I. A LIMITED KINETIC STUDY OF THE Mn(II) CATALYZED OXIDATION OF MALACHITE GREEN BY PERIODATE

II. SOME COMPARATIVE STUDIES ON DATA HANDLING PROCEDURES FOR THE VARIABLE TIME KINETIC METHOD OF ANALYSIS

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I. A LIMITED KINETIC STUDY OF THE Mn(II) CATALYZED OXIDATION OF MALACHITE GREEN BY PERIODATE

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PART I

A LIMITED KINETIC STUDY OF THE Mn(II) CATALYZED OXIDATION OF MALACHITE GREEN BY PERIODATE

CHAPTER I

INTRODUCTION

Kinetic methods of analysis are now becoming more widely accepted in analytical chemistry. Some of the possible advantages offered by kinetic methods in comparison to equilibrium methods are 1) selectivity, 2) speed of analysis, 3) sensitivity, and 4) a lower limit of detection. The basis for kinetic methods has long been known, but application has waited for improved instrumentation.

Kinetic methods are often employed for the determination of low concentrations of metal ions which have a catalytic effect on otherwise slow indicator reactions. Low concentrations of ligands or other rate modifying constituents can also be determined utilizing the catalytic reaction and the effects of chelate (metal-complex) catalysis, ligand promotion, and ligand inhibition (1). The reaction conditions are usually adjusted so that the reaction is first order in the monitored species and initial catalyst concentration, though not necessary, and pseudo zero order with respect to species other than the one(s) to be monitored.

Some kinetic methods of analysis have been developed under a set of reaction conditions without optimizing reaction parameters. Possible improvement of these methods with respect to sensitivity, selectivity, and limit of detection by varying experimental parameters becomes an area of interest. The kinetic studies of the reaction order with respect to reactants, stoichiometry, ionic strength effect, temperature effect, and product indentification are some of the more commonly employed studies to obtain insight into possible reaction mechanisms (2). With the insight provided by these studies, method improvements can be proposed and tried (3).

The reaction involving the oxidation of malachite green cation (MG^+) by periodate, catalyzed by Mn(II), was first employed by Fernandez, Sobel and Jacobs for the determination of submicrogram quantities of manganese. This method was applied to the determination of Mn(II) in human serum (4). This same reaction was later employed in other methods. Mottola (5) determined microgram amounts of aminopolycarboxylic acids by catalytic end point indication, Mottola and Harrison (6) increased the sensitivity and detectibility for Mn(II) in the original method by utilizing the accelerating effect that nitrilotriacetic acid (NTA) had on the original reaction. Mottola and Heath (7) utilized the accelerating effect of NTA for a variable time kinetic determination of microgram and submicrogram amounts of NTA. Fakasawa and Yamane (8) modified the original conditions and used the reaction to determine manganese in high-purity silicon, hydrofluoric acid, and nitric acid. Although the original reaction was widely employed, kinetic information available for providing insight into adjusting reaction conditions was sparse. A limited kinetic study was to be conducted as part of this research, with the hope of providing such information.

CHAPTER II

KINETIC STUDIES

Reagents

Malachite Green Perchlorate

Prepared by Bailey (6) by precipitation of the perchlorate by mass action from the hydrochloride.

Manganese Sulfate, Monohydrate

(J. T. Baker Chemical Company, Phillipsburg, N. J.) Reagent grade was used without further purification.

Sodium Meta Periodate

(Fisher Scientific Company, Fair Lawn, N. J.) Reagent grade was used without further purification.

Phosphate Buffer (pH 4.7)

68.40 gms NaH_2PO_4 and 1.00 gms Na_2HPO_4 were placed in a 500 ml volumetric flask and diluted to the mark. The NaH_2PO_4 was purified by precipitation from saturated aqueous solution.

Acetate Buffer (pH 5.0)

52.18 gms of sodium acetate and 21.02 ml of glacial acetic acid were placed in a 1000 ml volumetric flask and diluted to the mark.

Eastman Chromatogram Sheet for TLC

(Distillation Products Industries, Rochester, New York) type k301R (silica gel with fluorescent indicator).

Sodium Perchlorate, Anhydrous

(G. Frederick Smith Chemical, Columbus, Ohio) Reagent grade was used without further purification.

Potassium Iodide

(J. T. Baker Company, Phillipsburg, N. J.) Reagent grade was used without further purification.

Perchloric Acid ($\simeq$ 0.1N)

(Allied Chemical, Morristown, N. J.) Reagent grade 70% solution was used without further purification.

Chromotropic Acid Sodium Salt

(Fisher Scientific Company, Fair Lawn, N. J.) Reagent grade was used without further purification.

Benzidine Hydrochloride

(Harleco, Philadelphia, PA) Reagent grade was used without further purification.

Water

The water used throughout the investigation was purified by redistilling de-ionized distilled water through an all borosilicate still equipped with a quartz immersion heater.

Acetate and Phosphate Effects on the Mn(II) Catalyzed Oxidation of MG^+ by Periodate

The reaction for the periodate oxidation of malachite green cation, MG^+, catalyzed by low concentration of Mn(II), can be written as follows:

$$MG^+ + IO_4^- \xrightarrow{Mn(II)} \text{Colorless products} + IO_3^-$$

The reaction was monitored at 620 nm, the absorbance maximum for MG^+. In previous studies the buffer compositions and concentrations employed for the indicator reaction above have been varied. A pH 3.5 acetate-phosphate buffer system was employed by Mottola et al. (5) (7) for the determination of microgram and submicrogram amounts of NTA. Fukasawa and Yamane (8) employed the reaction for the determination of trace amounts of manganese in high purity silicon, hydrofluoric acid and nitric acid in 0.1 M pH 3.8 acetate buffer. Since acetate and phosphate ions are known complexing agents for Mn(II) and Mn(III) (9) (10) their role in the indicator reaction becomes a subject of interest.

Mottola (5) has observed in the catalytic end point determination of DTPA, EDTA, DCTA, and HEDTA, that the presence of phosphate leads to better developed curves and sharper end point indication with inherent good accuracy and reproducibility. Both acetate and phosphate were observed to promote the catalytic effect of Mn(II). The probable manganese species playing a role in the reaction could range from Mn(II) to Mn(VII), with Mn(III) being favored as having a reduction potential nearly equivalent to Mn(VII) and involving only a one-electron process. The observation of a brown precipitate or film on the tip of the burette used to deliver Mn(II) into the catalytic end point titration vessel was made in titrations performed in the absence of phosphate or acetate (5). At the pH of the determinations and the Mn(II) concentration employed, the only species of manganese that should precipitate would be Mn(III) or Mn(IV). With the concentration ranges of acetate and phosphate employed in previous methods as a guide, a systematic study of the individual acetate and phosphate concentration effects and reaction orders was undertaken. The changes in ionic strength and concentrations of phosphate and acetate were observed to have no effect on the rate of the uncatalyzed reaction. The contribution of the uncatalyzed reaction to the overall rate of reaction in the catalyzed studies were observed to be negligible relative to the catalyzed rate and therefore, not considered in later experiments and discussions.

Experimental Procedure for the Study of the Effects of Varying Acetate Concentration on the MG^+ Indicator Reaction

Variation of acetate concentration was achieved by varying the volume of 1.0 M - pH 4.98 acetate buffer solution added to the reaction mixture. Table I lists the experimental conditions. Five replicates were made for each acetate concentration.

The malachite green stock solution used in the kinetic runs was observed to decolorize to a small extent upon sitting over a two-day period and to an increasing extent the longer it sat on the lab counter. Kinetic runs that were made with a dye solution that was partially decolorized were observed to have an initial increase in absorbance, with the expected decrease in absorbance with time occurring later. Upon addition of pH 4.98 acetate buffer solution to a two-week old dye-stock solution, regeneration of color began immediately and appeared complete after a fifteen minute period. As a result of these observations malachite green stock solutions were prepared in 0.1 M - pH 4.98 acetate buffer and were observed to be stable for periods of several weeks. This stability was probably due to inhibition of the formation of carbinol base by the large concentration of H^+ present (11).

TABLE I

EXPERIMENTAL CONDITIONS FOR THE ACETATE STUDY

$C_{Acetate}^{*}$ = 0.01, 0.06, 0.11, 0.16, and 0.21 M. Ionic strength equals 1.0

$[MG^+] = 2.4 \times 10^{-5}$ M

$IO_4^- = 1.0 \times 10^{-3}$ M

pH = 4.98

$[Mn(II)] = 5.0 \times 10^{-6}$ M

$^{*}C_{Acetate}$ is the total analytical concentration of acetate.

The experimental procedure involved the pipetting of 2.0 ml of 2.4×10^{-5} $\underline{M}$ malachite green solution and 2.0 ml of the 5.0×10^{-5} $\underline{M}$ Mn(II) stock solution into a test tube labeled $\underline{A}$. In another test tube labeled $\underline{B}$, 2.0 ml of 1.0×10^{-2} $\underline{M}$ sodium periodate, an aliquot of the stock acetate buffer solution, the required amount of 2.0 $\underline{M}$ sodium perchlorate solution to make the ionic strength equal to unity, and the required volume of distilled water to make the total volume equal to 16.0 ml, were added. The test tubes were then placed in a temperature bath, maintained at $25.0 \pm 0.2^{o}C$ by a Lauda/Brinkman K-2/R circulator, and allowed to equilibrate. The reaction for a particular run was initiated by mixing the contents of tube labeled $\underline{A}$ with an appropriate tube labeled $\underline{B}$. The reaction mixture was then poured into a 5.0 cm quartz cell, which was then placed in a Cary 14 spectrophotometer. The decrease in absorbance of malachite green with time was monitored at a wavelength of 620 nm. The time required to mix the reaction solution and fill the spectrophotometer cell before beginning the monitoring of absorbance was thirty seconds.

Results and Discussion of the Acetate Effects

First order plots of MG^{+} concentration versus time were found to be linear for the various acetate concentrations employed. With the reaction rate first order with respect to the concentration of MG^{+} and the periodate concentration sufficiently high to be considered pseudo-zero order, the following treatment of data was made. A pseudo first order rate constant, k^{*}, for the oxidation of MG^{+} was obtained by the method of half-lives, assuming the rate expression shown below:

$$d[MG^{+}]/dt = k^{*}[MG^{+}] \qquad [1]$$

where

$$k^* = k(C_{\text{Acetate}})^n \qquad [2]$$

in which

$$k = k'[\text{Mn}^{+2}]^m \quad . \qquad [3]$$

The log of Equation [2] then gives

$$\log k^* = \log k + n \log (C_{\text{Acetate}}) \qquad [4]$$

Plotting log k^* versus log (C_{Acetate}) one then obtains a straight line with a slope, n, the experimental order in acetate. Figure 1 shows such a plot for the acetate concentrations employed. The data point for the 0.01 $\underline{M}$ acetate concentration is observed to fall off the line indicated by the other data points. The slope of the line was found to be 0.93, indicating the reaction is first order with respect to acetate for acetate concentrations $\geq$ .06 $\underline{M}$.

Mottola and Heath (12) (13) found the catalyzed indicator reaction to be first order with respect to initial Mn(II) concentration in an acetate phosphate buffer system and used this as the basis for determining manganese at the 10^{-7} $\underline{M}$ level. If m in Equation [3] is unity, a plot of initial Mn(II) concentration versus k^* should yield a straight line for a given acetate concentration. Runs using varying acetate concentrations and for Mn(II) concentrations of 2.0, 3.0, and 5.0 x 10^{-6} $\underline{M}$ were made. Plots of k^* versus initial Mn(II) concentration did yield straight lines for a given acetate concentration. It can then be concluded that the reaction is first order with respect to initial Mn(II) concentration and first order with respect to acetate concentration. The accelerating effect that acetate has on the reaction can be attributed to the stabilization of the catalytic manganese-containing

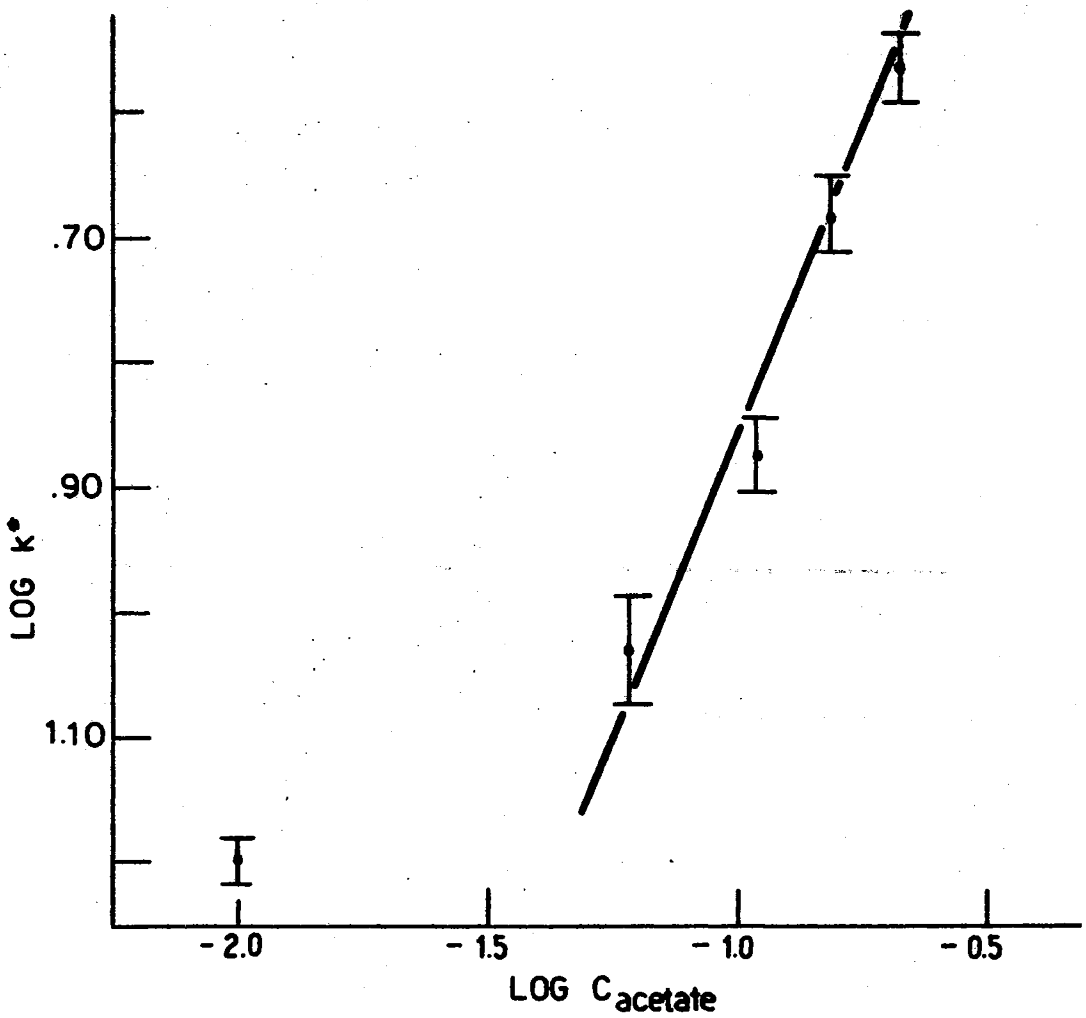

Figure 1. Plot of Log k* Versus Log ($C_{Acetate}$)

species, possibly Mn(III) or (IV) as proposed in reference (5).

Experimental Procedure for the Phosphate Concentration Effects

The phosphate concentration effects were studied under the same conditions and by the same procedure as the acetate study, with the exception that a pH 4.68 phosphate buffer ($C_{PO_4} = 1.0$ M) was used in place of acetate buffer. The malachite green stock solution was prepared in 0.1 M phosphate buffer to add to the stability of the solution.

Results and Discussion of the Ortho-Phosphate Study

Under the reaction conditions chosen for the acetate study summarized above, no observable induction period was encountered. The reaction carried out in the presence of phosphate under the reaction conditions chosen did have an induction period. The change in total phosphate concentration for the reaction runs was observed to affect the length of induction period, the order with respect to MG^+, and the rate of the reaction. When the total phosphate concentration is smaller than 0.11 M but greater than 0.035 M, plots of $1/[MG^+]$ versus time yielded straight lines suggesting a second-order dependence on MG^+. At phosphate concentrations greater than 0.11 M, the reaction is observed to be first order in MG^+. For a phosphate concentration less than, or equal to 0.035 M, neither first or second order plots yielded a straight line, and little of the catalytic effect of Mn(II) was observed.

The effect of total phosphate concentration (C_{PO_4}) upon the length of induction period is shown in Figure 2. From the figure, one can see that the length of induction period practically becomes independent of phosphate concentration at 0.11 M. Table II gives the rate proportionality constants for the oxidation of MG^+ for the various phosphate concentrations obtained by the method of half-lives.

The following rate expressions could be written for the phosphate concentration range where the rate order with respect to MG^+ concentration is second order.

$$d[MG^+]/dt = k_2^*[MG^+]^2 \qquad [5]$$

where

$$k_2^* = k_2(C_{PO_4})^n \qquad [6]$$

When the order with respect to MG^+ concentration is unity the following expression could be written:

$$d[MG^+]/dt = k_1^*[MG^+] \qquad [7]$$

where

$$k_1^* = k(C_{PO_4})^m \qquad [8]$$

Combining expressions [5], [6], [7], and [8], and then taking the log of the resulting expressions one obtains the following:

$$(MG^+ \text{ order} = 2) \quad \log k_2^* = \log k_2 + n \log (C_{PO_4}) \qquad [9]$$

$$(MG^+ \text{ order} = 1) \quad \log k_1^* = \log k_1 + m \log (C_{PO_4}) \qquad [10]$$

The slope of the log k^* versus log (C_{PO_4}) plots, where the appropriate order dependence is observed to apply, should then yield the experimental orders for phosphate under the two circumstances.

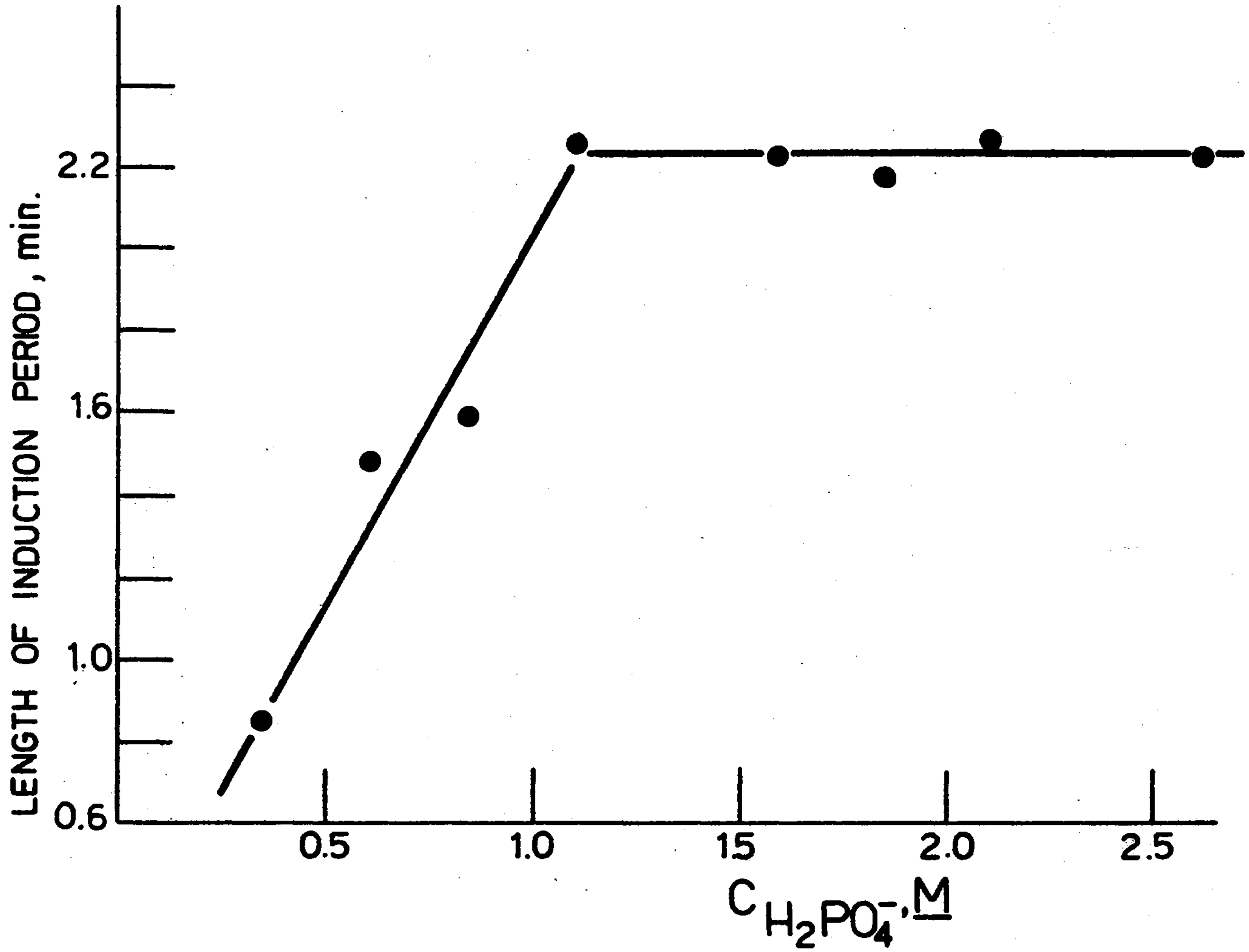

Figure 2. Length of Induction Period as a Function of C_{PO_4}

TABLE II

EFFECT OF $H_2PO_4^-$ ON THE RATE OF MANGANESE(II) CATALYZED OXIDATION OF MG+ BY PERIODATE ION

$C_{H_2PO_4^-}$	$t_{1/2}$, min.	k^* (a)
0.035	(b)	(b)
0.060	4.08	0.314 A^{-1} min^{-1}
0.085	6.08	0.210 A^{-1} min^{-1}
0.110	5.33	0.130 min^{-1}
0.135	5.33	0.130 min^{-1}
0.160	5.16_6	0.134 min^{-1}
0.185	4.80	0.144 min^{-1}
0.210	4.66_6	0.148 min^{-1}
0.260	4.25	0.163 min^{-1}

A: absorbance

(a) Calculated by the half-life method.

(b) Borderline case; cannot be treated as first or second order in MG^+.

Equations [11] and [12] represent the forms of the rate expression found utilizing the order-dependence information obtained from the mentioned plots:

$$(.035\ \underline{M} < C_{PO_4} < 0.11\ \underline{M}) \quad d[MG^+]/dt \ \alpha\ [MG^+]^2\ (C_{PO_4})^{-3/4} \qquad [11]$$

$$(C_{PO_4} < 0.11\ \underline{M}) \quad d[MG^+]/dt \ \alpha\ [MG^+]\ (C_{PO_4})^{1/3} \qquad [12]$$

Since the studies were carried out at a pH 4.68, the effect of phosphate is difficult to ascribe to any of the forms of phosphate existing in solutions except $H_2PO_4^-$ which predominates at this pH.

The initial accelerating effect of phosphate at concentrations less than or equal to 0.035 $\underline{M}$ could be attributed to the stabilization of the active catalytic species of manganese as similarly proposed in reference (5). The lengthening of induction period and the effect on the rate between phosphate concentration ranges of 0.035 and 0.11 $\underline{M}$ suggests that phosphate might be effectively competing for the active catalytic species but more probably complexing Mn(II), thus, subtracting it from the main catalytic cycle. At phosphate concentrations 0.11 $\underline{M}$, phosphate dominates the competition for active catalytic species during the time known as the induction period. Once the stabilized form of the active catalytic species has risen to a level where dissociation provides an effective level of catalytic psecies, catalysis is observed. The explanation above would indicate a rapid equilibrium step involving the active catalytic species and phosphate preceding the rate determining step.

Ionic Strength-Effect on the Pseudo-First Order Rate Constant of the MG^+ Indicator Reaction

The ionic-strength effect on reaction rates is used as a means of choosing between possible reaction mechanisms. To the analytical chemist, using a reaction in a kinetic determination, the empirical trend that increasing ionic strength has on the reaction rate is enough to indicate what adjustments or control of ionic strength should be made to make the rate more reliable and more responsive to the component of interest.

The equations employed for evaluating ionic-strength effects on experimental rate constants come from applying the Debye-Hückel limiting equations, or its modified forms, for ionic-strength correction to the Brønsted rate expression for a transition state complex. This complex is assumed to be the only species involved in the rate determining step of a reaction. Of the equations available for calculating ionic-strength effects (14), Equation [13] was observed to give the best linearity.

$$\log k - \log k_o = 2\ A\ Z_A \cdot Z_B\,(I)^{\frac{1}{2}} \qquad [13]$$

Where k is the rate constant found at the ionic strength I, k_o is the intercept for I = 0, A is the Debye-Hückel coefficient with a value of 0.50 for water at 25°C, and $Z_A \cdot Z_B$ is the product of the charges of the chemical species involved in the formation of the transition state complex. A plot of log k versus $I^{\frac{1}{2}}$ should be a straight line with a slope equal to the product of $Z_A \cdot Z_B$.

Experimental Procedure for the Ionic-Strength Study

The reaction conditions that were chosen to study the ionic-strength effect are listed in Table III. A test tube A and a test tube B were employed as in the acetate and phosphate studies. In test tube A, a total volume of 4.0 ml resulted from the addition of 2.0 ml of 2.4×10^{-5} M MG^+ and 2.0 ml of 5.0×10^{-5} M Mn(II). Tube B contained a total volume of 16.0 ml resulting from the addition of 2.0 ml of 1.0×10^{-2} M periodate, 2.0 ml of pH 4.98 acetate buffer ($C_{Acetate} = 1.0$), the volume of 2.0 M $NaClO_4$ necessary to bring the ionic strength to the value desired, and an amount of water that would bring the total volume of tube B to 16.0 ml. The procedure from this point on was the same as that reported for acetate and phosphate studies.

TABLE III

REACTION CONDITIONS FOR STOICHIOMETRIC STUDIES* AND IONIC STRENGTH STUDIES**

$[MG^+] = 2.4 \times 10^{-6}$ M

$C_{Acetate} = 0.002$ M

pH = 4.98

$[Mn^{++}] = 5.0 \times 10^{-6}$ M

$[IO_4^-]_o = 4.8 \times 10^{-5}$ M; 6.0×10^{-5} M; and 7.2×10^{-5} M

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*Total volume of reaction mixture was 20.0 ml and the solutions were allowed to react for 12 hours.

**$[IO_4]_o = 1 \times 10^{-3}$; $C_{Acetate} = 0.1$ M

Results and Discussion of the Ionic-Strength Effect

Most ionic-strength effect studies are ordinarily made at low ionic strengths where the equations provided by theory are applicable. In the case of this study, the previous analytical procedures employing the MG^+ indicator reaction involved ionic strengths on the order of 0.50 and this fact suggested that the ionic-strength study should also be in this range. The speudo first-order rate constant, k^*, for MG^+ oxidation was determined by using the method of half-lives.

A plot of k^* versus $I^{1/2}$ is shown in Figure 3. The value of the product $Z_A \cdot Z_B$ determined from the slope of line is -2.0. This then indicates that the possible species in the rate-determining step must have charges of +1 and -2 or +2 and -1. A possible step that meets the charge criteria for species known to be in solution is given below:

$$Mn^{+2} + IO_4^- \rightleftarrows Mn(IO_4)^+$$

However, this appears as unacceptable since Simpson (15) has given evidence indicating that the rate constant for the forward reaction is of the order of 10^8 $\underline{M}^{-1}$ seconds^{-1} and it is not the rate determining step. Since the ionic strength study is outside the range indicated by theory for the equation employed, one is left with the alternative of concluding that the slope of the plot is meaningless (16).

The Stoichiometry Regarding Moles of IO_4^- Consumed Per Mole of MG^+

When the question of how to determine the stoichiometry of periodate

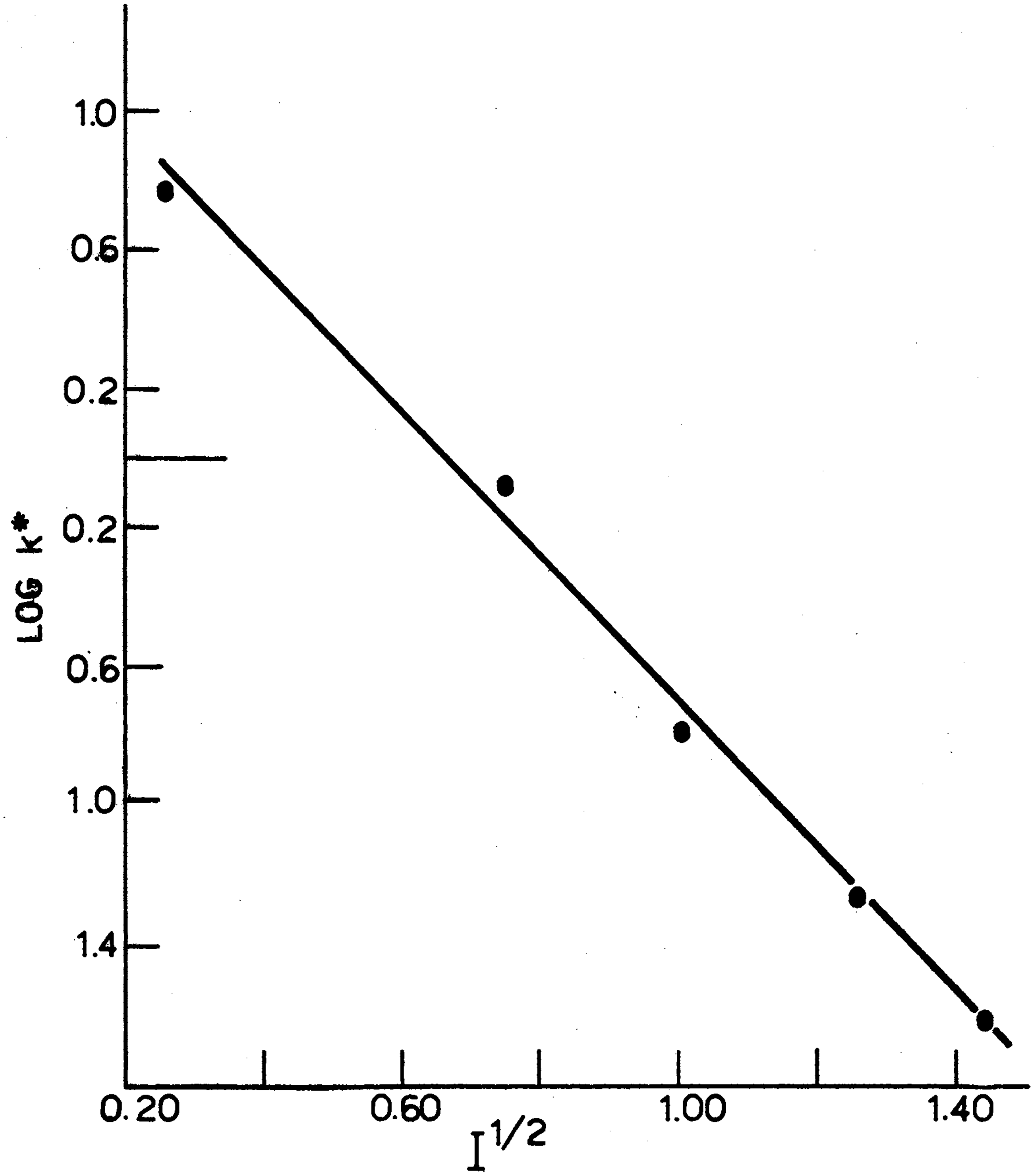


Figure 3. Plot of Log k^* Versus $(I)^{\frac{1}{2}}$

with respect to MG^+ first was encountered, the most obvious initial route was to add an amount of periodate that would only partially oxidize a given concentration of MG^+ and to measure the residual absorption of MG^+ at its absorbance maximum of 620 nm. Thus, knowing the amount of periodate added and the amount of MG^+ consumed, the stoichiometry could be found. This method, however, had to meet the following criterion. The oxidation of MG^+ must be rapid and complete before any of the reaction products could compete for oxidant. If the criterion was not met, experimental values for the stoichiometry employing different amounts of periodate would yield different stoichiometric values. The stoichiometric values found did vary with the amount of periodate added. This then indicated that this method could not be employed. The next step was to find a method to estimate periodate consumed.

Under the reaction conditions chosen for the kinetic study, the periodate that was consumed yielded iodate and water. The amount of periodate consumed when in excess under similar oxidizing conditions is usually determined utilizing the following reactions:

$$7I^- + IO_4^- + 8H^+ \rightleftarrows 4I_2 + 4H_2O \qquad [14]$$

$$5I^- + IO_3^- + 6H^+ \rightleftarrows 3I_2 + 3H_2O \qquad [15]$$

$$I_3^- + 2S_2O_3^= \rightleftarrows 3I^- + S_4O_6^= \qquad [16]$$

This is the sequence of reactions commonly employed in the differentiation of aldoses and ketoses (17).

The basis for the method is the difference in the number of moles of I_2 generated between reaction [14] and [15]. Using an excess of periodate to take the reaction of interest to completion, one then acidifies and adds an excess of KI to generate a stoichiometric amount of

I_3^-. This solution is then titrated with a standard thiosulfate solution, with starch indicator used for end point detection. Using a blank solution containing the same amount of periodate, the above procedure is repeated. The number of moles of periodate can than be calculated using the Equation [17]

$$\text{moles } IO_4^- \text{ consumed} = \frac{N_{Na_2S_2O_3}(V_{B1} - V_R)}{2} \qquad [17]$$

where $N_{Na_2S_2O_3}$ is the normality of the thiosulfate solution, V_{B1} is the volume of $Na_2S_2O_3$ to titrate the blank and V_R the volume of $Na_2S_2O_3$ to titrate the reaction solution.

Under the reaction conditions employed up to this time, the largest MG^+ concentration employed was 1.2×10^{-5} M. For the titrimetric method above to be feasible for the system to be studied, however, the concentration of malachite green was increased tenfold. The number of moles of periodate in the reaction vessel was initially made to be twice the number of moles of MG^+ present. The pH was held constant at 4.98 with 0.11 M acetate buffer, and the Mn(II) concentration was 1.0×10^{-6} M. Under these conditions the oxidation of MG^+ was incomplete, so the number of moles of periodate present was changed to a sixfold excess. This, however, resulted in the co-precipitation of the malachite green periodate and perchlorate ion-pairs. The constituents of the ion-pairs were identified by thin layer chromatography on starch using acetone and 3N aqueous ammonia in a 7:3 ratio as the mobile phase (18).

With the elimination of the possibility of adjusting reaction conditions to titrate successfully with thiosulfate, the previous procedure was modified so that a spectrophotometric estimation of stoichiometry could be made. The method still involved reactions [14] and [15] with the I_2 generated combining with an excess of I^- to form I_3^-. The concentration of I^- was chosen so that the formation of I_3^- was quantitative and the absorbance was then measured at a wavelength of 353 nm using a Cary 14 spectrophotometer. The amount of periodate consumed could then be estimated in the following manner. If all of the periodate in a given sample were converted to IO_3^- it would then have 3/4 of the absorbance of a blank sample containing the same amount of periodate treated in the same manner. By running a blank, one then knew that the maximum change in absorbance, ΔA_{max} is given by

$$\Delta A_{max} = (A_{Blank} - 3/4\, A_{Blank}) = 1/4\, A_{Blank}$$

With the periodate in excess for the sample, the sample runs had an absorbance change, ΔA_{sample}, less than ΔA_{max} and ΔA_{sample} was found using the relationship

$$\Delta A_{sample} = (A_{Blank} - A_{sample}) \; .$$

The change in periodate concentration, $\Delta[IO_4^-]$ was then found employing the following relationship

$$\Delta[IO_4^-] = [IO_4]_o\,(\Delta A_{sample}/\Delta A_{max})$$

where $[IO_4^-]_o$ equals the initial IO_4^- concentration. The stoichiometry of periodate with respect to MG^+ was then found by dividing $\Delta[IO_4^-]$ by the change in MG^+ concentration, $\Delta[MG^+]$, which was equal to the initial concentration of MG^+ since periodate was in excess.

Stoichiometry Procedure and Results

The stoichiometry was determined using three different IO_4^- concentrations and the conditions shown with Table III. The procedure used for I_3^- color development for each of the runs is given in Table IV. The absorbance was measured in the Cary 14 at a wavelength of 353 nm and with conditions chosen to make the absorbance of the blank near 2.0, so that the difference between blank and sample absorbance would be large enough to make the contribution of experimental errors from sample preparation minimal. Table V shows the results of the study for the IO_4^- concentration employed. The average ratio of moles periodate per mole of MG^+ was found to be 7.52 ± 0.22.

TABLE IV

CONDITIONS EMPLOYED FOR THE STOICHIOMETRIC REDUCTION OF IO_4^- TO I_3^-

$[IO_4^-]_o$	ml of Reaction mixture	ml .1 M $HClO_4$	ml 2.4×10^{-1} M KI	ml H_2O
4.8×10^{-5}	1.00	1.00	1.00	0.00
6.0×10^{-5}	1.00	1.00	1.00	0.50
7.2×10^{-5}	1.00	1.00	1.00	1.00

TABLE V

RESULTS OF STOICHIOMETRY STUDY

$[IO_4^-]_o$	$\Delta[IO_4^-]$	moles IO_4^-/moles MG^+
4.8×10^{-5}	1.87×10^{-5}	7.78
6.0×10^{-5}	1.78×10^{-5}	7.42
7.2×10^{-5}	1.76×10^{-5}	7.36

Identification of MG^+ Oxidation Products

Previous studies identifying the oxidation products of MG^+ are found in the literature. Matrka (19), used an excess of permanganate ion in acid solution to oxidize 0.34 gm of the dye and identified benzoic acid as an oxidation product; a transient product, the diphenoquinone-(4,4)-bis-dimethyl ammonium salt, was almost totally decomposed due to the oxidative action of excess oxidizing agent. Adams and Galus (20) used cyclic voltametry to perform the oxidation and also identified the oxidized form of NNN'N'-tetramethylbenzidine (TMB) and benzoic acid as products. The oxidation products of TMB can be inferred from references (21) and (22) to be diphenoquinone imines in the tri, di, and mono methyl forms. The methyl groups that were cleaved were found to go to formaldehyde under mild oxidizing conditions.

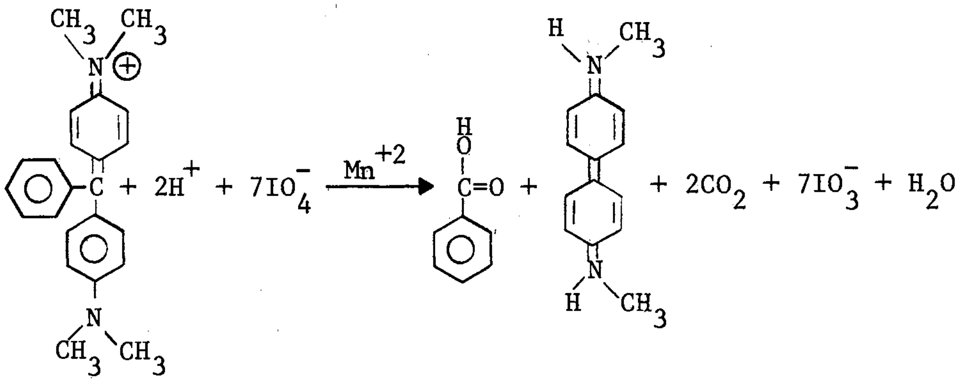
With the results of earlier studies as a guide, the oxidation of

MG^+ was carried out in a 100 ml volumetric flask with $[MG^+] = 1.2 \times 10^{-5}$ $\underline{M}$, $[IO_4^-] = 5.2 \times 10^{-3}$ $\underline{M}$, $C_{Acetate} = .01$ $\underline{M}$, pH = 5.0, and $[Mn^{++}] = 5.0 \times 10^{-6}$ $\underline{M}$. The oxidation products were then acidified with HCl and extracted with ether. The ether was evaporated and the residue was taken up into acetone and spotted on an Eastman silica-gel TLC sheet with a fluorescent indicator. Spots of benzoic acid and benzidine hydrochloride were also spotted. As the sample of benzidine was spotted, a high intensity "sun lamp" was used to dry the spot. The spot was observed to turn brown during this process. After eluting with the mobile phase (23) (Ethanol, 6 $\underline{M}$ aqueous ammonia, and H_2O in a ratio of 20:3:1) a visible brown spot in the extracted product had the same retention factor, R_f, as the benzidine spot. Upon examination of the chromatogram under a UV-light another spot in the extract was observed to have an R_f value of 0.85, the same as that for benzoic acid.

The reaction was repeated, and the reaction mixture was tested for the presence of formaldehyde using the chromotropic acid test (24). The results of the test were negative. The remaining reaction solution was then acidified and extracted with ether. The ether was back extracted with a small volume of 0.1 $\underline{M}$ NaOH. The test for formic acid was then run by mixing a drop of the basic extract in a test tube with a drop of 6 $\underline{M}$ HCl and by adding magnesium powder until no further hydrogen liberation was observed. The sample was then tested using chromotropic acid. The results of this test were also negative. The concentrations of formic acid and formaldehyde that would be expected from the reaction are at least an order of magnitude above the limit of detection for the tests employed.

Results of the Stoichiometric and Product Identification Study

The stoichiometric study indicated that besides the initial formation of benzoic acid and the postulated TMB (19), the TMB must be undergoing further oxidation. After elimination of the possibility of cleaved methyl groups being oxidized to formaldehyde or formic acid by excess oxidant, one might then conclude that the oxidation of any cleaved methyl groups did not stop at these intermediates, but probably continued until CO_2 was formed. With these products in mind the overall reaction below can be proposed:



for a stoichiometric ratio of 7 for moles of IO_4^- consumed per mole of MG^+. This ratio of 7 is agreeable with the experimentally determined ratio of 7.52 ± .22 .

CHAPTER III

SUMMARY OF RESULTS AND DISCUSSION

The observation that there was no catalytic effect in the absence of complexing agents in the Mn(II) catalyzed oxidation of MG^+ by periodate indicated that the buffer components not only served to maintain a constant pH, but also played an important role in the stabilization of the catalytic species of manganese. The most probable catalytic species of manganese would be Mn(III) because of its high redox potential (E_o = 1.51 volts (25)) and the process of its generation requiring only one electron.

At a constant ionic strength of 1.0, an increase in the acetate concentration was observed to cause an increase in the pseudo-first order rate constant, k^*, which was directly proportional to the total concentration of acetate. The functional form of the line being, $k^* = 0.1 \times C_{Acetate}$, at $I = 1.0$. An increase in ionic strength, however, was observed to cause a decrease in the value of k^*. The functional form which best fit the data for k as a function of ionic strength at a constant acetate concentration of 0.11 $\underline{M}$ is given in Equation [18].

$$\log k^*/k_o = -2(I)^{\frac{1}{2}}, \text{ at } C_{Acetate} = 0.11\ \underline{M} \qquad [18]$$

In a kinetic method, one would like to maximize the sensitivity for the species to be determined. For the case of the MG^+ indicator system, it is known that k^* is proportional to the initial concentration of Mn(II) (10). If one could then optimize the rate constant k^*, the sensitivity for the determination of Mn(II) would also be optimized. The following discussion will present a method tried for predicting the acetate concentration that would optimize k^*.

If one increases the acetate concentration in a system where the ionic strength is not held constant there should be a two fold effect on k^*. An increase in the concentration of acetate would increase the rate while also increasing the ionic strength which decreases the rate. Combining the information from the acetate study, Equation [18], and the knowledge that for a given acetate concentration, the ionic strength cannot drop below the contribution from acetate, which is 0.636 x C_{Acetate}, one obtains the following equation:

$$\log \frac{0.1\ (C_{\text{Acetate}})}{k_{\text{max}}} = -2(1.0 - 0.636 \times C_{\text{Acetate}})^{\frac{1}{2}} \qquad [19]$$

Where k_{max} is the maximum rate constant possible considering the ionic strength contribution coming from the acetate buffer. Rearranging and solving for k_{max} one obtains,

$$k_{\text{max}} = (0.1\ C_{\text{Acetate}})\, e^{\,4.606(1.0 - 0.636\ C_{\text{Acetate}})^{\frac{1}{2}}} \qquad [20]$$

If k_{max} has a maximum, with respect to C_{Acetate}, the maximum can be found by taking the first derivative, with respect to C_{Acetate}, setting the resulting equation equal to zero, and solving for k_{max}. Taking the derivative, setting it equal to zero and simplifying the following equation results.

$$0.10 - (0.1465 \times C_{\text{Acetate}})(1.0 - 0.636\ C_{\text{Acetate}})^{\frac{1}{2}} = 0 \qquad [21]$$

Equation [20] was found to have a maximum at an acetate buffer concentration of 0.55 M.

To check to see if the method could be used to optimize k_{max}, a set of runs were made in which the concentration of acetate was varied. The maximum value of k^* was found to occur between a total acetate concentration of 0.11 and 0.31. The primary reason for the failure of both values to agree could be attributed to the observations that ionic-strength effects are not independent of the ion used to adjust the ionic strength (16). The treatment did, however, predict that a maximum should occur.

The product identification and stoichiometric studies showed that deviation of first order MG^+ plots from linearity as the reaction proceeded could be attributed to secondary oxidation steps competing for catalyst. This reaffirms the need to use an early portion of the reaction profile when employing an indicator reaction in a kinetic method in which product competition does occur.

The result of the studies suggested some possible reasons for earlier adjustments in the reaction conditions found necessary for the MG^+ indicator reaction and indicated possible changes that could be made to improve existing methods. Mottola (5) had observed that at a pH of 3.5 addition of acetate or phosphate was necessary to obtain well developed and reproducible curves. Fukasawa and Yamane (8) observed that at a constant acetate concentration, an increase in pH gave a marked increase in the rate of reaction. Both of these observations are consistent with the information from the acetate and phosphate studies. These studies showed that at a constant ionic strength and pH an increase in total concentration of acetate or phosphate would increase the rate of reaction. These results show a direct relation to Mottola's observations (5). A direct relation to Fukasawa's and Yamane's (8) results is seen when one realizes that the higher the pH the larger the fraction of acetate present as the anion available to complex manganese intermediates.

Proposed modifications of reaction conditions for optimization of reaction sensitivity with respect to the species of interest in references (5) and (8) would involve empirically finding the value of k_{max}. This could be done by varying the buffer concentration and looking at the resulting value of k.

It should also be noted that when a phosphate buffer is employed for the MG^+ reaction the phosphate concentration should be above the minimum concentration required to make the reaction first order in MG^+ and the induction period constant. If the effects are caused by a particular species of phosphate, one would expect the value of this minimum to be pH dependent.

PART II

SOME COMPARATIVE STUDIES ON DATA HANDLING PROCEDURES FOR THE VARIABLE TIME KINETIC METHOD OF ANALYSIS

CHAPTER IV

INTRODUCTION

Most kinetic methods of analysis are usually applied to cases where one needs to determine low concentrations of a chemical species. The variable-time kinetic method is one of the commonly employed kinetic procedures (26). The method involves the measurement of the time interval, ΔT, required for the monitored species, R, to change between two preset concentration values. Under controlled experimental conditions, this time interval is a function of the concentration of the species to be determined. In most cases, $1/\Delta T$ is proportional to the concentration of the species of interest if it is a catalyst.

The most common method for obtaining $1/\Delta T$ is to record the signal from the device monitoring the species R with the help of a strip chart recorder. The time between two reference values of R can then be measured with a ruler or by the calibration on the chart along the time axis. The inverse is then taken. The first automated reciprocal time measurement system was developed by Pardue et al. (27). The system involved electronically switching a motor on at the first reference potential of the transducer. The shaft of the motor was connected to a linear potentiometer which was the input resistance for an inverting operational amplifier having a constant feedback resistor, R_f. When the transducer potential reached a second reference potential the motor was switched off. With an initial potentiometer resistance of zero, the final resistance of the potentiometer was directly proportional to ΔT. The output voltage of the operational amplifier was then proportional to $1/\Delta T$. Stehl et al. (28) later developed an all-electronic variable-time ratemeter, which measured ΔT, computed the log ΔT, and then differentiated the expression to yield a voltage directly proportional to $1/\Delta T$. A similar approach was taken by James and Pardue (29). A reciprocal time meter which eliminated the use of logarithmic elements has been reported by Crouch (30). In this system a voltage proportional to ΔT is obtained after the transversal between the transducer reference switching potentials is complete. A voltage-to-frequency converter then converted the resulting voltage to a frequency which was proportional to ΔT. The period of this frequency was then measured with a period meter. The value of the period is thus proportional to $1/\Delta T$.

In previous studies in this laboraotry (13) (31) the strip chart method and an electronic switching network have been employed to obtain ΔT values. The electronic switching network was designed to switch on an electromechanical timer at one transducer reference potential and to switch the timer off at a second reference potential. A third method has been employed in this study involving the measurement of the time interval by electronically starting and stopping an electronic clock at the two respective reference potentials.

In order to evaluate these alternative methods of obtaining time intervals, two systems were chosen for study. The first system studied was a simulation circuit whose output voltage varied lagarithmically with time. The circuit was designed so that the time interval for the output simulation signal to transverse between the two reference potentials was an inverse function of the ramp voltages used to generate simulated curves. The second system was the malachite green-periodate indicator reaction catalyzed by Mn(II) (4). 1,10-Phenanthroline was observed (32) to have an accelerating effect on the catalytic reaction which then allowed its determination as a function of $1/\Delta T$. The three methods of obtaining time intervals mentioned earlier were then employed to obtain time intervals for the two systems. The data was subjected to least square analysis and the precision of the methods evaluated in a manner similar to that indicated in reference (33). The replication error for the data points for each method was also determined.

CHAPTER V

EXPERIMENTAL APPARATUS

Spectrophotometric

The spectrophotometric unit used in the study consisted of an especially assembled modular unit. High values of radiant power, short and long range stability, and the use of as few as possible auxiliary optical units (considering that a rather high degree of resolution is not critical in rate measurements involving relative changes) are aspects that were considered in the construction of the unit. It comprises the following modular parts: a) light source: a (MP)*-1021 six volts light source powered by an MP-1026 regulated power supply; b) Monochromator: a Jarrell-Ash, ¼ meter monochromator covering the 180 to 860 nm wavelength range, with an aperture of f/3.5; c) Cell holder house: designed to accomodate a variety of conventional cell holders. This unit contains all necessary connections for air magnetic stirring, external cell flow circulation, electrical control of stirring velocities, and temperature control of reaction vessels; d) Cell holder with air magnetic stirring: described and reported earlier (34); e) Temperature control: a Lauda/Brinkman Model K-2/R circulator in conjunction with a circulating water-bath was used to maintain the

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*The operational amplifiers, power supply, and reference potential sources were modular electronic components from McKee-Pederson Instruments, (Danville, California).

temperature at 25.0 ± 0.2°C; and f) Detector: a UD-500 UV PIN silicon photodiode from United Detector Technology, Inc. was employed. A diagram of the apparatus is shown in Figure 4.

The output of the photodiode was then fed into the input of a MP-1031 chopper stabilized operational amplifier with a gain of one with $Z_i = Z_f = 1$ M ohm which served to match impedence and isolate the detector from auxilary circuits. The output of the operational amplifier was then fed into the appropriate circuit for the desired treatment of the signal.

Simulation

As it was mentioned previously, the purpose of the investigation was to compare some of the methods of obtaining elapsed times in the variable time kinetic method of analysis. The simulation of the first order curves were accomplished by employing a linear voltage ramp coupled with a log ratio amplifier.

The major components of the simulating circuit are, the spectrophotometer, a 1.35 Volt Mallory Duracell Battery, a Clarostat 62JA 10 K ohm variable resistor with centertap, MP-1032 and MP-1031 operational amplifiers, a MP-1012 integrator, a MP-1004A log ratio amplifier, and a Heath Universal Digital Instrument (UDI,EU-805). See Figure 5. A console (MP-1001) housed the power supply (MP-1002) for the amplifiers employed.

The spectrophotometer was used as the potential reference for the initial ramp voltage by turning the light source on setting the monochromator on a wavelength of 620 nm, and then adjusting the shutter on the light source until the initial output of the log ratio amplifier is

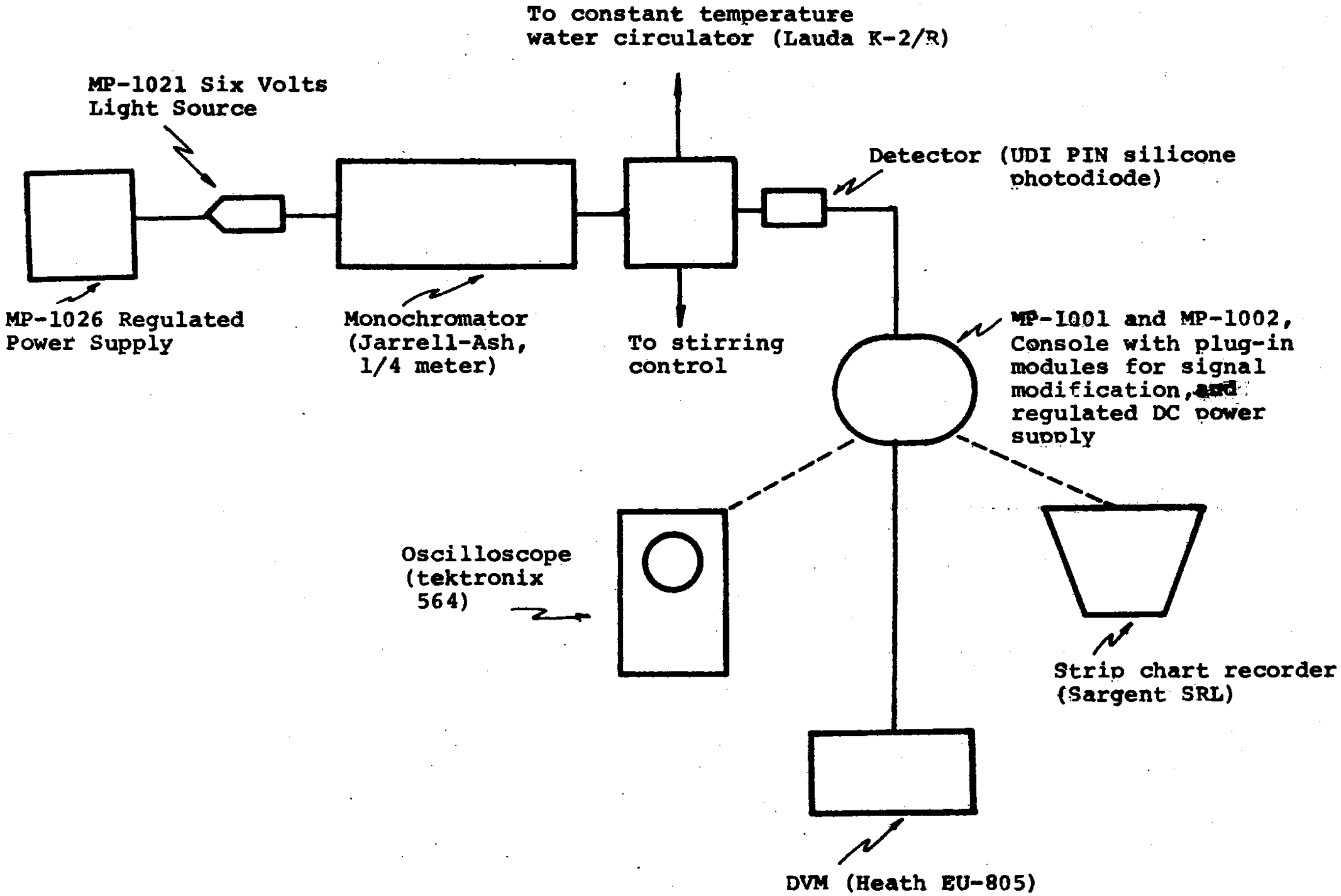
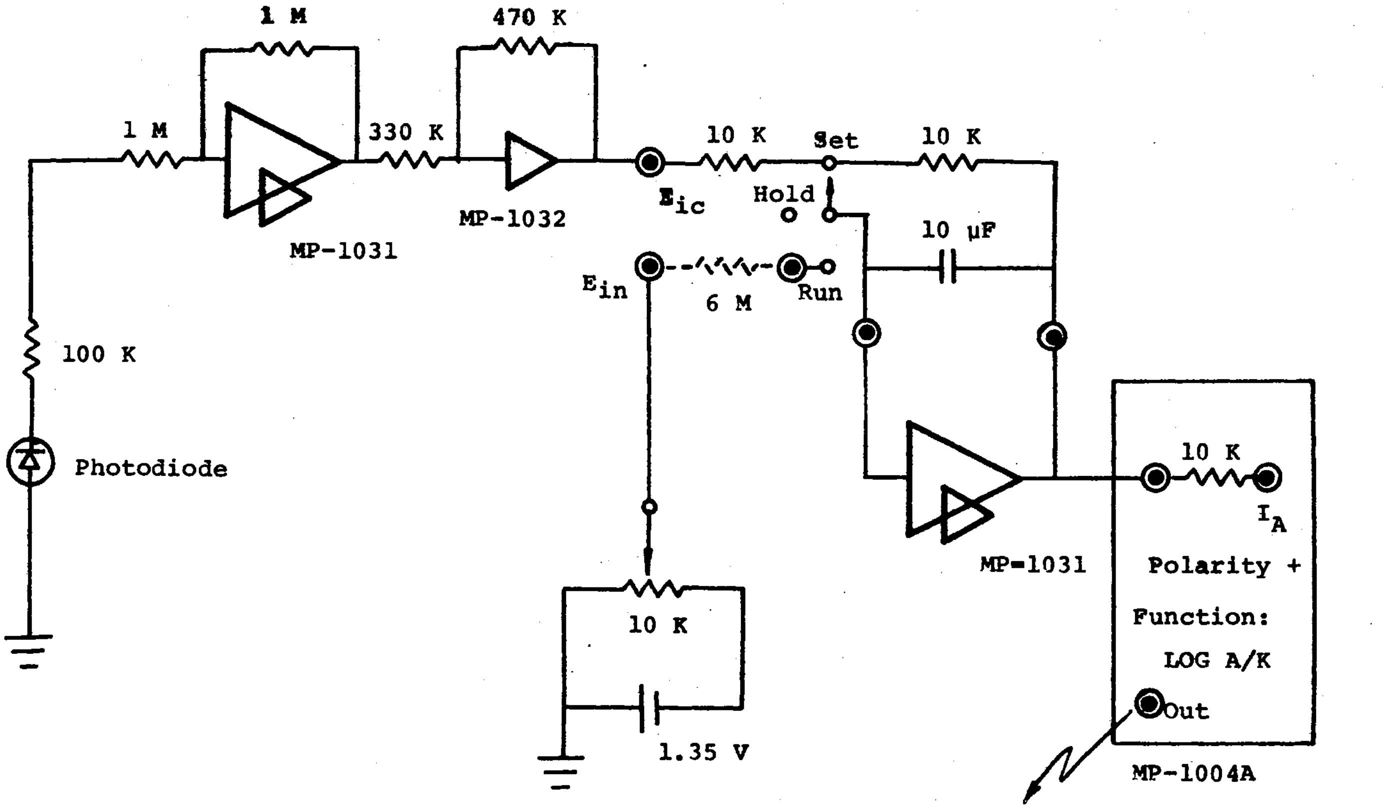


Figure 4. Block Diagram of the Spectrophotometer Arrangement



Output is fed into appropriate circuit for desired treatment

Figure 5. Schematic of Simulation Circuit

zero. The MP-1032 operational amplifier was used to reduce the output voltage of the MP-1031 operational amplifier with the light source shutter open so that the output voltage would approximately be the value to make the output of the log ratio amplifier equal to zero with a minimum of adjustment of the shutter. The integrator served the function of providing a linear voltage ramp which, with the 6M ohm input resistor and 10 Microfarad feedback capacitor had a time constant of 1 minute for each integral addition of the input ramp voltage. The log ratio amplifier converted the log of the ramp voltage with time to a first order plot for output voltage versus time. The ramp voltage was varied by adjusting the variable 10 K ohm resistor until the desired input ramp voltage was read on the UDI.

The log ratio amplifier was used in its log A/K mode. In this mode the unit provides a reference current, I_k, of 10^{-5} amps. If the current I_a is adjusted so it is also 10^{-5} amps. the output will be log 1 or zero. If the input voltage is such that I_A is 10^{-4} the output will be -100 mV. The unit is designed to give 100 mV per decade. In the simulated runs the initial input voltage was -100 mV which, through the 10 K ohm resistor, gave an initial current of 10^{-5} amps. and thus zero output voltage. When the integrator was switched to run, the initial voltage was incremented with the ramp voltage causing the current I_A to increase giving a negative output voltage for the log ratio amplifier which varied logarithmically as a function of time.

Time Interval Measurement

The three devices used for measuring time intervals were a strip chart recorder, an electromechanically switched timer, and an electronically switched electronic timer. The data which was gathered for the two methods was subjected to a least-square analysis to compare the reliability of the time interval measuring systems.

Strip Chart Recorder

The major components of the strip chart recorder were a MP-1031 operational amplifier, MP-1008 millivolt sources and a Sargent SRL Recorder (linear gears) (E. H. Sargent and Company, Chicago, Illinois). The output of the isolating and impedence matching circuit was fed into the input of a MP-1031 OA. For the circuit schematic see Figure 6.

The input resistance for the second operational amplifier (Figure 6) was 470 K ohm and the feedback resistance was 330 K ohm. The resistances were chosen to give a gain of less than one to reduce the signal from the spectrophotometer so that it would fall in the 100 mV variable range of the recorder. The output voltage of the photodiode was found to be linear with concentration, but the output voltage was maximum at zero absorbance. To make the input voltage to the recorder zero at zero absorbance, a bucking voltage from the millivolt source was summed into the summing point of the operational amplifier through a 10 K ohm resistor until a zero output voltage at zero absorbance was attained. The recorder was then zeroed, and a solution of malachite green of known absorbance introduced into the spectrophotometer. The millivolt range dial on the recorder was then adjusted until the correct absorbance value was read. The zeroing and the adjustment procedure was repeated until consistent zero and absorbance readings were obtained without adjustment of the recorder.

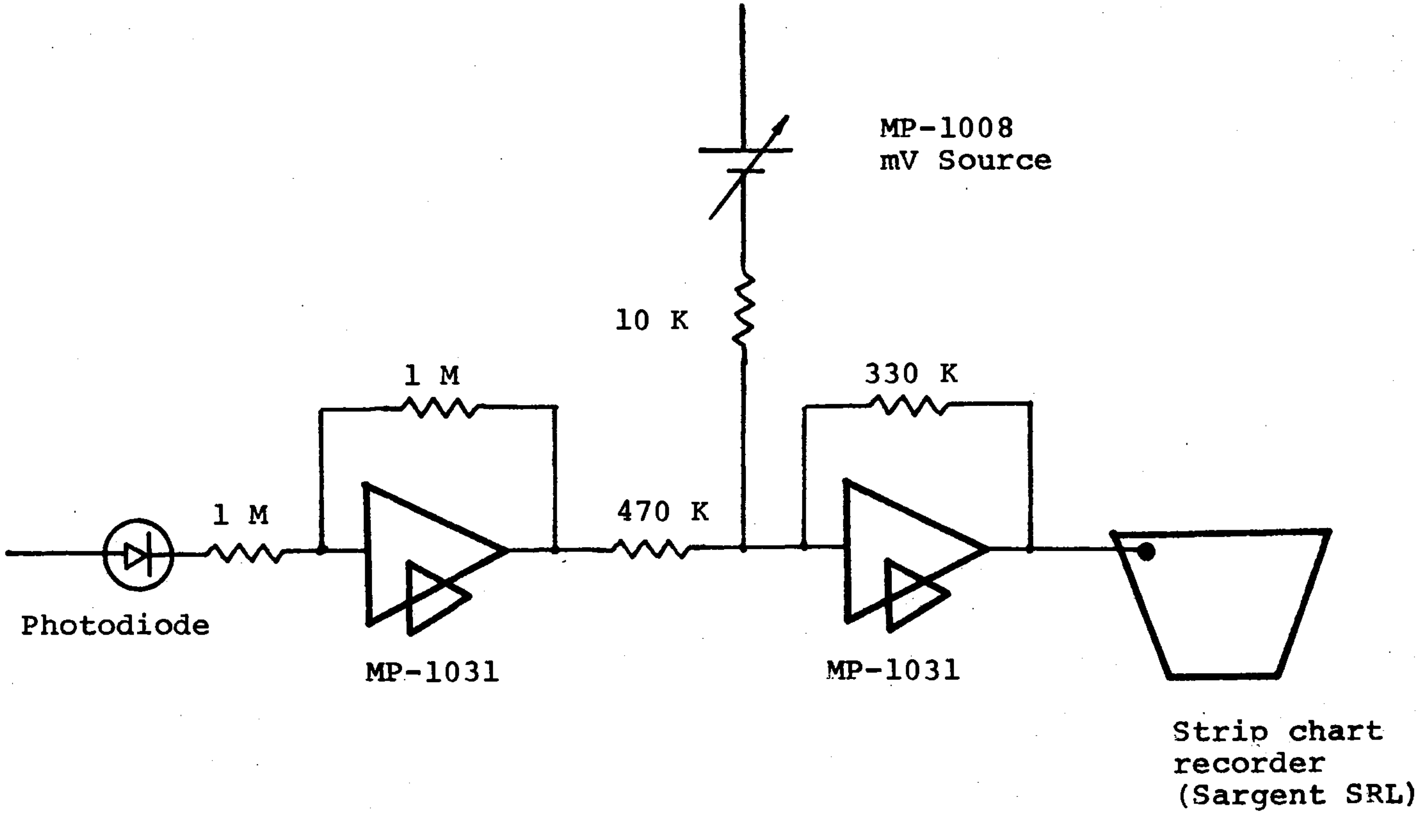


Figure 6. Circuit Schematic for the Strip Chart Recorder-Spectrophotometer System

Electromechanical Timer

The components of the double switch network for the mechanical timer were two MP-1006A operational amplifiers (OA), a MP-1031 OA; digital timer (MP-1029), 1N5223, 1N5221-zener diodes, and the MP-1008 millivolt source. See Figure 7 for details. The function of the circuit components and a description of how the circuit functions is given in references (13) and (31).

Electronic Timer

The major components for the switching networks for the electronic timing circuit were two MP-1006A OA's, the MP-1008 mV sources, and the Heath Universal Digital Instrument, UDI, EU-805. Figure 8 shows a schematic of this system. The operational amplifiers were used in the open loop mode with the desired switching potential from the millivolts source fed into the non-inverting input. The signal from the simulating circuit or spectrophotometer, whichever was in use, was introduced into the inverting input of the operational amplifiers. The comparators for channels A and B of the UDI were triggered by a change in slope of the output of the switching potential, E_r, greater than the input from the source potential, E_s, initially, the amplifier was saturated at a potential of +14.0 Volts. When the magnitude of E_s became greater than E_r, the operational amplifier then saturated at -20.0 Volts. The controls on the panel of the UDI for the channel to be switched were then set to switch on a negative slope with an attenuation setting to accomodate a 20 volt DC signal and to switch at the zero level by putting the level

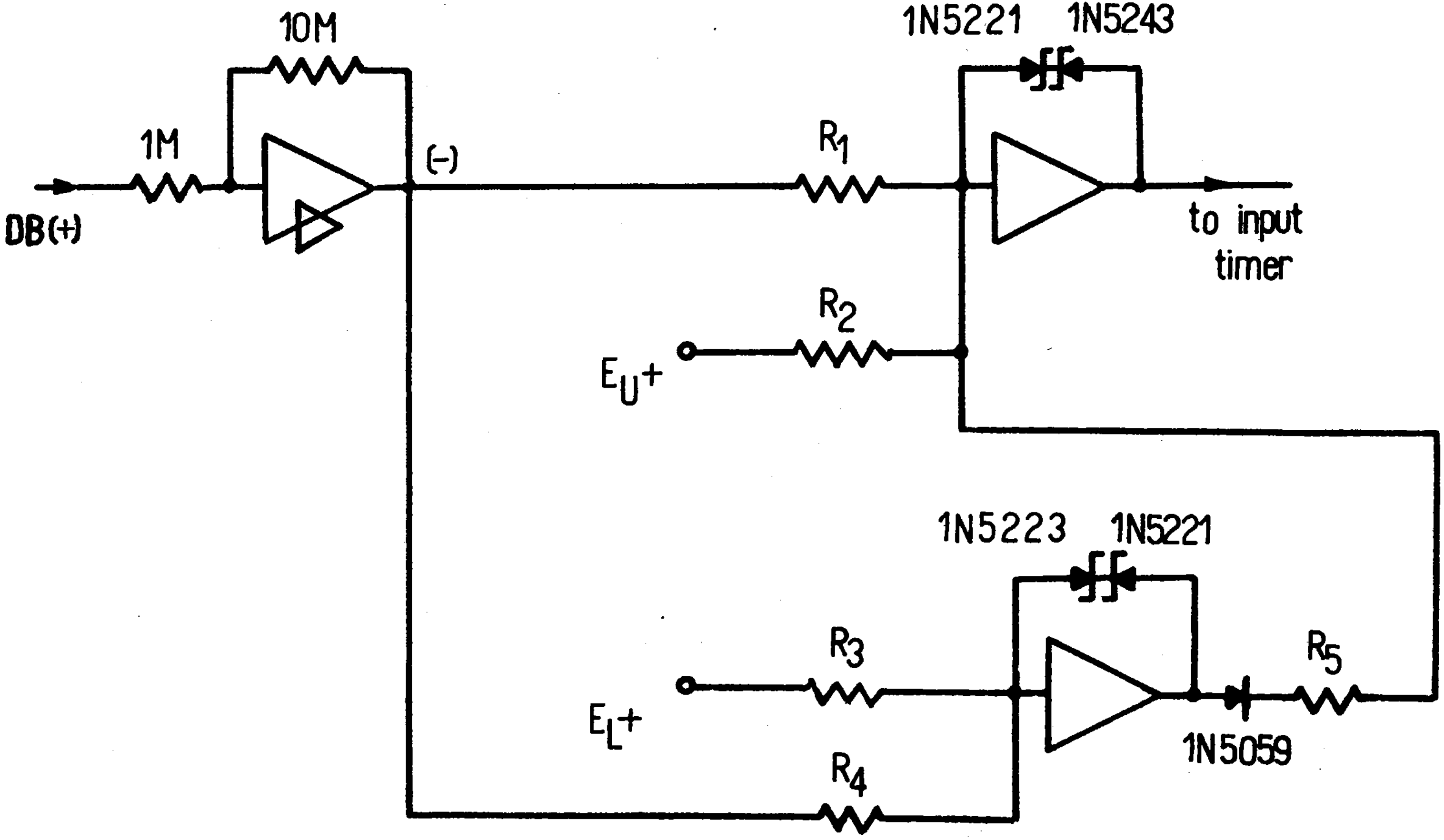


Figure 7. Double Switch Network. E_U and E_L are the reference potential sources from an MP-1008 millivolt source. R_1 to R_5: 10 K

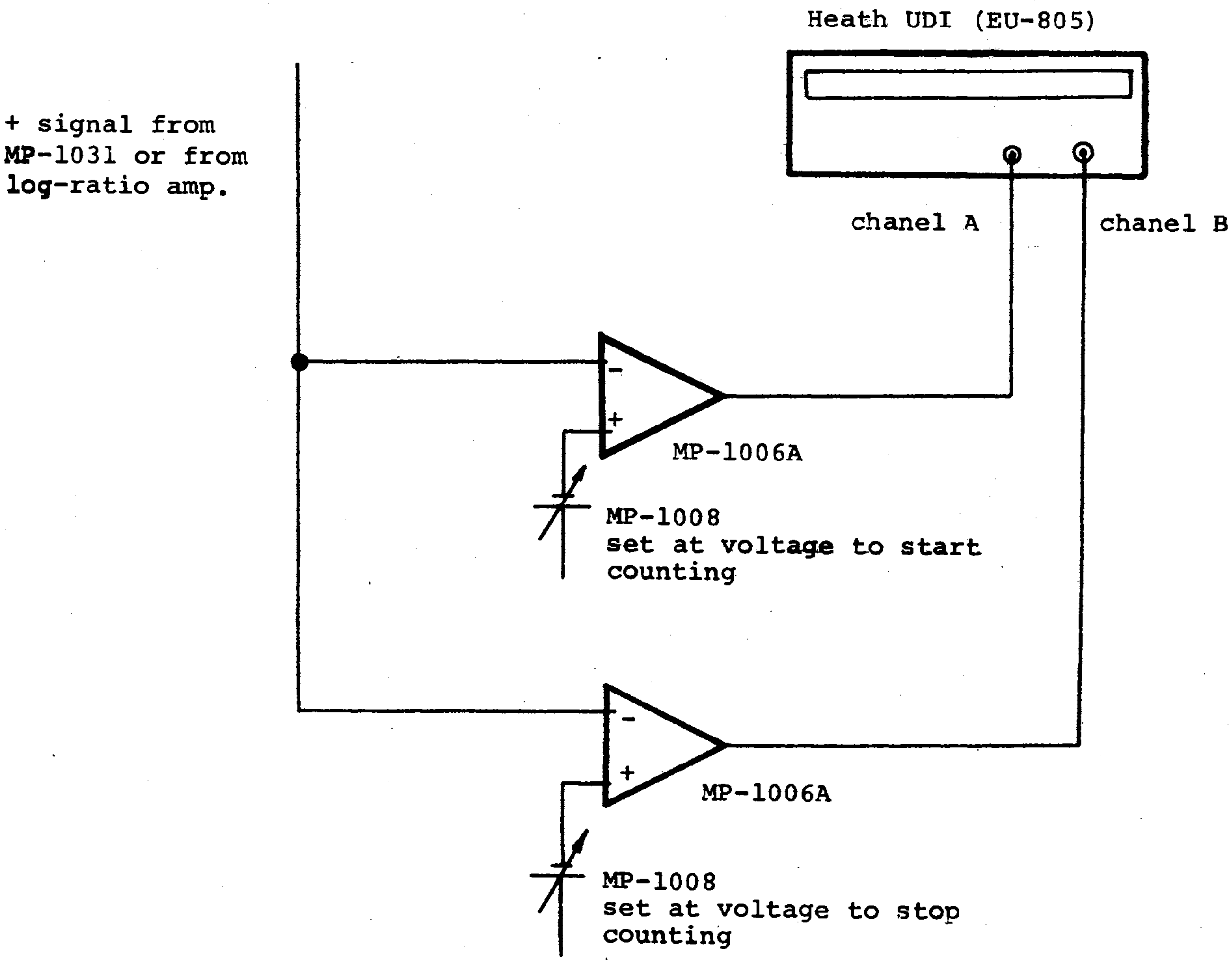


Figure 8. Switching Circuit for the UDI

control in "auto". With the UDI function switch on Time AB, the change in slope at the input of channel A started the timer, and the change in slope at input of channel B then switched the timer off. The time elapsed was then read from the display of the UDI. For a more complete description of the functioning of the UDI, see reference (35) or (36).

CHAPTER VI

THEORY

Chemical

A development and discussion of the theory for the employment of the variable time kinetic method of analysis for the determination of catalytic and catalytic-modifying species is given in reference (31). In the case of a catalyst the following general reaction could be written:

$$R + X \xrightarrow{C} \text{Products}$$

Where R is the reactant whose concentration is monitored with time; X is also a reactant, but is added in excess to create a pseudo-zero order dependence on it, and C is a catalyst. By rearranging and integrating between $[R]_1$ and $[R]_2$ and t_1 and t_2 of the rate equation for the general reaction, the following equation for C_o is obtained if first order in R and C is observed:

$$[C]_o = \frac{K}{\Delta T k c} - \frac{ku}{kc} \qquad [22]$$

Where K is a constant equal to the $\ln[R]_1/[R]_2$, and kc and ku are the catalyzed and uncatalyzed rate proportionality constants, respectively. A linear relationship between $[C]_o$ and $1/\Delta T$ is observed.

When addition of a complexing (chelating) agent causes acceleration of the rate of a catalyzed reaction, two possible explanations arise;

(1) true metal complex catalysis; and (2) ligand promotion. In a case where the catalyst concentration, C_o, is less than the ligand concentration, C_L, both (1) and (2) could apply. The rate expression for these conditions is given in Equation [23].

$$\frac{-d[R]}{dt} = ku[R] + k\acute{c}[C^{*}L]_o\,[R] \qquad [23]$$

Upon rearrangement and integration between $[R]_1$ and $[R]_2$ and t_1 and t_2 of the rate expression for these conditions the following expression can be shown to result.

$$C_L \;\alpha\; [C^{*}L] \;=\; \frac{K}{\Delta T k\acute{c}} - \frac{ku}{k\acute{c}} \qquad [24]$$

Where $C^{*}L$ is the transition metal complex, and $k\acute{c}$ is the new catalyzed proportionality rate constant. Linearity between C_L and $1/\Delta T$ can be seen from Equation [24].

For the experimental conditions where the catalyst concentration is greater than the ligand concentration, explanation (1) seems more probable. The rate expression using this rationale is given in Equation [25].

$$\frac{-d[R]}{dt} = ku[R] + kc[C][R] + k\acute{c}\,[C^{*}L]\,[R] \qquad [25]$$

After rearrangement and integration between $[R]_1$ and $[R]_2$ and t_1 and t_2 of the rate expression it can be shown that:

$$C_L = \frac{K}{\Delta T k\acute{c}} - k^{\prime} \qquad [26]$$

where K and $k^{\prime}$ are constants obtained by combining other constant terms. Again in this case the linearity between C_L and $1/\Delta T$ can be seen. The results of Equation [24] and [26], thus, show the feasibility of using the variable time kinetic method for the determination of low concentrations of complexing agents in solution where the conditions described are found to exist.

Simulation

The theory for the functioning of the simulation circuit is explained in the following manner. A voltage that varied linearly with time, E, from a linear voltage ramp was introduced into a log ratio amplifier. The value of this input voltage at any time, t, could be determined by using the following equation:

$$E = E_o + \left(\frac{E_R}{t_i}\right) \times t \qquad [27]$$

where E_o was the initial voltage of the ramp, t_i was the time for one increment of ramp voltage, E_R. The voltage from the ramp was then introduced into the log ratio amplifier through a resistor, R_A, which then gave resulting current, I_A. The following relationship gives I_A in terms of the ramp parameters.

$$I_A = \frac{E_o}{R_A} + \left(\frac{E_R}{R_A \times t_i}\right) \times t \qquad [28]$$

The output voltage of the log ratio amplifier, E_s, was then given by:

$$E_s = \log\ (I_A/I_K) \qquad [29]$$

where I_K is a constant internal current reference of the log ratio unit.

A relationship for the difference in two reference potentials, E_{s1} and E_{s2} is then given by:

$$E_{s2} - E_{s1} = \log\left[\left(\frac{E_o}{R_A} + \frac{E_R \times t_2}{R_A \times t_i}\right) / I_K\right] - \log\left[\left(\frac{E_o}{R_A} + \frac{E_R \times t_1}{R_A \times t_i}\right) / I_K\right] \qquad [30]$$

Rearranging [30] and letting $K = E_r/t_i$, one obtains,

$$10^{(E_{s2} - E_{s1})} = \left(\frac{E_o + K \times t_2}{I_K \times R_A}\right) - \left(\frac{E_o + K \times t_1}{I_K \times R_A}\right) \qquad [31]$$

Simplifying and factoring [31], one obtains,

$$10^{(E_{s2} - E_{s1})} = \frac{k(t_2 - t_1)}{I_K R_A} \qquad [32]$$

For a given set of experimental conditions, $(E_{s1} - E_{s2})$, I_K, R_A, and t_i are all constant. Incorporating these into a new constant, $k' = (10^{(E_{s2} - E_{s1})} \times t_i)/(I_K \times R_A)$, Equation [33] results:

$$k' = E_R(t_2 - t_1) \qquad [33]$$

Letting $(t_2 - t_1) = \Delta t$, and solving for E_R, one obtains,

$$E_R = k'/\Delta T \qquad [34]$$

A linear relationship between E_R and the inverse of time interval necessary to transverse between the two simulation reference voltages is observed.

CHAPTER VII

EXPERIMENTAL PROCEDURE

Reagents

Malachite Green Perchlorate

Prepared by Bailey (6) by precipitation of the perchlorate by mass action from the hydrochloride.

Manganese Sulfate, Monohydrate

(J. T. Baker Chemical Company, Phillipsburg, N. J.) Reagent grade was used without further purification.

Sodium Meta Periodate

(Fisher Scientific Company, Fair Lawn, N. J.) Reagent grade was used without further purification.

Phosphate-Acetate Buffer (pH 3.5)

35 grams of NaH_2PO_4 and 15 ml of glacial acetic diluted to 500 ml with purified water and the pH adjusted with 1.0 $\underline{M}$ NaOH.

1,10-Phenanthroline

(Eastman Kodak Co., Rochester, N. Y.) Reagent grade was used without further purification.

Bathocuproinedisulfonic Acid, Disodium Salt

(Aldrich Chemical Co., Milwaukee, Wisconsin) Reagent grade was used without further purification.

Neocuproine Hydrochloride (Monohydrate)

(Aldrich Chemical Co., Milwaukee, Wisconsin) Reagent grade was used without further purification.

4,7-Diphenyl-1,10-Phenanthroline

(Aldrich Chemical Co., Milwaukee, Wisconsin) Reagent grade was used without further purification.

Procedure

The methods chosen for evaluating the data collection procedures for the variable time kinetic method of analysis were the simulated reaction curves and the 1,10-phenanthroline effect on the MG^+-periodate-indicator reaction.

The simulated circuit was set up as described in the section on experimental apparatus. The ramp voltages were set by varying the resistance of the 10 Kohm potentiometer in Figure 5 until the desired voltage was read on the DVM function of the UDI. In the first series of runs the output of the simulating circuit was fed into a strip chart recorder. In the following two series of runs the output was fed into the summing points of the switching circuits for the method being evaluated. Seven determinations were made for each ramp voltage setting.

The determination of 1,10-phenanthroline by its effect on the periodate oxidation of MG^+, catalyzed by small amounts of Mn(II), was carried out in a one centimeter quartz cell with magnetic stirrer. All reactions were carried out at a MG^+ concentration of 1.2×10^{-5} $\underline{M}$ and in 0.10 $\underline{M}$ pH 3.5 acetate-phosphate buffer. The reagents were placed in the titration vessel in the following sequence: (1) 1.5 ml of purified water, (2) 0.3 ml of 1.0 $\underline{M}$ acetate-phosphate buffer, (3) 0.3 ml of 1,10-phenanthroline solution, (4) 0.3 ml of 1.0×10^{-6} $\underline{M}$ Mn(II), and (5) 0.3 ml of MG^+ solution. The cell was then placed in the spectrophotometer in the cell holder with magnetic stirrer. The stirrer was set at a constant stirring rate, and 0.3 ml of 5.2×10^{-2} $\underline{M}$ $NaIO_4$ was injected with a 0.5 ml syringe through an injection port in the lid of the spectrophotometer. The signal from the spectrophotometer was fed into the appropriate circuit for the timing method being evaluated. Seven determinations were made for each concentration of 1,10-phenanthroline tested.

The reference switching potentials were set using the UDI and were observed to be constant. The potential of the spectrophotometer was observed to change from day to day for a given absorbance value. The calibration curve of potential versus absorbance showed that the slope of the curve was remaining constant and only the intercept was changing. By using the value of the potential at zero absorbance and the calibration curve, it was then easy to determine the gate voltage level of a particular absorbance.

CHAPTER VIII

RESULTS AND DISCUSSION

The three methods for obtaining values of ΔT for the simulated and kinetic runs were to be evaluated on the basis of the variation of the slope of the calibration line provided by a first order least square analysis of ramp voltage and of 1,10-phenanthroline concentrations on $1/\Delta T$. The kinetic runs, however, did not afford a linear calibration curve for 1,10-phenanthroline concentrations above a concentration of 5.0×10^{-6} $\underline{M}$. The presence of a leveling effect at concentrations above this level caused the deviations in linearity of the 1,10-phenanthroline versus $1/\Delta T$ curves. Least square analysis for higher orders found the best fit occurring for a fourth order polynomial. Since seven replicas were made for each 1,10-phenanthroline concentration, the replication error for the three switching methods could still be compared. A first order analysis was still performed for the concentrations less than or equal to 5.0×10^{-6} $\underline{M}$. The simulated curves gave the expected first order dependence.

Treatment of Data

The value of the slope of the calibration line was obtained using the standard regression analysis formulas (37) for a line of the form $Y = mX + b$, where m is given by Equation [35].

$$m = \frac{N\Sigma X_i\bar{Y}_i - \Sigma X_i\Sigma\bar{Y}_i}{N\Sigma X_i^2 - (\Sigma X_i)^2} \quad [35]$$

where N is the number of points, X_i is the concentration of 1,10-phenanthroline or the ramp voltage and $\bar{Y}_i$ in the average ΔT value of the seven determinations. The standard error of regression, S_{er}, given by Equation [36], can then be employed to give an estimate of the variability of the value of X_i predicted from the least squares fit by dividing S_{er} by m to yield the standard deviation of X, S_x (33).

$$S_{er} = (\Sigma(\delta Y_i)^2/(N-2))^{\frac{1}{2}} \quad [36]$$

where $\delta Y_i = Y_i - (b + mX_i)$. The standard deviation of replication for the points on the calibration curve for a method were then pooled (38) by use of Equation [37]

$$S_p = \left(\frac{\Sigma n_i (S_i)^2}{\Sigma n_i}\right)^{\frac{1}{2}} \quad [37]$$

where n_i is the number of degress of freedom for the standard deviation, S_i.

Comparison of Data Handling Methods for the Simulated Runs

The switching potentials for the simulated runs were chosen at 14 millivolts to initiate the timing and at 25 mV to stop timing. These potentials were chosen because the curve shapes were very similar to those of the kinetic runs between these values. The calibration curves of ramp voltages versus 1/ΔT are shown in Figure 9 for the three methods. The lines are those indicated by the least square

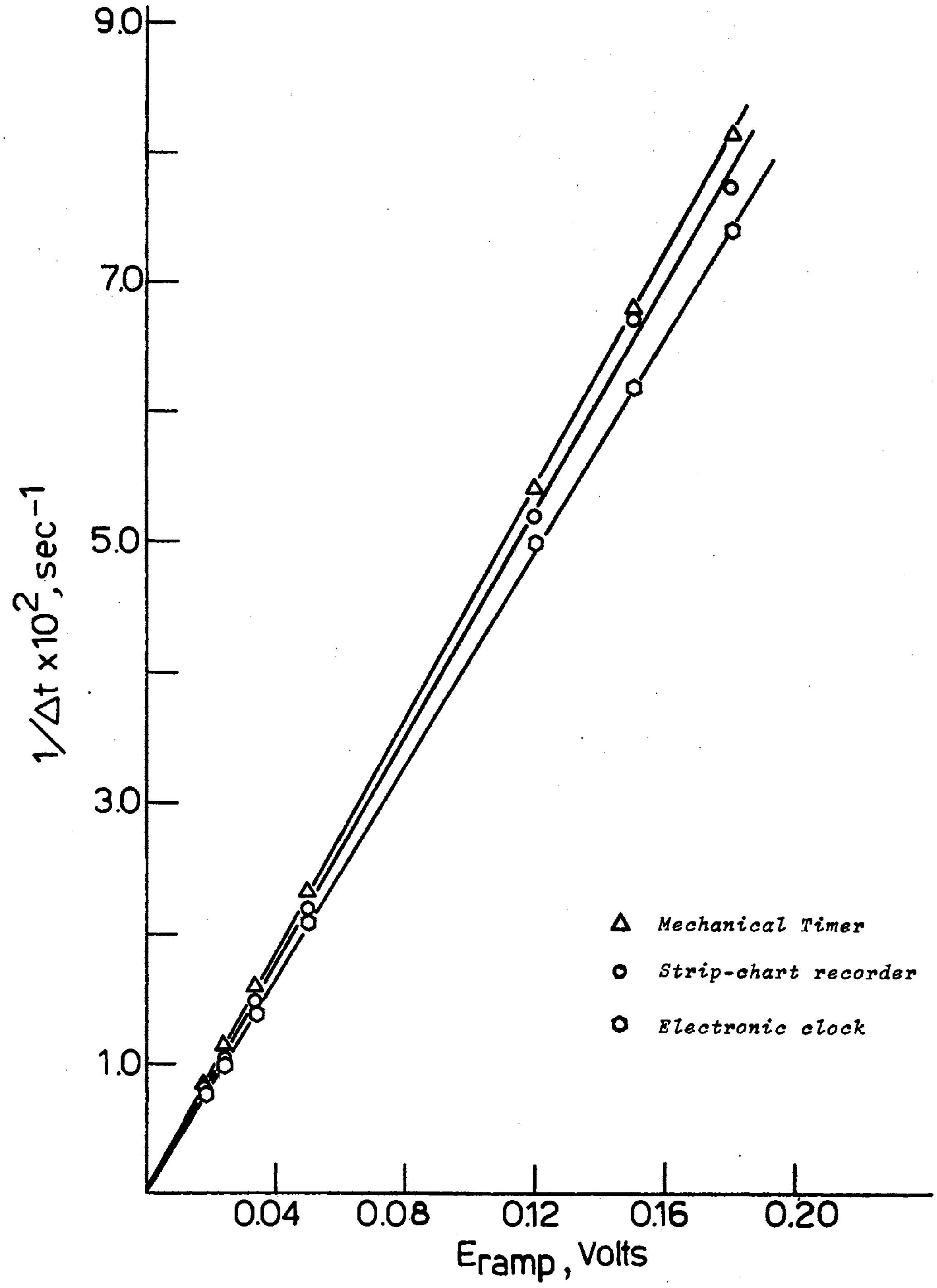


Figure 9. Calibration Curves for Ramp Voltages Versus 1/ΔT

analysis. Table VI lists the standard deviations of predicted X_i values and the pooled replication error, S_p.

TABLE VI

S_x AND S_p FOR THE THREE METHODS USING SIMULATED CURVES

Method	S_x	S_p
Strip Chart	2.5×10^{-3}	1.4×10^{-3}
Electromechanical	2.6×10^{-4}	2.7×10^{-4}
Electronic Clock	6.53×10^{-4}	8.6×10^{-5}

The values for the standard deviation of predicted ramp voltages of the least squares line indicated that the electromechanical timer system provided the best line. The pooled standard deviations indicated that the electronic clock system was the method which provided the greatest reproducibility.

Comparison of the Data Handling Systems for the Kinetic Runs

The switching potentials for the kinetic runs were chosen at values for absorbances of 0.700 and 0.600 for the strip chart system. The references for the electromechanical timer and electronic clock systems were chosen at 0.667 and 0.597. The calibration curves obtained for

1,10-phenanthroline versus $1/\Delta T$ are shown in Figures 10, 11, and 12. The line is that indicated by the least squares analysis of the first five points. The values of the standard deviations of predicted 1,10-phenanthroline concentrations for the first five points and the pooled standard deviations for the seven data points for each method are given in Table VII.

The values of the standard deviation of predicted 1,10-phenanthroline concentrations indicated that the best calibration line was provided by the electromechanical timer system. The pooled standard deviation was a minimum for the electronic clock system showing that reproducibility for this system was greatest.

Effects of 1,10-Phenanthroline Analogs on the MG^+ Indicator Reaction

Some analogs of 1,10-phenanthroline were tested for their effect on the MG^+ indicator reaction under the same conditions employed for the variable time study. The analogs studied and the values of the pseudo-first-order rate proportionality constants, k^*, are listed in Table VIII. The results indicated that the only compound of the analogs tested that enhanced the catalytic effect was 4,7-diphenyl-1,10-phenanthroline. This enhancement was approximately one-half of the enhancement exhibited by 1,10-phenanthroline.

Conclusions

The results of the experiments indicated that the system employing the electromechanical timer yielded the best calibration curves while the electronic clock system had the best reproducibility. For the

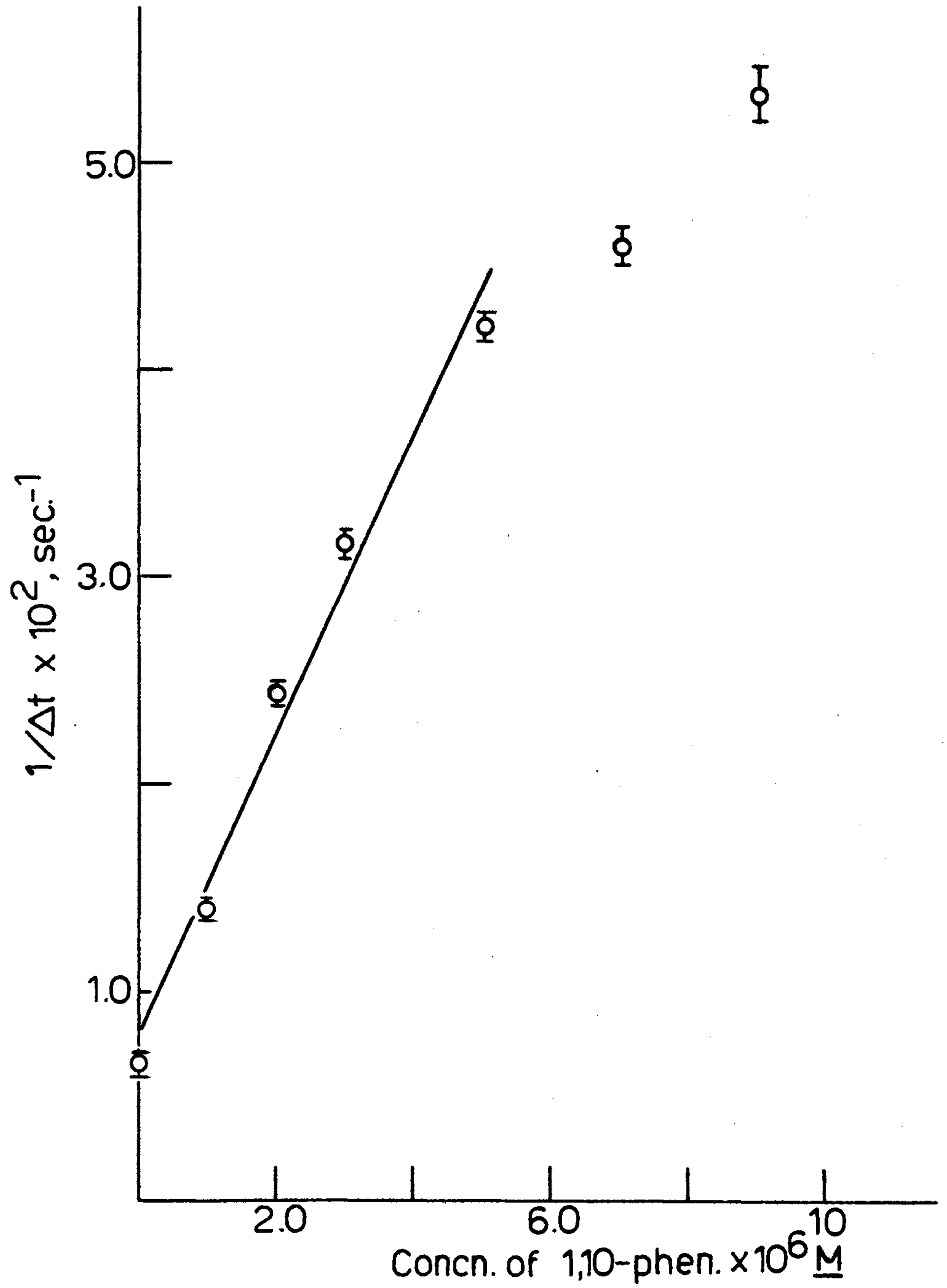


Figure 10. Calibration Curve for 1,10-Phenanthroline Using the Electronic Clock

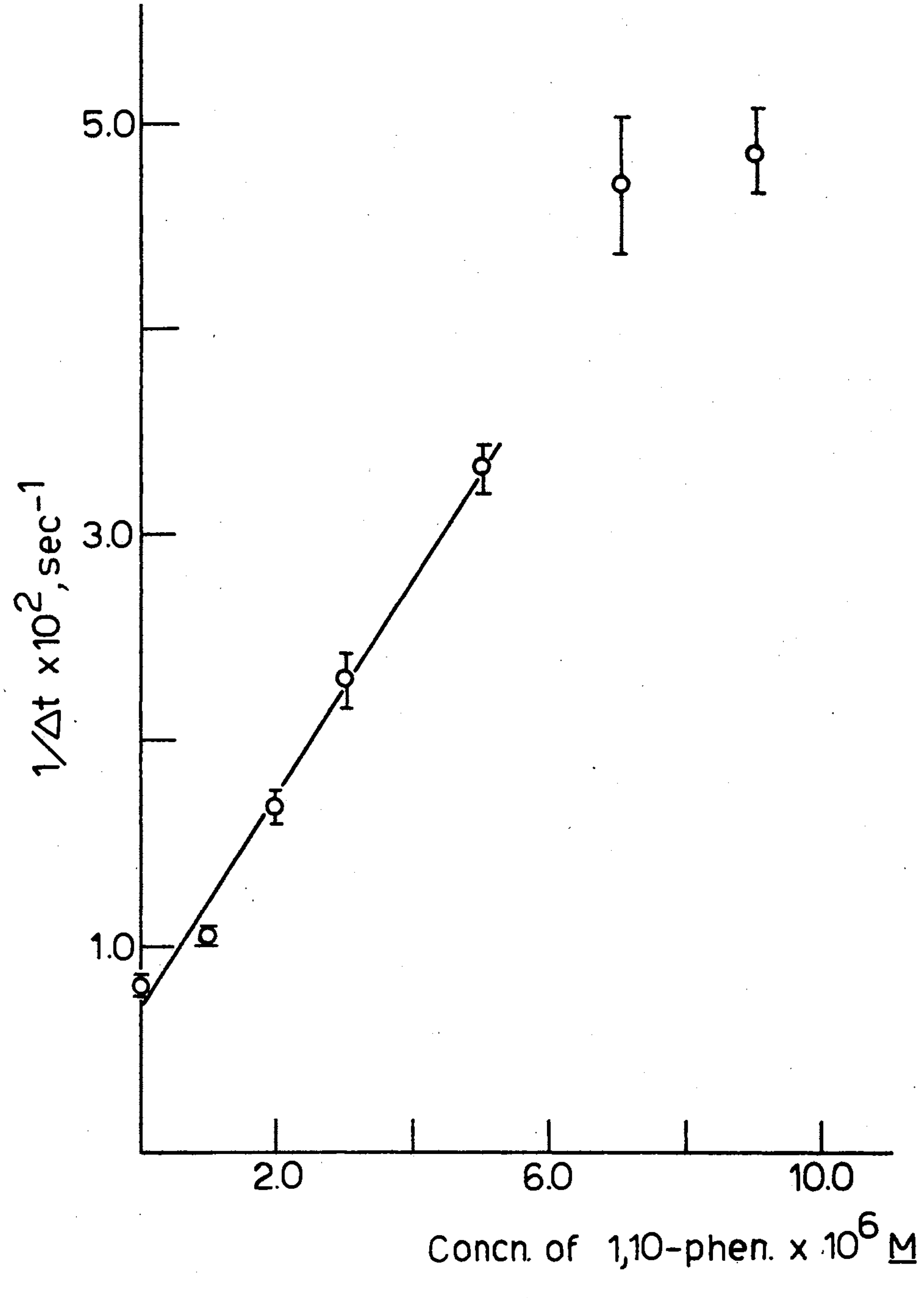


Figure 11. Calibration Curve for 1,10-Phenanthroline Using the Electromechanical Timer

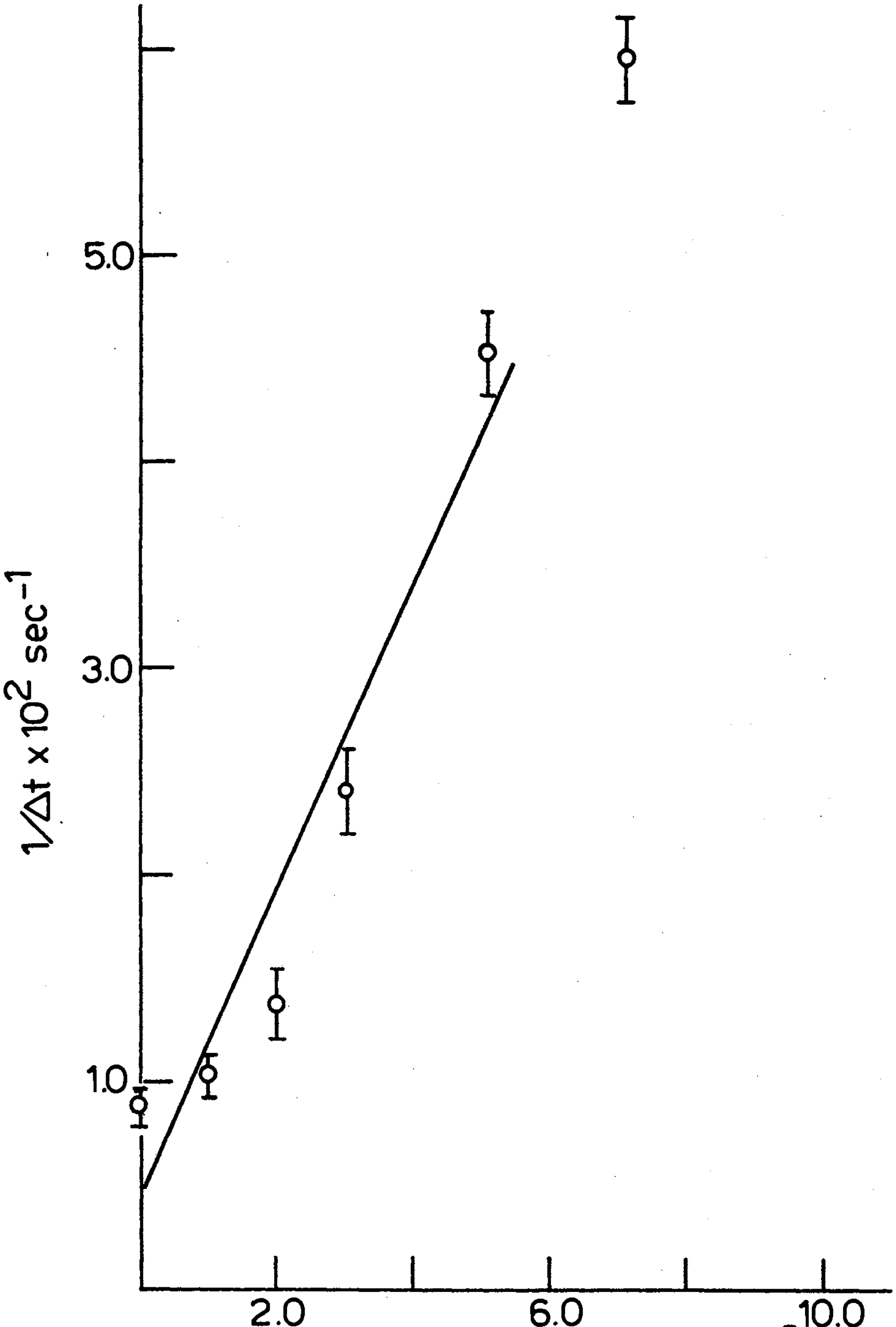


Figure 12. Calibration Curve for 1,10-Phenanthroline Using the Strip Chart Recorder System

TABLE VII

S_x AND S_p FOR THE THREE METHODS USING KINETIC RUNS

Method	S_x	S_p
Strip Chart	4.7×10^{-9}	2.2×10^{-3}
Electromechanical	4.1×10^{-10}	1.7×10^{-3}
Electronic Clock	1.1×10^{-9}	7.5×10^{-4}

TABLE VIII

COMPARATIVE PSEUDO-FIRST ORDER RATE PROPORTIONALITY CONSTANTS OF SOME ANALOGS OF 1,10-PHENANTHROLINE. ANALYTICAL CONCENTRATION 10^{-5} M

	$k^*(\text{min}^{-1})$
Blank	0.24
Neocuproine	0.24
Bathocuproinedisulfonic Acid	0.24
4,7-diphenyl-1,10-phenanthroline	0.44
1,10-phenanthroline	0.74

limited number of replications performed, one could reasonably conclude that for a larger population the calibration lines from the two methods could converge to yield the same accuracy. It should also be noted that the values for the strip chart system are not representative of the results that would be obtained for recorders other than the model employed in this study since chart speed, sensitivity, drift, etc. vary from model to model.

In deciding which method one would employ for their particular circumstances, there are a number of points to consider. The accuracy, reproducibility, and simplicity of the switching network are definite advantages of the electronic clock system. If one considers time of analysis, the automated systems have a decided advantage over the recorder system. For reaction conditions involving very fast reactions, the electronic clock system is the only one versatile enough in the millisecond range. If cost is a major criteria for judgement and rather slow reactions are to be monitored, one might choose the strip chart system since most laboratories have a recorder available and no further cost need be incurred.

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VITA 2

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Master of Science

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II. SOME COMPARATIVE STUDIES ON DATA HANDLING PROCEDURES FOR THE VARIABLE TIME KINETIC METHOD OF ANALYSIS

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