A KINETIC STUDY OF THE REACTIONS OF POLY-L-LYSINE WITH 1-HALO-2,4-DINITROBENZENES

BY

RAYMOND KENNETH RAKOWITZ

A THESIS

PRESENTED IN PARTIAL FULFILLMENT

OF THE REQUIREMENTS FOR THE DEGREE

OF

MASTER OF SCIENCE IN CHEMICAL ENGINEERING

AT

NEW JERSEY INSTITUTE OF TECHNOLOGY

ABSTRACT

The kinetics of the reactions of poly-L-lysine with 1-Fluoro, 1-Chloro, 1-Bromo, and 1-Iodo-2,4-Dinitrobenzenes have been studied. The investigation was done at 25°C as a function of pH and concentration. In all cases the rate of reaction increased as pH increased. In the reactions of Chloro, Bromo, and Iododinitrobenzenes with Polylysine the reaction rate is the composite of two rates; one associated with the random coil and one associated with the helical conformation. For the reaction of Fluorodinitrobenzene with Polylysine the reaction rate is the composite of four rates; two associated with the random coil and two associated with the helical conformation. Base catalysis was observed only in the reaction of Fluorodinitrobenzene with Polylysine. The order of reactivity was found to be $F \gg Br \geq Cl > I$.

APPROVAL OF THESIS

A KINETIC STUDY OF THE REACTIONS OF POLY-L-LYSINE WITH 1-HALO-2,4-DINITROBENZENES

BY

RAYMOND KENNETH RAKOWITZ

FOR

THE DEPARTMENT OF CHEMICAL ENGINEERING

NEW JERSEY INSTITUTE OF TECHNOLOGY

BY

THE FACULTY COMMITTEE OF

APPROVED: ______________________________

____

____

NEWARK, NEW JERSEY

MAY, 1978

PREFACE

This work was undertaken to investigate unanswered questions that have been brought up by previous works performed by members of the College. This work by no means answers all questions and in turn, has raised some new ones.

ACKNOWLEDGMENTS

I would like to thank those people who helped me achieve my goal of Master of Science in Chemical Engineering. First, Dr. Richard Parker, my advisor, who gave me many hours of his time and help.
Dr. David Kristol, who offered many useful comments.
Dr. Ernest Bart, whose calculator programs helped me complete the analysis of the data. And finally, I would like to thank my friends and family, especially Linda; without their encouragement I would never have completed this work.

Table of Contents

Page

Abstract..ii

Approval..iii

Preface...iv

Acknowledgments...................................v

List of Figures...................................vii

List of Tables....................................x

Introduction......................................1

Experimental Technique............................9

Kinetic Procedures................................14

Discussion and Analysis of Results................16

Conclusions and Recommendations...................48

Bibliography......................................52

Appendix I..54

Appendix II.......................................108

Appendix III......................................127

List of Figures

Figure

Structure of Polylysine 1

The Various Conformations of Poly-L-Lysine 2

Structures of the 1-Halo-2,4-Dinitrobenzenes 3

Simple Nucleophilic Aromatic Substitution 4

Reaction of 2,4-Dinitrofluorobenzene with Aniline 5

Structure of Poly (2,4-dinitrophenyllysine) 6

Plots of Optical Density vs. Time for the reactions of FDNB (5.748×10^{-4}M) with Polylysine (1.626×10^{-3}M) and Tham Buffer (0.05M); FDNB (5.748×10^{-4}M) with Tham Buffer (0.05M); and the Resulting Reaction Rate of FDNB (5.748×10^{-4}M) with Polylysine (1.626×10^{-3}M) 7

Net Rates of Reaction vs. pH for the Reactions of:

Polylysine with Fluorodinitrobenzene 8

Polylysine with Chlorodinitrobenzene 9

Polylysine with Bromodinitrobenzene 10

Polylysine with Iododinitrobenzene 11

Plot of Optical Density vs. Time for the Reactions of Polylysine (4.878×10^{-3} and 2.439×10^{-3}M) with FDNB (1.1496×10^{-3}M) 12

Plot of Optical Density vs. Time for the Reactions of Polylysine (1.626×10^{-3}M) with FDNB (1.1496×10^{-4} and 5.748×10^{-4}M) 13

List of Figures

	Figure
Plot of Θh, Fraction of Polylysine in Helical Conformation vs. pH	14
Plot of the formation of Poly (2,4-dinitrophenyllysine) per unit concentration of coil free amine and ClDNB vs. ratio of helix to coil free amine	15
Plot of the formation of Poly (2,4-dinitrophenyllysine) per unit concentration of coil free amine and BrDNB vs. ratio of helix to coil free amine	16
Plot of the formation of Poly (2,4-dinitrophenyllysine) per unit concentration of coil free amine and IDNB vs. ratio of helix to coil free amine	17
Plot of Net Reaction Rate per unit concentration of FDNB vs. pH as a whole and broken down into its component kinetic parts.	18
Plot of Net Reaction Rate per unit concentration of ClDNB vs. pH as a whole and broken down into its component kinetic parts.	19
Plot of Net Reaction Rate per unit concentration of BrDNB vs. pH as a whole and broken down into its component kinetic parts.	20

List of Figures

	Figure
Plot of Net Reaction Rate per unit concentration of IDNB vs. pH as a whole and broken down into its component kinetic parts.	21
Plot of Net Reaction Rate per unit concentration of FDNB vs. pH as a whole and broken down into its component kinetic parts which are extrapolated beyond the experimental limits.	22
Plot of Optical Density vs. Time at pH 10.5 for the reaction of Polylysine (1.626×10^{-3}M) with ClDNB (5.74×10^{-4}M, BrDNB (5.74×10^{-4}M), and IDNB (5.75×10^{-4}M).	23
Plot of Optical Density vs. Time for the reaction of ClDNB (5.74×10^{-4}M) with Polylysine (1.626×10^{-3}M) at pH's of 10.0 and 11.0.	24

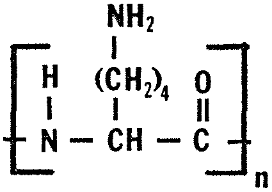
List of Tables

Table

Rate Constants I

INTRODUCTION

Presently, extensive research is being done in the area of Biocatalysis. Biocatalysis is simply defined as catalysis by enzymes, which are polyamino acids. When work first began on protein (polyamino acid) reactions, only interactions between the macromolecules were considered. Further studies, however, looked at the factors that were involved in the macromolecular reactions. These factors were found to be amino acid residues, bases, and the physical and chemical environment of the proteins and their reactants. Such is the case in the reaction of proteins with deoxyribonucleic acids which occur in living organisms. The results of this investigation strongly indicate stacking interaction between bases in DNA and, for example, phenylalanine residues in poly ($Lys^{58}Phe^{42}$).[1]



$$\left[\begin{array}{c} H \\ | \\ N \end{array} - \begin{array}{c} NH_2 \\ | \\ (CH_2)_4 \\ | \\ CH \end{array} - \begin{array}{c} O \\ \| \\ C \end{array} \right]_n$$

Figure 1: Poly-L-lysine

---

[1] Santella, R.M. and Li, H.J., "Studies on Interaction between Poly (L-lysine^{58}, L-phenylalanine^{42}) and Deoxyribonucleic Acids," Biochemistry, Vol. 14, No. 16, 1975, pp. 3604-3611.

Poly-L-lysine (Figure 1), like proteins, is a polyamino acid. Unlike most proteins it can exist in a purely helical or purely random coil conformation depending upon the pH of its aqueous medium. Polylysine has been shown to have several biological applications such as the activation of prothrombin[2], the ability to increase resistance to enzymatic hydrolysis of the interferon inducer polyinosinic acid-polycytidylic acid[3] and the elucidation of the conformations of such glycosaminoglycans as the important blood anticoagulant heparin.[4]

In addition, the active sites in some enzymes, e.g. ribonuclease, acetoacetate decarboxylase and glutamate dehydrogenase, involve nucleophilic attack by a lysine residue. Studies of the nucleophilic reactivity of polylysine can possibly answer some questions concerning the mechanisms of enzyme reactions involving lysine at the

---

[2] Vogel, C. N., Butkowski, R. J., Mann, K. G. and Lundblad, R. L., "Effect of Polylysine on the Activation of Prothrombin. Polylysine Substitutes for Calcium Ions and Factor V in the Factor Xa Catalyzed Activation of Prothrombin," Biochemistry, Vol. 15, No. 15, 1976, pp. 3265.

[3] Bektemirov, T. A., Burgosova, M. P. and Andzharparidze, O. G., "Interferon Induction in Monkeys by Poly (I) Poly (C) Complex with Poly-L-lysine," Voprosnii Virusologica, No. 5, 1976, pp. 536-539.

[4] Schodt, K. P., Gelman, R. A. and Blackwell, J., "The Effect of Changes in Salt Concentration and pH on the Interaction Between Glycosaminoglycans and Cationic Polypeptides," Biopolymers, Vol. 15, No. 10, 1976, pp. 1965-1977.

active site.[5]

The rate of reaction of polylysine with p-nitrophenyl acetate[6] and iodoacetamide[7] has been found to vary with pH since more free amine is present at higher pH values. In the case of iodoacetamide it was possible to determine from experimental data the separate rate constants for the SN2 reactions of the helical free amine and the coil free amine.

At a pH of less than eight polylysine is in a completely random chain configuration possessing a pk_a of 9.76. As the pH increases, the chain becomes more ordered. At a pH of about 12 the chain is completely helical and the pk_a is 10.20.[8,9] This can be better illustrated by

---

[5] Parker, R. C., Rakowitz, R. K. and Kristol, D. S., "The Reaction of Polylysine with 2,4-Dinitrofluorobenzene," International Journal of Biochemistry, Vol. 9, 1978, pp. 117-119.

[6] Kristol, D. S., Krautheim, P., Stanley, S. and Parker, R. C., "The Reaction of p-Nitrophenyl Acetate with Lysine and Lysine Derivatives," Bioorganic Chemistry, Vol. 4, 1975, pp. 299-304.

[7] Parker, R. C., Stanley, S. and Kristol, D. S., "The Reaction of Iodoacetamide with Polylysine, -Acetyllysine and -Acetyllysine," International Journal of Biochemistry, Vol. 6, 1975, pp. 863-866.

[8] Stanley, S. J., "A Kinetic Study of Lysine and Lysine Derivatives," A Thesis Presented in Partial Fulfillment of the Requirements for the Degree of Master of Science in Chemical Engineering at Newark College of Engineering, p. 46.

[9] Parker, R. C., Slutsky, L. J. and Applegate, K. R., "Ultrasonic Absorption and the Kinetics of Conformational Change in Poly-L-lysine," Journal of Physical Chemistry, Vol. 72, No. 9, 1968, pp. 3177-3186.

Figure 2. It has been proven that for polylysine as the pk_a increases so does the rate of reaction.[10]

The problem that was undertaken in this study was the investigation of the reactions of polylysine with 1-Fluoro, 1-Chloro, 1-Bromo, and 1-Iodo-2,4-Dinitrobenzenes. (Figure 3)

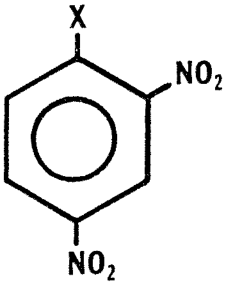


Figure 3: 1-halo-2,4-dinitrobenzene

This reaction is an example of Nucleophilic Aromatic Substitution. (Figure 4)

"There are two essential steps: Attack of a nucleophilic reagent upon the ring to form a carbanion (III), and the expulsion of halide ion from the carbanion to yield the product."[11]

A more complex mechanism has been reported to occur

---

[10] Stanley S. J., "A Kinetic Study of Lysine and Lysine Derivatives."

[11] Morrison, R. T. and Boyd, R. N., Organic Chemistry, Third Edition. Boston, Massachusetts: Allyn and Bacon Inc., 1973, p. 829.

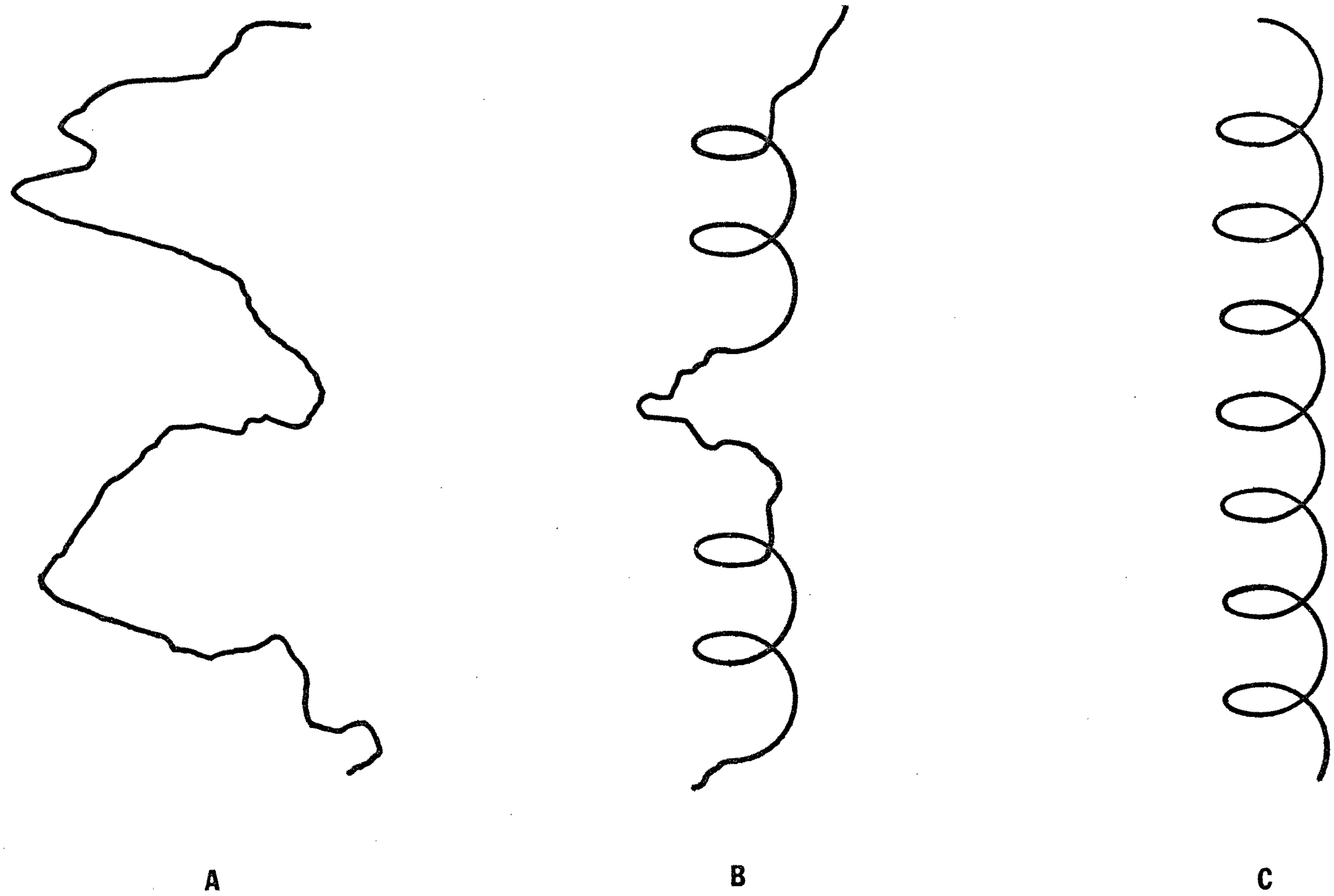


Figure 2: Poly-L-lysine in its Various Conformations

A: Completely Random Coil B: Helix-Coil C: Completely Helix

in the reactions of 1-Fluoro-2,4-Dinitrobenzene with aniline and piperdine[12] and with imidazole.[13]
(Figure 5)

In this case attack by the amine leads to a charged intermediate (IV) which can yield the product, a substituted aniline (VI) by either a spontaneous loss of H+ and F^- or by catalytic removal of H^+ by base with a concomitant loss of F^-. In the case where fluoride ion is displaced from a nitro activated aromatic substrate $(K_2/K_{-1} \ll 1)$[14] it is possible to observe base catalysis. In some cases where a halogen ion other than fluoride is displaced from a nitro activated aromatic substrate $(K_2/K_{-1} \gg 1)$[15] it is not possible to observe base catalysis. In this case they would follow the reaction path of a simple Nucleophilic Aromatic Substitution. (Figure 4)

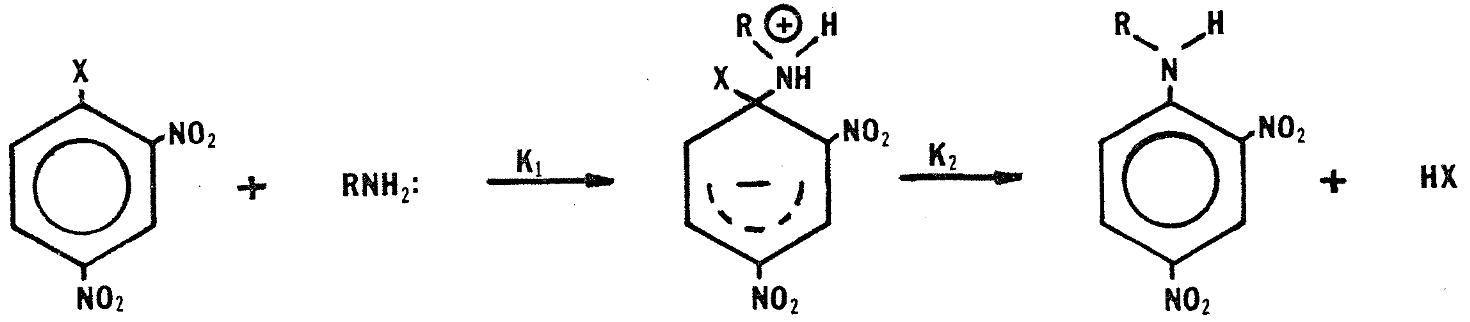
---

[12] De Rossi, R. H. and Rossi, A. R., "Kinetics of Reactions of 1-Substituted 2,4-Dinitrobenzenes, with Aniline and Piperdine in Acetone," Journal of Organic Chemistry, Vol. 39, No. 24, 1974, pp. 3486-3488.

[13] De Rossi, R. H., Rossi, A. R. and Gimenez, F. N. R., "Reaction of 2,4-Dinitrohalobenzenes with Imidazole in Nonpolar Aprotic Solvents," Journal of Organic Chemistry, Vol. 41, No. 19, 1976, pp. 3163-3166.

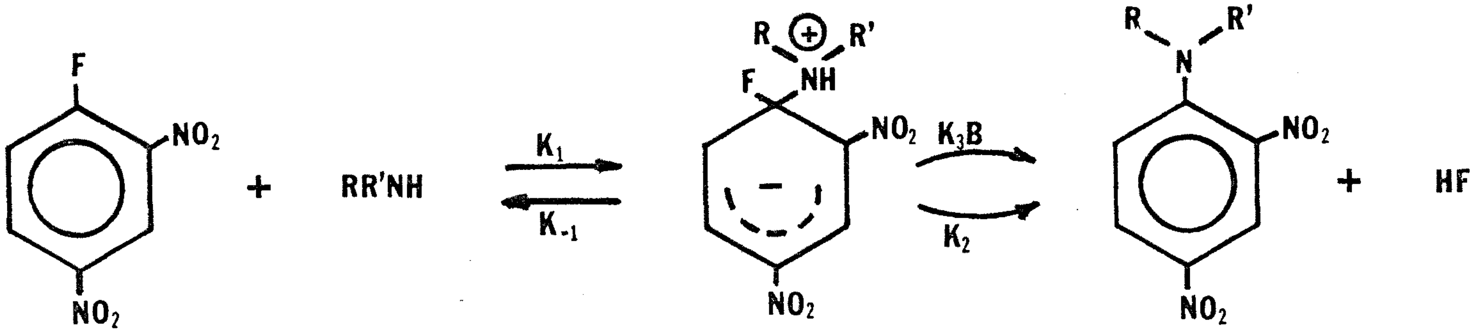
[14] De Rossi, R. H., Rossi, A. R. and Gimenez, F. N. R., "Reaction of 2,4-Dinitrohalobenzenes with Imidazole in Nonpolar Aprotic Solvents."

[15] De Rossi, R. H. and Rossi, A. R., "Kinetics of Reactions of 1-Substituted 2,4-Dinitrobenzenes, with Aniline and Piperdine in Acetone."



III

Figure 4: Simple Nucleophilic Aromatic Substitution



II IV VI

Figure 5: Reaction of 2,4-Dinitrofluorobenzene with Aniline

It was, therefore, felt that a fruitful study could be generated by investigating the reactions of polylysine with 1-halo-2,4-dinitrobenzenes. Because of the areas of desired investigation it was necessary to determine the rates of these reactions as a function of pH and concentration at constant temperature. In summary, this work was undertaken to investigate:

1) The effect of pH on the rates of reaction.

2) To determine by which mechanism each reaction occurs, whether catalyzed or uncatalyzed.

3) And to determine the rate constants for both the uncatalyzed and base catalyzed reactions of the 2,4-dinitrohalobenzenes with the helix and coil amines of poly-L-lysine.

EXPERIMENTAL SECTION

All reactions were run at a constant temperature of 25°C with varying pH and, in some cases, varying concentration. Attempts were made to investigate the reactions at higher temperatures; however, the reaction times were of such short duration that they could not be followed by the spectrophotometric method employed here.

The temperature was maintained with the aid of a Forma-Scientific constant temperature bath that circulated water through the thermo-spacers of the Beckman DU spectrophotometer. For long-term reactions an Eberbach constant temperature bath was used.

The pH range at which the reactions were run was from 8.50 to 11.00. Measurements were taken at pH values of 8.50, 9.00, 9.50, 10.00, 10.50, and 11.00 for the reaction of polylysine with the cholor-, bromo-, and iodo-dinitrobenzenes. For the reaction of fluoro-dinitrobenzene with polylysine measurements were taken at pH's of 8.50, 9.00, 9.50, 10.00, 10.35, 10.65, and 10.85.

The buffered solutions were adjusted to the proper pH's by using a Leeds and Northrop pH meter with a glass electrode and calomel reference electrode. The meter was calibrated using a standard Fischer Scientific Co. pH 9.18

buffer salt dissolved in the proper amount of boiled distilled water.

In Figure 4 the reaction of polylysine with 2,4-Dinitrohalobenzene compound is shown to give two products, the hydrohalic acid and Poly(2,4-dinitrophenyllysine) (Figure 6). This polymer was found to have a λ max of 365 nm.

As the reaction proceeds and product is produced more radiation is absorbed. This enables one to follow the course of the reaction by measuring the absorbance of the product at 365 nm as a function of time. The extinction coefficient, ϵ =2753 OD/(mole/l), of the 2,4-dinitrophenyl derivative of the model monomer unit N-α-acetyl-L-lysine-N-methylamide was used in the coversion of optical density to concentration.

To keep the hydrohalic acid from changing the pH as the reaction proceeded the solution had to be buffered. Due to the large pH range used one buffer was deemed insufficient to cover the whole range. Therefore, two different buffers were used. Tris (Hydroxymethyl) Aminomethane better known as THAM obtained from the Fischer Scientific Company was used for the pH range from 8.50 to 10.00. Sodium Borate, also obtained from Fischer Scientific Company was used in the pH range of 9.50 to 11.00. Both buffers were prepared at 0.05 molar concentration.

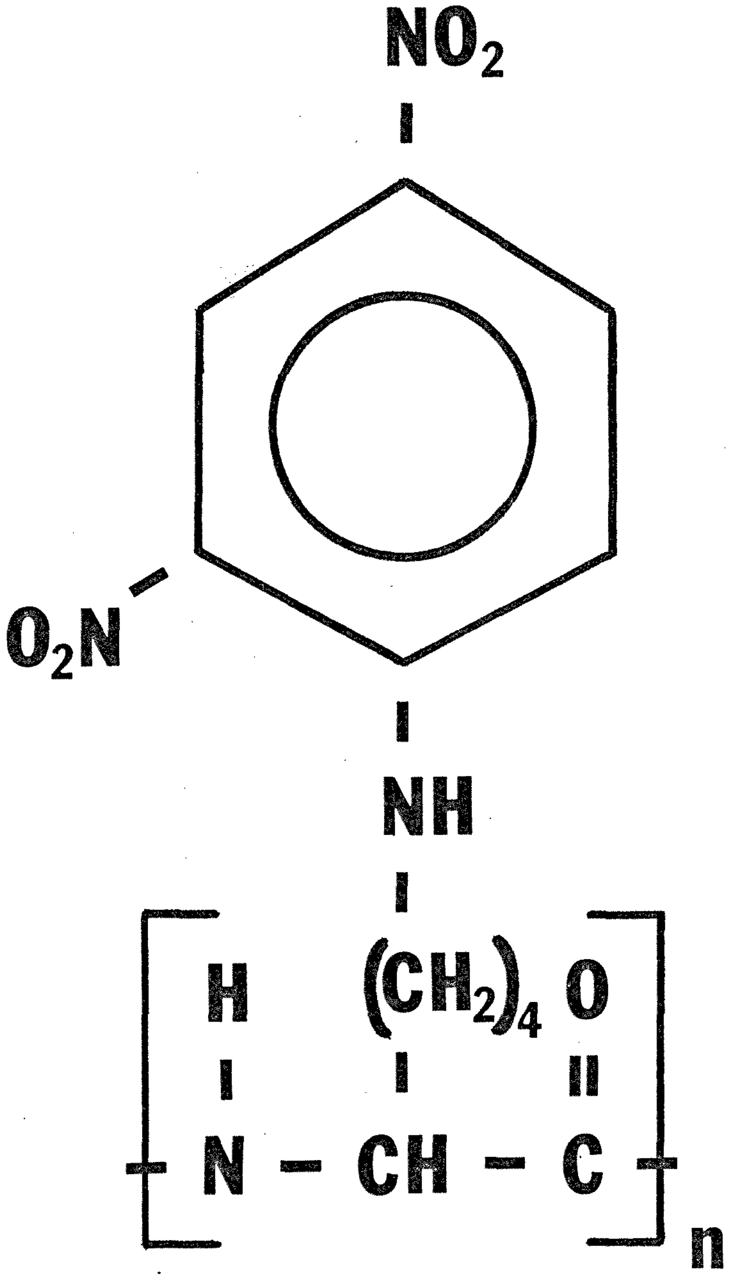


Figure 6: Poly(2,4-dinitrophenyllysine)

Constant ionic strength was maintained in order to minimize the effect of changing ionic strength on the rates of reaction. For this reason Sodium Nitrate, obtained from Brothers Chemical Company, was added to the buffer solutions at a concentration of 0.10 molar. Two liters of each of the Buffer-Sodium Nitrate solutions were prepared to insure uniformity throughout the experiment.

Poly-L-lysine Hydrobromide, Lot #L-71, of molecular weight 125,000 was obtained from the Pilot Chemical Company. This was mixed with buffer solution to prepare two hundred and fifty milliliters of a polylysine solution of 0.00492 molar, with respect to lysine residues, for each of the two buffers. The polylysine solutions were mixed by means of a magnetic stirrer for seven days to insure that all the polymer had gone into solution. Two hundred and fifty milliliters of each polylysine solution was calculated to be sufficient to complete the experiments.

Initial trials with the polylysine solutions showed them to be too concentrated. Therefore, small quantities of the polylysine solutions were diluted to one-third their original concentration with the buffer solutions. This gave a working concentration of 0.00166 molar.

The 2,4-Dinitrohalobenzene solutions were prepared using Eastman Kodak Chemical Company Acetonitrile (spectrograde) as the solvent. The Fluorodinitrobenzene was obtained from the Aldrich Chemical Company and was used without further purification at a concentration of 0.0696 molar. The Chlorodinitrobenzene was obtained from the Eastman Kodak Chemical Company and was recrystallized using absolute Ethanol as a solvent. The Chlordinitrobenzene concentration was 0.0694 molar. The Bromodinitrobenzene was also obtained from the Eastman Kodak Chemical Company and was also used without further purification at a concentration of 0.0695 molar. The Iododinitrobenzene was obtained from the Fairfield Chemical Company and was used as purchased at a concentration of 0.0696 molar.

All reactions were run in spectrophotometer cells with a light path of 10mm. The runs that had short reaction times were carried out in Quartz cells with teflon stoppers. The long-term reactions were carried out in disposable cells obtained from Markson Science, Inc. Parafilm was used to seal these cells. Each disposable cell was used only once for a reaction then discarded.

Approximately two hundred runs were performed taking anywhere from a few seconds to a few weeks to complete the run. The concentration of the nucleophile was always in large excess over that of the 2,4-Dinitrohalobenzene substrates. Most reactions were followed to completion and the net rates were calculated on a Hewlett-Packard 9100 calculator programmed for least squares linear regression.

KINETIC PROCEDURES

A typical run would be performed using the following sequence.

The polylysine solution is first diluted with the buffer solution to the working concentration of 0.00166M. The pH is then adjusted and three milliliters of the polylysine solution is placed in each cell. Duplicates are run of each reaction. In the short reaction time runs only three Quartz cells are filled. In the long reaction time runs as many as thirty disposable cells are filled.

The spectrophotometer is allowed to warm up for at least one hour before the absorbance readings are taken using the hydrogen lamp as the ultraviolet light source. When the instrument is properly warmed the three cells and a reference are placed in the cell holder which is then put into the spectrophotometer's constant temperature chamber, making sure the reference cell is directly in front of the photocell.

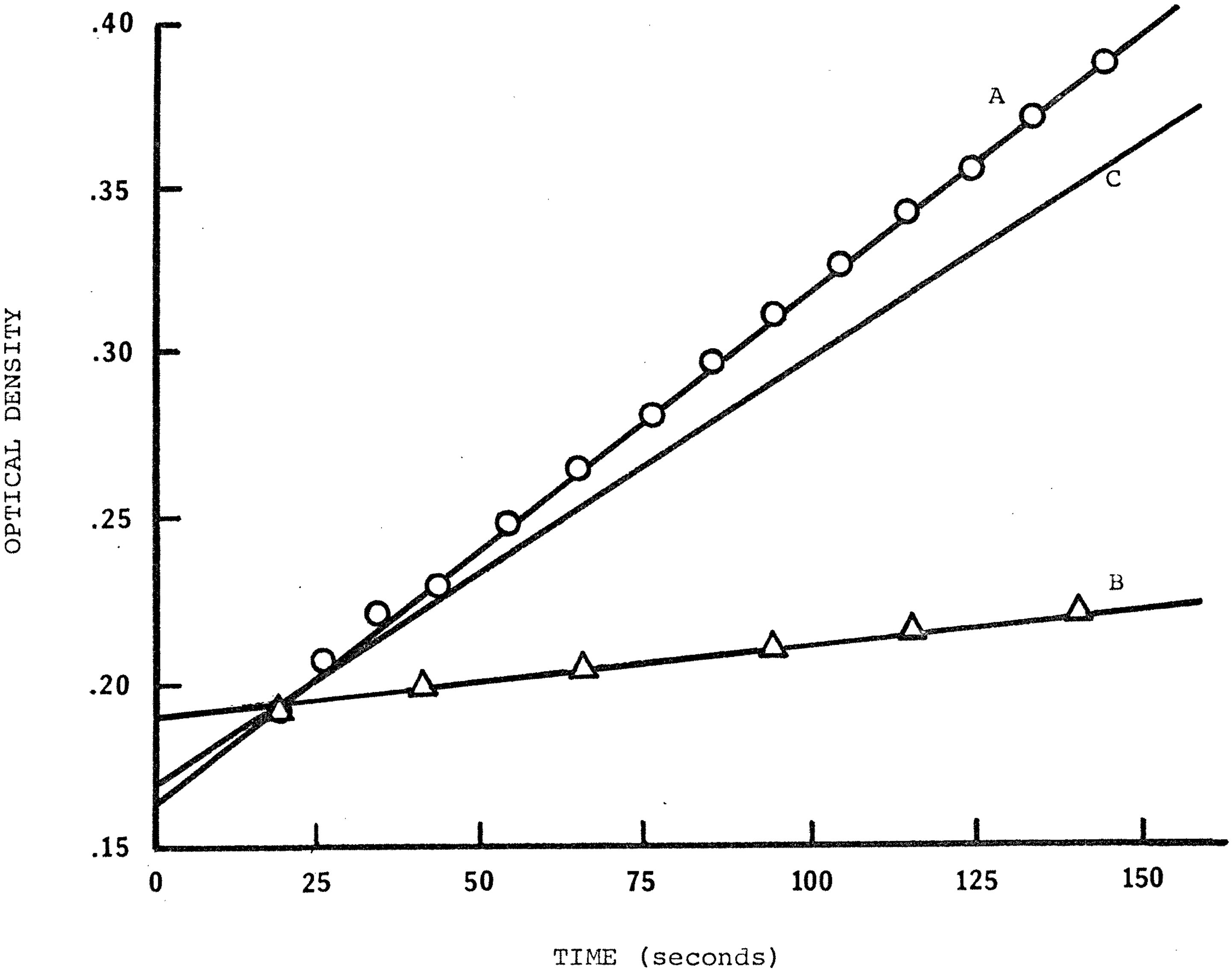
When thermal equilibrium has been attained the spectrophotometer dial is zeroed using distilled water as a reference. The kinetic runs are then ready to be initiated. The cell holder is brought out of the machine and the tops are removed from the cells in which the runs are going to take place. Twenty-five microliters of the 2,4-Dinitrohalobenzene solution are injected into the reaction cells using a Hamilton microsyringe. The tops are replaced and the cells are shaken to insure thorough mixing. The holder is placed back in the spectrophotometer and the absorbance of each reaction cell can be read. If the reaction time is short, the cells remain in the constant temperature chamber and readings are taken at selected intervals of time. For the very slow reactions the cells are checked once a day over a period of two weeks and are stored in a constant temperature bath between readings. The raw data for the kinetic reactions can be found in Appendix I.

DISCUSSION AND ANALYSIS OF RESULTS

A complete tabulation of data for all the runs may be found in Appendix I. An example of the typical type of kinetic data generated may be found in Table A40 of Appendix I. This specific run will be used to illustrate how the final results were obtained. In this example the concentrations of polylysine and 1-Fluoro-2,4-Dinitrobenzene (FDNB) are 1.626×10^{-3}M and 5.748×10^{-4}M respectively. This run was performed at 25°C at a pH of 9.50. A plot of this data is shown in Figure 7 as the upper curve A. A linear regression analysis was performed on this data using a program prepared by Dr. Ernest Bart for the Hewlett-Packard 9100 calculator. The results of this analysis give the slope of the best fit line for the rate of formation of poly (2,4-dinitrophenyllysine) for the given pH, temperature and reactant concentrations. This rate has the units of optical density (OD) per second.

Two reactions take place in the reaction cell upon the addition of FDNB. The one of interest is the reaction of FDNB with polylysine. The second is the reaction of FDNB with the buffer component. It therefore becomes necessary to break up the total reaction rate into its two individual rates. This was accomplished by measuring the rate of reaction of FDNB with the buffer component alone at the same pH, temperature and buffer concentration

Figure 7: Optical Density vs. Time for
A: The Reaction of FDNB (5.748×10^{-4}M) with Polylysine (1.626×10^{-3}M) and Tham Buffer (0.05M)
B: The Reaction of FDNB (5.748×10^{-4}M) with Tham Buffer (0.05M)
C: The Resulting Reaction Rate of FDNB (5.748×10^{-4}M) with Polylysine (1.626×10^{-3}M)



as the polylysine solution. These reactions generated a new set of curves. The run with buffer at the same conditions as the example is shown on Figure 7 as the lower curve B. Least squares analysis of this data was also performed and the results are found in Appendix II. The rates due to the reaction of FDNB and polylysine alone can now be calculated by subtracting the slope of the buffer run from that of the polylysine run and the resulting curve can be seen as the middle curve C in Figure 7.

For each concentration, pH and 2,4-dinitrohalobenzene at least two runs were performed. The rates of each were obtained and averaged. The average value of the reaction of the buffer alone was then subtracted to give an average net reaction rate. These have been tabulated in Appendix II and the resulting graphs of pH vs. reaction rate can be seen in Figure 8 for FDNB, in Figure 9 for ClDNB, in Figure 10 for BrDNB, and in Figure 11 for IDNB. It should be pointed out that at pHs 9.50 and 10.00 the curves in Figures 9, 10, and 11 are discontinuous as a result of switching from THAM to Sodium Borate buffer. This discontinuity is possibly due to the buffer salts' different abilities to catalyze the OH^- displacement of halogen from the benzene ring. For the reactions of FDNB with polylysine only THAM buffer was used and the curve in Figure 8 is continuous.

Figure 8: pH vs. Net Reaction Rate for the Reaction FDNB Polylysine (1.626×10^{-3}M) with FDNB (5.748×10^{-4}M)

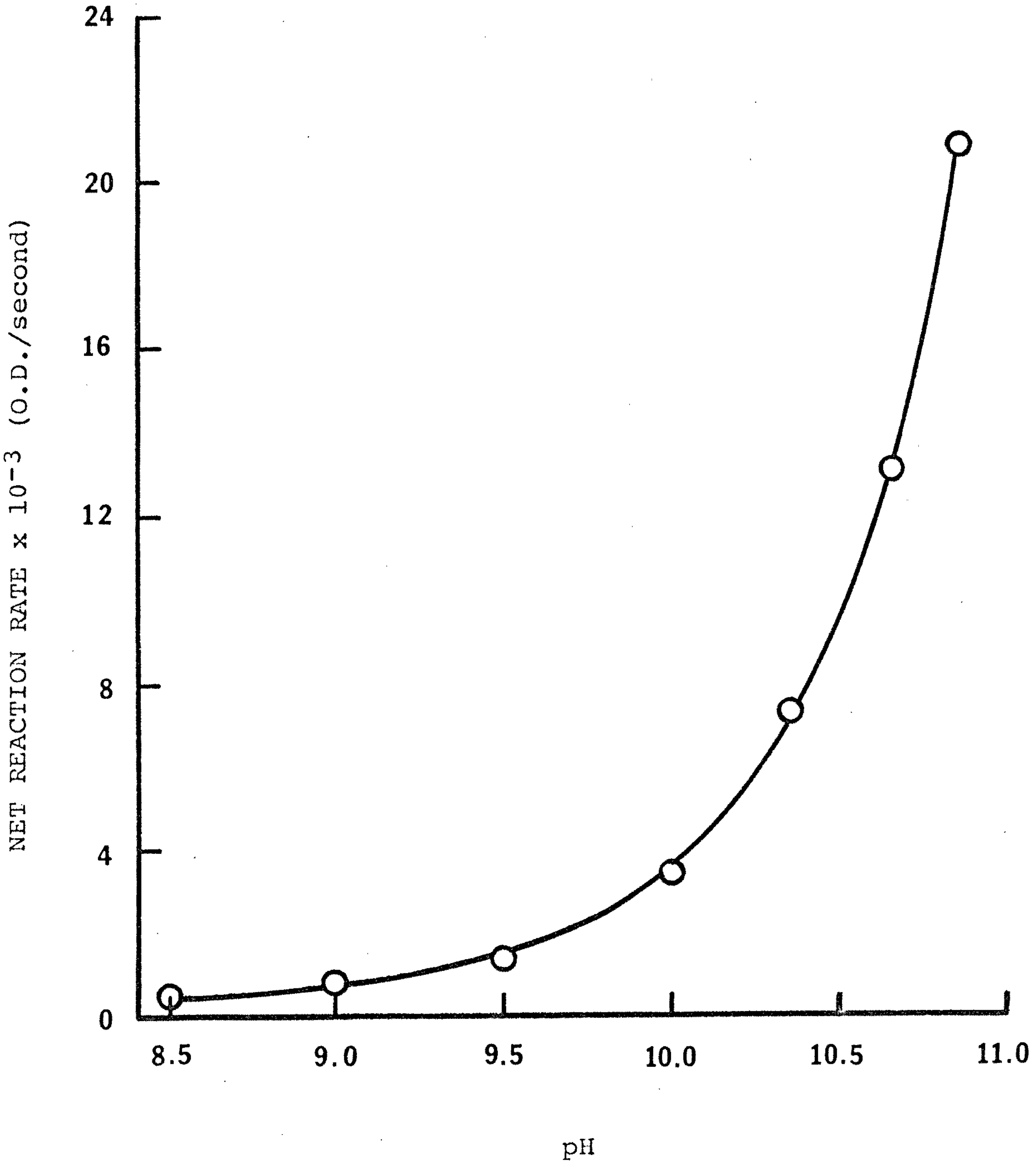


Figure 9: pH vs. Net Reaction Rate for the Reaction of Polylysine (1.626×10^{-3}M) with ClDNB (5.735×10^{-4}M)
△ in Borax Buffer
○ in Tham Buffer

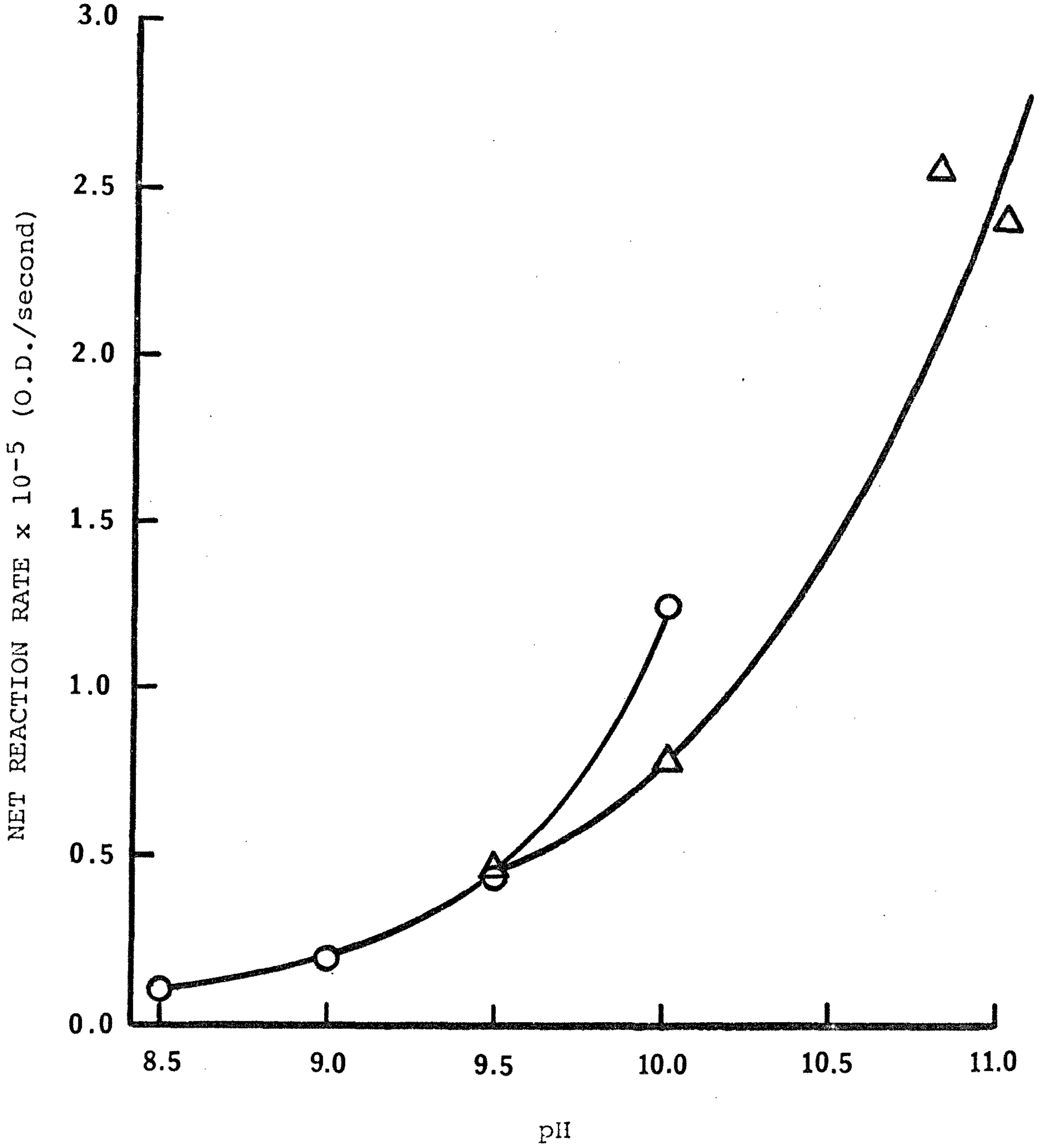


Figure 10: pH vs. Net Reaction Rate for the Reaction of Polylysine (1.626×10^{-3}M) with BrDNB (5.741×10^{-4}M)
△ in Borax Buffer
○ in Tham Buffer

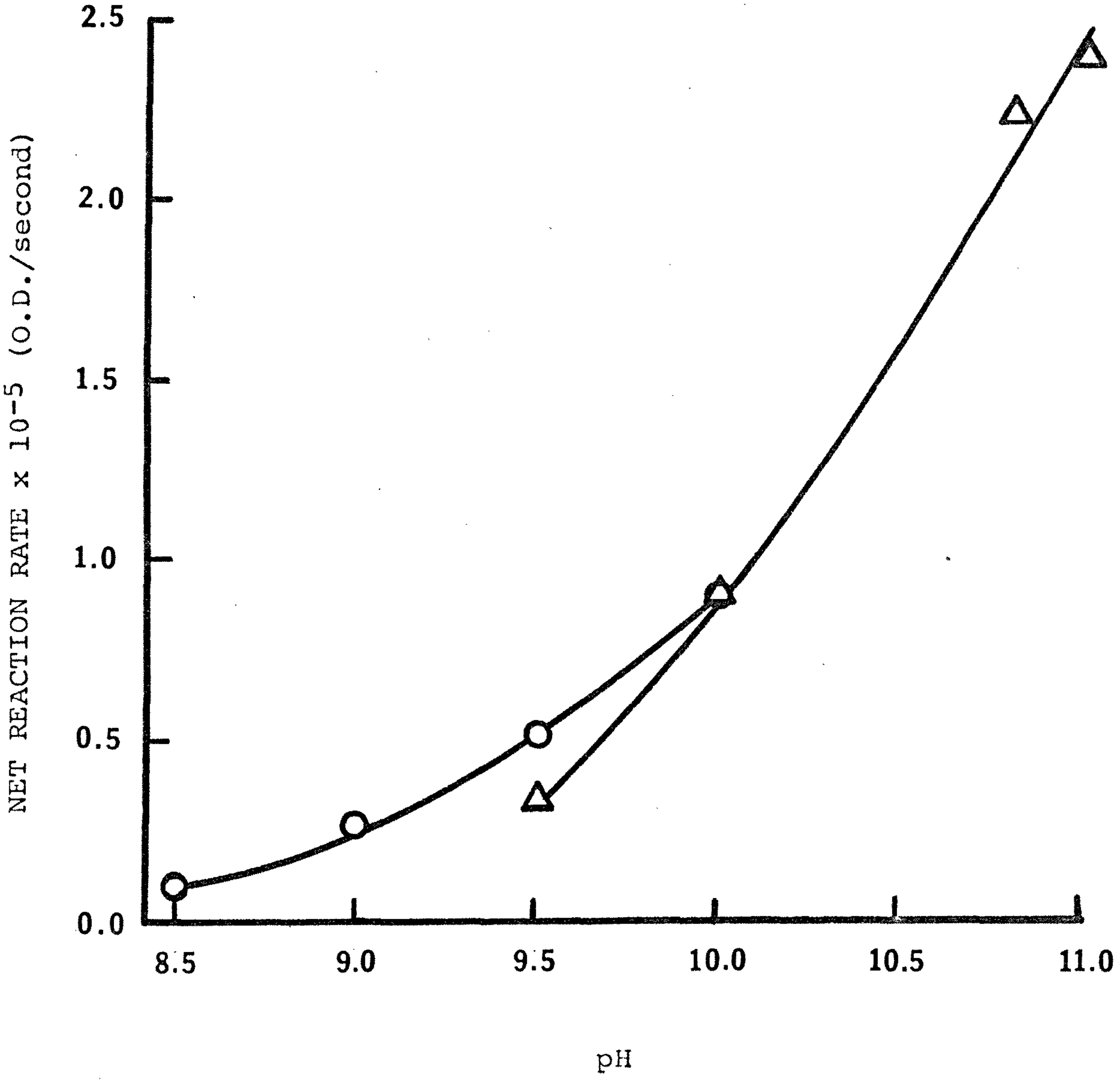
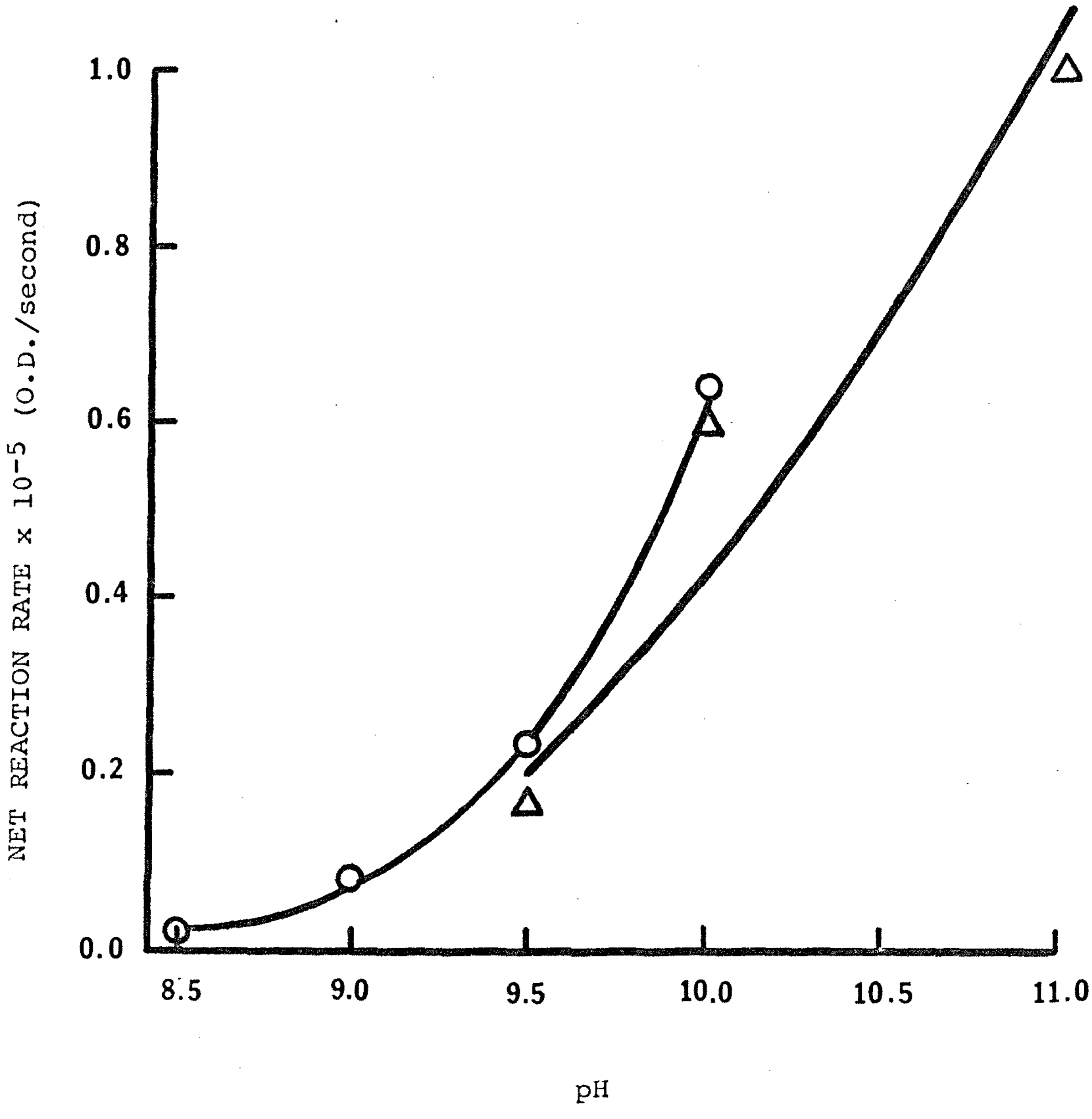


Figure 11: pH vs. Net Reaction Rate for the Reaction of Polylysine (1.626×10^{-3}M) with IDNB (5.748×10^{-4}M)
△ in Borax Buffer
○ in Tham Buffer



The reaction rates in the units of OD/time must be converted to more useful units. This is done by multiplying the average net rate at each pH by the extinction coefficient ϵ . The extinction coefficient was determined experimentally by measuring the optical density of a known concentration of the final product. The extinction coefficient was found to be ϵ =2753 optical density units/(mole/liter).

For the example the reaction rate was found to be 2.367×10^{-3}OD/second. The buffer reaction rate, $3,250 \times 10^{-4}$OD/second, was subtracted giving a total net reaction rate of 2.042×10^{-3}OD/second. This was converted to the units of (moles/liter)/second by use of the extinction coefficient to give a net rate of 7.417×10^{-7} (moles/liter)/second. The net rates in the units of (moles/liter)/seconds for all runs has been tabulated in Appendix II.

The reactions of polylysine with the 2,4-dinitrohalobenzenes were found to be first order with respect to polylysine concentration and first order with respect to 2,4-dinitrohalobenzene concentrations. This can be seen from Figures 12 and 13, where the reaction rates or slopes of the lines involving reactions of twice the normal concentrations of the reactants are double those involving the normal concentrations.

Figure 12: Optical Density vs. Time for
A: The Reaction of Polylysine (4.878×10^{-3}M) with FDNB (1.1496×10^{-3}M)
B: The Reaction of Polylysine (2.439×10^{-3}M) with FDNB (1.1496×10^{-3}M)

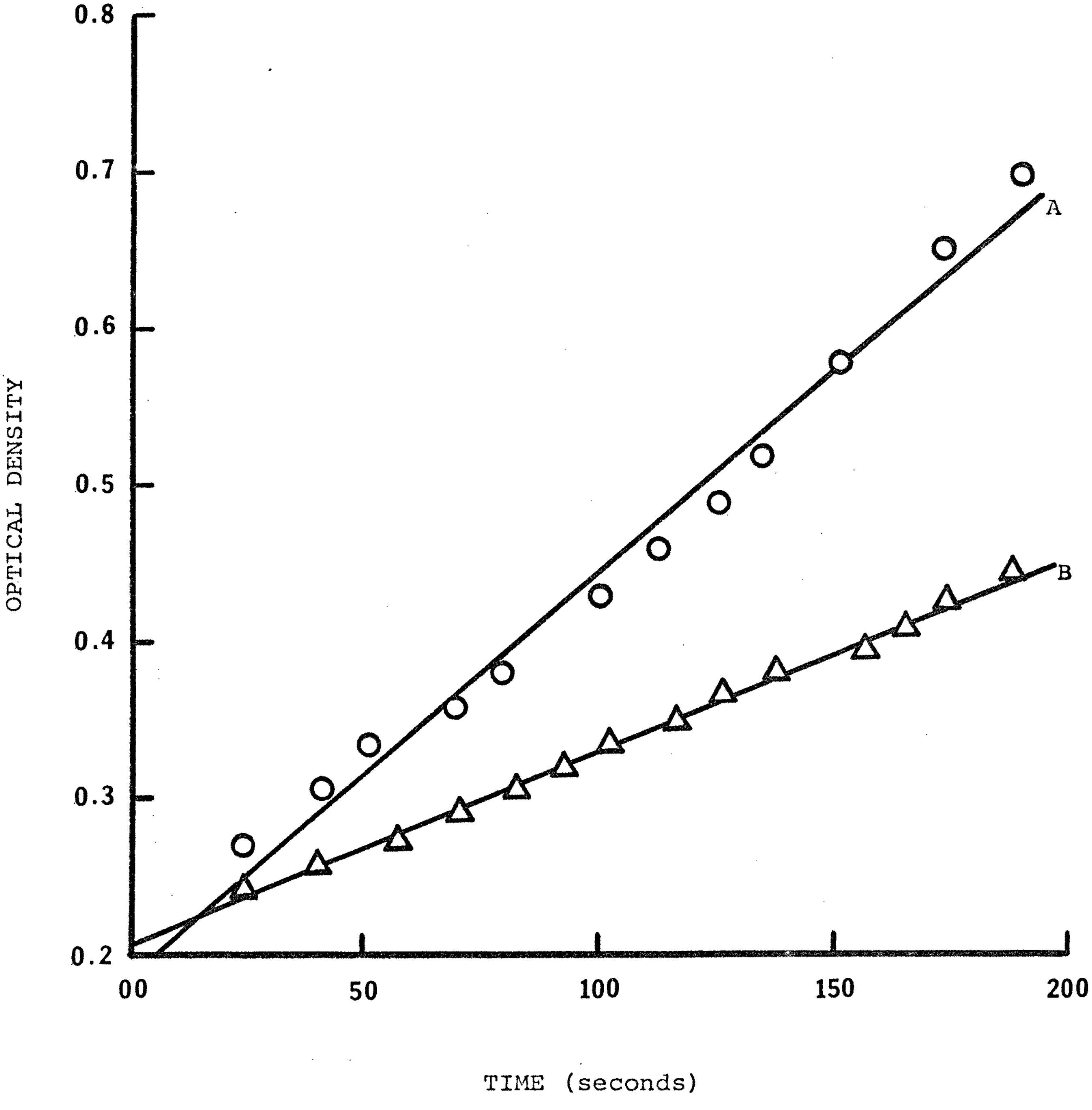
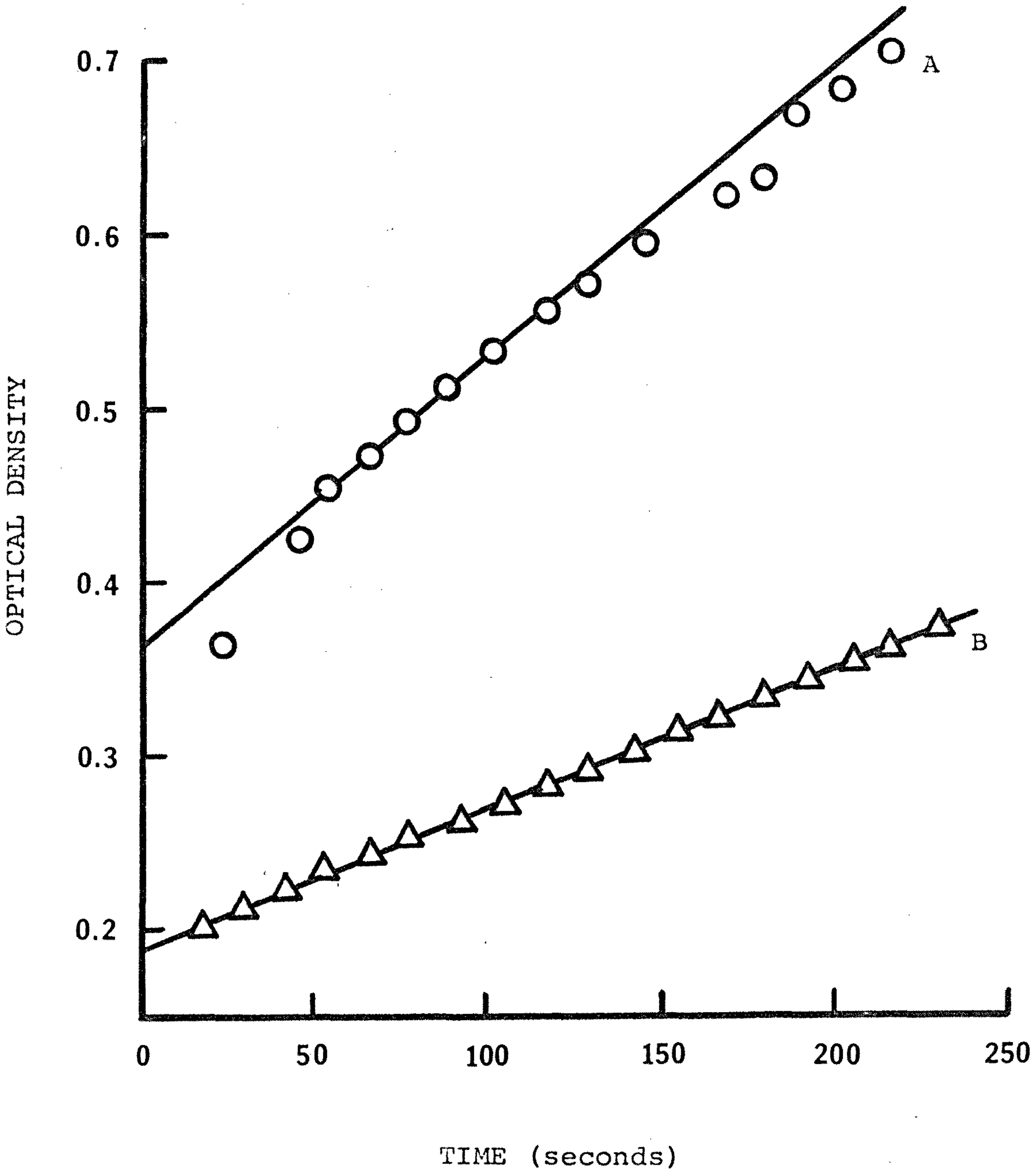


Figure 13: Optical Density vs. Time for the Reactions of
A: Polylysine (1.626 x 10^{-3}M) with FDNB (1.496 x 10^{-4}M)
B: Polylysine (1.626 x 10^{-3}M) with FDNB (5.748 x 10^{-4}M)



The rate expression that has been suggested includes all possible factors that might have an effect on the reaction rate and is Equation A.[16]

$$\text{A} \quad \frac{\text{Rate}}{[\text{X}]} = k_1[\text{NH}_2]_c + k_2[\text{NH}_2]_h + k_3[\text{NH}_2]_c[\text{OH}^-] + k_4[\text{NH}_2][\text{OH}^-] + k_5[\text{NH}_2]^2 + k_6[\text{NH}_2]^2$$

Where Rate = the rate of formation of poly(2,4-dinitrophenyl)lysine
$[X]$ = concentration of the 2,4-dinitrohalobenzene
$[NH_2]_c$ = free amine concentration in the coil configuration
$[NH_2]_h$ = free amine concentration in the helix configuration
$[OH^-]$ = concentration of hydroxide ion
k_1 = the rate constant associated with the coil amine
k_2 = the rate constant associated with the helix amine
k_3 = the rate constant associated with base catalysis of the coil amine
k_4 = the rate constant associated with base catalysis of the helix amine
k_5 = the rate constant associated with self catalysis of the coil amine
k_6 = the rate constant associated with self catalysis of the helix amine

The $[NH_2][OH^-]$ terms represent hydroxide catalysis and the $[NH_2]^2$ terms represent self catalysis.

Although self catalysis has been observed with some amines, the reaction here was found to be first order with respect to polylysine. On a molecular level this is not unreasonable since the charged intermediate formed during

---

[16] Parker, R. C., Rakowitz, R. K. and Kristol, D. S., "The Reaction of Polylysine with 2,4 Dinitroflurobenzene."

the initial attack by polylysine is still attached to the large polylysine molecule and therefore would be hindered to further attach by the bulky polylysine molecule. This precludes any significant contribution of the self-catalysis terms reduces Equation A to Equation B.

$$\text{B} \quad R=\frac{\text{Rate}}{[F]} = k_1[NH_2]_c + k_2[NH_2]_h + k_3[NH_2]_c[OH^-] + k_4[NH_2]_h[OH^-]$$

Before any of the rate constants could be obtained the concentration of the coil free amine $[NH_2]_c$ and the helix free amine $[NH_2]_h$ had to be calculated. This was done by solving two simultaneous equations. Equation D which is the equation for the equilibrium of the amine group.

$$\text{D} \quad -NH_3^+ \rightleftharpoons -NH_2 + H^+$$

And Equation E which states that the total amine concentration whether it be free amine or protonated amine must equal the total polylysine concentration.

$$\text{E} \quad [NH_2] + [NH_3^+] = [PL]$$

$[NH_3^+]$ = concentration of protonated amine
$[NH_2]$ = concentration of free amine
$[PL]$ = concentration of Polylysine

If the concentration of polylysine is broken down into the concentrations of each of its two configurations; helix and coil, Equation E would change to Equations F1 and F2.

$$\text{F1} \quad [NH_2]_c + [NH_3^+]_c = \theta_c\ [PL]$$

F2 $[NH_2]_h + [NH_3^+]_h = \theta_h\ [PL]$

where $\theta_c\ [PL] + \theta_h\ [PL] = [PL]$ and θ_c and θ_h are the fractions of polylysine in coil and helix configurations respectively.[17] Figure 14 is a graph of θ_h vs. pH.

Equation D can also be broken down to coil and helix polylysine with Equations G1 and G2 resulting.

G1 $-NH_3{}^+{}_c \rightleftharpoons -NH_{2c} + H^+$

G2 $-NH_3{}^+{}_h \rightleftharpoons -NH_{2h} + H^+$

The resulting equilibrium expressions for these two reactions are given by

H1 $k_c = \dfrac{[NH_2]_c\ [H^+]}{[NH_3^+]_c}$ H2 $k_h = \dfrac{[NH_2]_h\ [H^+]}{[NH_3^+]_h}$

These Equations can be further simplified by taking the log of both sides

$\log k_c = \log H^+ \log \dfrac{[NH_2]_c}{[NH_3^+]_c}$ $\log k_h = \log H^+ \log \dfrac{[NH_2]_h}{[NH_3^+]_h}$

substituting the identities $-\log k - pk$ and $-\log H^+ = pH$ gives the final Equations I1 and I2

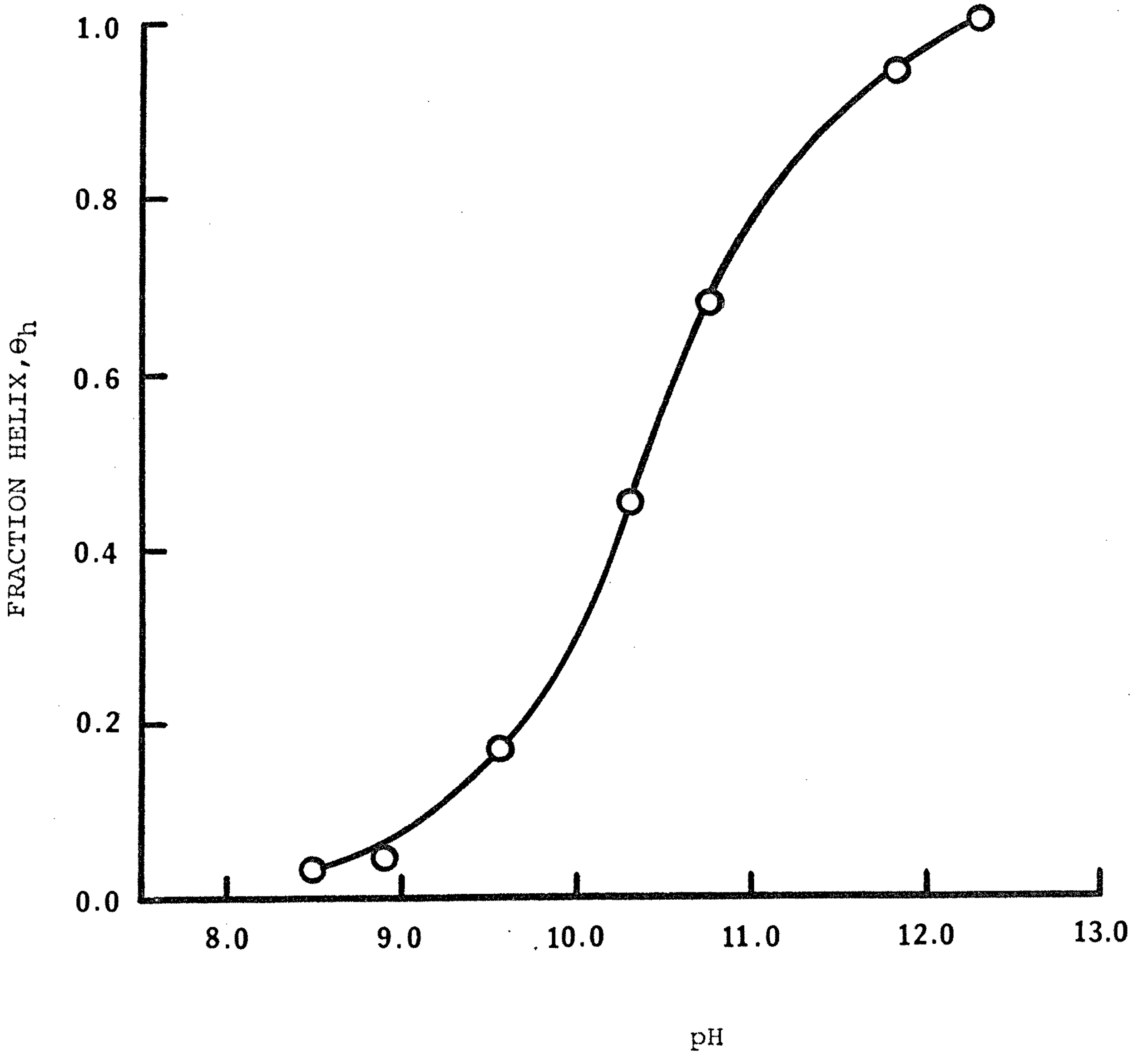
I1 $\dfrac{[NH_3^+]_c}{[NH_2]_c} = 10^{pkc-ph}$ I_2 $\dfrac{[NH_3^+]_h}{[NH_2]_h} = 10^{pkh-ph}$

now with two equations and two unknowns for both the helix and coil configurations, the unknowns can be found by solving the two equations simultaneously.

---

[17] Parker, R. C., Slutsky, L. J. and Applegate, K. R., "Ultrasonic Absorption and the Kinetics of Conformational Change in Poly-L-lysine."

Figure 14: Θ_h, Fraction of Polylysine in Helical Conformation vs. pH



For the example of the reaction of FDNB with polylysine at pH 9.50 and 25° C the polylysine concentration [PL] is 1.626×10^{-3} moles/liter. The pk for coil and helix polylysine are 9.76 and 10.20 respectively.[18] From Figure 14 the fraction of polylysine in the helix form O_h is 0.16 and the fraction of polylysine in the coil form is simply $(1-O_h)$ or 0.84. Substituting these into Equations I1, I2, F1, and F2 gives the following results:

$[NH_2]_c = 4.84 \times 10^{-4}$ M $[NH_3^+]_c = 8.82 \times 10^{-4}$ M

$[NH_2]_h = 3.63 \times 10^{-5}$ M $[NH_3^+]_h = 2.24 \times 10^{-4}$ M

These and all the other free amine calculation results can be found in Appendix II.

The rate constants in Equation B can now be determined. The reactions rates were obtained by experiment. The 2,4-dinitrohalobenzene concentrations and pH are known, and the hydroxide ion concentration and free amine concentrations for both the helical and coil amines can be calculated. Calculating the rate constants involves using a program designed by Dr. Ernest Bart for the Hewlett-Packard 9820 Calculator. This program involves finding all the necessary summations and a 4 x 4 matrix inversion to solve for the four rate constants. The program is listed

---

[18] Parker, R. C., Slutsky, L. J. and Applegate, K. R., "Ultrasonic Absorption and the Kinetics of Conformational Change in Poly-L-lysine."

in Appendix III.

This program worked well for the reactions of FDNB with polylysine but was unable to give a satisfactory fit of the rate data for any of the other reactions. Therefore, a new model was considered which involved no base catalysis; it generated a new rate expression. (Equation C)

$$\text{C} \quad \frac{\text{Rate}}{[X]} = k_1 \, [NH_2]_c + k_2 \, [NH_2]_h$$

This expression says that there is no observed base catalysis. The lack of base catalysis here can be attributed to the fact that Cl, Br, and I bonds to benzene rings are weaker than F-benzene bonds. Hence, k_{-1} is very small, and $k_2/k_{-1} >> 1$. The constants k_1 and k_2 could now be found by plotting $\frac{\text{Rate}}{[X]\,[NH_2]_c}$ vs. $\frac{[NH_2]_h}{[NH_2]_c}$ as in Figures 15, 16, and 17 for Chloro, Bromo and Iododinitrobenzenes. The resulting slope is the rate constant k_2 and the y-intercept is the rate constant k_1. This rate expression worked well for the remaining reactions and the results are tabulated for all the reactions in Table I.

Figures 18, 19, 20, and 21 show the total corrected rate resolved into its component parts for the reactions of polylysine with FDNB, ClDNB, BrDNB, and IDNB. In all

Table I

Reaction	k_1	k_2	k_3	k_4	Goodness of Fit
FDNB + Polylysine	9.18×10^{-1}	5.19	4.79×10^{3}	7.29×10^{3}	-
ClDNB + Polylysine	5.40×10^{-3}	1.21×10^{-2}	-	-	0.996
BrDNB + Polylysine	5.79×10^{-3}	1.22×10^{-2}	-	-	0.998
IDNB + Polylysine	2.23×10^{-2}	5.31×10^{-3}	-	-	0.983

Figure 15: Rate of formation of Poly (2,4-dinitrophenyllysine) per unit concentration of coil free amine and ClDNB vs. ratio of helix to coil free amine

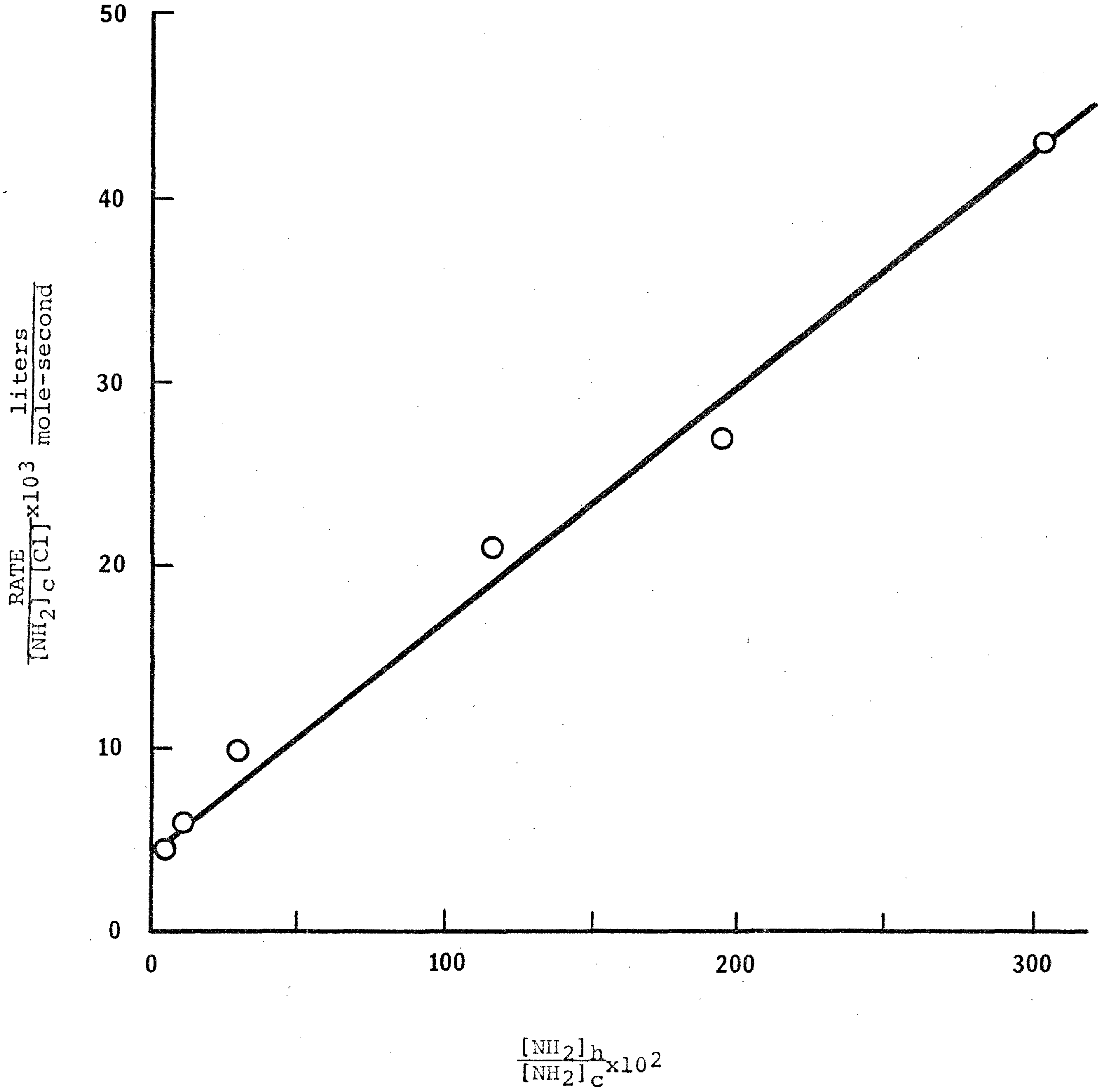


Figure 16: Rate of formation of Poly (2,4-dinitrophenyllysine) per unit concentration of coil free amine and BrDNB vs. ratio of helix to coil free amine

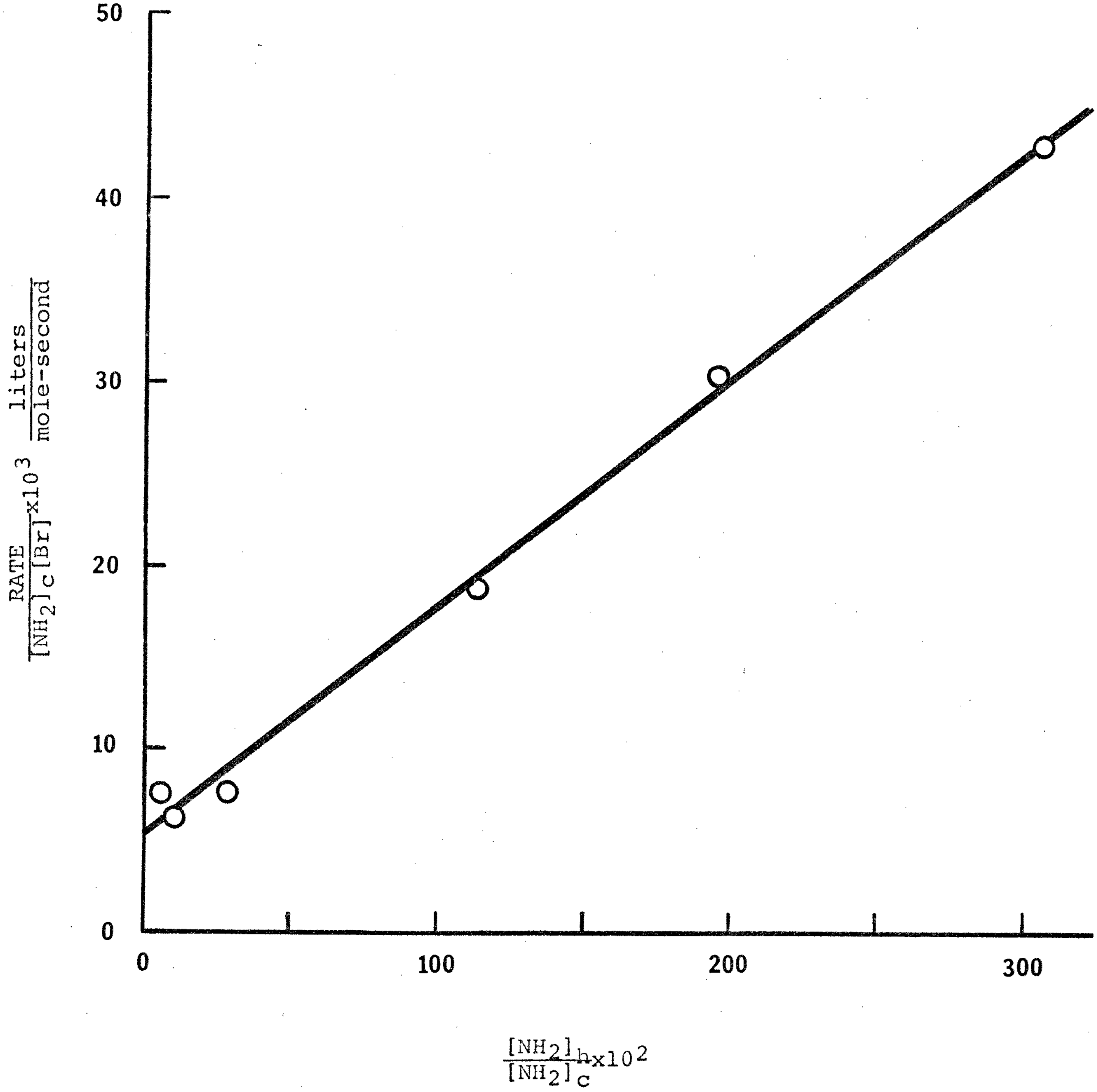


Figure 17: Rate of formation of Poly (2,4-dinitrophenyllysine) per unit concentration of coil free amine and IDNB vs. ratio of helix to coil free amine

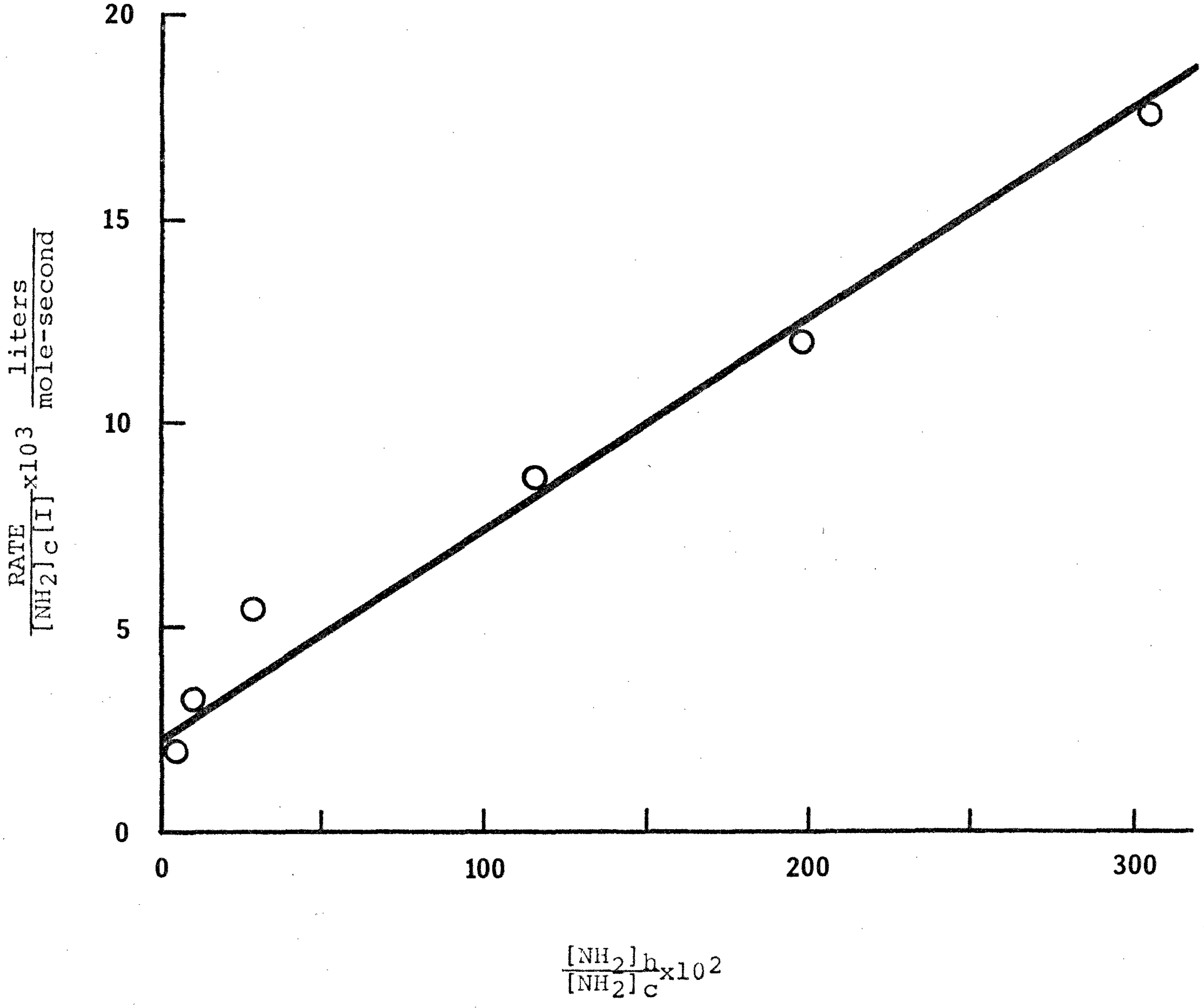


Figure 18: Net Reaction Rate per unit concentration of FDNB vs. pH as a whole and broken down into its component kinetic parts

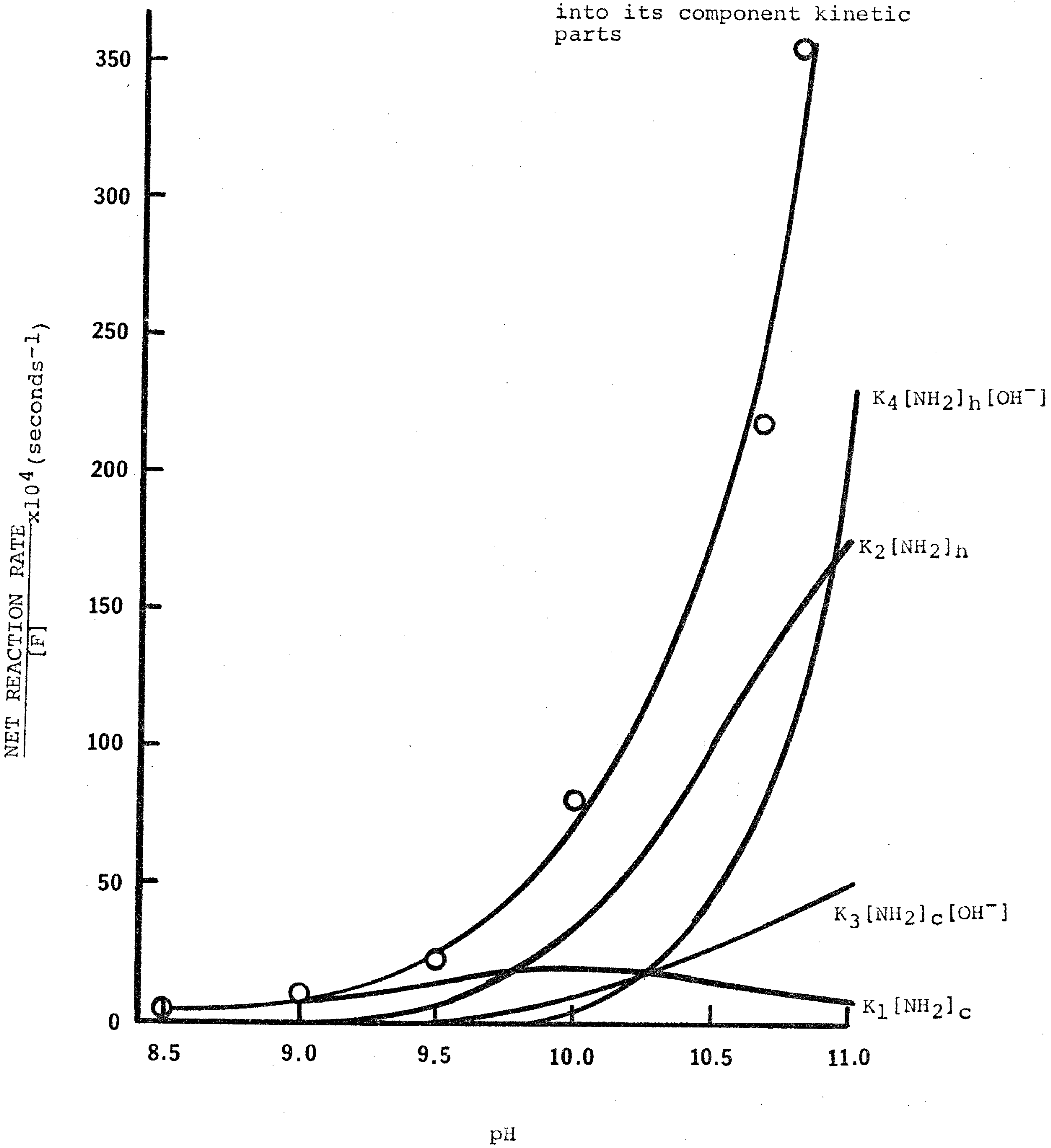


Figure 19: Net Reaction Rate per unit concentration of ClDNB vs. pH as a whole and broken down into its component kinetic parts

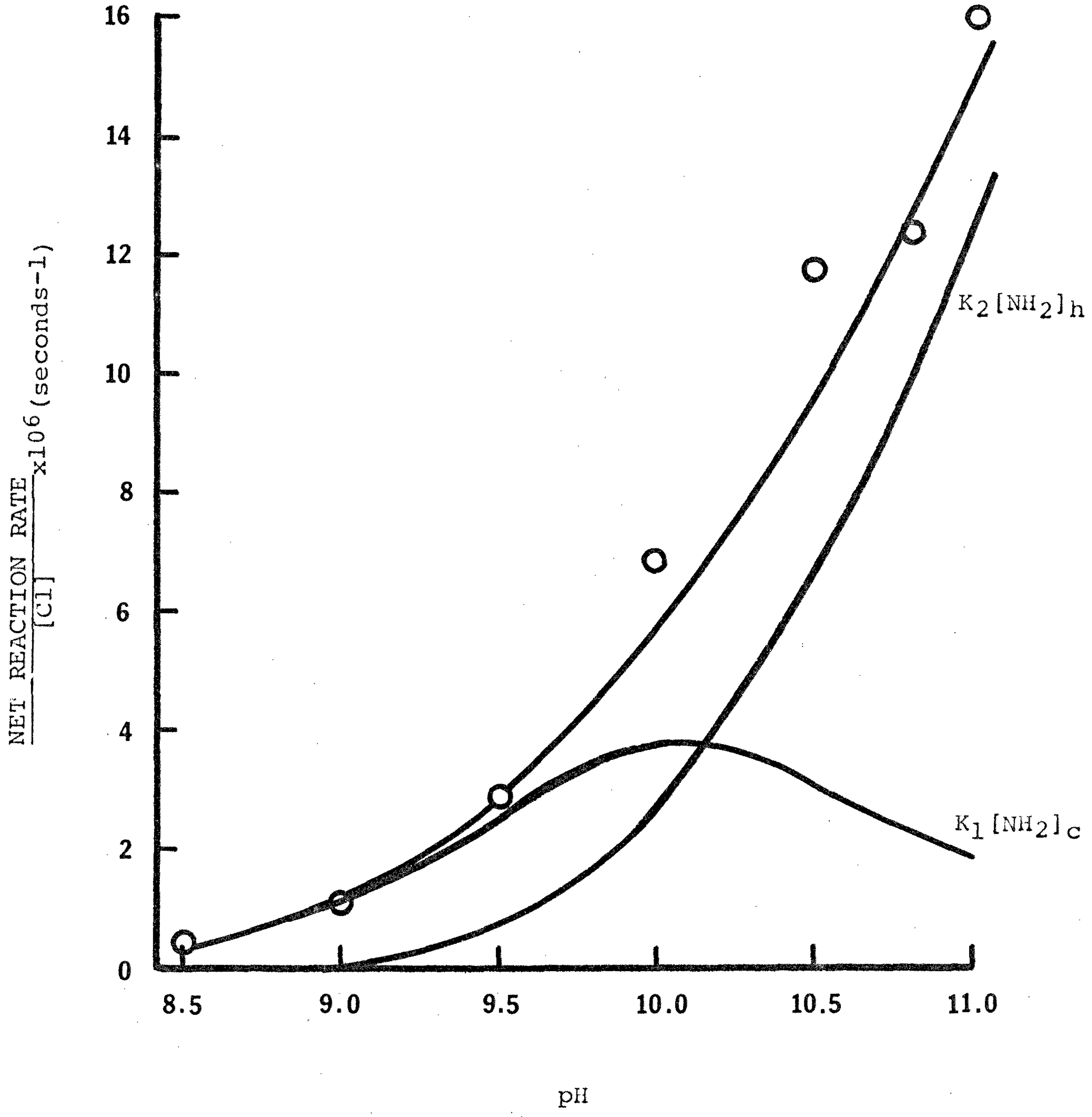


Figure 20: Net Reaction Rate per unit concentration of BrDNB vs. pH as a whole and broken down into its component kinetic parts

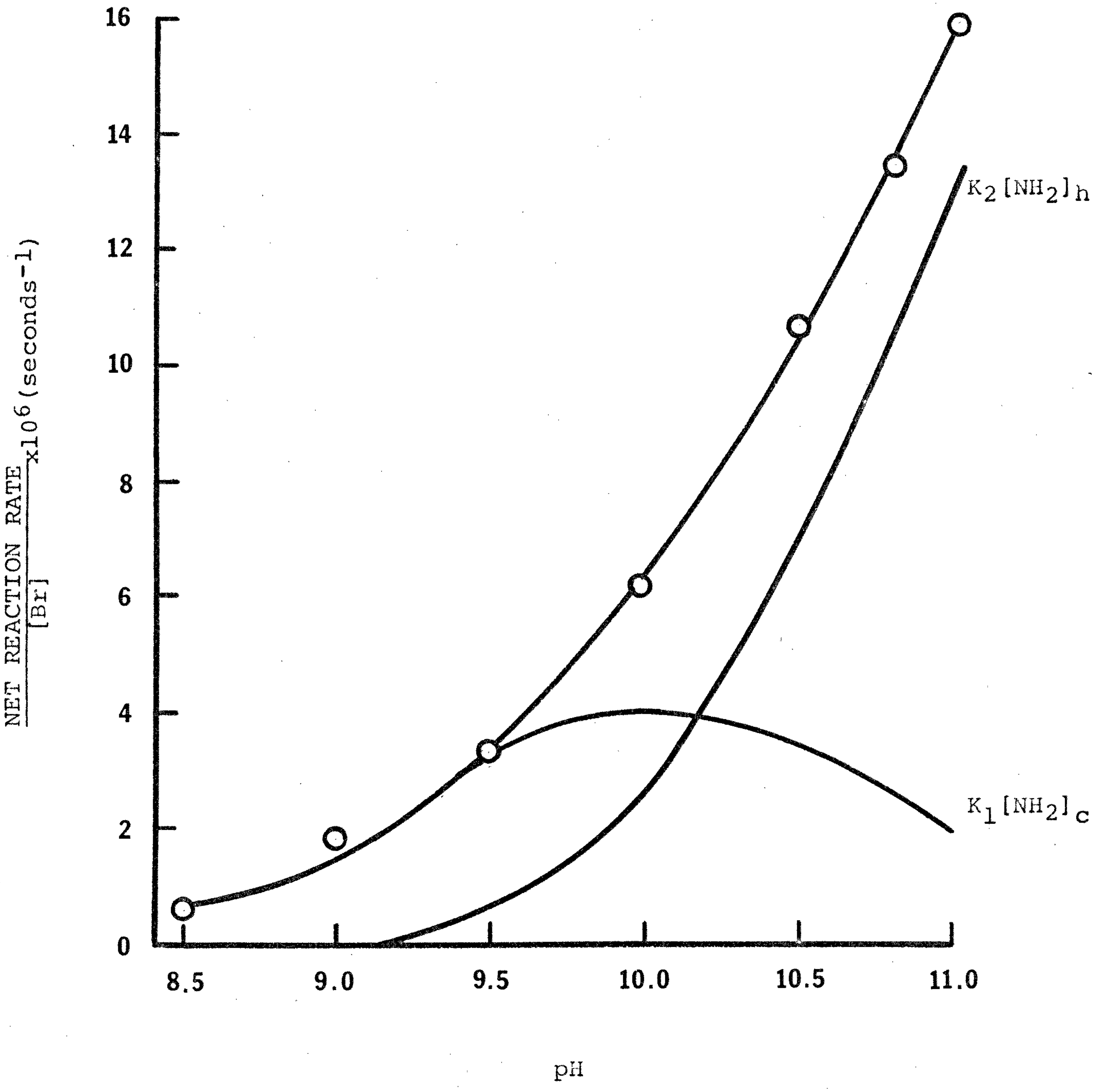
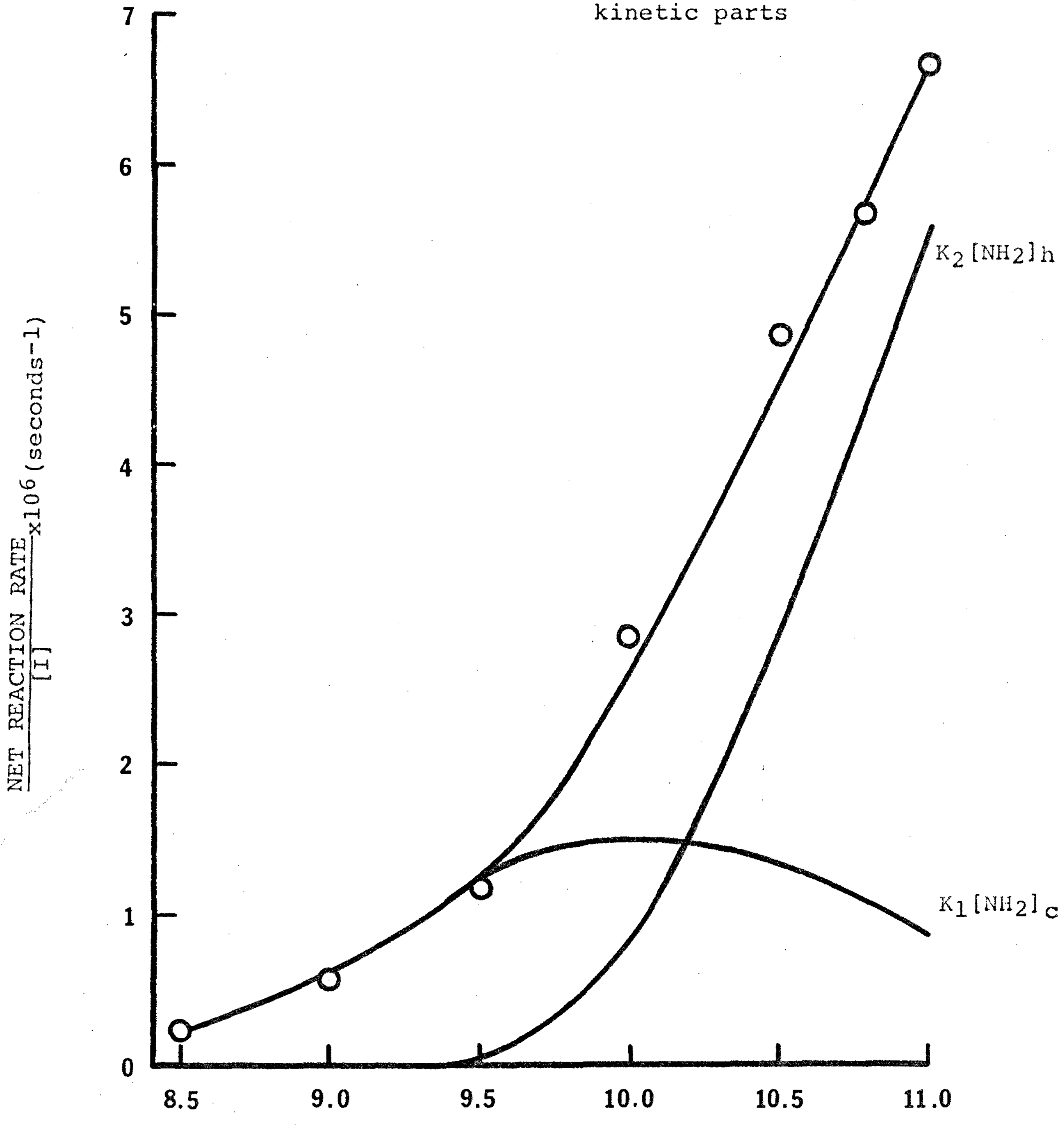


Figure 21: Net Reaction Rate per unit concentration of IDNB vs. pH as a whole and broken down into its component kinetic parts



pH

cases the coil amine reaction rate is the predominant reaction below pH 9.50. It then exhibits a maximum value near pH 10.0 due to a combination of two factors. With increasing pH there is a greater fraction of coil amine in the uncharged form. However, increasing pH also reduces the fraction of the polylysine in the coil form. Thus, the maximum concentration of coil free amine residues occurs near pH 10.0, and at sufficiently high pH values the coil free amine concentration would approach zero resulting in a minimal contribution by this kinetic term to the overall rate.

The helix amine reaction rate exhibits different behavior. It increases rapidly with pH because both the fraction of the amino groups in the helix which is uncharged, and the fraction of the polylysine which is in the helix configuration increase with increasing pH. At sufficiently high pH's where virtually all the helical amino groups are unprotonated and virtually all the polylysine is in the helical form, the rate will approach a maximum limiting value.

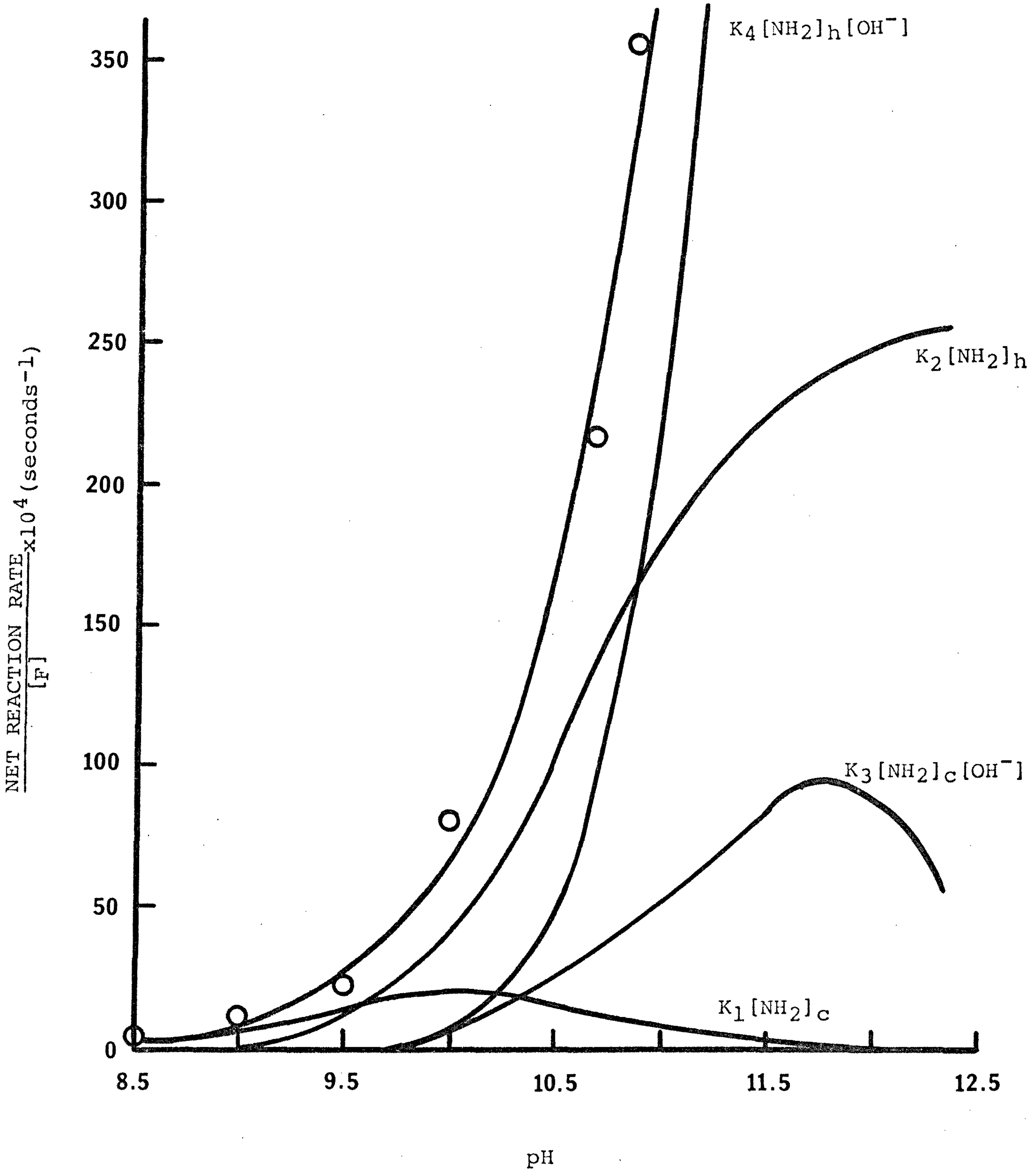
In the reaction of FDNB with polylysine there are two other factors involved in the reaction rate. They are the catalyzed amine reaction rates for both the coil and helix amines.

The catalyzed coil amine reaction rate increases with pH in the pH range studied because [OH$^-$] is increasing dramatically. However, extrapolation of the curves by calculation (Figure 22) predicts that a maximum in the rate will occur near pH 11.8 due to the effect of the decreasing coil free amine concentration above pH 10.00.

The catalyzed helix amine reaction rate increases more sharply with pH than does either the uncatalyzed helix amine reaction rate or the catalyzed coil amine reaction rate. This is because the fraction of free amine in the helix configuration, the fraction of the helix in the polylysine and the [OH$^-$] are all increasing in the pH range studied. Above pH 11.2 where virtually all the polylysine is in the unprotonated helical form, the rate will increase logarithmically with increasing pH and the catalyzed helix amine becomes the predominant factor in the reaction.

Since the helical amine rate constant is greater than the coil amine rate constant in both catalyzed and uncatalyzed reactions, the major contributor to the total rate depends upon the concentrations of the available amines and the [OH$^-$]. Therefore, as seen in Figures 18, 19, 20, 21, and 22, the uncatalyzed coil amine reaction predominates below pH 9.7, the uncatalyzed helix

Figure 22: Net Reaction Rate per unit concentration of FDNB vs. pH as a whole and broken down into its component kinetic parts which are extrapolated beyond the experimental limits



amine reaction predominates above pH 9.7, and in the case of base catalysis (Figures 21 and 22 only), the OH^- catalyzed helix amine reaction predominates above pH 10.8.

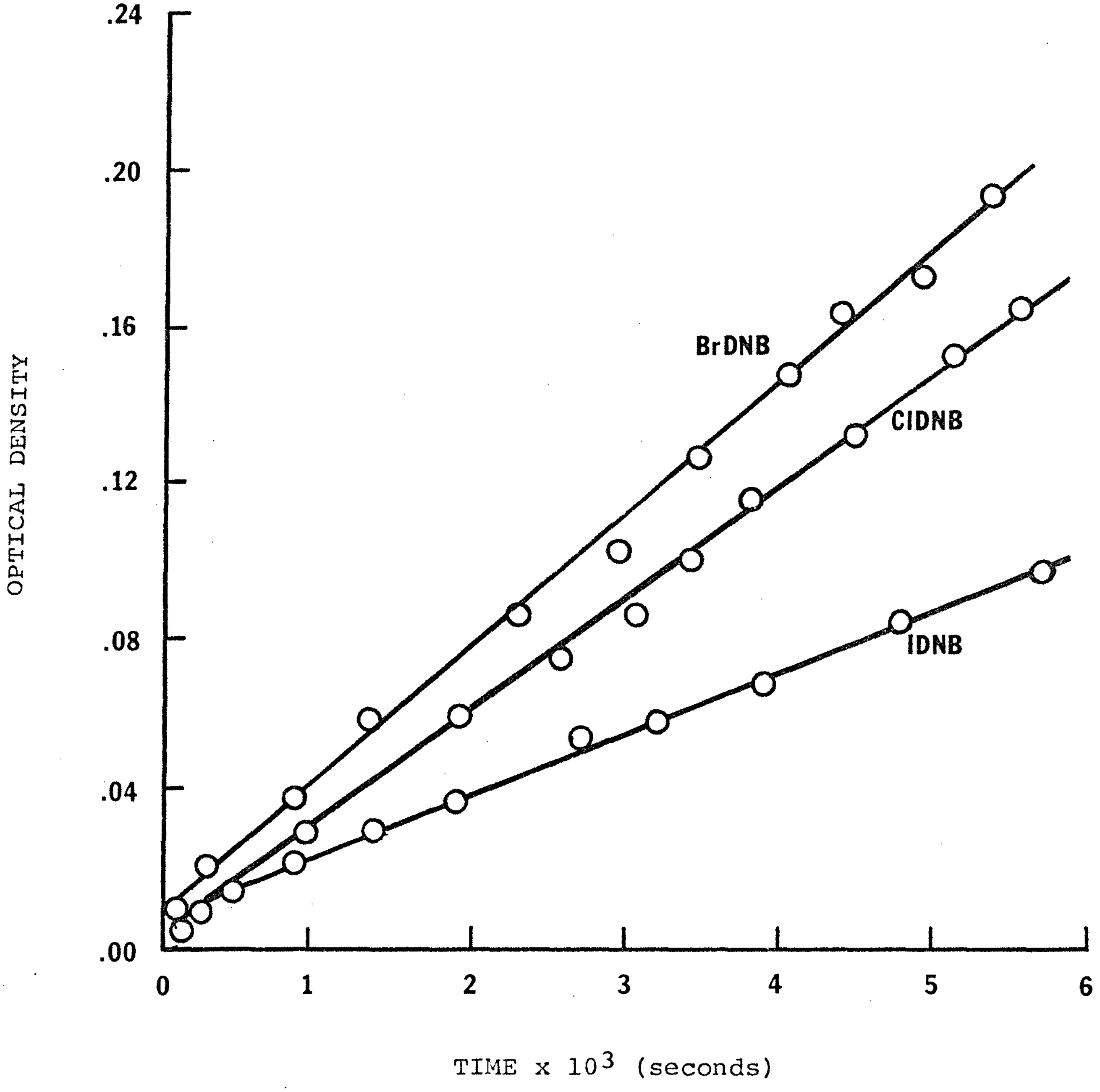
A comparison of the reaction rates of the 2,4-dinitrohalobenzene can best be demonstrated by a graph of the raw data for a give pH of 10.5, temperature of 25°C, polylysine concentration of $3.252 \times 10^{-3}M$ and a 2,4-dinitrohalobenzene concentration of about $5.74 \times 10^{-4}M$ (Figure 23). The reaction of FDNB with polylysine is excluded from this Figure 22 because it would run almost parallel to the y-axis with the scale used.

Figure 23 shows the reaction rates of ClDNB and BrDNB with polylysine to be about the same, with BrDNB slightly faster. The reaction of IDNB with polylysine is considerably slower. This observed order F>>Br>Cl>I is not unique since it had been previously observed by R. E. Parker and T. O. Read in their investigation of the reactions of Picryl Halides with Aniline.[19] Their explanation of why the order of ease of halogen displacement is F>>Br>Cl>I, and not F>>Cl>Br>I is that the accelerative London-force interaction between the reagent and the

---

[19] Parker, R. E. and Read, T. O., "The Mechanism of Displacement Reactions. Part I. Kinetics of the Reactions of the Four Picryl Halides,1,2,3,5-Tetranitrobenzene, and 1,2,4-Trinitrobenzene with Aniline in Ethanol," Chemical Society Journal, 1962, pp. 9-18.

Figure 23: Optical Density vs. Time at pH 10.5 for the Reaction of Polylysine (1.626 x 10^{-3}M) with ClDNB (5.74 x 10^{-4}M) BrDNB (5.74 x 10^{-4}M) and IDNB (5.75 x 10^{-4}M)



displaced group will be greater for Bromine than for the less polarisable Chlorine.

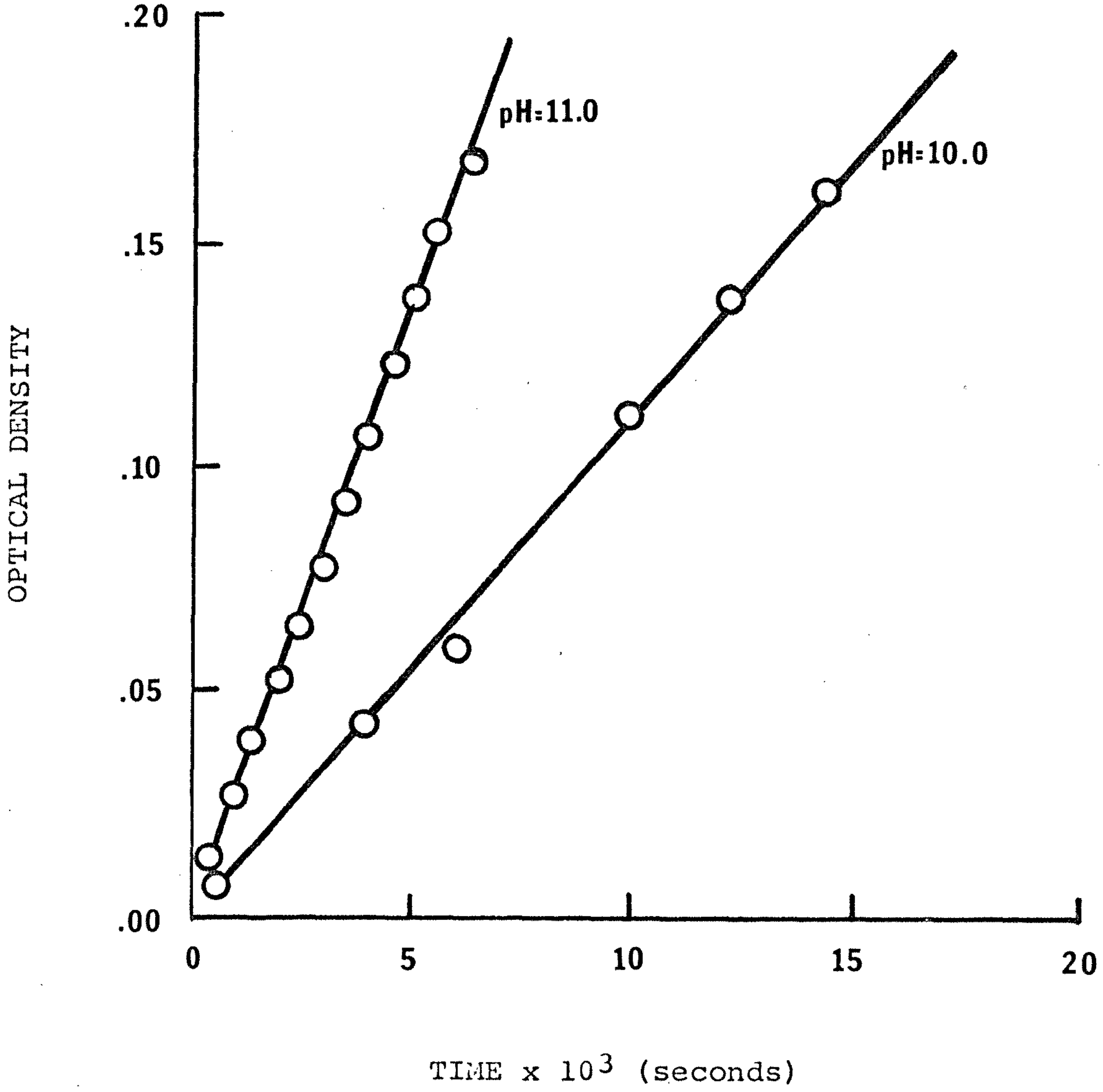
Figure 24 shows how the reaction rate is affected by pH for a constant ClDNB concentration of 5.74×10^{-4}M and polylysine concentration of 1.626×10^{-3}M. The reaction at pH 10.0 is illustrated by the lower curve and the reaction at pH 11.0, the upper. This demonstrates the effects of the coil and helical configurations on the reaction rate (Figure 18). At pH 10.0 the effect on the reaction rate brought on by the coil amine has reached its maximum; it is at this pH that the the effect of the helix amines begins approaching that of the coil amine. Above pH 10 the helix effect seems to be dominating the shape of the curve. At pH 11.0 the coil amine has lost all its effect on the reaction rate and the helical amine determines its shape completely.

This is not unexpected since in the reaction of polylysine with Iodoacetamide, S. J. Stanley found this to be true.[20] The explanation is that in the random coil region of polylysine the amines are less nucleophilic and are pointing in all different directions including inward. This would hinder a reaction with a molecule such as Iodoacetamide. In the helical

---

[20] Stanley, S. J., "A Kinetic Study of Lysine and Lysine Derivatives."

Figure 24: Optical Density vs. Time for the Reaction of ClDNB (5.74×10^{-4}M) with Polylysine (1.626×10^{-3}M) at pH's of 10.0 and 11.0



conformation of polylysine the amines are more nucleophilic and are pointing outward from the backbone of the helix making them quite free to react. This accounts for polylysine in the helix configuration's greater reactivity than that of the coil.

CONCLUSIONS AND RECOMMENDATIONS

The following conclusions have been drawn:

1. It has been shown that the rate of reaction for polylysine with 1-halo-2,4-dinitrobenzenes increases as the pH increases. In the cases where ClDNB, BrDNB or IDNB reacts with polylysine the reaction rate is the composite of two rates: one associated with the random coil and one associated with the helical conformation. For the reaction of FDNB with polylysine the total reaction rate is the summation of four rates; two associated with the random coil and two associated with the helical conformations. The final rate expressions for the reactions of polylysine with the 1-halo-2,4-dinitrobenzenes are:

   $$\frac{\text{Rate}}{[F]} = 9.18 \times 10^{-1} [NH_2]_c + 5.19 [NH_2]_h + 4.79 \times 10^{-3} [NH_2]_c [OH^-] + 7.29 \times 10^{-3} [NH_2]_h [OH^-]$$

   for Fluoro,

   $$\frac{\text{Rate}}{[Cl]} = 5.40 \times 10^{-3} [NH_2]_c + 1.21 \times 10^{-2} [NH_2]_h$$

   for Chloro,

   $$\frac{\text{Rate}}{[Br]} = 5.79 \times 10^{-3} [NH_2]_c + 1.22 \times 10^{-2} [NH_2]_h$$

   for Bromo, and

   $$\frac{\text{Rate}}{[I]} = 2.23 \times 10^{-2} [NH_2]_c + 5.31 \times 10^{-3} [NH_2]_h$$

   for Iodo.

2. The order of reactivity for the reactions of 1-halo-2,4-dinitrobenzenes with polylysine is FDNB>>BrDNB$\geq$ ClDNB>IDNB. The FDNB reacted much faster than the other 1-halo-2,4-dinitrobenzenes, the ClDNB and BrDNB were about equal with BrDNB taking a slight edge and the IDNB reacted slower than all the others.

3. Base catalysis was observed in the reactions of FDNB with polylysine and no base catalysis was observed in the reactions of ClDNB, BrDNB and IDNB with polylysine. This is why the rate expression for the reaction of FDNB with polylysine contains the two base catalysis terms $k_3[NH_2]_c[OH^-]$ and $k_4[NH_2]_h[OH^-]$ and the other rate expressions do not. No self catalysis was observed.

4. In all cases the conformation of the polylysine molecule has an effect on the reaction rate. At low pH's polylysine in the random coil conformation exhibits slow reaction rates and with increasing pH the polylysine molecule becomes more uniform as a helix and the reaction rate increases.

The following are recommendations for further work:

1. Copolymers of lysine with other amino acids tried to check the steric effects exhibited by the polymer.

In polylysine only one site is active. In copolymers of lysine other active sites could be checked for their reactivity.

2. The concentration of the free Amine should be varied while keeping the fraction helix constant to look for the nucleophilic effects and not conformation effects as was done in this work by varying the pH.

3. Other substrates could be studied instead of the 1-halo-2,4-dinitrobenzenes used in this experiment; e.g., 1-halo-2,6-dinitrobenzene. Where the halogen is located directly between the nitro groups one could check the steric effect of the nitro groups.

4. Another buffer should be used in future studies such as CAPS (Cyclohexylaminopropane Sulfonic Acid) which can buffer a greater pH range. This eliminates any error that might be involved in using multiple buffers.

5. Other reactions in which self-catalysis is involved might be of interest. In this work we found our experiment to proceed by base catalysis for Fluoro and no catalysis for the Chloro, Bromo, and Iodo dinitrobenzenes. One might work on a way to predict catalysis before hand.

6. The reactions of the 1-halo-2,4-dinitrobenzene with polylysine should be investigated at different

temperatures to determine the activation parameters for these reactions.

BIBLIOGRAPHY

Bektemirov, T. A., Burgosova, M. P. and Andzharparidze, O. G., "Interferon Induction in Monkeys by Poly (I) Poly (C) Complex with Poly-L-lysine," Voprosnii Virusologica, No. 5, 1976, pp. 536-539.

De Rossi, R. H. and Rossi, A. R., "Kinetics of Reactions of 1-Substituted 2,4-Dinitrobenzenes, with Aniline and Piperdine in Acetone," Journal of Organic Chemistry, Vol. 39, No. 24, 1974, pp. 3486-3488.

De Rossi, R. H., Rossi, A. R. and Gimenez, F. N. R., "Reaction of 2,4-Dinitrohalobenzenes with Imidazole in Nonpolar Aprotic Solvents," Journal of Organic Chemistry, Vol. 41, No. 19, 1976, pp. 3163-3166.

Kristol, D. S., Krautheim, P., Stanley, S. and Parker, R. C., "The Reaction of p-Nitrophenyl Acetate with Lysine and Lysine Derivatives," Bioorganic Chemistry, Vol. 4, 1975, pp. 299-304.

Morrison, R. T. and Boyd, R. N., Organic Chemistry, Third Edition. Boston, Massachusetts: Allyn and Bacon Inc., 1973, p. 829.

Parker, R. C., Rakowitz, R. K. and Kristol, D. S., "The Reaction of Polylysine with 2,4-Dinitrofluorobenzene," International Journal of Biochemistry, Vol. 9, pp. 117-119, 1978.

Parker, R. C., Slutsky, L. J. and Applegate, K. R., "Ultrasonic Absorption and the Kinetics of Conformational Change in Poly-L-lysine," Journal of Physical Chemistry, Vol. 72, No. 9, 1968, pp. 3177-3186.

Parker, R. E. and Read, T. O., "The Mechanism of Displacement Reactions. Part I. Kinetics of the Reactions of the Four Picryl Halides,1,2,3,5-Tetranitrobenzene, and 1,2,4-Trinitrobenzene with Aniline in Ethanol," Chemical Society Journal, 1962, pp. 9-18.

Parker, R. C., Stanley, S. and Kristol, D. S., "The Reaction of Iodoacetamide with Polylysine, -Acetyllysine and -Acetyllysine," International Journal of Biochemistry, Vol. 6, 1975, pp. 863-866.

Santella, R.M. and Li, H.J., "Studies on Interaction between Poly (L-lysine^{58}, L-phenylalanine^{42}) and Deoxyribonucleic Acids," Biochemistry, Vol. 14, No. 16, 1975, pp. 3604-3611.

Schodt, K. P., Gelman, R. A. and Blackwell, J., "The Effect of Changes in Salt Concentration and pH on the Interaction Between Glycosaminoglycans and Cationic Polypeptides," Biopolymers, Vol. 15, No. 10, 1976, pp. 1965-1977.

Stanley, S. J., "A Kinetic Study of Lysine and Lysine Derivatives," A Thesis Presented in Partial Fulfillment of the Requirements for the Degree of Master of Science in Chemical Engineering at Newark College of Engineering, p. 46.

Vogel, C. N., Butkowski, R. J., Mann, K. G. and Lundblad, R. L., "Effect of Polylysine on the Activation of Prothrombin. Polylysine Substitutes for Calcium Ions and Factor V in the Factor Xa Catalyzed Activation of Prothrombin," Biochemistry, Vol. 15, No. 15, 1976, pp. 3265.

APPENDIX I

RAW DATA

All Table Numbers are the same
as their respective Run Numbers

APPENDIX I

RAW DATA

Reaction Cuvette Condition		Table Numbers
3 ml. of THAM Buffer solution of 0.05M concentration + 50μl of 6.9554 x 10^{-2}M FDNB at 25°C and a pH of:	8.50	A1
	9.00	A2 - A4
	9.50	A5 - A6
3 ml. of THAM Buffer solution of 0.05M concentration + 25μl of 6.9554 x 10^{-2}M FDNB at 25°C and a pH of:	8.50	A7
	9.00	A8 - A13
	9.50	A14 - A15
	10.00	A16 - A17
3 ml. of THAM Buffer solution of 0.05M concentration + 10μl of 6.9554 x 10^{-2}M FDNB at 25°C and a pH of:	10.35	A18 - A19
	10.65	A20 - A21
	10.85	A22 - A23
3 ml. of THAM Buffer solution of 0.05M concentration + 50μl of 6.9554 x 10^{-2}M FDNB at 38°C and a pH of:	9.50	A24 - A25
3 ml. of THAM Buffer solution of 0.05M concentration + 25μl of 6.9554 x 10^{-2}M FDNB at 38°C and a pH of:	9.50	A26 - A27
3 ml. of 4.92 x 10^{-3}M Polylysine in 0.05M THAM + 50μl of 6.9554 x 10^{-2}M FDNB at 25°C and a pH of:	8.50	A28 - A30
3 ml. of 4.92 x 10^{-3}M Polylysine in 0.05M THAM + 25μl of 6.9554 x 10^{-3}M FDNB at 25°C and a pH of:	8.50	A31 - A32
3 ml. of 2.46 x 10^{-3}M Polylysine in 0.05M THAM + 50μl of 6.9554 x 10^{-3}M FDNB at 25°C and a pH of:	8.50	A33 - A35

3 ml. of 1.23 x 10^{-3}M Polylysine in 0.05M THAM + 50μl of 6.9554 x 10^{-3}M FDNB at 25°C and a pH of:	8.50	A36 - A37
3 ml. of 1.64 x 10^{-3}M Polylysine in 0.05M THAM + 25μl of 6.9554 x 10^{-3}M FDNB at 25°C and a pH of:	9.00	A38 - A39
	9.50	A40 - A41
3 ml. of 3.28 x 10^{-3}M Polylysine in 0.05M THAM + 25μl of 6.9554 x 10^{-3}M FDNB at 25°C and a pH of:	9.50	A42 - A43
3 ml. of 1.64 x 10^{-3}M Polylysine in 0.05M THAM + 25μl of 6.9554 x 10^{-3}M FDNB at 25°C and a pH of:	8.50	A44 - A46
	9.00	A47 - A49
	9.50	A50 - A51
	10.00	A52 - A53
3 ml. of 0.82 x 10^{-3}M Polylysine in 0.05M THAM + 10μl of 6.9554 x 10^{-3}M FDNB at 25°C and a pH of:	10.35	A54 - A57
	10.65	A58 - A61
	10.85	A62 - A63
3 ml. of 1.64 x 10^{-3}M Polylysine in 0.05M THAM + 50μl of 6.9554 x 10^{-3}M FDNB at 38°C and a pH of:	9.50	A64 - A66
3 ml. of 3.28 x 10^{-3}M Polylysine in 0.05M THAM + 25μl of 6.9554 x 10^{-2}M FDNB at 38°C and a pH of:	9.50	A67 - A68
3 ml. of 1.64 x 10^{-3}M Polylysine in 0.05M THAM + 25μl of 6.9554 x 10^{-2}M FDNB at 38°C and a pH of:	9.50	A69 - A70
3 ml. of THAM Buffer solution of 0.05M concentration + 25μl of 6.9395 x 10^{-2}M ClDNB at 25°C and a pH of:	8.50	A71 - A72
	9.00	A73 - A74
	9.50	A75 - A76
	10.00	A77 - A78

3 ml. of Sodium Borate (BORAX) Buffer solution of 0.05M concentration + 25μl of 6.9395 x 10^{-2}M ClDNB at 25°C and a pH of:	9.50	A79 - A80
	10.00	A81 - A82
	10.50	A83 - A84
	10.80	A85 - A86
	11.00	A87 - A88
3 ml. of 3.252 x 10^{-3}M Polylysine in 0.05M Sodium Borate + 25μl of 6.9395 x 10^{-2}M ClDNB at 25°C and a pH of:	10.50	A89 - A92
	10.80	A93 - A94
3 ml. of 1.626 x 10^{-3}M Polylysine in 0.05M THAM + 25μl of 6.9395 x 10^{-2}M ClDNB at 25°C and a pH of:	8.50	A95 - A96
	9.00	A97 - A100
	9.50	A101 - A104
	10.00	A105 - A107
3 ml. of 1.626 x 10^{-3}M Polylysine in 0.05M Sodium Borate + 25μl of 6.9395 x 10^{-2}M ClDNB at 25°C and a pH of:	9.50	A108 - A109
	10.00	A110 - A111
	10.80	A112 - A114
	11.00	A115 - A118
3 ml. of THAM Buffer solution of 0.05M concentration + 25μl of 6.947 x 10^{-2}M BrDNB at 25°C and a pH of:	8.50	A119 - A120
	9.00	A121 - A122
	9.50	A123 - A124
	10.00	A125 - A126
3 ml. of Sodium Borate (BORAX) Buffer solution of 0.05M concentration + 25μl of 6.947 x 10^{-2}M BrDNB at 25°c and a pH of:	9.50	A127 - A128
	10.00	A129 - A130
	10.50	A131 - A132
	10.80	A133 - A134
	11.00	A135 - A136

3 ml. of 3.252 x 10^{-3}M Polylysine in 0.05M Sodium Borate + 25μl of 6.947 x 10^{-2}m BrDNB at 25°C and a pH of:	10.50	A137 - A138
	10.80	A139 - A140
3 ml. of 1.626 x 10^{-3}M Polylysine in 0.05M THAM + 25μl of 6.947 x 10^{-2}M BrDNB at 25°C and a pH of:	8.50	A141 - A142
	9.00	A143 - A144
	9.50	A145 - A146
	10.00	A147 - A148
3 ml. of 1.626 x 10^{-3}M Polylysine in 0.05M Sodium Borate + 25μl of 6.947 x 10^{-2}M BrDNB at 25°C and a pH of:	9.50	A149 - A150
	10.00	A151 - A152
	10.80	A153 - A155
	11.00	A156 - A157
3 ml. of THAM Buffer solution of 0.05M concentration + 25μl of 6.9644 x 10^{-2}M IDNB at 25°C and a pH of:	8.50	A158 - A159
	9.00	A160 - A161
	9.50	A162 - A163
	10.00	A164 - A165
3 ml. of Sodium Borate (BORAX) Buffer of 0.05M concentration + 25μl of 6.9644 x 10^{-2}M IDNB at 25°C and a pH of:	9.50	A166 - A167
	10.00	A168 - A169
	10.50	A170 - A171
	11.00	A172 - A173
3 ml. of 3.252 x 10^{-3}M Polylysine in 0.05M Sodium Borate + 24μl of 6.9644 x 10^{-2}M IDNB at 25°C and a pH of:	10.50	A174 - A175
3 ml. of 1.626 x 10^{-3}M Polylysine in 0.05M THAM + 25μl of 6.9644 x 10^{-2}M IDNB at 25°C and a pH of:	8.50	A176 - A177
	9.00	A178 - A179
	9.50	A180 - A181
	10.00	A182 - A185

3 ml. of 1.626 x 10^{-3}M Polylysine in 0.05M Sodium Borate + 25 μl of 6.9644 x 10^{-2}M IDNB at 25°C and a pH of:

9.50	A186 - A187
10.00	A188 - A189
11.00	A190 - A191

TABLE A1

Time in Seconds	Optical Density
31	.190
100	.197
200	.207
300	.211
400	.226
500	.228
625	.236
700	.240
800	.250
900	.255
1000	.261
1100	.274
1200	.280
1300	.285
1400	.290
1500	.302

TABLE A2

Time in Seconds	Optical Density
24	.205
110	.221
220	.226
300	.244
420	.280
510	.294
600	.330
700	.350
800	.373
900	.420
970	.441
1035	.470
1100	.470
1200	.515
1300	.530

TABLE A3

Time in Seconds	Optical Density
110	.237
200	.261
363	.316
401	.310
450	.316
500	.329
553	.343
600	.362
655	.379
700	.398
753	.432
810	.449
855	.456
905	.510
955	.551
1010	.691

TABLE A4

Time in Seconds	Optical Density
116	.170
209	.303
269	.309
320	.336
372	.329
426	.340
505	.371
552	.408
622	.411
669	.432
740	.461
798	.499
854	.560

TABLE A5

Time in Seconds	Optical Density
35	.350
56	.360
86	.370
117	.380
150	.390
178	.400
302	.440
344	.450
376	.460
406	.470
433	.480
462	.490
491	.500
522	.510
552	.520

TABLE A6

Time in Seconds	Optical Density
72	.315
87	.320
102	.325
117	.330
134	.335
147	.340
163	.345
177	.350
192	.355
209	.360
222	.365
239	.370
252	.375
268	.380
283	.385
301	.390
315	.395
333	.400

TABLE A7

Time in Seconds	Optical Density
100	.101
200	.103
300	.106
400	.109
500	.106
600	.114
700	.120
800	.122
900	.129
1000	.131
1100	.134
1200	.140
1300	.143
1400	.149
1500	.152

TABLE A8

Time in Seconds	Optical Density
26	.123
104	.146
154	.150
208	.152
264	.163
306	.168
357	.173
406	.181
475	.187
545	.189
600	.196
650	.207
700	.212
750	.219
800	.221
850	.223
900	.229

TABLE A9

Time in Seconds	Optical Density
35	.133
110	.131
155	.138
203	.141
253	.149
307	.158
353	.163
400	.170
455	.175
510	.179
556	.186
610	.188
650	.199
703	.200
756	.207
800	.215
902	.221

TABLE A10

Time in Seconds	Optical Density
102	.152
190	.158
250	.163
356	.172
408	.176
465	.181
535	.192
580	.196
652	.202
700	.209
780	.215
840	.221
893	.228
934	.229
1068	.250

TABLE A11

Time in Seconds	Optical Density
100	.283
180	.295
241	.300
311	.310
430	.314
536	.335
602	.352
682	.375
750	.386
817	.405
926	.440
992	.471
1095	.536

TABLE A12

Time in Seconds	Optical Density
110	.266
191	.276
256	.281
328	.285
443	.293
550	.318
617	.327
695	.354
764	.360
832	.381
939	.408
1006	.430
1113	.453

TABLE A13

Time in Seconds	Optical Density
120	.221
205	.231
269	.241
343	.250
456	.248
566	.276
634	.284
709	.303
779	.311
849	.320
953	.350
1024	.370
1127	.381

TABLE A14

Time in Seconds	Optical Density
23	.210
36	.215
60	.220
85	.225
104	.230
132	.235
157	.240
179	.245
207	.250
235	.255
260	.260
285	.265
310	.270
334	.275
356	.280
383	.285
405	.290
428	.295
450	.300

TABLE A15

Time in Seconds	Optical Density
18	.190
41	.200
65	.205
94	.210
114	.215
140	.220
164	.225
187	.230
211	.235
236	.240
261	.245
285	.250
309	.255
331	.260
355	.265
376	.270
402	.275
425	.280
450	.285
472	.290

TABLE A16

Time in Seconds	Optical Density
27	.315
42	.325
60	.335
81	.345
97	.355
114	.365
130	.375
148	.385
164	.395
180	.405
202	.420
216	.430
231	.440
244	.450
259	.460
272	.470
287	.480
302	.490
316	.500

TABLE A17

Time in Seconds	Optical Density
23	.230
35	.240
54	.250
72	.260
91	.270
109	.280
127	.290
144	.300
161	.310
176	.320
191	.330
206	.340
221	.350
235	.360
249	.370
262	.380
278	.390
292	.400
308	.410
326	.420

TABLE A18

Time in Seconds	Optical Density
142	.170
203	.210
260	.245
334	.290
393	.325
517	.395
556	.420
592	.440
627	.460
682	.490

TABLE A19

Time in Seconds	Optical Density
162	.130
223	.170
280	.205
355	.255
413	.290
496	.315
535	.365
573	.385
607	.405
648	.430

TABLE A20

Time in Seconds	Optical Density
186	.190
239	.265
315	.375
377	.460
433	.535
451	.560
467	.580
481	.600

TABLE A21

Time in Seconds	Optical Density
12	.125
43	.170
75	.215
100	.250
133	.295
169	.345
234	.440
248	.460
262	.480

TABLE A22

Time in Seconds	Optical Density
28	.235
57	.315
86	.395
116	.475
151	.570
186	.660
198	.690
210	.720
222	.750

TABLE A23

Time in Seconds	Optical Density
18	.175
30	.205
38	.225
47	.250
57	.275
67	.300
77	.325
87	.350
97	.375
107	.400

TABLE A24

Time in Seconds	Optical Density
29	.310
43	.325
53	.335
62	.345
71	.355
81	.365
91	.375
100	.385
110	.395
123	.410
132	.420
142	.430
151	.440
160	.450
170	.460
180	.470

TABLE A25

Time in Seconds	Optical Density
19	.260
32	.275
42	.285
52	.295
61	.305
70	.315
79	.325
89	.335
98	.345
107	.355
116	.365
125	.375
134	.385
143	.395
148	.400

TABLE A26

Time in Seconds	Optical Density
18	.290
28	.300
44	.310
61	.320
78	.330
110	.350
125	.360
141	.370
156	.380
174	.390
188	.400
202	.410
218	.420
232	.430
247	.440
262	.450

TABLE A27

Time in Seconds	Optical Density
16	.210
26	.220
43	.230
59	.240
78	.250
94	.260
113	.270
133	.280
152	.290
169	.300
187	.310
205	.320
223	.330
241	.340
259	.350
277	.360
294	.370

TABLE A28

Time in Seconds	Optical Density
25	.275
41	.310
52	.335
70	.360
80	.380
100	.430
113	.460
124	.490
135	.520
152	.580
174	.650
189	.700

TABLE A29

Time in Seconds	Optical Density
20	.250
31	.265
40	.290
51	.310
60	.330
70	.350
82	.380
91	.400
101	.420
108	.440
117	.460
125	.480
132	.500

TABLE A30

Time in Seconds	Optical Density
21	.255
34	.280
41	.300
51	.320
59	.340
67	.360
75	.380
81	.400
90	.420
96	.440
104	.460
112	.480
118	.500

TABLE A31

Time in Seconds	Optical Density
24	.150
41	.165
60	.180
74	.195
88	.210
102	.225
118	.240
134	.255
146	.270
166	.285
178	.300
200	.325

TABLE A32

Time in Seconds	Optical Density
24	.135
36	.145
46	.155
57	.165
68	.175
81	.185
91	.195
99	.205
109	.215
135	.250
145	.260
153	.270

TABLE A33

Time in Seconds	Optical Density
23	.265
37	.285
55	.305
70	.315
88	.345
109	.385
119	.400
127	.415
137	.430
146	.445
154	.460
161	.475
169	.490
178	.505
185	.520

TABLE A34

Time in Seconds	Optical Density
24	.245
44	.260
57	.275
70	.290
82	.305
92	.320
103	.335
117	.350
126	.365
138	.380
157	.395
165	.410
175	.425
187	.440

TABLE A35

Time in Seconds	Optical Density
35	.245
54	.265
69	.280
79	.295
90	.310
109	.325
121	.340
133	.365
162	.385
179	.420
203	.440

TABLE A36

Time in Seconds	Optical Density
23	.190
40	.205
80	.220
96	.235
115	.245
148	.255
168	.265
186	.275
205	.285
224	.295
246	.305
265	.315
281	.325
305	.335
321	.345
338	.355

TABLE A37

Time in Seconds	Optical Density
21	.185
48	.195
66	.205
89	.215
105	.225
127	.235
144	.245
163	.255
187	.265
209	.275
226	.285
246	.295
266	.305
284	.315
296	.325
323	.335

TABLE A38

Time in Seconds	Optical Density
20	.436
39	.480
53	.510
63	.530
73	.550
91	.570
105	.590
126	.610
136	.630
159	.650
168	.670
181	.690
192	.710

TABLE A39

Time in Seconds	Optical Density
21	.343
42	.420
56	.450
66	.470
76	.490
92	.510
102	.530
116	.550
129	.570
144	.590
168	.620
179	.640
189	.660
201	.680
217	.700

TABLE A40

Time in Seconds	Optical Density
16	.400
23	.420
32	.440
40	.460
49	.480
57	.500
65	.520
74	.540
83	.560
91	.580
100	.600

TABLE A41

Time in Seconds	Optical Density
30	.390
36	.410
44	.430
52	.450
60	.470
68	.490
83	.530
91	.550
98	.570
106	.590
113	.610

TABLE A42

Time in Seconds	Optical Density
20	.290
26	.310
34	.330
41	.350
48	.370
56	.390
63	.410
70	.430
77	.450
84	.470
96	.500
103	.520
110	.540
117	.560

TABLE A43

Time in Seconds	Optical Density
24	.280
30	.300
40	.320
48	.340
56	.360
65	.380
74	.400
82	.420
91	.440
99	.460
108	.480
116	.500
124	.520
132	.540

TABLE A44

Time in Seconds	Optical Density
15	.175
33	.190
53	.195
83	.200
99	.205
118	.210
135	.215
151	.220
170	.225
186	.230
206	.235
222	.240
239	.245
257	.250
274	.255
292	.260
311	.265

TABLE A45

Time in Seconds	Optical Density
15	.175
34	.180
52	.185
67	.190
87	.195
105	.200
123	.205
143	.210
160	.215
179	.220
196	.225
213	.230
233	.235
251	.240
269	.245
288	.250
307	.255
325	.260

TABLE A46

Time in Seconds	Optical Density
29	.150
45	.155
64	.160
84	.165
102	.170
119	.175
139	.180
159	.185
176	.190
195	.195
214	.200
233	.205
251	.210
259	.215
284	.220
304	.225
322	.230
342	.235

TABLE A47

Time in Seconds	Optical Density
20	.210
31	.220
43	.230
59	.240
71	.250
85	.260
98	.270
111	.280
126	.290
138	.300
152	.310
166	.320
178	.330
193	.340
206	.350
218	.360
231	.370

TABLE A48

Time in Seconds	Optical Density
19	.200
29	.210
41	.220
53	.230
66	.240
76	.250
91	.260
104	.270
116	.280
129	.290
141	.300
154	.310
167	.320
179	.330
191	.340
204	.350
216	.360
229	.370

TABLE A49

Time in Seconds	Optical Density
26	.175
36	.185
49	.195
61	.205
74	.215
86	.225
98	.235
110	.245
122	.255
135	.265
147	.275
159	.285
171	.295
183	.305
196	.315
208	.325
220	.335
232	.345

TABLE A50

Time in Seconds	Optical Density
16	.190
25	.205
34	.220
44	.235
54	.250
64	.265
74	.280
84	.295
94	.310
104	.325
114	.340
124	.355
133	.370
143	.385

TABLE A51

Time in Seconds	Optical Density
19	.250
26	.265
37	.285
46	.300
55	.315
66	.330
76	.345
86	.360
96	.375
105	,390
115	.405
125	.420
135	.435
144	.450
153	.465
162	.480

TABLE A52

Time in Seconds	Optical Density
17	.31
22	.33
27	.35
32	.37
38	.39
43	.41
47	.43
53	.45
58	.47
63	.49
67	.51
71	.53
76	.55
80	.57
84	.59

TABLE A53

Time in Seconds	Optical Density
13	.35
17	.37
22	.39
27	.41
32	.43
38	.45
43	.47
48	.49
53	.51
58	.53
63	.55
67	.57
72	.59
76	.61

TABLE A54

Time in Seconds	Optical Density
10	.110
18	.125
32	.150
37	.160
43	.170
48	.180
53	.190
58	.200
63	.210
69	.220
74	.230
79	.240
84	.250

TABLE A55

Time in Seconds	Optical Density
7	.110
13	.120
20	.130
28	.140
35	.150
42	.160
49	.170
56	.180
63	.190
77	.200

TABLE A56

Time in Seconds	Optical Density
25	.170
30	.185
36	.200
42	.215
48	.230
54	.245
60	.260
66	.275
72	.290
78	.305

TABLE A57

Time in Seconds	Optical Density
21	.150
27	.165
33	.180
40	.195
46	.210
53	.225
59	.240
65	.255
71	.270
77	.285
83	.300

TABLE A58

Time in Seconds	Optical Density
21	.135
25	.150
29	.165
32	.180
37	.195
41	.210
45	.225
48	.240
52	.255
56	.270
60	.285
64	.300

TABLE A59

Time in Seconds	Optical Density
24	.170
33	.210
36	.225
40	.255
45	.265
50	.290
54	.310
58	.325
62	.345
72	.400
76	.420
80	.440

TABLE A60

Time in Seconds	Optical Density
12	.150
17	.170
22	.190
27	.210
32	.230
37	.250
42	.270
46	.290
51	.310
56	.330
60	.350
65	.370

TABLE A61

Time in Seconds	Optical Density
22	.170
27	.190
32	.210
37	.230
42	.250
47	.270
51	.290
56	.310
61	.330
65	.350
70	.370

TABLE A62

Time in Seconds	Optical Density
18	.165
22	.190
25	.210
29	.235
33	.260
37	.280
40	.305
44	.325
47	.350
51	.370
55	.400
58	.420
63	.450
66	.470

TABLE A63

Time in Seconds	Optical Density
21	.175
25	.195
30	.225
34	.250
38	.275
42	.300
46	.325
50	.350
54	.375
58	.400
62	.425
65	.450
69	.475
73	.500

TABLE A64

Time in Seconds	Optical Density
15	.550
21	.600
26	.650
30	.700
35	.750
39	.800
46	.900

TABLE A65

Time in Seconds	Optical Density
15	.450
20	.500
25	.550
29	.600
34	.650
38	.700
41	.750
44	.800
48	.850
50	.900

TABLE A66

Time in Seconds	Optical Density
13	.450
19	.500
21	.525
24	.550
28	.600
33	.650
38	.700
42	.750
46	.800
50	.850
53	.900

TABLE A67

Time in Seconds	Optical Density
17	.325
21	.350
24	.375
27	.400
34	.450
40	.500
47	.550
53	.600
60	.650
66	.700
72	.750
78	.800
84	.850
89	.900

TABLE A68

Time in Seconds	Optical Density
14	.275
17	.300
21	.325
24	.350
27	.375
30	.400
34	.425
37	.450
40	.475
43	.500
46	.525
50	.550
53	.575
56	.600
59	.625

TABLE A69

Time in Seconds	Optical Density
19	.325
27	.365
34	.400
39	.425
45	.450
50	.475
55	.500
60	.525
65	.550
69	.575
74	.600

TABLE A70

Time in Seconds	Optical Density
20	.275
25	.300
30	.325
36	.350
41	.375
47	.400
52	.425
57	.450
62	.475
67	.500
73	.525
77	.550
86	.575

TABLE A71

Time in Seconds	Optical Density
Start	.118
8.64×10^4	.140
1.728×10^5	.146
2.592×10^5	.145
3.456×10^5	.149
5.184×10^5	.124
6.048×10^5	.139
6.912×10^5	.130
7.776×10^5	.141
8.712×10^5	.138
12.186×10^5	.150
13.032×10^5	.129

TABLE A72

Time in Seconds	Optical Density
Start	.121
8.64×10^4	.143
1.728×10^5	.151
2.592×10^5	.145
3.456×10^5	.146
5.184×10^5	.123
6.048×10^5	.137
6.912×10^5	.126
7.776×10^5	.127
8.712×10^5	.125
12.186×10^5	.148
13.032×10^5	.127

TABLE A73

Time in Seconds	Optical Density
Start	.097
86400	.148
172800	.155
259200	.173
345600	.161
518400	.139
604800	.157
691200	.154
777600	.157
871200	.159
1218600	.184
1303200	.158

TABLE A74

Time in Seconds	Optical Density
Start	.105
86400	.108
172800	.143
259200	.132
345600	.135
518400	.136
604800	.142
691200	.164
777600	.151
871200	.154
1218600	.171
1303200	.159

TABLE A75

Time in Seconds	Optical Density
Start	.124
86400	.128
172800	.162
259200	.154
345600	.155
518400	.176
604800	.196
691200	.215
777600	.196
871200	.206
1218600	.251
1303200	.254

TABLE A76

Time in Seconds	Optical Density
Start	.130
86400	.131
172800	.174
259200	.164
345600	.182
518400	.179
604800	.207
691200	.230
777600	.209
871200	.224
1218600	.267
1303200	.262

TABLE A77

Time in Seconds	Optical Density
Start	.120
86400	.172
172800	.184
259200	.225
345600	.237
518400	.257
604800	.314
691200	.356
777600	.394
871200	.399
1218600	.441
1303200	.449

TABLE A78

Time in Seconds	Optical Density
Start	.140
86400	.169
172800	.170
259200	.229
345600	.229
518400	.245
604800	.292
691200	.330
777600	.360
871200	.362
1218600	.411
1303200	.401

TABLE A79

Time in Seconds	Optical Density
Start	.117
86400	.157
172800	.125
259200	.142
345600	.120
518400	.147
604800	.148
691200	.150
777600	.149
871200	.155
1218600	.153
1303200	.156

TABLE A80

Time in Seconds	Optical Density
Start	.100
86400	.113
172800	.123
259200	.158
345600	.117
518400	.123
604800	.125
691200	.147
777600	.142
871200	.164
1218600	.152
1303200	.146

TABLE A81

Time in Seconds	Optical Density
Start	.118
86400	.128
172800	.156
259200	.201
345600	.175
518400	.188
604800	.226
691200	.244
777600	.250
871200	.279
1218600	.303
1303200	.312

TABLE A82

Time in Seconds	Optical Density
Start	.119
86400	.129
172800	.159
259200	.163
345600	.187
518400	.228
604800	.229
691200	.229
777600	.239
871200	.259
1218600	.301
1303200	.314

TABLE A83

Time in Seconds	Optical Density
Start	.125
86400	.166
172800	.232
259200	.292
345600	.337
518400	.371
604800	.480
691200	.509
777600	.562
871200	.600
1218600	.788
1303200	.810

TABLE A84

Time in Seconds	Optical Density
Start	.115
86400	.159
172800	.233
259200	.292
345600	.335
518400	.374
604800	.470
691200	.510
777600	.565
871200	.609
1218600	.784
1303200	.812

TABLE A85

Time in Seconds	Optical Density
36	.128
100	.138
350	.147
550	.150
1050	.162
1550	.167
2550	.168
3550	.172
4550	.173
5850	.180
6550	.187
7500	.188
8400	.192
10200	.192
12800	.195
14000	.202

TABLE A86

Time in Seconds	Optical Density
300	.197
400	.205
600	.207
1100	.219
1600	.227
2600	.228
3600	.232
4600	.233
5900	.242
6590	.250
7525	.251
8440	.254
10250	.255
12800	.257
14020	.267

TABLE A87

Time in Seconds	Optical Density
Start	.134
86400	.302
172800	.498
259200	.678
345600	.835
518400	1.05
604800	1.36
691200	1.46
777600	1.63
871200	1.76

TABLE A88

Time in Seconds	Optical Density
Start	.110
86400	.293
172800	.497
259200	.686
345600	.820
518400	1.02
604800	1.31
691200	1.38
777600	1.48
871200	1.66

TABLE A89

Time in Seconds	Optical Density
440	.156
1618	.191
2140	.205
2624	.220
3537	.251
3920	.261
4942	.296
7785	.400
13562	.680
14950	.745
16532	.873
17438	.920

TABLE A90

Time in Seconds	Optical Density
455	.156
1638	.181
2150	.193
2633	.207
3534	.232
3935	.235
4972	.264
7793	.364
13580	.560
14958	.608
16552	.691
17468	.720

TABLE A91

Time in Seconds	Optical Density
1295	.122
1650	.133
2120	.153
3130	.195
3740	.215
4230	.232
4610	.249
4960	.264
5660	.289
6290	.310
6690	.328
7600	.363

TABLE A92

Time in Seconds	Optical Density
1300	.194
1680	.202
2140	.218
3140	.249
3750	.264
4240	.276
4620	.294
4965	.309
5665	.324
6300	.344
6700	.356
7615	.384

TABLE A93

Time in Seconds	Optical Density
50	.158
150	.170
250	.172
350	.179
800	.190
900	.196
1300	.208
1800	.218
2400	.240
2750	.248
3150	.263
3650	.274
4150	.287

TABLE A94

Time in Seconds	Optical Density
100	.248
800	.278
1000	.287
1500	.305
2500	.354
2650	.368
3260	.392
3460	.398
3950	.422
4600	.443
4850	.450

TABLE A95

Time in Seconds	Optical Density
10800	.172
81000	.215
100800	.255
169200	.303
255600	.376
342000	.431

TABLE A96

Time in Seconds	Optical Density
10800	.182
81000	.227
100800	.258
169200	.301
255600	.368
342000	.423

TABLE A97

Time in Seconds	Optical Density
10800	.187
81000	.313
100800	.333
169200	.456
255600	.607
342000	.783

TABLE A98

Time in Seconds	Optical Density
10800	.182
81000	.285
100800	.329
169200	.430
255600	.589
342000	.732

TABLE A99

Time in Seconds	Optical Density
Start	.145
16200	.212
86400	.356
105300	.385

TABLE A100

Time in Seconds	Optical Density
Start	.137
16200	.190
86400	.345
105300	.396

TABLE A101

Time in Seconds	Optical Density
Start	.158
16200	.239
86400	.571
105300	.697

TABLE A102

Time in Seconds	Optical Density
Start	.169
16200	.238
86400	.551
105300	.666

TABLE A103

Time in Seconds	Optical Density
223	.200
18603	.291
86038	.503
94000	.573
172800	.838

TABLE A104

Time in Seconds	Optical Density
227	.173
18606	.265
86037	.526
94005	.598
172780	.837

TABLE A105

Time in Seconds	Optical Density
360	.214
3595	.236
18700	.454

TABLE A106

Time in Seconds	Optical Density
360	.158
3818	.189
5903	.204
9703	.255
11906	.280
14066	.306
17114	.343
18869	.367
20340	.387
23143	.408

TABLE A107

Time in Seconds	Optical Density
350	.155
3817	.191
5898	.205
9700	.262
11900	.281
14071	.311
17109	.354
18871	.378
20359	.400
23160	.421

TABLE A108

Time in Seconds	Optical Density
Start	.181
16200	.256
86400	.507
105300	.646

TABLE A109

Time in Seconds	Optical Density
Start	.167
16200	.232
86400	.549
105300	.678

TABLE A110

Time in Seconds	Optical Density
270	.144
3671	.180
5765	.195
9597	.235
11762	.246
13980	.269
17124	.286
18886	.313
20275	.321
23150	.338

TABLE A111

Time in Seconds	Optical Density
270	.149
3681	.188
5765	.187
9596	.221
11761	.244
13980	.261
17130	.282
18893	.304
20273	.318
23150	.327

TABLE A112

Time in Seconds	Optical Density
1110	.184
1350	.201
1550	.189
2150	.207
2650	.217
3480	.240
4000	.250
4310	.267
4775	.278
5490	.287
6910	.325
7725	.332

TABLE A113

Time in Seconds	Optical Density
150	.102
240	.107
600	.119
1040	.128
1650	.131
2400	.148
2920	.158
3200	.168
3776	.180
4500	.206
5820	.237
6910	.264

TABLE A114

Time in Seconds	Optical Density
80	.151
470	.157
950	.175
1915	.200
2530	.210
3015	.225
3399	.234
3745	.247
4445	.259
5085	.274
5477	.281
6415	.306

TABLE A115

Time in Seconds	Optical Density
110	.158
450	.172
900	.185
1230	.187
1600	.197
2120	.207
2470	.217
2770	.226
3195	.242
3470	.246

TABLE A116

Time in Seconds	Optical Density
85	.191
425	.207
875	.218
1215	.221
1516	.223
2100	.238
2445	.248
2735	.257
3165	.274
3505	.273

TABLE A117

Time in Seconds	Optical Density
186	.161
860	.175
1377	.187
1980	.201
2477	.213
3033	.226
3542	.241
4004	.255
4550	.272
5070	.287
5520	.301
6118	.320

TABLE A118

Time in Seconds	Optical Density
255	.173
875	.187
1400	.199
1990	.213
2488	.226
3046	.242
3557	.257
4017	.270
4562	.286
5100	.312
5531	.318
6137	.340

TABLE A119

Time in Seconds	Optical Density
Start	.153
86400	.167
172800	.170
259200	.172
518400	.221

TABLE A120

Time in Seconds	Optical Density
Start	.161
86400	.181
172800	.166
259200	.181
578400	.198

TABLE A121

Time in Seconds	Optical Density
Start	.193
86400	.205
172800	.189
259200	.209
518400	.223

TABLE A122

Time in Seconds	Optical Density
Start	.149
86400	.159
172800	.172
259200	.216
518400	.210

TABLE A123

Time in Seconds	Optical Density
Start	.172
86400	.199
172800	.226
259200	.242
518400	.301

TABLE A124

Time in Seconds	Optical Density
Start	.191
86400	.216
172800	.226
259200	.242
518400	.271

TABLE A125

Time in Seconds	Optical Density
Start	.262
86400	.355
172800	.452
259200	.556
518400	.990

TABLE A126

Time in Seconds	Optical Density
Start	.274
86400	.364
172800	.438
259200	.482
518400	.566

TABLE A127

Time in Seconds	Optical Density
Start	.179
86400	.182
172800	.192
259200	.206
518400	.224

TABLE A128

Time in Seconds	Optical Density
Start	.163
86400	.165
172800	.169
259200	.173
518400	.203

TABLE A129

Time in Seconds	Optical Density
Start	.190
86400	.219
172800	.251
259200	.251
518400	.307

TABLE A130

Time in Seconds	Optical Density
Start	.192
86400	.210
172800	.236
259200	.261
518400	.333

TABLE A131

Time in Seconds	Optical Density
Start	.307
86400	.463
172800	.548
259200	.667
518400	1.02

TABLE A132

Time in Seconds	Optical Density
Start	.298
86400	.428
172800	.546
259200	.671
518400	1.05

TABLE A133

Time in Seconds	Optical Density
250	.118
1400	.128
1910	.130
2910	.132
4150	.135
5050	.137
7600	.141
8880	.145

TABLE A134

Time in Seconds	Optical Density
550	.234
1360	.239
1880	.248
2880	.246
4150	.249
5050	.250
7600	.256
8750	.262

TABLE A135

Time in Seconds	Optical Density
Start	.335
86400	.566
172800	.730
259200	.831
345600	.861
691200	.935

TABLE A136

Time in Seconds	Optical Density
Start	.243
86400	.608
172800	.921
259200	1.32
345600	1.83

TABLE A137

Time in Seconds	Optical Density
160	.174
435	.184
975	.200
1455	.217
2415	.246
3075	.274
3525	.288
4115	.308
4465	.324
4995	.336
5560	.355
6305	.383

TABLE A138

Time in Seconds	Optical Density
120	.179
395	.189
955	.207
1430	.228
2375	.256
3035	.283
3490	.296
4080	.319
4430	.334
4965	.345
5520	.366
6270	.396

TABLE A139

Time in Seconds	Optical Density
220	.173
1350	.229
1700	.245
2000	.266
2450	.289
2650	.301
3200	.316
3500	.332
4000	.360
4350	.378

TABLE A140

Time in Seconds	Optical Density
200	.318
1325	.372
1550	.385
1990	.411
2250	.419
2600	.438
3050	.452
3430	.460
3800	.478
4300	.508

TABLE A141

Time in Seconds	Optical Density
10800	.198
81000	.245
100800	.275
169200	.320
255600	.408
342000	.484

TABLE A142

Time in Seconds	Optical Density
10800	.175
81000	.257
100800	.285
169200	.329
255600	.398
342000	.465

TABLE A143

Time in Seconds	Optical Density
10800	.178
81000	.326
100800	.382
169200	.530
255600	.757
342000	1.11

TABLE A144

Time in Seconds	Optical Density
10800	.194
81000	.339
100800	.386
169200	.534
255600	.753
342000	1.21

TABLE A145

Time in Seconds	Optical Density
227	.187
18608	.301
86039	.627
94005	.700
172760	1.15

TABLE A146

Time in Seconds	Optical Density
307	.186
18662	.303
86090	.597
94035	.652
172740	1.15

TABLE A147

Time in Seconds	Optical Density
365	.176
2065	.202
3910	.216
5733	.241
11540	.287
13277	.308
14936	.323
16782	.340
18660	.358
20610	.381
22496	.412
24665	.428

TABLE A148

Time in Seconds	Optical Density
387	.175
2088	.193
3933	.214
5760	.231
11570	.286
13300	.303
14960	.322
16807	.338
18682	.357
20630	.378
22520	.399
24700	.424

TABLE A149

Time in Seconds	Optical Density
Start	.294
86400	.560
172800	.828
259200	1.30
345600	1.87

TABLE A150

Time in Seconds	Optical Density
Start	.288
86400	.562
172800	.823
259200	1.32

TABLE A151

Time in Seconds	Optical Density
400	.176
2115	.193
3950	.211
5780	.228
11587	.282
13320	.297
14975	.314
16828	.332
18700	.350
20642	.369
22537	.392
24722	.417

TABLE A152

Time in Seconds	Optical Density
460	.167
2220	.183
4084	.201
5870	.216
11664	.264
13393	.282
15070	.303
16920	.324
18809	.335
20730	.351
22640	.373
24840	.392

TABLE A153

Time in Seconds	Optical Density
150	.192
600	.217
800	.218
1400	.243
1900	.250
2750	.264
3250	.280
3575	.288
4100	.297
4800	.312
6200	.342
7150	.363

TABLE A154

Time in Seconds	Optical Density
125	.182
560	.202
800	.203
1370	.223
1870	.220
2720	.236
3210	.247
3540	.254
4065	.265
4765	.277
6160	.302
7110	.318

TABLE A155

Time in Seconds	Optical Density
75	.045
350	.054
915	.075
1400	.093
2330	.115
2995	.138
3485	.152
4030	.161
4385	.173
4925	.184
5480	.199
6225	.215

TABLE A156

Time in Seconds	Optical Density
100	.098
275	.104
730	.118
1080	.121
1420	.130
1975	.143
2280	.154
2610	.160
3005	.180
3370	.188

TABLE A157

Time in Seconds	Optical Density
150	.208
200	.212
760	.217
1000	.224
1420	.235
1890	.247
2290	.263
2520	.271
2990	.284
3300	.288

TABLE A158

Time in Seconds	Optical Density
Start	.385
86400	.333
172800	.309
259200	.295
518400	.276

TABLE A159

Time in Seconds	Optical Density
Start	.288
86400	.276
172800	.273
259200	.276
518400	.294

TABLE A160

Time in Seconds	Optical Density
Start	.268
86400	.269
172800	.259
259200	.263
518400	.261

TABLE A161

Time in Seconds	Optical Density
Start	.388
86400	.298
172800	.294
259200	.294
518400	.293

TABLE A162

Time in Seconds	Optical Density
Start	.460
86400	.412
172800	.410
259200	.362
518400	.345

TABLE A163

Time in Seconds	Optical Density
Start	.331
86400	.303
172800	.303
259200	.304
518400	.318

TABLE A164

Time in Seconds	Optical Density
Start	.313
86400	.329
172800	.352
259200	.367
518400	.396

TABLE A165

Time in Seconds	Optical Density
Start	.344
86400	.335
172800	.347
259200	.371
518400	.460

TABLE A166

Time in Seconds	Optical Density
Start	.295
86400	.270
172800	.257
259200	.256
518400	.262

TABLE A167

Time in Seconds	Optical Density
Start	.271
86400	.253
172800	.257
259200	.264
518400	.262

TABLE A168

Time in Seconds	Optical Density
Start	.270
86400	.261
172800	.258
259200	.274
518400	.281

TABLE A169

Time in Seconds	Optical Density
Start	.351
86400	.305
172800	.288
259200	.293
518400	.303

TABLE A170

Time in Seconds	Optical Density
Start	.262
86400	.280
172800	.312
259200	.342
518400	.402

TABLE A171

Time in Seconds	Optical Density
Start	.371
86400	.356
172800	.365
259200	.379
518400	.459

TABLE A172

Time in Seconds	Optical Density
Start	.782
86400	1.07
172800	1.24
259200	1.47

TABLE A173

Time in Seconds	Optical Density
Start	.792
86400	.942
172800	1.20
259200	1.42

TABLE A174

Time in Seconds	Optical Density
208	.355
830	.375
1380	.384
1840	.388
2600	.401
3210	.411
3885	.422
4795	.439
5750	.458
6940	.477
7850	.493

TABLE A175

Time in Seconds	Optical Density
170	.276
790	.293
1341	.301
1820	.307
2650	.326
3170	.328
3845	.339
4750	.354
5705	.368
6910	.385
7815	.396

TABLE A176

Time in Seconds	Optical Density
10800	.433
81000	.446
100800	.451
169200	.449
255600	.453
342000	.465

TABLE A177

Time in Seconds	Optical Density
10800	.376
81000	.338
100800	.351
169200	.350
255600	.386
342000	.400

TABLE A178

Time in Seconds	Optical Density
10800	.384
81000	.405
100800	.418
169200	.451
255600	.510
342000	.567

TABLE A179

Time in Seconds	Optical Density
10800	.435
81000	.514
100800	.546
169200	.565
255600	.656
342000	.737

TABLE A180

Time in Seconds	Optical Density
300	.445
18657	.508
86106	.672
94040	.693
172720	.842

TABLE A181

Time in Seconds	Optical Density
297	.459
18657	.533
86115	.665
94050	.678
172700	.828

TABLE A182

Time in Seconds	Optical Density
410	.466
3660	.505
18724	.603

TABLE A183

Time in Seconds	Optical Density
404	.472
3658	.521
18725	.612

TABLE A184

Time in Seconds	Optical Density
478	.476
2245	.498
4117	.516
5900	.528
11690	.556
13428	.582
15095	.581
16943	.587
18827	.598
20760	.606
22674	.609
24867	.632

TABLE A185

Time in Seconds	Optical Density
500	.469
2265	.488
4137	.508
5932	.522
11710	.549
13452	.562
15122	.572
16964	.581
18850	.592
20782	.601
22690	.606
24890	.624

TABLE A186

Time in Seconds	Optical Density
Start	.581
86400	.580
172800	.672
259200	.752
345600	.877
691200	1.37

TABLE A187

Time in Seconds	Optical Density
Start	.662
86400	.644
172800	.788
259200	.950
345600	1.14

TABLE A188

Time in Seconds	Optical Density
160	.423
2140	.445
3225	.452
4181	.455
6304	.468
7728	.482
9715	.496
11527	.504
13340	.510
15666	.526
17530	.531
19520	.542

TABLE A189

Time in Seconds	Optical Density
180	.264
810	.276
1360	.283
1840	.293
2770	.305
3270	.312
3890	.319
4770	.329
5710	.342
6930	.350
7700	.366

TABLE A190

Time in Seconds	Optical Density
145	.278
780	.289
1355	.298
1810	.306
2740	.314
3245	.319
3855	.329
4735	.336
5680	.346
6895	.353
7670	.385

APPENDIX II

Analysis of Raw Data

All Runs analyzed were performed at a temperature of 25°C. The results that have been obtained from these tables were used in the final analysis of the Reactions of Poly-L-lysine with 1-Halo-2,4-dinitrobenzenes.

Reaction	Table Numbers
1-Fluoro-2,4-dinitrobenzene with Poly-L-lysine	B1-B6
1-Chloro-2,4-dinitrobenzene with Poly-L-lysine	B7-B10
1-Bromo-2,4-dinitrobenzene with Poly-L-lysine	B11-B14
1-Iodo-2,4-dinitrobenzene with Poly-L-lysine	B15-B18

TABLE B1

Run Number	pH	Buffer	[F] (mole/l)	[PL] (mole/l)	$[NH_2]_c$ (mole/l)	$[NH_2]_h$ (mole/l)
A28	8.50	THAM	$1.140x10^{-3}$	$4.839x10^{-2}$	$2.432x10^{-4}$	$3.312x10^{-6}$
A29	8.50	THAM	$1.140x10^{-3}$	$4.839x10^{-2}$	$2.432x10^{-4}$	$3.312x10^{-6}$
A30	8.50	THAM	$1.140x10^{-3}$	$4.839x10^{-2}$	$2.432x10^{-4}$	$3.312x10^{-6}$
A31	8.50	THAM	$5.748x10^{-4}$	$4.879x10^{-2}$	$2.452x10^{-4}$	$3.339x10^{-6}$
A32	8.50	THAM	$5.748x10^{-4}$	$4.879x10^{-2}$	$2.452x10^{-4}$	$3.339x10^{-6}$
A33	8.50	THAM	$1.140x10^{-3}$	$2.420x10^{-2}$	$1.216x10^{-4}$	$1.656x10^{-6}$
A34	8.50	THAM	$1.140x10^{-3}$	$2.420x10^{-2}$	$1.216x10^{-4}$	$1.656x10^{-6}$
A35	8.50	THAM	$1.140x10^{-3}$	$2.420x10^{-2}$	$1.216x10^{-4}$	$1.656x10^{-6}$
A36	8.50	THAM	$1.140x10^{-3}$	$1.210x10^{-2}$	$6.080x10^{-5}$	$8.279x10^{-7}$
A37	8.50	THAM	$1.140x10^{-3}$	$1.210x10^{-2}$	$6.080x10^{-5}$	$8.279x10^{-7}$
A44	8.50	THAM	$5.748x10^{-4}$	$1.626x10^{-2}$	$8.173x10^{-5}$	$1.113x10^{-6}$
A45	8.50	THAM	$5.748x10^{-4}$	$1.626x10^{-2}$	$8.173x10^{-5}$	$1.113x10^{-6}$
A46	8.50	THAM	$5.748x10^{-4}$	$1.626x10^{-2}$	$8.173x10^{-5}$	$1.113x10^{-6}$
A38	9.00	THAM	$1.140x10^{-3}$	$1.613x10^{-2}$	$2.221x10^{-4}$	$6.701x10^{-6}$
A39	9.00	THAM	$1.140x10^{-3}$	$1.613x10^{-2}$	$2.221x10^{-4}$	$6.701x10^{-6}$
A47	9.00	THAM	$5.748x10^{-4}$	$1.626x10^{-2}$	$2.239x10^{-4}$	$6.755x10^{-6}$

TABLE B2

Run Number	pH	Net Rate O.D./sec	$\frac{\text{Net Rate}}{[F][NH_2]_c}$ $\frac{\text{O.D./sec}}{(\text{mole/l})^2}$	Avg. $\frac{\text{Net Rate}}{[F][NH_2]_c}$ $\frac{\text{O.D./sec}}{(\text{mole/l})^2}$	Avg. $\frac{\text{Net Rate}}{[F][NH_2]_c}$ $\frac{\text{mole/l}\cdot\text{sec}}{(\text{mole/l})^2}$	$\frac{[NH_2]_h}{[NH_2]_c}$
A28	8.50	2.480×10^{-3}	8.945×10^{3}			
A29	8.50	2.170×10^{-3}	7.827×10^{3}			
A30	8.50	2.477×10^{-3}	8.934×10^{3}			
A31	8.50	9.560×10^{-4}	6.783×10^{3}			
A32	8.50	1.012×10^{-3}	7.180×10^{3}			
A33	8.50	1.506×10^{-3}	1.086×10^{4}			
A34	8.50	1.156×10^{-3}	8.339×10^{3}	7.284×10^{3}	2.646	1.362×10^{-2}
A35	8.50	1.108×10^{-3}	7.993×10^{3}			
A36	8.50	4.324×10^{-4}	6.238×10^{3}			
A37	8.50	4.299×10^{-4}	6.202×10^{3}			
A44	8.50	2.481×10^{-4}	5.281×10^{3}			
A45	8.50	2.365×10^{-4}	5.034×10^{3}			
A46	8.50	2.384×10^{-4}	5.075×10^{3}			
A38	9.00	1.152×10^{-3}	4.550×10^{3}			
A39	9.00	1.371×10^{-3}	5.415×10^{3}			
A47	9.00	5.979×10^{-4}	4.646×10^{3}	4.972×10^{3}	1.806	3.017×10^{-2}

TABLE B3

Run Number	pH	Buffer	[F] (mole/l)	[PL] (mole/l)	$[NH_2]_c$ (mole/l)	$[NH_2]_h$ (mole/l)
A48	9.00	THAM	5.748×10^{-4}	1.626×10^{-2}	2.239×10^{-4}	6.755×10^{-6}
A49	9.00	THAM	5.748×10^{-4}	1.626×10^{-2}	2.239×10^{-4}	6.755×10^{-6}
A50	9.50	THAM	5.748×10^{-4}	1.626×10^{-2}	4.844×10^{-4}	4.327×10^{-5}
A51	9.50	THAM	5.748×10^{-4}	1.626×10^{-2}	4.844×10^{-4}	4.327×10^{-5}
A42	9.50	THAM	5.748×10^{-4}	3.252×10^{-2}	9.688×10^{-4}	8.654×10^{-5}
A43	9.50	THAM	5.748×10^{-4}	3.252×10^{-2}	9.688×10^{-4}	8.654×10^{-5}
A40	9.50	THAM	1.140×10^{-3}	1.626×10^{-2}	4.844×10^{-4}	4.327×10^{-5}
A41	9.50	THAM	1.140×10^{-3}	1.626×10^{-2}	4.844×10^{-4}	4.327×10^{-5}
A52	10.00	THAM	5.748×10^{-3}	1.626×10^{-2}	7.020×10^{-4}	2.013×10^{-4}
A53	10.00	THAM	5.748×10^{-3}	1.626×10^{-2}	7.020×10^{-4}	2.013×10^{-4}
A54	10.35	THAM	2.311×10^{-4}	8.130×10^{-3}	3.055×10^{-4}	2.455×10^{-4}
A55	10.35	THAM	2.311×10^{-4}	8.130×10^{-3}	3.055×10^{-4}	2.455×10^{-4}
A56	10.35	THAM	2.311×10^{-4}	8.130×10^{-3}	3.055×10^{-4}	2.455×10^{-4}
A57	10.35	THAM	2.311×10^{-4}	8.130×10^{-3}	3.055×10^{-4}	2.455×10^{-4}
A58	10.65	THAM	2.311×10^{-4}	8.130×10^{-3}	2.590×10^{-4}	3.950×10^{-4}
A59	10.65	THAM	2.311×10^{-4}	8.130×10^{-3}	2.590×10^{-4}	3.950×10^{-4}

TABLE B4

Run Number	pH	Net Rate O.D./sec	$\frac{\text{Net Rate}}{[F][NH_2]_c}$ $\frac{\text{O.D./sec}}{(\text{mole/l})^2}$	Avg. $\frac{\text{Net Rate}}{[F][NH_2]_c}$ $\frac{\text{O.D./sec}}{(\text{mole/l})^2}$	Avg. $\frac{\text{Net Rate}}{[F][NH_2]_c}$ $\frac{\text{mole/l}\cdot\text{sec}}{(\text{mole/l})^2}$	$\frac{[NH_2]_h}{[NH_2]_c}$
A48	9.00	$6.499\text{x}10^{-4}$	$5.050\text{x}10^{3}$			
A49	9.00	$6.694\text{x}10^{-4}$	$5.201\text{x}10^{3}$			
A50	9.50	$1.312\text{x}10^{-3}$	$4.712\text{x}10^{3}$			
A51	9.50	$1.357\text{x}10^{-3}$	$4.874\text{x}10^{3}$			
A42	9.50	$2.552\text{x}10^{-3}$	$4.583\text{x}10^{3}$	$4.313\text{x}10^{3}$	1.567	$8.933\text{x}10^{-2}$
A43	9.50	$2.159\text{x}10^{-3}$	$3.877\text{x}10^{3}$			
A40	9.50	$2.042\text{x}10^{-3}$	$3.698\text{x}10^{3}$			
A41	9.50	$2.283\text{x}10^{-3}$	$4.134\text{x}10^{3}$			
A52	10.00	$3.486\text{x}10^{-3}$	$8.639\text{x}10^{2}$	$8.515\text{x}10^{2}$	$3.093\text{x}10^{-1}$	$28.675\text{x}10^{-2}$
A53	10.00	$3.386\text{x}10^{-3}$	$8.391\text{x}10^{2}$			
A54	10.35	$1.292\text{x}10^{-3}$	$1.830\text{x}10^{4}$			
A55	10.35	$0.811\text{x}10^{-3}$	$1.149\text{x}10^{4}$	$2.063\text{x}10^{4}$	7.492	$80.35\text{x}10^{-2}$
A56	10.35	$1.920\text{x}10^{-3}$	$2.720\text{x}10^{4}$			
A57	10.35	$1.801\text{x}10^{-3}$	$2.551\text{x}10^{4}$			
A58	10.65	$2.465\text{x}10^{-3}$	$4.118\text{x}10^{4}$			
A59	10.65	$3.390\text{x}10^{-3}$	$5.664\text{x}10^{4}$	$4.756\text{x}10^{4}$	17.276	$152.5\text{x}10^{-2}$

TABLE B5

Run Number	pH	Buffer	[F] (mole/l)	[PL] (mole/l)	$[NH_2]_c$ (mole/l)	$[NH_2]_h$ (mole/l)
A60	10.65	THAM	2.311×10^{-4}	8.130×10^{-3}	2.590×10^{-4}	3.950×10^{-4}
A61	10.65	THAM	2.311×10^{-4}	8.130×10^{-3}	2.590×10^{-4}	3.950×10^{-4}
A62	10.85	THAM	2.311×10^{-4}	8.130×10^{-3}	2.205×10^{-4}	4.884×10^{-4}
A63	10.85	THAM	2.311×10^{-4}	8.130×10^{-3}	2.205×10^{-4}	4.884×10^{-4}

TABLE B6

Run Number	pH	Net Rate	$\frac{\text{Net Rate}}{[F][NH_2]_c}$	Avg. $\frac{\text{Net Rate}}{[F][NH_2]_c}$	Avg. $\frac{\text{Net Rate}}{[F][NH_2]_c}$	$\frac{[NH_2]_h}{[NH_2]_c}$
		O.D./sec	$\frac{\text{O.D./sec}}{(\text{mole/l})^2}$	$\frac{\text{O.D./sec}}{(\text{mole/l})^2}$	$\frac{\text{mole/l}\cdot\text{sec}}{(\text{mole/l})^2}$	
A60	10.65	2.754×10^{-3}	4.601×10^{4}			
A61	10.65	2.778×10^{-3}	4.641×10^{4}			
A62	10.85	3.752×10^{-3}	7.363×10^{4}	7.326×10^{4}	26.609	221.5×10^{-2}
A63	10.85	3.714×10^{-3}	7.288×10^{4}			

TABLE B7

Run Number	pH	Buffer	[Cl] (mole/l)	[PL] (mole/l)	$[NH_2]_c$ (mole/l)	$[NH_2]_h$ (mole/l)
A95	8.50	THAM	5.735×10^{-4}	1.626×10^{-3}	8.173×10^{-5}	1.113×10^{-6}
A96	8.50	THAM	5.735×10^{-4}	1.626×10^{-3}	8.173×10^{-5}	1.113×10^{-6}
A99	9.00	THAM	5.735×10^{-4}	1.626×10^{-3}	2.239×10^{-4}	6.755×10^{-4}
A100	9.00	THAM	5.735×10^{-4}	1.626×10^{-3}	2.239×10^{-4}	6.755×10^{-4}
A97	9.00	THAM	5.735×10^{-4}	1.626×10^{-3}	2.239×10^{-4}	6.755×10^{-4}
A98	9.00	THAM	5.735×10^{-4}	1.626×10^{-3}	2.239×10^{-4}	6.755×10^{-4}
A101	9.50	THAM	5.735×10^{-4}	1.626×10^{-3}	4.844×10^{-4}	4.327×10^{-5}
A102	9.50	THAM	5.735×10^{-4}	1.626×10^{-3}	4.844×10^{-4}	4.327×10^{-5}
A103	9.50	THAM	5.735×10^{-4}	1.626×10^{-3}	4.844×10^{-4}	4.327×10^{-5}
A104	9.50	THAM	5.735×10^{-4}	1.626×10^{-3}	4.844×10^{-4}	4.327×10^{-5}
A108	9.50	BORAX	5.735×10^{-4}	1.626×10^{-3}	4.844×10^{-4}	4.327×10^{-5}
A109	9.50	BORAX	5.735×10^{-4}	1.626×10^{-3}	4.844×10^{-4}	4.327×10^{-5}
A106	10.00	THAM	5.735×10^{-4}	1.626×10^{-3}	7.020×10^{-4}	2.013×10^{-4}
A107	10.00	THAM	5.735×10^{-4}	1.626×10^{-3}	7.020×10^{-4}	2.013×10^{-4}
A105	10.00	THAM	5.735×10^{-4}	1.626×10^{-3}	7.020×10^{-4}	2.013×10^{-4}
A110	10.00	THAM	5.735×10^{-4}	1.626×10^{-3}	7.020×10^{-4}	2.013×10^{-4}

TABLE B8

Run Number	pH	Net Rate O.D./sec	$\frac{\text{Net Rate}}{[\text{Cl}][\text{NH}_2]_c}$ $\frac{\text{O.D./sec}}{(\text{mole/l})^2}$	Avg $\frac{\text{Net Rate}}{[\text{Cl}][\text{NH}_2]_c}$ $\frac{\text{O.D./sec}}{(\text{mole/l})^2}$	Avg $\frac{\text{Net Rate}}{[\text{Cl}][\text{NH}_2]_c}$ $\frac{\text{mole/l}\cdot\text{sec}}{(\text{mole/l})^2}$	$\frac{[\text{NH}_2]_h}{[\text{NH}_2]_c}$
A95	8.50	$7.993\text{x}10^{-7}$	17.053	16.357	$5.941\text{x}10^{-3}$	$1.362\text{x}10^{-2}$
A96	8.50	$7.340\text{x}10^{-7}$	15.660			
A99	9.00	$2.171\text{x}10^{-6}$	16.907			
A100	9.00	$2.237\text{x}10^{-6}$	17.421			
A97	9.00	$1.737\text{x}10^{-6}$	13.527	15.118	$5.491\text{x}10^{-3}$	$3.017\text{x}10^{-2}$
A98	9.00	$1.620\text{x}10^{-6}$	12.616			
A101	9.50	$4.901\text{x}10^{-6}$	17.642			
A102	9.50	$4.546\text{x}10^{-6}$	16.364			
A103	9.50	$3.546\text{x}10^{-6}$	12.764	15.3685	$5.582\text{x}10^{-3}$	$8.933\text{x}10^{-2}$
A104	9.50	$3.766\text{x}10^{-6}$	13.555			
A108	9.50	$4.135\text{x}10^{-6}$	14.885			
A109	9.50	$4.722\text{x}10^{-6}$	16.998			
A106	10.00	$1.212\text{x}10^{-5}$	30.105			
A107	10.00	$1.199\text{x}10^{-5}$	29.782			
A105	10.00	$1.325\text{x}10^{-5}$	32.911	26.529	$9.637\text{x}10^{-3}$	$28.675\text{x}10^{-2}$
A110	10.00	$8.349\text{x}10^{-6}$	20.738			

TABLE B9

Run Number	pH	Buffer	[Cl] (mole/l)	[PL] (mole/l)	$[NH_2]_c$ (mole/l)	$[NH_2]_h$ (mole/l)
A111	10.00	BORAX	5.735×10^{-4}	1.626×10^{-3}	7.020×10^{-4}	2.013×10^{-4}
A91	10.50	BORAX	5.735×10^{-4}	3.252×10^{-3}	1.128×10^{-3}	1.278×10^{-3}
A92	10.50	BORAX	5.735×10^{-4}	3.252×10^{-3}	1.128×10^{-3}	1.278×10^{-3}
A89	10.50	BORAX	5.735×10^{-4}	3.252×10^{-3}	1.128×10^{-3}	1.278×10^{-3}
A90	10.50	BORAX	5.735×10^{-4}	3.252×10^{-3}	1.128×10^{-3}	1.278×10^{-3}
A93	10.80	BORAX	5.735×10^{-4}	3.252×10^{-3}	9.239×10^{-4}	1.793×10^{-3}
A94	10.80	BORAX	5.735×10^{-4}	3.252×10^{-3}	9.239×10^{-4}	1.793×10^{-3}
A112	10.80	BORAX	5.735×10^{-4}	1.626×10^{-3}	4.619×10^{-4}	8.967×10^{-4}
A113	10.80	BORAX	5.735×10^{-4}	1.626×10^{-3}	4.619×10^{-4}	8.967×10^{-4}
A114	10.80	BORAX	5.735×10^{-4}	1.626×10^{-3}	4.619×10^{-4}	8.967×10^{-4}
A115	11.00	BORAX	5.735×10^{-4}	1.626×10^{-3}	3.536×10^{-4}	1.081×10^{-3}
A116	11.00	BORAX	5.735×10^{-4}	1.626×10^{-3}	3.536×10^{-4}	1.081×10^{-3}
A117	11.00	BORAX	5.735×10^{-4}	1.626×10^{-3}	3.536×10^{-4}	1.081×10^{-3}
A118	11.00	BORAX	5.735×10^{-4}	1.626×10^{-3}	3.536×10^{-4}	1.081×10^{-3}

TABLE B10

Run Number	pH	Net Rate O.D./sec	$\frac{\text{Net Rate}}{[Cl][NH_2]_c}$ $\frac{\text{O.D./sec}}{(\text{mole/l})^2}$	Avg. $\frac{\text{Net Rate}}{[Cl][NH_2]_c}$ $\frac{\text{O.D./sec}}{(\text{mole/l})^2}$	Avg. $\frac{\text{Net Rate}}{[Cl][NH_2]_c}$ $\frac{\text{mole/l}\cdot\text{sec}}{(\text{mole/l})^2}$	$\frac{[NH_2]_n}{[NH_2]_c}$
A111	10.00	$7.694x10^{-6}$	19.111			
A91	10.50	$3.918x10^{-5}$	60.568			
A92	10.50	$2.987x10^{-5}$	46.175	56.615	$20.565x10^{-3}$	$113.313x10^{-2}$
A89	10.50	$4.433x10^{-5}$	68.530			
A90	10.50	$3.311x10^{-5}$	51.185			
A93	10.80	$2.615x10^{-5}$	49.360			
A94	10.80	$3.955x10^{-5}$	74.651			
A112	10.80	$1.846x10^{-5}$	69.687	72.965	$26.504x10^{-3}$	$194.118x10^{-2}$
A113	10.80	$1.896x10^{-5}$	71.574			
A114	10.80	$2.012x10^{-5}$	71.948			
A115	11.00	$2.279x10^{-5}$	112.370			
A116	11.00	$2.173x10^{-5}$	107.146	118.88	$43.182x10^{-3}$	$305.60x10^{-2}$
A117	11.00	$2.511x10^{-5}$	123.812			
A118	11.00	$2.681x10^{-5}$	132.194			

TABLE B11

Run Number	pH	Buffer	[Br] (mole/l)	[PL] (mole/l)	$[NH_2]_c$ (mole/l)	$[NH_2]_h$ (mole/l)
A141	8.50	THAM	$5.741x10^{-4}$	$1.626x10^{-3}$	$8.173x10^{-5}$	$1.113x10^{-6}$
A142	8.50	THAM	$5.741x10^{-4}$	$1.626x10^{-3}$	$8.173x10^{-5}$	$1.113x10^{-6}$
A143	9.00	THAM	$5.741x10^{-4}$	$1.626x10^{-3}$	$2.239x10^{-4}$	$6.755x10^{-6}$
A144	9.00	THAM	$5.741x10^{-4}$	$1.626x10^{-3}$	$2.239x10^{-4}$	$6.755x10^{-6}$
A145	9.50	THAM	$5.741x10^{-4}$	$1.626x10^{-3}$	$4.844x10^{-4}$	$4.327x10^{-5}$
A146	9.50	THAM	$5.741x10^{-4}$	$1.626x10^{-3}$	$4.844x10^{-4}$	$4.327x10^{-5}$
A149	9.50	BORAX	$5.741x10^{-4}$	$1.626x10^{-3}$	$4.844x10^{-4}$	$4.327x10^{-5}$
A150	9.50	BORAX	$5.741x10^{-4}$	$1.626x10^{-3}$	$4.844x10^{-4}$	$4.327x10^{-5}$
A147	10.00	THAM	$5.741x10^{-4}$	$1.626x10^{-3}$	$7.020x10^{-4}$	$2.013x10^{-4}$
A148	10.00	THAM	$5.741x10^{-4}$	$1.626x10^{-3}$	$7.020x10^{-4}$	$2.013x10^{-4}$
A151	10.00	BORAX	$5.741x10^{-4}$	$1.626x10^{-3}$	$7.020x10^{-4}$	$2.013x10^{-4}$
A152	10.00	BORAX	$5.741x10^{-4}$	$1.626x10^{-3}$	$7.020x10^{-4}$	$2.013x10^{-4}$
A137	10.50	BORAX	$5.741x10^{-4}$	$3.252x10^{-3}$	$1.128x10^{-3}$	$1.278x10^{-3}$
A138	10.50	BORAX	$5.741x10^{-4}$	$3.252x10^{-3}$	$1.128x10^{-3}$	$1.278x10^{-3}$
A139	10.80	BORAX	$5.741x10^{-4}$	$3.252x10^{-3}$	$9.239x10^{-4}$	$1.793x10^{-3}$
A140	10.80	BORAX	$5.741x10^{-4}$	$3.252x10^{-3}$	$9.239x10^{-4}$	$1.793x10^{-3}$

TABLE B12

Run Number	pH	Net Rate O.D./sec	$\frac{\text{Net Rate}}{[\text{Br}][\text{NH}_2]_c}$ $\frac{\text{O.D./sec}}{(\text{mole/l})^2}$	Avg $\frac{\text{Net Rate}}{[\text{Br}][\text{NH}_2]_c}$ $\frac{\text{O.D./sec}}{(\text{mole/l})^2}$	Avg $\frac{\text{Net Rate}}{[\text{Br}][\text{NH}_2]_c}$ $\frac{\text{mole/l}\cdot\text{sec}}{(\text{mole/l})^2}$	$\frac{[\text{NH}_2]_h}{[\text{NH}_2]_c}$
A141	8.50	$8.074\text{x}10^{-7}$	17.208	16.574	$6.020\text{x}10^{-3}$	$1.362\text{x}10^{-2}$
A142	8.50	$7.479\text{x}10^{-7}$	15.939			
A143	9.00	$2.660\text{x}10^{-6}$	20.694	21.437	$7.787\text{x}10^{-3}$	$3.017\text{x}10^{-2}$
A144	9.00	$2.851\text{x}10^{-6}$	22.180			
A145	9.50	$5.317\text{x}10^{-6}$	19.119			
A146	9.50	$5.249\text{x}10^{-6}$	18.875	17.98	$6.531\text{x}10^{-3}$	$8.933\text{x}10^{-2}$
A149	9.50	$4.437\text{x}10^{-6}$	15.960			
A150	9.50	$2.908\text{x}10^{-6}$	10.460			
A147	10.00	$9.083\text{x}10^{-6}$	22.537			
A148	10.00	$9.068\text{x}10^{-6}$	22.500	22.71	$8.249\text{x}10^{-3}$	$28.675\text{x}10^{-2}$
A151	10.00	$9.454\text{x}10^{-6}$	23.460			
A152	10.00	$9.002\text{x}10^{-6}$	22.340			
A137	10.50	$3.262\text{x}10^{-5}$	50.367	51.100	$18.525\text{x}10^{-3}$	$113.313\text{x}10^{-2}$
A138	10.50	$3.565\text{x}10^{-5}$	51.834			
A139	10.80	$4.634\text{x}10^{-5}$	87.370			
A140	10.80	$3.910\text{x}10^{-5}$	73.720	83.00	$30.149\text{x}10^{-3}$	$194.118\text{x}10^{-2}$

TABLE B13

Run Number	pH	Buffer	[Br] (mole/l)	[PL] (mole/l)	$[NH_2]_c$ (mole/l)	$[NH_2]_h$ (mole/l)
A153	10.80	BORAX	5.741×10^{-4}	1.626×10^{-3}	4.619×10^{-4}	8.967×10^{-4}
A155	10.80	BORAX	5.741×10^{-4}	1.626×10^{-3}	4.619×10^{-4}	8.967×10^{-4}
A156	11.00	BORAX	5.741×10^{-4}	1.626×10^{-3}	3.536×10^{-4}	1.087×10^{-3}
A157	11.00	BORAX	5.741×10^{-4}	1.626×10^{-3}	3.536×10^{-4}	1.087×10^{-3}

TABLE B14

Run Number	pH	Net Rate O.D./sec	$\frac{\text{Net Rate}}{[\text{Br}][\text{NH}_2]_c}$ $\frac{\text{O.D./sec}}{(\text{mole/l})^2}$	Avg. $\frac{\text{Net Rate}}{[\text{Br}][\text{NH}_2]_c}$ $\frac{\text{O.D./sec}}{(\text{mole/l})^2}$	Avg. $\frac{\text{Net Rate}}{[\text{Br}][\text{NH}_2]_c}$ $\frac{\text{mole/l}\cdot\text{sec}}{(\text{mole/l})^2}$	$\frac{[\text{NH}_2]_h}{[\text{NH}_2]_c}$
A153	10.80	2.035×10^{-5}	76.740			
A155	10.80	2.497×10^{-5}	94.157			
A156	11.00	2.414×10^{-5}	118.905	118.437	43.021×10^{-3}	305.6×10^{-2}
A157	11.00	2.395×10^{-5}	117.969			

TABLE B15

Run Number	pH	Buffer	[I] (mole/l)	[PL] (mole/l)	$[NH_2]_c$ (mole/l)	$[NH_2]_h$ (mole/l)
A176	8.50	THAM	$5.748x10^{-4}$	$1.626x10^{-3}$	$8.173x10^{-5}$	$1.113x10^{-6}$
A177	8.50	THAM	$5.748x10^{-4}$	$1.626x10^{-3}$	$8.173x10^{-5}$	$1.113x10^{-6}$
A178	9.00	THAM	$5.748x10^{-4}$	$1.626x10^{-3}$	$2.234x10^{-4}$	$6.755x10^{-6}$
A179	9.00	THAM	$5.748x10^{-4}$	$1.626x10^{-3}$	$2.234x10^{-4}$	$6.755x10^{-6}$
A180	9.50	THAM	$5.748x10^{-4}$	$1.626x10^{-3}$	$4.844x10^{-4}$	$4.327x10^{-5}$
A181	9.50	THAM	$5.748x10^{-4}$	$1.626x10^{-3}$	$4.844x10^{-4}$	$4.327x10^{-5}$
A186	9.50	BORAX	$5.748x10^{-4}$	$1.626x10^{-3}$	$4.844x10^{-4}$	$4.327x10^{-5}$
A187	9.50	BORAX	$5.748x10^{-4}$	$1.626x10^{-3}$	$4.844x10^{-4}$	$4.327x10^{-5}$
A182	10.00	THAM	$5.748x10^{-4}$	$1.626x10^{-3}$	$7.020x10^{-4}$	$2.013x10^{-4}$
A183	10.00	THAM	$5.748x10^{-4}$	$1.626x10^{-3}$	$7.020x10^{-4}$	$2.013x10^{-4}$
A184	10.00	THAM	$5.748x10^{-4}$	$1.626x10^{-3}$	$7.020x10^{-4}$	$2.013x10^{-4}$
A185	10.00	THAM	$5.748x10^{-4}$	$1.626x10^{-3}$	$7.020x10^{-4}$	$2.013x10^{-4}$
A189	10.00	BORAX	$5.748x10^{-4}$	$1.626x10^{-3}$	$7.020x10^{-4}$	$2.013x10^{-4}$
A174	10.50	BORAX	$5.748x10^{-4}$	$3.252x10^{-3}$	$1.128x10^{-3}$	$1.278x10^{-3}$
A175	10.50	BORAX	$5.748x10^{-4}$	$3.252x10^{-3}$	$1.128x10^{-3}$	$1.278x10^{-3}$

TABLE B16

Run Number	pH	Net Rate O.D./sec	$\frac{\text{Net Rate}}{[I][NH_2]_c}$ $\frac{\text{O.D./sec}}{(\text{mole/l})^2}$	Avg. $\frac{\text{Net Rate}}{[I][NH_2]_c}$ $\frac{\text{O.D./sec}}{(\text{mole/l})^2}$	Avg. $\frac{\text{Net Rate}}{[I][NH_2]_c}$ $\frac{\text{mole/l} \cdot \text{sec}}{(\text{mole/l})^2}$	$\frac{[NH_2]_h}{[NH_2]_c}$
A176	8.50	7.801×10^{-8}	1.661	2.153	0.782×10^{-3}	1.362×10^{-2}
A177	8.50	12.43×10^{-8}	2.646			
A178	9.00	5.694×10^{-7}	4.424	5.855	2.029×10^{-3}	3.017×10^{-2}
A179	9.00	8.683×10^{-7}	6.747			
A180	9.50	2.293×10^{-6}	8.235			
A181	9.50	2.071×10^{-6}	7.438	6.561	2.383×10^{-3}	8.933×10^{-2}
A186	9.50	1.326×10^{-6}	4.762			
A187	9.50	1.617×10^{-6}	5.808			
A182	10.00	6.976×10^{-6}	17.289			
A183	10.00	6.942×10^{-6}	17.204			
A184	10.00	5.654×10^{-6}	14.012	15.482	5.624×10^{-3}	28.675×10^{-2}
A185	10.00	5.758×10^{-6}	14.270			
A189	10.00	5.905×10^{-6}	14.634			
A174	10.50	1.718×10^{-5}	26.448	24.887	9.040×10^{-3}	113.313×10^{-2}
A175	10.50	1.509×10^{-5}	23.275			

TABLE B17

Run Number	pH	Buffer	[I] (mole/l)	[PL] (mole/l)	$[NH_2]_c$ (mole/l)	$[NH_2]_h$ (mole/l)
A189	11.00	BORAX	$5.748\text{x}10^{-4}$	$1.626\text{x}10^{-3}$	$3.536\text{x}10^{-4}$	$1.081\text{x}10^{-3}$
A190	11.00	BORAX	$5.748\text{x}10^{-4}$	$1.626\text{x}10^{-3}$	$3.536\text{x}10^{-4}$	$1.081\text{x}10^{-3}$

TABLE B18

Run Number	pH	Net Rate	$\frac{\text{Net Rate}}{[F][NH_2]_c}$	Avg. $\frac{\text{Net Rate}}{[I][NH_2]_c}$	Avg. $\frac{\text{Net Rate}}{[I][NH_2]_c}$	$\frac{[NH_2]_h}{[NH_2]_c}$
		O.D./sec	$\frac{\text{O.D./sec}}{(\text{mole/l})^2}$	$\frac{\text{O.D./sec}}{(\text{mole/l})^2}$	$\frac{\text{mole/l}\cdot\text{sec}}{(\text{mole/l})^2}$	
A189	11.00	$1.037\text{x}10^{-5}$	51.02	49.575	$18.008\text{x}10^{-3}$	$305.60\text{x}10^{-2}$
A190	11.00	$0.976\text{x}10^{-5}$	48.015			

APPENDIX III

Calculator Program Written By Dr. Ernest Bart To Calculate The Rate Constants For A Four Parameter Rate Expression Using A Hewlett-Packard 9820 Calculator

```
0:
ENT "NO. OF RUNS=?",PRT "NO. OF RUNS=",A;SPC 4;1→B⊢
1:
IF 4>A;PRT "TO LITTLE DATA";GTO 19⊢
2:
0→RB;B+1→B; IF B=21;GTO 2⊢
3:
1→B⊢
4:
PRT "RUN NO.=",B;SPC 1;ENT "X",X,"Y",Y,"Z",C,"W",R0⊢
5:
ENT "RATE",R21;PRT X,Y,C,R0;SPC 1;PRT R21;SPC 4⊢
6:
XX+R1→R1;XY+R2→R2;XC+R3→R3;XR0+R4→R4;XR21=R5→R5⊢
7:
YY+R7→R7;YC+R8→R8;YR0+R9→R9;YR21+R10→R10⊢
8:
CC+R13→R13;CR0+R14→R14;CR21+R15→R15⊢
9:
R0R0+R19→R19;R0R21+R20→R20;B+1→B;IF B≤A;GTO 4⊢
10:
SPC 3;R2→R6;R3 R11;R4→R16;R8→R12;R9→R17;R14→R18⊢
11:
1→A;0→C;2→B⊢
12:
R(B+5A)-R(B+5C)R(C+1+5A)/R(6C+1)→R(B+5A)⊢
13:
IF B=5;B+1→B;GTO 12⊢
14:
IF A=3;A+1→A;C+2→B;GTO 12⊢
15:
IF C=2;C+1→C;C+1→A;C+2→B;GTO 12⊢
16:
R20/R19→X; (R15-XR14)/R13→C;(R10-XR9-CR8)/R7→B⊢
17:
(R5-XR4-CR3-BR2)/R1→A⊢
18:
PRT "A1=",A,"A2=",B,"A3=",C,"A4=",X⊢
19:
SPC 8;END⊢
R58
```