A KINETIC STUDY OF LYSINE AND LYSINE DERIVATIVES

BY

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Abstract

The kinetics of the reaction of iodoacetamide with lysine, polylysine, N-α-Acetyl lysine, and N-ε-Acetyl lysine, have been studied. The investigation was done at 23°C $\pm$ 1°C, as a function of pH, and concentration. In all cases it was noted that the rate of reaction increased as the pH increased. The rate data was analyzed in order to obtain the second order rate constants. In addition, the effects of the reactive site's environment on the rate was demonstrated.

APPROVAL OF THESIS

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STEPHEN JOSEPH STANLEY

FOR

THE DEPARTMENT OF CHEMICAL ENGINEERING

NEWARK COLLEGE OF ENGINEERING

BY

THE FACULTY COMMITTEE OF

APPROVED: _____

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Preface and Acknowledgments

This work was undertaken to further investigate a series of problems that remained unanswered upon the completion of a previous work performed by members of the College. It by no means has completed the work in this area, and has itself raised questions that will be the subject of future works.

At this time I would like to thank those people who helped greatly in the preparation of this work. To Dr. Richard Parker, my advisor, who gave many hours of time, and much needed advice and encouragement. To Dr. David Kristol, whose comments were very helpful. Finally to my family, and most especially to Gail, for their encouragement, and help.

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Introduction

At this point in time much work is being done in the area of biocatalysis. Most simply defined, biocatalysis is generally concerned with the study of the catalytic effects of protein chains on chemical reactions within living organisms. Proteins are composed of amino acids. In many instances the specific amino acid residues which are found on the reactive sites of the protein chains have been determined. An example of such an exzymetic process is the catalytic breakdown of food proteins in the digestive tract. The enzyme trypsin is found to be one of the major digestive enzymes.[1]

It is generally believed that in their catalytic activity enzymes utilize free electron pairs located at the base centers along the protein chains. It has been shown that the free electron pairs are identical with the side chain functional groups of some amino acids.[2] The amine group which is the reactive site on the amino acid lysine, is an example of this analogy. Other examples that may be considered are the imidazole groups of histidine, the phenolic group of tyrosine, and the sulphydryl groups of cysteine.

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[1] Arthur L. Lehninger, Biochemistry (Worth Publishing Company, New York, 1970) p. 434.

[2] K. N. Shivaram, K. Wallenfels, "Reactions of Amino Acids, Peptides and Related Compounds with Electrophilic Reagents," Journal of Biochemistry (1968).

Because of these numerous parallels, much effort is being undertaken to determine the kinetic and thermodynamic properties for the reaction of various amino acids with a number of different substrates. Some of the amino acids most frequently used are: glycine, serine, cysteine, histidine, and lysine. The substrates are generally such compounds as paranitrophenylacetate, 1-fluoro-2,4-dinitrobenzene, and iodoacetamide. Studies have been conducted on the reactivity of the nucleophilic center of both amino acids and polypeptides.[3,4,5]

In most of these studies the reactions were carried at an approximate blood pH, and about 37°C, body temperature. This is done to better correlate the data taken, and the conclusions drawn, with reactions of protein chains in the body.

It is hoped that a study of these reactions will eventually yield enough kinetic and thermodynamic data that selective and specfic modification of protein chains will become possible.

The problem that was undertaken in this study was to determine the role of the amine groups, on the amino acid lysine, in their reaction with the commonly used substrate iodoacetamide (IAA).

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[3] ibid.

[4] C. C. Price, H. Akimoto, R. Ho, "Relative Reactivity of Nucleophilic Centers in Some Monopeptides," Journal of Organic Chemistry, (1973) Vol. 38, no. 8.

[5] C.C. Price, P. Gaucher, P. Koneru, Biochemistry, Vol. 166 (1968).

The reaction of amino acids with IAA is an example of bimolecular nucleophilic substitution.[6] This is know as an SN2 type reaction. Very briefly, an SN2 reaction proceeds as in figure one; with the nucleophile reacting with carbon and replacing the attached halogen. In this study the nucleophile is the amine group on the particular lysine species, and the halogen is the iodine atom on the IAA. Figure two illustrates the reaction of lysine with IAA. As can be seen from the figure, one of the products of the reaction is iodide ion. As will be discussed in the experimental section of the work this provides a most convenient method to follow the progress of the reaction.

Four species of lysine were chosen for use in this study. These are lysine, polylysine, N-α-Acetyl lysine, and N-ε-Acetyl lysine. The structures of these four are shown in figures three thru six. It should, at this time, be noted that lysine has two amine groups, both of which can act as reactive sites. One of these is located on the alpha carbon, while the other is located on the epsilon carbon. As indicated each has its characteristic pKa value; that is the pH at which 50% of the hydrogen ion dissociates from the amine groups and goes into the solution. This point is of importance because only after an amine dissociates is it free to react. It may be noted from figure three that the pKa for the alpha amine is 8.95, and for the epsilon amine it is 10.53.[7]

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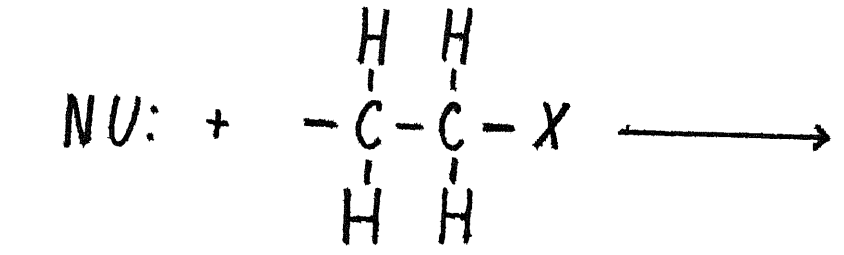
[6] C. K. Ingold, "Structures and Mechanisms in Organic Chemistry" (Cornell University Press, Ithaca, New York, 1953).

[7] The Chemical Rubber Company, Handbook of Biochemistry and Molecular Biology, (Cleveland Ohio, 1970).



where X is the Halogen and NU is the attacking nucleophile

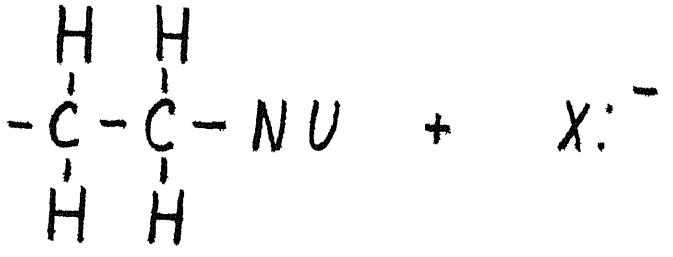
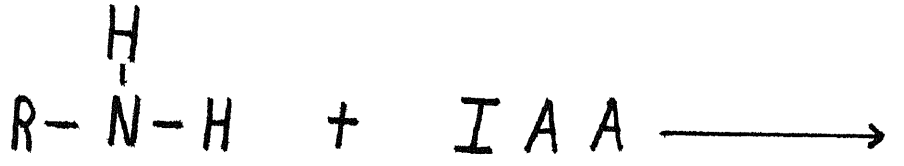


Figure I: One step SN2 Reaction



where R is the functional group of lysine

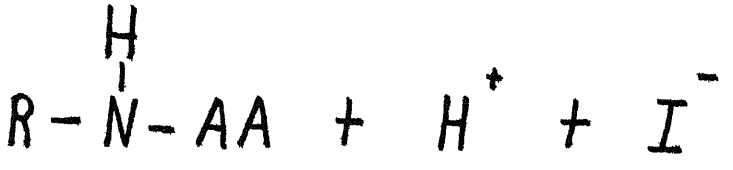


Figure 2: Reaction of Lysine Species with IAA

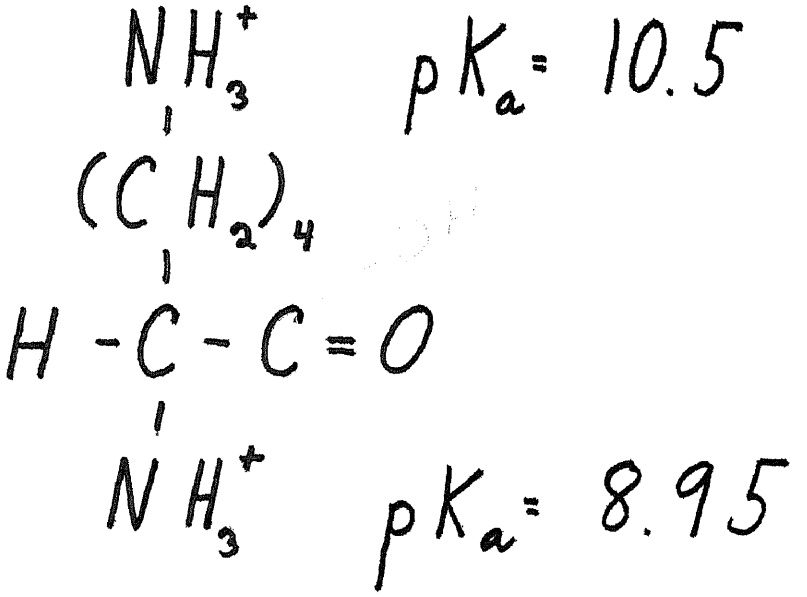


Figure 3: Structure of Lysine

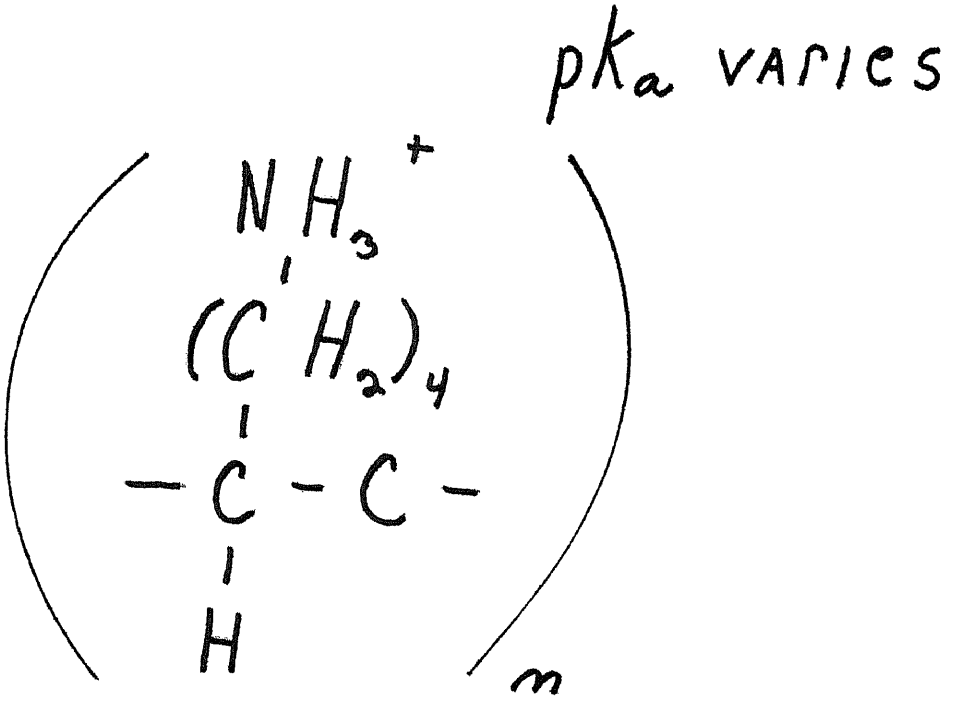


Figure 4: Structue of Polylysine

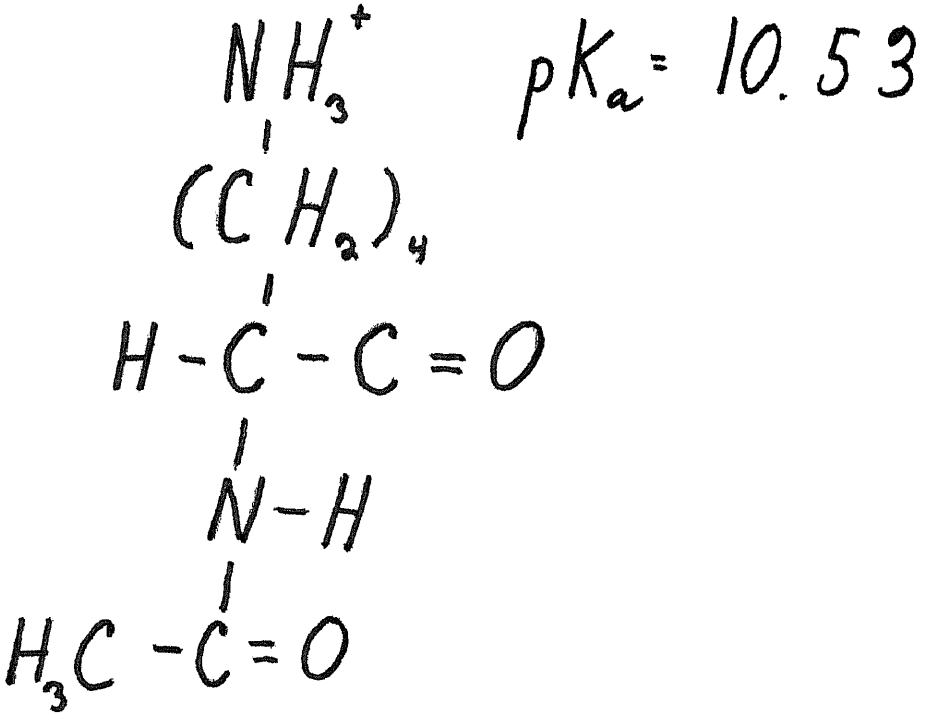


Figure 6: Structure of N-α-Acetyl lysine

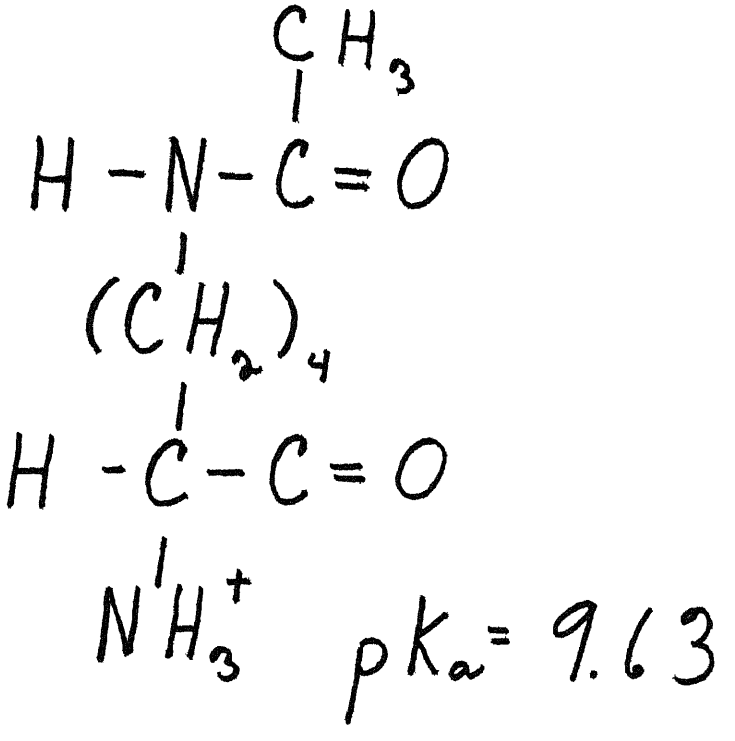


Figure 7: Structure of N-ε-Acetyl lysine

Polylysine, in figure four has only one free amine per monomer unit. Its pKa value varies as a function of its helical conformation.[8] This will be discussed in greater detail at a later time.

It was desirable in this study to selectively block either the alpha amine group or the epsilon amine group of lysine. As can be seen from figure five and six, this is accomplished by attaching an acetyl group to the alpha or epsilon amines. By doing this the pKa values are changed from 10.5 in the alpha position, and to 9.63 in the epsilon position.[9]

It will prove useful to discuss the considerations examined to determine what lysine species to use in the experiments.

At low pH the polymer of lysine is in a random chain configuration. The amines are scattered randomly in space, and there may or may not be some steric hindrance to the reaction taking place. As the pH increases the chain becomes less random until at about pH 12 it is completely helical.[10] It was desirable to measure the rate of reaction as a function of pH, and determine how, if at all, the orientation of the amine groups in space influence the reaction. It has also been shown by Parker, Kristol, Stanley, and Krautheim[11] that the rate of

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[8] J. Hermans, "Experimental Free Energy and Enthalpy fo Formation of the Alpha Helix" Journal of Physical Chemistry, Vol. 70, no. 510 (1966) pp. 510-514.

[9] R.B. Freedman, G.K. Roda, Journal of Biochemistry, Vol. 108 (1968).

[10] R.C. Parker, L.T. Slutsky, K.R. Applegate "Ultrasonic Absorption and the Kinetics of Conformation Change in Polysine" Journal of Physical Chemistry, Vol. 72 3177 (1968)

reaction is a function of the pKa value. Generally as the pKa increases the rate also increases. In the case of polylysine their work indicated that the rate might also be a function of the local environment occupied by the free amine. Obviously, as the polymer changes from the random chain to the helical configuration this environment must also change. However that work could not clearly determine what this effect is. It is therefore the intention of this work to determine this effect. Furthermore we will show what the effect of the pKa is on the rate. For these reasons polylysine was chosen for study.

By blocking off the amine groups on both the alpha and epsilon positions with the acetyl groups several characteristics could be measured.

First the effect of the pKa on the rate of reaction could be further looked into.

Second the effect of environment on the rate could also be further examined. This is possible because different atoms and functional groups surround the amines in each case.

Finally, a direct comparison between lysine with its pKa value, and the substituted groups could be made to gain still more information on the effect of pKa on the rate of reaction.

It was therefore felt that a reasonable study could be generated by studying the reactions of IAA with, lysine, polylysine, N-α-Acetyl lysine, and N-ϵ-Acetyl lysine. Because of the areas of desired

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[11]Paper accepted for publication.

investigation it was necessary to investigate these reactions as a function of pH and concentration, at a constant temperature. In summary this work was undertaken to investigate:

1. The effect of the pKa value associated with an amine on the rate of reaction,
2. The effect of the environment of the amine group on the rate,
3. And to determine the rate expression, with a rate constant, for all the compounds studied in their reaction with IAA.

Experimental Section

As stated previously the reactions were to be run at constant temperature, with varying pH, and varying concentration. In many studies the temperature of the reaction vessel is maintained at about 37°C which is body temperature. In this study a temperature as high as 37°C was found to be unfeasible, because at this temperature the reaction went to completion almost immediately above a pH of 9.5. This made it impossible to obtain kinetic measurements. Fortunately, for the objectives of the work it mattered not what temperature was chosen, but only that this temperature was kept constant, for the measurement period. For convenience sake 23°C was chosen since it is room temperature, and no preheating was necessary of the reacting fluids.

The reactions were run in a pH range from 7.4 to 11.2. It was desirable to choose an appropriately wide range so as to encompass all the pH values where any percentage of free amine would be available for reaction. It was found that at a pH below 7.4 no measurement of reaction progress could be observed within experimental error. Furthermore it was found that above a pH of 11.2 the reaction proceeded too quickly toward completion to be properly measured. Measurements were taken at pH values ($\pm$ 0.03) of; 7.4, 8.0, 8.5, 9.0, 9.5, 10.0, 10.5, 11.0 and 11.2.

The pH measurements were made using a Beckman pH meter with a glass electrode, and calomel reference electrode. The meter was calibrated using a standard buffer solution pH 9.18 obtained from the Aldrich Chemical Company.

In figure two the formulation of the lysine's species reaction with IAA is given. As can be seen from this formulation not only is the lysine-carbon bond formed, but also hydrogen and iodide ions are liberated. Because of this liberation there are two convenient methods available to monitor the progress of the reaction. It was decided to try both methods and determine the best of the two. A description of each follows.

Using the first method the formation of hydrogen ion could be followed. This could be accomplished through the use of an automatic titrimeter. This is a device that automatically adds enough base to maintain a present pH value. The amount of base added could be measured as a function of time and from this a rate could be determined.

This method proved undesirable for several reasons. First the reaction was so rapid at higher pH value that the lag time of the instrument proved too great to obtain a reaction rate with confidence. Second, due to the physical set up of the electrodes at least 150 ml. of reacting solution would have been necessary. This would have proved to be economically unfeasiable because of the cost of the electrodes. Third, since the solutions used in this method could not be buffered it was felt that they were subject to random pH changes due to adsorption of CO_2 from the air. This problem could have been solved by running the reaction in a nitrogen enviornment but the physical problems involved would have been complex, and costly, to solve. Finally pH and concentration corrections would have to be made because of the addition of the base to the solution.

The second available method proved to be more practical. This was to moniter the formation of the iodide ion. This was accomplished through the use of an Orion Specific Ion Meter. This device works under a principle similar to that of a pH meter, except that it can measure other then hydrogen ions in solution. A recommended double junction electrode was used along with an Orion iodide electrode. It was possible to measure iodide concentrations in the range from 2×10^{-7} to 1×10^{0} molar. Care had to be taken to use high quality distilled water since concentration of sulfur ion could not exceed 10^{-7} molar, without interfering with the iodide measurements.

As opposed to the first method it was necessary to use only 50 ml. of solution in the reaction vessel, and more importantly there was no lag time in the instrument enabling measurements to be taken at higher pH values. However since hydrogen is one of the products of the reaction being investigated, it was necessary to buffer the reaction solution. Since the pH range was too wide for one buffer, two were chosen. THAM, a commerical buffer, obtained from Aldrich Chemical Company, was used in the pH range from 7.4 to 10.0. Triethylamine-acetate was the buffer used in the range from 10.0 to 11.2. This buffer was prepared in a manner suggested by Pocker and Beug.[12] Equal molar amounts of triethylamine and glacial acetic acid, both obtained from Aldrich Chemical Company, were mixed with high quality distilled water. By an appropriate calculation it was determined that at completion the total hydrogen ion yielded by reaction would be about 10^{-3} molar. The buffer was therefore prepared at 10^{-2} molar.

It was recommended by the Orion Company that a constant ionic strength be maintained in the reaction vessel. This was accomplished by the addition of a salt solution, sodium nitrite, to the buffer solution. The sodium nitrite, obtained from Aldrich Chemical Company was added in 0.01 molar strength.

Six liter batches of the THAM, sodium nitrite, distilled water solutions were made up to insure uniformity throughout all the experiments. The lysine, N-α-Acetyl lysine, N-ϵ-Acetyl lysine, and polylysine solutions were prepared from these batches.

In order to determine the proper concentrations of the lysine, and lysine derivatives, that would give a measureable rate, 50 ml. batches were prepared over a range of molarities. At a test pH of 8.5 it was determined that a solution of 0.01 molar would proceed at a reasonable rate. Since it was necessary to do a concentration study it was decided to run lysine at 0.01 molar, and 0.05 molar, concentrations. The same concentrations were chosen for the N-ϵ-Acetyl lysine because both were readily available and inexpensive. This, however was not the case for the polylysine, and the N-α-Acetyl lysine. Therefore these were run at concentrations of 0.005 M and 0.01 M for the polylysine, and 0.01 M and 0.02 M for the N-α-Acetyl lysine.

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[12]Pocker Y., M. W. Beug,"Kinetic Study of Bovine Carbonic Anhydrase Catalyzed Hydrolyses of Para Phenly Esters"; Biochemistry Vol. 11, nO. 5 (1972)

The lysine and polylysine were obtained from Pilot Chemicals, and the N-α-Acetyl lysine and N-ε-Acetyl lysine were obtained from the Aldrich Chemical Company.

It was necessary to add a small volume of IAA to initate the reaction to avoid any concentration correction. However to avoid pseudo order kinetics it was necessary to add approximately equal molar amounts. Since the lysine IAA ratio was two to fifty, calculations showed it necessary to prepare a 0.513 molar solution of IAA. The IAA was obtained from the Aldrich Chemical Company. The IAA solution was prepared in small amounts with high quality distilled water. It had to be stored, frozen, between measurements to prevent decomposition.

Approximately two hundred runs were carried out each taking about two hours each to perform. A typical run followed this sequence.

The particular lysine derivative was prepared at the desired molarity in the buffer-sodium nitrite solution. This was mixed by the magnetic stirrer anywhere from eight hours for the lysine, up to two weeks for the polylysine. Once fully dissolved the solution was ready to run. The IAA that was to be used that day was prepared and stirred for ten minutes. A 50 ml. sample was withdrawn from the lysine solution and its pH was adjusted to its desired value by the addition of small amounts of acid or base.

The Specfic Ion Meter was standardized in the range of 10^{-4} to 10^{-3} molar with sodium iodide solution of known strength. The electrodes of the

meter were brought into contact with the solution. After the IAA was added to the reaction vessel the timer was started, and readings were taken at appropriate intervals. The mixture was stirred to prevent instrument lag time. The raw data is found in the appendix section.

Discussion and Analysis of Results

A Complete tabulation of all the data for all the runs may be found in the appendix. An example of the typical type of kinetic data generated may be found in table two, for a 0.01 M solution of lysine, in 0.1 M THAM, at a pH of 10.0, and a temperature of 23°C. A plot of this data is shown in figure seven. A least squares analysis was performed on this type of data using a program prepared for use on a Wang calculator. The slope of the best fit line is the rate of formation of iodide ion for a given pH, and reactant concentration conditions. The rate has units of moles per liter-second.

Two reactions took place upon the addition of IAA to the reaction vessel. The one of interest is the reaction of IAA with lysine. The second reaction was that of IAA with the buffer component. It therefore became necessary to separate these two rates from the total rate obtained when the least squares was performed. This was accomplished by measuring the rate of reaction by adding the IAA to a solution containing only the buffer component. The reaction was run at a constant temperature of 23°C and, a buffer concentration of 0.1 M, over the pH range of each of the two buffers. The reactions generated a set of curves similar to figure seven. From the least squares analysis of this set of curves, the rate due only to the reaction of IAA and the buffer was obtained. The results of this procedure are in table three and figure eight. It may be pointed out that at pH 10.0 the curve in figure eight is discontinous. This is the result of switching from THAM to triethylamineacetate at pH 10.0.

Table 3: Summary of Blank Runs

pH	Rate of Reaction Mole/Liter-sec Set 1	Set 2	Average $\times 10^{-9}$
7.4	1.58	1.58	1.58
8.0	3.85	3.76	3.80
8.5	8.69	7.56	8.13
9.0	12.6	13.1	12.9
9.5	13.2	15.8	14.5
10.0*	15.9	15.5	15.7
10.5	88	82	84
11.0	115	123	119
11.2	359	351	355

*This rate is for THAM

Table 2: Raw Data

Time in Seconds	I^- Conc. in Molarity $\times 10^{-4}$
100	.135
200	.210
300	.285
400	.360
500	.435
600	.510
800	.660
900	.740
1000	.820
1100	.900
1200	.980

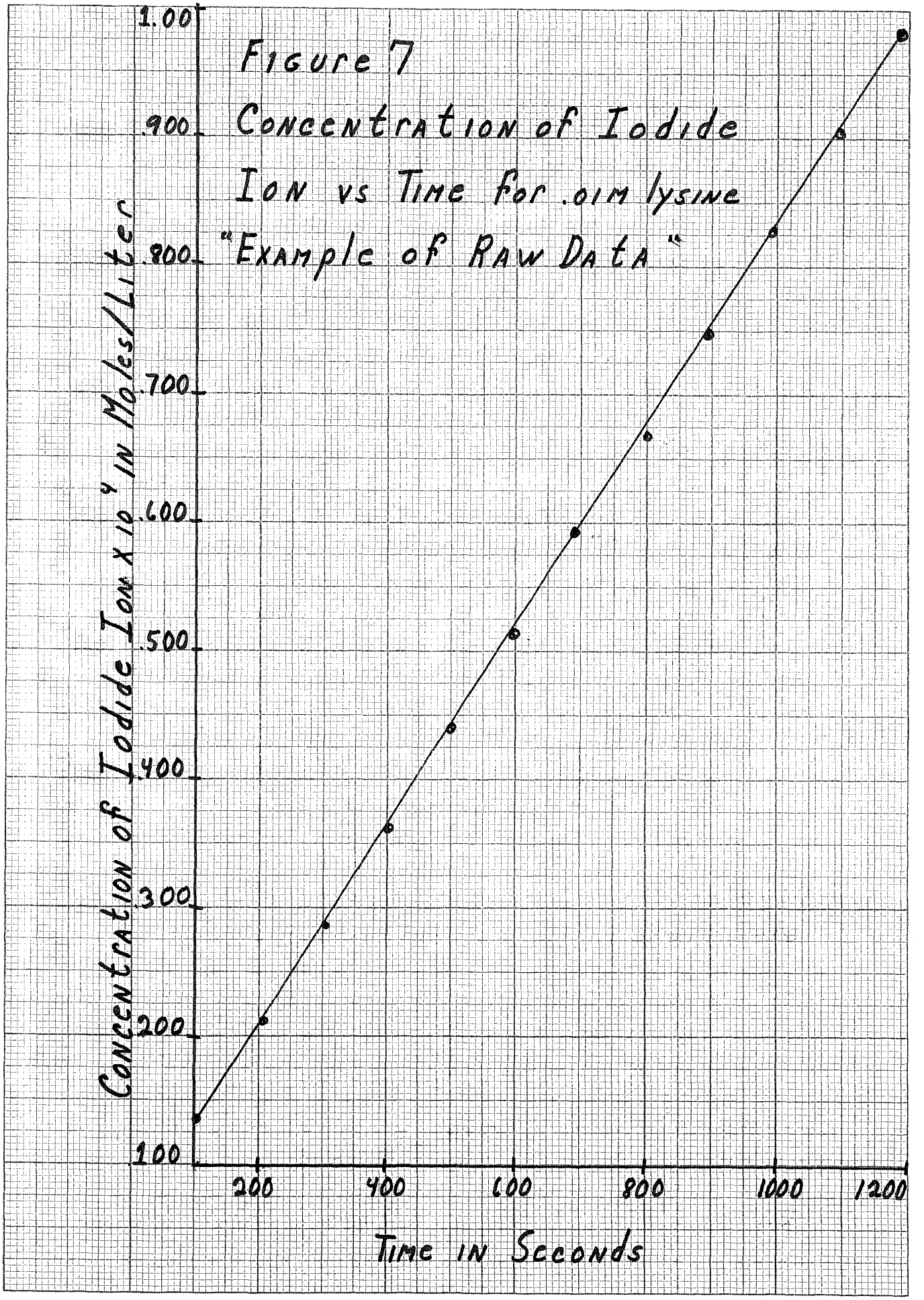
Figure 7
Concentration of Iodide Ion vs Time for .01M lysine
"Example of Raw Data"
Concentration of Iodide Ion x 10⁴ in Moles/Liter
1.00
.900
.800
.700
.600
.500
.400
.300
.200
.100
200
400
600
800
1000
1200
Time in Seconds

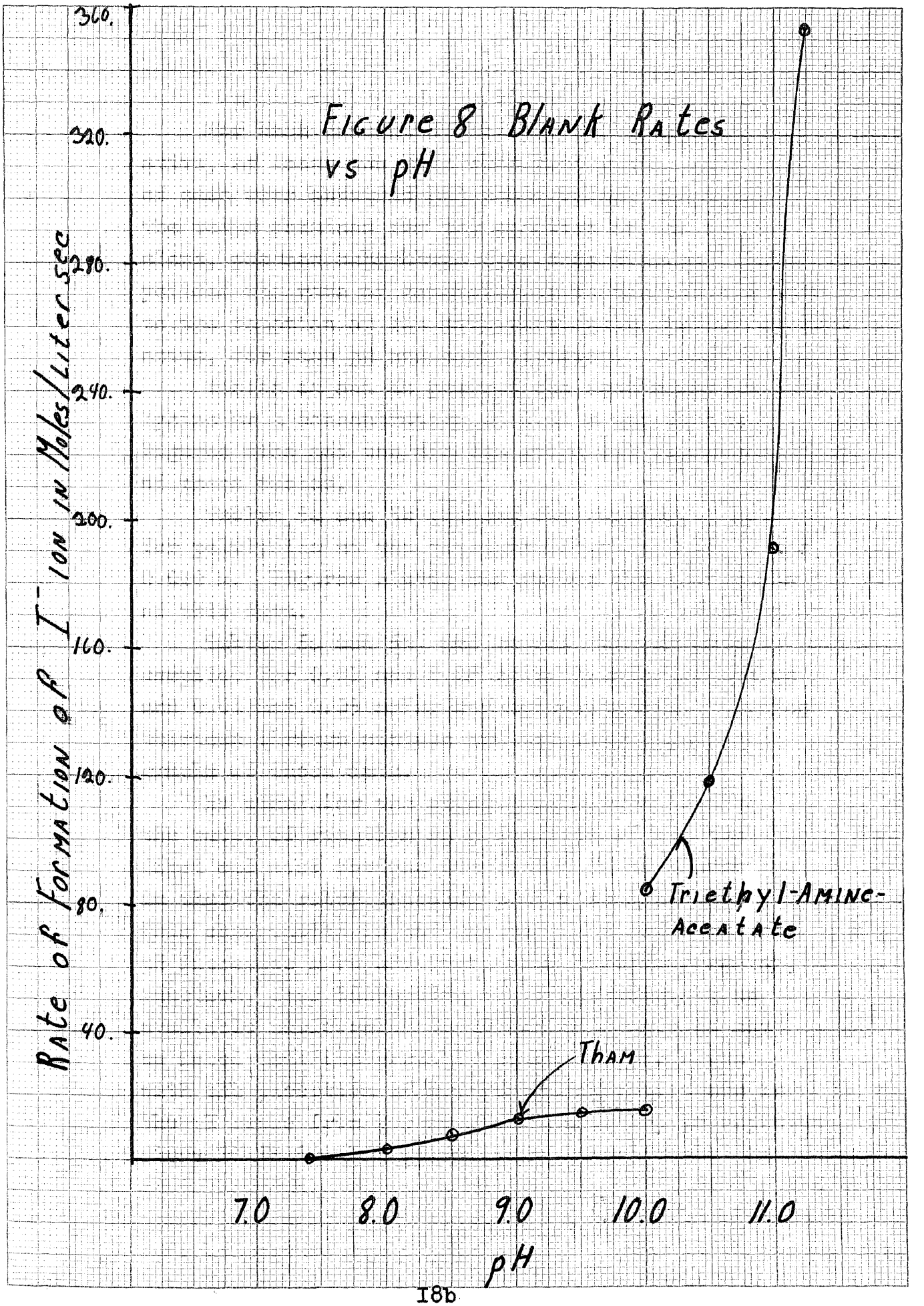
Figure 8 Blank Rates vs pH
Rate of Formation of I⁻ ion in Moles/Liter sec
360.
320.
280.
240.
200.
160.
120.
80.
40.
Triethyl-Amine-Acetate
Tham
7.0
8.0
9.0
10.0
11.0
pH

For each concentration, pH, and lysine deriverative, at least two runs were performed. The rates of each were obtained and these values averaged. The value obtained for the reaction of the buffer alone, was then subtracted from this average. This yeilded the rate of formation of iodide ion due only to the reaction of IAA with particular lysine deriverative, for each pH and concentration.

Tables 4a and 4b present the data compiled for lysine at a concentration of 0.01 M and 0.05 M. Figures nine and ten are the graphical representations of these tables.

Tables 5a and 5b present the summary of data for polylysine at the concentrations of 0.005 M and 0.010 M. Figures eleven and twelve are the graphs of this data.

Tables 6a and 6b, and 7a and 7b, the data compiled for 0.01 and 0.02 molar N-α-Acetyl lysine and 0.01 and 0.05 molar N-ϵ-Acetyl lysine. Figures thirteen through sixteen are the corresponding graphs of these data.

By examining the eight graphs representing the rates of reaction as a function of pH, the first and most obvious conclusion to be reached is that the rates increase as the pH increases. This observation is not unreasonable since as the pH increases more and more amine becomes free for reaction, thus increasing the probability of an available nucleophile to attack the IAA. It may be noted that the greatest change in the slope, with the exception of N-ϵ-Acetyl lysine, comes in the pH range from 9.5 to 10.5. This too is expected since it is in this range where there is the

Table 4a: Results for .01M lysine

Rate of Reaction x 10^{+9}

	Mole/Liter-sec		Average
pH	Set 1	Set 2	- Blank
7.4	6.28	6.28	4.70
8.0	13.3	13.8	9.80
8.5	35.1	35.9	27.3
9.0	58.3	66.7	49.7
9.5	121	122	107
10.0	153	153	137
10.5	359	-	240
11.0	586	613	406
11.2	769	-	408

Table 4b: Results for .05M lysine

Rate of Reaction x 10^{+9}

	Mole/Liter-sec		Average
pH	Set 1	Set 2	- Blank
7.4	9.00	10.8	7.84
8.0	22.0	23.2	18.87
8.5	84.5	60.0	64.1
9.0	146	150	135
9.5	375	342	344
10.0	625	630	611
10.5	1922	-	1803
11.0	2647	-	2454
11.2	2810	2810	2455

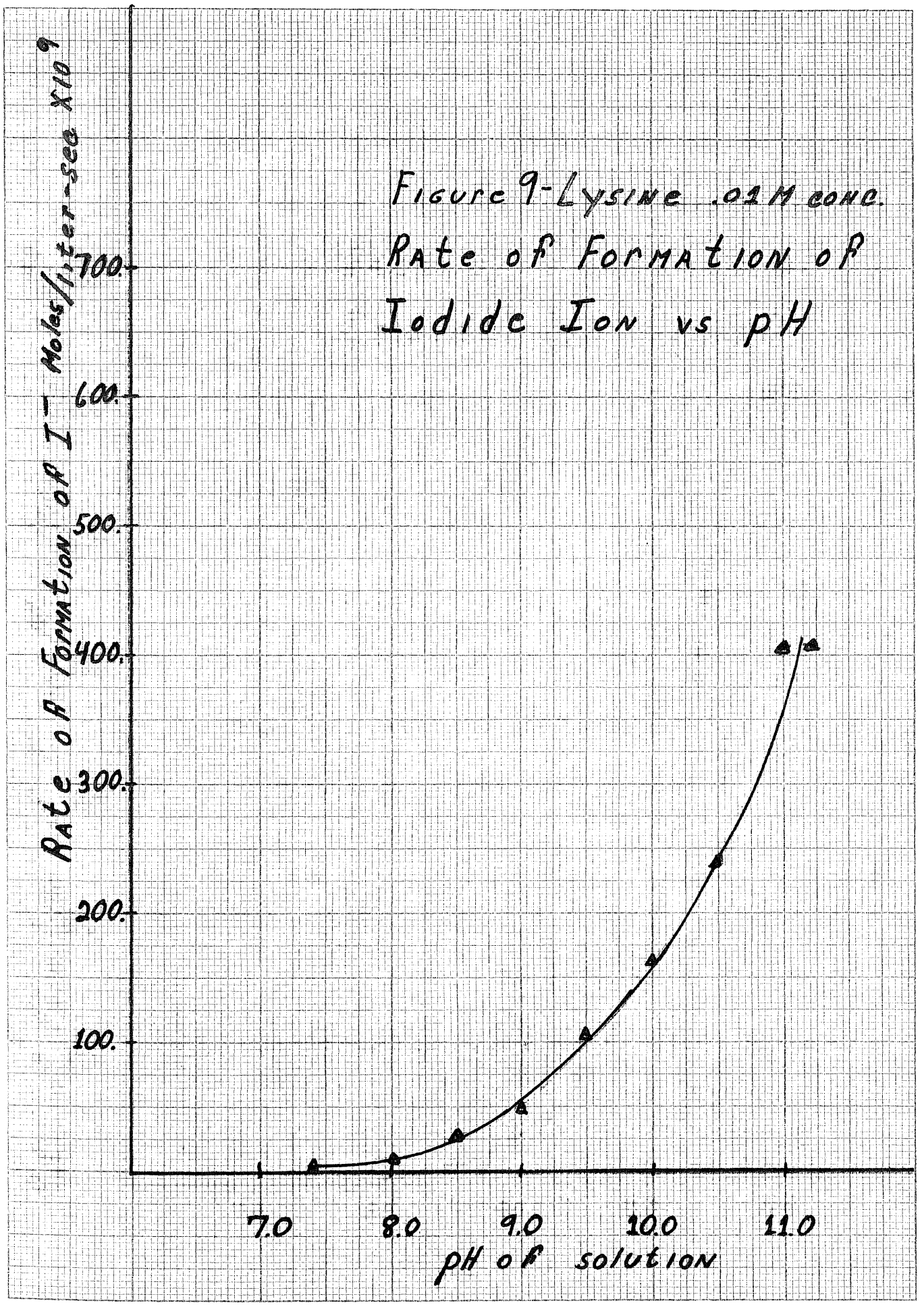

Figure 9-Lysine .01 M conc.
Rate of Formation of
Iodide Ion vs pH

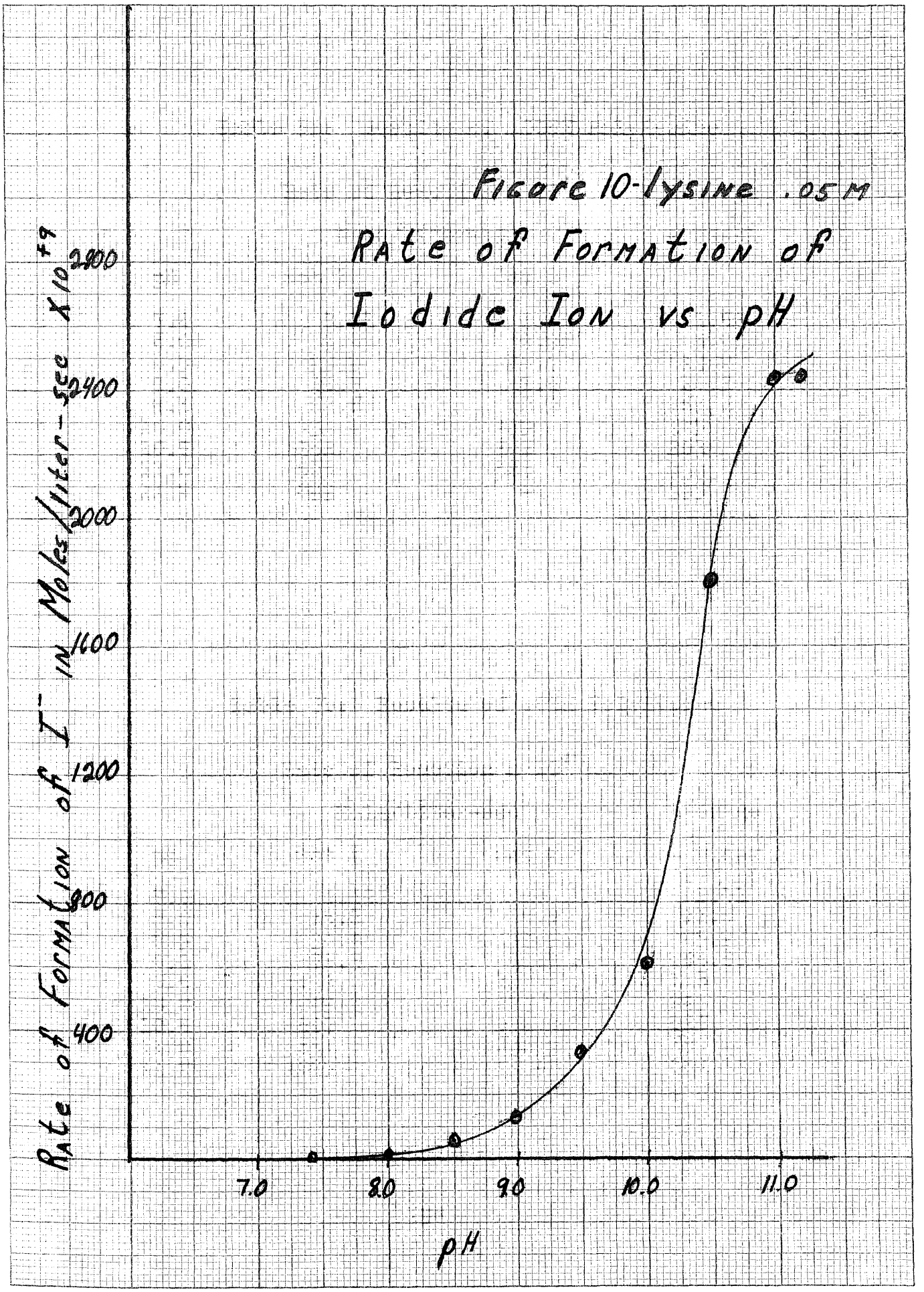
Figure 10-lysine .05 M
Rate of Formation of
Iodide Ion vs pH
Rate of Formation of I⁻ in Moles/liter-sec x 10⁻⁹
2800
2400
2000
1600
1200
800
400
7.0
8.0
9.0
10.0
11.0
pH

Table 5a: Results of .005M Polylysine

pH	Rate of Reaction x 10^{+9} Mole/Liter-sec. Set 1	Set 2	Average - Blank
7.4	3.11	3.11	1.53
8.0	9.44	9.96	5.90
8.5	23.9	18.7	13.2
9.0	32.5	41.6	24.2
9.5	69.6	82.9	61.6
10.0	134	149	125
10.5	382	382	263
11.0	525	525	333
11.2	699	-	335

Table 5b: Results of .01M Polylysine

pH	Rate of Reaction x 10^{+9} Mole/Liter-sec Set 1	Set 2	Average - Blank
7.4	4.49	-	2.91
8.0	15.2	15.1	11.4
8.5	34.4	33.8	25.9
9.0	56.3	58.9	44.8
9.5	133	133	118
10.0	249	273	245
10.5	647	654	527
11.0	851	842	657
11.2	1025	-	661

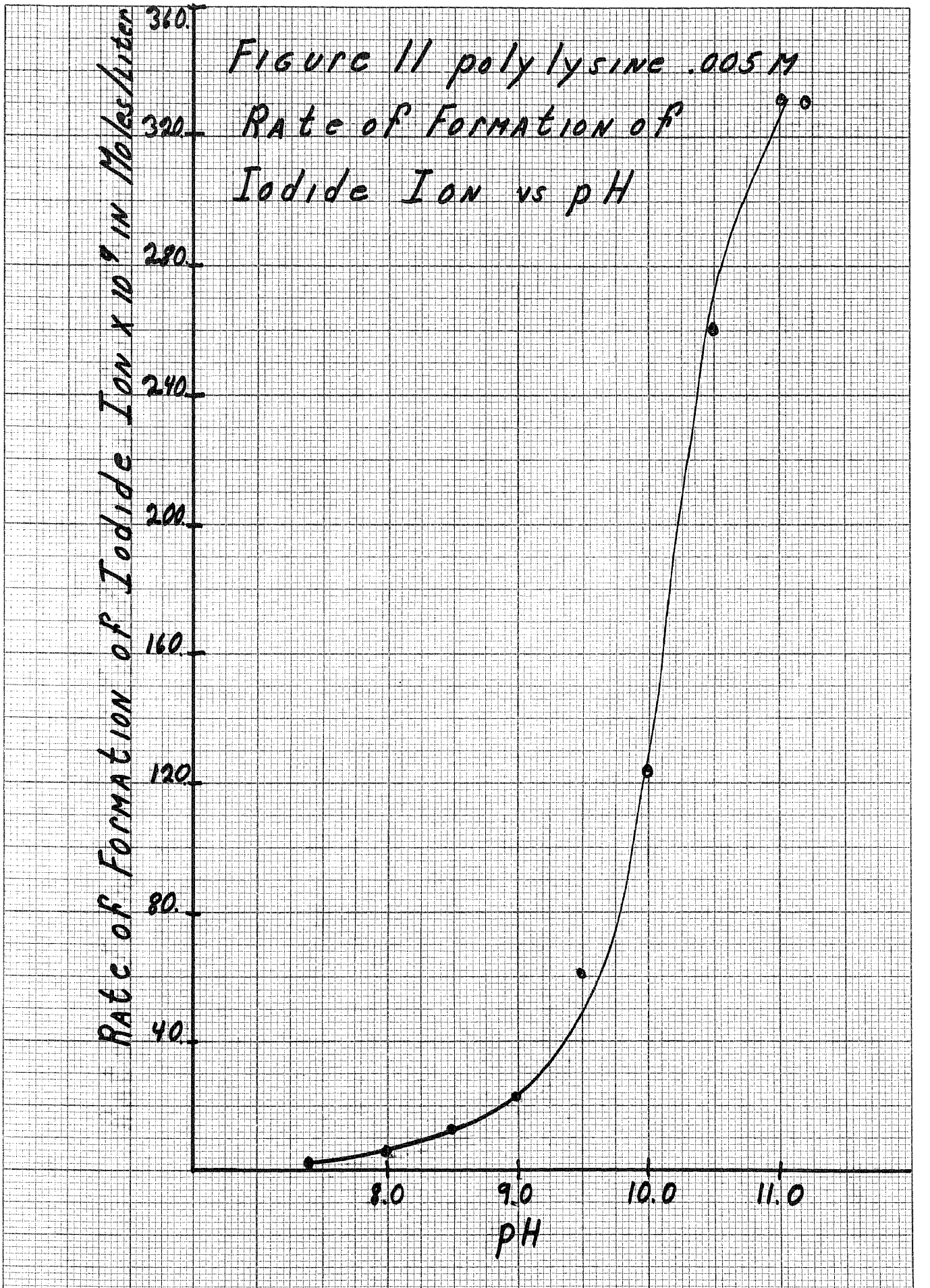
Figure 11 polylysine .005 M
Rate of Formation of
Iodide Ion vs pH
Rate of Formation of Iodide Ion x 10⁹ in Moles/Liter
360.
320.
280.
240.
200.
160.
120.
80.
40.
8.0
9.0
10.0
11.0
pH

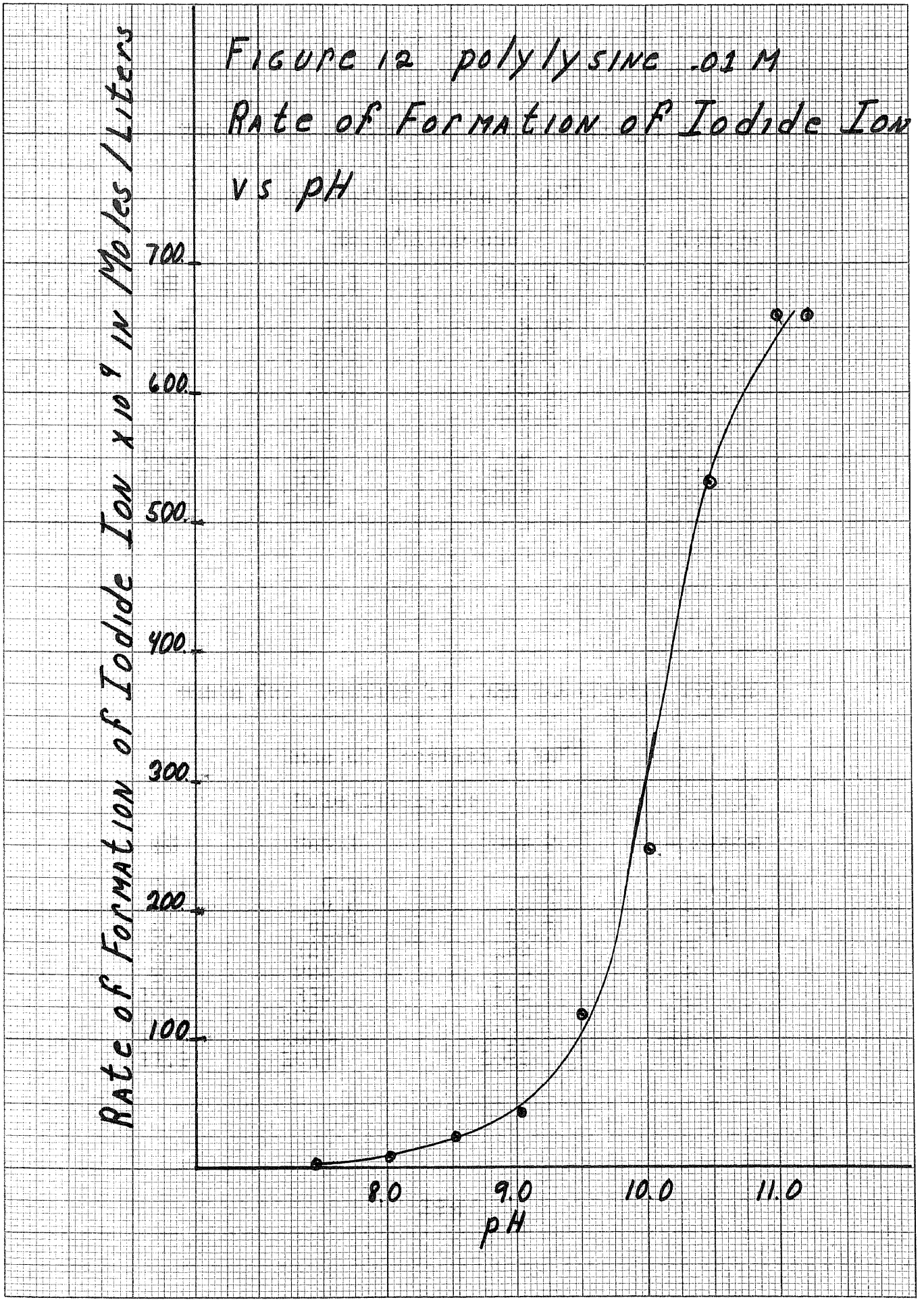
Figure 12 polylysine .01 M
Rate of Formation of Iodide Ion
vs pH
Rate of Formation of Iodide Ion x 10⁹ in Moles/Liters
700
600
500
400
300
200
100
8.0
9.0
10.0
11.0
pH

Table 6a: Results of .01M N-α-Acetyl lysine

	Rate of Reaction x 10^{+9} Mole/Liter-sec		Average
pH	Set 1	Set 2	- Blank
7.4	4.97	5.00	3.39
8.0	10.5	11.2	6.67
8.5	30.0	31.3	21.8
9.0	62.2	60.5	49.4
9.5	116	115.2	102
10.0	184	-	169
10.5	600	-	481
11.0	916	910	723
11.2	1094	1097	730

Table 6b: Results of .02M N-α-Acetyl lysine

	Rate of Reaction x 10^{+9} Mole/Liter-sec		Average
pH	Set 1	Set 2	- Blank
7.4	8.10	7.50	6.25
8.0	17.6	16.3	13.8
8.5	51.8	54.3	43.7
9.0	123	122	109
9.5	224	-	210
10.0	338	342	321
10.5	1081	-	962
11.0	1628	1635	1435
11.2	1833	1821	1468

Table 7a: Results of .01M N-ε-Acetyl lysine

	Rate of Reaction x 10^{+9} Mole /Liter-sec		Average
pH	Set 1	Set 2	- Blank
7.4	1.78	-	.200
8.0	5.32	6.13	1.58
8.5	13.2	13.0	3.94
9.0	27.0	30.9	16.1
9.5	51.7	-	37.2
10.0	67.1	72.4	54.0
10.5	174	-	56.6
11.0	264	258	69.5
11.2	434	439	70.3

Table 7b: Results of .05M N-ε-Acetyl lysine

	Rate of Reaction x 10^{+9} Mole/Liter-sec		Average
pH	Set 1	Set 2	- Blank
7.4	2.80	2.9	1.22
8.0	11.4	11.5	7.62
8.5	30.2	25.5	19.7
9.0	93.8	-	80.3
9.5	191	200	180
10.0	256	276	250
10.5	395	403	275
11.0	538	523	345
11.2	711	-	347

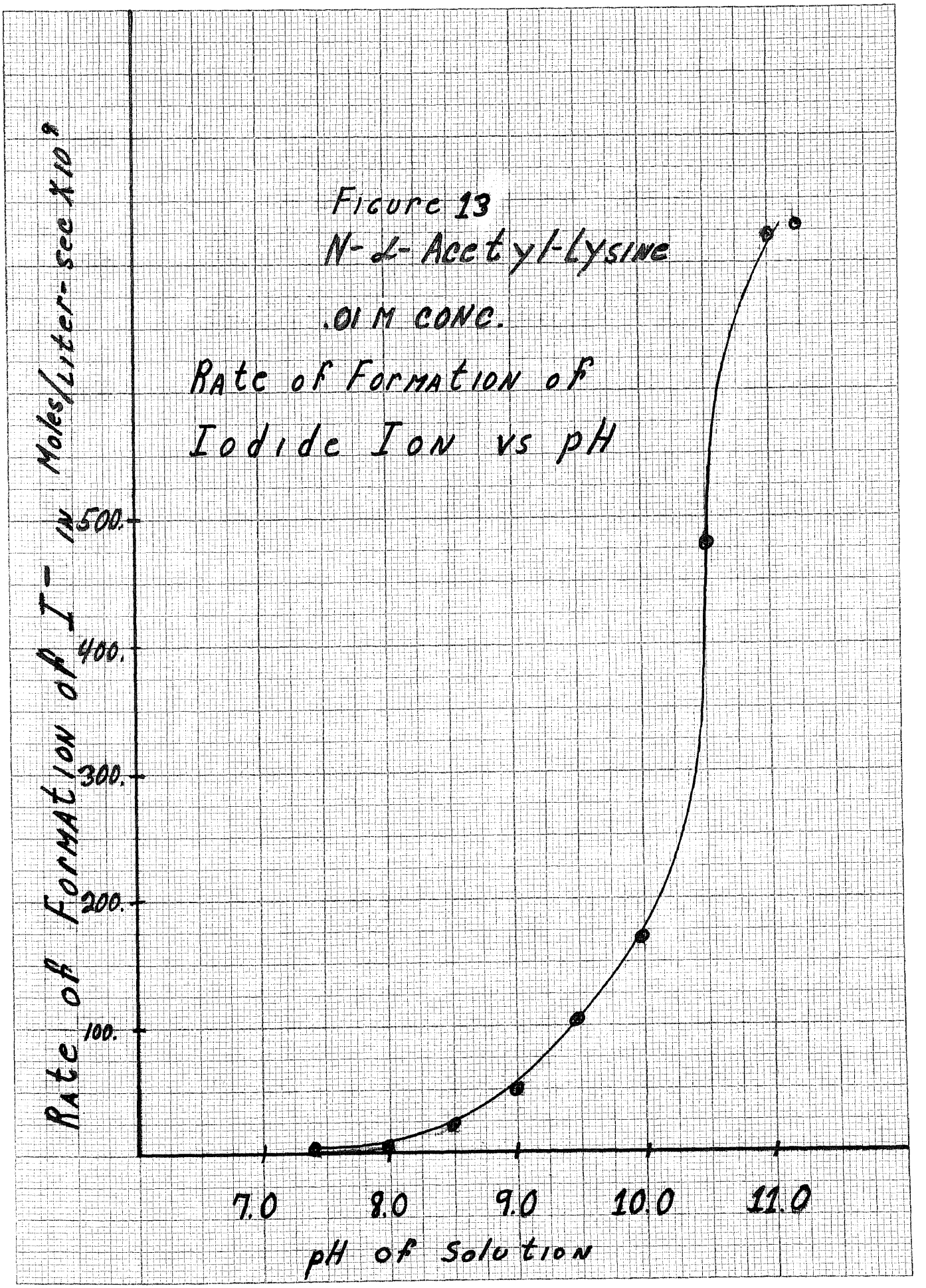
Figure 13
N-ℓ-Acetyl-Lysine
.01 M conc.
Rate of Formation of
Iodide Ion vs pH
Rate of Formation of I⁻ in Moles/Liter-sec X 10⁹
500.
400.
300.
200.
100.
7.0
8.0
9.0
10.0
11.0
pH of Solution

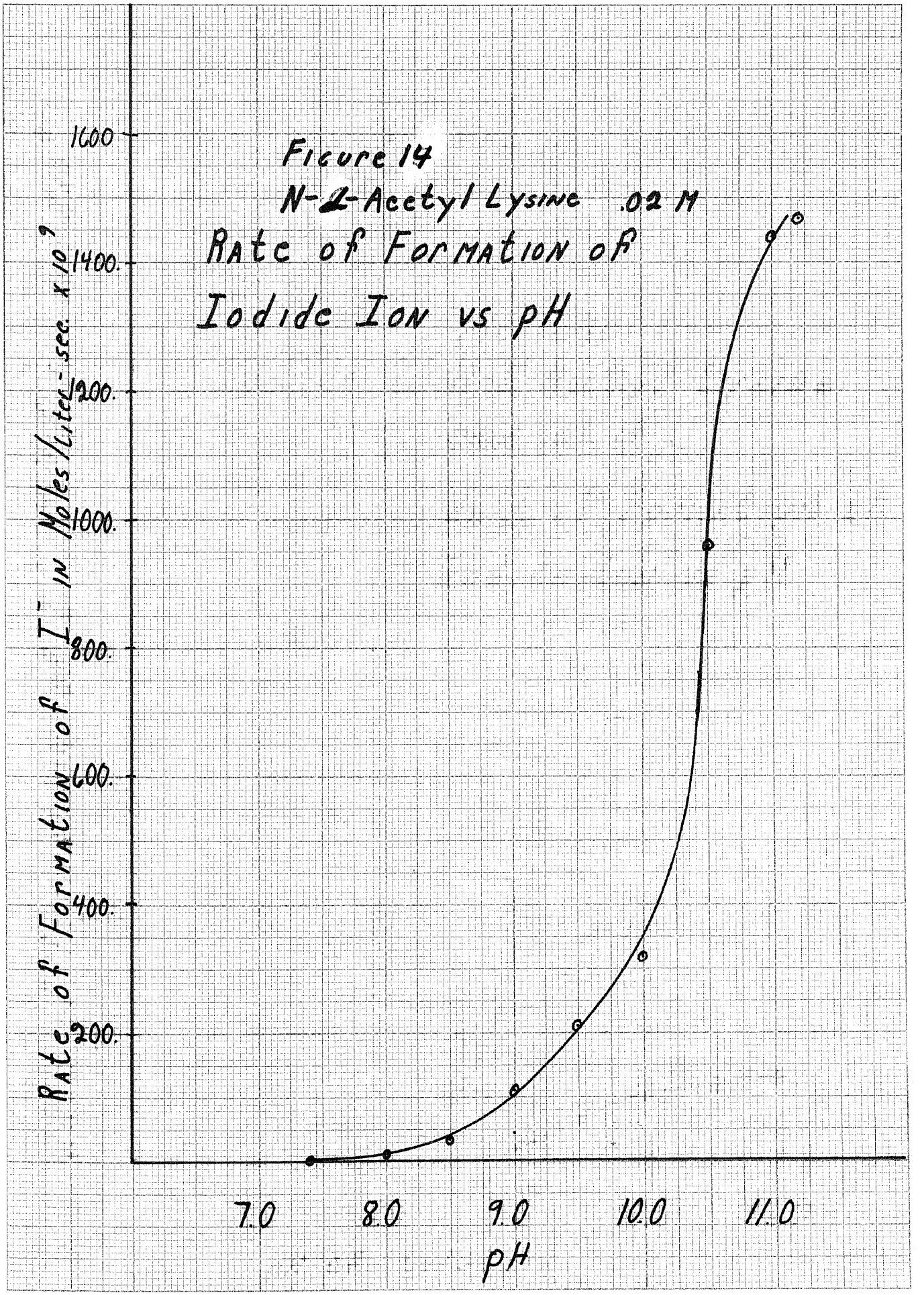
Figure 14
N-α-Acetyl Lysine .02 M
Rate of Formation of
Iodide Ion vs pH
Rate of Formation of I⁻ in Moles/Liter-sec. x 10⁹
1600
1400.
1200.
1000.
800.
600.
400.
200.
7.0
8.0
9.0
10.0
11.0
pH

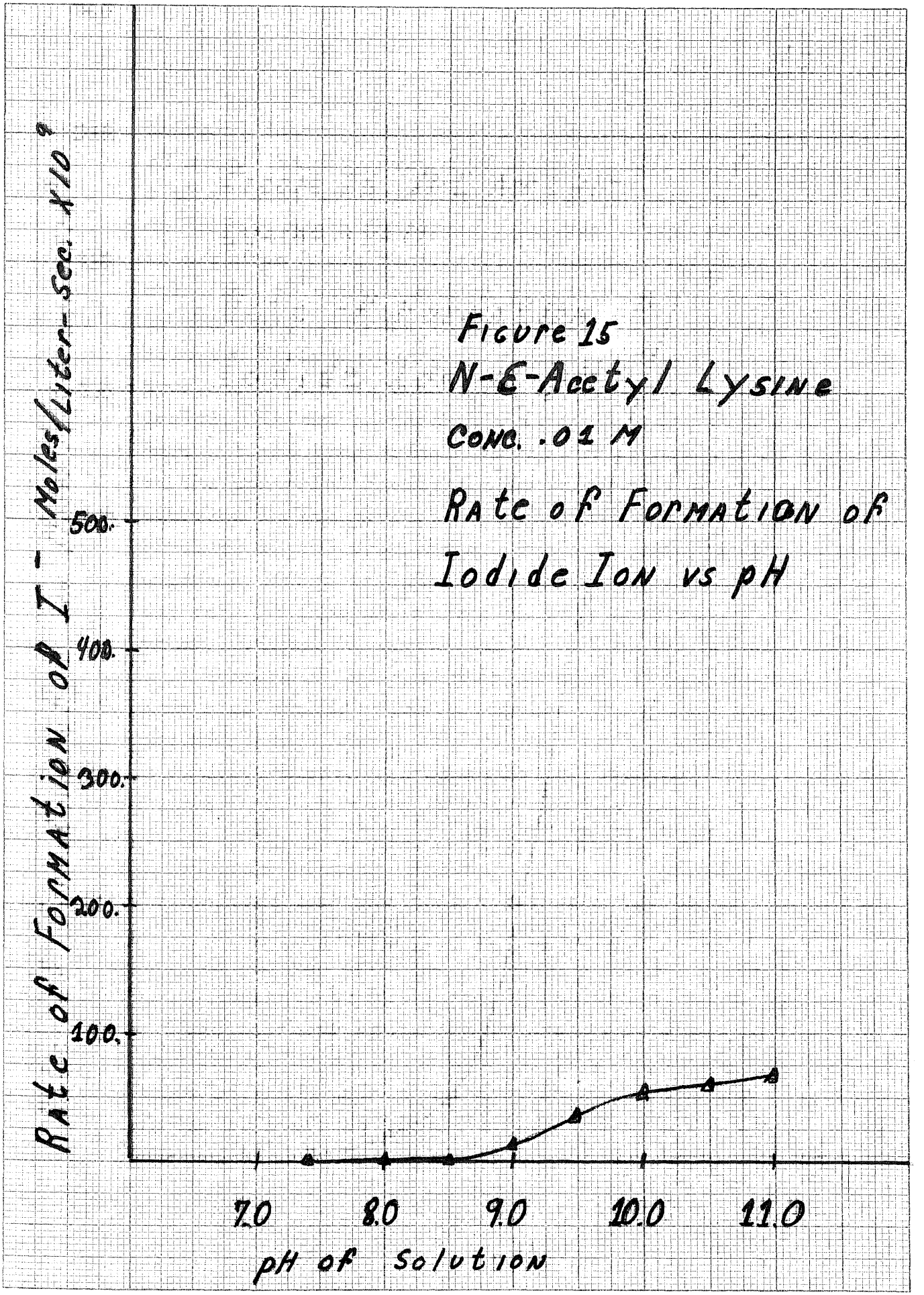
Figure 15
N-ε-Acetyl Lysine
Conc. .01 M
Rate of Formation of
Iodide Ion vs pH
Rate of Formation of I⁻ - Moles/Liter-Sec. X10⁹
500.
400.
300.
200.
100.
7.0
8.0
9.0
10.0
11.0
pH of Solution

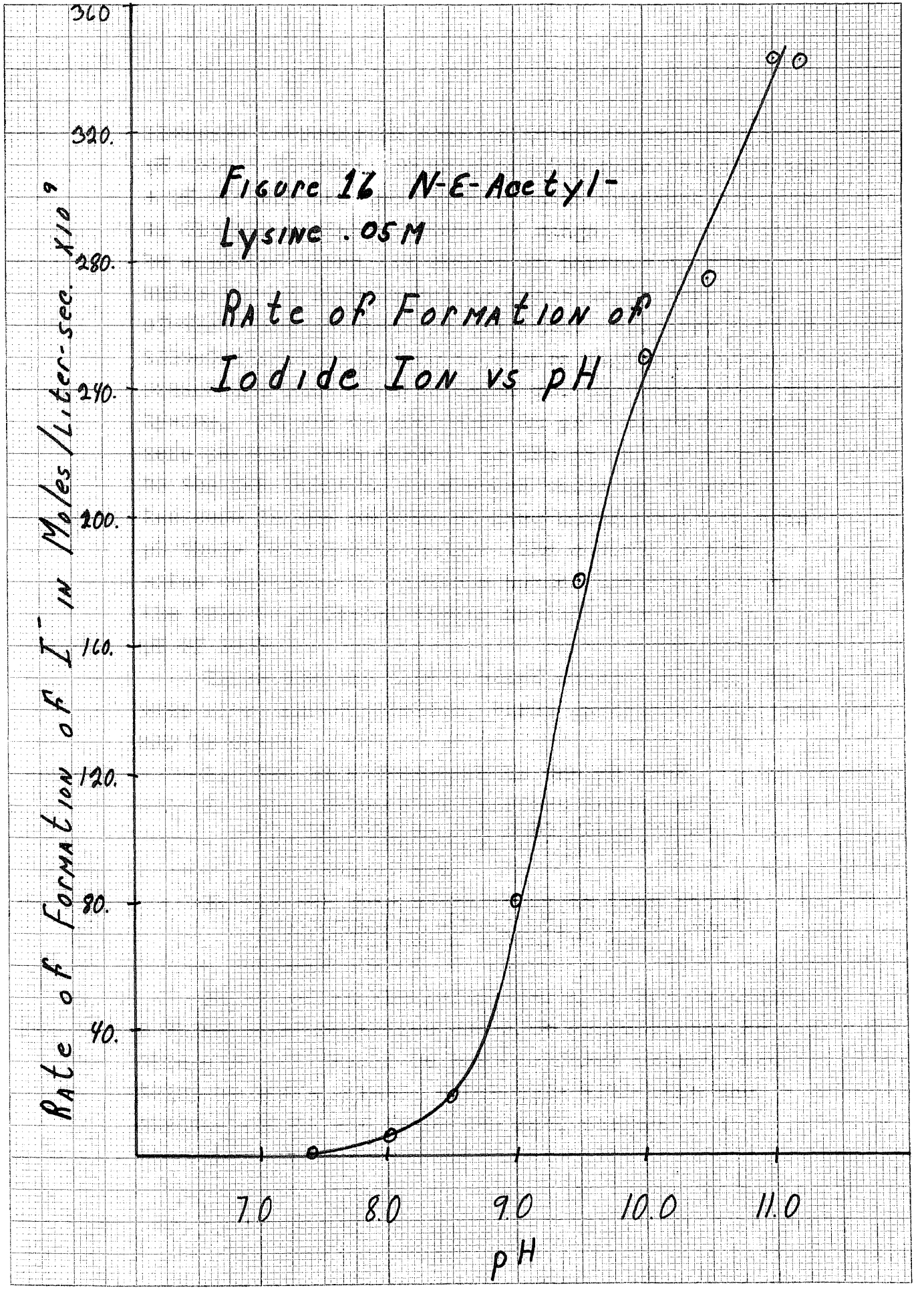
Figure 16 N-ε-Acetyl-
Lysine .05M
Rate of Formation of
Iodide Ion vs pH
Rate of Formation of I⁻ in Moles/Liter-sec. X10⁹
360
320.
280.
240.
200.
160.
120.
80.
40.
7.0
8.0
9.0
10.0
11.0
pH

sharpest increase in free amine concentration per pH unit. It may also be generally noted that above a pH of 10.5 the rate continues to increase but not as sharply as in the lower pH range. This can also be expected since the change in percent of free amine per pH unit is not as great as in the range of the sharp increase in the rate. This can best be illustrated by refering to figure eighteen, which presents a plot of free amine vs. pH, for N-α-Acetyl lysine. It can be seen that at pH 7 there is about zero percent free amine. As the pH corresponding to the pKa point is approached there is a sharp increase in the percent of free amine until at this pH there is 50% free amine. The amount of free amine continues to increase beyond this point but the rate of increase begins to fall off above a pH of 10.5.

Therefore by just a brief examination of figures nine through sixteen, three general conclusions may be drawn.

1. The rate increases as the pH does,
2. The pH range of sharpest increase in the rate is the same range where there is the sharpest increase in free amine, and
3. As the rate of increase in free amine concentration decreases, so does the rate of increase in the formation of iodide ion.

As stated in the introduction it was felt that a three point objective could be accomplished by this work. It would be best to first examine the third of these points, since this will make the explanation of the first two much easier.

The third point was the generation of the rate equation, and the determination of K, the rate constant, for each of the lysine derivatives studied.

By performing the concentration runs it has been demonstrated that for all lysine derivatives the reation is first order with respect to the lysine derivatives, and first order with respect to the IAA. For the case of lysine itself, the reaction is not observed to be first order with respect to lysine. Rather, the concentration data shows that the order varies as a function of pH. As shown in figure seventeen, the order of the reaction is about 0.32 at pH 7.4. It increases linearly as pH increases until at about pH 10.5 the reaction is first order, and at pH 11.0 and 11.2, the order is 1.11. This variation of the order will be discussed more fully at a latter point.

Shivaram, and Wallenfels proposed that the reaction follows an elementary mechanism for all amino, and polyamino-acids when reacted with IAA.[13] Furthermore it is known that the particular elementary mechanism is an SN2 type. At any given pH or concentration the rate expression proposed is:

$$\text{Rate} = K_1(\text{IAA})(NH_2)_\alpha + K_2(\text{IAA})(NH_2)_\varepsilon \quad \text{where:}$$

Rate = the rate of formation of iodide ion.

IAA = Concentration of IAA = 0.02 M

NH_2 = Free alpha or free epsilon amine concentration in moles per liter.

K_1 = The rate constant associate with the alpha amine.

K_2 = The rate constant associate with the epsilon amine.

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[13] op. cit 2

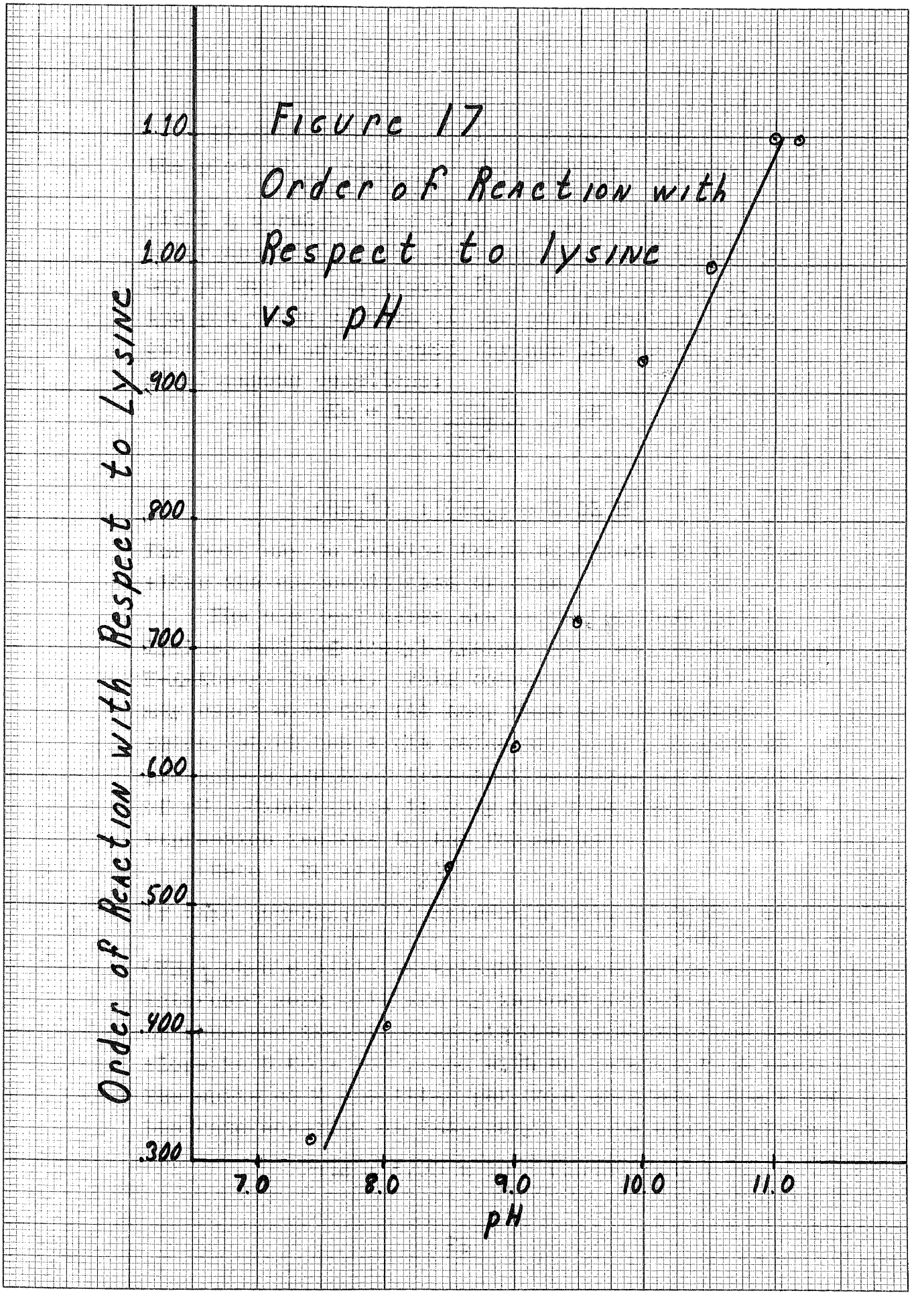


Figure 17
Order of Reaction with Respect to lysine vs pH
Order of Reaction with Respect to Lysine
1.10
1.00
.900
.800
.700
.600
.500
.400
.300
7.0
8.0
9.0
10.0
11.0
pH

If this rate expression is the correct one the plot of $\frac{\text{Rate}}{(IAA)(NH_2)}$ vs. $\frac{(NH_2\varepsilon)}{(NH_2\alpha)}$ should yield a straight line with a slope of K_2 and a intercept of K_1. The free amine concentration at any pH may be calculated from the knowledge of the pKa value of each of the amine groups. This calculation is performed through the use of the simultaneous equations:

$$pKa = \log \frac{(NH_3^+)}{(NH_2)} + pH$$

$$(NH_3^+) + (NH_2) = 0.01 \text{ M}$$

The best way to examine these equations is to consider the compounds case by case.

The simplest cases are those for N-α-Acetyl lysine, and N-ε-Acetyl lysine. Let us first examine N-α-Acetyl lysine. This compound has one amine that may react with the IAA and, this is the epislon amine, which has a pKa value of 10.53. Figure 18 presents a graph of free epsilon amine as a function of pH. Since there is only one free amine the rate expression reduces to the following:

$$\text{Rate} = K(IAA)(NH_2)_\varepsilon$$

If this expression is correct a plot of $\frac{\text{Rate}}{(IAA)}$ vs. $(NH_2)_\varepsilon$ should yield a straight line of slope K and intercept zero. Figure 19 is such a plot and it may be observed that it does yield a straight line whose slope is 5.00×10^{-3}, and with an intercept of zero. The units of K are liter per mole seconds. Therefore the rate equation of N-α-Acetyl lysine may be given as;

$$\text{Rate} = 5.00 \times 10^{-3} (IAA)(NH_2)_\varepsilon$$

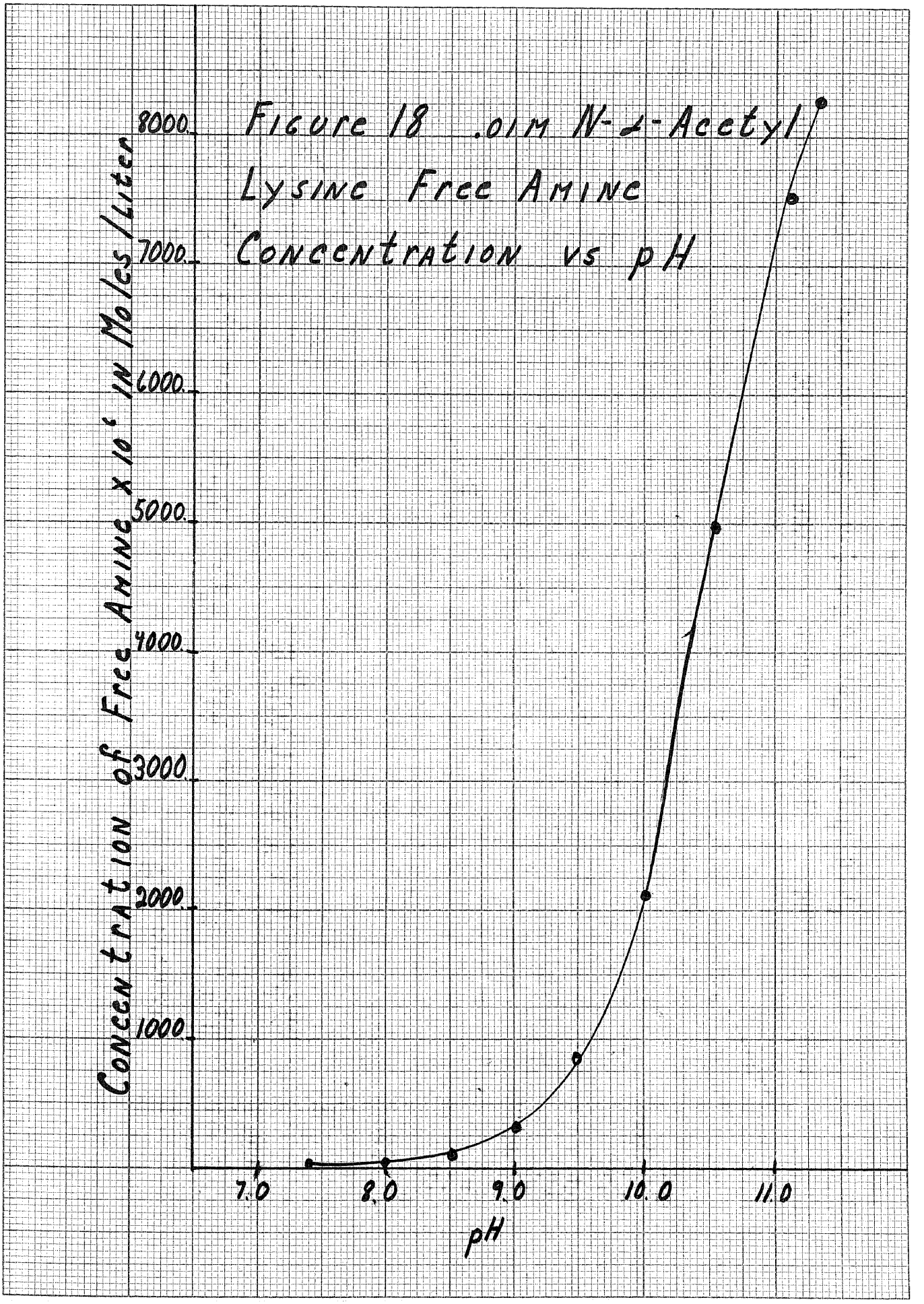


Figure 18 .01M N-ε-Acetyl Lysine Free Amine Concentration vs pH
Concentration of Free Amine x 10⁶ in Moles/Liter
8000
7000
6000
5000
4000
3000
2000
1000
7.0
8.0
9.0
10.0
11.0
pH

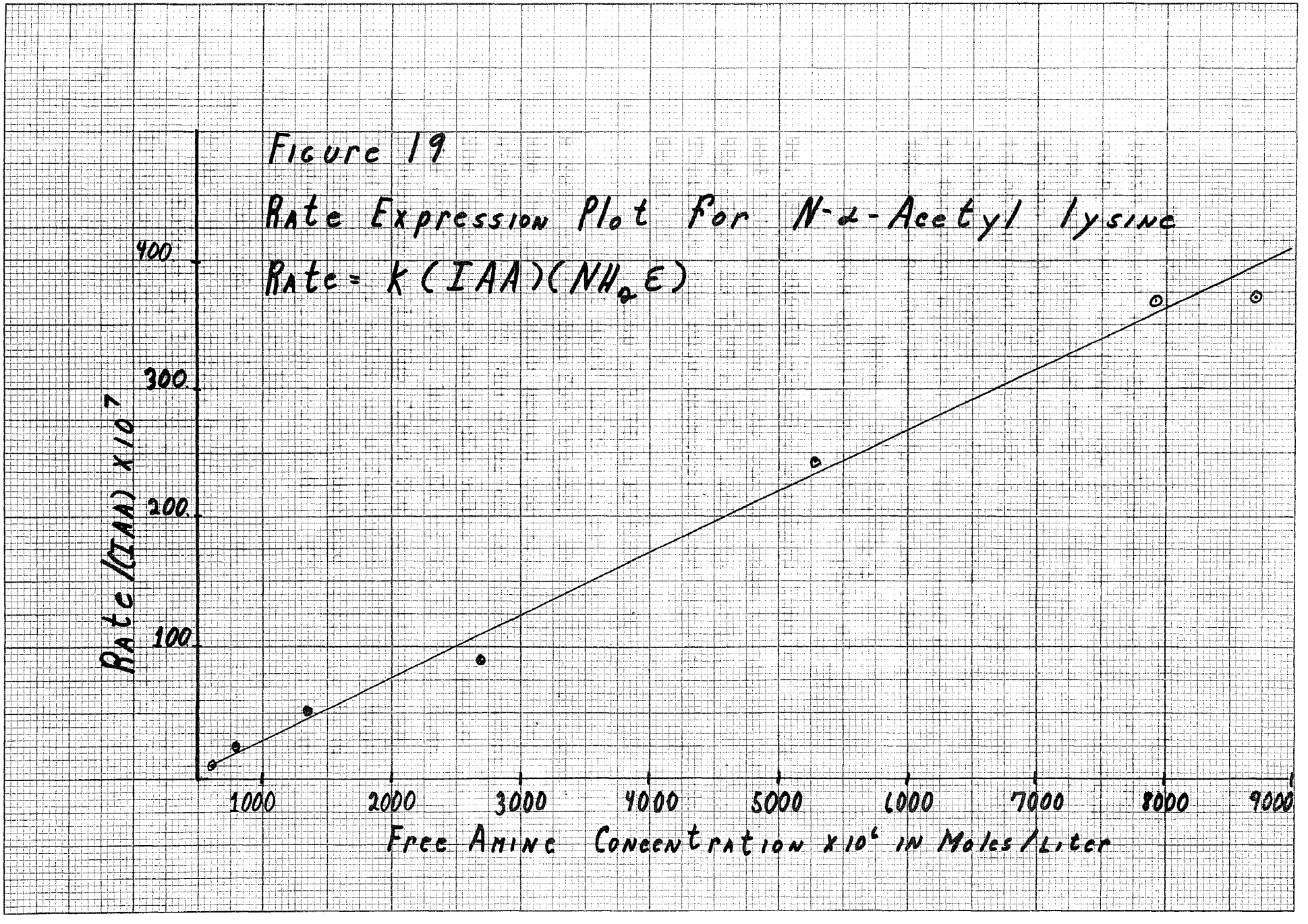


Figure 19
Rate Expression Plot For N-α-Acetyl lysine
Rate = k(IAA)(NH2 E)
400
300
200
100
Rate/(IAA) x 10^7
1000
2000
3000
4000
5000
6000
7000
8000
9000
Free Amine Concentration x 10^6 in Moles/Liter

The case of N-ε-Acetyl lysine is quite similar. Again, there is only one free amine group per molecule, except in this case it is on the alpha carbon, and has a pKa value of 9.63. In a manner similar to the N-α-Acetyl lysine the free amine concentration is calculated as a function of pH. These values are plotted in figure 20. Again there is only one term in the rate expression. The proposed equation is the same as for N-α-Acetyl lysine, that is:

$$\text{Rate} = K(\text{IAA})(\text{NH}_2)_\alpha$$

If this expression holds a plot of $\frac{\text{Rate}}{(\text{IAA})}$ vs. $(\text{NH}_2)_\alpha$ should yield a straight line of slope K and intercept zero. As can be seen in figure 21 the slope is 3.75×10^{-3}, and the intercept is zero. Therefore the rate expression for N-ε-Acetyl lysine is given as:

$$\text{Rate} = 3.75 \times 10^{-3}(\text{IAA})(\text{NH}_2)_\alpha$$

The next most difficult case to arrive at is a rate expression for lysine. In this case there are two free amine groups per molecule; one on alpha carbon and the other on the epsilon carbon. The alpha amine has a pKa value of 8.95, while the epsilon carbon has a pKa of 10.5. The same procedure as in the first two cases is used to find the free amine as a function of pH, except in this case the calculations are made for both alpha and epsilon positions. These results are plotted in figure 22.

In light of the previous results a similar equation is proposed, that is:

$$\text{Rate} = K_1(\text{IAA})(\text{NH}_2)_\alpha + K_2(\text{IAA})(\text{NH}_2)_\varepsilon$$

If this expression is the correct one then a

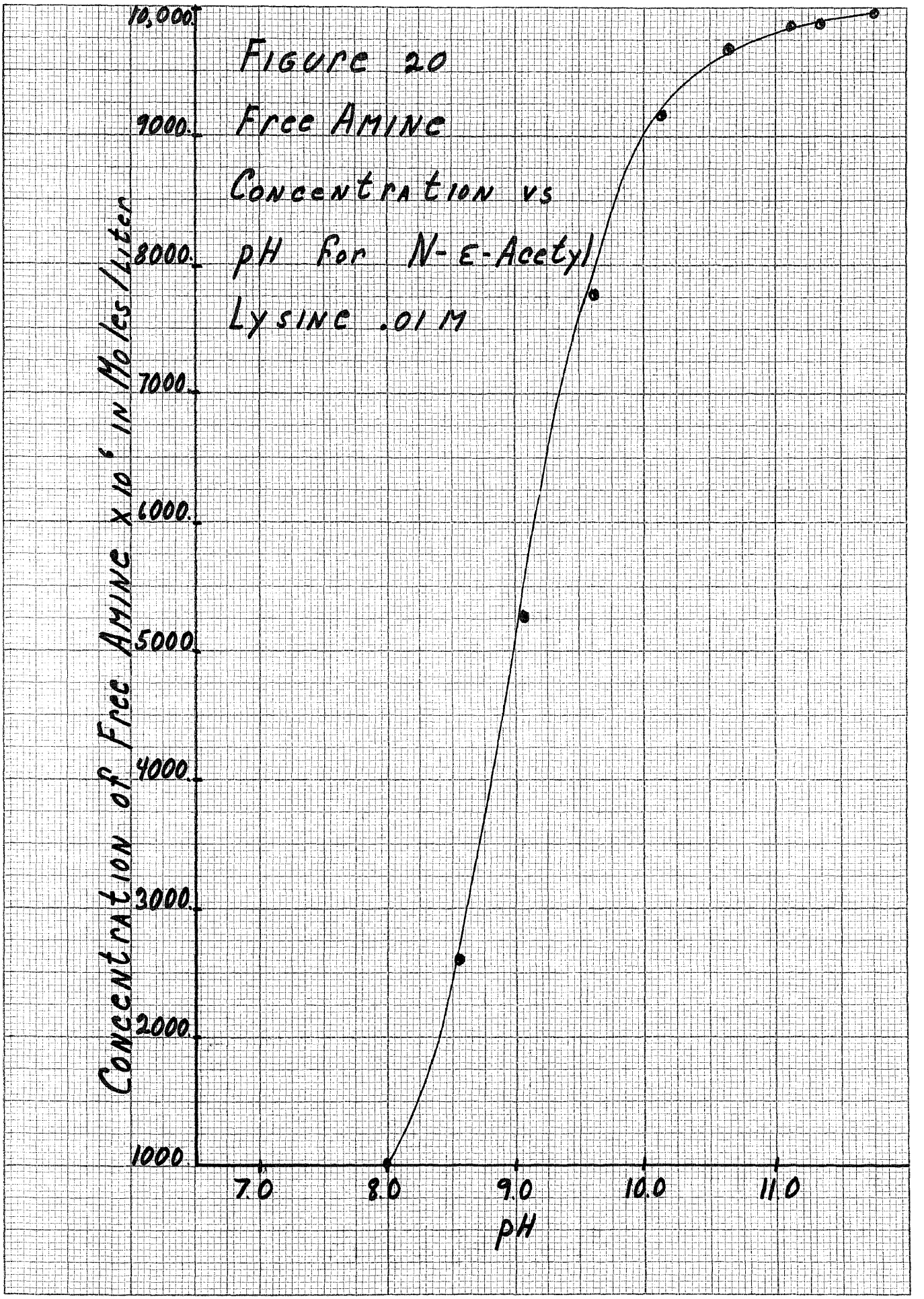


FIGURE 20
FREE AMINE
CONCENTRATION VS
pH FOR N-ε-ACETYL
LYSINE .01 M

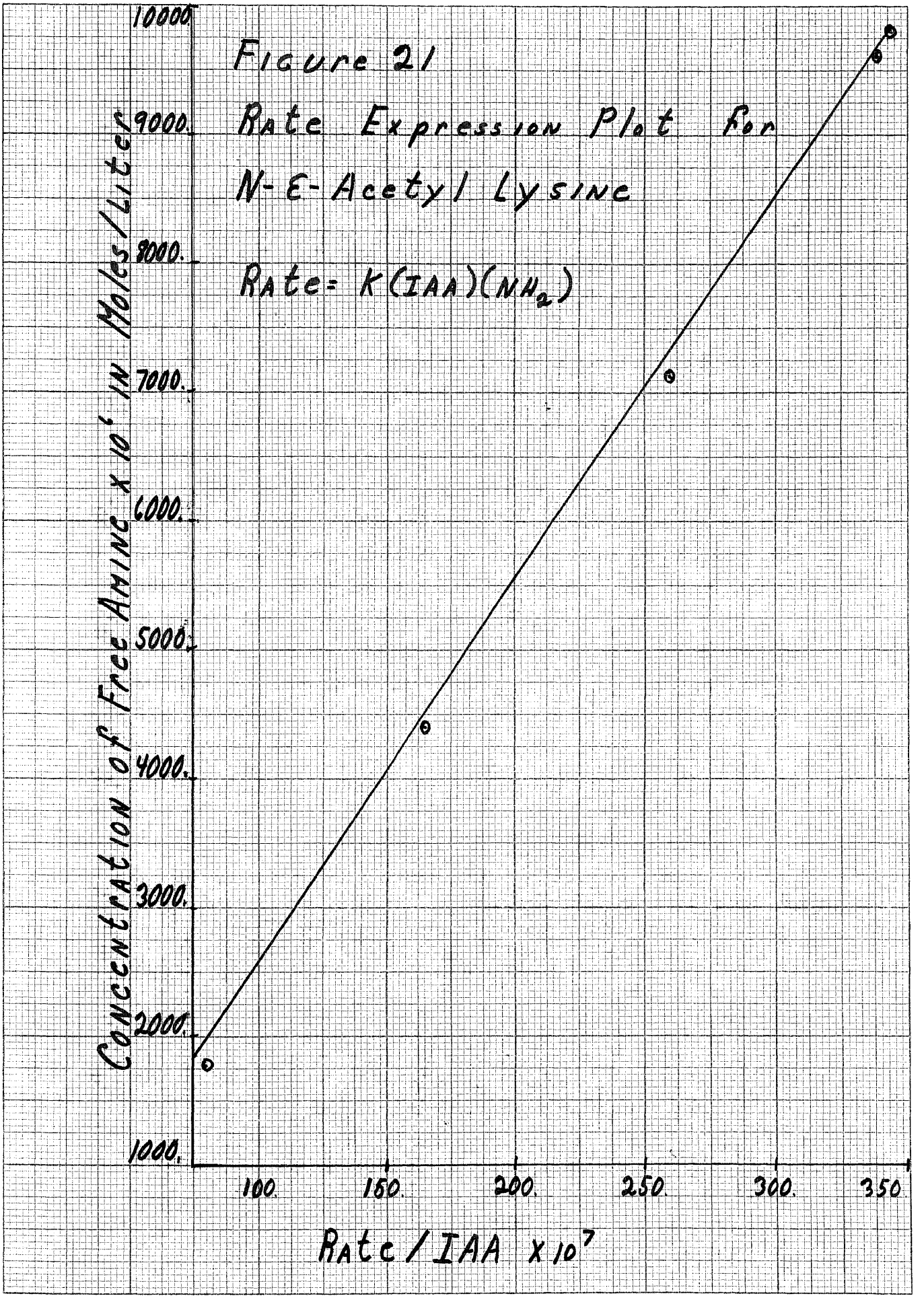


Figure 21
Rate Expression Plot for
N-ε-Acetyl Lysine
Rate = K(IAA)(NH2)
Concentration of Free Amine x 10⁴ in Moles/Liter
10000.
9000.
8000.
7000.
6000.
5000.
4000.
3000.
2000.
1000.
100.
150.
200.
250.
300.
350
Rate/IAA x 10⁷

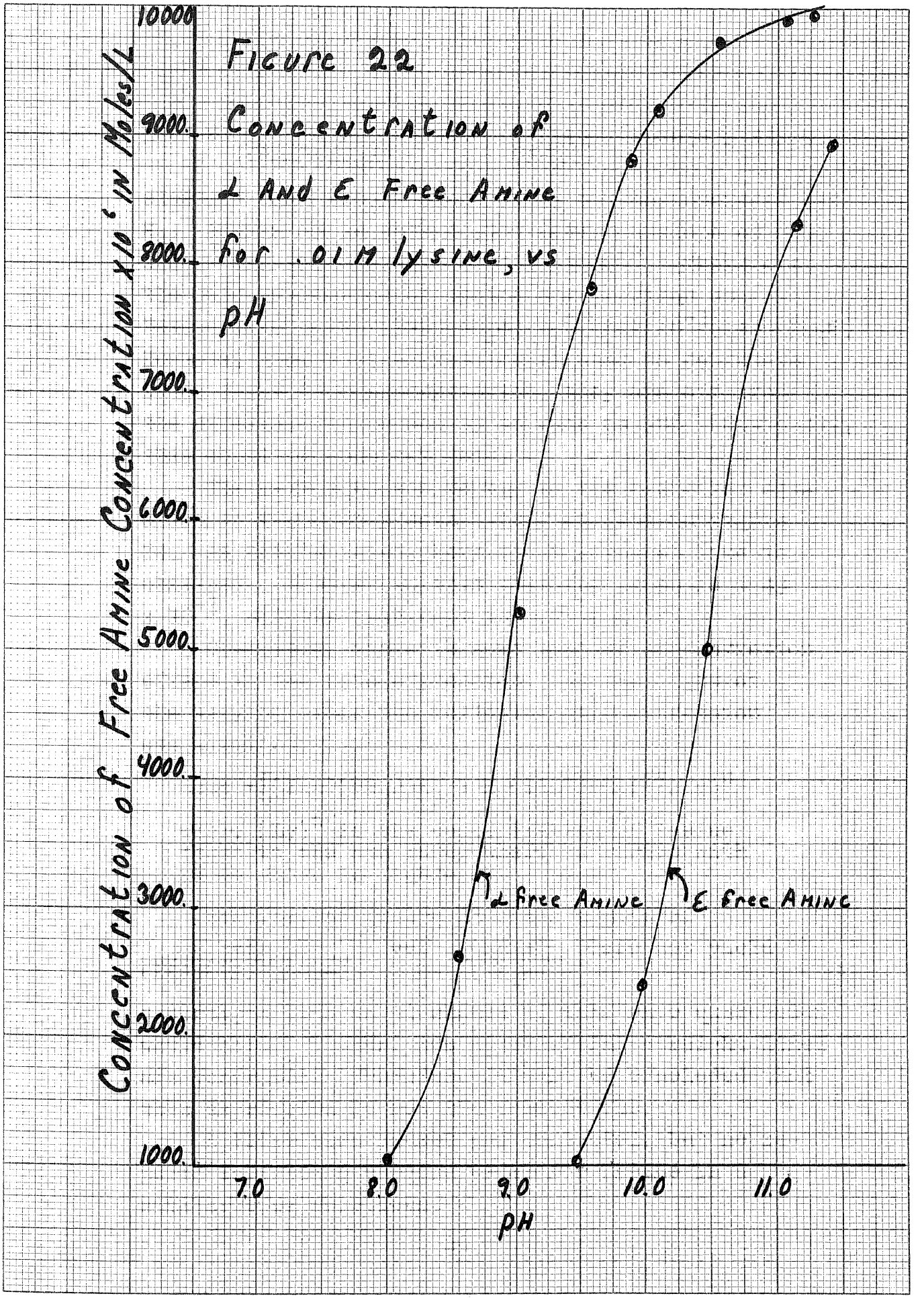


Figure 22
Concentration of
α And ε Free Amine
for .01 M lysine, vs
pH
Concentration of Free Amine Concentration x10⁶ in Moles/L
10000
9000.
8000.
7000.
6000.
5000.
4000.
3000.
2000.
1000.
α Free Amine
ε Free Amine
7.0
8.0
9.0
10.0
11.0
pH

plot of $\frac{\text{Rate}}{(IAA)(NH_2\alpha)}$ vs. $\frac{(NH_2\varepsilon)}{(NH_2\alpha)}$ should yield a straight line with a slope of K_2 and an intercept of K_1. Figure 23 is such a plot having a slope of 8.75×10^{-3} and an intercept of 1.05×10^{-3}. Therefore the rate expression of lysine may be given as:

$$\text{Rate} = 1.05 \times 10^{-3}(IAA)(NH_2\alpha) + 8.75 \times 10^{-3}(IAA)(NH_2\varepsilon)$$

However, when this expression is used to calculate rates for 0.05 M concentration, they are off from the experimental values by as much as 50%. Obviously, this equation does not hold.

The next step was to try to fit a similar equation to the 0.05 M data. This is done in figure 24. As can be seen, here too a straight line is obtained. However in this case the slope is 1.43×10^{-3}, and the intercept is 0.06×10^{-3}.

Obviously, there is some type of concentration effect in the rate expression. In the light of the varying order the following rate expression was examined.

$$\text{Rate} = [(IAA)(NH_2\alpha)K_1 + K_2(IAA)(NH_2\varepsilon)](Ly)^n$$

where $(Ly)^n$ is the concentration of lysine taken to the nth power where n varies with pH as shown in figure 17.

However, as figure 25 shows a plot of $\frac{\text{Rate}}{(IAA)(Ly)^n(NH_2\alpha)}$ vs. $\frac{(NH_2\varepsilon)}{(NH_2\alpha)}$ does not yield a straight line as it should if this rate expression would hold. Rather, two intersecting straight lines are generated. Therefore it must be concluded that this expression is not the correct one.

A literature search was conducted, but no reports indicted a varying order for lysine in a similar type reactions.

Figure 23

Rate Expression for .01 M lysine

Rate = $(IAA)(NH_2\alpha)\,k_1 + (IAA)(NH_2\varepsilon)\,k_2$

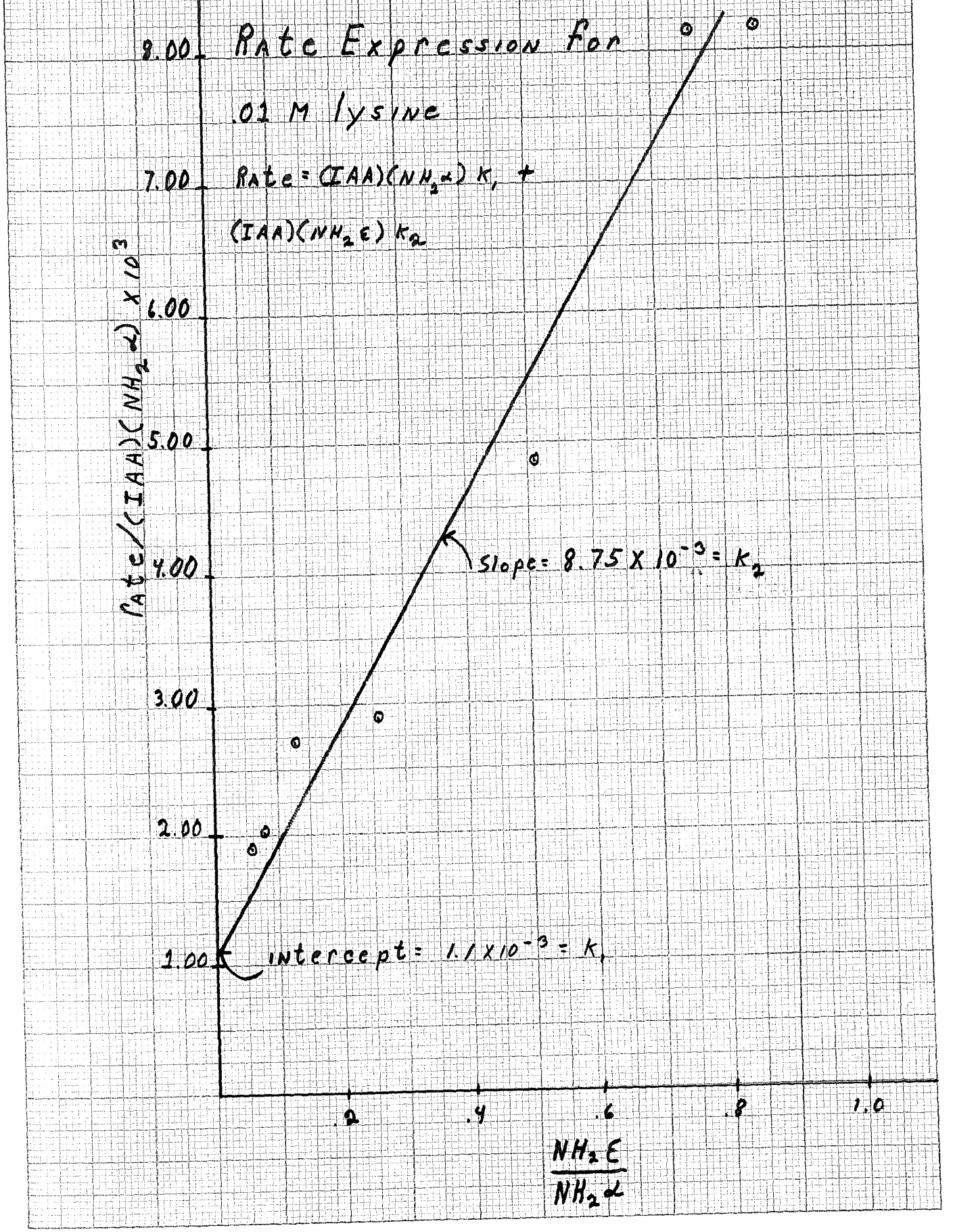
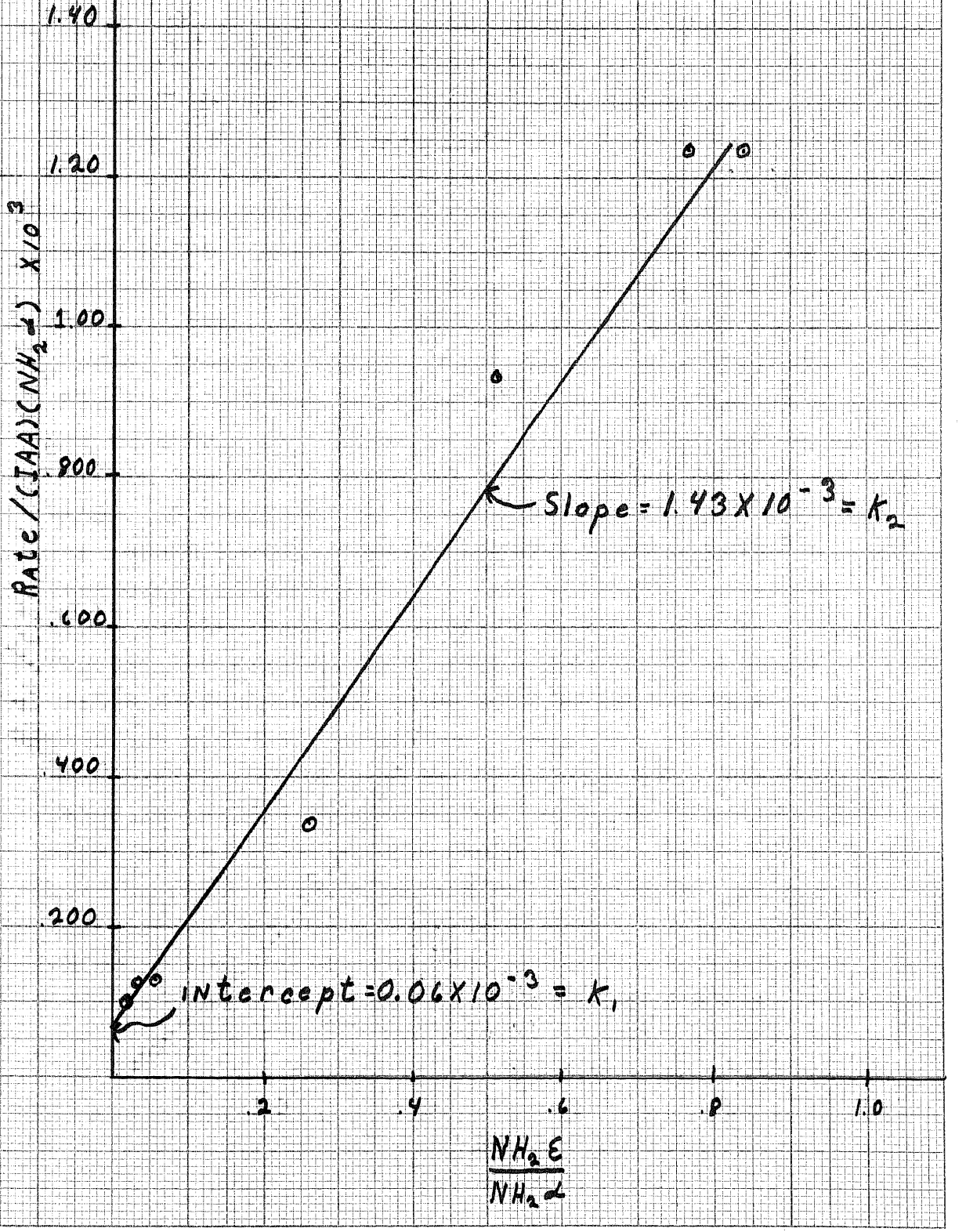


Figure 24

Rate Expression for .05 M Lysine

$$\text{Rate} = (IAA)(NH_2\alpha)\,k_1 + (IAA)(NH_2\varepsilon)\,k_2$$



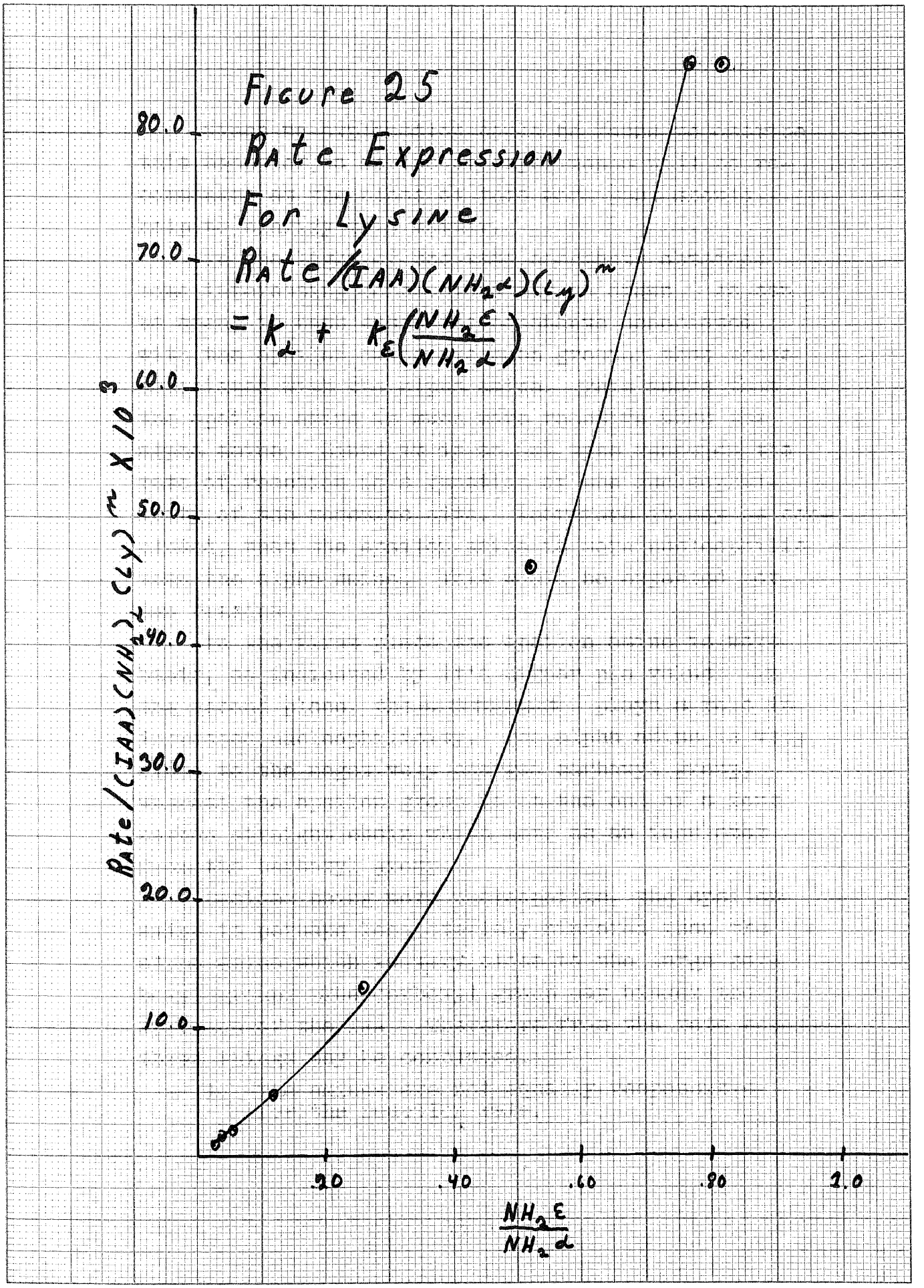


Figure 25
Rate Expression
For Lysine
Rate/(IAA)(NH2α)(Ly)^m
= k2 + kε(NH2ε/NH2α)
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
Rate/(IAA)(NH2α)2(Ly)m X 10^3
.20
.40
.60
.80
1.0
NH2ε/NH2α

Since no explanation could be found to decribe this behavior, a series of experiments will be recommended at the close of this work that should help to clarify this matter.

In the light of the rate constants obtained some interesting conclusions may be drawn. As can be seen, in both cases the constants associated with the epsilon amine are much greater than those associated with the alpha amine. This means that the epsilon amine is much more reactive than the alpha amine. Since it is an SN2 type reaction the rate of reaction is a function of the nucleophilicity of the reactive site. Therefore it may be concluded, that the epsilon amine is more nucleophilic than the alpha amine. This would be expected for two reasons.

In the first place the epsilon amine is more basic, as indicated by it's higher pKa value. In the second place, the environment plays a role in increased nucleophilicity. The alpha amine is very close to the negatively charged carboxyl group, whereas the epsilon group is at the end of a four carbon straight chain which acts as an insulator from the carboxyl group.

Besides being more nucleophilic, the epsilon amine is perhaps less stericly hindered than the alpha amine which is very close to the large carboxyl group. The carboxyl group may hinder the approach of the IAA. Therefore from the rate equation three things may be concluded.

1. The amines are not equally responsible for the reaction rate,

2. The amines are not additive in their effect on the rate and,

3. The epsilon amine is more reactive than the alpha amine.

The last and most difficult analysis is that of the rate expression for polylysime. Unlike lysine, N-α-Acetyl lysine, and N-ε-Acetyl lysine, which have invariant pKa values, polylysine's pKa value is a function of thr fraction of the molecule in helical conformation.[14] At low pH the polymer exists in a random coil; as the pH increases regions start to assume a helical conformation, until at about pH 12.5 it is completely in the helical structure.[15]

Because of the varying pKa, the calculation of amine concentration is no longer a simple matter. Jan Hermans determined the value of the apparent pKa as a function of alpha, that is the fraction of polymer that is in helical conformation.[16] Figure 26 reproduces his findings. Since it is of interest to find the pKa value as a function of pH, it is necessary to find the fraction helix as a function of pH. Parker, Applegate, and Slutsky, have done this.[17] Their finding have been reproduced in figure 27. By combining these two works it becomes possible to generate figure 28, that is, pH vs. apparent pKa. Using this figure it is possible to calculate the concentration of free amine at any pH, by using the same method as in the previous cases.

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[14] op. cit. 8

[15] op. cit. 10

[16] op. cit. 8

[17] op. cit. 10

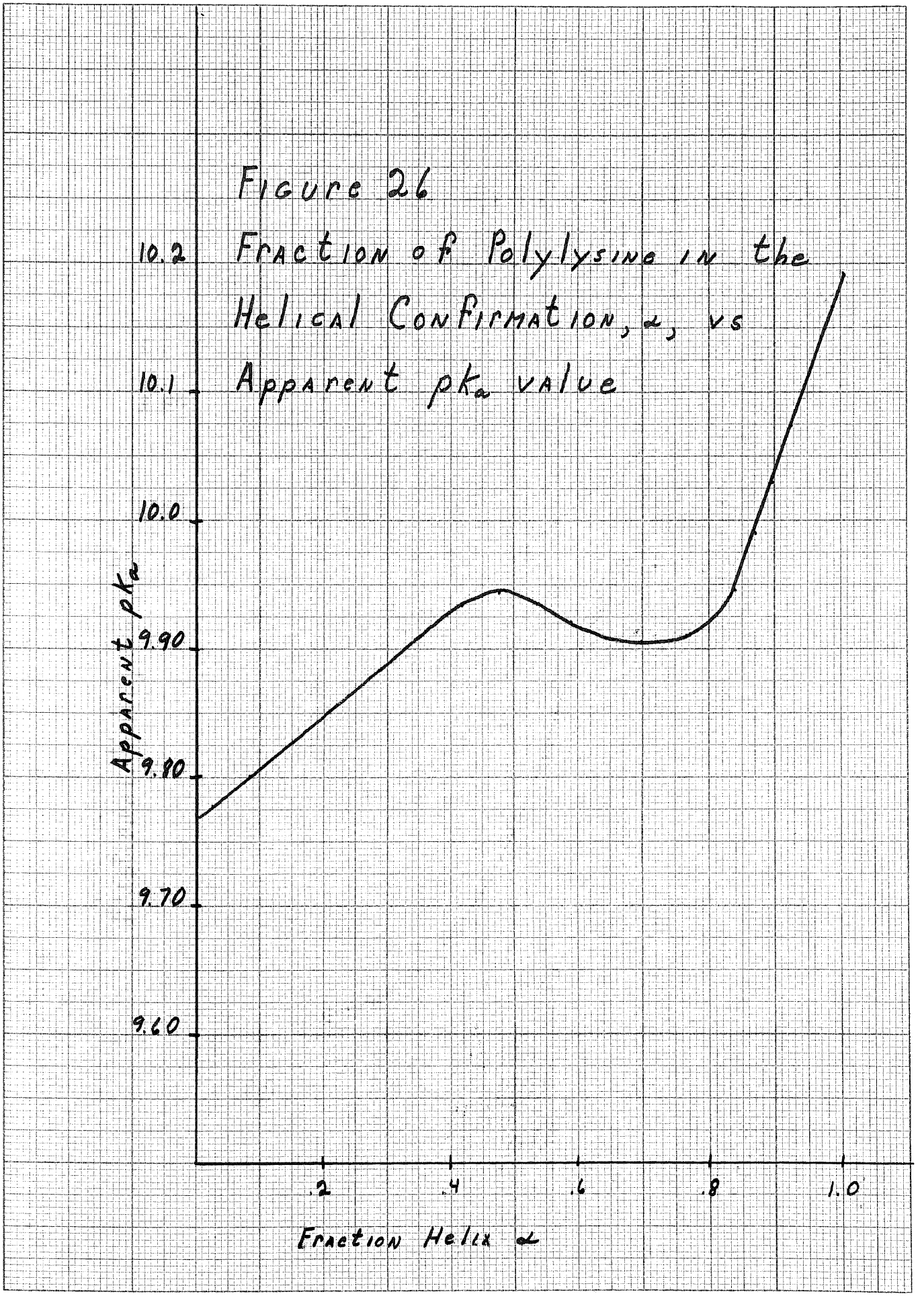


Figure 26
Fraction of Polylysine in the Helical Confirmation, α, vs Apparent pka value
10.2
10.1
10.0
9.90
9.80
9.70
9.60
Apparent pka
.2
.4
.6
.8
1.0
Fraction Helix α

Figure 27

α, Fraction of Polylysine in Helical Conformation, vs. pH

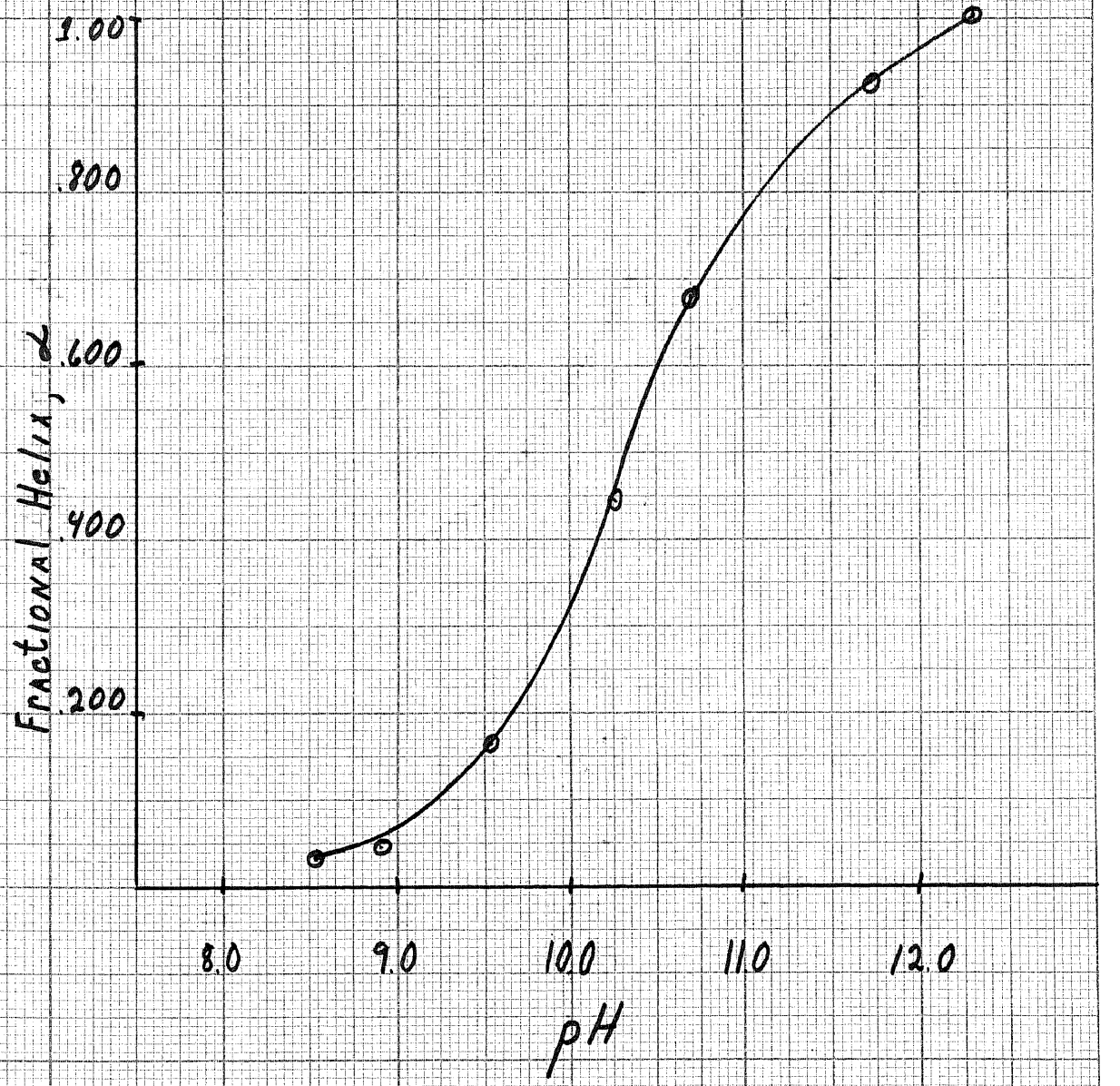
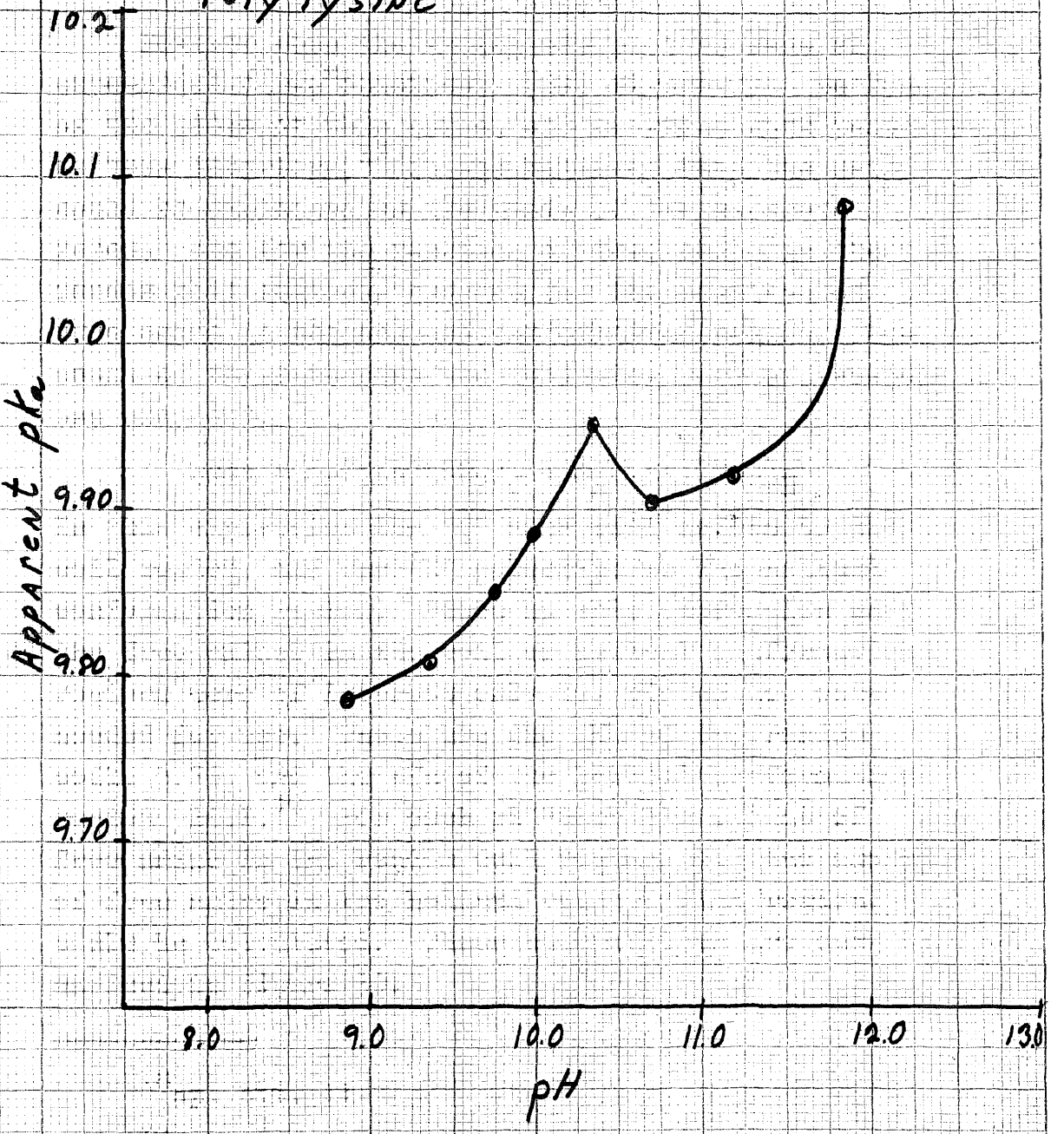


Figure 28

pH vs Apparent pka for Poly lysine



Based upon all the other rate expressions it was assumed that the following expression would hold:

$$\text{Rate} = K(\text{IAA})(NH_2)$$

However when the approprate plot is made an expoential curve[15] obtained. (See figure 29) Because of the slope of this curve, and previous work performed in this labortory,[18] it was believed that the data could be explained on the basis of two separate rates; one for the reaction of free amine in the random coil regions, and one for the reaction of free amine in the helical conformation region. Because of these reasons the following rate expression was proposed:

$$\text{Rate} = K_C(\text{IAA})(NH_2)_C + K_H(\text{IAA})(NH_2)_H$$

In this expression the "C" subscript refers to the coil region, and the "H" subscript to the helical conformation region. Since in this expression only the free amines in the coil or helical conformation regions were of interest only two pKa values were needed for use. These are the pKa of the random coil, 9.76, and the pKa of the helical conformation region, 10.2.[19] For each pH value the amount of free amine in the random coil region, and the amount of free amine in the helical conformation region, could be calculated. These calculations are summarized in figure 30.

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[18] op. cit. 11

[19] op. cit. 8

Figure 29

Simple Rate Expression

For Polylysine

Rate = $K(IAA)(NH_2)$

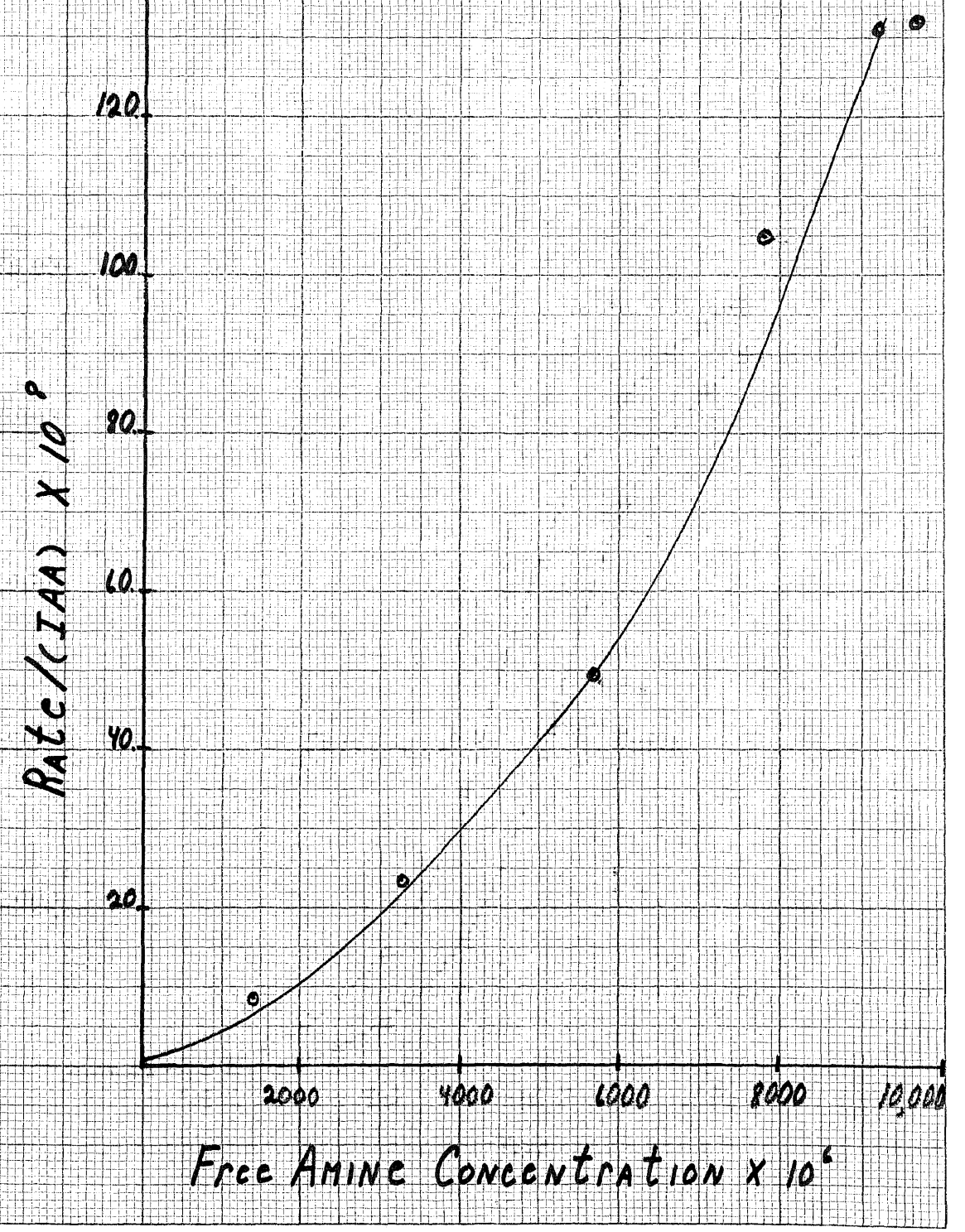


Figure 30

Free Amine Concentration for Polylysine using Coil and Helix pKa Values

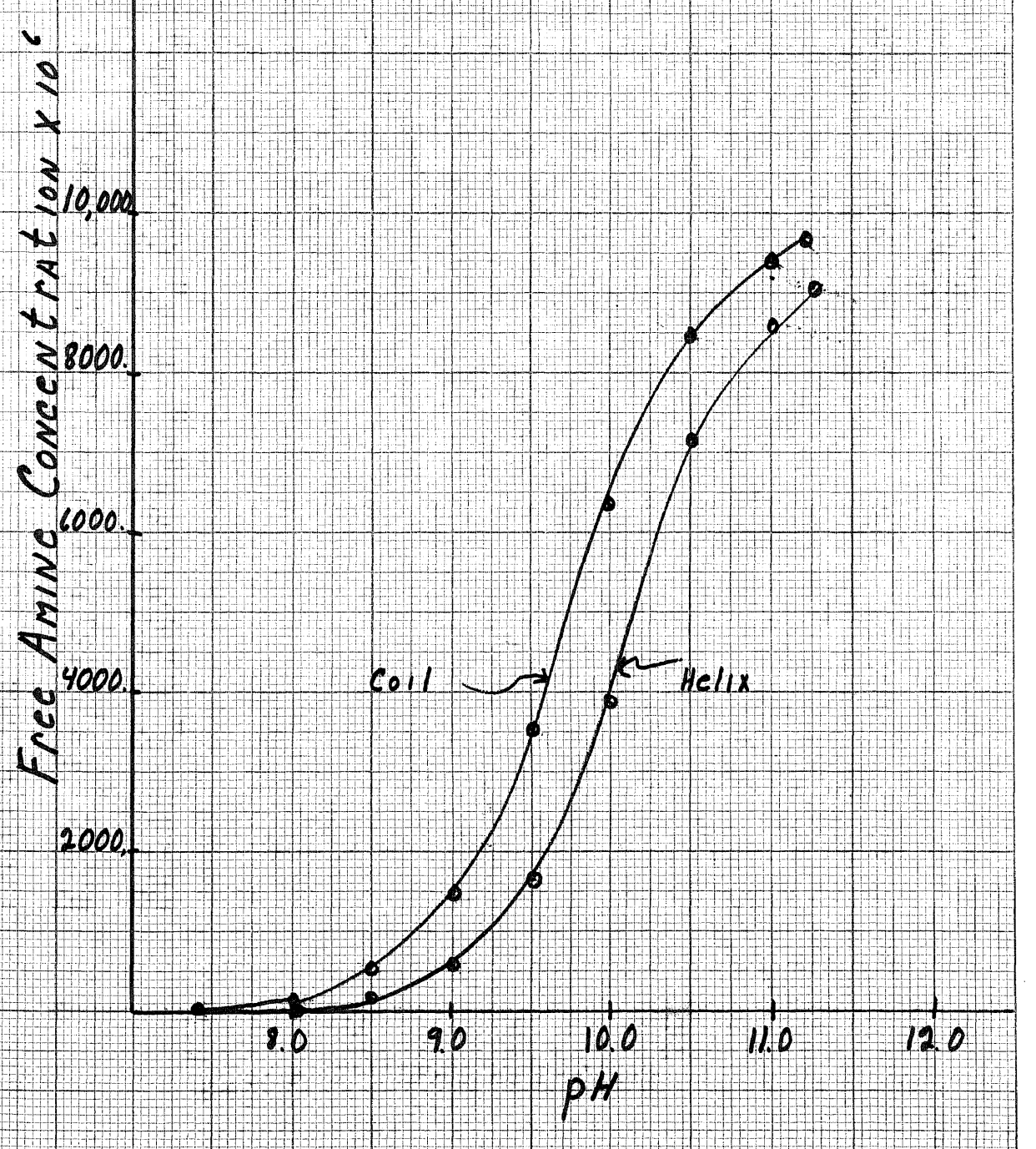
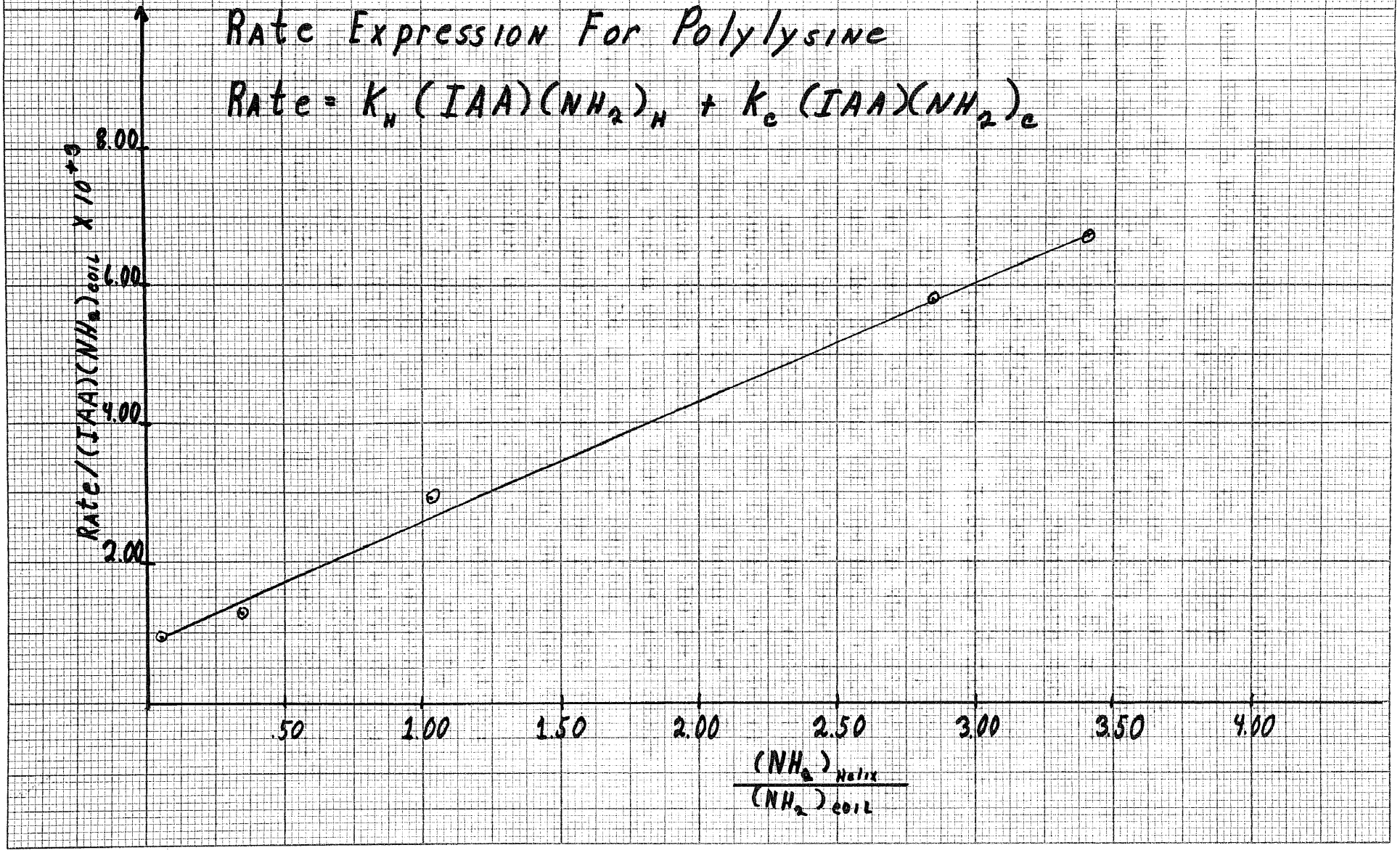


Figure 31

Rate Expression For Polylysine

$Rate = K_H (IAA)(NH_2)_H + K_C (IAA)(NH_2)_C$



If the proposed rate equation is the correct one then a plot of $\frac{\text{Rate}}{(IAA)(NH_2)_C}$ vs. $\frac{(NH_2)_H}{(NH_2)_C}$ should yield a straight line of slope K_H, and with an intercept of K_C. Figure 3 is the plot that does meet these conditions giving a slope of 1.81×10^{-3} and an intercept of 0.650×10^{-3}. Therefore the rate expression for polylysine becomes:

$$\text{Rate} = 0.650 \times 10^{-3}(IAA)(NH_2)_C + 1.81 \times 10^{-3}(IAA)(NH_2)_C$$

From this rate expression some interesting conclusions may be drawn. As mentioned in the introduction it was felt that the formation of the helix might make those amines in this conformation more reactive. However up to now this effect had not been determined. It was the intention of this work to try to observe this increased reactivity. This has been done and, it has been shown that those amines in the helical conformation region are about three times more reactive than those in the random coil region.

One of the possible explainations for this increased reactivity is that while in the random coil region the amines are pointing in all directions and some of these may be pointing inward toward the coil, stericlly hindering the reaction. On the other hand those amines in the helical conformation region are very well ordered and point outward from the backbone of the helix, and are quite free to react. This, then, could account for their increased reactivity.

Conclusions and Recommendations

The following conclusions have been drawn:

1) It has been shown in general that the rate of reaction for lysine, and it's derivatives, with IAA, increases as the pH increases, because of the increase in free amine concentration. In the case of N-α-Acetyl lysine, and, N-ϵ-Acetyl lysine, this increase is proportional to the concentration of free amine. The proportionality constants are the rate constants. In the case of lysine the rate of reaction is the summation of two proportionality terms. One of these is associated with the alpha amine while, the other, is associated with the epsilon amine. Furthermore there appears to be some unexplainable concentration term. In the case of polylysine, the rate of reaction is again the summation of two proportionality constants, one associated with the random coil region, while the other is associated with the helical conformation region.

2) It has been demonstrated that the two amines on the lysine are not equally responsible for the total rate of reaction. Rather the epsilon amine has been shown to be more reactive than the alpha amine. It has also been shown that these amines are not additive in their effect, on the reaction.

3) We can conclude that the enviornment of the free amine plays a large role in the determination of its reactivity. This has been shown in two cases. The first of these is in the case of lysine. The epsilon amine which is at the end of a four carbon straight chain, is more reactive than the alpha amine. This could probably be attributed to two causes. The first of these is the major cause, that is, the epsilon amine is more nucleophilic than the alpha amine. The second of these is that

the epsilon amine is not sterically hindered. The second of these illustrations is in the case of polylysine. It has been shown that those free amines in the helical conformation region are about three times more reactive than those in random coil region. This increased reactivity effect is probably caused because, the free amines in the helical conformation region are not sterically hindered, as those are in the random coil region.

4) It may be concluded that all the species studied obey a rate expression of the following form:

$$\text{Rate} = (\text{IAA})(\text{NH}_2)_{\alpha}K_1 + (\text{IAA})(\text{NH}_2)_{\epsilon}K_2$$

In the cases of N-α-Acetyl lysine and N-ϵ-Acetyl lysine, there is only one free amine group so the second term drops out. In the case of lysine K_1 K_2 are properties associated with the alpha and epsilon amines. The (NH_2) terms are the concentrations of the alpha amine alone, and the epsilon amine alone. There also is a concentration term which could not be arrived at in this work. For the case of polylysine K_2 and K_1 are associated with those free amines in the helical conformation region, and those free amines in the random coil region.

5) The table on the following page compares the pKa value of each of the species with the corresponding rate constant. Some interesting points may be concluded from this table.

Compound	pKa	K x 10^{+3}	pKa	K x 10^{+3}
N-α-Acetyl lysine	10.53	5.00		
N-ε-Acetyl lysine	9.63	3.75		
lysine 0.01 M	8.95	1.05	10.50	8.75
0.05 M	8.95	0.06	10.50	1.43
polylysine	9.76	0.65	10.29	1.81

If we first compare N-α-Acetyl lysine and N-ε-Acetyl lysine, one will find that as the pKa increases, so does the amine's reactivity. In this case it may be concluded that the higher the pKa the more nucleophilic that amine is. However, these conclusions cannot be extended for the cases of lysine and polylysine, because there are different effects associated with their reactivity.

In the case of lysine there seems to be some unexplained concentration effect. However, it may be noted that within the molecule, the higher the pKa value, the more reactive that amine is.

In the case of polylysine there is the effect of the helical conformation on the rate of reaction. However, as in the case of lysine, within the molecule the more reactive the amine is the higher it's pKa value.

Therefore it may be generally concluded that within a molecule the higher the pKa value of the amine the more reactive that amine is. Furthermore it may be noted that this conclusion is also valid when comparing species, except when factors other then increased nucleophilicity, are partially responsible for the rate.

The following are the recommendations of this work.

1) To further examine the effect of transistion from random coil to helical conformation, it is suggested that studies be made in a system where the fraction of polylysine in the helical conformation is maintained constant and the concentration of free amine is varied. This would permit the conformation effect to be fully separated from the nucleophilic effect.

2) More information could be gained about this effect by looking at the IAA reaction with trilysine, and tetralysine. The length of a helical segment in polylysine is 3.6 lysyl units. By using these compounds one could perhaps elucidate any assistance effects of one amine to the other as the helix is formed.

3) The steric effects could be closely examined by using a larger substrate then IAA in the reaction. Such a substrate could be Iododinitrobenzene. Also environmental effects could be examined by following IAA's reaction with dilysine, trilysine, and tetralysine.

4) Since this work could not explain the molecular basis for a concentration effect in the lysine reaction, it is recommended that a series of concentrations be run. Also that Iododinitrobenzene be used as a substrate instead of IAA since this too is an SN2 type reaction, and should yield a similar concentration effect.

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APPENDIX

The appendix is made up of tables of the raw data taken during the work. Each table is made up of two columns. The one on the right is concentration of Iodide ion in solution in moles per liter x 10^4. The left column is the time, in seconds, at which this concentration occurs. For convience the discription of what each table conditions are, is listed below.

Reaction Vessel Condition		Table Numbers
50 ml. of .01 lysine, in .1 M THAM + 2 ml. of .513 M IAA at 23°C at pH of:	7.4	A1
	8.0	A2 - A7
	8.5	A3 - A8
	9.0	A4 - A9
	9.5	A5 - A10
	10.0	A6 - A11
50 ml. of .05 M lysine in .1 M THAM + 2ml. of .513 M IAA at 23°C at pH of:	7.4	A12
	8.0	A13 - A18
	8.5	A14 - A19
	9.0	A15 - A20
	9.5	A16 - A21
	10.0	A17 - A22
50 ml. of .05 M lysine in .1 M THAM + 2 ml. of .513 M IAA at 23°C, and a pH value of:	7.4	A23 - A29
	8.0	A24 - A30
	8.5	A25 - A31
	9.0	A26 - A32
	9.5	A27 - A33
	10.0	A28 - A34
50 ml. of .01 M polylysine in .1 M THAM + 2 ml. of .513 M IAA at 23°C, and a pH value of:	7.4	A35
	8.0	A36 - A41
	8.5	A37 - A42
	9.0	A38 - A43
	9.5	A39 - A44
	10.0	A40 - A45

50 ml. of .005 M polylysine in .1 M THAM + 2 ml. of .513 M IAA at 23°C and a pH value of:	7.4	A46
	8.0	A47 - A52
	8.5	A48 - A53
	9.0	A49 - A54
	9.5	A50 - A55
	10.0	A51 - A56
50 ml. of .01 M N-ε-Acetyl lysine in .1 M THAM + 2 ml. of .513 IAA at 23°C and a pH value of:	7.4	A57
	8.0	A59 - A64
	8.5	A60 - A65
	9.0	A61 - A66
	9.5	A62 - A67
	10.0	A63 - A68
50 ml. of .05 M N-ε-Acetyl lysine in .1 M THAM + 2 ml. of .513 M IAA, at 23°C, and a pH value of:	7.4	A69
	8.0	A70 - A75
	8.5	A71 - A76
	9.0	A72 - A77
	9.5	A73 - A78
	10.0	A74 - A79
50 ml. of .01 M N-ℓ-Acetyl lysine in .1 M THAM + 2 ml. of .513 M IAA, at 23°C, and a pH value of:	7.4	A80
	8.0	A81 - A87
	8.5	A82 - A88
	9.0	A83 - A89
	9.5	A84 - A90
	10.0	A85 - A91
50 ml. of .02 M N-ℓ-Acetyl lysine in .1 M THAM + 2 ml. of .513 M IAA, at 23°C, and a pH value of:	7.4	A92
	8.0	A93 - A98
	8.5	A94 - A99
	9.0	A95 - A100
	9.5	A96 - A101
	10.0	A97 - A102

50 ml. of .1 M triethylamine - acetate (TEA) + 2 ml. of .513 M IAA, at 23° C, and a pH value of:	10.0	A103
	10.5	A104
	11.0	A105
	11.2	A106
50 ml. of .1 M TEA with .01 M lysine + 2 ml. of .513 M IAA, at 23° C, and a pH value of:	10.5	A107
	11.0	A108
	11.2	A109
50 ml. of .05 M lysine in .1 M TEA + 2 ml. of .513 M IAA at 23° C, and a pH value of:	10.5	A110
	11.0	A111
	11.2	A112
50 ml. of .005 M polylysine in .1 M TEA + 2 ml. of .513 M IAA at 23° C, and a pH value of:	10.5	A113
	11.0	A114
	11.2	A114
50 ml. of .01 M polylysine in .1 M TEA + 2 ml. of .513 M IAA at 23° C and a pH value of:	10.5	A115
	11.0	A116
	11.2	A117
50 ml. of .01 M N-ε-Acetyl lysine in .1 M TEA + 2 ml. of .513 M IAA at 23° C and a pH of:	10.5	A118
	11.0	A119
	11.2	A120
50 ml. of .05 M N-ε-Acetyl lysine in .1 M TEA + 2 ml. of .513 M IAA, at 23 C, and a pH of:	10.5	A121
	11.0	A122
	11.2	A123

50 ml. of .01 M N-*d*-Acetyl lysine in .1 M TEA + 2 ml. of .513 M IAA, at 23 C, and a pH value of:	10.5	A124
	11.0	A125
	11.2	A126
50 ml. of .02 M N-*d*-Acetyl lysine in .1 M TEA + 2 ml. of .513 IAA, at 23 C, and a pH value of:	10.5	A127
	11.0	A128
	11.2	A129

TABLE A1

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
4000	.104
4500	.112
5000	.121
5500	.129
6000	.136
6500	.145
7000	.153
7500	.159
8000	.167

TABLE A2

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
2000	.103
2100	-
2300	.115
2400	.118
2500	-
2600	.125
2800	.135
3000	.143
3200	.150
3400	.158

TABLE A3

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
900	.105
1000	.114
1100	.122
1200	.131
1300	.140
1400	.149
1500	.159
1600	.168
1700	.177
1800	.186

TABLE A4

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
600	.106
700	.118
800	.129
900	.141
1000	.152
1100	.165
1200	.179
1300	.191
1400	.203
1500	.218

TABLE A5

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
400	.103
500	.120
600	.137
700	-
800	.169
900	.187
1000	.203
1200	.240
1400	.275
1600	.311

TABLE A6

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
400	.100
500	.114
600	.129
700	.142
800	.158
900	.171
1000	.185
1100	.200
1200	.218
1300	.232

TABLE A7

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
2000	.104
2200	.111
2400	.118
2600	.126
2800	.133
3000	.140
3200	.149
3400	.156
3600	.164
3800	-

TABLE A8

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
700	.101
800	.108
900	.115
1000	.122
1100	-
1200	.134
1300	.146
1400	.152
1500	.161
1600	.169

TABLE A9

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
400	.104
500	.116
600	.128
700	.140
800	.153
900	.165
1000	.179
1100	.193
1200	.207
1300	.221

TABLE A10

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
500	.102
600	.116
700	.130
800	.143
900	.158
1000	.171
1100	.184
1200	.198
1300	.212
1400	.229

TABLE A11

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
400	.102
500	-
600	.131
700	.144
800	.160
900	.174
1000	.191
1100	.206
1200	.221
1300	.236

TABLE A12

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
1900	.100
2000	.103
2200	.110
2400	.116
2600	-
2700	.125

TABLE A13

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
800	.102
900	.108
1000	.114
1100	.121
1200	.128
1300	.134
1400	.140
1500	.145
1600	-
1700	.160

TABLE A14

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
500	.167
600	.183
700	.196
800	.212
900	.228
1000	.248
1100	.268
1200	.284
1300	.300
1400	.318

TABLE A15

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
250	.131
300	.147
350	.162
400	.178
450	.189
500	.201
600	.235
700	.261
800	.301
900	.328

TABLE A16

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
100	.141
125	.158
150	.176
200	.211
250	.250
300	.285
350	.321
400	.362
450	.400
500	.438

TABLE A17

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
100	.203
125	.230
150	.261
175	.295
200	.330
225	.390
250	.420
275	.450
300	.480
325	.515

TABLE A18

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
600	.103
700	.112
800	.116
900	.122
1000	.131
1100	.138
1200	.143
1300	.150
1400	.156
1500	.162

TABLE A19

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
200	.107
300	.126
400	.144
500	.162
600	.180
700	.197
800	.211
900	.233
1000	.250
1100	.270

TABLE A20

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
100	.123
200	.156
300	.189
400	.222
500	.255
600	.289
700	.321
800	.354
900	.389
1000	.421

TABLE A21

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
400	.305
500	.380
600	.440
700	.500
800	.560
900	.620
1000	.680
1100	.740
1200	.805
1300	.860

TABLE A22

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
100	.135
200	.210
300	.285
400	.360
500	.435
600	.510
700	-
800	.660
900	.740
1000	.820

TABLE A23

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
2000	.100
2200	.109
2400	.118
2600	.127
2800	.135
3000	.144

TABLE A24

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
500	.120
600	.135
700	.151
800	.166
900	.181
1000	.197
1100	.212
1200	.231
1300	-
1400	.261

TABLE A25

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
200	.119
300	.159
400	.199
500	.240
600	.281
700	-
800	.362
900	.405
1000	.450
1100	.495

TABLE A26

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
200	.196
300	.278
400	.359
500	.440
600	.520
700	.620
800	.690
900	.775
1000	.860
1100	.943

TABLE A27

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
100	.200
125	.242
150	.285
175	.330
200	.370
225	.415
250	.460
275	.505
300	.550
325	.595

TABLE A28

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
57	.200
69	.240
71	.280
99	.340
111	.380
124	.420
137	.460
149	.500
162	.540
174	.580

TABLE A29

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
2000	.100
2200	.111
2400	.122
2600	.133
2800	.142
3000	.153
3200	.165

TABLE A30

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
500	.100
600	.116
700	.132
800	.146
900	.160
1000	.173
1100	.188
1200	.201
1300	.216
1400	.230

TABLE A31

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
400	.129
500	.160
600	.190
700	.220
800	.250
900	.280
1000	.310
1100	.340
1200	.370
1300	.400

TABLE A32

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
150	.173
172	.190
196	.210
220	.230
245	.250
270	.270
296	.290
321	.310
347	.330
362	.340

TABLE A33

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
125	.269
144	.300
155	.320
166	.340
177	.360
188	.380
199	.400
210	.420
222	.440
234	.460

TABLE A34

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
80	.290
95	.340
107	.380
119	.420
131	.460
143	.500
156	.540
169	.580
181	.620
194	.660

TABLE A35

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
530	1.5
1390	4.2
2240	6.9
3110	9.6

TABLE A36

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
200	.112
300	.119
400	.128
500	.135
600	.141
700	.150
800	.158
900	.165
1000	.172
1100	.181

TABLE A37

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
200	.133
250	.141
300	.150
350	-
400	.167
450	.175
500	.182
550	.190
600	.199
650	.208

TABLE A38

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
150	.137
200	.155
250	.171
300	.188
350	.205
400	.220
450	.237
500	.252
550	.268
600	.285

TABLE A39

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
100	.151
200	.230
250	.270
300	.305
350	.340
400	.375
450	.410
500	.445
550	.480
600	.515

TABLE A40

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
85	.260
96	.290
104	.310
113	.330
125	.360
134	.380
142	.400
151	.420
160	.440
169	.460

TABLE A41

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
200	.110
300	.118
400	.128
500	.137
600	.143
700	.150
800	.156
900	.166
1000	.173
1100	.180

TABLE A42

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
200	.130
250	.140
300	.150
350	.160
400	.170
450	.176
500	.183
550	.191
600	.201
650	.211

TABLE A43

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
86	.140
103	.150
119	.160
137	.170
155	.180
173	.190
191	.200
208	.210
225	.220
261	.250

TABLE A44

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
112	.250
124	.270
138	.290
146	.300
158	.320
172	.340
186	.360
200	.380
212	.400
229	.420

TABLE A45

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
69	.240
76	.260
83	.280
91	.300
98	.320
105	.340
112	.360
125	.400
132	.420
138	.440

TABLE A46

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
650	.100
880	.110
1200	.120
1530	.130
1950	.140
2350	.150

TABLE A47

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
118	.110
189	.120
260	.130
337	.140
404	.150
471	.160
542	.170
618	.180
703	.190
790	.200

TABLE A48

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
129	.130
170	.140
210	.150
254	.160
290	.170
326	.180
367	.190
404	.200

TABLE A49

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
84	.150
109	.160
134	.170
158	.180
208	.200
231	.210
254	.220
279	.230
307	.240
329	.250

TABLE A50

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
102	.200
113	.210
124	.220
136	.230
148	.240
159	.250
171	.260
182	.270
194	.280
205	.290

TABLE A51

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
99	.260
112	.280
123	.300
136	.320
149	.340
161	.360
172	.380
185	.400
199	.420
210	.440

TABLE A52

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
200	.112
300	.122
400	.134
500	.143
600	.152
700	.161
800	.170
900	.180
1000	.189
1100	.197

TABLE A53

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
150	.127
200	.140
300	.163
400	.188
500	.212
600	.235
700	.258
800	.280

TABLE A54

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
100	.137
200	.173
300	.209
400	.242
500	.274
600	.305
700	.338
800	.369
900	.400
1000	.430

TABLE A55

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
113	.190
127	.200
142	.210
156	.220
171	.230
187	.240
200	.250
215	.260
229	.270
244	.280

TABLE A56

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
100	.235
110	.250
118	.260
132	.280
147	.300
162	.320
176	.340
192	.360
205	.380
220	.400

TABLE A57

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
1500	.103
1700	.108
1900	.112
2100	.117
2300	.122
2500	.126
2700	.131
2900	.135
3100	.139

TABLE A59

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
700	.101
900	.112
1100	.120
1300	.126
1500	.137

TABLE A60

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
600	.110
700	.120
800	.128
900	.139
1000	.150
1100	.159
1200	.169

TABLE A61

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
300	.134
400	.156
500	.180
600	.193
700	.217
800	.239
900	.261
1000	.281

TABLE A62

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
250	.138
300	.158
400	.200
500	.244
600	.289
700	.331
800	.375
900	.418
1000	.460
1100	.502

TABLE A63

Time in Seconds	Iodide ion Concentration x10^{+4} M/L
200	.230
250	.255
300	.290
350	.320
400	.350
450	.375
500	.400
550	.430

TABLE A64

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
700	.105
900	.117
1100	.121
1300	.131
1500	.142
1700	.154
1900	.166

TABLE A65

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
500	.102
600	.112
700	.120
800	.128
900	.135
1000	.142
1100	.150
1200	.159
1300	.165
1400	.175

TABLE A66

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
200	.118
300	.143
400	.168
500	.191
600	.219
700	.244
800	.270
900	.294
1000	.317

TABLE A67

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
250	.138
300	.158
400	.200
500	.244
600	.289
700	.331
800	.375
900	.418
1000	.460
1100	.502

TABLE A68

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
300	.200
350	.230
400	.260
450	.290
500	.320
550	.355
600	.387
650	.420
700	.450

TABLE A69

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
1450	.100
1800	.103
1950	.112
2200	.119
2350	.123
2550	.129
2750	.134
2950	.139

TABLE A70

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
500	.127
600	.140
700	.151
800	.165
900	.177
1000	.188
1100	.199
1200	.210
1300	.221

TABLE A71

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
250	.131
300	.145
350	.161
400	.189
500	.205
550	.221
600	.235
650	.251
700	.267

TABLE A72

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
150	.118
200	.148
300	.205
350	.232
400	.263
450	.291
500	.320
550	.349
600	.375
650	.402

TABLE A73

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
107	.220
118	.240
130	.260
140	.280
151	.300
161	.320
170	.340
190	.380
199	.400
210	.420

TABLE A74

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
70	.200
83	.230
97	.260
110	.290
121	.320
133	.350
149	.390
161	.420
176	.460
190	.500

TABLE A75

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
500	.120
600	.133
700	.142
800	.160
900	.170
1000	.180
1100	.191

TABLE A76

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
200	.116
250	.132
300	.147
350	.161
400	.175
450	.189
500	.195
550	.200
600	.211
650	.223

TABLE A77

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
122	.150
148	.170
183	.200
202	.220
222	.240
242	.260
264	.280
285	.300
306	.320
330	.340

TABLE A78

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
89	.210
99	.230
110	.250
121	.270
131	.290
142	.310
152	.330
163	.350
173	.370
182	.390

TABLE A79

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
80	.240
87	.260
95	.280
103	.300
110	.320
117	.340
124	.360
132	.380
144	.420
160	.460

TABLE A80

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
135	.130
320	.140
505	.150
909	.170

TABLE A81

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
92	.100
180	.110
270	.120
360	.130
455	.140
550	.150
645	.160
763	.170

TABLE A82

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
71	.150
108	.160
148	.170
184	.180
226	.190
257	.200
290	.210
326	.220
362	.230
402	.240

TABLE A83

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
35	.150
61	.160
85	.170
105	.180
136	.190
156	.200
184	.210
203	.220
228	.230
252	.240

TABLE A84

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
46	.170
57	.180
68	.190
81	.200
92	.210
103	.220
115	.230
127	.240
139	.250
151	.260

TABLE A85

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
70	.285
79	.300
89	.320
100	.340
111	.360
122	.380
131	.400
143	.420
154	.440
166	.460

TABLE A87

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
90	.100
173	.110
275	.120
371	.130
450	.140
553	.150
640	.160

TABLE A88

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
60	.140
100	.150
140	.160
180	.170
220	.180
250	.190
290	.200

TABLE A89

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
25	.140
55	.150
75	.160
100	.170
125	.180
155	.190
180	.200

TABLE A90

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
40	.150
60	.160
70	.170
85	.180
90	.190
105	.200
120	.210

TABLE A91

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
60	.160
70	.180
79	.200
91	.220
101	.240
111	.260
119	.280
131	.300
141	.320

TABLE A92

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
140	.120
330	.140
490	.160
700	.180
980	.200

TABLE A93

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
80	.120
170	.140
260	.160
350	.180
450	.200
540	.220
630	.240

TABLE A94

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
80	.140
118	.160
158	.180
196	.200
246	.220
277	.240
310	.260

TABLE A95

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
117	.120
135	.130
155	.140
174	.150
192	.160
211	.170
231	.180
250	.190

TABLE A96

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
60	.150
69	.160
77	.170
85	.180
94	.190
102	.200
110	.210
119	.220
126	.230
135	.240

TABLE A97

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
43	.210
53	.220
59	.230
70	.240
82	.250
93	.260
105	.270

TABLE A98

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
80	.120
170	.140
260	.160
351	.180
430	.200
520	.210
610	.220

TABLE A99

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
60	.120
91	.140
122	.160
150	.180
179	.200
211	.220
245	.240
275	.260

TABLE A100

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
101	.110
122	.120
141	.130
161	.140
181	.150
200	.160
219	.170

TABLE A101

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
50	.110
59	.120
69	.130
79	.140
88	.150
98	.160
107	.180
117	.190
127	.200
138	.210

TABLE A102

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
40	.220
50	.250
60	.270
69	.300
81	.340
91	.380
106	.420

TABLE A103

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
50	.150
62	.160
74	.170
86	.180
97	.190
108	.200
120	.210
132	.220
145	.230

TABLE A104

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
76	.160
85	.170
93	.180
103	.190
110	.200
121	.210
138	.220
146	.230
155	.240

TABLE A105

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
40	.180
53	.200
67	.220
78	.240
89	.260
94	.280
110	.300
118	.320
126	.340
135	.360

TABLE A106

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
25	.200
32	.250
39	.300
46	.350
53	.400
61	.460
69	.520
76	.580
84	.640
91	.700

TABLE A107

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
44	.180
49	.200
55	.220
60	.240
65	.260
70	.280
74	.300
82	.344
92	.380
99	.400

TABLE A108

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
27	.160
35	.200
42	.240
49	.280
56	.320
63	.360
69	.400
77	.440
83	.480
86	.500

TABLE A109

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
20	.150
27	.200
35	.250
42	.300
49	.360
54	.400
59	.440
67	.500
72	.540
78	.600

TABLE A110

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
21	.200
28	.300
35	.400
41	.500
47	.600
52	.700
60	.800
65	.900
69	1.00
79	1.20

TABLE A111

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
39	.700
42	.800
47	.900
51	1.00
59	1.20
65	1.40
72	1.60
79	1.80
86	2.00
93	2.20

TABLE A112

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
39	.700
43	.800
47	.900
51	1.00
58	1.20
66	1.40
72	1.60
79	1.80
85	2.00
96	2.20

TABLE A113

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
33	.200
58	.300
70	.350
83	.400
95	.450
106	.500
116	.540
131	.600
144	.660
153	.700

TABLE A114

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
19	.200
27	.250
35	.300
42	.350
51	.400
57	.440
67	.500
78	.560
83	.600
92	.640

TABLE A114

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
23	.250
31	.300
38	.350
44	.400
50	.440
59	.500
67	.560
74	.600
87	.700
95	.760

TABLE A115

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
64	.400
82	.500
113	.700
128	.800
144	.900
159	1.00
173	1.10
188	1.20
203	1.30
216	1.40

TABLE A116

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
42	.400
54	.500
66	.600
78	.700
90	.800
102	.900
115	1.00
126	1.10
138	1.20
149	1.30

TABLE A117

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
40	.400
50	.500
61	.600
72	.700
82	.800
92	.900
102	1.00
112	1.10
122	1.20
132	1.30

TABLE A118

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
19	.210
23	.240
28	.300
34	.330
39	.370
47	.400
55	.440
61	.480
66	.520
72	.560

TABLE A119

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
18	.320
24	.400
36	.520
41	.600
48	.680
56	.780
64	.880
72	.980
82	1.10
88	1.20

TABLE A120

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
30	.500
39	.600
46	.700
54	.800
62	.900
70	1.00
78	1.10
85	1.20
93	1.30
100	1.40

TABLE A121

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
27	.300
35	.380
43	.460
51	.540
58	.620
65	.700
73	.780
80	.860
88	.940
94	1.00

TABLE A122

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
27	.500
34	.600
40	.700
46	.800
53	.900
59	1.00
65	1.10
72	1.20
78	1.30
83	1.40

TABLE A123

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
25	.500
30	.600
36	.700
42	.800
47	.900
53	1.00
58	1.10
64	1.20
69	1.30
75	1.40

TABLE A124

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
103	.160
121	.180
133	.200
148	.220
157	.240
173	.260
186	.280
198	.300
209	.320
236	.340

TABLE A125

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
43	.140
53	.160
63	.180
72	.200
81	.220
89	.240
101	.260
108	.280
115	.300
125	.320

TABLE A126

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
47	.160
52	.180
58	.200
64	.220
71	.240
78	.260
83	.280
88	.300
94	.320
100	.340

TABLE A127

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
54	.150
60	.170
66	.190
72	.210
78	.230
83	.250
89	.270
95	.290
100	.310
106	.330

TABLE A128

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
41	.200
52	.250
62	.300
72	.350
82	.400
92	.450
101	.500
110	.550
119	.600
128	.650

TABLE A129

Time in Seconds	Iodide ion Concentration x 10^{+4} M/L
27	.150
37	.200
46	.250
55	.300
63	.350
71	.400
80	.450
87	.500
95	.550
103	.600