM TTY.
01201  1377          TAD (AS1
01202  4776'         JMS POOH
01203  4105  OPTN1,  JMS LISN
01204  3115          DCA OP1          /STORE FOR LPT.
01205  1115          TAD OP1
01206  1375          TAD (-301
01207  7450          SNA              /IS IT AN A?
01210  5322          JMP A1
01211  1374          TAD (-1
01212  7450          SNA              /IS IT A B?
01213  5325          JMP B1
01214  1374          TAD (-1
01215  7450          SNA              /IS IT A C?
01216  5330          JMP C1
01217  1374          TAD (-1
01220  7450          SNA              /IS IT A D?
01221  5333          JMP D1
01222  1374          TAD (-1
01223  7450          SNA              /IS IT AN E?
01224  5773'         JMP E1
01225  5772'         JMP WOPT1        /IS IT NONE?
01226  1371  OPT2,   TAD (AS2
01227  4776'         JMS POOH
01230  4105  OPTN2,  JMS LISN
01231  3116          DCA OP2          /STORE FOR LPT.
01232  1116          TAD OP2
01233  1375          TAD (-301
01234  7450          SNA
01235  5770'         JMP A2
01236  1374          TAD (-1
01237  7450          SNA
01240  5767'         JMP B2
01241  1374          TAD (-1
01242  7450          SNA
01243  5766'         JMP C2
01244  1374          TAD (-1
01245  7450          SNA
01246  5765'         JMP D2
01247  1374          TAD (-1
01250  7450          SNA
01251  5764'         JMP E2
01252  5763'         JMP WOPT2
01253  1362  OPT3,   TAD (AS3
01254  4776'         JMS POOH
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598   01255  4105  OPTN3,  JMS LISN
599   01256  3117          DCA OP3
600   01257  1117          TAD OP3
601   01260  1375          TAD (-301
602   01261  7450          SNA
603   01262  5761'         JMP A3
604   01263  1374          TAD (-1
605   01264  7450          SNA
606   01265  5760'         JMP B3
607   01266  1374          TAD (-1
608   01267  7450          SNA
609   01270  5757'         JMP C3
610   01271  1374          TAD (-1
611   01272  7450          SNA
612   01273  5756'         JMP D3
613   01274  5755'         JMP WOPT3
614   01275  1354  OPT4,   TAD (AS4
615   01276  4776'         JMS POOH
616   01277  4105  OPTN4,  JMS LISN
617   01300  3120          DCA OP4
618   01301  1120          TAD OP4
619   01302  1375          TAD (-301
620   01303  7450          SNA
621   01304  5753'         JMP A4
622   01305  1374          TAD (-1
623   01306  7450          SNA
624   01307  5752'         JMP B4
625   01310  1374          TAD (-1
626   01311  7450          SNA
627   01312  5751'         JMP C4
628   01313  1374          TAD (-1
629   01314  7450          SNA
630   01315  5750'         JMP D4
631   01316  1374          TAD (-1
632   01317  7450          SNA
633   01320  5747'         JMP E4
634   01321  5746'         JMP WOPT4
635   01322  1345  A1,     TAD (2
636   01323  3022          DCA CLCT
637   01324  5226          JMP OPT2
638   01325  1344  B1,     TAD (12
639   01326  3022          DCA CLCT
640   01327  5226          JMP OPT2
641   01330  1343  C1,     TAD (62
642   01331  3022          DCA CLCT
643   01332  5226          JMP OPT2
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644   01333  1342  D1,     TAD (144
645   01334  3022          DCA CLCT
646   01335  5226          JMP OPT2
647   01342  0144
648   01343  0062
649   01344  0012
650   01345  0002
651   01346  1471
652   01347  1466
653   01350  1463
654   01351  1460
655   01352  1455
656   01353  1452
657   01354  4504
658   01355  1446
659   01356  1443
660   01357  1440
661   01360  1435
662   01361  1432
663   01362  4477
664   01363  1426
665   01364  1423
666   01365  1420
667   01366  1415
668   01367  1412
669   01370  1407
670   01371  4472
671   01372  1403
672   01373  1400
673   01374  7777
674   01375  7477
675   01376  4000
676   01377  4100
677          1400  PAGE
678   01400  1377  E1,     TAD (1750
679   01401  3022          DCA CLCT
680   01402  5776'         JMP OPT2
681   01403  7300  WOPT1,  CLA CLL          /WRONG INPUT.
682   01404  1375          TAD (AS10
683   01405  4774'         JMS POOH
684   01406  5773'         JMP OPTN1
685   01407  1372  A2,     TAD (1
686   01410  3024          DCA APD
687   01411  5771'         JMP OPT3
688   01412  1370  B2,     TAD (3
689   01413  3024          DCA APD
690   01414  5771'         JMP OPT3
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691   01415  1367  C2,      TAD (12
692   01416  3024            DCA APD
693   01417  5771'           JMP OPT3
694   01420  1366  D2,      TAD (36
695   01421  3024            DCA APD
696   01422  5771'           JMP OPT3
697   01423  1365  E2,      TAD (144
698   01424  3024            DCA APD
699   01425  5771'           JMP OPT3
700   01426  7300  WOPT2,   CLA CLL          /WRONG INPUT.
701   01427  1375            TAD (AS10
702   01430  4774'           JMS POOH
703   01431  5764'           JMP OPTN2
704   01432  1372  A3,      TAD (1
705   01433  3025            DCA TID
706   01434  5763'           JMP OPT4
707   01435  1367  B3,      TAD (12
708   01436  3025            DCA TID
709   01437  5763'           JMP OPT4
710   01440  1365  C3,      TAD (144
711   01441  3025            DCA TID
712   01442  5763'           JMP OPT4
713   01443  1377  D3,      TAD (1750
714   01444  3025            DCA  TID
715   01445  5763'           JMP OPT4
716   01446  7300  WOPT3,   CLA CLL          /WRONG INPUT.
717   01447  1375            TAD (AS10
718   01450  4774'           JMS POOH
719   01451  5762'           JMP OPTN3
720   01452  1367  A4,      TAD (12
721   01453  3026            DCA NDP
722   01454  5275            JMP PTD
723   01455  1361  B4,      TAD (62
724   01456  3026            DCA NDP
725   01457  5275            JMP PTD
726   01460  1365  C4,      TAD (144
727   01461  3026            DCA NDP
728   01462  5275            JMP PTD
729   01463  1360  D4,      TAD (764
730   01464  3026            DCA NDP
731   01465  5275            JMP PTD
732   01466  1377  E4,      TAD (1750
733   01467  3026            DCA NDP
734   01470  5275            JMP PTD
735   01471  7300  WOPT4,   CLA CLL          /WRONG INPUT.
736   01472  1375            TAD (AS10
737   01473  4774'           JMS POOH
738   01474  5757'           JMP OPTN4
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739   01475  1356  PTD,    TAD (AS5
740   01476  4774'         JMS POOH
741   01477  3121  PTD1,   DCA TD1
742   01500  3122          DCA TD2
743   01501  3123          DCA TD3
744   01502  3124          DCA TD4
745   01503  3125          DCA DITD
746   01504  3755'         DCA MX1
747   01505  3754'         DCA MX2
748   01506  3753'         DCA MX3
749   01507  3752'         DCA MX4
750   01510  4105          JMS LISN        /GET FIRST DIGIT.
751   01511  3121          DCA TD1
752   01512  1121          TAD TD1
753   01513  4751'         JMS WNUM
754   01514  4105          JMS LISN        /GET SECOND DIGIT.
755   01515  3122          DCA TD2
756   01516  1122          TAD TD2
757   01517  4751'         JMS WNUM
758   01520  4105          JMS LISN        /GET THIRD DIGIT.
759   01521  3123          DCA TD3
760   01522  1123          TAD TD3
761   01523  4751'         JMS WNUM
762   01524  4105          JMS LISN        /GET FOURTH DIGIT.
763   01525  3124          DCA TD4
764   01526  1124          TAD TD4
765   01527  4751'         JMS WNUM
766   01530  4105          JMS LISN        /FIFTH CHAR MUST BE SPACE OR 0.
767   01531  1350          TAD (-240
768   01532  7450          SNA             /IS IT A SPACE?
769   01533  5747'         JMP C5BN        /YES-DONE.
770   01534  1346          TAD (-20
771   01535  7450          SNA             /IS IT A ZERO?
772   01536  5745'         JMP FVDIG
773   01537  7300          CLA CLL         /NO-START OVER.
774   01540  1344          TAD (AS11
775   01541  4774'         JMS POOH
776   01542  5277          JMP PTD1
777   01544  5164
778   01545  1604
779   01546  7760
780   01547  1631
781   01550  7540
782   01551  1610
783   01552  1727
784   01553  1717
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785   01554  1707
786   01555  1700
787   01556  4511
788   01557  1277
789   01560  0764
790   01561  0062
791   01562  1255
792   01563  1275
793   01564  1230
794   01565  0144
795   01566  0036
796   01567  0012
797   01570  0003
798   01571  1253
799   01572  0001
800   01573  1203
801   01574  4000
802   01575  5151
803   01576  1226
804   01577  1750
805          1600  PAGE
806   01600  7100  FRDIG,  CLL
807   01601  1377          TAD (5400
808   01602  3037          DCA EN
809   01603  5231          JMP C5BN
810   01604  7100  FVDIG,  CLL
811   01605  1376          TAD (5300
812   01606  3037          DCA EN
813   01607  5231          JMP C5BN
814   01610  0000  WNUM,   0
815   01611  1375          TAD (-240
816   01612  7450          SNA             /IS IT A SPACE?
817   01613  5200          JMP FRDIG       /YES-DONE.
818   01614  1374          TAD (-20        /CONVERT TO BINARY NUMBER.
819   01615  7500          SMA
820   01616  5223          JMP CKPOS
821   01617  7300  DBAD,   CLA CLL         /NOT A PROPER DIGIT.
822   01620  1373          TAD (AS10
823   01621  4772'         JMS POOH
824   01622  5771'         JMP PTD1
825   01623  1370  CKPOS,  TAD (-12
826   01624  7500          SMA
827   01625  5217          JMP DBAD
828   01626  7300          CLA CLL
829   01627  2125          ISZ DITD
830   01630  5610          JMP I WNUM
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831   01631  1125  C5BN,   TAD DITD        /HOW MANY DIGITS IN
832   01632  1367          TAD (-1         /POST-TRIGGER DELAY?
833   01633  7450          SNA             /IS IT ONE DIGIT?
834   01634  5245          JMP DITD1
835   01635  1367          TAD (-1
836   01636  7450          SNA             /IS IT TWO DIGITS?
837   01637  5250          JMP DITD2
838   01640  1367          TAD (-1
839   01641  7450          SNA             /IS IT 3 DIGITS?
840   01642  5255          JMP DITD3
841   01643  7300          CLA CLL
842   01644  5264          JMP DITD4       /MUST BE 4 DIGITS.
843   01645  1366  DITD1,  TAD (1
844   01646  3300          DCA MX1
845   01647  5274          JMP CPTD
846   01650  1365  DITD2,  TAD (12
847   01651  3300          DCA MX1
848   01652  1366          TAD (1
849   01653  3307          DCA MX2
850   01654  5274          JMP CPTD
851   01655  1364  DITD3,  TAD (144
852   01656  3300          DCA MX1
853   01657  1365          TAD (12
854   01660  3307          DCA MX2
855   01661  1366          TAD (1
856   01662  3317          DCA MX3
857   01663  5274          JMP CPTD
858   01664  1363  DITD4,  TAD (1750
859   01665  3300          DCA MX1
860   01666  1364          TAD (144
861   01667  3307          DCA MX2
862   01670  1365          TAD (12
863   01671  3317          DCA MX3
864   01672  1366          TAD (1
865   01673  3327          DCA MX4
866   01674  1121  CPTD,   TAD TD1         /CALCULATE POST-TRIGGER DELAY.
867   01675  1362          TAD (-260
868   01676  7421          MQL
869   01677  7405          MUY
870   01700  0000  MX1,     0
871   01701  7721          CLA SWP
872   01702  3036          DCA MTCC
873   01703  1122          TAD TD2
874   01704  1362          TAD (-260
875   01705  7421          MQL
876   01706  7405          MUY
877   01707  0000  MX2,     0
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878   01710  7721          CLA SWP
879   01711  1036          TAD MTCC
880   01712  3036          DCA MTCC
881   01713  1123          TAD TD3
882   01714  1362          TAD (-260
883   01715  7421          MQL
884   01716  7405          MUY
885   01717  0000  MX3,     0
886   01720  7721          CLA SWP
887   01721  1036          TAD MTCC
888   01722  3036          DCA MTCC
889   01723  1124          TAD TD4
890   01724  1362          TAD (-260
891   01725  7421          MQL
892   01726  7405          MUY
893   01727  0000  MX4,     0
894   01730  7721          CLA SWP
895   01731  1036          TAD MTCC
896   01732  7041          CIA
897   01733  3036          DCA MTCC
898   01734  1361          TAD (AS6        /GET # OF PUSHES
899   01735  4772'         JMS POOH        /PER SYRINGE FILLING.
900   01736  4105  NPUF,   JMS LISN
901   01737  3126          DCA OP6
902   01740  1126          TAD OP6
903   01741  1362          TAD (-260
904   01742  7500          SMA
905   01743  5760'         JMP CKPL
906   01744  5757'         JMP NBAD
907   01757  2000
908   01760  2004
909   01761  4777
910   01762  7520
911   01763  1750
912   01764  0144
913   01765  0012
914   01766  0001
915   01767  7777
916   01770  7766
917   01771  1477
918   01772  4000
919   01773  5151
920   01774  7760
921   01775  7540
922   01776  5300
923   01777  5400
924          2000' PAGE
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925   02000  7300  NBAD,   CLA CLL
926   02001  1377          TAD (AS10
927   02002  4776'         JMS POOH
928   02003  5775'         JMP NPUF
929   02004  1374  CKPL,   TAD (-10
930   02005  7500          SMA
931   02006  5200          JMP NBAD
932   02007  1373          TAD (10
933   02010  7041          CIA
934   02011  3033          DCA MNPSH
935   02012  1372          TAD (3
936   02013  1033          TAD MNPSH
937   02014  3073          DCA MPLFT
938   02015  1076          TAD KPL
939   02016  7440          SZA
940   02017  5226          JMP RONR
941   02020  6664  PAGN,   6664
942   02021  4771'         JMS TM1
943   02022  6674          6674
944   02023  4771'         JMS TM1
945   02024  2073          ISZ MPLFT
946   02025  5220          JMP PAGN
947   02026  7200  RONR,   CLA           /RINSE OR NOT?
948   02027  1370          TAD (AS7
949   02030  4776'         JMS POOH
950   02031  4105  RON,    JMS LISN
951   02032  1367          TAD (-261
952   02033  7450          SNA           /IS IT A 1?
953   02034  5244          JMP YRINS
954   02035  1366          TAD (-1
955   02036  7450          SNA           /IS IT A 2?
956   02037  5252          JMP NRINS
957   02040  7300          CLA CLL
958   02041  1377          TAD (AS10
959   02042  4776'         JMS POOH
960   02043  5231          JMP RON
961   02044  1032  YRINS,  TAD MNFSH
962   02045  3046          DCA MFSH
963   02046  6664          6664
964   02047  4771'         JMS TM1
965   02050  6674          6674
966   02051  5254          JMP BOSR
967   02052  7040  NRINS,  CMA
968   02053  3046          DCA MFSH
969   02054  1365  BOSR,   TAD (AS8     /BLANK OR SAMPLE RUN?
970   02055  4776'         JMS POOH
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 971   02056  4105  BOS,    JMS LISN
 972   02057  1367          TAD (-261
 973   02060  7450          SNA              /IS IT A 1?
 974   02061  5271          JMP BLRN
 975   02062  1366          TAD (-1
 976   02063  7450          SNA              /IS IT A 2?
 977   02064  5273          JMP SMRN
 978   02065  7300          CLA CLL          /IT IS NEITHER.
 979   02066  1377          TAD (AS10
 980   02067  4776'         JMS POOH
 981   02070  5256          JMP BOS
 982   02071  3027  BLRN,   DCA SOB
 983   02072  5275          JMP NFPR
 984   02073  7040  SMRN,   CMA
 985   02074  3027          DCA SOB
 986   02075  1364  NFPR,   TAD (AS9        /# OF SYRINGE FILLINGS PER RUN?
 987   02076  4776'         JMS POOH
 988   02077  4105  NSFR,   JMS LISN
 989   02100  3127          DCA OP9
 990   02101  1127          TAD OP9
 991   02102  1363          TAD (-260
 992   02103  7500          SMA
 993   02104  5311          JMP CKPP
 994   02105  7300  NWNG,   CLA CLL
 995   02106  1377          TAD (AS10
 996   02107  4776'         JMS POOH
 997   02110  5277          JMP NSFR
 998   02111  1374  CKPP,   TAD (-10
 999   02112  7500          SMA
1000   02113  5305          JMP NWNG
1001   02114  1373          TAD (10
1002   02115  3021          DCA FLS
1003   02116  1362          TAD (AS12       /# OF DATA PUSHES PER RUN?
1004   02117  4776'         JMS POOH
1005   02120  4105  DPSH,   JMS LISN
1006   02121  3130          DCA OP10
1007   02122  1130          TAD OP10
1008   02123  1367          TAD (-261
1009   02124  7450          SNA
1010   02125  5346          JMP DP1
1011   02126  1366          TAD (-1
1012   02127  7450          SNA
1013   02130  5351          JMP DP2
1014   02131  1366          TAD (-1
1015   02132  7450          SNA
1016   02133  5761'         JMP DP3
1017   02134  1366          TAD (-1
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1018   02135  7450          SNA
1019   02136  5760'         JMP DP4
1020   02137  1366          TAD (-1
1021   02140  7450          SNA
1022   02141  5757'         JMP DP5
1023   02142  7300          CLA CLL
1024   02143  1377          TAD (AS10
1025   02144  4776'         JMS POOH
1026   02145  5320          JMP DPSH
1027   02146  1366  DP1,    TAD (-1
1028   02147  3040          DCA MNGP
1029   02150  5756'         JMP BIP
1030   02151  1355  DP2,    TAD (-2
1031   02152  3040          DCA MNGP
1032   02153  5756'         JMP BIP
1033   02155  7776
1034   02156  2210
1035   02157  2206
1036   02160  2203
1037   02161  2200
1038   02162  5211
1039   02163  7520
1040   02164  5123
1041   02165  5070
1042   02166  7777
1043   02167  7517
1044   02170  5027
1045   02171  2265
1046   02172  0003
1047   02173  0010
1048   02174  7770
1049   02175  1736
1050   02176  4000
1051   02177  5151
1052          2200' PAGE
1053   02200  1377  DP3,    TAD (-3
1054   02201  3040          DCA MNGP
1055   02202  5210          JMP BIP
1056   02203  1376  DP4,    TAD (-4
1057   02204  3040          DCA MNGP
1058   02205  5210          JMP BIP
1059   02206  1375  DP5,    TAD (-5
1060   02207  3040          DCA MNGP
1061   02210  1374  BIP,    TAD (AS13
1062   02211  4773'         JMS POOH
1063   02212  4105          JMS LISN
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1064   02213  6652          6652              /ACTIVATE VALVE FLIP-FLOPS.
1065   02214  7300          CLA CLL
1066   02215  1020          TAD SLD           /INITIALIZING PARAMETERS.
1067   02216  3017          DCA DLOC
1068   02217  1021          TAD FLS
1069   02220  7041          CIA
1070   02221  3041          DCA MNFL
1071   02222  1022          TAD CLCT
1072   02223  7041          CIA
1073   02224  3042          DCA MCC
1074   02225  1024          TAD APD
1075   02226  3772'         DCA NAPD
1076   02227  1024          TAD APD
1077   02230  7041          CIA
1078   02231  3043          DCA MNAP
1079   02232  1025          TAD TID           /TID=TIME INTERVAL BETWEEN DATA
1080   02233  7041          CIA               /POINTS IN UNITS OF THE TIME
1081   02234  3044          DCA MNEP          /SPAN OF ONE DATA POINT.
1082   02235  1026          TAD NDP
1083   02236  7041          CIA
1084   02237  3045          DCA MNDP
1085   02240  7300          CLA CLL           /FLUSH THREE TIMES.
1086   02241  2046  SFSH,   ISZ MFSH
1087   02242  5244          JMP .+2
1088   02243  5327          JMP GO
1089   02244  1033          TAD MNPSH
1090   02245  3053          DCA MPSH
1091   02246  6661          6661
1092   02247  4265          JMS TM1
1093   02250  6662          6662
1094   02251  4306          JMS TM2
1095   02252  6671          6671
1096   02253  4265          JMS TM1
1097   02254  6672          6672
1098   02255  4265          JMS TM1
1099   02256  6664  FSPSH,  6664
1100   02257  4265          JMS TM1
1101   02260  6674          6674
1102   02261  4265          JMS TM1
1103   02262  2053          ISZ MPSH
1104   02263  5256          JMP FSPSH
1105   02264  5241          JMP SFSH
1106   02265  0000  TM1,    0
1107   02266  7300          CLA CLL
1108   02267  6133          CLAB
1109   02270  1034          TAD MNC1
1110   02271  3063          DCA MC1
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1111    02272   1023            TAD TKHZ
1112    02273   6132            CLOE                    /START CLOCK
1113    02274   6135            CLSA                    /CLEAR CLOCK FLAG.
1114    02275   6131    CF1,    CLSK
1115    02276   5275            JMP .-1
1116    02277   6135            CLSA
1117    02300   2063            ISZ MC1
1118    02301   5275            JMP CF1
1119    02302   7340            CLA CLL CMA
1120    02303   6130            CLZE
1121    02304   7200            CLA
1122    02305   5665            JMP I TM1
1123    02306   0000    TM2,    0
1124    02307   7300            CLA CLL
1125    02310   6133            CLAB
1126    02311   1035            TAD MNC2
1127    02312   3064            DCA MC2
1128    02313   1023            TAD TKHZ
1129    02314   6132            CLOE
1130    02315   6135            CLSA                    /CLEAR CLOCK FLAG.
1131    02316   6131    CF2,    CLSK
1132    02317   5316            JMP .-1
1133    02320   6135            CLSA
1134    02321   2064            ISZ MC2
1135    02322   5316            JMP CF2
1136    02323   7340            CLA CLL CMA
1137    02324   6130            CLZE
1138    02325   7200            CLA
1139    02326   5706            JMP I TM2
1140    02327   7300    GO,     CLA CLL
1141    02330   1041            TAD MNFL                /MNFL=MINUS # FILLINGS.
1142    02331   3052            DCA MFL
1143    02332   1033    FILL,   TAD MNPSH               /MNPSH=MINUS # OF PUSHES.
1144    02333   7001            IAC
1145    02334   3053            DCA MPSH                /MPSH=MINUS(#PUSHES-1).
1146    02335   1040            TAD MNGP
1147    02336   3054            DCA MGP
1148    02337   6661            6661                    /SET FF1.
1149    02340   4265            JMS TM1
1150    02341   6662            6662                    /SET FF2
1151    02342   4306            JMS TM2
1152    02343   6671            6671                    /CLEAR FF1
1153    02344   4265            JMS TM1
1154    02345   6672            6672                    /CLEAR FF2.
1155    02346   4265            JMS TM1
1156    02347   6664            6664                    /SET FF3.
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1157   02350  4265          JMS TM1
1158   02351  6674          6674            /DON'T TAKE DATA ON FIRST PUSH.
1159   02352  4265          JMS TM1
1160   02353  5771'         JMP PUSH
1161   02371  2400
1162   02372  2466
1163   02373  4000
1164   02374  5244
1165   02375  7773
1166   02376  7774
1167   02377  7775
1168          2400' PAGE
1169   02400  6664  PUSH,   6664            /SET FF3
1170   02401  6654          6654            /SKIP ON S.F. TRIGGER.
1171   02402  5201          JMP .-1
1172   02403  1036          TAD MTCC
1173   02404  7450          SNA
1174   02405  5221          JMP ADCVT
1175   02406  6133          CLAB            /SET CLOCK COUNTER.
1176   02407  7200          CLA
1177   02410  1037          TAD EN
1178   02411  6132          CLOE            /START CLOCK.
1179   02412  6135          CLSA            /CLEAR CLOCK FLAG.
1180   02413  6131          CLSK            /SKIP ON CLOCK FLAG.
1181   02414  5213          JMP .-1
1182   02415  7340          CLA CLL CMA
1183   02416  6130          CLZE
1184   02417  7200          CLA
1185   02420  5221          JMP ADCVT       /POST TRIGGER DELAY IS OVER.
1186   02421  3056  ADCVT,  DCA APL
1187   02422  3055          DCA APM
1188   02423  1045          TAD MNDP        /MNDP=MINUS # OF DATA POINTS.
1189   02424  3051          DCA MDP
1190   02425  1042          TAD MCC
1191   02426  6133          CLAB            /SET CLOCK COUNTER.
1192   02427  7200          CLA
1193   02430  1023          TAD TKHZ
1194   02431  6132          CLOE            /START CLOCK.
1195   02432  6135          CLSA            /CLEAR CLOCK FLAG.
1196   02433  7200          CLA
1197   02434  1043  SAP1,   TAD MNAP        /MNAP=MINUS # OF ANALOG
1198   02435  3047          DCA MAP         /POINTS PER DATA POINT.
1199   02436  1044          TAD MNEP        /MNEP=MINUS # OF
1200   02437  3050          DCA MEP         /EMPTY DATA POINTS.
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1201   02440  6131  FLG1,   CLSK
1202   02441  5240          JMP .-1
1203   02442  6135          CLSA            /CLEAR CLOCK FLAG.
1204   02443  7300          CLA CLL
1205   02444  6531          6531            /A-D CONVERSION:
1206   02445  6532          6532            /TAKING ANALOG POINTS.
1207   02446  5245          JMP .-1
1208   02447  6534          6534
1209   02450  1031          TAD XFM         /CONVERT TO POSITIVE BINARY.
1210   02451  7100          CLL
1211   02452  1056          TAD APL         /DEPOSIT ANALOG POINTS IN
1212   02453  3056          DCA APL         /TEMPORARY LOCATION.
1213   02454  7430          SZL
1214   02455  2055          ISZ APM         /CAVEAT: CAN'T HANDLE
1215   02456  7100          CLL             />4096 ANALOG POINTS
1216   02457  2047          ISZ MAP         /PER DATA POINT.
1217   02460  5240          JMP FLG1
1218   02461  7447          SWBA            /TAKE THE AVERAGE OF THE
1219   02462  1056          TAD APL         /SET OF ANALOG POINTS
1220   02463  7421          MQL             /TO GET THE VALUE OF
1221   02464  1055          TAD APM         /A DATA POINT.
1222   02465  7407          DVI
1223   02466  0000  NAPD,   0
1224   02467  7721          CLA SWP
1225   02470  3057          DCA HPT         /DEPOSIT DATA POINT TEMPORARILY.
1226   02471  3055          DCA APM
1227   02472  3056          DCA APL
1228   02473  1027          TAD SOB         /BLANK OR SAMPLE RUN?
1229   02474  7450          SNA
1230   02475  5306          JMP DBD         /BLANK-JMP TO DBD
1231   02476  1030          TAD BLNK        /SAMPLE-GET DATA POINT
1232   02477  3302          DCA DVBK        /AND DIVIDE BY BLANK.
1233   02500  1057          TAD HPT         /(EASIEST WAY TO CHECK IF
1234   02501  7407          DVI             /SAMPLE %T>BLANK %T)
1235   02502  0000  DVBK,   0
1236   02503  7430          SZL
1237   02504  5353          JMP SGB         /SAMPLE %T>BLANK %T.
1238   02505  7621          CLA MQL         /CLEAR AC & MQ-DON'T USE.
1239   02506  1057  DBD,    TAD HPT
1240   02507  6211  DSD,    CDF 10          /CHANGE TO DATA FIELD 1.
1241   02510  3417          DCA I DLOC
1242   02511  6201          CDF 00
1243   02512  2051          ISZ MDP         /ENOUGH DATA POINTS?
1244   02513  5337          JMP SDP2        /NO.
1245   02514  7040          CMA
1246   02515  6130          CLZE            /STOP CLOCK.
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1247   02516  7200          CLA
1248   02517  6674          6674              /CLEAR FF3
1249   02520  4777'         JMS TM1
1250   02521  2053          ISZ MPSH          /INCREMENT PUSH COUNTER.
1251   02522  5324          JMP .+2
1252   02523  5334          JMP MOFIL
1253   02524  2054          ISZ MCP           /MORE DATA PUSHES?
1254   02525  5200          JMP PUSH
1255   02526  6664  NDPSH,  6664
1256   02527  4777'         JMS TM1
1257   02530  6674          6674
1258   02531  4777'         JMS TM1
1259   02532  2053          ISZ MPSH          /MORE PUSHES?
1260   02533  5326          JMP NDPSH
1261   02534  2052  MOFIL,  ISZ MFL           /MORE FILLINGS?
1262   02535  5776'         JMP FILL
1263   02536  5775'         JMP ENRN          /END OF RUN.
1264   02537  2050  SDP2,   ISZ MEP          /TIME FOR NEXT DATA POINT?
1265   02540  5342          JMP SAP2          /NO-TAKE SOME EMPTY POINTS.
1266   02541  5234          JMP SAP1          /YES-GET MORE POINTS.
1267   02542  1043  SAP2,   TAD MNAP         /RESET THE # OF ANALOG
1268   02543  3047          DCA MAP           /POINTS PER DATA POINT.
1269   02544  6131  FLG2,   CLSK
1270   02545  5344          JMP .-1
1271   02546  6135          CLSA              /CLEAR CLOCK FLAG.
1272   02547  7300          CLA CLL
1273   02550  2047          ISZ MAP
1274   02551  5344          JMP FLG2
1275   02552  5337          JMP SDP2
1276   02553  7621  SGB,    CLA MQL           /CLEAR AC & MQ.
1277   02554  1030          TAD BLNK
1278   02555  5307          JMP DSD
1279   02575  2600
1280   02576  2332
1281   02577  2265
1282          2600  PAGE
1283   02600  7300  ENRN,   CLA CLL           /END OF RUN-OUTPUT DATA.
1284   02601  1045          TAD MNDP
1285   02602  3051          DCA MDP
1286   02603  1027          TAD SOB           /BLANK OR SAMPLE RUN?
1287   02604  7440          SZA
1288   02605  5251          JMP SOUT          /JMP OUTPUT SAMPLE DATA.
1289   02606  7300          CLA CLL
1290   02607  1020          TAD SLD
1291   02610  7041          CIA
1292   02611  1017          TAD DLOC
1293   02612  3060          DCA NPTS
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1294   02613  1060          TAD NPTS
1295   02614  7041          CIA
1296   02615  3061          DCA MNPTS
1297   02616  1061          TAD MNPTS
1298   02617  3062          DCA MPTS
1299   02620  3065          DCA BLKL
1300   02621  3066          DCA BLKM
1301   02622  1020          TAD SLD
1302   02623  3017          DCA DLOC
1303   02624  7100          CLL
1304   02625  6211  LOOPB,  CDF 10          /CHANGE TO DATA FIELD 1.
1305   02626  1417          TAD I DLOC      /GET BLANK DATA POINTS.
1306   02627  6201          CDF 0           /BACK TO DATA FIELD 0.
1307   02630  1065          TAD BLKL
1308   02631  3065          DCA BLKL
1309   02632  7430          SZL
1310   02633  2066          ISZ BLKM        /CAVEAT:CAN'T HANDLE >
1311   02634  7100          CLL             /4096 DATA POINTS.
1312   02635  2062          ISZ MPTS        /MORE POINTS?
1313   02636  5225          JMP LOOPB       /YES.
1314   02637  1060          TAD NPTS        /NO-CALC AVG.
1315   02640  3245          DCA DVP
1316   02641  1065          TAD BLKL
1317   02642  7421          MQL
1318   02643  1066          TAD BLKM
1319   02644  7407          DVI
1320   02645  0000  DVP,    0
1321   02646  7721          CLA SWP
1322   02647  3030          DCA BLNK
1323   02650  5777'         JMP RONR
1324   02651  7300  SOUT,   CLA CLL
1325   02652  6651          6651            /DEACTIVATE FLIP-FLOPS.
1326   02653  1376          TAD (6061       /PRINT OUT OPTIONS.
1327   02654  3100          DCA TSFA
1328   02655  1375          TAD (6066
1329   02656  3102          DCA TLSA
1330   02657  1374          TAD (AS18
1331   02660  4773'         JMS POOH
1332   02661  1115          TAD OP1
1333   02662  4325          JMS TOPN
1334   02663  1116          TAD OP2
1335   02664  4325          JMS TOPN
1336   02665  1117          TAD OP3
1337   02666  4325          JMS TOPN
1338   02667  1120          TAD OP4
1339   02670  4325          JMS TOPN
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1340   02671  1125          TAD DITD
1341   02672  7041          CIA
1342   02673  3125          DCA DITD
1343   02674  1121          TAD TD1
1344   02675  4334          JMS DOP5
1345   02676  1122          TAD TD2
1346   02677  4334          JMS DOP5
1347   02700  1123          TAD TD3
1348   02701  4334          JMS DOP5
1349   02702  1124          TAD TD4
1350   02703  4334          JMS DOP5
1351   02704  1037          TAD EN
1352   02705  1372          TAD (-5300
1353   02706  7440          SZA
1354   02707  5312          JMP DOP6
1355   02710  1371          TAD (260
1356   02711  4077          JMS TYPE
1357   02712  1370  DOP6,   TAD (254
1358   02713  4077          JMS TYPE
1359   02714  1367          TAD (240
1360   02715  4077          JMS TYPE
1361   02716  1126          TAD OP6
1362   02717  4325          JMS TOPN
1363   02720  1127          TAD OP9
1364   02721  4325          JMS TOPN
1365   02722  1130          TAD OP10
1366   02723  4325          JMS TOPN
1367   02724  5766'         JMP POOP
1368   02725  0000  TOPN,   0
1369   02726  4077          JMS TYPE
1370   02727  1370          TAD (254
1371   02730  4077          JMS TYPE
1372   02731  1367          TAD (240
1373   02732  4077          JMS TYPE
1374   02733  5725          JMP I TOPN
1375   02734  0000  DOP5,   0
1376   02735  4077          JMS TYPE
1377   02736  2125          ISZ DITD
1378   02737  5341          JMP .+2
1379   02740  5312          JMP DOP6
1380   02741  5734          JMP I DOP5
1381   02766  3056
1382   02767  0240
1383   02770  0254
1384   02771  0260
1385   02772  2500
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1386   02773  4000
1387   02774  6250
1388   02775  6066
1389   02776  6061
1390   02777  2026
1391          3000   PAGE
1392   03000  0000   STSF,   0                /USE PREVIOUS 0 & 100 %T LEVELS?
1393   03001  3076           DCA KPL
1394   03002  6521           6521             /SET MUX TO CHANNEL 0.
1395   03003  7200           CLA
1396   03004  1377           TAD (AS21
1397   03005  4776'          JMS POOH
1398   03006  4105           JMS LISN
1399   03007  1375           TAD (-261
1400   03010  7450           SNA              /IS IT A 1?
1401   03011  5221           JMP SVLVL
1402   03012  1374           TAD (-1
1403   03013  7450           SNA              /IS IT A 2?
1404   03014  5600           JMP I STSF
1405   03015  7200           CLA
1406   03016  1373           TAD (AS10
1407   03017  4776'          JMS POOH
1408   03020  5201           JMP STSF+1
1409   03021  1372   SVLVL,  TAD (177         /SAVE 0 AND 100 %T LEVELS
1410   03022  3017           DCA DLOC         /AND OFFSET PARAMETERS.
1411   03023  6211           CDF 10
1412   03024  1417           TAD I DLOC
1413   03025  6201           CDF 0
1414   03026  3771'          DCA UDAS
1415   03027  6211           CDF 10
1416   03030  1417           TAD I DLOC
1417   03031  6201           CDF 0
1418   03032  3074           DCA FCTR
1419   03033  6211           CDF 10
1420   03034  1417           TAD I DLOC
1421   03035  6201           CDF 0
1422   03036  3075           DCA FEXP
1423   03037  6211           CDF 10
1424   03040  1417           TAD I DLOC
1425   03041  6201           CDF 0
1426   03042  3770'          DCA UPLVL
1427   03043  6211           CDF 10
1428   03044  1417           TAD I DLOC
1429   03045  6201           CDF 0
1430   03046  3070           DCA ZRLVL
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1431   03047  6211           CDF 10
1432   03050  1417           TAD I DLOC
1433   03051  6201           CDF 0
1434   03052  3030           DCA BLNK
1435   03053  7040           CMA
1436   03054  3076           DCA KPL
1437   03055  5767'          JMP OPTNS
1438   03056  1366   POOP,   TAD (AS19        /PRINTOUT OFFSET PARAMETERS.
1439   03057  4776'          JMS POOH
1440   03060  1771'          TAD UDAS
1441   03061  4303           JMS TPRM
1442   03062  1074           TAD FCTR
1443   03063  4303           JMS TPRM
1444   03064  1075           TAD FEXP
1445   03065  4303           JMS TPRM
1446   03066  1770'          TAD UPLVL
1447   03067  4303           JMS TPRM
1448   03070  1070           TAD ZRLVL
1449   03071  4303           JMS TPRM
1450   03072  1030           TAD BLNK
1451   03073  4303           JMS TPRM
1452   03074  1365           TAD (215
1453   03075  4077           JMS TYPE
1454   03076  1364           TAD (212
1455   03077  4077           JMS TYPE
1456   03100  1364           TAD (212
1457   03101  4077           JMS TYPE
1458   03102  5763'          JMP CHFT
1459   03103  0000   TPRM,   0               /CNVT TO ASCII & TYPE--SUBROUTINE.
1460   03104  3346           DCA HPRM
1461   03105  1346           TAD HPRM
1462   03106  0362           AND (0007
1463   03107  1361           TAD (260
1464   03110  3347           DCA PR1
1465   03111  1346           TAD HPRM
1466   03112  7012           RTR
1467   03113  7010           RAR
1468   03114  0362           AND (0007
1469   03115  1361           TAD (260
1470   03116  3350           DCA PR2
1471   03117  1346           TAD HPRM
1472   03120  7002           BSW
1473   03121  0362           AND (0007
1474   03122  1361           TAD (260
1475   03123  3351           DCA PR3
1476   03124  1346           TAD HPRM
1477   03125  7002           BSW
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1478   03126  7012          RTR
1479   03127  7010          RAR
1480   03130  0362          AND (0007
1481   03131  1361          TAD (260
1482   03132  4077          JMS TYPE
1483   03133  1351          TAD PR3
1484   03134  4077          JMS TYPE
1485   03135  1350          TAD PR2
1486   03136  4077          JMS TYPE
1487   03137  1347          TAD PR1
1488   03140  4077          JMS TYPE
1489   03141  1360          TAD (254
1490   03142  4077          JMS TYPE
1491   03143  1357          TAD (240
1492   03144  4077          JMS TYPE
1493   03145  5703          JMP I TPRM
1494   03146  0000  HPRM,    0
1495   03147  0000  PR1,     0
1496   03150  0000  PR2,     0
1497   03151  0000  PR3,     0
1498   03157  0240
1499   03160  0254
1500   03161  0260
1501   03162  0007
1502   03163  3200
1503   03164  0212
1504   03165  0215
1505   03166  6262
1506   03167  1200
1507   03170  0706
1508   03171  0704
1509   03172  0177
1510   03173  5151
1511   03174  7777
1512   03175  7517
1513   03176  4000
1514   03177  6505
1515          3200  PAGE
1516   03200  1377  CHFT,    TAD (204
1517   03201  3017           DCA DLOC
1518   03202  1030           TAD BLNK
1519   03203  6211           CDF 10
1520   03204  3417           DCA I DLOC
1521   03205  6201           CDF 0
1522   03206  1376           TAD (6041
1523   03207  3100           DCA TSFA
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1524   03210  1375          TAD (6046
1525   03211  3102          DCA TLSA
1526   03212  1374          TAD (AS20       /SET-UP THE FILE NAME.
1527   03213  4773'         JMS POOH
1528   03214  4226  SFF,    JMS GTO
1529   03215  3301          DCA NAME
1530   03216  4226          JMS GTO
1531   03217  3302          DCA NAME+1
1532   03220  4226          JMS GTO
1533   03221  3303          DCA NAME+2
1534   03222  4105          JMS LISN
1535   03223  5256          JMP ESFF
1536   03224  0000  HCR,    0
1537   03225  0000  HDC,    0
1538   03226  0000  GTO,    0
1539   03227  4105          JMS LISN
1540   03230  4241          JMS QUE
1541   03231  0372          AND (0077
1542   03232  7002          BSW
1543   03233  3224          DCA HCR
1544   03234  4105          JMS LISN
1545   03235  4241          JMS QUE
1546   03236  0372          AND (0077
1547   03237  1224          TAD HCR
1548   03240  5626          JMP I GTO
1549   03241  0000  QUE,    0
1550   03242  3225          DCA HDC
1551   03243  1225          TAD HDC
1552   03244  1371          TAD (-277
1553   03245  7440          SZA
1554   03246  5253          JMP OKAY
1555   03247  7300  TAGN,   CLA CLL
1556   03250  1370          TAD (AS10
1557   03251  4773'         JMS POOH
1558   03252  5214          JMP SFF
1559   03253  7300  OKAY,   CLA CLL
1560   03254  1225          TAD HDC
1561   03255  5641          JMP I QUE
1562   03256  1367  ESFF,   TAD (-256
1563   03257  7440          SZA
1564   03260  5247          JMP TAGN
1565   03261  1366          TAD (1          /CHAIN TO FORTRAN.
1566   03262  6201          CDF 0
1567   03263  6212          CIF 10
1568   03264  4765          JMS I (7700
1569   03265  0002          2
```

```
1570   03266  3301  BLK,    NAME
1571   03267  0000          0
1572   03270  7402          HLT
1573   03271  7200          CLA
1574   03272  1266          TAD BLK
1575   03273  3300          DCA ARG1
1576   03274  6201          CDF 0
1577   03275  6212          CIF 10
1578   03276  4765          JMS I (7700
1579   03277  0006          6
1580   03300  0000  ARG1,   0
1581   03301  2016  NAME,   FILENAME PNF.SV
1582   03302  0600
1583   03303  0000
1584   03304  2326
1585   03305  7700
1586   03306  0001
1587   03307  7522
1588   03310  5151
1589   03311  7501
1590   03312  0077
1591   03313  4000
1592   03314  6303
1593   03315  6046
1594   03316  6041
1595   03317  0204
1596          4000          *4000
1597   04000  0000  POOH,   0              /ROUTINE TO TYPE OUT
1598   04001  3245          DCA CADR        /A CHARATER STRING
1599   04002  7100          CLL             /PACKED AS 6-BIT ASCII.
1600   04003  6046          TLS
1601   04004  1250  GCHR,   TAD ZZ1         /TAD -2
1602   04005  3246          DCA PM2
1603   04006  1645          TAD I CADR      /GET TWO CHARACTERS.
1604   04007  7002          BSW
1605   04010  0251  MSK,    AND ZZ2         /AND 0077.
1606   04011  7450          SNA             /END OF CHARACTER STRING
1607   04012  5600          JMP I POOH      /SIGNALED BY ZERO.
1608   04013  1252          TAD ZZ3         /TAD -40
1609   04014  7500          SMA
1610   04015  5230          JMP TUE
1611   04016  1253          TAD ZZ4         /PUT BACK THE 40,PLUS 300.
1612   04017  3247          DCA CRHD
1613   04020  1247          TAD CRHD
1614   04021  1254          TAD ZZ5         /CHECK FOR CRLF.
1615   04022  7450          SNA
1616   04023  5240          JMP CRLF
```

```
1617   04024  7300          CLA CLL
1618   04025  1247          TAD CRHD
1619   04026  4077          JMS TYPE
1620   04027  5232          JMP OHLF
1621   04030  1255  TUE,    TAD ZZ6          /PUT BACK THE 40, PLUS 200.
1622   04031  4077          JMS TYPE
1623   04032  2246  OHLF,   ISZ PM2
1624   04033  5236          JMP PMSK
1625   04034  2245          ISZ CADR
1626   04035  5204          JMP GCHR
1627   04036  1645  PMSK,   TAD I CADR
1628   04037  5210          JMP MSK
1629   04040  1256  CRLF,   TAD ZZ7
1630   04041  4077          JMS TYPE
1631   04042  1257          TAD ZZ8
1632   04043  4077          JMS TYPE
1633   04044  5232          JMP OHLF
1634   04045  0000  CADR,   0
1635   04046  0000  PM2,    0
1636   04047  0000  CRHD,   0
1637   04050  7776  ZZ1,    7776
1638   04051  0077  ZZ2,    0077
1639   04052  7740  ZZ3,    7740
1640   04053  0340  ZZ4,    0340
1641   04054  7441  ZZ5,    7441
1642   04055  0240  ZZ6,    0240
1643   04056  0215  ZZ7,    0215
1644   04057  0212  ZZ8,    0212
1645          4100          *4100
1646   04100  3737  AS1,    3737
1647   04101  4040          4040
1648   04102  4040          4040
1649   04103  4040          4040
1650   04104  4040          4040
1651   04105  4040          4040
1652   04106  4040          4040
1653   04107  4040          4040
1654   04110  4040          4040
1655   04111  4040          4040
1656   04112  4040          4040
1657   04113  4040          4040
1658   04114  5555          5555
1659   04115  5523          5523
1660   04116  0503          0503
```

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 616 | 750 | 754 | 758 | 762 | 766 | 900 | 950 | 971 | 988 |
| | 1005 | 1063 | 1398 | 1534 | 1539 | 1544 | | | | |
| LLFU | 318 | 321# | | | | | | | | |
| LLM | 265 | 280 | 288 | 316 | 325# | | | | | |
| LL1 | 287# | 296 | | | | | | | | |
| LL2 | 282 | 292# | | | | | | | | |
| LOL1M | 183 | 264# | 320 | | | | | | | |
| LOLVL | 184 | 279 | 284 | 293 | 311 | 312 | 328# | | | |
| LOOPB | 1304# | 1313 | | | | | | | | |
| LSR | 10# | 525 | | | | | | | | |
| LVFG | 234# | 250 | | | | | | | | |
| LVL | 222 | 244 | 245 | 254 | 261# | | | | | |
| LVM | 223 | 247 | 256 | 262# | | | | | | |
| LXDG | 269# | 304 | 307 | | | | | | | |
| MAP | 40# | 1198 | 1216 | 1268 | 1273 | | | | | |
| MCC | 35# | 1073 | 1190 | | | | | | | |
| MC1 | 52# | 1110 | 1117 | | | | | | | |
| MC2 | 53# | 1127 | 1134 | | | | | | | |
| MDP | 42# | 1189 | 1243 | 1285 | | | | | | |
| MEP | 41# | 1200 | 1264 | | | | | | | |
| MFL | 43# | 1142 | 1261 | | | | | | | |
| MFSH | 39# | 962 | 968 | 1086 | | | | | | |
| MGP | 45# | 1147 | 1253 | | | | | | | |
| MNAP | 36# | 1078 | 1197 | 1267 | | | | | | |
| MNC1 | 29# | 1109 | | | | | | | | |
| MNC2 | 30# | 1126 | | | | | | | | |
| MNDP | 38# | 1084 | 1188 | 1284 | | | | | | |
| MNEP | 37# | 1081 | 1199 | | | | | | | |
| MNFL | 34# | 1070 | 1141 | | | | | | | |
| MNFSH | 27# | 961 | | | | | | | | |
| MNGP | 33# | 1028 | 1031 | 1054 | 1057 | 1060 | 1146 | | | |
| MNPSH | 28# | 934 | 936 | 1089 | 1143 | | | | | |
| MNPTS | 50# | 1296 | 1297 | | | | | | | |
| MOFIL | 1252 | 1261# | | | | | | | | |
| MPLFT | 60# | 937 | 945 | | | | | | | |
| MPSH | 44# | 1090 | 1103 | 1145 | 1250 | 1259 | | | | |
| MPTS | 51# | 1298 | 1312 | | | | | | | |
| MRGN | 108# | 123 | 134 | 150 | | | | | | |
| MRG1 | 58# | 109 | 110 | 144 | 152 | 157 | 159 | 164 | | |
| MRG2 | 59# | 113 | 114 | 153 | 158 | 162 | 170 | | | |
| MSK | 1605# | 1628 | | | | | | | | |
| MTCC | 31# | 872 | 879 | 880 | 887 | 888 | 895 | 897 | 1172 | |
| MULM | 334 | 353 | 368 | 380 | 399# | | | | | |
| MUY | 9# | 166 | 172 | 869 | 876 | 884 | 892 | | | |
| MX1 | 746 | 844 | 847 | 852 | 859 | 870# | | | | |
| MX2 | 747 | 849 | 854 | 861 | 877# | | | | | |

# APPENDIX E

## FORTRAN PROGRAM, PNF401.FT, CALCULATES ABSORBANCE AND THE FIRST DERIVATIVE OF ABSORBANCE

This program is chained to from PNSF1.PA (Appendix D). Absorbance and the first derivative of absorbance are calculated from intensity data passed from PNSF1.PA. The absorbance data is automatically stored on a magnetic disk for future use. The calculated values can then be plotted and a link can be made to another program if desired.

```
C       PROGRAM:  PNF401.FT  6/13/76
C       PHILLIP KENT NOTZ
C       MICHIGAN STATE UNIVERSITY
C
C         THIS PROGRAM ANALYZES ABSORBANCE DATA TAKEN BY THE
C       PAL8 PROGRAN, PNSF1.PA.  THE ABSORBANCE PARAMETERS; K1,
C       K2, K3, K4, K5, KBLNK AND THE DATA ARRAY, IAR, ARE PASSED
C       FROM PNSF1.PA.
C
C       FUNCTIONS: CALCULATES ABSORBANCE VALUES, STORES ABSORBANCE
C       DATA, CALCULATES THE FIRST DERIVATIVE (SAVITSKY-GOLAY), AND
C       PLOTS ABSORBANCE AND/OR THE DERIVATIVE.
C
C         THE PROGRAN UTILIZES SUBROUTINE,PNPR1.FT TO PERFORM
C       THE PLOTTING.
C
        COMMON K1,K2,K3,K4,K5,KBLNK,IAR,T,W,SDW,Y
        DIMENSION IAR(2000),T(100),W(100),SDW(100),Y(100)
        WRITE(1,1000)
1000    FORMAT(///'PEANUTS---STORES DATA, CALCS SLOPES, & PLOTS!'//
        1'YOU MUST HAVE 100 DATA POINTS PER PUSH!'//
        2'TERMINATE ALL RESPONSES WITH "NEW LINE".'//)
C
C               INPUT INFORMATION
C
        READ(1,1010)N,T0,TINC
1010    FORMAT('NO. OF DATA PUSHES PER FILL? ',I2,/
        2'TIME AT FIRST POINT (SEC)? ',E10.0,/
        3'INCREMENT ON THE TIME AXIS (SEC)? ',E10.0)
7       MFIL=20/N
        XIP=IDP-1
        TS0=T0+XIP*TINC
        XFP=LDP-1
        TSF=T0+XFP*TINC
        WRITE(1,1030)MFIL,
1030    FORMAT('NUMBER OF FILLS USED(1-',I2)
        READ(1,1040)IFIL
1040    FORMAT(')? ',I2)
```

```
      WRITE(1,999)
999   FORMAT(//)
C
C              CALCULATE ABSORBANCE VALUES
C
S     CLA CLL CML RAR
S     DCA \I1
      XK1=K1
      XK2=K2+I1
      XK2=XK2+2048.
      XKB=KBLNK+I1
      XK5=K5+I1
      TR=XK1*XK2*2.**K3+XKB-XK5
      RR=XKB+2048.
      CPT=1.-RR/TR
      JR=IFIL*N
      READ(1,1052)FLNM
1052  FORMAT('ABSORBANCE DATA FILENAME (6 CHARACTERS)= ',A6)
      CALL OOPEN('RKB0',FLNM)
      WRITE(1,999)
      TM=T0-TINC
      DO 20 I=1,100
      SUM=0.
      SUM2=0.
      NUM=0
      DO 10 L=1,JR
      NUM=NUM+1
      IL=100*(L-1)+I
      X=IAR(IL)+I1
      A=-ALOG(CPT+(X+2048.)/TR)/2.3026
```

```
C
C       AVERAGE ABSORBANCE POINTS AND CALC STD DEV
C
        SUM2=SUM2+A*A
10      SUM=SUM+A
        XNUM=NUM
        TM=TM+TINC
        T(I)=TM
        W(I)=SUM/XNUM
        VAR=ABS(SUM2-SUM*SUM/XNUM)/(XNUM-1.)
20      SDW(I)=SQRT(VAR)
C
C               STORE ABSORBANCE DATA ON THE DISK
C
        WRITE(4,1058)(T(I),W(I),SDW(I), I=1,100)
1058    FORMAT(3E13.6)
        CALL OCLOSE
C
C       CALCULATE FIRST DERIVATIVE:  11-POINT SAVITZKY-GOLAY
C
        SM=-(W(1)*5.+W(2)*4.+W(3)*3.+W(4)*2.+W(5))
        SP=W(7)+W(8)*2.+W(9)*3.+W(10)*4.+W(11)*5.
        ST=SM+SP
        Y(6)=ST/(110.*TINC)
        DO 50 I=1,89
        SM=ST+W(I)*5.-(W(I+1)+W(I+2)+W(I+3)+W(I+4)+W(I+5))
        ST=SM-(W(I+6)+W(I+7)+W(I+8)+W(I+9)+W(I+10))+W(I+11)*5.
50      Y(I+6)=ST/(110.*TINC)
        READ(1,1060)IPL
1060    FORMAT('PLOT DATA?  1=YES, 2=NO: ',I1)
        WRITE(1,999)
        IF(IPL-1)55,55,85
55      CALL PNPR1
85      READ(1,1069)PFN
1069    FORMAT('WHICH PAL PROGRAM DO YOU WISH TO CHAIN BACK TO? ',A6)
        CALL CHAIN(PFN)
        END
```

```
      SUBROUTINE PNPR1
C
C       THIS SUBROUTINE IS CALLED BY PNF401.
C
C     FUNCTION:  SCALES THE ABSORBANCE AND/OR THE FIRST
C     DERIVATIVE DATA FOR INPUT TO THE PLOTTING PROGRAM,
C     ADDPLT, WHICH CONTAINS THE HANDLERS FOR THE ADDS
C     TERMINAL AND EXECUTES THE PLOT.
C
      COMMON K1,K2,K3,K4,K5,KBLNK,IAR,T,W,SDW,Y
      DIMENSION IAR(2000),T(100),W(100),SDW(100),Y(100)
      DIMENSION IT(100),IA(100),IPLOT(21,32),ICHAR(21)
55    READ(1,1066)IPAD
1066  FORMAT('PLOT ABSORBANCE DATA?  1=YES, 2=NO: ',I1)
      IF(IPAD-1)130,140,130
130   WRITE(1,1067)
1067  FORMAT('PLOT RATE DATA?  1=YES, 2=NO, 0=PLOT IT WITH ABS DATA')
      READ(1,1068)IPAD
1068  FORMAT('WHICH? ',I2)
      IF(IPAD-1)140,137,60
137   IPAD=2
140   READ(1,1020)IDP,LDP
1020  FORMAT('NO. OF THE FIRST POINT TO BE PLOTTED? ',I2,/
     1'NO. OF THE LAST POINT TO BE PLOTTED? ',I3)
      IF(IPAD-1)8,7,7
```

```
C
C                SCALE THE DATA: 0 TO 2047
C
7       TMIN=T(IDP)
        TMAX=T(LDP)
        TF=2047./(TMAX-TMIN)
8       I2DP=IDP+1
        IF(IPAD-1)11,9,11
9       AMAX=W(IDP)
        AMIN=W(IDP)
        DO 24  I=I2DP,LDP
        IF(W(I)-AMIN)14,16,16
14      AMIN=W(I)
16      IF(AMAX-W(I))22,24,24
22      AMAX=W(I)
24      CONTINUE
        AF=2047./(AMAX-AMIN)
        DO 30  I=IDP,LDP
30      IA(I)=(W(I)-AMIN)*AF
        GO TO 31
11      IF(IDP-6)111,112,112
111     IDP=6
        WRITE(1,1111)
1111    FORMAT('CANNOT CALCULATE RATE FOR POINTS 1-5, SO PLOT'/
        1'OF RATE DATA STARTS WITH POINT 6.')
112     IF(95-LDP)113,114,114
113     LDP=95
        WRITE(1,1113)
1113    FORMAT('CANNOT CALCULATE RATE FOR POINTS 96-100, SO PLOT'/
        1'OF RATE DATA ENDS WITH POINT 95.'//)
114     RX=ABS(Y(IDP))
        RN=ABS(Y(IDP))
        DO 19  I=I2DP,LDP
        IF(ABS(Y(I))-RN)13,15,15
13      RN=ABS(Y(I))
15      IF(RX-ABS(Y(I)))17,19,19
17      RX=ABS(Y(I))
19      CONTINUE
        RTF=2047./(RX-RN)
        DO 21  I=IDP,LDP
21      IA(I)=(ABS(Y(I))-RN)*RTF
        IF(IPAD-1)310,31,31
```

```
C
C       PASS DATA AND PLOTTING PARAMETERS TO ADDPLT
C
31      CALL PINT(IPLOT)
310     CALL AXES(IPLOT)
        READ(1,1033)IPEN
1033    FORMAT('1=PLOT WITH PEN DOWN, 2=PLOT POINTS.  WHICH? ',I1)
        IF(IPEN-1)33,33,32
32      IPEN=-1
33      CONTINUE
        DO 35 I=IDP,LDP
        IX=(T(I)-TMIN)*TF
        IY=IA(I)
        CALL PLT(IPEN,IX,IY,IPLOT)
35      CONTINUE
C
C               LABEL AXES
C
        CALL TXTRAN(ICHAR)
S       TEXT /ABS OR RATE/
S       0
        CALL PEND2(IPLOT,ICHAR)
        IF(IPAD-1)45,42,44
42      WRITE(1,1099)AMIN,AMAX,TMIN,TMAX
1099    FORMAT('ABS=',F8.5,' TO ',F8.5,4X,'TIME=',F10.4,' TO ',F10.4)
        GO TO 131
44      WRITE(1,1100)RN,RX,TMIN,TMAX
1100    FORMAT('/RATE/=',E11.4,' TO ',E11.4,4X,'TIME=',F10.4,' TO ',F10.4)
        GO TO 131
45      WRITE(1,1101)AMIN,AMAX,RN,RX
1101    FORMAT('ABS=',F8.5,' TO ',F8.5,4X,'/RATE/=',E11.4,' TO ',E11.4)
        WRITE(1,1102)TMIN,TMAX
1102    FORMAT(12X,'TIME=',F10.4,' TO ',F10.4)
131     IF(IPAD-1)60,130,60
60      READ(1,1400)IMPL
1400    FORMAT('MORE PLOTS WITH THIS DATA? 1=YES, 2=NO: ',I1)
        IF(IMPL-1)55,55,85
85      RETURN
        END
```

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