AUTOMATED STOPPED-FLOW INSTRUMENTATION AND FAST REACTION RATE STUDIES OF THE FORMATION OF 12-MOLYBDOPHOSPHORIC ACID

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THESIS

This is to certify that the

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presented by

Paul Michael Beckwith

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Major professor

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ABSTRACT

AUTOMATED STOPPED-FLOW INSTRUMENTATION AND FAST REACTION RATE STUDIES OF THE FORMATION OF 12-MOLYBDOPHOSPHORIC ACID

By

Paul M. Beckwith

The development of an automated stopped-flow spectrophotometer has made possible the utilization of fast reactions for reaction rate methods of analysis. The stopped-flow system features a vertical flow system to minimize problems with air bubbles, pneumatically actuated valves for directing the liquid flow and dispelling waste solutions, and a spring loaded stopping syringe. The entire operating cycle of the system is controlled by a digital sequencing system or by manual switches. The dead time of the flow system was determined to be 5 ± 1 msec. Mixing was also found to be 99% complete approximately 5 msec after stopping the flow. Reaction rate determinations of Fe(III) with the Fe(III)-SCN^- reaction and of phosphate with the formation reactions of 12-molybdophosphoric acid are used to evaluate the performance of the instrument. Approximately 1000 phosphate samples can be analyzed per hour with the stopped-flow system.

The development of new reaction rate methods of analysis should be based on a thorough knowledge of the kinetics and equilibria involved in the analytical reaction. The kinetics of the formation of 12-molybdophosphoric acid (12-MPA) in perchloric acid and sulfuric acid solutions has been studied by stopped-flow spectrophotometry. Mechanisms are proposed from the reaction rate data which involve an initial reaction between phosphate ion and Mo(VI) followed by polymerization to form 12-MPA. The rate law has been derived and is in agreement with the experimentally observed rates, and rate constants have been evaluated for the measurable steps. The results are compared to those of previous studies on the kinetics and equilibria involved in the formation of 12-MPA. The results also provide sound experimental evidence on which new phosphate analyses may be based.

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I. INTRODUCTION

A. Reaction Rate Methods of Analysis

Reaction rate methods of analysis have become increasingly popular in recent years. Their application to analytical problems in several areas of chemistry has been the subject of books (1,2) and numerous review articles (3-10). Rate methods utilize the kinetics of reactions rather than reaction stoichiometries to provide analytical information.

There are basically two types of analyses based on reaction kinetics. Reaction conditions are usually chosen so that the reaction is either first order or pseudo-first order for the species of interest. For a reaction of this type, the rate of disappearance of a reactant, A, with time is

$$-\frac{d[A]}{dt} = k_A[A] \tag{1}$$

where k_A is the first order or pseudo-first order rate constant. Integrating this equation yields a relationship between the concentration of A at any time, t, and the initial concentration of A, $[A]_o$, where $[A]_o$ is the desired result of a kinetic measurement.

$$[A]_t = [A]_o \exp(-k_A t) \tag{2}$$

If equation (2) is substituted into equation (1), the reaction rate is expressed in terms of $[A]_o$.

$$-\frac{d[A]_t}{dt} = k_A[A]_o \exp(-k_A t) \qquad (3)$$

Equation (2) forms the basis of one type of kinetic method of analysis. By making a measurement of the concentration of A at any time, t, the initial concentration, $[A]_o$, can be determined.

The other type of kinetic method, based on equation (3), is more correctly called a reaction rate method because it involves a measurement of the change in concentration of A, ΔA, which occurs in a given time interval, $\Delta t = t_2 - t_1$. If the measurements of ΔA are performed during the initial portion of the reaction, the procedure is termed an initial reaction rate method of analysis. In this case the exponential term in equation (3) is approximately unity. Conditions for which this approximation is valid have been discussed by Ingle and Crouch (11). In the parts of this thesis which follow, a reaction rate method will be considered to be based upon actual rate measurement (Equation (3)).

One advantage of kinetic methods based on initial reaction rates is that measurements can be made in a small fraction of the time required for the reaction to reach equilibrium. Reaction rate measurements may be obtained within a few minutes after initiation of the reaction, even if the reaction has a half life of a few hours. The time necessary for an equilibrium method based on the same reaction would be prohibitively long for routine analytical procedures.

Because reaction rate measurements are usually obtained during the initial stages of the reaction, very complicated reactions, reactions with unfavorable equilibrium constants, or nonstoichiometric reactions can often be employed. Although the equilibria for these reactions may be complicated, the initial reaction rates are often straightforward and can be used for obtaining quantitative analytical information.

Another advantage of rate methods is that they are often more specific than the corresponding equilibrium based methods. Specificity can result in additional savings of time since separations can often be avoided. In addition, simultaneous analysis of complex mixtures by differential reaction rate measurements can often be accomplished. If a sample contains two or more substances which react with a common reagent, reaction rate differences as well as thermodynamic differences can be exploited for analysis. By measuring the rate of the reaction when the species of interest is the major contributor, specificity can be achieved. If the reaction were allowed to reach equilibrium, other substances which react with the reagents would interfere with the analysis for the species of interest. Multicomponent analyses can be easily performed by making several rate measurements during time periods when different species are reacting (1).

One of the most important advantages of kinetic methods of analysis is that they involve relative measurements. For a first-order or pseudo-first-order reaction, the quantity of interest in a kinetic method is the change in the value of some parameter (proportional to the concentration of a reactant or product) with time. The absolute value of that parameter need not be determined accurately. Therefore, the rate methods may afford freedom from those interferences which contribute to the absolute value of the parameter but do not enter into the reaction and, hence, do not contribute to the rate of change of the parameter. For the specific case of spectrophotometric measurements, the absolute value of the absorbance of a solution at the analytical wavelength depends on factors such as the presence of impurities which absorb radiation at that wavelength, turbidity in the solution, and cell imperfections. In a kinetic method, these factors do not interfere if they do not change as the reaction proceeds (12).

Kinetic methods of analysis are also subject to several limitations. One is imposed by the rate of the reaction. In order for a reaction to be analytically useful, it must occur at a measurable rate. To date the fastest reactions which have been used for analyses have half lives of several milliseconds (13,14). The use of reactions with half lives of more than a few hours is clearly undesirable.

Since initial rate methods depend on the precise measurement of the reaction rate, a second limitation is that experimental conditions must be carefully controlled. The rate of a reaction is dependent on factors such as pH, ionic strength, and temperature. If these factors are not carefully controlled, reliable results will not be obtained. These factors are often not as important in stoichiometric methods.

Another limitation of rate methods is that lower signal-to-noise ratios are obtained than with equilibrium based analyses since only a small portion of the total available signal is measured. In many cases only very small changes in signal in a given measurement time are monitored. Thus, the detection system must have very high sensitivity.

In summary, it should be pointed out that for selected reactions the advantages of reaction rate measurements can far outweigh the limitations of control of reaction conditions and lower signal-to-noise ratios. In addition, new improvements in instrumental detection systems and the introduction of computerized instrumentation are rapidly minimizing the limitations of rate methods.

B. Fast Reaction Rate Methods of Analysis

Reaction rate methods have been used in analysis for several years, and new applications are being published frequently. Most of the existing methods utilize slow reactions in which the reaction can be studied by using conventional mixing methods and standard instrumentation. For most automated analyses in laboratories where large numbers of samples are involved, it is desirable to keep measurement times relatively short.

Recently, reports of reaction rate analyses based on rapid reactions have been published (13,14). To measure rates of reactions with half lives from a few milliseconds to approximately ten seconds, it is necessary to use rapid mixing and observation techniques. A very useful method is the stopped-flow method which allows observation of the course of a reaction within a few milliseconds after initiation of the reaction. Commercial instrumentation has recently become available for stopped-flow methods. However, these instruments are manually operated and are unattractive for routine analyses, where large numbers of samples are involved. With automation of the sample handling steps, however, the stopped-flow method is well adapted to analytical procedures because reliable data can be obtained very rapidly.

Because of the potential applications of fast reaction rate methods in analytical chemistry, this present work was undertaken. In order to overcome the limitations of commercial stopped-flow mixing systems, an automated stopped-flow spectrophotometer was designed and evaluated.

The automated stopped-flow spectrophotometer features a vertical flow system to minimize problems with air bubbles, pneumatically actuated valves for directing the liquid flow and dispelling waste solutions, and a spring-loaded stopping syringe. The entire operating cycle of the system is controlled by a digital sequencing system or by manual switches. The dead time of the flow system was determined to be 5 ± 1 msec. Mixing was found to be 99% complete within 5 msec after stopping the flow.

Some initial work has also been done on the feasibility of placing the entire stopped-flow system under control of a small digital computer. Thus the sample handling steps as well as the data acquisition and data treatment would be under program control.

Analytical procedures which utilize fast reactions should be based upon a thorough knowledge of the equilibria and kinetics of the primary reactions and potential interfering reactions. Having such thermodynamic and rate data, the analytical chemist can design analytical procedures that are rapid, accurate, and selective. The formation of 12-molybdophosphoric acid (12-MPA) from phosphate and molybdate in strong acid solutions is the basis for the vast majority of phosphate analyses (15). Spectrophotometric measurements of the amount of 12-MPA produced or the amount of heteropoly blue formed from reduction of 12-MPA are utilized for phosphate determinations in clinical samples, soils, polluted water, steels, and many other matrices. These methods are often based upon empirically derived procedures and suffer from many interferences. Silicate, arsenate, and germanium all form similar heteropoly acids and heteropoly blues which can interfere with the analysis of phosphate.

Because fast reaction rate measurements based upon the rapid formation of 12-MPA appeared to be highly attractive for rapid and selective phosphate analysis, a study of the mechanism of formation of 12-MPA in strong acid solutions was undertaken. The kinetics of 12-MPA formation was studied in perchloric and sulfuric acid solutions under conditions used in analysis. Important insight into the mechanism of formation of 12-MPA and into the influence of chemical parameters upon the rate was gained through this study. The reaction was found to be first order in phosphate in the two different acids. However, the molybdate and acid dependencies are quite complex and different in the two solutions. Rate constants for the initial step in the reaction were obtained in both media and compared to those previously obtained in nitric acid solution. In addition, rate data have been correlated with prior studies of the equilibria of 12-MPA formation in these strong acid solutions (16). Analytical implications of both the rate data and the thermodynamic information are discussed.

II. HISTORICAL

A. Techniques for the Study of Fast Reactions in Solution

1. Introduction

The study of fast reactions in solution first began in 1923 with the pioneering work of Hartridge and Roughton (17-19), which has subsequently led to the development of many fast reaction techniques (20-22). Prior to the introduction of the continuous flow method, the study of kinetics was limited to reactions with half lives of several minutes. Rate studies were performed with manual mixing methods and slow detection systems. The continuous flow technique made it possible to investigate the kinetics and mechanisms of many reactions which were previously considered instantaneous. A general rule for reactions initiated by mixing reagents is that the time taken for mixing and observation should be shorter than the half life of the reaction.

The term "fast reaction" is relative and imprecise, but the concept is important. In one sense, fast reaction can be taken to mean that the half life of the reaction is short relative to the time needed for mixing and observation by conventional means. Based on this definition, a reaction having a half life of one second or less under normal conditions is certainly to be considered fast. However, the rate of this same reaction can often be drastically reduced by conducting the reaction at lower temperatures or by using lower concentrations. Thus a better definition of a fast reaction is a reaction that is too fast for conventional mixing methods if conducted at ordinary temperatures and concentrations.

A more quantitative idea of the meaning of fast reactions can be gained from a consideration of reaction half lives (24). When conventional mixing methods are employed, rate measurements cannot be made accurately on reactions with half lives much less than 10 seconds. Under ideal circumstances this range may be extended to one second. This corresponds to a first-order rate constant of 1.0 sec^{-1}. Several of the fast reaction techniques are capable of measuring half lives down to 10^{-7} seconds, and this corresponds to a first-order rate constant of 10^{7} sec^{-1}. Thus, a reaction may be considered fast if it has a first-order rate constant greater than 0.1 or 1.0 sec^{-1}.

Experimental studies of the kinetics of fast reactions involve several difficulties which include the adjustment of parameters to make the time necessary for mixing and observation shorter than the half life of the reaction. Several types of detection systems are available that require only very short measurement times; hence the limitation is reduced to the time for mixing the reactants and the rate of the chemical reaction. Mixing efficiencies have been vastly improved with the introduction of jet mixers. Complete mixing can now be readily attained in less than one millisecond (23). In addition to mixing efficiency, practical limitations on flow velocities and stopping time restrict techniques which involve physical mixing of reactants to the study of reactions with half lives of at least a few milliseconds.

In addition to the mixing techniques mentioned previously, other fast reaction methods involve systems that are initially at equilibrium (24-26). In these methods, mixing time is not a limitation because the reaction is perturbed by a rapid change in some parameter upon which the equilibrium depends. Changes in temperature, pressure, electric field, and concentration are common for perturbation of equilibria. Therefore, faster reactions can be studied by these techniques.

Since the introduction of the flow methods by Hartridge and Roughton in 1923, numerous rapid measurement procedures have been reported. At a 1954 meeting of the Faraday Society, these techniques were first discussed and found to encompass a wide variety of measurement principles which were applied to a wide range of reaction rates (21). Many of these techniques, however, lacked versatility and were encumbered with several experimental problems; therefore these techniques were not immediately popular for the study of chemical systems. Much research in recent years has been involved with the elimination of these problems and the development of more versatility and more sophisticated instrumentation for many of these methods. Noyes has classified rapid reaction methods into mixing methods, sprung, periodic, and steady state methods (27). The last three are aptly termed non-mixing methods.

2. Mixing Methods

Mixing methods involve physical contact between reagents. Since no previously established equilibrium is disturbed, these methods can be applied to both reversible and irreversible chemical systems. This category of rapid reaction methods includes the capacity flow method, thermal methods, the quenching method, and flow methods. Each of these methods will be discussed in this section.

a. Capacity Flow Method. The capacity flow method has been used for the study of reactions with half lives in the range of 1-4000 seconds. The technique requires simple instrumentation and is versatile because it is applicable to both slow and moderately fast reactions. Reagents are continually added to a reaction cell with adequate stirring, and the subsequent outflow occurs at a known uniform rate. A constant and uniform steady state concentration is rapidly attained inside the reaction chamber. Rate constants can be calculated from the initial concentrations of the reagents, the concentration of the effluent, the volume of the reaction chamber, and the rate of flow. The accuracy of the rate constants is dependent on the uniformity of the flow rate. Accuracies of 1% have been reported (28). The volumes required, which depends on the size of the reaction cell, the stirring efficiency, and the flow rate, vary from a few milliliters to several hundred milliliters. Applications of the capacity flow technique include the isolation and study of transient intermediates (29), the hydrolysis of esters (30), the bromination of acetone (28), and the iodination of 2,4-dichlorophenol (31).

b. Thermal Methods. Thermal methods can be used to study reactions which have a minimum half life of about 0.2 second. The reaction is conducted under adiabatic and under nonadiabatic conditions. Under adiabatic conditions, the temperature attains a value, T_o, inside an insulated reaction cell. Under nonadiabatic conditions, exothermic systems are allowed to lose heat to the surroundings at a carefully controlled rate, which results in a maximum in the curve of temperature versus time. Endothermic systems show a minimum in the temperature-time curve. The rate constants for the reactions are qualitatively related to the ratio of the temperature maximum or minimum, T_m, to the steady state value, T_o.

One serious experimental limitation of thermal methods is the accurate measurement of temperature. The measured temperature changes must be accurate to 0.01°C, and the response of the temperature sensor must be rapid. Recently small, stable, and fast response temperature sensors have become readily available. Thermocouples with response times of approximately 50 microseconds are obtainable (32). A further disadvantage is that the chemical system being studied must be of known order if rate constants are to be evaluated. Despite these limitations, however, thermal methods involve simple instrumentation and experimental procedures. Some applications have been in the study of hydrolysis of esters, acid anhydrides, and acid chlorides, the hydration of aldehydes, and neutralization of nitroparaffins (24).

c. Quenching Method. The quenching method, or the chemical method, makes use of a multiple mixing system (24). The first mixer is used to mix the reactants, and the reaction then proceeds for a short time. This is usually accomplished by allowing the solution to flow through a tube. The reaction is then stopped rapidly by mixing the reaction mixture with a quenching solution in the second mixer. The reaction time is calculated from the volume between the two mixers and the rate of flow of the solutions from one mixer to the other. Results with a precision of $\pm$ 7% have been reported for a reaction with a half life of 0.4 second (33).

Although the quenching method requires simple and inexpensive instrumentation, it does have several limitations. The method requires a minimum of about 20 milliliters of reagents. Observation may be made at leisure, but an absolute measurement is needed rather than a relative measurement. One great limitation is that rapid quenching reactions are rare. In addition, reactions must have half lives greater than 0.01 second. Applications of quenching methods include isotope exchange reactions involving electron transfer (34), halogenation of hydrogen peroxide (35,36), and solvent exchange reactions (37).

d. Flow Methods. Flow methods can be divided into three categories: the continuous flow; the accelerated flow; and the stopped-flow method. The regenerative flow method is a special variation of the stopped-flow method for applications which involve solutions of widely different viscosities.

Flow methods all utilize initially separated reactants which flow in two or more different streams. The reactants are mixed rapidly in a mixing chamber. For the continuous flow method,observation can be made at leisure. The accelerated and stopped-flow methods require rapid observation systems.

In the continuous flow method (17,38), the two reactants flow at the rate of several meters per second through a mixing chamber into a cylindrical tube. The initial observation point is a short distance from the mixing chamber. To follow the progress of the reaction, several observations must be made at various points along the cylindrical tube. The reaction time for each point is calculated from the flow velocity and the distance from the mixing chamber. The flow of the solutions must be turbulent in order to assure good mixing and to maintain uniform composition in the reaction tube.

The fastest reaction that can be studied by the continuous flow method is limited by the efficiency of the mixer. Because the observation can be made near the mixing chamber, reactions with half lives on the order of 1 msec can be studied (17,39). The slowest reactions accessible to the method are determined by the minimum velocity required to maintain turbulent flow.

The experimental apparatus for the continuous flow method is relatively simple compared to that of the other flow methods. Several different types of detection methods have been reported. Roughton and coworkers (40-43) employed thermal detection to the study of reactions with half lives of 2 msec and obtained 7% reproducibility for the rate constants. Dalziel (44-46) and Davis (47) have adapted Beckman spectrophotometers for spectrophotometric detection and were able to follow reactions with half lives of 5 msec. Rate constants were measured with 2-3% reproducibility. Reactions with half lives from 10 msec to a few seconds have been successfully studied by Pearson and coworkers who used conductivity measurements (48).

There are some experimental limitations for the continuous flow method. A major limitation is the large volume of reagents usually necessary. Solution requirements, which depend on how fast the observation is made, range from a few milliliters (39) to a few liters (17). The solutions must be flowing at a high flow velocity for a time which is sufficiently long for the observation to be made. In general, the continuous flow method is applied to chemical systems where high concentrations and large volumes of reactants are available. A second problem with the continuous flow method is that in order to record the entire reaction curve, measurements must be taken at several points along the reaction tube since each point corresponds to a different reaction time. In spite of these limitations, the continuous flow method has been successfully employed in studies of enzyme systems (49), of hemoglobin and related reactions (17,44-46), of proton transfer and of oxidation-reduction reactions (50-55).

The accelerated flow method (43,56-59) is also based on rapid mixing of solutions which initially flow in separate streams. In this method, however, the flow velocity is continuously varied, while rapid observation is made at a point close to the mixing chamber. Solution requirements are minimal (about 0.1 ml) since the observation point is only a short distance from the mixer. The accelerated flow method is thus applicable to the study of chemical systems where concentrations and amounts of reagents are limited. Enzymatic reactions often involve expensive reagents which are available in limited amounts. Chance (56-58) originally developed the accelerated flow method for the study of enzyme reactions. Reactions with half lives of 0.3 msec have been the fastest reactions studied by this technique. The third type of flow method, the stopped-flow method, will be discussed separately in Section B of this chapter.

The designs of flow systems for the continuous flow, accelerated flow and stopped-flow methods readily allow equal volumes of reactants of roughly the same viscosities to be mixed. To obtain sufficient volume for this type of mixing can be a difficult matter as in the case of some enzymes, where isolation of materials of reasonable purity is a major problem. Dilution of these materials can create further problems. The regenerative flow apparatus designed by Chance (60,61) is a modification which allows mixing of unequal volumes of reagents. An 18-jet, 2-stage mixing chamber is employed, which provides a 100:1 mixing volume ratio of reagents. The system can be operated in either of two modes. In the continuous mode, reagents flow into the mixing chamber and then into the observation chamber where the reaction mixture is retained long enough for complete reaction. In the intermittent mode, reagents are delivered by batch to the mixer and observation tube. The materials that flow from the observation tube are collected in a syringe and are either pumped back continuously or are collected and driven back to the delivery syringe.

Regenerative flow principles have been used in conjunction with spectrophotometric detection systems (60,61) for studies of labile enzyme intermediates. Brill (62,63) employed this technique with a Rankine balance for the detection of volume magnetic susceptibility of primary enzyme substrate intermediates. An apparatus used for electron spin resonance measurements has been reported for mixing 100:1 volume ratios of enzyme to substrate (64).

The baffle method (65) can also be classified as a flow method since it involves mixing two reagents which are initially flowing in separate streams. The two reactants are initially separated by a baffle which is suddenly displaced from its position. Mixing proceeds and is 90% complete within about 15 msec. Progress of the reaction is followed with a suitable detection system. Reactions with half lives on the order of 100 milliseconds can be studied by the baffle method.

3. Relaxation Methods

For the study of reactions with half lives shorter than a few milliseconds, nonmixing or relaxation methods can sometimes be employed. In these methods, the reactants are mixed and allowed to equilibrate. The equilibrium is then suddenly perturbed, and the system readjusts to the same or a new state of equilibrium through a process known as relaxation. The reaction is studied during the relaxation time, the time between equilibrium states. The relaxation time is related to the forward and reverse rate constants for the reaction. The displacement of equilibrium can be accomplished by small alterations of pressure (66-68), temperature (66-68), or electric field (66,68,69). Relaxation techniques are limited to reversible chemical reactions.

The "sprung" methods are those in which the nonequilibrium state is produced by a stepwise perturbation of one of the experimental parameters. Periodic methods obviously involve periodic disturbances on the system. Use of relaxation methods allows the measurement of first order rate constants of up to 10^{12} liter mole^{-1} minute^{-1} or half lives of 10^{-9} second. These values are typical for fast ionic reactions.

For even faster reactions, the equilibrium may be continuously disturbed, which causes the production of a steady state different from the original equilibrium (24). This method has limited application due to difficulties in producing efficient and uniform continuous perturbations.

B. The Stopped-Flow Method

1. Introduction

The stopped-flow method has become an increasingly popular technique for fundamental studies of rapid reactions and is now the most widely used of the flow methods because the simple instrumentation and rapid mixing features of the continuous flow method are incorporated with the small reagent volumes of the accelerated flow method. In addition to the applications in fundamental studies, the stopped-flow technique has recently been shown to have considerable potential for quantitative analytical determinations based upon rapid reaction rate measurements (13,14). One of the more attractive features of the method is the extremely short analysis time which can be achieved using moderately fast reactions.

The stopped-flow method involves forcing the two reactants through the mixing chamber at high flow velocity into an observation cell. The flow of the solution is abruptly stopped, after which measurements are made rapidly on the still mixture. The more sudden the stopping of the flow, the faster the reaction that can be observed. The fastest reaction that can be observed is limited by the time necessary for physical mixing, which is on the order of a few milliseconds. To insure that complete mixing has occurred, the observation cell is placed a short distance from the mixing chamber. The efficiency of a stopped-flow mixing system is dependent on the flow velocity, mixer design, and the distance between the mixer and the observation cell.

2. Advantages of Stopped-Flow Methods

There are several advantages of the stopped-flow method over the continuous and accelerated flow methods. The most obvious improvement over the continuous flow method is the decreased volume of reagents required. The continuous flow method requires from several milliliters to a few liters of solution, whereas most stopped-flow and accelerated flow procedures involve less than one milliliter of each solution.

The slowest reaction time accessible to the continuous flow method is limited by volume requirements and the minimum velocity for turbulent flow. This sets the practical upper limit in the region of 100 msec for the half life of the reaction. The lower limit is determined by the efficiency of mixing. With the accelerated flow technique, the practical limits are 1-10 msec. The range is determined by the requirement that rapid observation must be made while the solution flow velocity is varied. In the stopped-flow procedure, however, the time range extends from about one millisecond to several minutes. The lower limit is determined by mixing efficiency, and the upper limit, if any, depends on the stability of the detection system.

Another advantage of the stopped-flow method is that the entire reaction curve is easily obtained from a single observation. The complete reaction can be observed for a single volume element of solution. In order to obtain the complete reaction curve from a continuous flow experiment, observations at several different points along the reaction tube must be made.

The continuous flow method is also extremely sensitive to solution inhomogeneities within the reaction tube, particularly if spectrophotometric detection is employed. In stopped-flow experiments this is not so large a problem because there is usually sufficient time between stopping the flow and the observation to allow the solution to become homogeneous.

3. General Considerations in Stopped-Flow Instruments

The first stopped-flow mixing system was reported by Chance in 1940 (56-58). Since that time several reports have appeared describing improvements in the basic components of that instrument. Several new stopped-flow systems have been designed. A general block diagram of a stopped-flow mixing system is shown in Figure 1. The basic apparatus consists of a reagent delivery system, drive mechanisms, mixing chamber, observation cell, stopping device and a suitable detection system.

a. Reagent Delivery System. The reagent delivery system consists of the reagent reservoirs, reagent syringes, and valves of some type. The reagent reservoirs may be bottles of stock solutions, large syringes, or microbeakers in a sample turntable. The reagent syringes vary in volume from one milliliter (60) to fifty milliliters (70). The most convenient device to connect the reagent syringes with either the reagent reservoirs or with the mixing chamber is a three-way stopcock. Several instruments have made use of manually operated stopcocks (56,70,71). Gibson (72) has used a more elaborate and laborious valve system. Four manually operated valves were involved in the design. Two valves connected the reagent syringes with the reservoirs, and the other two connected the reagent syringes to the mixing chamber. Javier and coworkers employed a motor-driven double 3-way stopcock (14). This device is more adaptable to automation because both reagents are handled with a single operation.

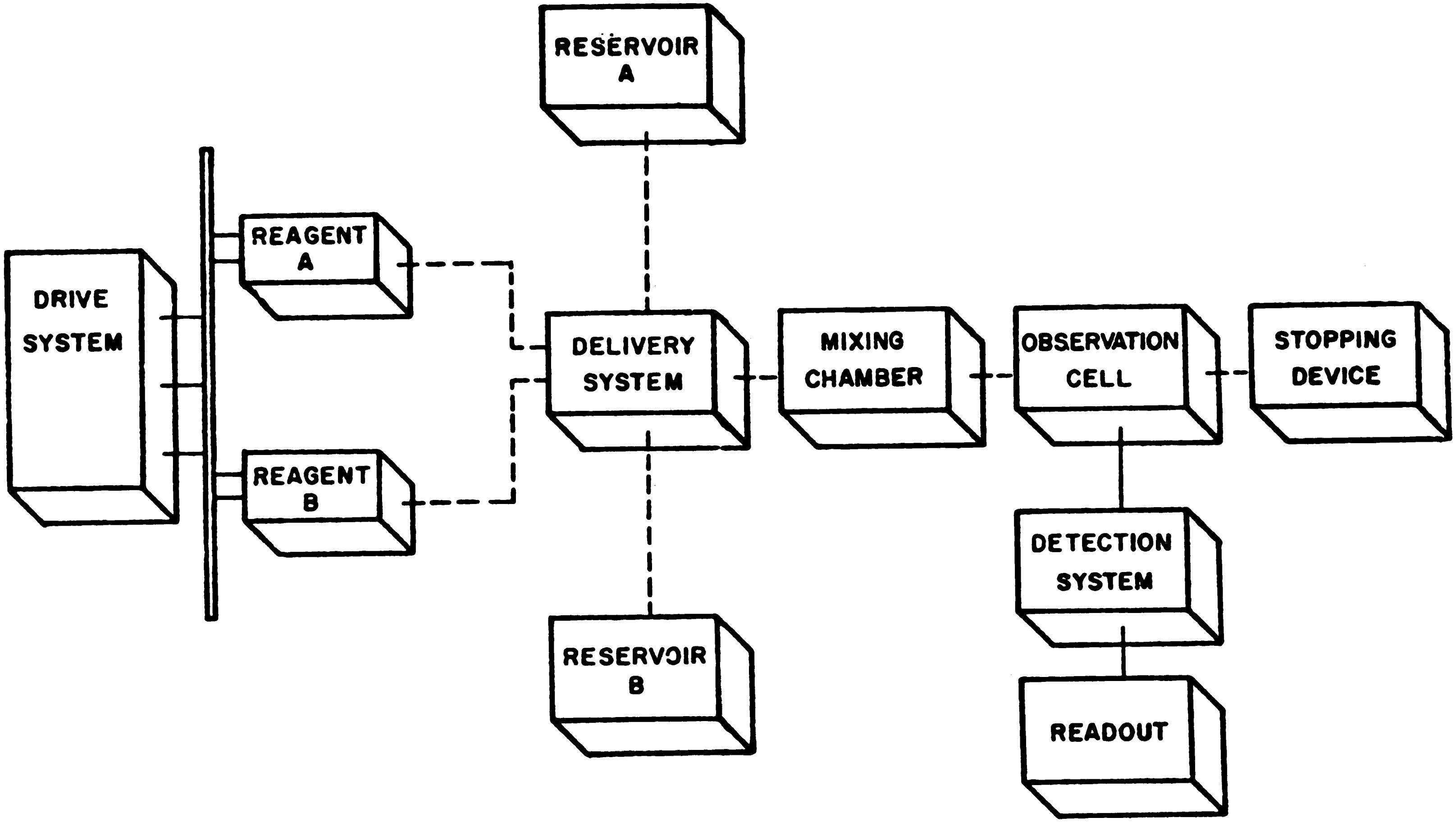


Figure 1. General block diagram of a stopped-flow mixing system.

b. Drive Mechanism. The purpose of the drive mechanism is to produce a high solution flow rate to force the reagents rapidly through the mixing chamber. Syringe plungers can be driven by compressed gases at high pressures (45,73),manual force (56-59), hydraulic cylinders (74,75), or electric motors (50-55,76). Many of these methods are subject to experimental problems. Compressed gas drive involves mechanical problems such as maintaining constant pressure, opening gauges simultaneously, and synchronizing with the stopping device. However, variable flow rates are easily accessible. The chief problem with manual and hydraulic systems is lack of reproducibility in initiating flow. Pressure exerted on reagent syringes depends on the speed and the strength of the actuating force. Another problem with these systems is that mechanical vibrations may be introduced, which may cause erroneous rate measurements. Electric motor drives eliminate many of the mechanical problems associated with the other drive systems but introduce some additional problems. One problem is obtaining a motor capable of producing high flow rates with small solution volumes. Vibration and electronic noise are also relatively serious drawbacks. Another problem is that a motor does not start or stop instantaneously; hence, the flow rate is not constant.

c. Mixing Chamber. The mixing chamber must be designed in such a way that the reactants are 99% mixed within the chamber itself. Mixers have been constructed from plastic (88,70,77), Teflon (78), or stainless steel (14,73), Pyrex (79,80), and quartz (81). The choice of mixer material depends upon the nature of the chemical systems to be studied, the availability of materials, and the skill of the machinist. Teflon and nylon exhibit good chemical and physical properties, such as corrosion resistance, heat exchange, and photochemical sensitivity. Most stainless steels are attacked by chlorides and strong acids, but special stainless steels, such as AISI 316, are exceptionally resistant to chemicals of this type. Glass mixing chambers have been used, but are very difficult to construct.

Most of the mixing chambers which have been designed for stopped-flow systems employ tangentially offset jets. This design produces high mixing efficiency by causing rotary motion of the liquid. In his early work with flow systems, Roughton made use of a glass T-joint with a 1.5 millimeter bore (79). Berger (73) has made use of the turbulent wake behind a sphere as a mixing mechanism instead of jets. One mechanism for improving mixing efficiency is to cascade mixing chambers. By increasing the number of mixing chambers, however, the range of reaction times accessible to the method is decreased. There are several considerations which must be taken into account in the design of efficient mixers. These considerations will be discussed in detail in the experimental section of this thesis.

d. Stopping Device. The function of the stopping device is to arrest the flow of the solutions in the shortest possible time. The rate at which the flow is stopped is one of the factors that determines the lower limit of reaction times which can be studied by stopped-flow. The most common, and simplest, stopping device is a syringe whose piston comes to rest against a fixed stopping block. Sturtevant (82) found tapered pins more efficient. The tapered pin fits into a tapered hole in a metal plate. Berger and coworkers (83) developed a high speed stop valve using pneumatic triggers.

e. Observation Cell. The observation cell is commonly a cylinder of small volume. Rectangular cells are not entirely suitable especially for continuous and accelerated flow systems because of the uncertainty in the flow characteristics. In the stopped-flow method, the flow is sufficiently turbulent for adequate mixing, even in the corners of a rectangular tube, but they have not become popular for stopped-flow systems. The volume of the observation cell should be small for minimum system dead volume.

4. Complete Stopped-Flow Mixing Systems

In this section some of the most recent advances in complete stopped-flow systems are discussed. Several of the units are now available from commercial instrument manufacturers. The design features, limitations, and advantages of each of the systems are pointed out to put into perspective the state-of-the-art systems which were available prior to this present work.

a. Apparatus of Chance. The flow system of Chance's apparatus (56-58) was constructed completely of glass. The mixing chamber was made of glass capillary tubes which formed a Y-tube going into the observation cell. The other side of the mixer was attached to a pair of one milliliter tuberculin syringes. The flow system was mounted vertically in a thermostatted housing, which attached to the monochromator. The drive system consisted of a pushing block taped to a pulley mounted on the shift of the potentiometer for the flow velocity measurement circuit. Stopping was provided by the friction drag, which was provided by a slider and track and a spring mounted between the pulley and the frame. The detection system was a spectrophotometer in which light intensity was controlled by means of a compensating photocell. Results were recorded with an oscillograph. The overall performance of this apparatus was very good. The stopping efficiency of this system was relatively low compared to later systems.

b. Design of Gibson and Milnes. Another stopped-flow mixing system was reported by Gibson and Milnes (72) in 1964. An improved version of this system is now available commercially from Durrum Instrument Corporation, Palo Alto, California. One of these systems was used extensively in a portion of this research; therefore, a detailed description of this system will be reported in a later chapter.

c. High Speed Stopped-Flow System. The high speed stopped-flow apparatus described by Berger and coworkers (83) makes use of both a high speed and a low speed mode of operation. A commercial instrument based upon this design is available from American Instrument Company, Silver Spring, Maryland. The system is vertical with the observation cell and stop valve at the top. The reagent syringes are concentric and are filled from reservoirs by means of a separate pneumatic system. This concentric arrangement reduces the mechanical forces on the syringe walls and promotes thermal exchange between the reactants. Pneumatic triggers are employed to start the flow rapidly and to stop the flow. A piston, contained in a gas accumulator and seated on an O-ring seal, has a differential area between the skirt and the follower piston head. This volume is vented to the atmosphere so that as pressure in the accumulator is raised, the piston is pressed against the seal. Motion is initiated by energizing the trigger solenoid and moving the trigger rod up. This action closes the vent and opens the gas inlet, thus equalizing the pressure across the seal. The piston breaks contact with the follower piston head and moves up under the pressure in the accumulator and drives the solutions through the mixer and observation tube. The follower piston is the driving mechanism in the low speed mode and a hydraulic lock in the high speed mode. The follower piston moves up by hydraulic pressure to seal off the accumulator after the first piston has driven the syringe plungers in the high speed mode. In the low speed mode, the follower piston is driven by hydraulic pressure to move the syringe plungers.

The mixing chamber is designed to utilize the capabilities of the drive system. The two reactants flow directly into the mixer, impinge on each other, and move around a hemispherical surface towards the observation cell. Efficient, rapid mixing results from the impinging jets and turbulence behind the spherical surface.

The stop valve is constructed similarly to the drive system. Solution enters the stop valve, follows a groove around the piston, flows through small holes into the central part of the valve, and then to the exit port. The piston is accelerated to high velocity and, when the desired velocity is reached, the flow is sealed off.

Berger's system (83) has provision for thermal, spectrophotometric, and fluorimetric detection systems. The measured dead time of the system was 270 microseconds, and the mixing time was about 90 microseconds when the system was operated in the high speed mode. One disadvantage of this system is the relatively large volume requirements. At least 10 milliliters of each solution are required with about 1 milliliter of each reagent needed per trial.

d. Design of Morrow. The second stopped-flow system that is available from American Instrument Company was designed by Morrow in 1970 (78). The vertical flow system consists of reservoir syringes which are connected to the drive syringes via Hamilton valves, drive syringes, stopping syringe, and flow cell. The syringe drive mechanism is a pneumatic cylinder. When actuated, the drive cylinder pushes the plungers of the drive syringes and forces the reactants into a dual path length flow cell where the reactants are mixed and observed. The flow is stopped when the plunger of the stopping syringe contacts the stop, which is a micrometer with an oscilloscope trigger attached to the tip. After observation is complete, the waste release valve is opened, and solution is expelled to the drain.

The mixing chamber and observation cell of the flow cell consists of a Teflon input chamber, a Teflon exhaust chamber, two quartz windows, and a quartz plate all clamped together onto a mounting plate. The reactants enter the input chamber through separate Teflon tubes and come together about five millimeters before the entrance to the observation cell. The mixed reactants make two 90° turns and are split into two streams which recombine at the point where observation begins. The reactants pass through the observation cell and into the exhaust chamber which is connected to the stopping syringe. The system is set up for spectrophotometric detection with a 10 mm or a 2 mm path length. A mixing time of 1.5 msec and a dead time of about 4 msec were obtained. The complete system has some good features such as the vertical flow system, the pneumatic drive system, and the small mixing and dead times. However, its greatest disadvantage is that it requires manual operation of the valves, an operation that becomes extremely tedious for large numbers of samples.

e. The Design of Harvey. American Instrument Company also manufactures a variable ratio microflow apparatus, which is based on the design reported by Harvey (84). The fluid delivery system is a modification of the design published by Strittmatter (77). The solutions are driven into a mixing chamber situated in the base of a 3 mm square observation cell. The mixture transverses the mixing chamber and enters the observation area where it displaces a float that fits into the cell. The flow is stopped by a block which is attached to the drive system. This block allows only a given volume of reagents to flow before the block comes to rest against another block. In the commercial system, the syringe drive mechanism is a pneumatic cylinder. The dead time of the flow system is 3 msec. One feature of this flow system that is not present on other stopped-flow systems is the variable ratio feature. With the commercial system, it is possible to make use of seven different volume ratios which range from 1:1 to 100:1 merely by changing a dial setting. Observation may be accomplished with either a spectrophotometric or a fluorimetric detection system.

f. Variations on Stopped-Flow Methods. Recently some interesting variations on the implementation of the stopped-flow method have been reported. Stewart and Lum (85) have combined the techniques of stopped-flow and temperature-jump to investigate fast reactions which occur in the time range from microseconds to seconds. The first applications of this technique were reported by Erman and Hammes (86). Stopped-flow, temperature-jump instruments are available commercially from both American Instrument Company and Durrum Instrument Corporation (87,88).

The stopped-flow mixing system in the instrument utilized by Stewart and Lum (85) is based on the design of Gibson and Milnes (72) with a few modifications, such as the replacement of the observation cell with a much larger observation cell. A telemicroscope is used to inspect the solution in the cell for bubbles before the temperature perturbation is applied. The temperature jump is provided by a high voltage spark. The reaction is initiated by rapid mixing, and the system is allowed to equilibrate. The temperature perturbation is then applied, and the course of the reaction is observed during relaxation. This technique extends the time limit for the stopped-flow method down to approximately 20 microseconds.

There are some restrictions on the chemical systems which can be investigated by the stopped-flow, temperature-jump technique. First of all, the use of the commercial instruments is primarily restricted to aqueous ionic solutions since the temperature jump is produced by an electrical discharge. Secondly, the chemical systems must be reversible, and adequate amounts of reagents must be available. Finally, the chemical system must have a nonvanishing enthalpy of reaction.

Another interesting variation on the stopped-flow method was described by Gibson and coworkers (89). A double stopped-flow mixing system was designed to allow the addition of a third reagent within about twenty five milliseconds after initial mixing. The apparatus is basically the same as that of Gibson and Milnes (72) with the addition of a second mixer, a second observation cell, and a delay line between the first observation cell and the stopping syringe. The plunger of the stopping syringe is attached to the plunger of the syringe which contains the third reagent. The reaction is started by mixing two reagents as in a normal stopped-flow experiment, and an observation is made in the first observation cell. When the solution enters the stopping syringe and forces the plunger toward the stopping block, the third reagent is added, and observation is made in the second observation cell. Details of the performance of the flow system were not given.

5. Detection Systems

a. UV-Visible Spectrophotometry. The most common detection system for the stopped-flow method is UV-visible spectrophotometry. These systems are used because rapid response photodiodes and photomultiplier tubes are readily available at relatively low cost. The Zeiss PMQ II spectrophotometer was adapted to a stopped-flow mixing system by French, Benkovic, and Bunice (90). Durrum Instrument Company offers an adapter kit to allow the use of a Beckman DU spectrophotometer with their stopped-flow system (91). The kit consists of a support assembly to firmly align the DU monochromator with the mirror box on the mixing system and a spacer assembly which mounts between the exit slit of the monochromator and the DU sample changer. The function of the spacer is to divert the light beam from the normal light path of the DU to the mirror box on the mixing system.

A significant amount of research has brought about the development of more sensitive and more stable detection systems for stopped-flow experiments. A major effort has been devoted to the development of intense light sources to increase the signal-to-noise ratio of the results. Gibson (92) has utilized a modulated Xenon arc as an intense light source but noticed intensity variations of up to 10%. Through negative feedback, this variation was reduced to about 2%. Pardue (93) has employed quartz iodine lamps to gain more intensity over normal tungsten lamps.

Stability in the detection system has usually been gained by increasing the stability of the lamp power supply toward short term fluctuations. One popular method is to regulate the line voltage, because line voltage changes can cause changes in the light intensity comparable to the measured reaction rate. Weichselbaum and coworkers (94) have described an electronic regulator circuit to provide this stabilization. The circuit compensates for changes in light bulb resistance and is thus superior to circuits which only regulate the applied voltage or the applied current. This lamp power supply was not originally designed for use with a stopped-flow system, but the design is very good for this application. Another method of stabilizing the light intensity is to monitor the intensity of the source with a second detector. A signal from this detector is fed back to the power supply. A system of this type was described by Loach and Loyd (95).

DeSa and Gibson (92) introduced a dual beam spectrophotometric detection system to be employed with the rapid mixing system of Gibson and Milnes (72). The unit employs Xenon arc lamps as light sources, modulated to produce square light pulses at 5000 Hz. Two wavelengths of light are selected by monochromators, and the light is delivered to the observation port of the stopped-flow mixing system via a fiber optical Y-lightpipe. Current and light intensity feedback are utilized to control lamp intensity. Simple transistor choppers demodulate the photomultiplier output, and a second photomultiplier is used in a difference mode to reduce effects of residual lamp instability. The instrument provides time resolution at least five times greater and reagent economy at least twenty times greater than other available dual wavelength spectrophotometers.

The detection systems described up to this point have involved spectrophotometric measurements at one or two wavelengths. In some cases, it is desirable to observe changes in the spectrum of the reactants or products of fast reactions during the course of the reaction. For this purpose, Dye and Feldman (80) designed a system using a rapid scan monochromator. With this system, the spectrum from 400 to 1100 nm could be scanned from 3 to 150 times per second. In this manner, several species could be followed simultaneously. Recent work by Pardue and coworkers (96) has made use of a different type of rapid scan system. The basic detector element is a vidicon television camera pickup tube in which an active photosensitive surface is interrogated by an electron beam. With standard television scanning circuits, it is possible to obtain up to sixty spectra per second. Little work has been done on the use of this system, but it does appear to be a promising development for detection of results in stopped-flow experiments. Tektronix, Incorporated, Beaverton, Oregon has introduced a similar system for development (97).

b. Molecular Fluorescence Spectrometry. Another detection system for stopped-flow methods which is gaining popularity is that of molecular fluorescence, which is particularly useful for enzymatic reactions where many of the substrates fluoresce. To measure fluorescence, the observation cell has to be modified somewhat to include an observation port at 90° from the incident radiation. Durrum Instrument Corporation offers a modification of their basic mixing system used for fluorescence measurements (98).

The same observation system used for fluorescence measurements may also be used for light scattering. Measurement of light scattering yields information concerning the size and shape of particles suspended in a liquid medium. The technique shows potential application in studies of biological cells and membranes, and in the study of heterogeneous reactions (99,100).

Other applications of the same observation cell include a number of dual detector techniques. Possible techniques are dual wavelength measurements, optical rotation, polarization of fluorescence, and simultaneous measurement of absorption and fluorescence.

c. Non-Optical Methods. Non-optical methods of detection have application in various stopped-flow experiments. Solutions which are initially turbid are better observed by using thermal or electrochemical detection systems. Berger's apparatus has provision for thermal measurements as well as optical detection (83).

Thermal detection systems have physical advantages over spectrophotometric systems. With thermocouples, the observation point may be very close to the mixing chamber. Thermal sensors permit a more compact design. Also, the actual physical contact between solution and thermocouple eliminates uncertainties which arise in optical measurements. These uncertainties can arise from the spatial arrangement, such as optical alignment, air bubble effects, and light scattering.

The application of thermal methods of detection in stopped-flow procedures is no less demanding in sensitivity, and the engineering problems involved are more severe. Berger and Stoddart (101) have designed a thermal stopped-flow system. Included in this system is a provision for monitoring the course of the reaction simultaneously by optical means. The main problem with thermal detection systems is the difficulty of obtaining thermal sensors with response times on the order of a fraction of a millisecond. Balko and Berger (102) have measured response times of thermocouples down to 50 microseconds. The results were compared to those of theoretical calculations.

Electrochemical detection systems have also been reported for use with stopped-flow systems. Glass electrodes for the measurement of pH have been employed by Sirs (103). The chief limitation of this type of detection is the sluggish response of the electrode system. The electrode has a high internal resistance, which cannot be conveniently overcome by increasing electrode surface area because this would lead to a large increase in volume. A limitation due to the volume delivery from the flow system would be introduced. Through compromise, the best response time Sirs was able to achieve was 50 msec.

Electrical conductivity as a detection method for stopped-flow procedures has proved satisfactory. Suitable systems were described by Sirs (104) and Prince (105). The applications of this method have been mainly restricted to reactions involving changes in conductivity greater than 10%.

6. Readout Devices

Stopped-flow procedures have been developed using a wide variety of readout devices. Since the signal must be recorded rapidly, the most common readout device is a storage oscilloscope. The results are photographed, and rate data are obtained at leisure by treatment of the reaction curves. This procedure works well but is extremely tedious and subject to inherent inaccuracies. An improvement in this type of detection system was reported by Busch, et al. (106). They describe a simple and inexpensive apparatus to digitize the analog data presented on the photograph. The apparatus consists of a light source to scan the entire exposed photograph, a photo detector to generate a signal proportional to the intensity of the light reflected or transmitted by regions of the photograph, a digitizer, and a recorder to store the data.

For automated reaction rate analyses where large numbers of samples may be involved, the use of an oscilloscope as a readout device is totally inadequate. For this reason, various semiautomatic or automatic hardware rate measurement systems have been designed for use in single component analyses. James and Pardue (107) designed an analog computer for reaction rate analysis. Measurements and computations were performed automatically, and results were obtained on reaction times in the range from six milliseconds to one thousand seconds. Cordos, Crouch, and Malmstadt (108) designed a system based upon an integration procedure, which has high noise immunity. Instead of making the measurement between two points, two portions of the curve are integrated, subtracted, and the difference is automatically displayed on a digital readout. The readout is directly proportional to the reaction rate, which can be made proportional to the concentration of the species of interest. Ingle and Crouch (109) have described a fixed-time digital counting system for reaction rate methods. This instrument computes the voltage change over a fixed time interval by a digital integration procedure. The output voltage from a suitable reaction monitor is converted to a frequency by a voltage to frequency converter. The output is then gated to a seven decade up-down counter. The number of counts accumulated over a fixed time interval is subtracted from the number of counts obtained over a previous identical time interval by the up-down counter. This difference in counts is a digital number proportional to the reaction rate.

A transient recorder is another useful data-acquisition device for results of stopped-flow experiments. A commercial instrument is available from Biomation, Palo Alto, California (110). The recorder uses digital techniques to digitize and store a preselected section of an analog signal as it varies with time. The waveshape during the selected measurement time is digitized and held in the instrument until the operator instructs the instrument to record another waveform. After rapid data acquisition, the information can be observed at leisure on an oscilloscope, on a strip chart recorder, or be read out in digital form to a digital printer or a computer. The Biomation systems have sweep times from 10 microseconds to 20 seconds. The analog-to-digital converter in this system is, however, only a 6-bit converter which can limit the precision.

Further automation of data acquisition from stopped-flow experiments is possible through the use of small laboratory computers. The first work in this area was reported by DeSa and Gibson (111) using a stopped-flow instrument from Durrum Instrument Corporation and a Digital Equipment Corporation PDP 8/I computer. Samples of the output voltage from the spectrophotometer were digitized at arbitrarily chosen, accurately known time intervals after initiation of the reaction in the stopped-flow system. The sampling rate was chosen so that the entire reaction curve could be recorded with 400 data points. After digitizing, the data were converted to floating point, stored in core, and checked to make certain that full scale on the A-D converter had not been exceeded. Data points were then smoothed, changed to absorbance, and rates were calculated. The results were then printed out or stored for future reference. The program and interface system described seem quite flexible and could be used with many other laboratory instruments.

Willis, et al. (93) have also made use of on-line computer processing of stopped-flow data. A logic control system and interface were developed to link an optically stabilized spectrophotometer to a Hewlett-Packard laboratory computer. Data were acquired and processed with ensemble averaging and quadratic smoothing in real time, and various kinetic parameters were either displayed on an oscilloscope, or a permanent record was obtained on punched tape or a teletype. The optically stabilized spectrophotometer was used with a stopped-flow system from Durrum Instrument Corporation and also with another system.

C. The 12-Molybdophosphoric Acid Chemical System

The heteropolymolybdates of phosphorus, silicon, arsenic, and germanium have wide application in analytical chemistry. Many analytical procedures for the quantitative determination of these elements are based on the formation of the yellow heteropoly acids (15) or their subsequent reduction to the classical heteropoly blue species (15). Associated with these procedures are several experimental problems, which include length of analysis time, lack of stability of the heteropoly product, and chemical interferences. Definition of optimum conditions for the analysis for one of these elements has not been possible due to the lack of knowledge of the predominant equilibria present in solution. In this section, the chemistry of Mo(VI) in acid solutions will be reviewed. The formation of 12-MPA in acid solutions has been the subject of equilibrium studies (112,113) and kinetics studies (16), and the important results of these studies will be presented. Finally, analytical applications of the formation and reduction of 12-MPA will be discussed.

1. The Chemistry of Mo(VI) in Acid Solution

An important reason why the predominant equilibria involved in the formation of 12-MPA are not well known is the lack of knowledge of the chemistry of Mo(VI) in strong acid solutions. Acidification of alkaline solutions of molybdate ions, MoO_4^{-2}, results in the formation of polynuclear species (114). Most work has been done with solutions of pH 1.0 to pH 6.0. The techniques which have been employed to investigate the isopoly anions in solution were summarized in an article by Aveston, Anacker, and Johnson (115). The methods include dialysis and diffusion (116-121), conductometric titrations (122-124), pH titrations (125-130), light scattering (131), cryoscopy (132), ion exchange (133,134), ultraviolet spectrophotometry (135-138), ultracentrifugation (115), and Raman spectroscopy (115). Lindqvist reviewed the work on isopolymolybdates up to 1950 (139). It was concluded, mainly from the cryoscopic evidence of Bye (124) and analogy with solid compounds, that the ions shown by X-ray diffraction to be present in crystalline molybdates, $Mo_7O_{24}^{-6}$, and $Mo_8O_{26}^{-4}$, are also formed in solution according to the reactions

$$7MoO_4^{-2} + 8H^+ \rightleftarrows Mo_7O_{24}^{-6} + 4H_2O$$

$$8MoO_4^{-2} + 12H^+ \rightleftarrows Mo_8O_{26}^{-4} + 6H_2O$$

Through further spectrophotometric studies, Lindqvist (135) and Danielle (137) obtained additional evidence for these species. Tyree (140) also obtained similar results by light scattering. Sasaki and coworkers (129) found through potentiometric measurements that $Mo_7O_{24}^{-6}$ is the first polymeric species formed on acidification of molybdate solutions; it was proposed, however, that more highly protonated heptamers formed at increased acidities rather than the octamer according to Lindqvist (139).

Several authors have reported that the polymeric species which forms depends on the ratio of H^+ to MoO_4^{-2} concentrations. Glemser and coworkers (141) carried out ultraviolet spectrophotometric studies and found that at a H^+ to MoO_4^{-2} ratio of 1.14, the species formed was the heptamer, $Mo_7O_{24}^{-6}$, while at a ratio of 1.5, the octamer, $Mo_8O_{26}^{-4}$, was formed. Aveston, Anacker, and Johnson (115) found substantially the same results from ultracentrifugation, potentiometric acidity measurements, and Raman spectroscopy. These results were further substantiated by the work of Pungor and Halasz (142) who found that four processes occur at different H^+ to MoO_4^{-2} ratios: (1) formation of heptamolybdate; (2) protonation of heptamolybdate to $H_2Mo_7O_{24}^{-4}$; (3) the formation and protonatonation of octamolybdates by simultaneous processes; (4) formation of cations. Honig and Kustin (143) performed temperature jump studies on molybdate solutions and found that the rapid formation of heptamers could be explained in terms of a mechanism involving reactions of protonated monomeric molybdate.

Aveston and coworkers (115) found no evidence for the existence of isopolymolybdate species with a degree of polymerization intermediate between the monomer and the heptamer. In early work, however, Jander and coworkers (116) measured the diffusion coefficients of molybdate solutions and reported the existence of MoO_4^{-2}, $Mo_6O_{21}^{-6}$, $Mo_{12}O_{41}^{-10}$, and $Mo_{24}O_{78}^{-12}$ in solution. Other authors (144) have also found $Mo_6O_{21}^{-6}$ in acidic molybdate solutions. The species, $Mo_4O_{13}^{-2}$, has also been reported (134).

Further acidification of molybdate solutions results in the precipitation of molybdic acid, $MoO_3 \cdot H_2O$, at its isoelectric point, pH 0.9 (126). The precipitate dissolves upon further acidification, and cationic molybdenyl species are formed. The first species formed is the MoO_2^+ ion found in solutions of molybdenum trioxide dissolved in perchloric acid (145). It is also possible that the MoO^{+4} species exists (146), and polymeric cationic molybdenyl species such as $HMo_2O_6^+$ have been reported (147,148). In some recent work, Krumenacker and coworkers (149-151) studied isopolymolybdate formation in perchloric acid solutions (0.4-3 $\underline{M}$). An equilibrium was found to exist between monomeric and dimeric cations, A and A_2. The concentration of A_2 increased with increasing acid concentration due to a second equilibrium between the dimer cation and a protonated dimer cation, A_2H. It was shown via diffusion studies (152) that 78% of the molybdenum-containing cations are dimeric, either A_2 or A_2H. No evidence of higher order polymeric molybdenyl cations was found for perchloric acid solutions under ordinary conditions.

2. The Formation of 12-Molybdophosphoric Acid

The formation of 12-molybdophosphoric acid (12-MPA) was first observed by Berzelius in 1826 (153). In 1908, Miolati (154) made the first systematic attempt to understand the structure of heteropoly compounds when a structure based on the ionic theory and Werner's coordination theory was suggested.

The stoichiometry of the formation of 12-MPA has been investigated in nitric and sulfuric acid (112) and in perchloric acid solutions (113). These reactions are shown in Table 1. The reaction coefficients were determined from spectrophotometric studies that were conducted under conditions in which the concentration of 12-MPA formed in the reaction was very small relative to the initial concentrations of the reactants. The term, $Mo(VI)_t$,represents the total Mo(VI) concentration. The differences in reaction coefficients in the different acids may be due, in part, to experimental error since extreme measurement sensitivity is required. It is strongly believed that the reported reaction coefficient for H^+ in perchloric acid (113) is in error and should actually be nine (112).

Table 1

Stoichiometry of 12-MPA Formation

In 0.5-0.8 M HNO_3

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

In 0.3-0.5 M H_2SO_4

$$H_3PO_4 + 5Mo(VI)_t \rightleftarrows 12\text{-MPA} + 7H^+$$

In 0.2-0.8 M $HClO_4$

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

A mechanistic study of the formation of 12-MPA in nitric acid solutions was reported by Javier, Crouch, and Malmstadt (16). A stopped-flow spectrophotometer, designed by the same authors (14), was used to carry out the experiments. A mechanism was proposed from the kinetic data which involved an initial reaction between phosphate ions and Mo(VI) followed by polymerization to form 12-MPA:

$$H_3PO_4 + HMo(VI)_2^{+} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} PO_4 - Mo(VI)_2^{-3} + 4H^{+}$$

$$PO_4 - Mo(VI)_2^{-3} + HMo(VI)_2^{+} \xrightarrow{k_2} PO_4 - Mo(VI)_4^{-3} + H^{+}$$

$$PO_4 - Mo(VI)_4^{-3} + 4HMo(VI)_2^{+} \xrightarrow{\text{rapid}} \text{12-MPA} + 4H^{+}$$

From this mechanism the following rate law was derived:

$$\text{Rate} = \frac{d[\text{12-MPA}]}{dt} = \frac{k_1[H_3PO_4][HMo(VI)_2^{+}]}{\dfrac{k_{-1}[H^{+}]^4}{k_2[HMo(VI)_2^{+}]} + 1}$$

This rate law was in agreement with the experimental observations. Rate constants were evaluated from least squares analysis of the experimental data. The constant k_1 had an average value of 22.03 liter-mole^{-1} sec^{-1}. The ratio k_2/k_{-1} was found to be 15.38 mole^3-liter^{-3}. The constants k_2 and k_{-1} could not be evaluated independently because the concentration of the intermediate species was not known.

The results of the kinetics study do not indicate what the actual reactive species of Mo(VI) is in acid solutions, but a dimeric cation seems likely based on the stoichiometry (112). The proposed mechanism would then involve the reaction of phosphate with one dimer to form a

2/1 heteropoly acid in the first reversible step. It should be noted that the proposed mechanism gives the correct stoichiometry.

Unusual kinetic behavior was observed when the phosphate concentration was much greater than 10^{-3} $\underline{M}$. Log-log plots of initial rates versus phosphate began to deviate from linearity at high phosphate concentrations. A different heteropoly acid might be forming in addition to 12-MPA in solutions of comparable phosphate and Mo(VI) concentrations.

3. Analytical Applications of 12-Molybdophosphoric Acid

The formation of 12-molybdophosphoric acid is the basis for several analytical procedures for the quantitative determination of phosphorus in a wide variety of samples (15). Until recently, these analyses have involved the mixing of an acid solution of Mo(VI) with a solution containing the phosphate as orthophosphate. The solutions of 12-MPA are intensely yellow, and, thus, spectrophotometric observation can be made directly on this solution (15). The compound is greatly dissociated in acid solutions so the amount of color in the solution can be greatly increased by extraction of 12-MPA into organic solvents. Other analytical procedures are based on the reduction of 12-MPA to a heteropoly blue species (15). The oxidation potential of 12-MPA is comparable to that of chromic acid (155-158); hence, traces of relatively weak reducing agents, such as ascorbic acid and 1-amino-2-naphthol-4-sulfonic acid, can easily cause this reduction. This compound can also be extracted into organic solvents (15). The accuracy of methods based on both of these reactions is dependent upon the absence of interfering substances such as silicon, arsenic, and germanium. If any of these substances is present, erroneous results will be obtained.

Recently, reaction rate methods of analysis based on the formation of 12-MPA (14) and its subsequent reduction (12) have been reported. Javier, Crouch, and Malmstadt (14) developed an automated stopped-flow spectrophotometer to measure the rate of formation of 12-MPA. Applications of this method were made to the determination of phosphorus in blood serum and soil samples. Crouch and Malmstadt measured the rate of reduction of 12-MPA and used this for the quantitative determination of inorganic phosphate in the presence of adenosine triphosphate (159). In the reaction rate analyses, the interference of silicon, arsenic, and germanium is not great since these substances form heteropolymolybdates and reduced heteropolymolybdates at much slower rates. Javier (160) has studied the effect of arsenic and silicon on the rate of formation of 12-MPA and found that a thousand-fold excess of arsenic or an eighty-fold excess of silicon led to an error of only 1.5% in the phosphate determination. Ingle and Crouch (161) have utilized reaction rate differences to determine phosphate and silicate concentrations in the same sample. Excellent results were obtained.

III. AUTOMATED STOPPED-FLOW INSTRUMENTATION

The stopped-flow method has potential wide application in analytical chemistry because of the possibility of utilizing rapid reactions for reaction rate methods of analysis. Reliable data can be obtained in a relatively short amount of time, which makes the method attractive in situations where large numbers of samples must be analyzed, such as in clinical, environmental, and industrial analytical laboratories. With the instrumentation which is now commercially available, however, the sample handling and data acquisition operations are too time-consuming and tedious to be of general application in routine analysis. In this chapter an automated stopped-flow spectrophotometer is described. This instrument overcomes several of the limitations of the commercial systems and allows all sample handling and data acquisition steps to be controlled by a logic sequencing system. Before discussing the components of the automated stopped-flow spectrophotometer in detail, several considerations in the design of stopped-flow mixing systems will be reported. Results of evaluation tests and a discussion of some of the experimental problems encountered will then be presented.

A. Considerations in the Design of Stopped-Flow Mixing Systems

Several of the factors which influence the efficiency of a stopped-flow mixing system can be optimized if careful consideration is given to the design of the apparatus. The ultimate purpose of a stopped-flow apparatus is to provide complete mixing within a few milliseconds after the initial contact of the two reagents so that the course of a fast reaction may be observed. Some of the important factors are the character of the fluid flow, the design of the mixing chamber, the volume of the system, and the design of the stopping device.

1. Character of Flow

The flow in a stopped-flow mixing system must be turbulent to provide efficient mixing. The critical velocity of flow which produces turbulence can be calculated from the Reynolds equation:

$$u_c = \frac{N_R \eta}{\rho d}$$

where

u_c = critical velocity, cm/sec

N_R = Reynolds number (about 2000 for short tubes with streamline entry)

η = viscosity of the liquid, poises

ρ = density, gm/ml

d = diameter of the flow tube, cm

Thus high flow rates must be used to obtain a high degree of turbulence and rapid, efficient mixing. The syringe drive system, therefore, must be capable of producing a high flow rate. For water at 20°C, the critical velocity in a tube of two millimeters diameter is about 2 m/sec.

Turbulent flow has several advantages which add to the efficiency of the method (43). These advantages apply more to the continuous flow method but still should be noted in the stopped-flow method. The longitudinal motion along the reaction tube is accompanied by constant eddying which aids mixing and produces a more homogeneous mixture throughout the tube. In laminar flow, the solution at the center of the tube moves faster than the solution near the walls of the tube. There is thus a concentration difference within the tube since the concentration of a solution at a given distance from the mixer is related to the flow velocity. A second advantage is that in turbulent flow, the flow velocity is proportional to the square root of the driving pressure and is, therefore, less sensitive to variations in pressure than is laminar flow, where the velocity is directly proportional to the driving pressure. Another advantage is that although the critical velocity is higher when the viscosity is increased, the flow velocity, once the critical velocity is exceeded, becomes less and less dependent on the solution viscosity. In contrast, with laminar flow, the flow velocity is always inversely proportional to the viscosity of the liquid.

At high flow velocities there are considerable variations in the hydrostatic pressure of the streaming fluid at different parts of the apparatus, and the phenomenon of cavitation may occur. Cavitation consists of the formation of a stream of very fine bubbles which cause turbidity and, consequently, a sharp decrease in light transmission by the solution in the observation cell. The phenomenon can, therefore, be a serious limitation in measurements of reaction rates by optical methods and, to a lesser extent, in other detection methods. Cavitation can be avoided by various means (162). One of the most straightforward methods is to use reactant solutions which have been thoroughly degassed.

This leads to a minimization of cavitation about nucleation centers. Another method of avoiding cavitation is to employ a back pressure such that the pressure drop at any point in the stream never falls below the vaporization pressure of any component in the solution. Back pressure can be obtained in two ways. First of all it can be obtained by means of a constriction at the end of the observation cell. This method, the hydraulic method, has the advantage that the pressure rises at the point of constriction as does the pressure drop at the point of cavitation. Another method, the pneumatic method, utilizes the back pressure applied to the liquid flowing out of the observation cell. In a stopped-flow system, a back pressure sufficient to avoid cavitation is obtained when the flow is stopped.

2. Mixing Chamber Design

The efficiency of mixing, which defines the limit of the reaction times accessible to the stopped-flow technique, is highly dependent on the design of the mixer. The function of the mixing chamber is to start the distribution of the two solutions and to create turbulence. Mixing of the turbulent fluid then continues throughout the remainder of the flow system. High mixing efficiencies have been obtained using tangentially offset jets which promote rotary motion in the fluid. Berger (23) found that the optimum offset or distance between the jets, was equal to the jet diameter. The rotary or vortex motion allows eddying to persist in the flow system for a longer period of time. Eddies associated with longitudinal turbulence, however, die out in about one millisecond (43). Mixing efficiency is also dependent upon the diameter of the jets; the smaller the bore size, the higher the jet velocity.

The total area of the jets and observation tube should be made equal to avoid an excessive pressure drop in the mixer. Mixing performance is improved by increasing the number of jets, but to maintain the same relative area, the diameter of each of the jets must be made correspondingly smaller as the number of jets is increased.

Cavitation can be avoided by proper mixer design. The necessary turbulence may be obtained in the mixer by the impingement of one jet stream upon another without the need to pass the flow streams over sharp edges. The offset mixer must be constructed such that the offset is not too great to avoid cavitation due to vortex action. These mixer designs are consistent with the equipment for avoiding flow past sharp edges; however, the desirability of making an abrupt transition from the mixing chamber to the observation cell and thereby avoiding unnecessary dead space does require sharp edges.

3. Flow System Volume

Another factor in the design of stopped-flow mixing systems is the total volume of the flow system. The volume should be kept small so that the solution volume requirements are minimal. Another reason for small volume is that the time necessary for sweeping the old solution from the observation cell and replacing it with a new solution should be very small. This time, known as the dead time, is a parameter of the stopped-flow system and is one factor in determining the shortest reaction time accessible with the system. If there is a large change in the property being measured, it is possible to study reactions with half lives shorter than the dead time of the system. Reactions with half lives as short as 0.5 millisecond have been studied using a system with a dead time of two milliseconds (163).

Another consideration concerning the total volume of the flow system is the method of observation. For example, for spectrophotometric measurements, greater sensitivity is obtained if an observation cell with a long path length is employed. Thus, to maintain a small system volume, it is necessary to make observations along the flow axis. This problem also is present for some other detection methods.

4. Efficiency of Stopping Device

The efficiency of the stopping device is largely dependent on the rate at which the flow is stopped. This factor partially determines the lower limit of reaction times accessible to the stopped-flow method. Efficient stopping devices should give zero displacement to prevent fluid expansion in the observation tube. The flow should be abruptly stopped rather than slowly decelerated. If the latter case prevailed, the flow velocity would not be constant and the mixing efficiency would be lowered for the last portion of solution. Since high pressure gradients are developed from the rapid stopping, the material and the design of the stop assembly must be able to withstand the high mechanical stress. Berger (164) found that quartz breaks down at a pressure of 400 psi and a stopping time of 0.2 millisecond.

In summary, the design of stopped-flow mixing systems involves several trade-offs. A high solution flow rate is desirable to allow for turbulent flow and rapid mixing; however, if the flow rate becomes too high, the problem of cavitation affects the results. Another trade-off involves the total volume of the system. The volume of the system should be small, but many measurement systems require a long path length for optimum sensitivity.

B. The Automated Stopped-Flow Spectrophotometer

In this section a new automated stopped-flow spectrophotometer is described. The new system features solenoid-actuated valves for directing the liquid flow and eliminating waste solutions, and a pneumatic syringe drive system for rapid mixing. The flow system is vertical to minimize interferences due to bubble formation. The entire operating cycle can be actuated by pulses from a digital sequencing system or from push button switches. The system can be preset to deliver up to ten repetitive samples without refilling the drive syringes for ensemble averaging purposes. The stopped-flow mixing system is easily interfaced to a light source and monochromator or interference filter and photomultiplier tube for photometric observation. Rate information can be recorded on an oscilloscope, or initial reaction rates can be measured automatically with a fixed-time digital readout system (109).

1. Instrument Description

In Figure 2 a schematic diagram of the complete stopped-flow spectrophotometer is shown. A photograph of the flow system with the spectrophotometric detection system is shown in Figure 3. The operating cycle of the stopped-flow instrument is under complete control of a digital sequencing system which will be described in detail in a later section. By referring to Figure 2, the sequence of operations during a rate determination or a series of repetitive determinations can be followed.

Figure 2. Schematic diagram of automated stopped-flow spectrophotometer.

A. Solenoid actuated pneumatic drive cylinder

B. Solenoid actuated pneumatic cylinder controlling delivery valve

C. Reagent drive syringes

D. Double 3-way stopcock connecting reagent syringes with reservoirs or flow system

E. Port to reagent reservoirs

F. Mixing chamber

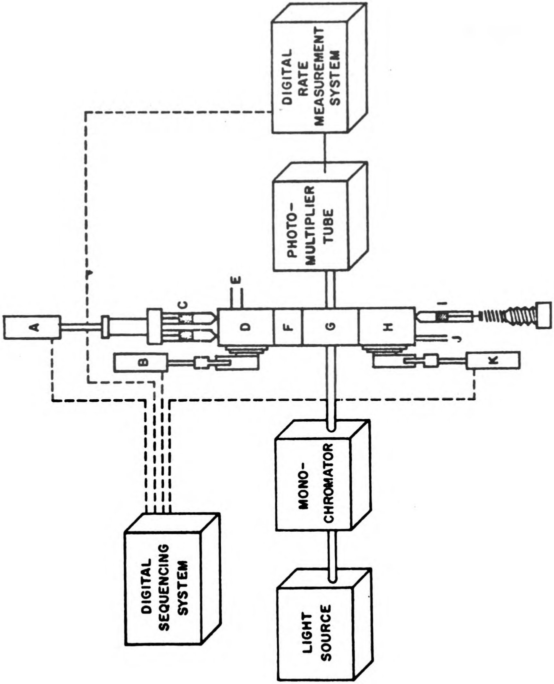
G. Observation cell

H. 3-way waste release valve

I. Spring-loaded stopping syringe

J. Port to drain

K. Solenoid-actuated pneumatic cylinder controlling waste release valve


DIGITAL RATE MEASUREMENT SYSTEM
PHOTO-MULTIPLIER TUBE
MONO-CHROMATOR
LIGHT SOURCE
DIGITAL SEQUENCING SYSTEM
A
B
C
D
E
F
G
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I
J
K

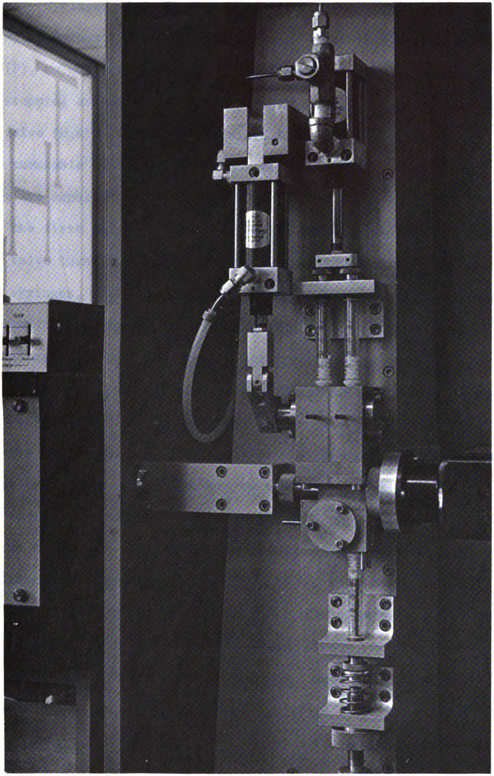


Figure 3. Photograph of automated stopped-flow system.

a. Sequence of Operations. The basic flow system consists of two delivery syringes (C in Figure 2), a pneumatic syringe drive system (A), a pneumatically operated delivery valve (B,D), a mixing chamber (F), an observation cell for spectrophotometric monitoring (G), and the stopping assembly (I) with a pneumatically operated waste release valve (H,K). Solutions contained in reservoir bottles or on a sample turntable can enter the system through port E, while waste solutions are expelled after the reaction through port J.

On the first pulse from the sequencing system, the double 3-way delivery valve turns to connect reagent and sample reservoirs to the delivery syringes, while closing off the remainder of the flow system. On the second pulse, the drive syringe piston moves upward and draws reagent and sample solutions into the syringes. On the next pulse, the delivery valve turns to connect the syringes to the mixing chamber and the observation cell. On the fourth pulse, air pressure is applied to the drive syringe piston, which remains stationary because the waste release valve is open, which disconnects the flow system from the stopping syringe and the drain. On the fifth pulse, the waste release valve turns to connect the flow system to the stopping syringe, and the syringe drive piston rapidly strokes forward and forces the solutions through the mixing chamber, observation cell, and stopping device. The flow is automatically stopped when the spring-loaded stopping syringe comes to rest against a mechanical stop. This same pulse triggers the digital readout system or an oscilloscope. After a variable time delay to allow for the collection of reaction rate data, the next pulse opens the waste release valve. This action connects the spring-loaded stopping syringe to the drain, and the old solution in the stopping syringe is forced out the drain port. The sequencing system can be operated in a single analysis mode or in a repetitive mode. If repetitive trials are desired for the same sample, the sequencer continues with steps five and six until the preset number of trials has occurred. The number of trials which can be obtained without refilling the syringes depends on the setting of the mechanical stop. With this system, it is easy to obtain up to ten analyses without refilling the syringes. This allows the user to average several results for high precision. The sequencing system can be modified to add additional steps to accommodate a sample turntable for automatic sample changing.

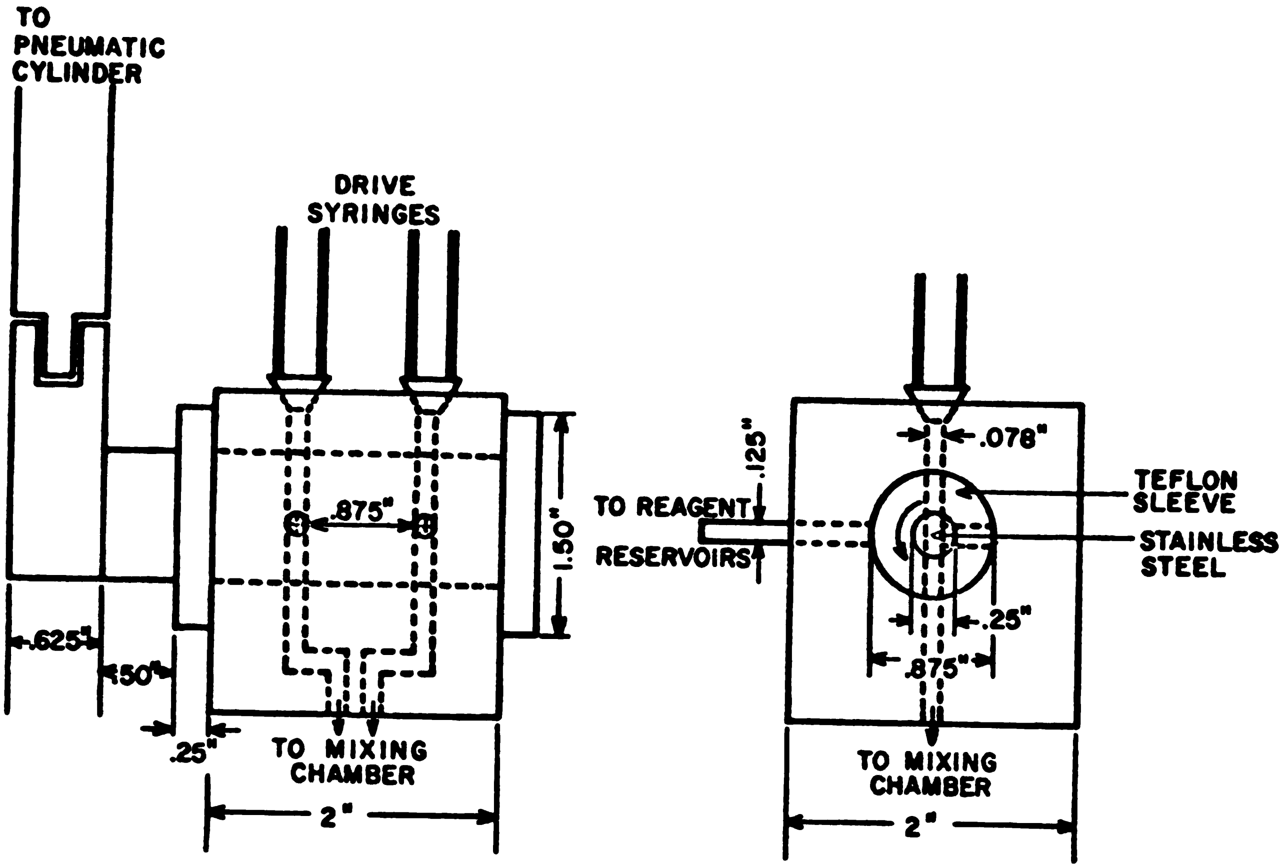
b. Syringe Drive System. The sample syringes are driven by an air piston actuated by a solenoid valve. The air piston is a Schrader Cylinder, No. 305-0014, from Scovill Manufacturing Company, and the solenoid valve is a Schrader solenoid, Scovill No. 41001-1115. The pneumatic drive system was chosen because of the high flow velocities which can be obtained and the ease of electronic actuation. The reagent syringes are filled from the reservoirs on the return stroke of the piston. A flow control valve, Schrader speed control valve, Scovill No. 3250, is attached to the air cylinder to make the return stroke for filling the syringes more slowly than the forward driving stroke. An air pressure of approximately 60 psi is applied to the air piston.

c. Reagent Syringes. The reagent syringes were modified from commercial syringes. The syringe barrels are glass and were obtained from Durrum Instrument Corporation, Part No. 13643. Teflon tips were constructed to attach the syringe barrels to the delivery valve block. The syringes are rigidly fastened into the apparatus by means of stainless steel bushings which are screwed into an aluminum support. A Teflon gasket is placed between the glass barrel and the stainless steel bushing. The syringe plungers have been the subject of several design changes which will be discussed later. The most satisfactory plunger, which is now in use, consists of a piston and rod machined from a single bar of AISI 316 stainless steel. A double Neoprene O-ring seal is employed, and the amount of leakage is minimal. As can be seen in Figure 3, the upper ends of the two syringe plungers are attached to the piston of the air cylinder by means of an aluminum pushing bar.

d. Delivery Valves. The double 3-way stopcock delivery valve is shown in Figure 4. Figure 4(a) shows a front view of the delivery valve block and illustrates the direction of liquid flow from the drive syringes to the mixing chamber. The delivery valve block is machined from AISI 316 stainless steel. Figure 4(b) shows a side view of the block and illustrates the connection of the valve to the reagent reservoirs. The moving portion of the valve is an AISI 316 stainless steel shaft in contact with a Teflon sleeve. The valve and air piston are designed such that a two-inch stroke of the air piston turns the stopcock the required 90°.

e. Mixing Chamber. A two-stage mixing chamber based on the design of Gibson and Milnes (72) is employed in this instrument. Previous work with this mixing chamber (14) has shown that rapid and efficient mixing can be obtained within a few milliseconds.

f. Observation Cell. A 2-millimeter diameter observation cell with a 2-centimeter path length is employed to provide high sensitivity for spectrophotometric monitoring and small volume for minimum system



a) front view

b) side view

Figure 4. Diagram of delivery valve block.

dead volume. The cell is made with quartz windows, 4 millimeters thick and 3 millimeters in diameter, to allow measurements in the ultraviolet and visible regions of the spectrum. The thickness of the windows is necessary to withstand the high mechanical stress which may develop when the flow is stopped. The windows are set in threaded Teflon holders which screw into the stainless steel block containing the observation cell. A Teflon gasket provides a seal between the window and the stainless steel. The arrangement of the observation cell is similar to that described by Gibson and Milnes (72). Thermostatted water can be circulated around the observation cell for temperature control.

g. Stopping Device and Waste Release Valve. Figure 5 shows the arrangement for stopping the liquid flow and expelling old solution. The stopping device is a 2.5 milliliter syringe (exactly like the drive syringes) which rapidly stops the flow when the shaft of the plunger comes to rest against the adjustable stopping block. The stopping syringe is mounted in place by means of a stainless steel bushing which screws into an aluminum support. The plunger of the stopping syringe is machined from AISI 316 stainless steel, and a seal is made with the walls of the syringe by means of a double O-ring. The plunger is spring-loaded so that waste solution will be expelled when the pneumatically actuated waste release valve is opened to the drain. The waste release valve is constructed similarly to the reagent delivery valve, except that it acts as a single 3-way stopcock.

The stop block is a stainless steel block attached to a threaded brass adjusting screw. A brass jam nut provides a lock on the stop position by being adjusted against an aluminum support. The position of the

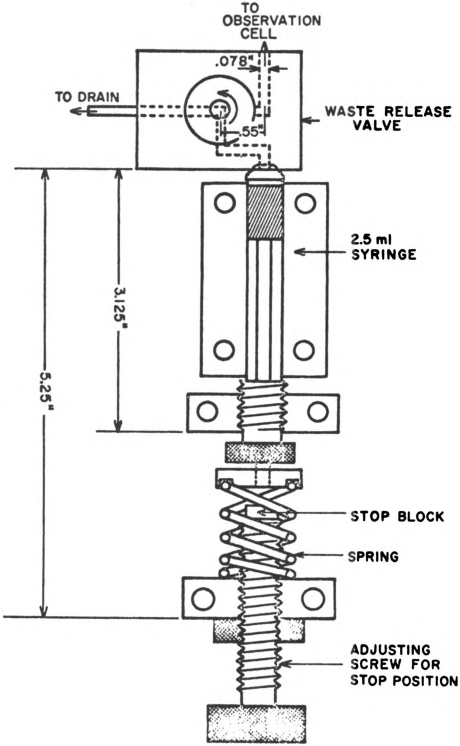


Figure 5. Stopping mechanism and waste release valve.

stopping block can be varied to change the amount of solution necessary for each trial. With the present system, the smallest number of trials which can be obtained before refilling the syringes is five and the largest practical number is twelve. The limiting number of samples must be chosen such that the volume of reagents delivered is sufficient to completely flush the old solution from the observation cell into the stopping syringe. The system is normally adjusted so that ten repetitive trials can be performed before the syringes must be refilled.

h. Detection and Readout System. Several different spectrophotometric detection systems have been utilized with the stopped-flow mixing system. For maximum versatility during preliminary studies, the Heath EU-701-50 light source module and EU-700 monochromator were used in conjunction with a 1P28A photomultiplier tube transducer. As can be seen from Figure 3, a simple brass light pipe was employed to transfer light from the exit slit of the monochromator to the entrance window of the observation cell. Light-tight seals are provided by Neoprene O-rings imbedded in brass jam nuts which can be tightened against the steel block around the observation cell. The photomultiplier tube is contained in a photomultiplier tube housing, Model No. 50, from Pacific Photometric Instruments, Berkeley, California. As will be shown later, the precision of reaction rate analyses using this system is severely limited by light throughput. Therefore, for high precision studies, the light level incident on the observation cell was increased by replacing the monochromator with a narrow band interference filter and inserting a lens at the entrance of the light pipe.

Results can be expressed either in terms of absorbance or transmittance. In the % transmittance mode, the current output of the photomultiplier tube was converted to voltage with a Philbrick SP2A premium parametric operational amplifier. The current-to-voltage converter has feedback resistors of 10K, 100K, 1M, 10M, and 100M ohms and capacitors to provide RC time constants from 10^{-8} second to 0.1 second. For direct absorbance readout a logarithmic amplifier, Teledyne Philbrick Nexus 4351, was constructed.

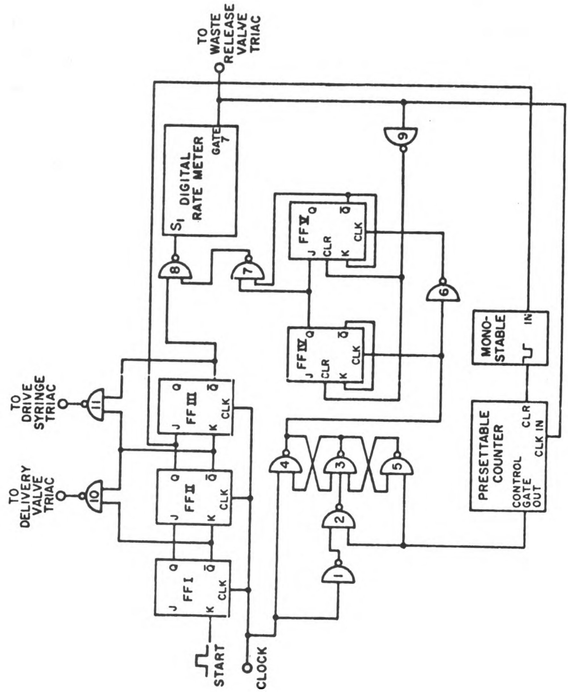
For preliminary studies, results were obtained from a Tektronix Type 564 Storage Oscilloscope with Type 2A63 Differential Amplifier and Type 2B67 Time Base. For automated analyses based on initial reaction rates, the fixed time digital rate meter recently described by Ingle and Crouch (109) was interfaced to provide digital readout of initial reaction rates with high noise immunity.

i. Digital Sequencer. The digital sequencing system, which controls all the operations of the stopped-flow spectrophotometer, is shown in Figure 6 along with the appropriate connections to the digital ratemeter.

The circuit cards listed in the caption of Figure 6 were inserted into a Heath EU-801A "Analog-Digital Designer" (ADD) which supplies the necessary power to operate the TTL logic circuits. The sequencer provides pulses at the appropriate times to operate three triac switches (RCA 40526), which gate the line voltage to the three solenoid actuated pneumatic cylinders. The sequencer also provides a pulse to start the digital ratemeter or to trigger an oscilloscope.

Figure 6. Circuit diagram of digital sequence.

NAND Gates	Heath NAND gate card (EU-800-JC)
Flip-Flops	Heath Dual J-K flip-flop card (EU-800-CB)
	Heath Quad J-K flip-flops card (EU-800-CC)
Monostable	Heath Dual Monostable card (EU-800-CA)
Presettable Counter	Made from Heath Dual J-K Flip Flop Cards and Heath NAND gate cards
Clock	1-2 Hz square wave from Heath Digital Timing Module


TO WASTE RELEASE VALVE TRIAC
DIGITAL RATE METER
S1
GATE 7
8
7
9
FF V
J
CLR
K
CLK
Q
Q̄
FF IV
J
CLR
K
CLK
Q
Q̄
6
TO DRIVE SYRINGE TRIAC
11
TO DELIVERY VALVE TRIAC
10
FF III
J
K
CLK
Q
Q̄
FF II
J
K
CLK
Q
Q̄
FF I
J
K
CLK
Q
Q̄
4
3
5
2
1
MONO-STABLE
IN
PRESETTABLE COUNTER
CLR
CLK IN
CONTROL GATE OUT
START
CLOCK

The sequencing system is clocked by a 1-2 Hz square wave from the ADD Digital Timing Module. The clock frequency is limited by the time necessary to refill the drive syringes. The operation of the sequencing system can be easily understood by referring to Figure 6. Flip flops I, II, and III form a 3-bit shift register, while flip flops IV and V and NAND gates 6 and 7 make up a single pulse gate. NAND gates 1-5 direct the pulses from the clock to the single pulse gate at the appropriate times. By observing the waveform diagram in Figure 7, the operation of the sequencer can be followed. Application of a pulse to the START input begins the sequence. The first 1-0 transition of the clock after the start pulse causes a logical 1 to appear at QI which shifts to QII and QIII on subsequent 1-0 clock transitions. NAND gate 10 becomes a logical 1 when either QI or QII is in the 1 state. Thus the delivery valve triac is turned on while QI or QII are logical 1's, the drive syringe piston returns, and the reagents are drawn into the syringes. When QII undergoes a 1-0 transition, a monostable multivibrator is triggered, which provides a short pulse to clear the 16-bit presettable counter. The 1-0 transition of QIII resets the fixed-time digital ratemeter (109). The output of gate 7 of the ratemeter is connected to the triac that controls the waste release valve. When the ratemeter is reset, the output of gate 7 becomes a logical 1, the waste release valve closes, and the first sample enters the mixing chamber and observation cell. The ratemeter provides an internal variable delay time before the measurement is started. After the measurement is complete, gate 7 returns to logical 0, and a count is registered in the presettable counter. Simultaneously, the waste release valve opens, and the single clock pulse which is allowed through the gate then resets

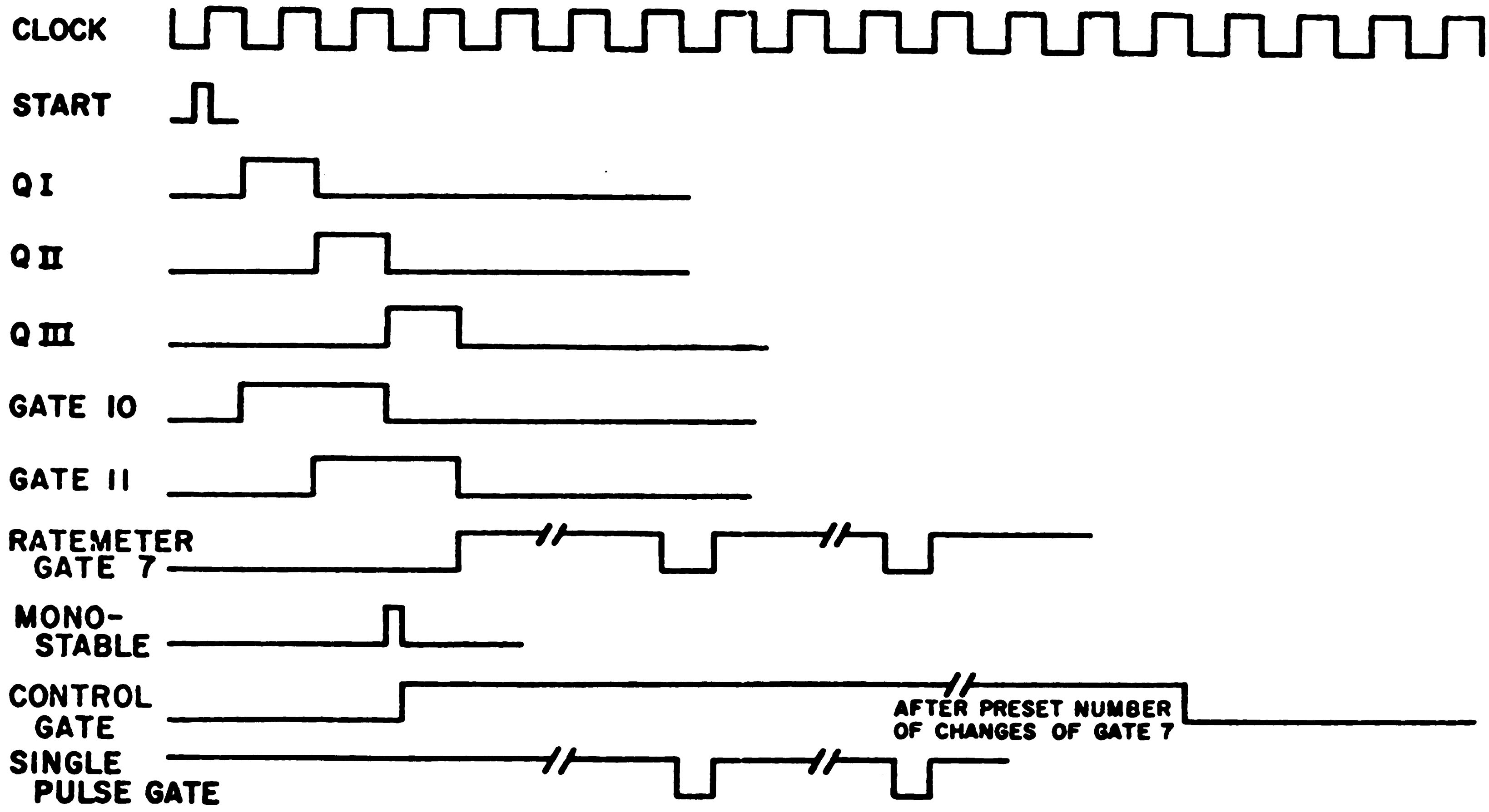


Figure 7. Waveform diagram.

the ratemeter, closes the waste release valve, and the second sample enters the observation cell. This process continues until the preset number of counts has been registered in the counter. When this number of trials has been reached, the output of the control gate on the counter becomes a logical 0, and the clock input to the single pulse gate is disabled. The system then remains off until another start pulse is applied. With the present logic system it is relatively easy to add steps to the sequence to provide pulses to control a sample turntable.

If automated operation of the stopped-flow system is undesirable, the logic sequencing system can be replaced by three manual switches to control TTL logic level signals to gate the triacs on and off at the appropriate times. This is often the case when preliminary studies are being performed.

j. Computer Automation of the Stopped-Flow Spectrophotometer. The study of kinetics with a stopped-flow spectrophotometer is a complex and tedious process when conventional oscilloscopic readout of rate information is used. Because large amounts of data can be obtained rapidly, the small laboratory computer is ideal for use with stopped-flow systems. A small computer can be used to provide on-line sample handling, on-line data acquisition, and on-line data treatment. Of these three applications, the procedures involving data acquisition and treatment are probably the most tedious and, therefore, are the logical problems to be attacked first. While the problem of sample handling is tedious, especially for large numbers of samples it is of lesser importance than the other processes.

Some preliminary work on the computerization of the data acquisition and data treatment has been completed. No specific experimental data were obtained, but it was demonstrated that the use of a laboratory computer led to the acquisition of reliable reaction rate data by a much less tedious procedure. The computer used in this work was a PDP LAB-8/E computer from Digital Equipment Corporation, Maynard, Massachusetts. The PDP LAB-8/E consists of a PDP 8/E computer with a special package of options which includes a real time clock, a 10-bit analog-to-digital (A/D) converter, and a 12-channel buffered digital I/O (165).

The software for the data acquisition process was written in BASIC/RT, an interactive language that contains the commands for operation of the real time clock and the A/D converter. The signal which is connected to the input of the A/D converter is obtained from the current-to-voltage converter. This same signal is observed on an oscilloscope. After initial conditions such as delay time, A/D converter sampling rate, number of A/D conversions per trial, offset voltage, and average 100% Transmittance are typed into the program, the program halts and waits for an interrupt which is provided by actuating the drive cylinder of the stopped-flow system. After the preset delay time has elapsed, the desired number of A/D conversions is calculated, and the slopes between successive points are performed. The time and slope are then printed out. If the trial is not acceptable for any number of reasons, the data are erased and the program again halts and waits for another interrupt which admits a new sample to the observation cell. A flow chart and listing of this program are presented in Appendix A.

The program for data acquisition has need for many improvements. A single value of the slope of the reaction curve can be obtained from a least squares calculation over the linear portion of the rate curve. The computer can also be programmed to make the decision concerning the acceptability of the data. An oscilloscope display of the reaction rate data that are stored in memory would be desirable. If software is written in BASIC/RT, however, some of these improvements are not possible due to the limited amount of core available. The ideal situation will be to rewrite the software in machine language. This will also make possible the use of the computer to control sample handling steps.

A further use of the computer in conjunction with a stopped-flow system is to monitor reaction conditions. One limitation of reaction rate methods of analysis is that the rate of the reaction is very dependent on such variables as temperature, pH, and ionic strength. If reliable data are to be obtained, these reaction conditions must be carefully controlled. The computer could be used to monitor these variables and then correct the experimental data for variations. A much simpler experiment would be the result.

Each of the two approaches to automation of stopped-flow kinetics which were described above have advantages in different situations. For an application where the same procedure is repeated for several samples, such as in the case of automated analyses, a hardware logic system is more than adequate. If, however, parameters must be changed often, such as in preliminary studies, the use of a computer is more applicable since, in most cases, it is much easier to change statements in the software than it is to rewire the hardware system. With regard to expense it is rather wasteful to involve an expensive computer system in performing a simple, invariable procedure which could be handled equally well by an inexpensive hardware logic system.

2. Experimental Evaluation

To evaluate the stopped-flow system for mixing effectiveness and the reliability of reaction rate measurements, several chemical tests were performed. Standard test reactions were employed to determine the instrument dead time and mixing efficiency. An initial rate determination of Fe(III) based on the Fe(III)-SCN^- reaction (93) was then carried out using oscilloscopic readout to test the accuracy and precision of the system on a rapid reaction under normal conditions. Finally, the reaction rate analysis of the phosphate (14) was performed using a digital integration readout system (109) and ensemble averaging to demonstrate the high precision which can be obtained when averaging techniques are utilized.

a. Reagents. For the determination of Fe(III), a 0.1 M stock solution of iron was prepared by dissolving reagent grade iron in aqua regia. Perchloric acid was added, and the solution was evaporated to dryness before dilution with distilled water. The working solutions of 1×10^{-4} M to 5×10^{-4} M were prepared by dilution of this stock solution and were made 0.05 M in H_2SO_4. The 0.01 M SCN^- solution was made by dissolving reagent grade KSCN in 0.05 M H_2SO_4.

For the determination of phosphate, reagent grade K_2HPO_4 was oven dried and dissolved in distilled water. This solution is stable and can be kept for months. The working solutions of 0.5 to 4.0 ppm P were made from this stock solution as needed. A 0.2 M aqueous stock solution of Mo(VI) was prepared by dissolving reagent grade $Na_2MoO_4 \cdot 2H_2O$ in distilled water. This stock solution was also stable for months. The working solution of 0.04 M Mo(VI) in 0.4 M HNO_3 was prepared from this stock as needed. Before the working solutions were used, they were filtered through a Millipore filter to eliminate dust and other particles prior to use in the stopped-flow system. Both the stock and working solutions were kept in polyethylene bottles to prevent leaching of silicon from the volumetric glassware.

b. Procedure. For the quantitative determination of Fe(III), equal volumes of Fe(III) and SCN^- were mixed in the stopped-flow system, and the progress of the reaction was followed spectrophotometrically at 450 nm using the monochromator as the wavelength isolation device. This reaction is quite rapid, and measurements were made within 50 milliseconds after the reaction was initiated. The output current of the photomultiplier tube, proportional to transmittance, was converted to voltage with an operational amplifier current-to-voltage converter described in a previous section. The results were recorded on film by photographing the trace on the storage oscilloscope.

Transmittance values during the initial portion of the reaction were converted to absorbance values manually, and the initial rates were obtained as $\Delta A/\Delta t$.

For the determination of phosphate, the formation of 12-molybdophosphoric acid (12-MPA) was followed at 400 nm using an interference filter to isolate a small band of wavelengths. Since this reaction is slower than the Fe(III)-SCN^- reaction, the fixed-time digital ratemeter (109) could be used to measure the initial reaction rates. An initial delay time of approximately 200 milliseconds was used before the up-down digital integration began. The total integration time was 400 milliseconds. Because of the large changes in transmittance which occurred during this measurement time, it was necessary to use a logarithmic amplifier to measure the true rate. The logarithmic amplifier employed in this study was constructed using the log diode, contained in an oven, from the Heath EU-20-28 log/linear current module in the feedback loop of a Philbrick PP25AU operational amplifier.

c. Determination of Dead Time. The flow dead time of a stopped-flow system is defined as the time for the solution to flow from the mixer to a point halfway through the observation cell. The procedure used here to measure the dead time is known as the extrapolation technique (166). This method allows determination of the flow dead time through a measurement of the amount of reaction which occurs before the first observation is made. A 5×10^{-4} $\underline{M}$ solution of Fe(III) in 0.05 $\underline{M}$ H_2SO_4 was mixed with a 0.01 $\underline{M}$ solution of KSCN in 0.05 $\underline{M}$ H_2SO_4, and the course of the reaction was followed spectrophotometrically at 450 nm using the monochromator and oscilloscopic readout. The dead time was determined by extrapolating the slope of the rate curve back to the initial absorbance of the reaction mixture. The dead time is the time between the intersection of the extrapolated curve with the initial absorbance line and the time of the first observation of the reaction curve. The flow dead time of the system was determined to be 5 ± 1 milliseconds. Similar measurements using other stopped-flow systems have shown comparable dead times (167).

d. Determination of Mixing Time. The mixing time of the stopped-flow system was determined by mixing 0.01 $\underline{M}$ NaOH with 0.01 $\underline{M}$ p-nitrophenol solutions. The course of this very rapid reaction was followed spectrophotometrically at 490 nm using the monochromator, and the results were obtained from an oscilloscope. The oscilloscope was triggered as the plunger of the stopping syringe came to rest against the adjustable stop by means of a microswitch and battery. The measured time for complete mixing was taken as the time between the beginning of the trace and the time at which a constant transmittance was attained. Mixing was found to be complete within approximately five milliseconds after the flow was stopped.

e. Determination of Iron. The results for the reaction rate determination of iron are given in Table 2. The results as listed were obtained from five repetitive trials on each sample solution. The relative standard deviations obtained are reasonable considering the small number of trials, the short reaction times involved, and the imprecisions in obtaining the initial reaction rates from photographs. Precision of the results could be improved by automating the collection of initial rate data and by emsemble averaging. Willis, *et al.* (93), have shown that high precision results can be obtained by using rate measurements at several different times during the reaction, instead of taking only one initial reaction rate measurement.

f. Determination of Phosphate. The results for the quantitative determination of phosphate are given in Table 3. The results as listed are the averages of fifty determinations, and the relative standard deviations are for averages of groups of ten results. Relative standard deviations of individual results are 1-3%, except for the lowest phosphate concentration. The results, while quite satisfactory, could be improved by the use of a more intense light source and a more efficient optical design. This problem will be discussed later.

Table 2. Reaction Rate Determination of Iron

Fe concentration, M X 10^4		Rel std	
Taken	Found[a]	dev, %	Rel error, %
1.0	1.00	6.0	0
2.0	....	2.6	....
3.0	3.08	5.9	+2.7
4.0	4.00	3.0	0
5.0	4.83	3.7	-3.6

[a] Averages of 5 results based upon calibration with 2 X 10^{-4} _M_ Fe(III).

Table 3. Automated Reaction Rate Analysis of Phosphate

Phosphorus concentration, ppm		Rel std	
Taken	Found[a]	dev,[b] %	Rel error, %
0.5	0.49	1.7	-2.0
1.0	1.01	0.9	+1.0
2.0	....	0.5	....
3.0	2.99	0.7	-0.3
4.0	3.95	0.5	-1.3

[a] Averages of 50 individual results based upon calibration with 2.0 ppm standard.

[b] Relative standard deviation of averages of 10 results.

3. Experimental Problems and Considerations

Several experimental problems were encountered in the course of the design and evaluation of the automated stopped-flow spectrometer. Some of these problems were due to basic design flaws in the mixing system while others were due to the measurement system. Some problems encountered in the use of other stopped-flow systems are not as important in this automated stopped-flow mixing system.

a. Leakage of Reagents. Leakage of reagents was a severe problem in the original design of the stopped-flow mixing system. Leakage occurred aroung the plungers of the reagent syringes and around the stopcocks of the reagent delivery valve and the waste release valve. This leakage resulted in premixing of the reagents in the reagent syringes and the loss of solutions to the drain and behind the heads of the plungers in the reagent syringes. Erroneous observations may have been obtained because the solution in the observation cell was actually flowing at a much slower flow rate. The reason for this leakage was traced to the tendency for Teflon to cold flow. The stopcocks of the delivery valve and the waste release valve were originally a solid piece of Teflon. These have since been replaced with stopcocks which consist of a Teflon sleeve and a small diameter stainless steel rod. The stainless steel rod is the movable part in the valve assembly. No evidence of leakage has been noted.

The original design of the syringe plungers consisted of a stainless steel shaft attached to a Teflon piston which was lowered to minimize leakage. Another unsatisfactory design involved a construction similar to the design of the plunger of a Hamilton gas-tight syringe. Louvers in the Teflon were forced against the walls of the syringe by means of an O-ring. Another design involved a Teflon plunger with concave edges. Seals with the walls of the syringes were made at the ends of the cylinder. This design also proved unsatisfactory. The most satisfactory design eliminated the use of Teflon altogether. The present design is a stainless steel plunger which makes use of a double Neoprene O-ring seal. Little evidence of leakage has been noted during the one year period this plunger has been in use.

b. Diffusion of Reagents. The stopped-flow technique is useful for the study of slow reactions as well as the fast reactions for which it is normally used. The upper limit for the application of the stopped-flow method has always been thought to be determined by the stability of the detection system. However, when the stopped-flow system was used for slower reactions with reagents of widely different densities, diffusion of the more dense solution into the observation cell occurred after about 30 seconds of reaction. Spectrophotometric observation was not possible after this diffusion occurred. Sturtevant (82) noted this diffusion problem in his stopped-flow system several years ago, but it has been largely ignored in the literature since then. When diffusion was first observed in our mixing system, the cause was unknown. The diffusion occurs because the mixing chamber is not closed off from the observation cell at any time. Thus unmixed reactants are present in the jets of the mixing chamber, and the more dense solution can diffuse into the observation cell. The vertical flow system promotes this diffusion. The immediate problem was eliminated by transforming the vertical stopped-flow system into a horizontal flow system when the system was used for the study of slow reactions. This configuration permitted the necessary long measurement times, but diffusion presented a different type of problem. Diffusion now caused each successive reaction trial to have a higher initial absorbance value than the previous trial. This effect is seen only if the length of time between successive samples is about one minute or longer. Insufficient work has been done at this point to determine if the change in initial absorbance of each trial has any significant effect on the rate or extent of reaction. In a recent paper, Gibson (168) discusses the occurrence of this problem in the apparatus designed by Gibson and Milnes (72). Based on his results, there does not appear to be a solution for this problem other than restoring the same initial absorbance by initiating several reaction trials in quick succession. This type of solution is clearly impossible for any automated procedure or for any reaction where reactants are available in limited quantities. A more attractive solution may be the installation of an electronically actuated valve between the mixing chamber and the observation cell.

c. Flow System Volume. The total volume of the tubes, valves, mixing chamber, and observation cell is approximately 0.25 milliliter. Thus the minimum volume of reagents which will just replace the solution in the observation cell is about 0.13 milliliter of each reagent. It is desirable to use more solution to assume that the old solution is completely flushed from the observation cell. A convenient volume to use per trial is 0.25 milliliter of each reagent; this corresponds to 10 trials before refilling the syringes.

d. Flow System Rinsing. The amount of rinsing of the flow system is a serious limitation for some reactions since it does determine the total amount of solution necessary for the analysis. In the original stopped-flow system, Tygon tubing, 15 inches long and 1/8 inch inside diameter, was used to connect the reservoirs with the inlets to the delivery valve. These tubes each contained a volume of approximately three milliliters. It was found that best results were obtained if two complete syringe cycles (5.0 ml) of solution were rinsed through the flow system and the third cycle (2.5 ml) used for analysis. Thus a total of about 8-10 milliliters of each reagent would be necessary. These Tygon tubes have been replaced with tubing which is 1/16 inch inside diameter. The volume of the tubing is reduced to about 0.7 milliliter; thus one syringe can be used for a rinse and the second for analysis. The required volume is then reduced to about 5-7 milliliters of each reagent.

e. Syringe Breakage. While this system has been in use, syringe breakage has not been a severe problem. The breakage of syringes is possible, however, due to the mechanical stresses exerted during operation of the system. For this reason, care should be taken to provide safety shields to cover the syringes to prevent injury from fragmented glass.

f. Air Bubbles. Interference from air bubbles is a serious problem in any stopped-flow apparatus. If air bubbles become trapped in the observation cell, erratic observations are the result. With the vertical flow system, however, air bubbles are not a significant problem since they rise to the tops of the reagent syringes and are forced out around the O-ring seals. Even with the use of our horizontal stopped-flow system, air bubbles have not been a significant problem.

g. Chemical Resistance. The stopped-flow mixing system is constructed such that the reagent solutions are in contact with glass, quartz, stainless steel, and Neoprene rubber. The stainless steel is AISI 316 stainless steel which was chosen for its high chemical resistance. Little evidence of chemical attack of the stopped-flow system has been observed. In some experiments, the Neoprene O-ring seals were attacked by one molar sulfuric acid.

h. Temperature Effects. Besides the obvious effect of temperature variations on the rate of the reaction, temperature also affects measurements in stopped-flow experiments in other ways. In the case of spectrophotometric measurements, Gibson (169) found that the reagents and the observation cell should be at nearly the same temperature. If not, a gradient of refractive index within the observation cell will be seen due to the dependence of refractive index on temperature. This effect can result in an erroneous observation. Other observation techniques, such as pH and conductivity measurements, are also temperature dependent.

Temperature changes during the time of flow and observation can arise from several sources. The overall effect, however, is small. Recent calculations (170) have shown that the effects of momentum and friction of the flowing solution and the heat of dilution of an acid solution would raise the temperature of the observation cell approximately 0.15 degrees. Calculations also show that in the time that is normally needed for the measurement to be made in a stopped-flow experiment (approximately 200 milliseconds), the solution will not be cooled to the initial temperature. It is necessary, therefore, to measure the temperature in the cell while the course of the reaction is being followed and correct the experimental data for the temperature change.

i. Light Intensity in the Measurement System. In performing the quantitative determination of phosphate, the precision of results was found to be a function of light intensity. Some of the preliminary work was performed using the Heath EU-701-50 light source module and the Heath EU-700 monochromator. At a wavelength of 400 nm and maximum slit width (spectral bandpass of 4 nm), the output current of the photomultiplier tube was about 10^{-8} ampere. A 2.00 ppm phosphorus solution gave a relative standard deviation of 5% under these conditions. Amplifying the output signal by a factor of two did not improve the signal-to-noise ratio. The light intensity was increased by the use of optical filters. A combination of a Corning 7-59 and a Corning 5-57 filter increased the signal level to about 10^{-5} A but was unsatisfactory because of the lower absorbance changes obtained. This filter combination was replaced by an interference filter which had a peak transmittance of 42% at 403 nm and a half-band width of 10 nm. The output current of the photomultiplier tube was again 10^{-5} A. A 2.0 ppm phosphorus solution yielded a relative standard deviation of 2.8%. Addition of a focussing lens to focus the radiation on the entrance window of the observation cell decreased the relative standard deviation to 1.7%.

The experiments above indicate that the precision of the reaction rate measurements is limited by photomultiplier shot noise. Attempts to increase the light level to the point where the mixing precision of the stopped-flow becomes limiting have thus far been unsuccessful. The light intensity could be increased by use of a high intensity quartz-iodine lamp as the source so that a monochromator can be used with high precision. The use of fiber optics could also be explored.

IV. KINETICS AND MECHANISMS OF THE FORMATION OF 12-MOLYBDOPHOSPHORIC ACID

In order to develop selective, sensitive, and accurate quantitative determinations based on the measurement of reaction rates, it is important to have detailed mechanistic information about the reaction. A complete knowledge of the equilibria and kinetics of the reactions used in quantitative determinations allows the analytical chemist to choose conditions for the analysis in a rational manner, rather than by the empirical methods which are often used to develop analytical procedures. Even for analytical methods based upon reaction stoichiometries, a detailed knowledge of the mechanisms of the reactions of interest and of reactions which may cause interferences is desirable in the development of selective methods.

The reaction of Mo(VI) with phosphate in acid solution which results in the formation of 12-molybdophosphoric acid (12-MPA) is the most widely used reaction for the quantitative determination of phosphate in a wide variety of materials. The product, 12-MPA, is yellow in acid solution and is itself the basis for analytical procedures. In addition, 12-MPA is an important intermediate in the widely used heteropoly blue method for phosphate analysis.

In this chapter results of stopped-flow reaction rate studies on the formation of 12-MPA in perchloric and sulfuric acid media are presented. The results have important consequences for users of analytical procedures based on the formation and/or reduction of 12-MPA. The rate laws obtained in the two acids are in agreement with mechanisms which involve the direct reaction of phosphate with Mo(VI) as the first step followed by several pH dependent polymerization steps. The rate studies provide important information about the influence of the acid anion on the reaction. Results of the present work will be compared with previous stopped-flow studies on the formation of 12-MPA in nitric acid solutions (16) in a later chapter which summarizes our present knowledge of the equilibria and rates of formation of 12-MPA in the most commonly used media.

A. The Measurement System

The stopped-flow mixing system employed in these experiments was a commercial unit obtained from Durrum Instrument Corporation, Palo Alto, California. The original design of the system was reported by Gibson and Milnes (72) in 1964. Several minor improvements were made in the commercial version, but the basic design is the same. Spectrophotometric detection was utilized with this mixing system, and results were obtained from oscilloscope traces of absorbance vs. time curves. This section describes the stopped-flow mixing system and the detection system. In order to perform these experiments, some modifications of the mixing system were necessary and will also be presented.

1. Mixing System

The Durrum stopped-flow mixing system is operated in the following manner. The reagents are placed in reservoir syringes and are transferred to the 2.5 ml driving syringes by opening the appropriate valves. These valves are then closed, and the two valves connecting the driving syringes with the mixing chamber and the remainder of the flow system are opened. The solutions are then forced together by actuating a pneumatic cylinder. The reactants pass through the mixing chamber, through the observation cell, and into the stopping syringe. When the plunger of the stopping syringe comes to rest against a stationary stop, the flow is abruptly stopped, and the progress of the reaction is observed spectrophotometrically. After observation is complete, the waste release valve is opened and the solutions are expelled to the drain.

In the original apparatus designed by Gibson and Milnes (72), the syringe drive system was hydraulic. The first systems manufactured by Durrum also made use of a hydraulic syringe drive system. A pneumatic syringe drive system has now been substituted by Durrum because it is easier to obtain a more rapid and more reproducible flow velocity.

The reagent or drive syringes are 2.5 milliliter syringe barrels which are connected to the delivery valve block by means of Teflon syringe tips. The plungers of these syringes are ceramic, which, in most cases,gives a good compromise between chemical resistance and expansion due to temperature changes. The syringes are immersed in a water bath through which water from a constant temperature bath is pumped.

The valves connecting the reservoir syringes with the delivery syringes and the delivery syringes with the mixing chamber are needle valves with 60° Teflon tips which seat into corresponding 60° depressions machined in the valve block. This elaborate arrangement was used in order to withstand the high hydrostatic pressures. The chief disadvantage with these valves is that they are manually controlled by means of a nut driver. The waste release valve is of the same design.

The mixing chamber in the stopped-flow system consists of eight tangentially offset jets, 0.5 millimeter in diameter, which open into a central chamber. The jets are so placed that the two series, each consisting of four jets, attempt to spiral the liquid in opposite directions. The purpose is to produce maximum turbulence while avoiding a violent spinning motion of liquid that might favor the onset of cavitation. Two such mixing chambers are used in series.

The observation cell is a cylinder 2 millimeters in diameter and 20 millimeters long. This configuration provides a long path length for spectrophotometric sensitivity but small volume for minimum system dead volume. The cell has quartz windows to allow measurements in either the ultraviolet or visible regions of the spectrum. The observation cell is provided with adequate temperature control by means of thermostatted water circulating around the cell. This control is necessary in order to avoid optical artifacts due to thermal effects in the observation cell.

The stopping device is a syringe similar to the drive syringes. The plunger of the stopping syringe comes to rest against a fixed stop block to stop the flow. The waste release valve is the same design as the other valves in the system.

2. Detection System

The method of detection employed with the Durrum stopped-flow mixing system was spectrophotometric. A 12-volt tungsten lamp, powered by a Heath Tungsten Lamp Power Supply, Model EU-44A, was used as the light source. The source was attached to a Farrand Grating Monochromator which was attached to the entrance optics system of the Durrum stopped-flow. The photomultiplier tube was supplied by Durrum and was an end window, S-20 response tube powered by a Heath High Voltage Power Supply, Model EU-42A. The current from the photomultiplier tube was converted to a voltage by two methods. In the transmittance mode, a Philbrick SP2A premium parametric operational amplifier was used. For direct absorbance readout a logarithmic amplifier, Teledyne Philbrick Nexus 4351, was employed. Results were obtained from a Tektronix Type 564 Storage Oscilloscope with a Type 2A63 Differential Amplifier and a Type 2B67 Time Base. A permanent record of the data was obtained by photographing the transmittance - or absorbance - time curves.

The overall performance of this stopped-flow mixing system was good. A mixing time of 2 milliseconds and a dead time of 2 milliseconds have been reported (171). In our studies, however, some problems with the system as received from Durrum Instrument Corporation were encountered. It was found that the monochromator must be firmly attached to the mixing system in order to obtain reproducible results. Without any attachment, vibration of the detection system relative to the mixing system caused irreproducible results. This problem was solved by anchoring both the mixing system and the observation system to an aluminum base. Another experimental problem occurred with the ceramic plungers of the reagent syringes. These tended to cause reduction of Mo(VI) when low acid concentrations were employed. The ceramic plungers were replaced with louvered Teflon plungers for chemical resistance. No other unusual or serious problems were encountered with the stopped-flow mixing system. The most serious drawback with the entire system is its completely manual operation. Each sample requires changing five valves manually, and, obviously, this can be quite tedious for large numbers of samples. The valve configuration also makes automation nearly impossible.

B. Preparation of Reagents

1. Phosphate Solutions

A phosphate stock solution (0.1 M) was prepared from oven dried, reagent grade K_2HPO_4. This solution, which was stored in a polyethylene bottle, was stable and was prepared as needed. The working solutions of 5×10^{-5} M to 1×10^{-3} M phosphate were prepared as needed by dilution of this stock solution.

2. Mo(VI) Solutions

Reagent grade $Na_2MoO_4 \cdot 2H_2O$ was used to prepare a 0.2 M stock solution by dissolving the appropriate amount in distilled water. The solution was prepared as needed and was stored in polyethylene bottles to avoid leaching silica from glass. The solution was allowed to age for at least 24 hours before use (112). The working concentrations of 0.005 M to 0.05 M Mo(VI) were prepared as needed by dilution of this stock solution.

3. Perchloric Acid and Sulfuric Acid Solutions

Working solutions of perchloric acid were prepared by dilution of AR grade 70% perchloric acid from G. Frederick Smith Chemical Company, Columbus, Ohio. The concentrated acid solution was standardized by titration with standard sodium hydroxide. Sulfuric acid solutions were prepared in the same manner from AR grade concentrated sulfuric acid.

4. Sodium Perchlorate Solutions

Sodium perchlorate solutions were used to adjust the ionic strength of the working solutions. A 6 M sodium perchlorate solution was prepared by dissolving AR grade $NaClO_4 \cdot H_2O$ in distilled water. This solution was also stored in a polyethylene bottle.

5. 12-Molybdophosphoric Acid Solutions

For molar absorptivity measurements, solutions were prepared from 12-molybdophosphoric acid, $H_3PMo_{12}O_{40} \cdot XH_2O$, from Climax Molybdenum Company. Attempts to oven dry the 12-MPA were unsuccessful and resulted in the reduction of the compound. Thus, the 12-MPA was analyzed gravimetrically to determine the actual amount of 12-MPA in the hydrated salt. Three waters of hydration were found in the material obtained from Climax.

C. Experimental Procedure

1. Determination of Molar Absorptivity

The initial rates of reactions were measured as the change in absorbance of the 12-MPA solution per unit time. To calculate the rate in concentration units, the molar absorptivity of 12-MPA and observation cell length are needed. Aqueous standards of 12-MPA were made up with distilled water, and their absorbance was measured at the wavelength, approximately 425 nm, where all the reaction rate measurements were taken. A molar absorptivity of 267 ℓ mole^{-1} cm^{-1} was obtained at that wavelength. Measurements were obtained from the stopped-flow spectrophotometer which has an optical cell length of 2 cm.

2. Rate Measurements

The rate of formation of 12-MPA from Mo(VI) and phosphate in perchloric and sulfuric acid solutions was determined by measuring the time necessary for a given small change in absorbance at the analytical wavelength of approximately 425 nm. The exact wavelength could not be determined with the monochromator employed in these studies; therefore, the wavelength was fixed, and all rate measurements were performed at that wavelength. Initial reaction rates were evaluated by measuring the time necessary for a given small change in absorbance. All measurements were made on the initial linear portion of the reaction rate curve where the concentration of 12-MPA was very small relative to the initial concentrations of the reagents. These initial reaction rate measurements were made from photographs of reaction rate curves. In most cases, five rate measurements were made per solution and averaged. Typically, initial rates were measured with a relative standard deviation of 2-5%.

3. Determination of Reaction Orders

Reaction orders were evaluated by carrying out experiments under conditions of variable acidity and concentrations of phosphate and molybdate. With the concentrations of two of the reagents kept constant, the concentration of the third reagent was varied. The logarithm of the initial rate was plotted against the logarithm of the concentration of the variable reagent, and the reaction order was evaluated from a slope of the plot.

The method of determining reaction orders is based upon the following general relationships. From previous studies of the stoichiometry (112) and kinetics of the formation of 12-MPA (16), it was known that the rate was dependent on the phosphate concentration, the Mo(VI) concentration, and the solution acidity. The general rate law for the reaction was expressed as

$$\text{Initial Rate} = \left(\frac{d[\text{12-MPA}]}{dt}\right)_i = K[H_3PO_4]_i^{\,x}[\text{Mo(VI)}_t]_i^{\,y}[H^+]_i^{\,z}$$

where K is a proportionality constant. The subscripts i represent initial values, and $Mo(VI)_t$ represents the total concentration of Mo(VI) in solution. When the logarithms of both sides of the general rate law are taken, the following equation results:

$$\log \text{(Initial Rate)} = \log K + x \log [H_3PO_4]_i + y \log [Mo(VI)_t]_i + z \log [H^+]_i$$

If the initial concentrations of two of the reagents, for example $Mo(VI)_t$ and H^+, are held constant and a series of initial rate measurements is made at varying initial concentrations of H_3PO_4, the equation simplifies to

$$\log \text{(Initial Rate)} = K' + x \log [H_3PO_4]_i$$

where $K' = \log K + y \log [Mo(VI)_t]_i + z \log [H^+]_i$. A plot of log (initial rate) vs. log $[H_3PO_4]_i$ should reveal the reaction order x in phosphate. Similarly, the reaction orders y and z can be determined by holding two other reagent concentrations constant and by obtaining the slopes of the log-log plots. If linear plots are not obtained throughout wide concentration ranges, changes in reaction orders are often the cause. In many cases, limiting forms of the rate law can be obtained from linear log-log plots at the extremes of concentration. In several cases noted later, it was not possible to isolate completely limiting forms of the rate law due to limitations in the ranges over which reagent concentrations could be varied. Also, some of the apparent reaction orders obtained by trying to isolate limiting forms of the rate law did not fit the entire rate vs. concentration curves in the intermediate ranges. Therefore, in order to obtain rate laws which gave good fits to experimental data over wide concentration ranges, two computer curve-fitting programs were employed.

The first program, known as KINFIT, was developed by Dye and Nicely (172). KINFIT is a general purpose curve fitting program which has three options available: (1) fit an arbitrary function of not more than 20 parameters and four variables (dependent plus independent) to a data set containing not more than 99 points; (2) solve arbitrary algebraic equations having not more than 20 unknowns; (3) minimize (or maximize) an arbitrary function of not more than twenty parameters. The user must specify ten control constants, the data (including estimates of relative variance), and a short subroutine in standard form which gives the equation to be used for the particular problem. The second curve-fitting program was written in BASIC for use with a PDP 8/E computer from Digital Equipment Corporation, Maynard, Massachusetts. This program calculates reaction rates and rate constants from concentration data which are typed into the program. A listing of the BASIC program is shown in Appendix B.

KINFIT was used to test the agreement of experimental data to a given rate law. The output from the program contained the values of the parameters (rate constants) which best fit the experimental data (initial reaction rates) as a function of the variables (reagent concentrations), a plot which compared the experimental and calculated rates, and a list of the experimental and calculated initial rates. This program was used to test a wide variety of possible rate laws. Different functional forms of the rate law were provided, and the best fit to the proposed rate law obtained from the KINFIT program. Upon inspection of the output,many of the proposed rate laws could be immediately discarded. In all cases a single rate law was found by KINFIT which gave a reasonable fit throughout the concentration ranges which were used experimentally. After the best fit rate law was obtained, the BASIC program was used to vary individual rate constants while other parameters were held constant.

4. Temperature Control

The large temperature dependence of reaction rates, which will be discussed later, necessitated the close control of reagent temperatures and the temperature in the mixing system. Temperature was controlled at 25.0 ± 0.1°C by circulating water from a constant temperature bath around the observation cell and the drive syringes. The reagents and samples were maintained at this same temperature prior to experiments by equilibration in the water bath.

5. Ionic Strength

The ionic strength of the working solutions was not controlled except in the experiments dealing specifically with the effect of ionic strength. Results of these experiments will be presented in a later section.

D. Results and Discussion

The only previous mechanistic study of the formation of 12-MPA was carried out in nitric acid solution (16). The present work studied the 12-MPA formation reaction in sulfuric and perchloric acid media, which are both important in analytical applications. In this section experimental results are presented which have led to rate laws for the formation of 12-MPA in perchloric and sulfuric acid. A reasonable mechanism which describes the polymerization process is proposed.

1. Rate Law in Perchloric Acid Solutions

a. Dependence on Acid Concentration. The dependence of the initial rate of formation of 12-MPA on the concentration of perchloric acid is shown in Figure 8. The concentration of perchloric acid was varied from 0.17 M to 1.13 M. At concentrations below 0.17 M, Mo(VI) begins to precipitate as MoO_3. At concentrations much greater than the upper limit, the reaction rate becomes immeasurably slow. Also, since increasing acidity leads to a greater dissociation of 12-MPA (173), the amount of product formed at concentrations greater than 1.13 M is very small. Large relative standard deviations were thus obtained at acidities greater than about 1.1 M.

As can be seen from Figure 8, the reaction rate becomes independent of acid concentration (zero-order) at low concentrations (< 0.25 M $HClO_4$), but becomes extremely concentration dependent as the acidity is increased. Attempts to determine the reaction order from the limiting slopes of log initial rate vs. log $[H^+]_i$ plots were unsuccessful because the hydrogen ion concentration could not be made large enough to isolate a limiting form of the rate law. In the study reported by Javier and coworkers (16), two limiting forms of the rate law were obtained. The reaction behavior at intermediate concentrations could be described adequately in terms of the two limiting cases. In the present study, the same type of approach was attempted, but reasonable results were not

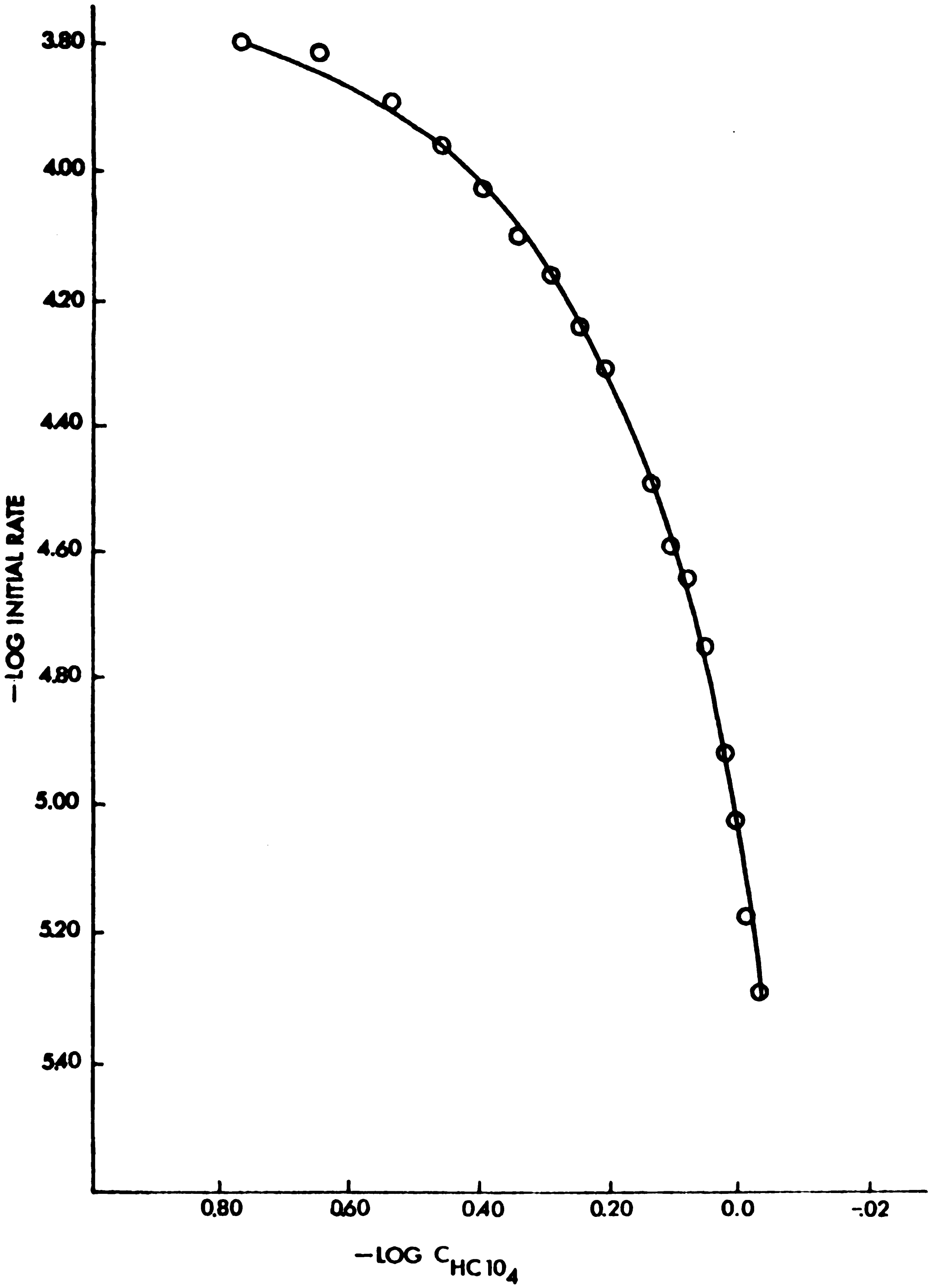


Figure 8. Effect of concentration of $HClO_4$ on initial reaction rate

$C_{PO_4^{-3}} = 1 \times 10^{-4}$ M

$C_{Mo(VI)_t} = 2 \times 10^{-2}$ M

$C_{HClO_4} = 0.17\text{-}1.13$ M

obtained. In the limiting cases, seemingly different results were obtained from day to day, even for the same set of solutions. This problem was particularly important in the determination of the high order with respect to the acid concentration where the differences in the rates obtained for successive concentrations of acid were often of the same order of magnitude as the variance in the rates obtained for the repetitive trials for the same solution. Therefore, to obtain an experimental value for the reaction order with respect to perchloric acid concentration, at least seven different sets of solutions containing various high concentrations of perchloric acid (0.8-1.2 M) were prepared and analyzed in duplicate or triplicate. The results showed enough variation so that an experimental determination of the high order acid dependence was not possible. Hence, the KINFIT routine was employed. In order to completely characterize the initial rate vs. initial concentration data, four acid dependent terms were found in the rate law obtained with the help of KINFIT. At very high acidities, an inverse eighth-order dependence on the acid concentration was found. The solid line in Figure 8 represents the best fit rate law calculated from the above acid orders and the rate constants whose determination is discussed later. The circles in Figure 8 represent the experimental points which are the results of at least fifteen individual trials.

b. Dependence on Phosphate Concentration. Figure 9 shows a plot of log initial rate vs. log $[H_3PO_4]_i$ at low perchloric acid concentration. At 0.17 M $HClO_4$, the slope of the log-log plot was found to be 0.97 ± 0.02 for phosphate concentrations below 3×10^{-4} M. At 1.008 M $HClO_4$, a non-integral slope was obtained from the raw experimental data. Since perchloric acid is known to contain trace amounts of phosphate and silicate, the deviation from first-order kinetics was attributed to a

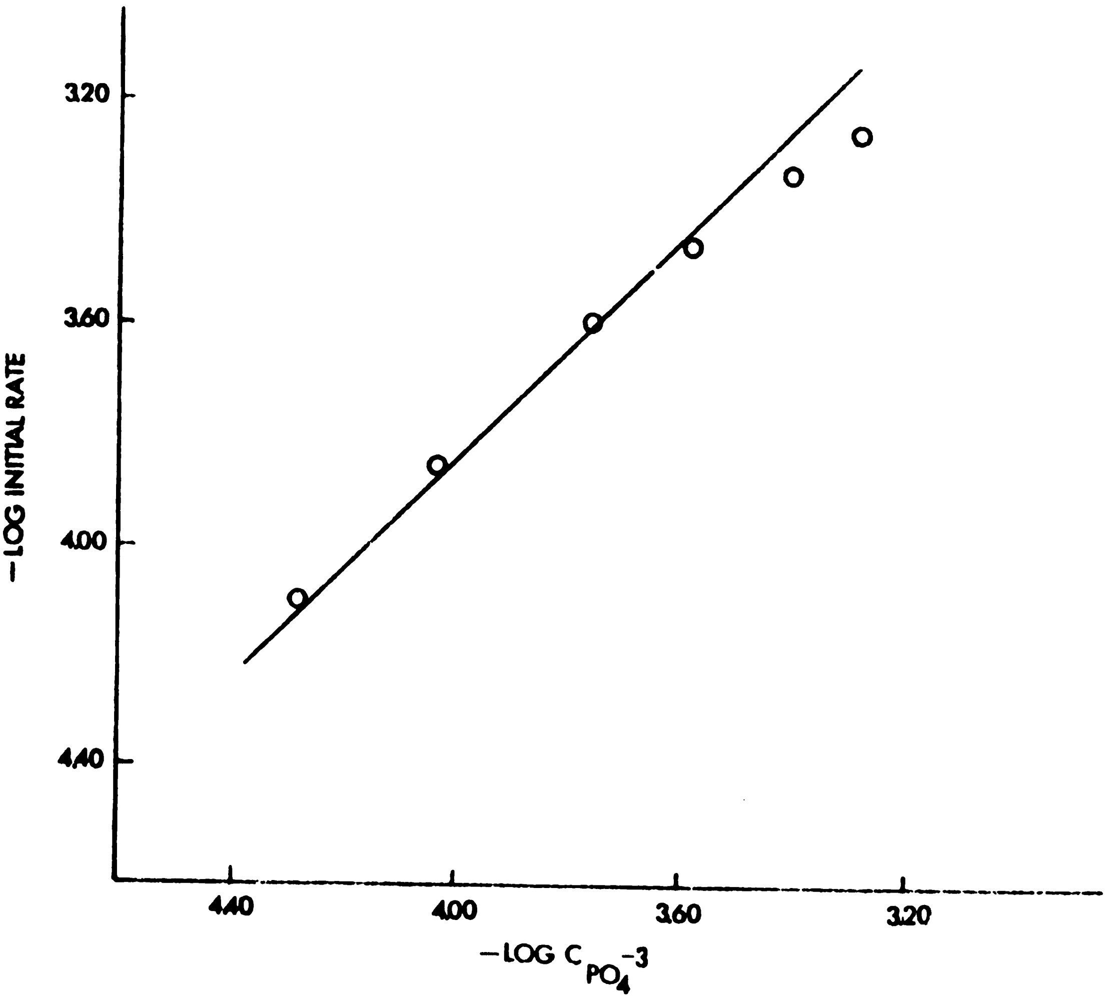


Figure 9. Effect of phosphate on initial reaction rate at low acid concentration.

$C_{HClO_4} = 0.17$ M

$C_{Mo(VI)_t} = 0.02$ M

$C_{PO_4^{-3}} = 5 \times 10^{-5} - 5 \times 10^{-4}$ M reagent blank. Experiments were performed in which no phosphate was added and, indeed, a significant reaction rate was found at 1.008 M $HClO_4$. By plotting the rate vs. concentration data on a linear scale, the reaction rate at zero added phosphate was determined experimentally by extrapolation. When the reagent blank was subtracted from the raw data points, the slope of the log initial rate vs. log $[H_3PO_4]_i$ plot was found to be 1.00 ± 0.05.

In both experiments to determine the phosphate concentration dependence, the reaction was first-order in phosphate concentration up to approximately 3×10^{-4} M phosphate. At higher concentrations, the log-log plot began to curve, and the reaction order became less than unity. The same effect has been observed in the work of Javier, et al. (16) on the formation of 12-MPA in nitric acid solutions. One possible reason for this deviation from first-order kinetics at high phosphate concentrations is that a different heteropoly anion may be forming when the phosphate and Mo(VI) concentrations become comparable. It is well established that large excesses of Mo(VI) over phosphate are necessary to form 12-MPA exclusively (174). Thus, almost all analytical procedures are carried out using excesses of 100-fold or more.

c. Dependence on Mo(VI) Concentration. The variation of the reaction rate with concentration of Mo(VI) is strongly dependent on the acid to Mo(VI) ratio. At high acid to Mo(VI) ratios, i.e. high perchloric acid concentration and low Mo(VI) concentration, the reaction order with respect to Mo(VI) is quite high, as can be seen in Figure 10. At low acid to Mo(VI) ratios, the reaction order with respect to Mo(VI) decreases and reaches a limiting value. The limiting reaction order at low acid to Mo(VI) ratios could be determined from

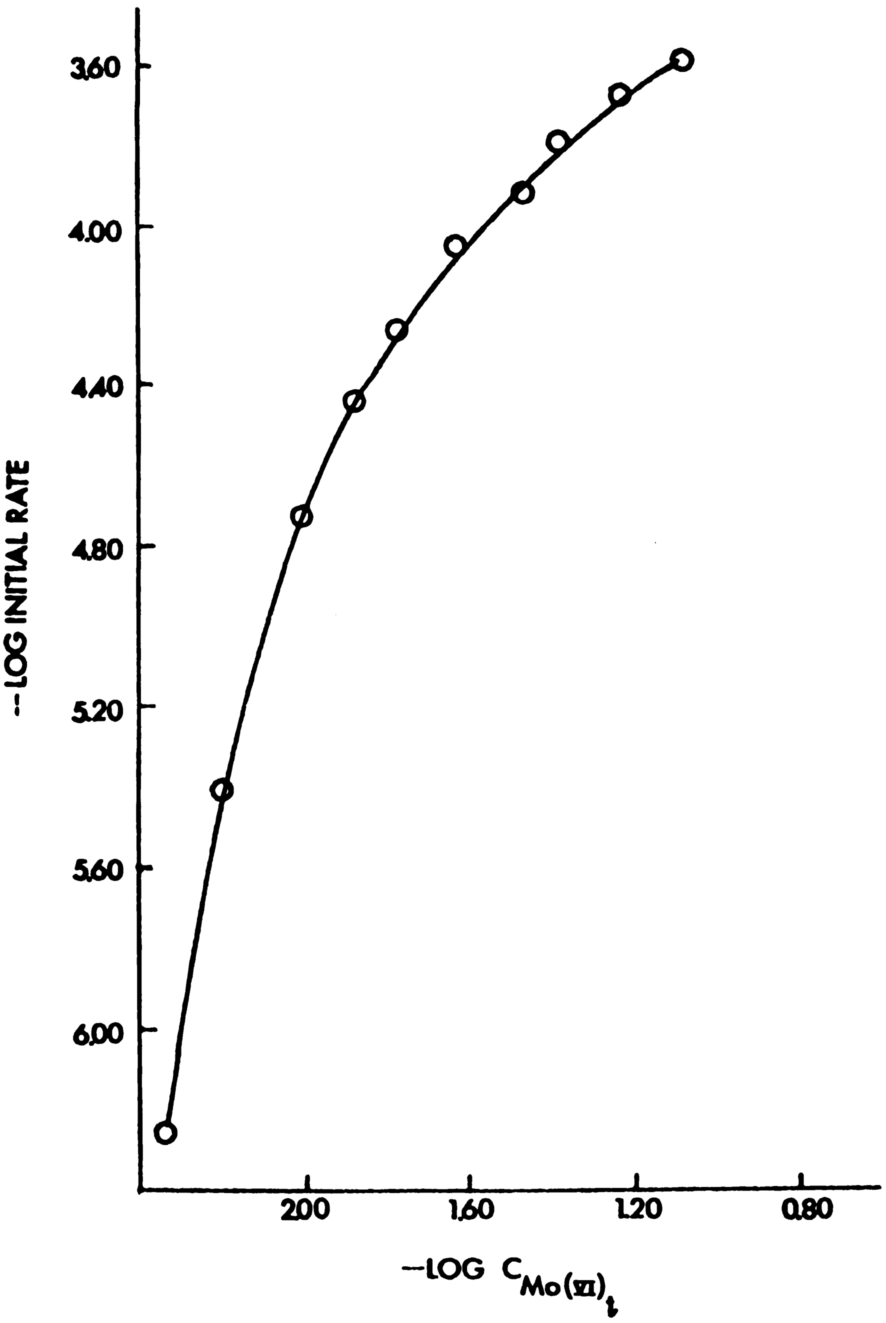


Figure 10. Effect of $Mo(VI)_t$ concentration on initial reaction rate.

$C_{HClO_4} = 0.565$ M

$C_{PO_4} = 1 \times 10^{-4}$ M

$C_{Mo(VI)_t} = 5 \times 10^{-3} - 5 \times 10^{-2}$ M the slope of the log initial rate vs. log $[\text{Mo(VI)}_t]_i$ plots. At an acid concentration of 0.678 M, the reaction order in Mo(VI) was found to be 1.02 ± 0.10 at Mo(VI) concentrations from 0.030 M to 0.050 M. The higher order Mo(VI) dependence at lower concentrations could not be obtained from log-log plots since limiting forms of the rate law could not be isolated. At this concentration of perchloric acid, measurable rates of formation of 12-MPA could not be obtained for Mo(VI) concentrations below 0.005 M, and, thus, the limiting region could not be isolated.

The curve-fitting programs were employed to obtain reaction orders for Mo(VI) which would best fit the experimental data. The data used in comparison were obtained at 0.565 M $HClO_4$ and are represented by the circles in Figure 10. The experimental values shown by the circles are the averages of the results of at least fifteen individual trials. Several different reaction orders for Mo(VI) were tested before a reasonable fit was obtained. At high acid to Mo(VI) ratios, a sixth order dependence was found to agree well with the experimental data, and at low ratios, a first order dependence on Mo(VI) concentration was found. At intermediate values of the acid to Mo(VI) ratio, a fifth-order dependence was determined. This Mo(VI) concentration dependence is represented in Figure 10 by the solid line. Initial Rates were calculated by using rate constants which were determined by a procedure discussed later.

d. Experimental Rate Laws. Based on the reaction orders for Mo(VI) and H^+ which were obtained from the curve-fitting programs, the following rate law was obtained for the formation of 12-MPA in perchloric acid solution:

$$\text{Initial Rate} = \left(\frac{d[\text{12-MPA}]}{dt}\right) = \frac{K_1[H_3PO_4][Mo(VI)_t]}{\dfrac{K_2[H^+]^8}{[Mo(VI)_t]^5} + \dfrac{K_3[H^+]^4}{[Mo(VI)_t]^4} + K_4[H^+]^2 + 1}$$

where $[Mo(VI)_t]$ represents the total concentration of Mo(VI) in solution, and K_1-K_4 are rate constants or ratios of rate constants.

e. Rate Constants. Rate constants were obtained from the curve-fitting programs. Based on the experimental data, the two sets of best fit rate constants or rate constant ratios were found. The values obtained from the acid dependence experiments at 25°C were:

$K_1 = 87.5\ \ell\ \text{mole}^{-1}\ \text{sec}^{-1}$

$K_2 = 2.2 \times 10^{-8}\ \ell^3\ \text{mole}^{-3}$

$K_3 = 7.0 \times 10^{-7}$

$K_4 = 3.6\ \ell^2\ \text{mole}^{-2}$

The other set of constants, which was obtained from the Mo(VI) concentration dependence at 25.0°C, was:

$K_1 = 87.5\ \ell\ \text{mole}^{-1}\ \text{sec}^{-1}$

$K_2 = 1.0 \times 10^{-8}\ \ell^3\ \text{mole}^{-3}$

$K_3 = 2.5 \times 10^{-7}$

$K_4 = 3.6\ \ell^2\ \text{mole}^{-2}$

Comparison of the two sets of rate constants shows that the two values for K_2 differ by a factor of approximately two, and the two K_3's differ by a factor of about three. The differences between these constants can probably be attributed to experimental error. These constants are extremely small, and in order to get the same value from two completely different experiments, extreme measurement reproducibility would be required. Also, if the constants K_2 and K_3 are ratios of rate constants, the differences in the reported constants could be due to extremely small errors in the values of the individual rate constants. Another possible reason for the differences in the two sets of rate constants is that the acid concentration actually present in the Mo(VI) solutions is lower than the value used in calculations because some acid is taken up in the formation of the reacting species of Mo(VI). The value of acid concentration used in the calculations was the amount of acid added.

Initial rates of formation of 12-MPA were calculated from the above rate constants or rate constant ratios and compared to the experimental data. The results for the dependence on acid concentration are shown in Figure 8 where the solid line is drawn through the logarithms of the calculated initial rates. The calculated and experimental values agree within an average of 4%. The Mo(VI) dependence values in Figure 10 agreed within an average of 2.8%. As can be seen in Figure 9, the phosphate dependence data at 0.17 $\underline{M}$ $HClO_4$ agree very well with the calculated initial rates. At 1.008 $\underline{M}$ acid, however, there is some discrepancy between the experimental data corrected for the reagent blank and the calculated values. The error here is probably introduced by the method used to determine the blank rate.

f. Effect of Ionic Strength. The rate law was obtained under conditions where ionic strength was not controlled. Since the concentrations of Mo(VI) and H_3PO_4 were quite small compared to the solution acidity, ionic strength variations only occur when the effect of acid on the reaction rate is studied. To determine if the rather high order in acid could be attributed to ionic strength variations, the rate law and rate constants for the formation of 12-MPA were determined at constant ionic strength. Sodium perchlorate was used to maintain the ionic strength constant at 1.5 M. Rate constants at low acid to Mo(VI) ratios where the reaction obeys second-order kinetics were found to be little influenced by ionic strength. However, rate constants obtained at high acid to Mo(VI) ratios show a definite dependence on ionic strength, which indicates that intermediate steps in the mechanism may involve the reaction of species of different charge. Similar reaction orders to those obtained previously when ionic strength was uncontrolled were obtained in the constant ionic strength experiments.

g. Proposed Mechanism. A mechanism which accounts well for the experimental observations and gives the previously determined stoichiometry (112) of the formation reaction of 12-MPA is given below:

$$H_3PO_4 + HMo_2^{+} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} H_2PMo_2^{-1} + 2H^{+}$$

$$H_2PMo_2^{-1} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} PMo_2^{-3} + 2H^{+}$$

$$PMo_2^{-3} + 4HMo_2^{+} \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} PMo_{10}^{-3} + 4H^{+}$$

$$PMo_{10}^{-3} + HMo_2^{+} \xrightarrow{k_4} PMo_{12}^{-3} + H^{+}$$

where the oxygen atoms in the intermediates above have been omitted because exact molecular formulae are not known. It is assumed that Mo(VI) is present in strong acid solutions as a protonated dimer. This form is used because the stoichiometry indicates that six Mo(VI) species react with one PO_4^{-3} to form 12-MPA, and recent experimental data have given strong evidence for the protonated dimer being present in strongly acidic solutions. Application of the steady state approximation to the intermediates leads to the following rate law:

$$\text{Rate} = \frac{d[\text{12-MPA}]}{dt} = \frac{k_1[H_3PO_4][HMo_2^{+}]}{\dfrac{k_{-1}k_{-2}k_{-3}[H^{+}]^8}{k_2k_3k_4[HMo_2^{+}]^5} + \dfrac{k_{-1}k_{-2}[H^{+}]^4}{k_2k_3[HMo_2^{+}]^4} + \dfrac{k_{-1}}{k_2}[H^{+}]^2 + 1}$$

where $k_1 = K_1$ from the experimental rate law and

$$K_2 = \frac{k_{-1}k_{-2}k_{-3}}{k_2k_3k_4}$$

$$K_3 = \frac{k_{-1}k_{-2}}{k_2k_3}$$

$$K_4 = \frac{k_{-1}}{k_2}$$

It should be noted that this rate law is the same as that which was derived from the experimental data.

h. Interpretation of Mechanism. The formation of 12-MPA in perchloric acid solutions is quite complex as the rate law shows. In the proposed mechanism the first step in the process is the reaction of phosphoric acid with a protonated Mo(VI) dimer to form a 2/1 heteropoly acid which is not completely deprotonated. The di-protonated species dissociates in the next step to form the 2/1 heteropoly anion intermediate. It is rather surprising that the dissociation of the di-protonated intermediate can be seen on the stopped-flow time scale since proton transfer reactions are normally quite rapid. However, the rate constant, k_2, may still be quite large since it is the competition between deprotonation of $H_2PMo_2^-$ and dissociation by acid to reform the starting materials that determines the kinetics. The rate constant ratio for this competition (k_{-1}/k_2) was found to be 3.6 ℓ^2 mole^{-2}. The addition of four more Mo(VI) dimers to the 2/1 heteropoly anion take place at approximately the same rate and cannot be distinguished experimentally. Finally, the 10/1 heteropoly anion reacts with one additional dimer to form the saturated 12/1 heteropoly anion (12-MPA).

i. Temperature Dependence. The dependence of the rate of formation of 12-MPA in perchloric acid solution on temperature was determined by measurement of the initial reaction rate at several different temperatures. The primary purpose of this study was to determine the extent to which temperature must be controlled in order to obtain precise and accurate analytical results. Also, an approximate value for the activation energy of the process was desired.

The experiments were carried out at low acid concentration (0.17 M) under conditions where the initial rate of formation of 12-MPA was first order in both phosphate and Mo(VI) concentrations or

$$\text{Rate} \approx K[H_3PO_4][HMo_2^+]$$

where K is the second-order rate constant. Under these conditions, the initial reaction rate doubled with a temperature increase of approximately 5°C. The second-order rate constants were evaluated at three different temperatures and are presented in Table 4. The natural logarithm of the rate constant was plotted against the reciprocal of the absolute temperature, and an activation energy of 20.55 ± 1.20 kcal/mole was obtained from the least squares slope of the plot.

2. Rate Law in Sulfuric Acid Solutions

a. Experimental Results. The results obtained on the kinetics of formation of 12-MPA in sulfuric acid solutions were similar in many aspects to those obtained in perchloric acid solutions. The dependence of the reaction rate on sulfuric acid concentration is shown in Figure 11. At acid concentrations below 0.20 N, the reaction rate is virtually independent of acid concentration. As in the experiments in perchloric acid solutions, the concentration of sulfuric acid could not be made large enough to isolate a limiting form of the rate law at high acidities. Thus, the curve-fitting programs were used to obtain a best fit acid dependence for sulfuric acid solutions. The best fit at high acid concentrations (0.70-0.88 N) was found to be an inverse sixth-order dependence and a zero-order dependence at low acid concentrations (< 0.20 N). The intermediate concentration range was best fit by the inclusion of terms for an inverse fourth-order dependence and an inverse second-order dependence. Initial rates were calculated by using these reaction orders and the rate constants which will be given later, and the results are plotted as the solid line in Figure 11. The circles represent the experimental points which are the result of at least

Table 4. Temperature Dependence of 12-MPA Formation in Perchloric Acid Solutions

Temperature, °C	K, ℓ mole^{-1} sec^{-1}
25.0	89.0
31.0	170.0
36.9	308.0

Reagent concentrations

$C_{PO_4^{-3}} = 1 \times 10^{-4}\ \underline{M}$

$C_{HClO_4} = 0.17\ \underline{M}$

$C_{Mo(VI)_t} = 0.02\ \underline{M}$

Results are averages of five trials with relative standard deviations of 1-2%.

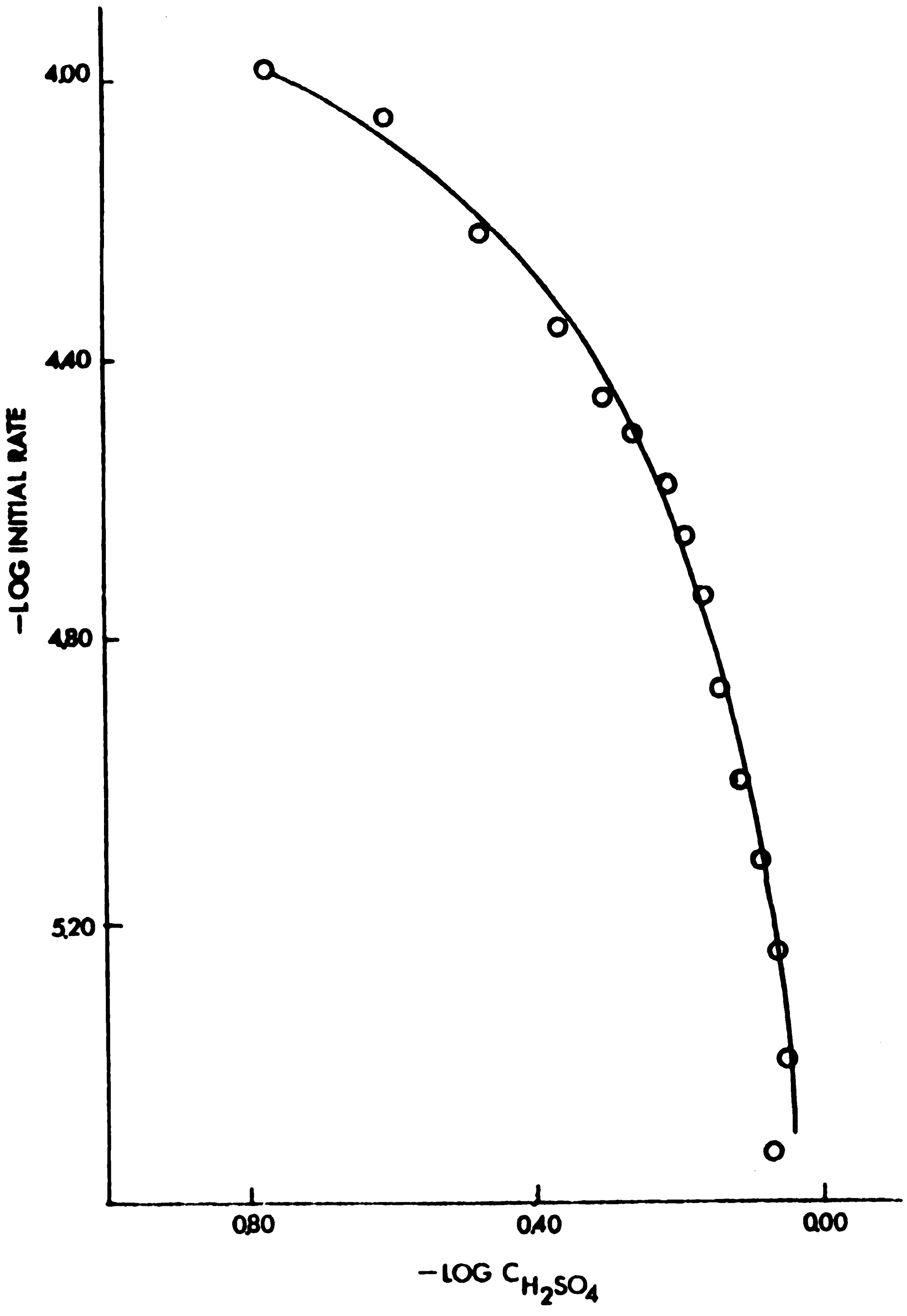


Figure 11. Effect of sulfuric acid concentration on initial reaction rate.

$C_{Mo(VI)_t} = 2 \times 10^{-2}$ M

$C_{PO_4} = 1 \times 10^{-4}$

$C_{H_2SO_4} = 0.176\text{-}0.881$ N

fifteen individual trials. The calculated and experimental initial rates agree within an average of 3.6%, except for the rate determined for the highest acid concentration (0.881 N), where the initial reaction rate and the total change in signal are both very small. Thus,it is very possible that the experimental rate is in large error.

The phosphate dependence at both low and high concentrations of sulfuric acid was found to be first order up to a concentration of 3×10^{-4} $\underline{M}$ phosphate. The results at 0.211 N H_2SO_4 are shown in Figure 12. In 0.211 N H_2SO_4, a reaction order of 0.96 ± 0.01 was obtained, and in 0.881 N H_2SO_4 an order of 1.02 ± 0.06 was determined. In contrast to the perchloric acid experiments, no blank rate was seen, and thus, correction of experimental results was not necessary. At phosphate concentrations greater than 3×10^{-4} $\underline{M}$, again, the reaction order was no longer unity.

The Mo(VI) concentration dependence was also somewhat different in H_2SO_4 solutions than in $HClO_4$ solutions. Due to experimental limitations, again, it was not possible to make measurements in concentration regions where limiting forms of the rate law describe the formation of 12-MPA. Curve-fitting procedures were employed to obtain a fifth-order dependence on Mo(VI) concentration at high acid to Mo(VI) ratios and a first-order dependence at low ratios. The intermediate range was best fit by inclusion of a term which involves a third-order Mo(VI) dependence. Initial rates calculated by means of these reaction orders and the rate constants which will be given later, are represented in Figure 13 by the solid line. The experimental data, represented by circles, were obtained at 0.44 N H_2SO_4. The calculated and experimental data agree within an average of 2.1%.

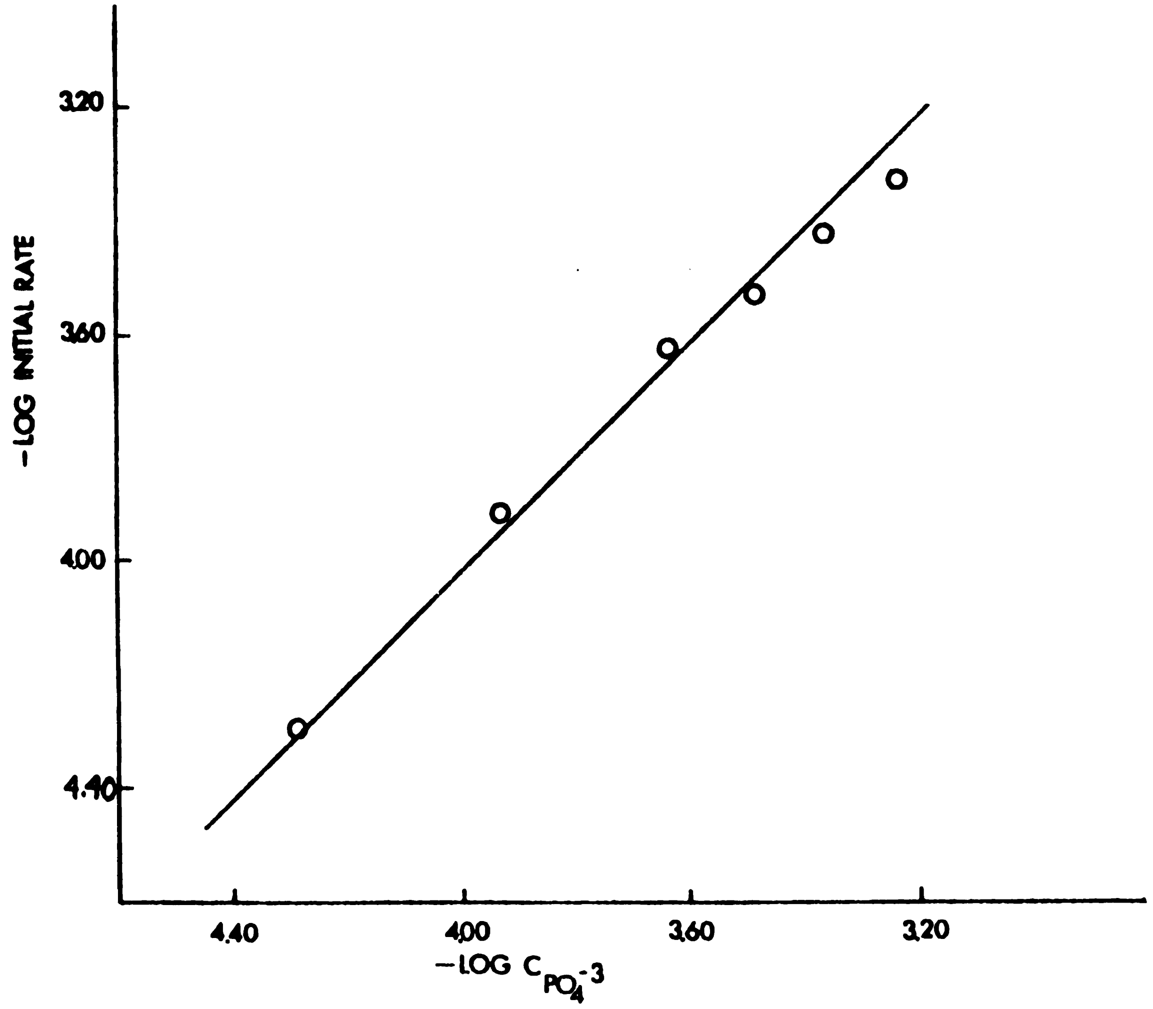


Figure 12. Effect of phosphate at low acid concentration.

$C_{Mo(VI)_t} = 2 \times 10^{-2}$ M

$C_{H_2SO_4} = 0.211$ N

$C_{PO_4} = 5 \times 10^{-5} - 5 \times 10^{-4}$

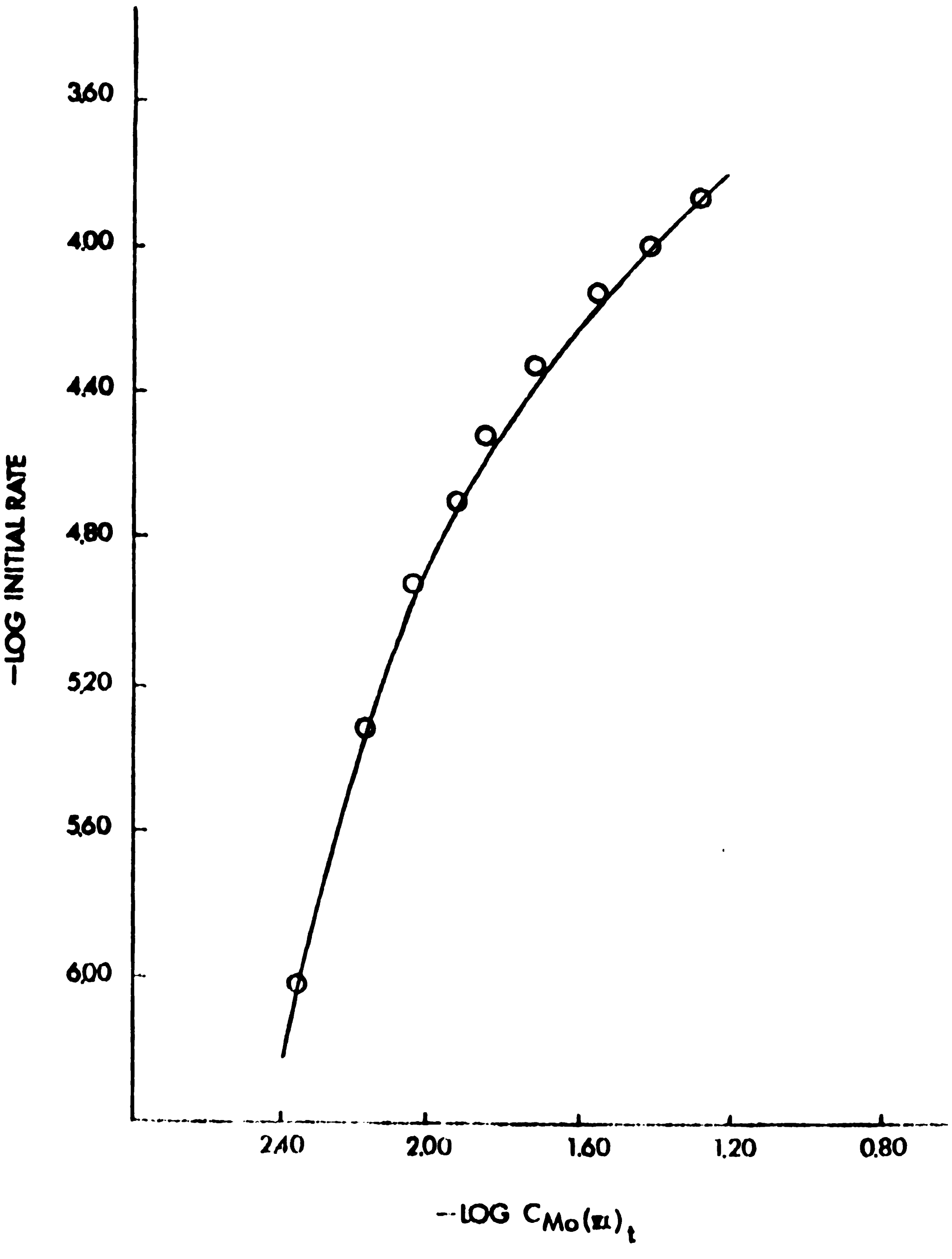


Figure 13. Effect of $Mo(VI)_t$ concentration on initial reaction rate.

$C_{H_2SO_4} = 0.440$ N

$C_{PO_4} = 1 \times 10^{-4}$ M

$C_{Mo(VI)_t} = 5 \times 10^{-3} - 5 \times 10^{-2}$ M

b. Proposed Rate Law. Based on the results of the curve fitting procedures, the following best fit rate law was obtained:

$$\text{Rate} = \frac{K_1[H_3PO_4][HMo(VI)_t]}{\dfrac{K_2[H^+]^6}{[HMo(VI)_t]^4} + \dfrac{K_3[H^+]^4}{[HMo(VI)_t]^2} + K_4\ [H^+]^2 + 1}$$

where K_1-K_4 are rate constants or ratios of rate constants.

c. Rate Constants. Two sets of best fit rate constants or ratios of rate constants at 25°C were obtained from the curve fitting programs. The first set was obtained from the acid dependence data:

$K_1 = 62.0\ \ell\ \text{mole}^{-1}\ \text{sec}^{-1}$

$K_2 = 5.7 \times 10^{-6}\ \ell^2\ \text{mole}^{-2}$

$K_3 = 2.0 \times 10^{-3}\ \ell^2\ \text{mole}^{-2}$

$K_4 = 6.0\ \ell^2\ \text{mole}^{-2}$

The other set was obtained from the data for the Mo(VI) dependence:

$K_1 = 57.0\ \ell\ \text{mole}^{-1}\ \text{sec}^{-1}$

$K_2 = 1.8 \times 10^{-6}\ \ell^2\ \text{mole}^{-2}$

$K_3 = 4.0 \times 10^{-3}\ \ell^2\ \text{mole}^{-2}$

$K_4 = 6.0\ \ell^2\ \text{mole}^{-2}$

Comparison of the two sets of rate constants or rate constant ratios shows that the two K_2's differ by a factor of three, and the two values of K_3 differ by a factor of two. These differences can be attributed to experimental error for the reasons discussed in a previous section.

d. Proposed Mechanism. A reasonable mechanism was derived to fit the rate law which was obtained from the curve fitting programs and is shown below:

$$H_3PO_4 + HMo_2^+ \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} H_2PMo_2^- + 2H^+$$

$$H_2PMo_2^{-1} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} PMo_2^{-3} + 2H^+$$

$$PMo_2^{-3} + 2HMo_2^+ \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} PMo_6^{-3} + 2H^+$$

$$PMo_6^{-3} + 2HMo_2^+ \xrightarrow{k_4} PMo_{10}^{-3} + 2H^+$$

$$PMo_{10}^{-3} + HMo_2^+ \xrightarrow{\text{fast}} \text{12-MPA} + H^+$$

The rate determining step in the mechanism is the formation of the 10/1 heteropoly anion intermediate which then reacts rapidly with one Mo(VI) species to form 12-MPA. Application of the steady state approximation to the intermediates yields the following rate law:

$$\text{Rate} = \frac{d[\text{12-MPA}]}{dt} = \frac{K_1[H_3PO_4][HMo_2^+]}{\dfrac{k_{-3}k_{-2}k_{-1}[H^+]^6}{k_4k_3k_2[HMo_2^+]^4} + \dfrac{k_{-2}k_{-1}[H^+]^4}{k_3k_2[HMo_2^+]^2} + \dfrac{k_{-1}}{k_2}[H^+]^2 + 1}$$

This is the same form as the rate law which was obtained as a best fit to the experimental data where

$$K_1 = k_1$$

$$K_2 = \frac{k_{-3}k_{-2}k_{-1}}{k_4k_3k_2}$$

$$K_3 = \frac{k_{-2}k_{-1}}{k_3k_2}$$

$$K_4 = \frac{k_{-1}}{k_2}$$

e. Interpretation of Results. The first step in the formation of 12-MPA in sulfuric solutions is a reaction between phosphoric acid and one Mo(VI) species, which is assumed to be a protonated dimeric cation, to form a di-protonated intermediate. The next step is a dissociation where the intermediate loses two protons. This step is then followed by two steps which occur at approximately the same rate. Each step is a reaction of a Mo/P intermediate with a single Mo(VI) species. Two more similar steps occur, and a 10/1 heteropoly anion intermediate is formed in a slow, rate-determining step. This species then reacts rapidly with another Mo(VI) dimer to form 12-MPA. It is also possible that 12-MPA is not formed in sulfuric acid solution but that the formation of 10-MPA occurs instead. Both the results of this work and the previously reported equilibrium studies (112) indicate this possibility. A comparison of the mechanisms of 12-MPA formation in sulfuric, perchloric, and nitric acid solutions will be made in the next chapter, and analytical implications of the mechanistic studies will be discussed.

f. Effect of Ionic Strength. The above experimental results were obtained with solutions in which ionic strength was not controlled. Results obtained from solutions which were adjusted to a constant ionic strength of 1.5 M with $NaClO_4$ were not significantly different from those obtained from solutions with no ionic strength control.

g. Temperature Dependence. The dependence of the initial rate of formation of 12-MPA on temperature was determined as described previously. The reaction rate doubled with an increase in temperature of approximately 5°C. The second order rate constants obtained from the data are presented in Table 5. An activation energy of 20.39 ± 1.32 kcal/mole was obtained from the least squares slope of the plot of ℓn k vs. 1/T. The activation energies obtained in the two different acid media are the same within the limits of experimental error.

Table 5. Temperature Dependence of 12-MPA Formation in Sulfuric Acid

Temperature, °C	K, ℓ mole^{-1} sec^{-1}
25.3	63.5
30.6	107.0
36.0	192.0

Reagent concentrations

$C_{PO_4^{-3}}$ 1 X 10^{-4} $\underline{M}$

$C_{H_2SO_4}$ 0.176 N

$C_{Mo(VI)_t}$ 0.02 $\underline{M}$

Results are averages of five trials with relative standard deviations of 1-2%.

V. THE 12-MOLYBDOPHOSPHORIC ACID FORMATION REACTION: COMPARISON OF KINETICS AND ANALYTICAL IMPLICATIONS

Complete equilibrium and reaction rate studies have now been carried out on the formation of 12-molybdophosphate in nitric acid, perchloric acid, and sulfuric acid solutions. The present work reports rate studies in perchloric and sulfuric acid media, while previous work has reported rate studies in nitric acid (16) and equilibrium studies in all three acids (112). The rate laws and mechanisms derived from the experimental results in each medium show several similarities as well as many differences. Some trends are also observed. The results also yield much information of value to analytical chemists since these three acids are the primary media for analytical procedures for the determination of phosphate. Based on the results of these rate and equilibrium studies, an analytical chemist who is faced with a phosphate analysis can choose the optimum reaction conditions for the particular analysis. The choice of reaction conditions can thus be based on sound experimental evidence rather than on empirical observations. In this chapter, a comparison of the rates and mechanisms of the formation of 12-MPA in the three different acid media will be presented along with a comparison of the rate information with previous equilibrium information. In addition, some of the analytical implications of the results will be presented.

A. Comparison of Results

1. Rate Laws and Proposed Mechanisms

The rate laws and proposed mechanisms for the formation of 12-MPA in the three different acids have many similarities as well as several striking differences. In all three acids, a simple second-order reaction is obtained at low acid concentrations. Thus at low acid concentrations, the rate determining step in all three acids is the same. This step appears to involve the reaction of one Mo(VI) dimeric species with phosphoric acid. This is an important observation since several previous workers had speculated that polymerization of Mo(VI) was necessary prior to reaction with phosphate. All the results obtained thus far indicate that the polymerization process occurs after the initial reaction of phosphoric acid with the Mo(VI) dimer.

The first intermediate formed in all three acids appears to be a 2/1 heteropoly anion, although the anion appears to be protonated in sulfuric and perchloric acids. This is postulated because the rate laws in these two acids contains an inverse second order acid term which is not present in the rate law in nitric acid. This inverse second order term, unlike the other acid terms, does not depend on the acid to Mo(VI) ratio. Since ratio terms in the denominator of a rate law indicate competition of two species with a reactive intermediate, the lack of such a ratio indicates either a dissociation step (deprotonation) or possibly competition of two protons with the solvent for the reactive intermediate. The lack of an inverse second order acid term in the nitric acid case means either the 2/1 tribasic anion is formed directly or the dissociation of its protonated form is extremely rapid compared to the reverse reaction of the first step, i.e. acid dissociation of the 2/1 intermediate to form the starting materials.

After formation of the tribasic 2/1 anion, all the proposed mechanisms involve the addition of five more Mo(VI) dimers to form the 12-molybdophosphate anion. In some of the media, the individual steps in the polymerization process can be observed and thus the rate laws are rather complex. In nitric acid, however, only the addition of the second Mo(VI) dimer to form the 4/1 intermediate can be seen. Thus the highest order in Mo(VI) is second-order, and the highest acid dependence is inverse fourth-order. The acid dependence in the rate law appears in the ratio $[H^+]^4/[Mo(VI)_t]$ in the denominator which indicates the competition of four protons with one Mo(VI) dimer for the 2/1 intermediate.

In perchloric acid and sulfuric acid media, the acid and Mo(VI) dependencies are more complex. An inverse fourth order acid term is observed in both rate laws. However, the molybdate dependence in the ratio is different in the two acids. The ratio $[H^+]^4/[Mo(VI)_t]^4$ appears in the perchloric acid rate law, while the term $[H^+]^4/[Mo(VI)_t]^2$ appears in the sulfuric acid rate law. In perchloric acid, these ratios are interpreted to indicate that four protons compete with four Mo(VI) dimers for the 2/1 intermediate in perchloric acid, while four protons compete with two Mo(VI) dimers for the 2/1 intermediate in sulfuric acid.

A final high order acid to Mo(VI) ratio term appears in the perchloric and sulfuric acid rate laws. The term is $[H^+]^8/[Mo(VI)_t]^5$ in perchloric acid and $[H^+]^6/[Mo(VI)_t]^4$ in sulfuric acid. Since the overall stoichiometry is most likely

$$H_3PO_4 + 6HMo_2^+ \rightarrow 12\text{-MPA} + 9H^+$$

these different dependencies are easily explained. In $HClO_4$, the final step, the addition of one Mo(VI) dimer to the 10/1 intermediate heteropoly anion, appears to be rate determining. Thus the highest acid dependence is inverse eighth order, and the highest Mo(VI) dependence is sixth order. In H_2SO_4, the formation of the 10/1 intermediate anion appears to be rate determining, while the final polymerization step appears rapid. Thus the highest acid dependence is inverse sixth-order, and the highest molybdate dependence is fifth order. An alternative explanation for the sulfuric acid reaction is that the product formed is not the 12/1 product but a 10/1 product with the inclusion of sulfate ions in the heteropoly structure. This latter explanation is supported by the experimentally determined stoichiometry in H_2SO_4, which involved only five Mo(VI) dimers instead of the expected six (112). However, further experiments would be necessary to test which of these explanations is correct.

2. Rates of Formation

The rates of formation of 12-MPA in the three different acid media were found to be different. Since the mechanisms in all three media involved an initial reaction of phosphoric acid with a single Mo(VI) dimer, it is convenient to compare the rates of formation of 12-MPA in terms of the second order rate constant for this step. The rate constants were determined under conditions of low acid concentrations where the acid dependence is zero-order. The rate constant previously obtained in nitric acid solutions was 22.0 ℓ $mole^{-1}$ sec^{-1} at 23.6°C (16). In sulfuric acid, two values were obtained, 57.0 ℓ $mole^{-1}$ sec^{-1} and 62.0 ℓ $mole^{-1}$ sec^{-1} from the Mo(VI) dependence and the acid dependence data, respectively at 25.0°C. In perchloric acid solutions, the value obtained was 87.5 ℓ $mole^{-1}$ sec^{-1} at 25.0°C. Since two different temperatures are involved, only relative magnitudes can be compared. Under conditions of zero-order acid dependence, formation of 12-MPA proceeds fastest in perchloric acid solutions and slowest in nitric acid solutions. Sulfuric acid appears to be intermediate.

3. Equilibrium Constants

Equilibrium studies on 12-MPA in nitric, sulfuric, and perchloric acid solutions were previously reported (112). Although equilibrium constants have not been reported because of a lack of knowledge of what the exact reactive species are, the reaction to form 12-MPA proceeds farthest in perchloric acid solution and least in sulfuric acid solution. It is interesting that the trend in equilibrium constants for 12-MPA formation is $HClO_4 > HNO_3 > H_2SO_4$, while the trend for second order rate constants is $HClO_4 > H_2SO_4 > HNO_3$.

4. Influence of Anion

One of the major reasons for undertaking the present study was to determine if the anion of the acid had any effect on the rate and mechanism of formation of 12-MPA. It appears that the anion of the acid has little effect on the mechanism of formation of 12-MPA, except the possible stabilization of some of the intermediates, probably through the formation of complexes. The anion, however, does appear to influence the second-order rate constant. One explanation could be the formation of cationic molybdenum complexes with the acid anion. This process should occur least in perchloric acid since the perchlorate ion does not readily form complexes. The process would be more important in nitric and sulfuric acids. It is possible that the formation of complexes would retard the rate of formation of 12-MPA. It is also possible that the final product formed in sulfuric acid is not 12-MPA but a 10/1 product.

5. Activation Energies

The activation energies obtained from the temperature dependence of the second order rate constant in perchloric and sulfuric acid solutions were the same within experimental error. This indicates that the first step of the mechanism involves the same energy barrier. Of course, this fact indicates nothing about the anion dependence of the remaining steps of the reaction.

B. Analytical Implications

The results of the rate studies on the formation of 12-MPA in nitric acid, sulfuric acid, and perchloric acid solutions yield several considerations of importance in the formulation of analytical procedures for the analysis of phosphate. The information is of value whether an initial reaction rate procedure or a conventional equilibrium based method is desired. In this section some of the important considerations with regard to reagent concentrations and the choice of acidic media in which to carry out the reaction will be presented. A discussion of for the choice of experimental conditions will follow.

1. Reagent Concentrations

Based on the results of the rate studies presented above, the actual reagent concentrations as well as the concentrations of reagents relative to each other must be carefully considered in analytical procedures. The acid concentration should be carefully controlled since the rate and equilibrium show a great and complex dependence on acid concentration. Procedures which involve solvent extraction of 12-MPA do not require such careful control since the extraction shifts the equilibrium to the right. Reaction rate analyses should be performed at low acid concentrations so that the maximum possible rate may be obtained. Also, control of solution acidity is not nearly so critical at low acid concentrations where the rate becomes independent of acidity. Equilibrium based analyses should also be performed at low acid concentrations because of a more favorable equilibrium.

For both equilibrium based and reaction rate methods of analysis, the choice of Mo(VI) concentration should be carefully considered. Reactions should be conducted under conditions of low acid to Mo(VI) ratio where the reaction proceeds rapidly and the equilibrium is favorable. Secondly, in reaction rate methods the Mo(VI) concentration should be present in at least a 100-fold excess over phosphate in order to assure that the reaction will exhibit a first order dependence on phosphate concentration. Unusual kinetic behavior was observed when the concentrations of phosphate and molybdate were of comparable magnitude. It is possible that a second heteropolymolybdate is formed at these levels.

2. Experimental Conditions

In order for reproducible and accurate data to be obtained from reaction rate methods of analysis, experimental conditions must be carefully controlled. The results of the rate studies on the formation of 12-MPA in strong acid solutions support this limitation. The rates of formation in perchloric acid and sulfuric acid solutions doubled with a temperature rise of approximately five degrees. Thus, small variations in temperature would yield a few percent error in the rates. The effect of ionic strength on the rates of formation of 12-MPA was not large. Therefore, the ionic strength does not have to be exactly equal in different samples.

Sample preparation is a relatively important experimental problem. Many samples must be dissolved in or treated with acids or bases to prepare the material for analysis. Since the formation of 12-MPA is very dependent on acid concentration, this concentration should be known exactly. Thus, if a sample preparation involves use of acid or base, the sample solution should be neutralized before it is subjected to analysis.

In summary, some generalizations about which acid medium to use in a given application can be made. The use of perchloric acid medium for the formation of 12-MPA is not recommended. In this acid, the greatest rate of formation is obtained for reaction rate analyses, and the greatest concentration of 12-MPA at equilibrium is found. However, perchloric acid also introduces a reagent blank for which compensation must be made. Thus, the choice of the acid to use involves either sulfuric or nitric acid. Sulfuric acid appears most suitable for reaction rate methods because faster rates are obtained in sulfuric acid than in nitric acid. Nitric acid appears to be the choice for equilibrium based methods because the equilibrium is more favorable.

VI. FUTURE PERSPECTIVES

A. General Considerations

In many analytical laboratories in fields such as clinical chemistry and environmental chemistry, chemists are faced with the analysis of a large number of real samples in a short amount of time. Often the results of the analysis are very critical and must be obtained almost immediately. Thus, conventional methods of analysis, which may include several separation and concentration procedures that are necessary to transform a substance into a form ready for analysis, are highly undesirable. Much of the current research in analytical chemistry has been directed toward the development of rapid and selective methods of analysis and the development of highly automated analytical instrumentation. This present work is well in accord with these trends.

Analytical procedures based on the measurement of the initial rate of a reaction have several advantages over the conventional equilibrium based methods of analysis. In many cases they are more rapid and more selective, and they also have the advantage of using relative measurements rather than absolute measurements. These advantages far outweigh the limitations, such as the necessity of careful control of reaction conditions, since several of the limitations can be overcome by the introduction of more sophisticated instrumentation. Initial reaction rate methods of analysis are widely used in fields such as clinical chemistry, but these applications are based on reactions with half lives of several minutes. For any large number of samples, these procedures are not desirable because of the relatively long measurement time. Therefore, one would prefer to develop reaction rate methods based on reactions with half lives of at most a few seconds, but for the study of these reactions, special rapid mixing techniques are required.

B. Improvements in Stopped-Flow Instrumentation

The stopped-flow method is suitable for the study of reactions with half lives down to a few milliseconds. Several commercial stopped-flow mixing systems have become available recently, but these require large numbers of manual operations and are quite tedious to use for any large number of samples. In order for the stopped-flow method to become more widely applicable in analytical chemistry, more highly automated stopped-flow instrumentation must be developed.

In the present work, an automated stopped-flow spectrophotometer, which represents a significant improvement over the commercial systems now available, was designed and evaluated. It features solenoid actuated pneumatic valves for directing the liquid flow and a pneumatic syringe drive system for rapid mixing. The entire operating cycle is actuated by pulses from a digital sequencer or, if desired, by manual switches. The flow system is vertical to minimize problems with air bubbles and has a mixing time and dead time comparable to those of instruments which are commercially available. One further advantage of this system is the ease of sample handling. This system has been shown to be useful for rate methods of analysis based on reactions of widely varying rates.

As a result of the work reported in this thesis, several improvements in the automation of the stopped-flow method for analytical purposes and for fundamental investigations have become apparent.

1. Computerization of Stopped-Flow Instruments

The most obvious of the improvements is the complete computerization of the stopped-flow system. The hardware sequencer which is used to control the valve changes, the flow initiation, and the measurement cycle can be either replaced by software or be controlled by the computer. The data acquisition and data treatment procedures should be placed under computer control. Both for fundamental studies and for analytical applications, the computer can be used to obtain initial reaction rates from least squares analysis of the raw acquired data. Ensemble averaging can easily be used to improve the signal-to-noise ratio with a computerized system. Finally, the computer can be used to make a significant contribution to the quality of the data. As previously mentioned, temperature fluctuations in the flow system remain an important concern. If the temperature in the observation cell can be measured at very nearly the same time spectrophotometric data are acquired, the computer can be used to correct the data for temperature variations. Likewise, variations in other parameters, such as light source fluctuations can be corrected for by computer techniques.

2. Reagent Premixing System

A second modification of the present stopped-flow system, which would increase its utility in fundamental studies and in analytical applications, would be the development of a computer-controlled premixing system, which would allow as many as four different reagent concentrations to be varied just prior to entering the drive syringes. Presently available stopped-flow systems allow the mixing of only two reagents, although some systems allow different volume ratios to be mixed. A more versatile approach, particularly with the present, fixed-volume apparatus is to premix reagents before initiating the reactions. Such a premixing unit should also find utility in using reagents of limited stability if premixing, filling the drive syringes, and initiation of reactions can be accomplished rapidly.

One attractive premixing system is an electromechanical system which uses stepping motors to drive syringes to add the reagents to the reservoir bottles. This system is attractive because of the ease with which it can be operated from digital signals provided by the computer. When operated under computer control, the preparation of reagents for the next stopped-flow run can be proceeding while data from the previous run are being acquired. Such a system should also allow much more efficient design of experiments. Use of factorial design schemes could easily be programmed, and the effects of variations in a wide variety of reaction conditions could be easily measured. In addition, entire rate studies could be accomplished, in principle, during one working time period.

3. Automated Sample Pick-Up System

For routine analytical applications of fast kinetics, the extreme versatility of the computer-controlled premixing system is probably unnecessary. Once the procedures have been established, conditions are normally arranged so that one composite reagent is made which contains everything but the analyte. A more useful sample handling system for routine applications would consist of a sample turntable with a pneumatically operated pick-up system. The hardware sequencer described in this work has provision for adding steps to move a sample turntable.

4. Improvements in Optics and Electronics

The preliminary results obtained from the stopped-flow spectrophotometer indicated that the measurements appeared to be significantly limited by shot noise in the detection system. Several methods of improving the precision of the results are possible through improvements in optics and electronics in the detection system.

Most of these improvements involve increasing the radiant power throughput which, for most stopped-flow systems, is low because small diameter observation cells are usually used for low dead volume, and illumination of the observation cell is rarely optimized. There are several important reasons for improving the radiant power. If shot noise turns out to be an important contributor to the total system noise, the signal-to-noise ratio will improve with increasing radiant power. If shot noise is the limiting noise source, the signal-to-noise ratio improves with the square root of the radiant power striking the photocathode. Also, if the incident flux to the transducer can be increased far enough to be in the useful region of a photodiode, an improvement in signal-to-noise ratio will result.

Several approaches may be utilized to increase the radiant power through the observation cell. Higher intensity sources can be used, the monochromator can be replaced by optical filters, or higher efficiency optical transfer systems can be developed. A quartz iodine lamp should provide sufficient radiance for high precision measurements, but since these sources often lack the stability of lower intensity sources, an optical feedback system of some type should be employed. For high efficiency transfer of the radiant power from the monochromator exit slit to the observation cell, quartz fiber optics or a tapered light pipe should be investigated.

C. New Fast Reaction-Rate Methods

Applications of reaction rate methods of analysis based on fast reactions are not widespread at the present time. Thus, much research should go into the development of more rapid reaction rate analyses. Typical examples of reactions which occur within the time range of the stopped-flow method are fast complexation reactions such as the formation of peroxy complexes of vanadium, chromium, and titanium and the formation of o-phenanthroline complexes of iron, copper, and certain other transition metals. Another interesting application for fast reaction rate methods would be the simultaneous determination of two or more substances which react with a common reagent. A good example is the determination of phosphorus and silicon based on the rates of formation of 12-MPA and 12-molybdosilicic acid (12-MSA). The rate of 12-MSA formation is much slower than 12-MPA formation; thus, reaction conditions could be chosen where the 12-MPA reaction is virtually complete before the 12-MSA reaction occurs to a significant extent. The development of these analytical procedures should not be based on empirical observations, as is often the case, but instead should be derived from a thorough knowledge of the kinetics and equilibria involved in the chemical system. The present work involves a study of the formation of 12-MPA in strong acid solution, and the results of this work provide sound experimental evidence from which new analytical procedures for phosphate analyses may be derived. In addition, the experimental approach which was used in this study can serve as a model for the type of study which should procede the development of new rapid reaction rate methods of analysis. The rate laws obtained in this study are essentially correct, but large differences between the calculated and experimental rates in some regions of the acid and Mo(VI) dependence curves are observed. It is possible that these regions could be best fit by the addition of more acid to Mo(VI) ratio terms. Further application of KINFIT should elucidate a more complete rate law and give a complete statistical analysis of the data.

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APPENDICES

APPENDIX A: BASIC/RT PROGRAM FOR DATA ACQUISITION FROM THE STOPPED-FLOW SPECTROPHOTOMETER

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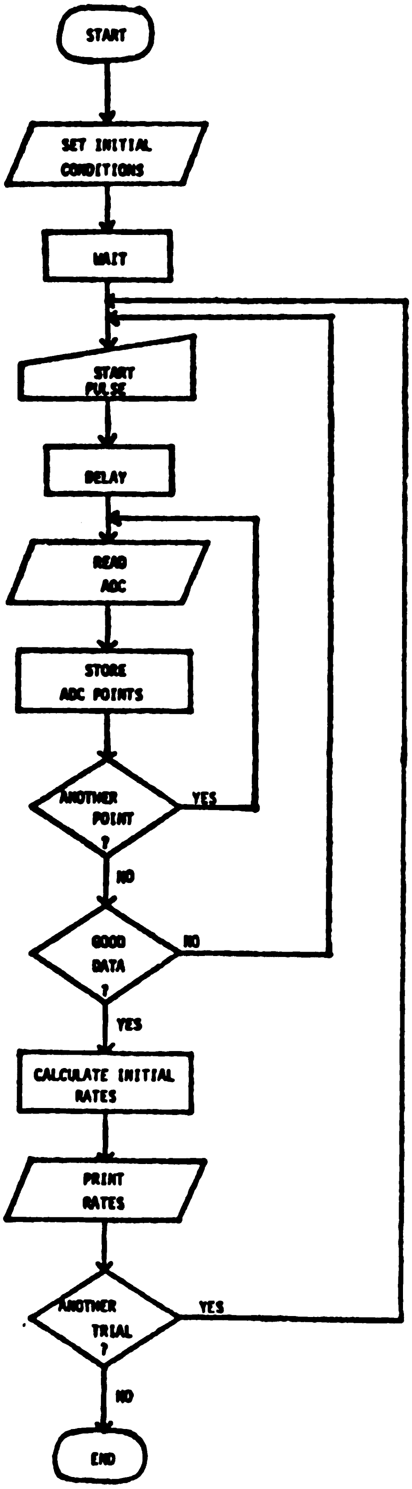
1 REM PROGRAM FOR STOPPED-FLOW KINETICS: DATA ACQUISITION
5 PRINT "DELAY TIME";
10 INPUT D,D1
15 PRINT "CLOCK RATE";
20 INPUT R
25 PRINT "ADC SAMPLING RATE";
30 INPUT S
35 PRINT "NUMBER OF ADC POINTS PER TRIAL";
40 INPUT B
45 PRINT "TIME BETWEEN ADC POINTS IN MSEC";
50 INPUT U
55 PRINT "VOLTAGE OFFSET";
60 INPUT Y
65 PRINT "AVERAGE 100% T = ";
70 INPUT C
73 PRINT
75 PRINT "OK---PUSH THE BUTTON!!"
100 SET RATE 1, 1
105 WAITC
110 SET RATE 7, 0
115 SET RATE D, D1
120 WAITC
125 SET RATE 7, 0
130 DIM E(50)
135 DIM B2(50)
140 REAL TIME E,1,1,B
145 ACCEPT
150 SET RATE R,S
155 IF TIM(0) B THEN 155
160 REJECT
161 PRINT
162 PRINT "IS THIS TRIAL GOOD";
163 INPUT G1
164 PRINT
165 IF G1=1 THEN 168
166 GO TO 75
167 PRINT
168  PRINT "FINAL TIME,SEC"," DA/DT"
169 PRINT
170 FOR K=0 TO B-1
175 LET B2(K)=0
180 LET B2(K)=B2(K)+C/(Y+ADB(1))
```

```
181 LET B2(K)=LOG(B2(K))/2.303
182 NEXT K
185 FOR K=0 TO B-2
190 LET B3=0
195 LET B3=B3+(B2(K+1)-B2(K))/((U*(K+1)-U*K)*1.000000E-03)
200 PRINT U*(K+1)*1.000000E-03,B3
205 NEXT K
206 PRINT
210 GO TO 75
500 END
```



Flow Chart of Data Acquisition Program

APPENDIX B: BASIC CURVE-FITTING PROGRAMS

```
10 REM CALCULATIONS USING RATE LAWS---ACID DEPENDENCE
20 PRINT "K1=";
21 INPUT A
25 PRINT "K2=";
26 INPUT B
30 PRINT "K3=";
31 INPUT C
35 PRINT "K4=";
36 INPUT D
40 PRINT "MO CONCENTRATION";
41 INPUT E
45 PRINT "ACID CONCENTRATION";
46 INPUT F
50 PRINT "HOW MANY CALCULATIONS";
60 INPUT G
65 PRINT
70 PRING "H", "REXP", "LOG RE", "RCAL", "LOG RC"
80 FOR H=1 TO G
90 READ V,Z
100 LET I=0
101 LET O=0
102 LET P=0
103 LET Q=0
111 LET O=O+B*V↑8/E↑4
112 LET P=P+C*V↑4/E↑2
113 LET Q=Q+D*V↑2
115 LET I=I+A*E/(O+P+Q+1)
120 PRINT V,Z*10000,LOG(Z*10000)/2.303,I,LOG(I)/2.303
130 NEXT H
202 DATA .176
203 DATA .264
204 DATA .352
205 DATA .44
206 DATA .484
207 DATA .428
208 DATA .572
209 DATA .616
210 DATA .66
211 DATA .704
212 DATA .748
213 DATA .793
214 DATA .837
215 DATA .881
500 END
```

```
10 REM CALCULATIONS USING RATE LAWS---MOLYBDATE DEPENDENCE
20 PRINT "K1=";
21 INPUT A
25 PRINT "K2=";
26 INPUT B
30 PRINT "K3=";
31 INPUT C
35 PRINT "K4=";
36 INPUT D
40 PRINT "MO CONCENTRATION";
41 INPUT E
45 PRINT "ACID CONCENTRATION";
46 INPUT F
50 PRINT "HOW MANY CALCULATIONS";
60 INPUT G
65 PRINT
70 PRINT "MO", "RCAL", "LOG RCAL"
80 FOR H=1 TO G
90 READ V
100 LET I=0
101 LET O=0
102 LET P=0
103 LET Q=0
104 LET O=O+B*F↑8/V↑4
105 LET P=P+C*F↑4/V↑2
106 LET Q=Q+D*F↑2
110 LET I=I+A*V/(O+P+Q+1)
120 PRINT V,I,LOG(I)/2.303
130 NEXT H
198 DATA 3.000000E-03
199 DATA 5.000000E-03
200 DATA 6.000000E-03
201 DATA 7.500000E-03
202 DATA .01
203 DATA .0125
204 DATA .015
205 DATA .02
206 DATA .025
207 DATA .03
208 DATA .035
209 DATA .04
210 DATA .045
211 DATA .05
500 END
```

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