A STUDY OF THE DECOMPOSITION OF MUREXIDE

by

JAMES HOWARD MOSER

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APPROVED:

[redacted]

Associate Professor of Chemistry

In Charge of Major

[redacted]

Chairman of Department of Chemistry

[redacted]

Chairman of School Graduate Committee

[redacted]

Dean of Graduate School

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TABLE OF CONTENTS

A STUDY OF THE DECOMPOSITION OF MUREXIDE[1]

I. INTRODUCTION

The determination of alkaline earth metals in low concentrations has always been a difficult problem. In 1949, Schwarzenbach and Gysling (35) found that murexide forms colored complexes with the common alkaline earth ions Mg, Ca, Sr and Ba, as well as with the ions Zn, Cd, Cu(II), Ni(II), Co(II), Fe(II,III), Mn(II) and Sn(II). The author (31) found that a complex ion is also formed with lithium and apparently with zirconium and hafnium. Since the complex ions have different spectral characteristics than the dye alone, it appears that murexide shows strong promise as a new colorimetric reagent for the analytical chemist.

However, in the development of a colorimetric method for the determination of calcium with murexide, the author (31; 41) was greatly impeded by the fact that this dye is unstable in solution. At that time, it was not feasible to investigate this instability extensively, and the analytical method finally developed for calcium effectively cancels errors caused by this factor. The absorption curves found by the author for the lithium-purpurate systems indicated that it should be feasible to develop a colorimetric method for the determination of

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[1] Ammonium purpurate ($C_8H_4O_6N_5NH_4$).

this ion, which has long resisted successful efforts of colorimetric analysis. However, experiments showed that the equilibrium constant for the formation of a lithium-purpurate complex ion in basic solution is not as great as that for calcium, and therefore the concentration of the dye would have to be known precisely in order that the developed method be of acceptable accuracy. At that time, it was not possible to control or predict the concentration of the murexide to a sufficient degree, for little was known about the specific rate of decomposition of the dye, or whether or not it would be possible to inhibit this reaction. Thus it was not possible to develop a reliable colorimetric method for the determination of lithium. Perhaps, as a result of the quantitative study of murexide decomposition, the above mentioned problems could be solved, thus enabling one to develop a colorimetric method for this ion at a later date.

Ostertag and Rinck (33), as well as this author, found that it was quite difficult to prepare a stable stock solution of murexide. It was found that the stability of the dye in solution seemed to vary with both the commercial source and the solvent used. Ostertag and Rinck also stated that a pure sample of the dye is stable for 15 days in aqueous solution, and that the spectral sensitivity of the dye with respect to calcium is the

greatest for the purest sample. However, these authors did not state their criteria for purity and stability. The present writer found no stable samples of murexide. Betz and Noll (12) overcame the difficulty of storage of murexide reagent for their titrimetric determination of calcium with disodium dihydrogen ethylenediaminetetra-acetate by using a dry mixture of the dye and sodium chloride. However, this technique is not suitable for more exact colorimetric procedures.

It may therefore be appreciated that a thorough investigation of the decomposition of murexide would enable one more readily to select optimum conditions for the development of analytical procedures, perhaps solve the problem of storing a stock reagent solution of the dye, and therefore increase the acceptability of murexide as an analytical reagent.

It has been known since the early part of the nineteenth century that murexide decomposes very rapidly in acid solution (2). It was not until 1949 that the decomposition in basic solution was mentioned in the literature (35). No quantitative studies on the instability of murexide have been reported to the present time, although the products of the decomposition in acid solution have been investigated. This latter subject will be discussed further in chapter III. It was thus

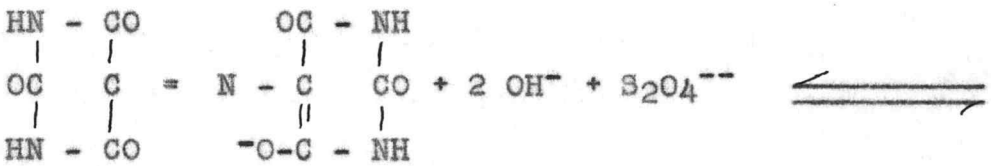
concluded that a study of the reaction kinetics involved in the decomposition of murexide under various solvent conditions would be very valuable. However, before this subject could be undertaken, it was necessary to investigate certain factors. One must prove that the reactions studied are of pure murexide, and if it is not possible to obtain such a sample, the kind, amount, and effect of impurities present must be known and controllable. It would also be very desirable to know the products of the decomposition of murexide in basic as well as acidic solutions, for then one would be able to make predictions about the probable nature of impurities present in samples of the dye, and perhaps, with the knowledge of rate laws, write reaction mechanisms. In view of this discussion, these subjects have been investigated and are presented in relevant order.

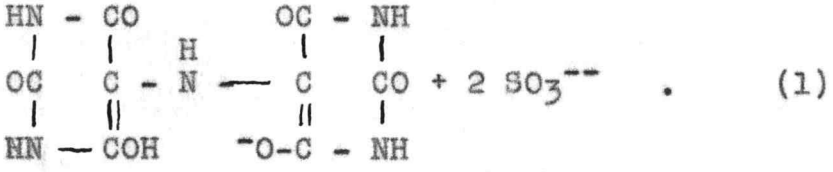
Due to the fact that this investigation covered a variety of topics, the discussions of the literature pertinent to each subject are presented in the appropriate chapters.

II. MUREXIDE ASSAY

In a previous work (31; 41), it was found that commercial samples of murexide obtained from different sources apparently varied quite widely in purity, as found by absorbance measurements. All samples available to this author were suspected of being impure, but it was found that no reliable method was available for murexide assay.

It was not possible to utilize absorptivity[2] for the colorimetric determination of murexide purity, for the absolute purity of the various murexide samples available was unknown, as was information on their rate of decomposition. Kuhn and Lyman (28) and Davidson (15) titrated murexide with sodium hyposulfite in the absence of oxygen. The reaction is





(1)

[2] All nomenclature in this report conforms with that suggested in reference 25.

A reproducibility of ± 8% was the best attainable by the present author with this titration. This large error was ascribed to the instability of hyposulfite in solution (30,p.69) and to inability to control to the desired degree the rate of reaction between hyposulfite and murexide. The stability of the hyposulfite and its oxidation potential increases with increasing pH, while the rate of the oxidation reaction with murexide decreases with increasing basicity.

Utilizing the standard oxidation potential of Kuhn and Lyman (28), an attempt was made to titrate murexide with ascorbic acid. It was found that the rate of the oxidation-reduction reaction in neutral solution was too slow for practical titration. Parenthetically, it is questionable whether the oxidation potential reported above is of value, for murexide decomposes quite rapidly at pH 4.7, which was the acidity for the determination of the standard potential.

Schwarzenbach and Gysling (35) show that the formation of calcium purpurate complexes result in proton release. If one represents the ion formed by the loss of five protons from purpuric acid by F^{-5}, these authors state that the reactions are

$$\left\{\begin{matrix} H_4F^- \\ H_3F^{--} \\ H_2F^{-3} \end{matrix}\right\} + Ca^{++} \longrightarrow \left\{\begin{matrix} (CaH_4F)^+ \\ (CaH_3F) \\ (CaH_2F)^- \end{matrix}\right\} + m\ H^+. \qquad (2)$$

Their work indicates that while the reaction is not strictly stoichiometric, for m above will not in general be an integer due to the equilibria involved, an acid-base titration might be feasible under strictly controlled conditions. However, the method would only be usable when murexide of known purity is available for the construction of an analytical curve. It was also foreseen that the presence of acidic or basic impurities would contribute an error. The above authors mentioned that precipitation was encountered above pH 9 during the titration of a solution containing calcium and murexide with base. This author carried out a similar titration. The resultant titration curve was that of a strong acid with a point of inflection at about pH 7. Formation of a green-black precipitate was noted near the inflection point, with more precipitate being formed as base was added. This precipitate was removed by filtration, washed with distilled water and dried at 110° C. Ignition of the precipitate at orange heat in a platinum crucible to constant weight resulted in an amount of calcium oxide that corresponded approximately to a

mole-to-mole ratio of calcium to purpurate. It was concluded that perhaps the formation of the calcium purpurate precipitate might lead to a gravimetric method for the determination of murexide. This approach was successfully carried out as described below.

In order to determine the optimum conditions for quantitative precipitation of calcium purpurate as quickly as possible, experiments were performed over various pH values, calcium concentrations, and amounts of murexide, with the relative completeness of precipitation evaluated by determining the amount of murexide remaining in solution colorimetrically after centrifugation of the precipitate.

EXPERIMENTAL PROCEDURE

Apparatus. A Beckman model H-2 pH meter with a pH 0-11, 5°-100° C. glass electrode was used. This instrument was standardized with a pH 7.00 buffer solution obtained from the same company and which in turn had been standardized against a Bureau of Standards buffer solution. Absorbance values were determined with a Beckman model B spectrophotometer equipped with 1 cm. matched Corex cells. A "clinical" type laboratory centrifuge fitted with 50 ml. tubes was utilized.

Reagents. C.P. calcium nitrate was weighed out on a trip scale to make a 1 M solution.

NaOH, 0.200 N, carbonate free.

Murexide, prepared by the method of Davidson (15) from alloxantin. The alloxantin was Eastman white label, used without further purification.

Procedure. The desired amount of murexide was weighed out to 0.1 mg. and placed in a 50 ml. beaker. The correct volume of 1 M calcium nitrate was then added from a graduated pipet. Distilled water was added, if necessary, to increase the volume to 20 ml. and the solution stirred until all the murexide was dissolved. The pH was then adjusted to the desired value by adding the NaOH slowly from a 5 ml. buret.

After adjustment of pH, the mixture was poured into a 50 ml. centrifuge tube and spun for 5 minutes at maximum speed. A portion of the resulting clear supernatant was then placed in a cuvette, and the absorbance determined at 505 mμ against distilled water in the spectrophotometer.

Two experiments were performed. In the first, 3.5, 7.0 and 20.0 ml. $Ca(NO_3)_2$ solution were added to 100 mg. murexide and the pH was adjusted to 9.0, 9.3 and 10.0 at each calcium level. The absorbances found are presented in table 1. It will be noted that the relative degree of precipitation decreases with increasing pH and calcium concentration. This is probably due to the neutralization of an additional proton with generation of

Table 1

ABSORBANCES OF SUPERNATANT AFTER CALCIUM PRECIPITATION

100 mg. murexide taken

ml. $Ca(NO_3)_2$	pH 9.0	pH 9.3	pH 10.0
3.5	0.348	2.24	3.50
7.0	3.50	3.50	4.0
20.0	3.5	(infinite)	(infinite)

a more soluble purpurate species (see reaction 2, page 7) in the former case, and to the formation of a more soluble calcium purpurate complex in the latter. On the basis of these data, another experiment was performed. Two quantities of murexide were taken, 40.0 and 100.0 mg. At each level of murexide, the pH was adjusted to 8.0, 8.5 and 9.0 for calcium volumes of 0.70, 1.75, and 3.5 ml. These volumes of calcium nitrate correspond to 2, 5 and 10 moles of Ca per mole murexide for 100 mg. murexide, respectively. The data are shown in table 2. Statistical analysis (5% significance level) of these data showed that the only significant difference detectable with the experimental design used was due to the varying amounts of calcium. The mean absorbance due to the 0.7 ml. level of calcium was significantly higher than either of the other two levels,

Table 2

ABSORBANCES OF SUPERNATANT AFTER CALCIUM PRECIPITATION

ml. $Ca(NO_3)_2$	pH 9.0	pH 8.5	pH 9.0
	--- 40 mg. murexide ---		
0.70	2.09	2.00	2.32
1.75	0.571	0.932	0.907
3.50	0.610	0.820	2.75
	--- 100 mg. murexide ---		
0.70	3.10	2.80	2.32
1.75	0.497	0.389	2.12
3.50	0.500	0.420	0.480

which did not differ significantly from each other. Although no significant difference among the absorbance means was found for the various pH values, it was decided that further adjustment of solutions to pH 9 would be undesirable in light of the trend shown in the first experiment (table 1).

It was concluded that if between 40 and 100 mg. of murexide are taken, the optimum conditions for precipitation of murexide as calcium purpurate are: 1) the adjusted pH value should lie between 8.0 and 8.5, and 2) between 1.75 and 3.50 millimoles of calcium should be

added per 20 ml. of solution before adjustment of pH.

Due to inability to obtain absolutely complete precipitation, it was proposed to determine the residual concentration of murexide in the solution colorimetrically, provided that this residual amount was very small. This was found to be the case.

Consequently, a tentative procedure was developed for the gravimetric assay of murexide. A preliminary determination on the sample of murexide obtained from Eastman gave a result of about 107% murexide, calculated on the basis of anhydrous calcium purpurate. The calcium content was found to be too low in the calcium purpurate precipitate for the anhydrous salt. The discrepancy was thought to be caused by a water of crystallization or constitution in the precipitate not removed at 110° C. This was to be supported by elemental analysis of the calcium purpurate and determination of the water.

Therefore an experiment was performed with a threefold purpose: 1) to test the applicability of the tentative procedure, 2) to assay five samples of murexide obtained from different sources, and 3) to determine the composition of the calcium purpurate precipitate. This work is discussed below and the results are presented in tables 3a and 3b.

Table 3a

PERCENT MUREXIDE FOUND FOR VARIOUS SAMPLES

Level of Murexide	Source of Murexide					Mean
	Eastman	Davidson	Hartley	Hach	CaM	
high	99.9	100.3	74.8	69.8	101.8	89.32
	(115.5)*	(110.6)	(111.4)	(111.1)	(100.2)	
low	100.4	101.0	74.1	70.3	102.9	89.74
	(71.9)	(71.4)	(71.4)	(72.5)	(59.4)	
means	100.2	100.7	74.45	70.05	102.4	

* The numbers enclosed in parentheses indicate the weight in milligrams of murexide taken for each determination.

Table 3b

ANALYSIS OF VARIANCE

Data of Table 3a

Variation Due to:	Sum of Squares	Degrees of Freedom	Mean Square	F	Remarks
Sample	2015.34	4	503.84	2239	Significant*
Murexide level	0.44	1	0.44	1.95	Not significant
error	0.90	4	0.225	---	
Pooled level plus error	1.34	5	0.268	---	

* The significance level used throughout this investigation was 5%.

PROCEDURE FOR MUREXIDE ASSAY

Apparatus. In addition to the spectrophotometer and pH meter described on page 8, small sintered glass filter crucibles of "fine" porosity were utilized.

Reagents. Calcium nitrate, 1 M, and 0.2 N NaOH as described on page 8 were used. Five samples of murexide were obtained from the following sources: 1) purchased from Eastman Organic Chemicals, Distillation Products Industries, Rochester 3, N. Y., 2) purchased from Hach Chemical Co., Ames, Iowa, 3) synthesized from alloxan (Eastman white label) according to the method of Hartley (20), in which alloxan or alloxantin is refluxed for several hours in anhydrous ethanol through which a stream of dry ammonia is being passed, 4) prepared by refluxing alloxantin (in this case, Eastman white label) several minutes with ammonium acetate dissolved in glacial acetic acid according to Davidson (15), and 5) by the metathetical reaction between calcium purpurate and ammonium oxalate. Murexide was prepared in this last manner as follows: Approximately 1 gram calcium purpurate was prepared from Eastman's murexide by the procedure to be given below. A 0.93 gram sample of this calcium purpurate was ground with 1.85 g. $(NH_4)_2C_2O_4 \cdot H_2O$, then stirred vigorously with 500 ml. distilled water for one hour at room temperature. The insoluble residue was removed by filtration through a

fine sintered glass filter funnel, the filtrate reduced to about one-half volume by distillation *in vacuo* (water aspirator) between 30 and 35 degrees centigrade. This solution was then cooled in an ice bath, the murexide filtered out and washed carefully with 95% ethyl alcohol. Each of five samples of the dye obtained in the above manners was dried to constant weight at 110° C. These five lots of murexide will hereafter be referred to as Eastman's, Hach's, Hartley's, Davidson's and "CaM", respectively.

The pH 9 buffer solution was prepared by dissolving 49.4 g. C.P. boric acid in 100 ml. distilled water, adding to this 852 ml. 0.200 N NaOH, followed by dilution of this solution to 2 liters.

ANALYTICAL METHOD. 1. *Precipitation*. The desired amount of murexide was weighed into a 50 ml. beaker, several drops of water were added, and this mixture made into a smooth paste with a flat-ended stirring rod (this technique was found to greatly decrease the time subsequently required for solution). Then 3.50 ml. 1 M $Ca(NO_3)_2$ and 16.5 ml. distilled water were placed in the beaker and the contents stirred until complete solution was attained. In the least pure samples, a light colored residue remained, so the solution was quantitatively filtered through paper and the residue discarded. The pH

of the solution was adjusted to 8.5 by means of the pH meter and the 0.2 N NaOH, with the solution being well stirred during the addition of the base. The material adhering to the electrodes was washed back into the beaker with a minimum amount of distilled water and the precipitate allowed to coagulate and settle by setting the beaker aside for five minutes at room temperature. Next, the precipitate was quantitatively filtered by means of suction with a sintered glass crucible, previously dried to constant weight at 110° C., with the filtrate and washings being caught in a clean suction flask. The precipitate was washed three times with distilled water and dried to constant weight at 110° C. in an air oven.

2. Determination of Murexide Remaining in Filtrate. The filtrate remaining in the suction flask (see immediately preceding paragraph) was quantitatively transferred into a 250 ml. volumetric flask, 50.00 ml. of the borate buffer solution added, then distilled water to the mark. The absorbance of this solution was determined at 485 mμ (see figure 1) in a 1 cm. cuvette against distilled water, and the total amount of the murexide contained in the filtrate (which should not exceed 5 mg.) was calculated by means of Beer's law, using a previously determined constant.

The Beer's law constant was obtained in the following manner: 125 mg. Eastman's murexide was dissolved in

250 ml. distilled water. Various exactly measured amounts of this solution were then added to 250 ml. volumetric flasks containing 50.00 ml. of the borate buffer and 0.5 ml. 0.200 N NaOH (approximately the volume required to adjust the pH to 8.5 as discussed above). The various solutions were diluted to the mark with distilled water and the absorbance of each solution determined as in the preceding paragraph. Beer's law was found to be obeyed up to a murexide concentration of 10 mg./250 ml., as determined by ten equally spaced points obtained in the described manner. The applicability of Beer's law to higher concentrations of the dye was not investigated at this time.

In view of the fact that the amount of murexide remaining in the filtrate was very small, it was contemplated that the Beer's law constant for pure murexide would have to be obtained by using the results of the gravimetric portion of the method and a series of successive approximations. However, this was not necessary in this case for the Eastman murexide sample was fortunately quite pure (table 3a).

3. Calculations. To determine the original weight of murexide taken, the weight of calcium purpurate found was multiplied by

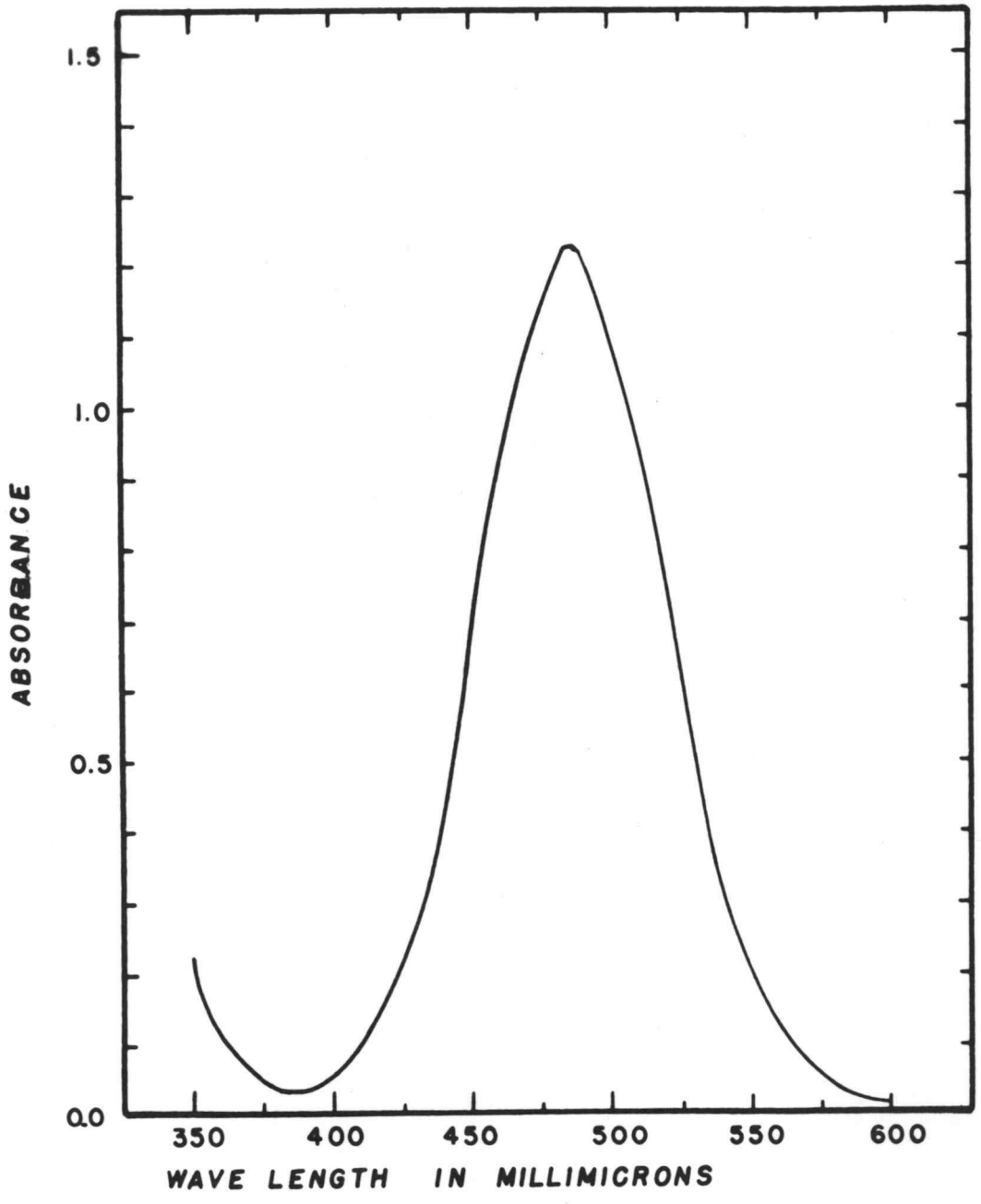


FIGURE 1

ABSORPTION SPECTRUM
OF
CALCIUM PURPURATE

4 mg. Murexide, 3.5 ml. 1M $Ca(NO_3)_2$, 250 ml. pH 9 Buffer

$$\frac{\text{molecular weight murexide}}{\text{molecular weight calcium purpurate monohydrate}} = 0.8792$$

and the amount of murexide remaining in the filtrate, calculated by Beer's law, was then added to this value.

ANALYSIS OF CALCIUM PURPURATE PRECIPITATE

1. Calcium. Except for the "CaM" precipitate, the duplicate residues remaining in the sintered glass crucibles were combined according to the original source of murexide, utilizing in addition, the calcium purpurate from the preliminary experiment discussed on page 14. The resulting five samples were then ignited to CaO in platinum crucibles under the full heat of a Meeker burner. Results. Percent calcium found was 12.45 with a standard error of 0.40 based on six determinations. The theoretical percent of calcium in the anhydrous salt, $CaC_8N_5O_6H_3$, is 13.13; and is 12.40 percent in the monohydrate, $CaC_8N_5O_6H_3 \cdot H_2O$.

2. Water. About 800 mg. calcium purpurate were prepared from Davidson's murexide according to the method outlined previously. This was dried at 110° C., ground to a fine powder, and redried to constant weight. Then 100 to 200 mg. portions were weighed into tared porcelain combustion boats, and dried in a drying pistol (Abderhalden) over P_2O_5 in vacuo (water aspirator) to constant weight. The reflux liquid was xylene, B.P. 137°-140° C. Results. Percent water found was 6.8 with a standard error of 0.5

based on six determinations. The theoretical water content for the monohydrate is 5.6%.

3. Total Nitrogen. An independent check on the composition of the precipitate was obtained by sending a sample of the calcium purpurate to the Microchemical Specialties Co., 1834 University Avenue, Berkeley 3, California. The results of the Dumas nitrogen analysis was 21.4 ± 0.3% nitrogen. The theoretical nitrogen content for the monohydrate is 21.67, and 22.95 for anhydrous calcium purpurate.

On the basis of calcium, water and nitrogen analyses, it was concluded that the precipitate was calcium purpurate monohydrate.

DISCUSSION OF RESULTS

The results of the experiment testing the proposed gravimetric method for the assay of murexide are presented in table 3a, and the analysis of variance of these data (table 3b) indicate that the method is quite reproducible. The results were not dependent upon the size of sample taken (level) within the weight limits of murexide investigated. The estimated standard deviation was 0.52 percent murexide (calculated from the last line, table 3b) with a corresponding 95% confidence interval of ± 1.3 percent murexide.

Utilizing Tukey's method (39), the assay results in the last line of table 3a may be divided as follows: The essentially pure Eastman and Davidson samples do not differ significantly, while the other three differ significantly among themselves and from the former two. Accordingly, the murexide prepared by Davidson's method was used for all remaining work unless otherwise specified since it was in largest supply. The high result of 102% murexide found for the "CaM" sample could have been caused by contamination of the sample with calcium oxalate. This sample was therefore discarded.

It was noticed in the water of crystallization determination that the calcium purpurate monohydrate was somewhat hygroscopic, which might explain the slightly high results. This was not apparent during the weighing of the salt on the sintered glass crucibles, which could presumably be explained by the fact that the precipitate was packed tightly on the mat, and thus a smaller surface area was exposed than in the case of the water determination.

It was thought advisable to use the monohydrate of calcium purpurate in the gravimetric procedure rather than the anhydrous salt because the latter compound was more hygroscopic than the monohydrate, and because the equipment for the removal of this water was not readily available.

It should be stated that while the water contained in the calcium purpurate is most likely present as water of crystallization, it could be contained as water of constitution. The latter possibility is weakly supported by Slimmer and Steiglitz (37), who contend that murexide adds a water of constitution when dissolved.

The assay results presented in table 3a were further verified in a relative manner by determination of the absorptivities for each sample. Thus, if the absorptivities of the various samples coincided within the experimental error after taking into account the fraction of murexide actually present, it could be concluded that the results in table 3a for the purity of the murexide samples are at least relatively correct. It should be realized that an assay of murexide by colorimetric means would be of doubtful value without an independent determination of purity, for results obtained in different laboratories with different instruments could be expected to vary.

Procedure. To each of nine 100 ml. volumetric flasks, 14.82 ml. 0.200 N NaOH and 25.00 ml. 0.200 M KH_2PO_4 were added (this buffer mixture results in a pH of 7.0 when diluted to the mark). Between 40 and 50 mg. murexide from each of the first four sources listed in table 3a was then weighed to the nearest 0.1 mg., transferred to 250 ml.

volumetric flasks, dissolved, and diluted to the mark with distilled water. A 10.00 ml. aliquot of the murexide solution was added to each of two 100 ml. flasks prepared as above, the solution diluted to volume, and the absorbances determined at 525 mμ (the absorption maximum of murexide under these conditions) in 1 cm. cells versus a blank prepared by diluting the ninth 100 ml. flask with distilled water. The absorptivity (a) of pure murexide for each of the five samples was computed according to the equation

$$a = 100\ A/bPw$$

where A is the absorbance at 525 mμ, w is the concentration of a sample in mg./ml. as calculated from the amount of material weighed out, b is the length of the light path through the solution, and P is the percent murexide as taken from table 3a.

The results are shown in table 4. Since the values for the absorptivities agree quite well, it was concluded that the proposed gravimetric method for the assay of murexide is acceptable. The advantage of the gravimetric method is that no errors due to standardization of reagents are introduced (as in the case of the redox procedures mentioned previously), and once the purity of one murexide sample has been quickly established, the colorimetric method may be utilized for other samples.

Table 4

ABSORPTIVITIES OF VARIOUS MUREXIDE SAMPLES

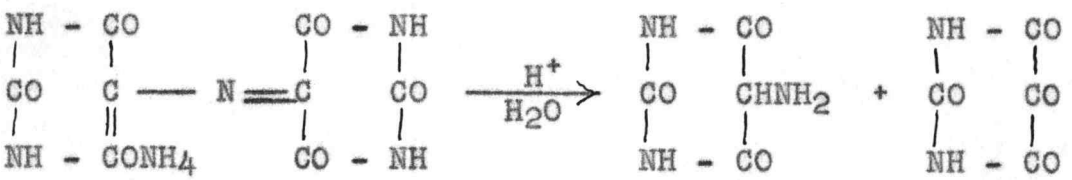
525 mμ pH 7.0

Source of Murexide	a	mean a
Eastman	49.1 48.5	48.8
Davidson	49.1 49.5	49.3
Hartley	49.2 49.6	49.4
Hach	48.6 49.0	48.8

III. THE DECOMPOSITION PRODUCTS

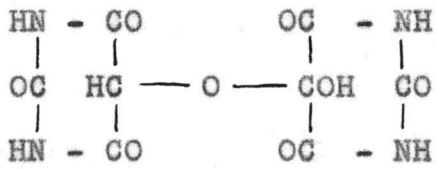
It would be very desirable for one to know the products of the decomposition of murexide in acid and basic solution, for then it would be possible to gain insight into what the impurities might be in a sample of murexide prepared according to a certain method, and also, one cannot write correct reactions without this type of information. It was found that the reactions occurring in basic solution are very complex, so the experimental work described in this chapter was meant to serve as a brief survey of the extent of the problem. Although a more thorough investigation was considered, it was thought that such an extensive study would not have been within the scope of the main objectives of this research.

Beilstein (2) found that murexide (I) decomposes in acid solution to uramil (II) and alloxan (III), which was in agreement with the earlier work of Gmelin, and which refuted the earlier suggestion of Liebig and Wöhler that the reaction products were alloxan, alloxantin, uramil, urea, and ammonium ion.



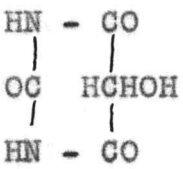
(I) murexide (II) uramil (III) alloxan

Davidson and Epstein (16) state, "The most conspicuous chemical property of murexide is its hydrolysis by acids, in which uramil and alloxan are obtained, accompanied more or less by alloxantin." (IV). It is a



(IV)

alloxantin



(V)

dialuric acid

matter of conjecture as to the meaning of "more or less alloxantin", for no experimental data on this subject were presented by these authors. However, they found that upon boiling uramil for 1-2 minutes with 6 N HCl, sufficient dialuric acid (V) was produced to yield a purple precipitate of barium dialurate on the addition of barium hydroxide. Davidson and Solaway (17) show that alloxan appears to be a true catalyst for the reaction

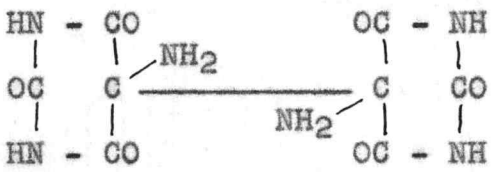
$$\text{uramil} + \text{water} \underset{H^+}{\overset{\text{alloxan}}{\rightleftharpoons}} \text{dialuric acid} + NH_4^+ .$$

They also report that alloxantin appears to be dissociated into alloxan and dialuric acid.

Thus, the decomposition of murexide in acid solution apparently results in the formation of alloxan and

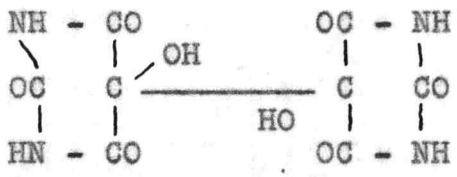
uramil as the primary reaction products, followed by the appearance of alloxantin and dialuric acid by the paths described.

Davidson and Epstein (16) doubt the correctness of the formulas given previously for murexide and alloxantin and propose the following:



(VI)

murexide



(VII)

alloxantin

(according to Davidson and Epstein)

This writer believes that formula VI is inconsistent with the properties of murexide, especially from the standpoint that the properties and composition of the potassium salt of purpuric acid as described by Beilstein (2) and by Schwarzenbach and Gysling (36) are incompatible with Davidson and Epstein's formula. The work on the preparation and composition of calcium purpurate described previously also supports this view, for an NH_4 group is replaced in the formation of the metal salts, whereas one would expect only hydrogen ion replacement if formula VI is correct.

Although the acid decomposition of murexide was well known in the eighteenth century, no mention of its instability in basic solutions was found in the literature until 1949. At that time, Schwarzenbach and Gysling (35) reported that the dye was unstable in alkaline solutions, but they apparently pursued the subject no further. This instability was also noted by the present writer in 1952 (31; 41). No other reports on this topic were found. Therefore, experiments were performed in an attempt to determine the products of the basic decomposition of murexide.

The problem was approached by assuming that uramil and alloxan could very likely be the primary reaction products. On this basis, the literature was studied in order to obtain information as to the fate of these two compounds in basic solution. In addition to the reactions discussed previously, dialuric acid is converted to alloxan by atmospheric oxidation (24), and uramil is subject to oxidation by air to violuric acid (VIII). Alloxan is very unstable in alkaline solution and decomposes to alloxanic acid (IX), which is oxidized by air to form parabanic acid (X), which then slowly decomposes to mesoxalic acid and urea (5; 21; 28). Uramil is attacked by strong, warm base to form aminomalonic acid, and thus would be expected to form this acid slowly in the presence

of cold dilute base.

CONHCONHCO:NOH	NHCONHCOC(OH)COOH	NHCONHCOCO
(VIII)	(IX)	(X)
violuric acid	alloxanic acid	parabanic acid

On the basis of this information, a series of qualitative tests (summarized in table 5) was developed for the above mentioned compounds, and each precipitate and solution obtained from the procedure discussed below was tested with it.

Procedure for attempted resolution of products of the basic decomposition of murexide. One-half gram of Davidson's murexide was dissolved in 500 ml. distilled water, five ml. of 1 N NaOH were added, the flask stoppered, and the solution allowed to stand at room temperature for 36 hours. After this time the pH had decreased to 9.5, so 5 ml. more of the 1 N NaOH were added. Twenty four hours later, the solution was clear and slightly yellow colored. By means of "Universal" pH indicator paper , the pH was adjusted to approximately 4 with 6 N HCl. This solution was concentrated to about 100 ml. by vacuum distillation (water aspirator) with the temperature of the water heating bath maintained between 30° and 40° C. The distillate containing only HCl was discarded. The residue in the distilling flask consisted of an orange colored

Table 5

QUALITATIVE TESTING SCHEME

(See next page for explanation of table)

Compound	Test								
	Tollen's	$Ba(OH)_2$	Fe^{++} NH_4OH	Biuret	Lead acetate	Heat, NH_4OH	HCl Heat, $Ba(OH)_2$	Ag^+	HNO_2
Alloxan	pos.	NR	blue	----	W	red	NR	W	--
Alloxantin	pos.	purple	blue	----	----	red	--	W	--
Dialuric acid	NR	purple	---	----	----	---	purple	--	--
Uramil	pos.	NR	NR	----	W	red	purple	--	gas
Aminomalonic acid	NR	NR	NR	----	W	NR	--	W	--
Mesoxalic acid	pos.	NR	---	----	----	NR	--	--	--
Urea	pos.	NR	---	pos.	NR	---	--	--	N_2
Alloxanic acid	NR	W	---	----	---	NR	W	--	--
Violuric acid	NR	red	---	----	dk. red	---	---	W	--
Parabanic acid	W	NR	NR	NR	NR	NR	---	W	--

EXPLANATION OF TABLE 5

Abbreviations

Pos. represents a positive reaction, NR means no reaction, W represents white precipitate formation, an underlined color indicates a precipitate of that color is formed, a color without an underline shows that a clear solution of that color is formed, N_2 represents nitrogen evolution, and a dashed line indicates that no information was available.

Sources of Data

Compound	References
Alloxan	7, 16, 21, 26
Alloxantin	11, 16, 21, 26
Dialuric acid	9, 21, 16, 26
Uramil	10, 16, 21
Aminomalonic acid	5
Mesoxalic acid	4
Urea	32
Alloxanic acid	3
Violuric acid	8
Parabanic acid	6

Alloxanic acid, violuric acid and parabanic acid were prepared by this author according to references 13, 1, and 14, respectively, and subjected to these tests.

solution and a small quantity of light buff colored precipitate. This mixture was cooled in an ice bath and the precipitate removed by filtration. The characterization of this precipitate (designated "precipitate IIa" hereafter) will be described in a later section. The filtrate, designated "filtrate IIb" was an orange-red color, which did not change upon addition of base to a small aliquot. A small portion of filtrate IIb turned water white on addition of sodium hyposulfite. Small volumes of filtrate IIb were then subjected to the test scheme of table 5, a distilled water blank being carried through all operations. Positive results are as follows: Tollen's reagent was reduced, and a white precipitate was formed, which did not appear to be totally composed of silver chloride. Barium hydroxide turned the solution pink, which is indicative of the presence of violurate ion, but no precipitate was formed. The addition of lead acetate solution caused the color of the solution to change to buff, and a slight amount of white precipitate was formed. On heating, this solution produced a voluminous white precipitate. The addition of nitrous acid solution resulted in gas evolution, and the addition of a few crystals of phenol had no effect. This indicated the presence of an alpha amino acid and/or urea, for the presence of a diazotizable amino group should have

incurred a color change on the addition of the phenol. Thus, the possible presence of compounds listed in table 5 was narrowed down to aminomalonic acid, mesoxalic acid, urea, violuric acid and parabanic acid.

The remaining volume of filtrate IIb was then extracted with three 25 ml. portions of ethyl ether, and the combined ether extracts evaporated to dryness. A slight residue was found, but not enough to enable further study. The extracted filtrate was then evaporated *in vacuo* to dryness, the temperature of the heating bath being kept at or below 40° C. The solid residue was extracted with 50 ml. alcohol by refluxing for 5 minutes, cooling to room temperature, filtering, then rinsing the flask and solid residue (residue IIb) with 25 ml. of the alcohol.

The alcohol extract was evaporated to a syrup, and after standing overnight, crystals were formed. The crystals were separated by filtration, washed with ether, and air dried (the filtrate was discarded). The biuret test on these crystals was negative, therefore urea was probably not present. Some of the white crystals seemed to appear cubic under the microscope, and may have been ammonium chloride. The melting block behaviour of the yellow crystals was as follows: Sublimation occurred at about 140° C.; at about 185° the yellow color changed to

tan, and at 230° much explosive decomposition took place.

The residue IIb was not dealt with further because it was realized that the presence of ammonium chloride would interfere with certain tests in the qualitative scheme. Accordingly, the whole procedure described above was repeated, but 6 N HNO_3 was substituted for the 6 N HCl, and all quantities were doubled. The results were approximately the same, the residue remaining after alcohol extraction of filtrate IIb (residue IIb') was subjected to the qualitative tests and the following results obtained: Silver nitrate produced a white precipitate. Barium hydroxide addition resulted in no reaction. Ferrous ammonium chloride plus ammonium hydroxide gave no reaction. Concentrated ammonium hydroxide produced no color change on heating. Tollen's reagent produced a gelatinous dark precipitate, and on heating, silver was reduced. The addition of lead acetate solution resulted in only very slight precipitate formation, and no further precipitation occurred on heating. The biuret test was negative. The addition of 1 M $Ca(NO_3)_2$ produced no reaction in the cold, but a white precipitate was formed on heating.

It thus appears from this preliminary survey that the major constituent of residue IIb' may have been parabanic acid, with perhaps some aminomalonic acid present.

Working on the assumption that the only compound present in residue IIb' that gave a precipitate with silver was parabanic acid, the silver precipitate obtained by addition of silver nitrate to a water solution of the residue was washed with distilled water, air dried, and ground in a mortar to a fine powder. Pure silver parabanate was then prepared from parabanic acid, which in turn was prepared by the method of Blitz and Schiemann (14). The infra-red spectra of a Nujol mull of these two materials were then obtained in an automatic recording Perkin-Elmer Model 12 C spectrophotometer, equipped with sodium chloride optics. The results were inconclusive.

Precipitate IIa was characterized as uramil by utilization of the qualitative scheme of table 5 and by comparison of its infrared spectrum with that of pure uramil. (A sample of purified uramil was kindly furnished by Professor B. E. Christensen, Chemistry Department, Oregon State College). The infrared spectra were obtained in the manner described above and are shown in figure 2. In the original recorder charts, the 1000 to 2000 wave number region was greatly expanded and the detail was quite clear. Ten transmittance minima for the uramil and precipitate IIa were found to check with an average deviation of 4 $cm.^{-1}$. The shoulder in the region of

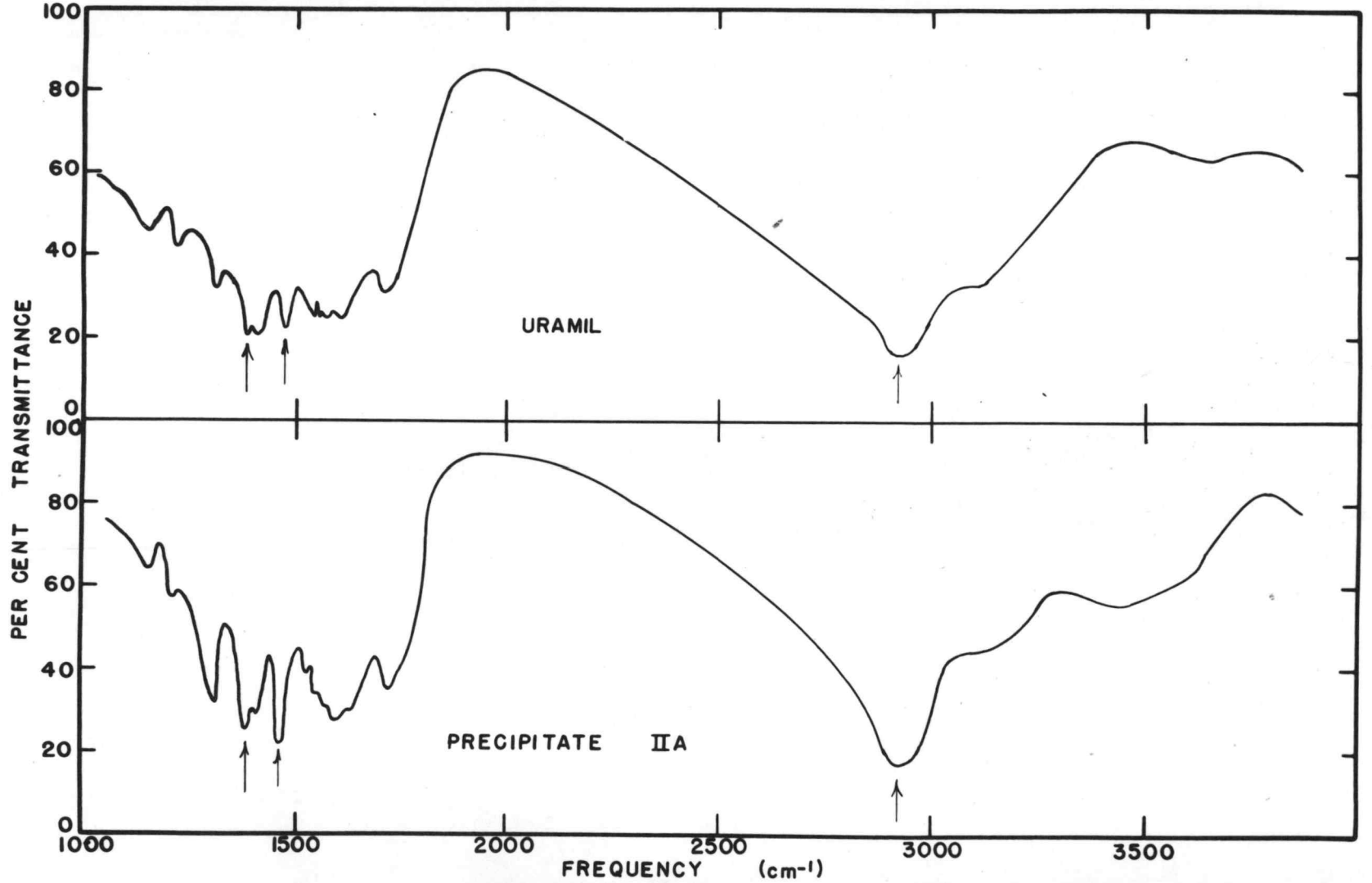


Figure 2 *INFRARED SPECTRA* **(Arrows indicate Nujol peaks.)**

3030-3130 cm.$^{-1}$ is due to uramil. The only point where the two curves did not correlate was at about 3430 wave numbers, and this was found to be caused by contamination of precipitate IIa with violuric acid. One would not expect to obtain exactly the same shape curves for the two samples, for with the qualitative technique used one could not control exactly the relative amounts of sample and Nujol.

The results of the qualitative tests (table 5) on precipitate IIa are as follows: No reduction was caused with Tollen's reagent, but a voluminous white precipitate was formed, as was formed with silver nitrate. No reaction was produced with barium hydroxide. When the precipitate was heated gently with concentrated ammonium hydroxide, a red color was formed. Addition of lead acetate solution to a solution of the precipitate resulted in a white precipitate. A purple precipitate was formed when the solid was boiled several minutes with 6 N HCl, cooled, and solid barium hydroxide added. The precipitate was insoluble in ether, and soluble in base. Inspection of table 5 shows that, of the compounds listed, these results are consistent for uramil, as is the infrared spectrum.

Discussion. The complexity and large number of decomposition products apparently formed in basic solution

make it difficult to isolate the individual compounds with the techniques utilized. Therefore, a more thorough investigation was not undertaken due to the reasons previously discussed. It has been shown that uramil is apparently formed to a certain extent, and it is quite likely that it is a primary reaction product. The identity of any other compounds could not be determined with the procedures used, although the tests on filtrate IIb and on residue IIb' do not exclude the possibility of the presence of parabanic acid. Since alloxan reacts with base to form alloxanic acid, and alloxanic acid is subject to air oxidation in the presence of base to parabanic acid, it is somewhat likely that alloxan could also be a primary reaction product.

IV. EFFECT OF IMPURITIES ON THE DECOMPOSITION OF MUREXIDE

Before the study of reaction rates can be undertaken, the effect of possible impurities on the decomposition reaction must be ascertained, for it might happen that one or more of the impurities could have a catalytic effect, and thus invalidate much of the data. Since the uncertainty of purity of the purest samples of murexide available is about one percent (see chapter II), one must be certain that presence of any foreign compounds, whatever they may be, have no catalytic properties or, if catalysts are present, their effect must be controllable.

Since it was not feasible to analyze the various samples of murexide for specific compounds present as impurities, an indirect approach was necessitated. If any catalytic phenomena exist, they may be separated into two general classes: oxidation-reduction and acid-base. The former may be investigated experimentally by determining the reaction rate of murexide with and without the presence of suspected redox catalysts in a buffered solution. The presence of protolytic or hydroxylytic substances could be determined in an impure lot of murexide by measurement of pH, with an additional check obtainable by titration.

Any impurities present in the murexide can conceivably originate from two sources: 1) from improper or incomplete separation of starting materials and products from side reactions during preparation of the dye, and 2) decomposition of the murexide in solution before the final crystallization, with some of the decomposition products being precipitated at that time. Since the starting material for the synthesis of murexide is generally alloxan or alloxantin, these compounds may be present as impurities. It was shown in chapter III that when alloxantin is placed in aqueous solution an equilibrium mixture is formed between alloxantin and alloxan plus dialuric acid, that dialuric acid reacts reversibly with ammonium ion to form uramil, and that murexide could decompose to produce uramil and alloxan. Therefore, the compounds most likely to be present as impurities are dialuric acid, uramil, alloxan and alloxantin. In Hartley's method of synthesis (20), absolute alcohol and ammonia gas are used, but the purification procedure practically excludes the presence of these materials in the final product. Davidson's method (15) utilizes glacial acetic acid and ammonium acetate, followed by salting out of the dye from an ammonium chloride solution. With this procedure, the possibility of the presence of acetic acid and ammonium acetate is

practically nil in the final product. If the final product were slightly contaminated with ammonium chloride, the pH decrease caused by the hydrolysis of this compound would be very small compared to that caused by hydrolysis of the ammonium ion of the murexide.

On the basis of this information, the following experiments were performed: 1) The relative rates of decomposition of Hach's murexide (70% pure) and of Davidson's murexide were compared in buffered solutions of pH 3.03, 6.98 and 11.49 at an ionic strength of 0.10 at 25.0° C. This should detect any significant redox catalysis of the impurities present in Hach's murexide. 2) Alloxantin and uramil were added to buffered solutions of Davidson's murexide at pH 5.11. The above discussion shows that dialuric acid and alloxan would automatically be formed in the solutions. 3) The pH of solutions of Eastman's, Davidson's and Hach's murexide were measured, and then titrated with standard base.

EXPERIMENTAL PROCEDURE

Apparatus. Absorbances and pH values were determined as described on page 8. The temperature of the reaction solutions was controlled by immersion in a water bath, maintained at 25.0 ± 0.2° C. The water bath temperature was controlled by means of a thermistor and the electronic thermoregulator of Sweeney (37,pp.46-52).

Solutions. Standardized 0.2 N "carbonate free" NaOH, 0.400 M KH_2PO_4, 0.400 M potassium acid phthalate, 0.200 N HCl, and 0.500 M KCl were prepared from C.P. chemicals. These chemicals contained no detectable quantities of calcium as determined by qualitative analysis with murexide in basic solution (31; 41).

The following solutions were freshly prepared just before use: Murexide solution A, 50.0 mg. Davidson's murexide dissolved in 250.0 ml. distilled water; Murexide solution B, 40 mg. Davidson's murexide dissolved in 100.0 ml. distilled water; Murexide solution C, 57.0 mg. Hach's murexide dissolved in 100.0 ml. distilled water; Alloxantin solution, 20 mg. Eastman white label alloxantin dissolved in about 100 ml. distilled water with gentle heating then transferred to a 250 ml. volumetric flask and diluted to the mark with distilled water; Uramil solution, 10.1 mg. uramil (a purified sample kindly furnished by Dr. B. E. Christensen) dissolved in 10.0 ml. of the 0.2 N NaOH, transferred to a 100 ml. volumetric flask, and diluted to the mark with distilled water.

Experiment 1: Comparison of rate of decomposition of Hach's and Davidson's murexide.

pH 6.98: To each of four 250 ml. volumetric flasks, 5.94 millimoles NaOH, 25.0 ml. 0.400 M KH_2PO_4 and 12.53 ml. 0.500 M KCl were added. Distilled water was

added to about 15 ml. below the mark, and the flasks were allowed to remain in the 25° water bath until temperature equilibrium was attained. Next, 10.00 ml. murexide solution B was pipetted into each of two of the flasks, and 10.00 ml. of murexide solution C was similarly delivered into each of the two remaining flasks. The flasks were diluted to volume, mixed, and replaced in the water bath. The time was recorded when the pipet was one-half drained. The pH of each solution was determined at the beginning and end of the experiment (about two weeks). The absorbances at 525 mμ were determined at approximately 24 hour intervals, at identical times from the start for each flask. Since this was the first rate experiment performed in this investigation, the experimental design used was analysis of variance. The results of the analysis of variance calculations made with the $\log_{10}$ of the absorbances are given in table 6. The logarithms of the absorbances were also plotted against time[3] on linear graph paper. Straight lines resulted, but there was a point of discontinuity, with the latter portion of the line having the same slope graphically as the first portion. It was found that this was caused by a sticking of the relay in the thermoregulator assembly, causing the

[3] See Appendix.

Table 6

ANALYSIS OF VARIANCE RELATIVE RATES OF DECOMPOSITION OF HACH'S AND DAVIDSON'S MUREXIDE AT pH 6.98 AND 25° C.

Observations converted to $\log_{10}$ A

Variation Due to:	Sum of Squares	Degrees of Freedom	Mean Square x 10^{-4}	F	Remarks
Murexide source	0.00020641	1	2.0641	2.63	Not significant
Time	0.63401126	10	634	----	----
Interaction	0.00011278	10	0.1128	0.10	Not significant
Error	0.00240155	22	1.0916	----	----
Pooled: Error plus Interaction	0.00251433	32	0.78573	----	----

bath temperature to rise temporarily to 32° C., and then to return permanently to 25° C. The slope of the first portion of line resulting from the log absorbance vs. time plot was evaluated by the method of least squares (18; chapter II) and is given in table 7. The relation between log A (where A is the absorbance) vs. time was tested statistically for linearity, and this hypothesis was accepted at the 5% significance level, for the F value was found to be 0.27 with 9 and 33 degrees of freedom. It was therefore concluded that the decomposition of murexide is

first order with respect to the dye under these conditions if the assumption is made that Beer's law is obeyed. It will be shown later that this assumption is valid.

Table 7

FIRST ORDER RATE CONSTANTS[4] FOR DAVIDSON'S AND HACH'S MUREXIDE AT VARIOUS pH VALUES

25° C., Ionic Strength 0.10

pH	Source of Murexide	k min.$^{-1}$	s_k* min.$^{-1}$	Degrees of Freedom
6.98	Davidson and Hach[5]	1.101×10^{-4}	1.15×10^{-6}	42
3.03	Davidson	4.195×10^{-2}	4.12×10^{-4}	43[6]
3.03	Hach	4.119×10^{-2}	2.44×10^{-4}	12
11.49	Davidson	3.348×10^{-3}	6.38×10^{-5}	12
11.49	Hach	3.304×10^{-3}	5.83×10^{-5}	12

[4] See Appendix.

[5] Rate constants combined, due to results shown in table 6.

[6] Taken from table 10, page 60.

* s_k represents the estimated standard error of the rate constant k.

pH 3.03: To each of four 100 ml. volumetric flasks, 10.16 ml. 0.200 N HCl, 12.50 ml. 0.400 M potassium acid phthalate, and 9.8 ml. 0.5 M KCl were added. Distilled water was added to the 90 ml. marks (previously made) and the flasks were placed in the 25° water bath and allowed to attain temperature equilibrium. Then 10.00 ml. aliquots of murexide solution B were added to each of two of the flasks, and the same volumes of murexide solution C were added to the other two flasks. Zero time was taken when the pipets were half empty. The flasks were replaced in the water bath, and the absorbances determined at 505 mμ at 5 minute intervals . The pH was measured at the end of each run. The plot of log A vs. time was linear (exemplified in figures 4 and 5), the points being less scattered about the line than for the pH 6.98 case. Therefore, the slope of the line and the estimated standard error of the slope were obtained by the method of least squares. These are reported in table 7.

pH 11.49: To each of four 100 ml. volumetric flasks, 0.400 milliequivalents of NaOH, 19.20 ml. 0.500 M KCl and distilled water to the 90 ml. mark were added. The rest of the procedure was the same as in the above paragraph with these exceptions: the absorbances were determined at 30 minute intervals, the wavelength was 525 mμ, and the pH was calculated with the mean activity

coefficient of 0.1 M KCl given by Latimer (30). (This is a fairly good approximation because the mean activity coefficient vs. the ionic strength curves for NaOH and KCl coincide up to an ionic strength of about 0.1). The results are shown in table 7.

Experiment 2. Effect of added impurities.

No compounds added: To each of two 100 ml. volumetric flasks, 2.39 milliequivalents NaOH and 12.50 ml. 0.500 M KCl were added. The remainder of the procedure was similar to that given above for pH 3.03, with murexide solution A (Davidson's) being used.

Uramil: The procedure was like that of the immediately preceding paragraph, with the amount of NaOH changed to 2.20 milliequivalents, and with the addition of 10.0 ml. of the uramil solution.

Alloxantin: The procedure was identical to that of "no compounds added" with the addition of 25.00 ml. of the alloxantin solution.

These concentrations of uramil and alloxantin are about equimolar with that of murexide. Higher concentrations were not feasible, due to the low solubilities of the two compounds.

The results of this experiment are reported in table 8.

Table 8

COMPARISON OF RATES OF
DECOMPOSITION WITH ADDED IMPURITIES

pH 5.11

Compound Added	k min.$^{-1}$	s_k min.$^{-1}$	Degrees of Freedom
None*	5.448×10^{-4}	3.55×10^{-6}	35
Alloxantin	5.582×10^{-4}	4.67×10^{-6}	14
Uramil	5.669×10^{-4}	4.56×10^{-6}	14

* Taken from table 10, page 60.

Experiment 3. Titration and pH measurement.

Additional apparatus: A Beckman model H-2 pH meter equipped with a pH 0-11 glass electrode was used. The standardized 0.2 N NaOH was delivered from a 5 ml. buret. Carbon dioxide was removed from the compressed air by passing through 20% potassium hydroxide, distilled water and glass wool, in the order given. The gas washing bottles were equipped with sintered glass tubes for fine dispersion of the bubbles. The 100 ml. titration beakers were covered with a card containing the suitable number of holes for introduction of apparatus.

Technique: It was first determined that 15 minutes sufficed to sweep the carbon dioxide from 50 ml. distilled water, as shown by attainment of a constant pH value of 7. As a check on the procedure, 1.00 ml. 0.200 N HCl plus 50 ml. distilled water was potentiometrically titrated. A "beautiful, textbook" curve resulted, with a point of inflection at pH 7 with the stoichiometric amount of base consumed (figure 3). Then 55.0 mg. samples of Eastman's, Davidson's and Hach's murexide were weighed into 100 ml. beakers and 50 ml. distilled water added. After solution, the pH was recorded, the CO_2 swept out for 15 minutes, the pH again recorded, and then the sample was potentiometrically titrated with the standard base. Duplicate determinations were made. The titration curves all had a point of inflection at pH 7, as shown in figure 3. The pH data are reported in table 9. From the milliequivalents of base used, and the assay results on Hach's murexide (70% pure), it was calculated that the overall equivalent weight of the impurities in this sample of dye was about 1500, which seems to indicate that some of the foreign materials present are neutral.

Discussion of Results.

Inspection of tables 6 and 7 show that the impurities present in Hach's murexide, whatever they may be, have no significant effect on the rate of decomposition

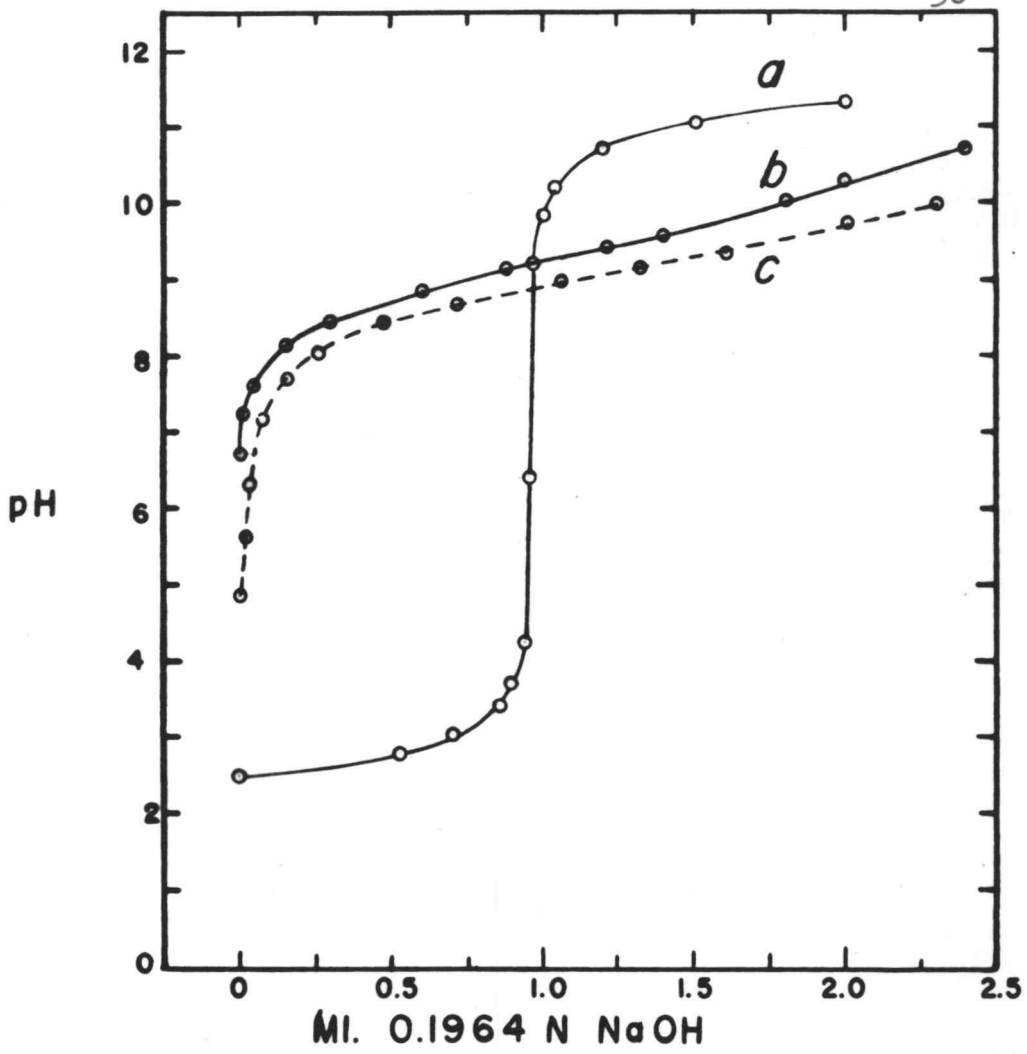


FIGURE 3

POTENTIOMETRIC TITRATION OF MUREXIDE

Curve a: HCl
b: 55mg. Davidson's murexide
c: " " Hach's "

Table 9

pH MEASUREMENTS ON VARIOUS MUREXIDE SAMPLES

pH measured:	Source of Murexide		
	Davidson	Eastman	Hach
before CO_2 removal	5.5	5.9	4.6
	5.5	5.9	4.6
after CO_2 removal	6.70	6.71	4.90
	6.70	6.82	5.03

under the conditions studied. It would appear that uramil and alloxantin could have a small effect on the rate constant, as seen in table 8. However, it was concluded that no appreciable effect exists, for the next chapter will show that an error of 0.05 in the determination of pH will cause about a 10 percent change in the rate constant in this pH region, and an accuracy of determination of about 0.05 pH unit is about the best obtainable with the measuring system used.

The next chapter will show that the rate of the decomposition increases with an increase in the acidity, in acid solution. As seen in table 9, it would be expected that a solution of Hach's murexide would decompose very much faster in an unbuffered solution than would the murexide obtained from the other two sources. Thus, the presence of any acidic impurities in a sample of murexide

would reduce the stability of the dye in unbuffered solution, and which would explain the observations of Ostertag and Rinck, and of this author, that the stability of murexide in unbuffered solutions apparently varies with commercial source (as discussed in the introductory chapter).

V. THE RATES OF REACTION

In view of the previous discussions, and the fact that no reports on quantitative studies of the decomposition of murexide were found in the literature, an investigation of this topic was undertaken.

In this section, the experimental procedures used and the resultant data are presented. The possible theoretical significance of the data obtained will be discussed in chapter VI.

The effect of pH, ionic strength, solvent and temperature on the rate of reaction was studied. Due to the fact that murexide was found to be insoluble in all of the readily available organic solvents, it was only possible to study the effect of solvent in various organic liquid-water solutions in which the organic liquid was completely miscible with water.

It was found that under constant conditions of solvent and temperature, the rate of decomposition was always first order with respect to the murexide concentration. This was determined by plotting $\log_{10} A$ (A = absorbance) against time for each set of experimental data obtained (see appendix). Two examples of the linear plots thus obtained are figures 4 and 5.

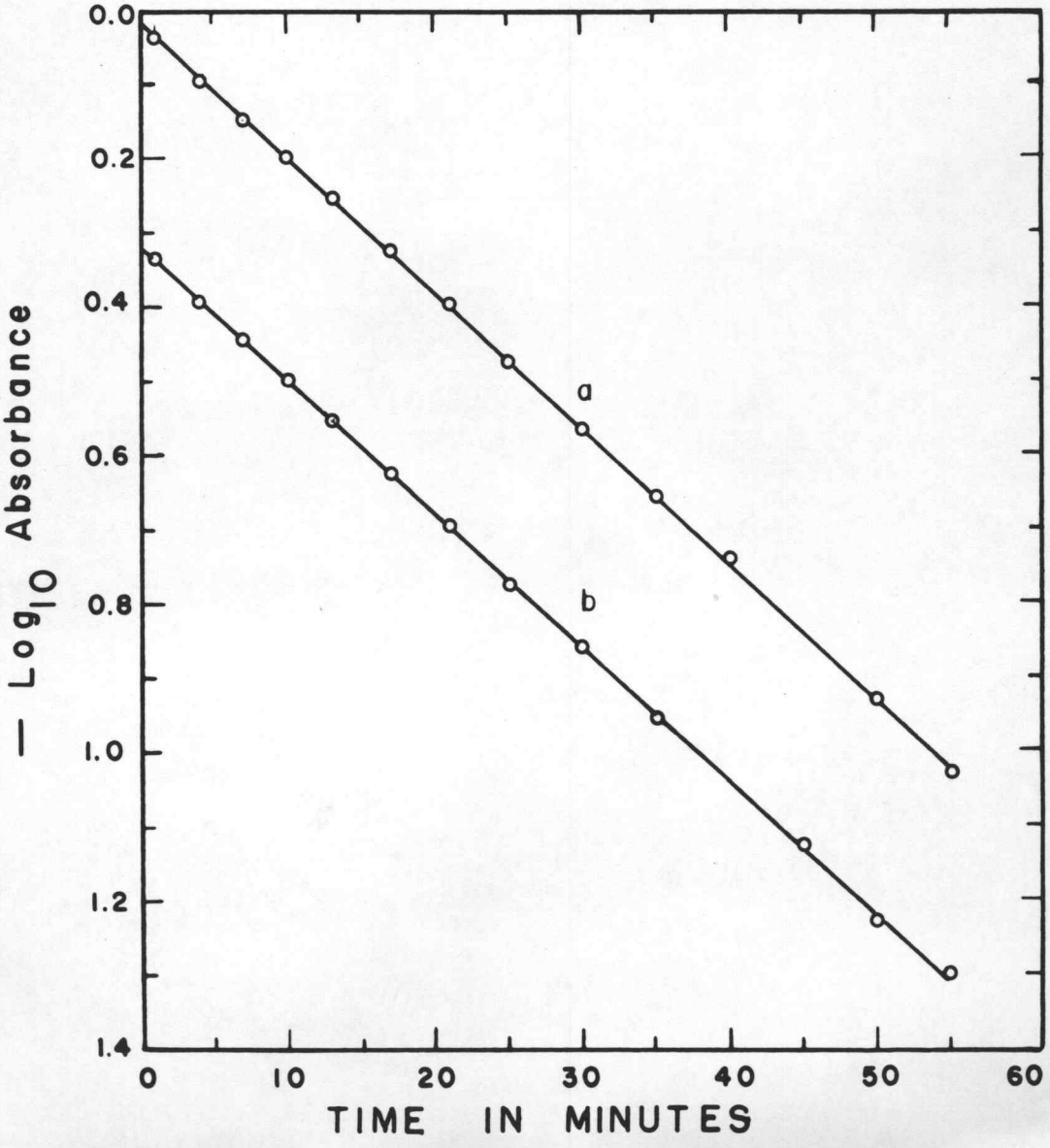


FIGURE 4

DECOMPOSITION OF MUREXIDE
AT pH 3.13
25°C. IONIC STRENGTH 0.10

Curve a: Initially 20 μg./ml. murexide.
b: " 10 " " " .

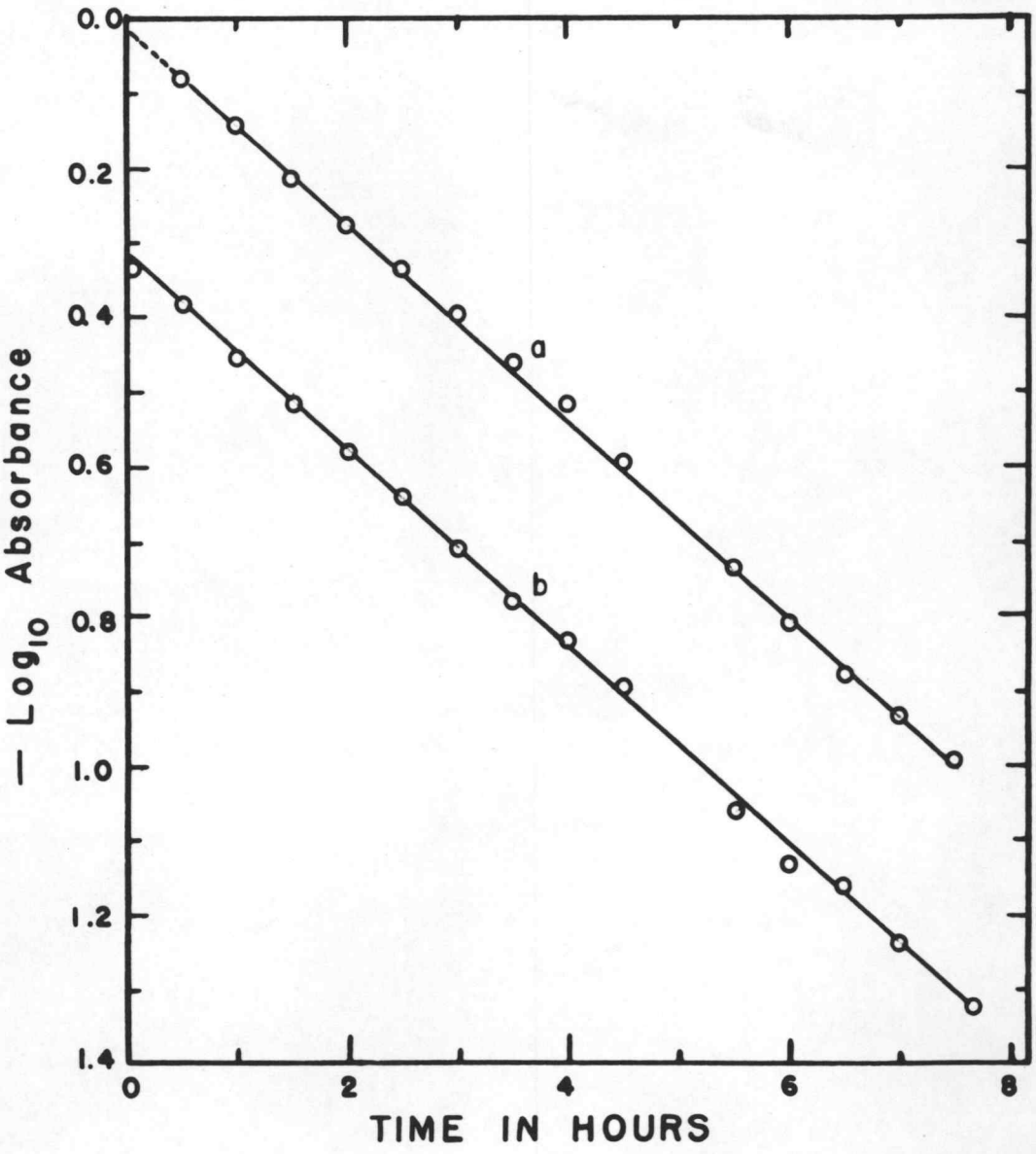


FIGURE 5

DECOMPOSITION OF MUREXIDE AT pH 11.88

25°C. IONIC STRENGTH 0.10

Curve a: Initially 16 μg./ml. murexide.
b: " 8 " " "

EXPERIMENTAL PROCEDURE

Apparatus: In addition to the equipment described before, a thermometer calibrated to the nearest 0.05° C. by the Thüringisches Staatsprüfamt was used for measurement of the water bath temperature, which did not deviate more than 0.3° C. from the mean during any run. A water-jacketed vessel through which water from the bath could be circulated was constructed for use in the measurement of pH at temperatures differing more than five degrees from that of the room. Time was determined with a precision stopwatch and a chronometer that was accurate to about 30 seconds per day. The reaction flasks were 100 ml. volumetric flasks, additionally marked at 90 and 95 ml. Five ml. pipets fitted with the proper size rubber stoppers were placed in each flask after the final solution mixing, and were used for sample withdrawal. The pipets were capped with rubber policemen for the forty and fifty degree experiments.

Reagents: The absence of calcium in all of the chemicals in the following list was verified by the method referred to previously. All reagents used were of C.P. or better grade unless otherwise specified.

0.200 M HCl
0.400 M Potassium acid phthalate
0.500 M Boric acid
0.200 M NaOH, "carbonate free", stored in borosilicate glass
0.400 M KH_2PO_4
0.200 M Piperidine, stored in borosilicate glass
0.500 M KCl
1 M $Ca(NO_3)_2$

Methyl alcohol, commercial, redistilled
Ethyl alcohol, commercial, absolute
n-Propyl alcohol, commercial, redistilled
Isopropyl alcohol, commercial, redistilled
Acetone, commercial, redistilled
Glycerol
Dioxane, purified

The dioxane was purified in the following manner: To 3 kg. dioxane, 40 ml. concentrated HCl and 200 ml. of water were added and the solution was allowed to stand at room temperature for 1.5 hours. 40 g. KI were added and the mixture was stirred well for 15 minutes. After this time, KOH pellets were added until the solution was saturated. The upper dioxane phase was filtered by decantation through paper and the filtrate was distilled from KOH pellets. B.P. 99.0-100.5° C., uncorrected. The tested peroxide-free dioxane was stored in the absence of light.

Two stock murexide solutions were prepared just before use from the dye (synthesized according to Davidson) and distilled water. One solution contained 20 mg. murexide per 100 ml, and the other contained 40 mg. per 100 ml.

General Procedure: Unless otherwise specified, 0.05 M buffer mixtures were used for all runs in the pH range 3 to 11.3. Except for the piperidine buffer system, the reagents were mixed in the porportions given by Willard, Merritt and Dean (40,p.136). The amount of NaOH required for adjustment of the piperidine buffer system to the desired pH (near 11) was determined by experiment, as it was for certain cases of mixed solvent, i.e., the borate system in 50 volume percent dioxane. For solutions of pH value greater than 11.3, NaOH was used, with the pH calculated according to the method given on page 46. Except for the study of the effect of ionic strength, all experiments in aqueous solution were made at an ionic strength of 0.10 moles/liter, adjustment being made to this value with the KCl solution. In the mixed solvent experiments, the ionic strength was adjusted with KCl (unless otherwise specified) to the value comparable to 0.10 in water by means of the Debye-Hückel limiting equation. In using this equation, the assumptions made were that there was no volume change on mixing, and that the dielectric constants of the mixtures were proportional to the mole fractions of the constituents. The values given by Kortüm and Bockris (27,pp.616-620) for the various physical constants were used. It is realized that this will only very approximately lead to constant activity

coefficient quotients comparable to one another in the various systems studied, but it is better than no correction at all. This use of the Debye-Hückel limiting law was necessitated by lack of activity coefficient data in the literature.

The general technique was as follows: The desired amount of pH controlling solutions, KCl, and organic liquid, if so desired, were added to 100 ml. volumetric flasks and distilled water was added up to the 90 or 95 ml. mark. The flasks were placed in the water bath, and after temperature equilibrium had been attained, 10.00 ml. of the 20 mg./ml. murexide solution or 5.00 ml. of the 40 mg./ml. murexide solution were added to each flask. The time was recorded when half of the murexide solution was delivered. Distilled water was added to the 100 ml. mark, the contents of the flasks well mixed, and the flasks replaced in the water bath. At various intervals, portions were withdrawn with the 5 ml. pipets, and the absorbance measured at the wavelengths given in table 10. (It was necessary to use various wavelengths for murexide is an acid-base indicator; therefore, the absorption maximum shifts with pH). For studies at temperatures other than 25° C., 5.00 ml. of the 40 mg./ml. murexide reagent was utilized.

Table 10

RATE CONSTANTS FOR THE DECOMPOSITION
OF MUREXIDE IN AQUEOUS SOLUTION
AT 25° C. AND IONIC STRENGTH 0.10

pH	k min.$^{-1}$	s_k* min.$^{-1}$	Degrees of Freedom	Wavelength of Measurement, mμ
3.03	4.195×10^{-2}	4.12×10^{-4}	43	505
4.04	4.905×10^{-3}	5.99×10^{-5}	36	510
5.11	5.448×10^{-4}	3.55×10^{-6}	35	510
6.05	1.381×10^{-4}	8.36×10^{-7}	32	510
6.57	1.096×10^{-4}	2.95×10^{-7}	18	520
7.00	1.167×10^{-4}	9.44×10^{-7}	34	520
7.82	2.878×10^{-4}	3.02×10^{-6}	26	520
8.90	9.392×10^{-4}	6.26×10^{-6}	32	520
9.80	1.946×10^{-3}	3.75×10^{-5}	44	520
10.52	3.118×10^{-3}	2.76×10^{-5}	7	525
10.92	3.634×10^{-3}	2.74×10^{-5}	7	525
11.00	4.106×10^{-3}	4.01×10^{-5}	44	525
11.25	4.101×10^{-3}	1.72×10^{-5}	7	525
11.70	4.124×10^{-3}	2.79×10^{-5}	12	525
11.89	5.063×10^{-3}	1.33×10^{-4}	54	535
12.14	6.256×10^{-3}	3.06×10^{-5}	44	530
12.39	8.609×10^{-3}	6.91×10^{-5}	32	530
12.67	1.235×10^{-2}	7.53×10^{-5}	40	530
12.89	1.819×10^{-2}	1.82×10^{-4}	52	530

* Estimated standard error of k.

The pH of each solution was measured at the end of the run at the temperature in question. As mentioned before, the pH meter was standardized against a buffer solution obtained from Beckman Instruments, Inc., which in turn was calibrated by that company against a U. S. Bureau of Standards solution at various temperatures. It is believed that the pH measurements were accurate to ± 0.08 pH unit.

Approximately 8 to 10 absorbance determinations over a period of about two half-lives were obtained for each solution prepared. In the first few runs, about 12 absorbance readings were made over a period of about three half-lives. However, measurements during the third half-life generally resulted in absorbance values below 0.1 with the number of significant figures obtainable changing from three to two, thus when the absorbances were subsequently converted to logarithms the error was greatly increased.

Specific Details.

Effect of pH: A thorough study was undertaken at 25° C. and ionic strength 0.10. Except for pH values of 6.44, 10.52, 10.92 and 11.25, four runs were made at each of the pH values listed in table 10, with two of the runs at a given pH value containing twice the initial amount of murexide as the other two. This use of two initial

concentrations of the dye served two purposes: 1) to verify the obeyance of Beer's law at each pH, and 2) to show that the half-life was independent of the initial concentration of the dye, which is true only for first order reactions. These conditions were well obeyed at all pH values and are exemplified in figures 4 and 5. The logarithm to the base 10 of the rate constant is plotted against pH in figure 6 (this rate constant, pseudo-first order with respect to murexide, will be designated by $\underline{k}$ throughout this report). The slope of the line at any pH on this curve gives the exponential dependence of the rate of reaction on hydrogen ion activity. This is shown by the following relations: Since it has been demonstrated that the decomposition reaction is first order with respect to the murexide concentration (see also the appendix), the observed first order rate constant $\underline{k}$ will be a function of solvent conditions. If all variables except the hydrogen ion activity (H^+) are held constant, suppose

$$k = k' (H^+)^n$$

where $\underline{n}$ is the order of the reaction with respect to (H^+) and k' is the specific rate constant, then

$$\log_{10} k = \log_{10} k' - n(pH)$$

and

$$\frac{d (\log_{10} k)}{d (pH)} = - n .$$

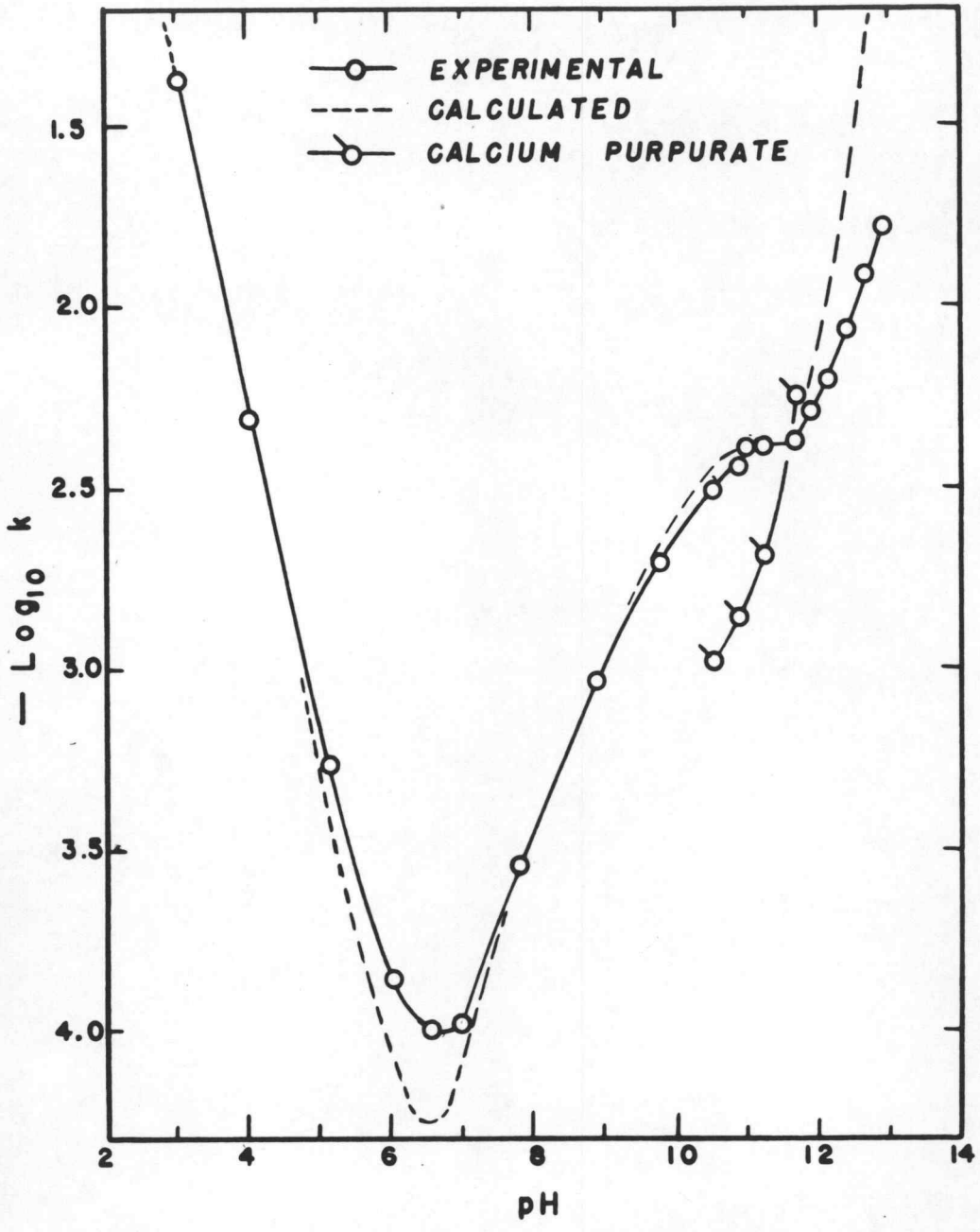


FIGURE 6

VARIATION OF k WITH pH

25° C. IONIC STRENGTH 0.10

Thus figure 6 shows that the reaction is first order with respect to hydrogen ion activity in the pH region of about 3 to 5, and is one-half order with respect to hydroxyl ion activity in the pH region of about 7 to 9. The "step" in this curve around pH 10 to 12 was quite unexpected, but its existence was verified by additional runs. An attempt will be made to explain this phenomenon in a later chapter.

Effect of Ionic Strength: It was mentioned in the previous paragraph that the decomposition of murexide is first order with respect to hydrogen ion activity in the pH range of about 3 to 5 and is one-half order with respect to hydroxyl ion activity in the pH region of about 7 to 9. Since it was not considered feasible to study the effect of ionic strength on the observed rate constants for the whole range of pH previously mentioned, the variation of the specific rate constants for these pH regions (k_1 and k_2, respectively) with ionic strength was studied. The data are given in tables 11 and 12, and are presented graphically in figures 7 and 8.

An approximate equation for the variation of $\underline{k}$ with pH will be presented on page 87. An attempt was made to study the variation of rates (k_3 and k_4) with ionic strength for the pH range of values greater than 9. The results were inconclusive, for k_3 and k_4 were

Table 11

VARIATION OF RATE OF DECOMPOSITION WITH IONIC STRENGTH IN AQUEOUS ACID SOLUTION

25° C.

Ionic Strength, moles/l.	pH	$k \times 10^2$ min.$^{-1}$	$s_k \times 10^4$ min.$^{-1}$	Degrees of Freedom	k_1 min.$^{-1}$ (moles/1)$^{-1}$
0.040	3.20	4.241	4.45	10	67.4
0.050	3.14	4.260	1.93	8	58.9
0.067	3.12	4.198	2.99	10	55.4
0.100	3.03	4.195	9.49	43	45.0
0.100	4.04	0.491	0.60	36	53.8
0.200	3.04	3.855	3.44	10	42.3

Table 12

VARIATION OF RATE OF DECOMPOSITION WITH IONIC STRENGTH IN WEAKLY ALKALINE SOLUTION

25° C.

Ionic Strength, moles/l.	pH	$k \times 10^4$ min.$^{-1}$	$s_k \times 10^6$ min.$^{-1}$	Degrees of Freedom	k_2 min.$^{-1}$ (moles/l.)$^{-1/2}$
0.0085	8.40	3.843	3.96	6	0.243
0.0219	8.38	3.937	11.1	6	0.254
0.0416	8.36	4.032	3.04	6	0.266
0.0650	8.48	5.084	3.62	6	0.293
0.1000*	---	---	---	---	0.351±0.02**

* k_2 is the weighted mean calculated from pH 7.00, 7.82 and 8.92 data in table 9.

** Indicates the range of calculated values.

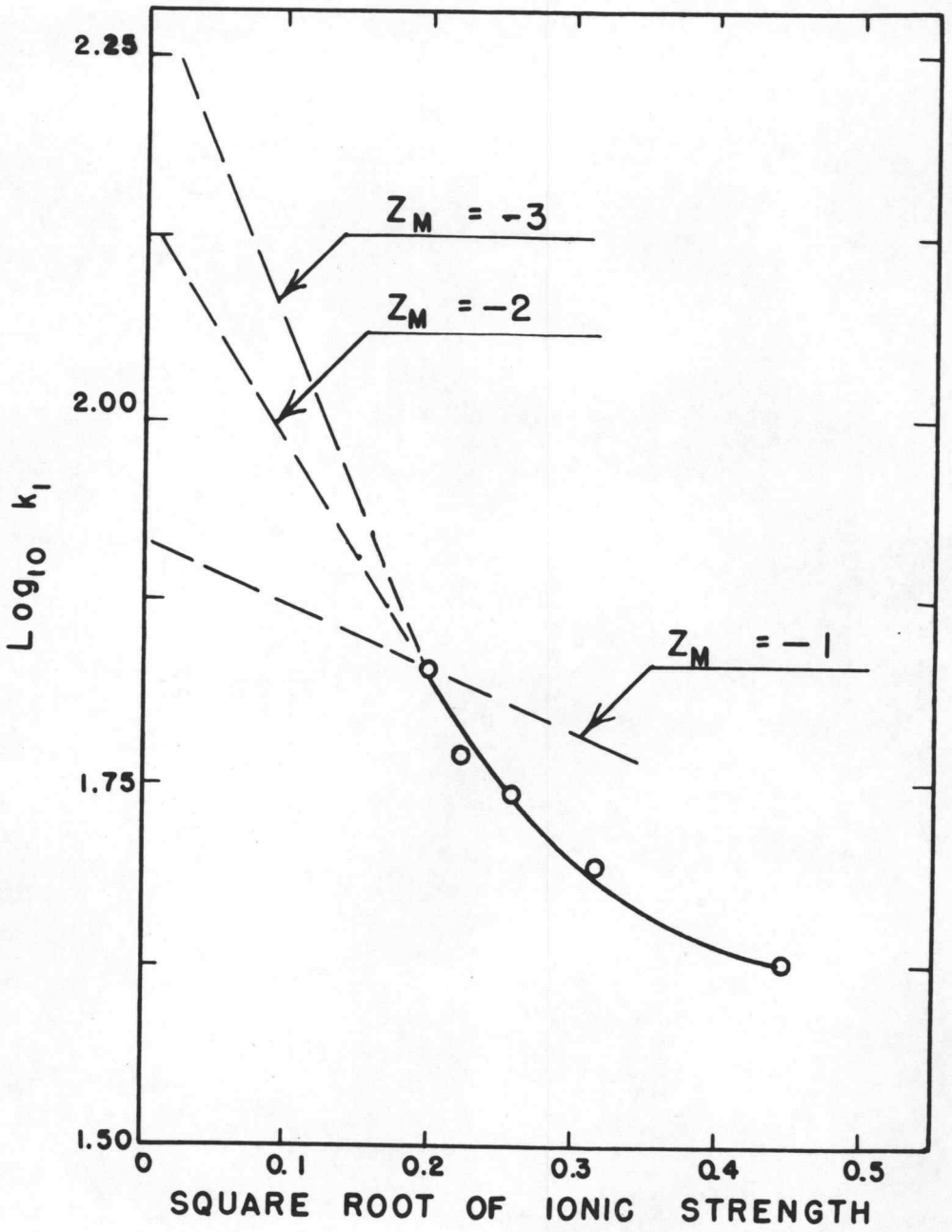


FIGURE 7

VARIATION OF k_1 WITH IONIC STRENGTH

25° C.

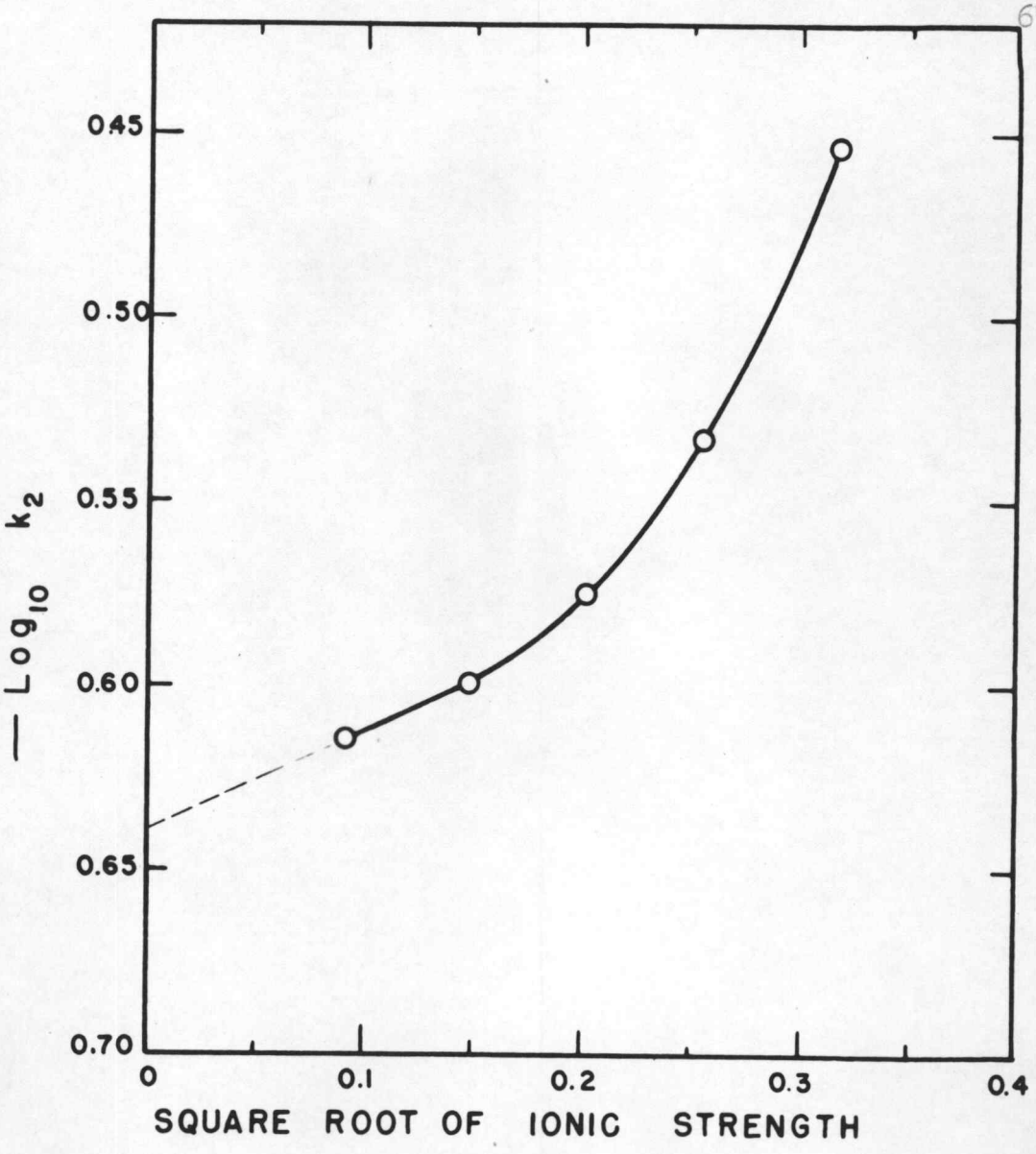


FIGURE 8

VARIATION OF k_2 WITH IONIC STRENGTH

25° C.

calculated from data obtained at pH ca. 11.2 and ca. 11.9, (calculations at these points apparently gave the best fit of the last term of equation I), and the scattered values were attributed to an inability to determine the pH of the solutions accurately near values of 11.2. However, the variation of k with ionic strength at pH 11.88 is presented in table 13.

Table 13

VARIATION OF RATE CONSTANT WITH IONIC STRANGTH AT pH 11.88 AND 25° C.

Ionic Strength moles/l.	$k \times 10^3$ $min.^{-1}$
0.0180	4.04
0.0310	4.30
0.0471	4.55
0.0666	4.64
0.1000	5.06

It can be seen that ionic strength is an important variable in the rate of decomposition of murexide. By utilization of the data presented here and equation I, it would be possible for one to calculate the optimum ionic strength for minimal decomposition of murexide, for the variations of k_1 and k_2 with ionic strength are of opposite direction. The variation of k_1 with ionic

strength will be discussed more fully in the following chapter.

Effect of Mixed Solvents: The solvents used, their concentrations, the observed first order rate constants, and the ionic strengths of each run are given in tables 14, 15, 16, 17 and 18. Since the objects of studying the mixed solvents systems were 1) perhaps to gain an insight into the mechanism and 2) to find a solvent in which the rate of decomposition of murexide could be greatly decreased compared with water, the rates were only studied in the pH range of about 3 to 9. It was then contemplated that the relative order of minimum decomposition for the various solvent systems could be calculated by means of an equation, to be presented. One will notice that in acid solution, the rate of reaction increases with increasing proportion of organic liquid, and in weakly alkaline solution, the rate of decomposition decreases with increasing organic solvent concentration. An attempt will be made to explain some of these phenomena in chapter VI.

In the case of the dioxane-water systems, it was not possible to adjust the ionic strength to the calculated values, due to the very low dielectric constant of the dioxane, and the fact that the various buffer concentrations could not be practically reduced to values less

Table 14

RATES OF DECOMPOSITION OF MUREXIDE IN VARIOUS ETHANOL-WATER MIXTURES

25° C.

Volume % Ethanol	pH	k min.$^{-1}$	s_k min.$^{-1}$	Degrees of Freedom	k_1 min.$^{-1}$ (moles/l.)$^{-1}$
20	3.14	4.262×10^{-2}	2.35×10^{-4}	18	58.9
40	3.14	5.061×10^{-2}	4.42×10^{-4}	18	69.9
50	3.12	6.360×10^{-2}	4.51×10^{-4}	16	84.0
20	4.16	5.466×10^{-3}	2.69×10^{-5}	16	62.6
40	4.13	5.538×10^{-3}	1.05×10^{-5}	7	74.5
40	3.95	8.451×10^{-3}	2.83×10^{-5}	7	75.2
50	4.09	7.292×10^{-3}	3.43×10^{-5}	14	89.5
					$k_2' \times 10^9$ min.$^{-1}$ (moles/l.)$^{1/2}$
20	8.50	2.060×10^{-4}	0.84×10^{-6}	10	11.6
20	8.83	3.295×10^{-4}	4.35×10^{-6}	10	12.6
40	8.51	8.287×10^{-5}	4.56×10^{-6}	22	4.62
50	8.52	6.127×10^{-5}	0.59×10^{-6}	22	3.38
20	9.10	4.363×10^{-4}	2.33×10^{-6}	18	12.2
40	8.95	1.422×10^{-4}	2.53×10^{-6}	18	4.74
50	8.92	1.010×10^{-4}	2.81×10^{-6}	18	3.49

Note: For 20, 40 and 50 volume percents ethanol, the ionic strengths were adjusted to 0.0845, 0.0676 and 0.0596 respectively.

Table 15

RATES OF DECOMPOSITION OF MUREXIDE IN VARIOUS ORGANIC LIQUID-WATER MIXTURES

25° C.

Solvent	Vol. %	pH	k min.$^{-1}$ x 10^2	s_k min.$^{-1}$ x 10^4	Deg. of Free.	Ionic Strength x 10^2	k_1 min.$^{-1}$ (moles/l.)$^{-1}$
Methyl alcohol	25	3.13	5.469	3.06	10	6.95	74.0
"	50	3.19	8.022	9.44	8	4.62	124.2
Ethyl alcohol*	20	3.14	4.262	2.35	18	8.45	58.9
"	50	3.12	6.360	4.51	16	6.76	84.0
n-Propyl alcohol	25	3.22	3.622	6.17	10	7.62	60.0
"	50	3.14	5.130	4.79	10	5.60	70.6
Isopropyl alcohol	25	3.15	3.686	2.40	10	7.45	53.3
"	50	3.16	4.389	3.18	10	5.24	63.4
Acetone	25	3.13	3.620	4.54	10	7.57	48.7
"	50	3.13	4.324	4.77	10	5.50	58.2
Ethylene glycol	25	3.12	4.559	1.83	10	8.24	60.1
"	50	3.13	5.773	6.03	10	6.75	77.7
Glycerol	25	3.13	4.062	4.88	10	8.09	54.8
"	50	3.14	4.483	3.91	10	6.19	61.8

* Taken from table 14.

Table 16

RATES OF DECOMPOSITION OF MUREXIDE
IN VARIOUS DIOXANE-WATER MIXTURES

25° C.

Buffer Composition	Volume % Dioxane	pH	k min.$^{-1}$	s_k min.$^{-1}$	Deg. of Free.	k_1 min.$^{-1}$ (moles/l.)$^{-1}$
0.05 M Potassium acid phthalate plus HCl	25	3.19	4.571×10^{-2}	2.60×10^{-4}	16	71.0
	50	3.04	1.190×10^{-1}	1.37×10^{-3}	10	130.6
	25	4.07	6.019×10^{-3}	2.58×10^{-5}	16	70.9
	50	4.13	9.455×10^{-3}	2.44×10^{-5}	18	127.8
						$k_2'\times10^9$ min.$^{-1}$ (moles/l.)$^{1/2}$
0.04 M KH_2PO_4 + NaOH	25	7.77	5.750×10^{-5}	4.28×10^{-7}	18	(7.43)*
	50	7.84	7.661×10^{-5}	1.22×10^{-6}	18	(9.10)*
	25	8.75	8.368×10^{-5}	6.38×10^{-7}	18	3.52
0.05 M H_3BO_3 + NaOH	50	8.82	3.705×10^{-5}	1.59×10^{-6}	18	1.44

* Calculated from this data and equation IIa, page 90.

Table 17

DECOMPOSITION OF MUREXIDE IN VARIOUS GLYCEROL-WATER MIXTURES (BASIC SOLUTION)

25° C. Ionic Strength 0.14

Volume %	pH	k min.$^{-1}$ x 10^4	s_k min.$^{-1}$ x 10^7	Degree of Freedom	k_2' min.$^{-1}$ x 10^8 (moles/l.)$^{1/2}$
25	7.70	1.455	14.5	2	2.05
50	7.84	1.327	4.15	2	1.59

Table 18

ADDITIONAL POINTS FOR 50 VOLUME PERCENT ETHANOL-WATER

0.05 M KH_2PO_4-K_2HPO_4 Buffer

Temperature degrees C.	pH	k min.$^{-1}$
25	7.15	3.56×10^{-5}
25	7.42	3.34×10^{-5}
25	7.81	3.59×10^{-5}
25	8.12	3.84×10^{-5}
40	3.76	6.00×10^{-2}
40	7.20	2.06×10^{-4}
40	7.43	2.06×10^{-4}
40	7.80	2.15×10^{-4}
40	8.11	2.45×10^{-4}
40	8.60	3.32×10^{-4}
40	9.08	4.25×10^{-4}

than 0.04 M. In addition, an attempt was made to study the effect of ethyl alcohol around pH 7 at 2.7° and at 40° C. This did not meet with success, for at 2.7° the murexide precipitated from solution and the glass electrode did not function properly in the solvent mixture at this temperature. The region of interest for this solvent system was near pH 7, and the ionic strength could not be adjusted to the desired value because of the necessitated use of phosphate buffers, so data were obtained at 40° and 25° for 50% ethanol in 0.05 M phosphate buffers with no KCl added (table 18). This was meant to serve as a semi-quantitative comparison of the rate of decomposition in water and in 50% alcohol at the pH of minimum decomposition.

Temperature Studies: The additional temperatures at which runs were made for aqueous solutions were: near 0°, 20°, 33°, 40°, and 50° C. The studies at about 0° and 40° were made over the entire pH range (3-13) with only one solution being prepared for each pH value, for the data of 25° indicated that sufficient accuracy could thus be obtained. For the experiments near zero degrees, a water-ice mixture was used for temperature control of the reactions with half-lives of less than about six hours. A refrigerator was utilized for the remaining runs, and the temperature was recorded from a thermometer immersed

in a 100 ml. volumetric flask (filled with water) each time a sample was withdrawn for an absorbance determination.

The resultant rate constants are presented in tables 19 and 20, and are plotted as a function of pH in figures 9 and 10. The ionic strength was adjusted to 0.10 in these studies, for insufficient activity data were obtainable to permit the proper temperature correction. Therefore, any activation energies found will be "apparent" activation energies. However, it is reasonable to expect that the change of activities of the various species involved with temperature would be very small compared with the change of rate constant. In order to investigate the temperature dependence of the observed rate constants (k) over the whole pH range studied, the logarithms of the observed rate constants taken from figures 6, 9, and 10 at each unit of pH were plotted against the reciprocal of the absolute temperature. The simple Arrhenius equation was apparently exactly obeyed for pH values of 8 or greater (for an example of the type of plot obtained see figure 12). At first it was doubted whether the Arrhenius equation was obeyed for solutions of pH 7 or less, so additional points for the specific rate constant (k_1) in acid solutions were obtained at 20, 33 and 50 degrees C. The resultant values of the observed

Table 19

RATE CONSTANTS FOR THE DECOMPOSITION
OF MUREXIDE IN AQUEOUS SOLUTION
AT LOW TEMPERATURES

Ionic Strength 0.10

Temperature degrees C.	pH	k min.$^{-1}$
0.0 ± 0.1	3.00	4.25×10^{-3}
3.5 ± 0.5	3.90	7.89×10^{-4}
2.7 ± 0.4	5.10	7.85×10^{-5}
2.7 ± 0.4	6.10	1.28×10^{-5}
2.7 ± 0.4	6.62	7.36×10^{-6}
2.7 ± 0.4	7.12	1.10×10^{-5}
2.7 ± 0.4	8.00	2.40×10^{-5}
2.7 ± 0.4	8.90	6.41×10^{-5}
2.7 ± 0.4	10.00	1.68×10^{-4}
2.7 ± 0.4	11.30	4.62×10^{-4}
0.0	11.88	5.75×10^{-4}
0.0	12.88	2.58×10^{-3}

Table 20

RATE CONSTANTS FOR THE DECOMPOSITION OF MUREXIDE IN AQUEOUS SOLUTION

40.0 ± 0.3° C. Ionic Strength 0.10

pH	k $min.^{-1}$
3.19	1.365×10^{-1}
4.09	1.586×10^{-2}
5.15	1.982×10^{-3}
6.02	7.527×10^{-4}
6.53	6.659×10^{-4}
7.02	7.875×10^{-4}
7.88	1.497×10^{-3}
8.87	4.951×10^{-3}
9.73	8.453×10^{-3}
10.45	1.291×10^{-2}
11.88	1.639×10^{-2}
12.88	5.153×10^{-2}

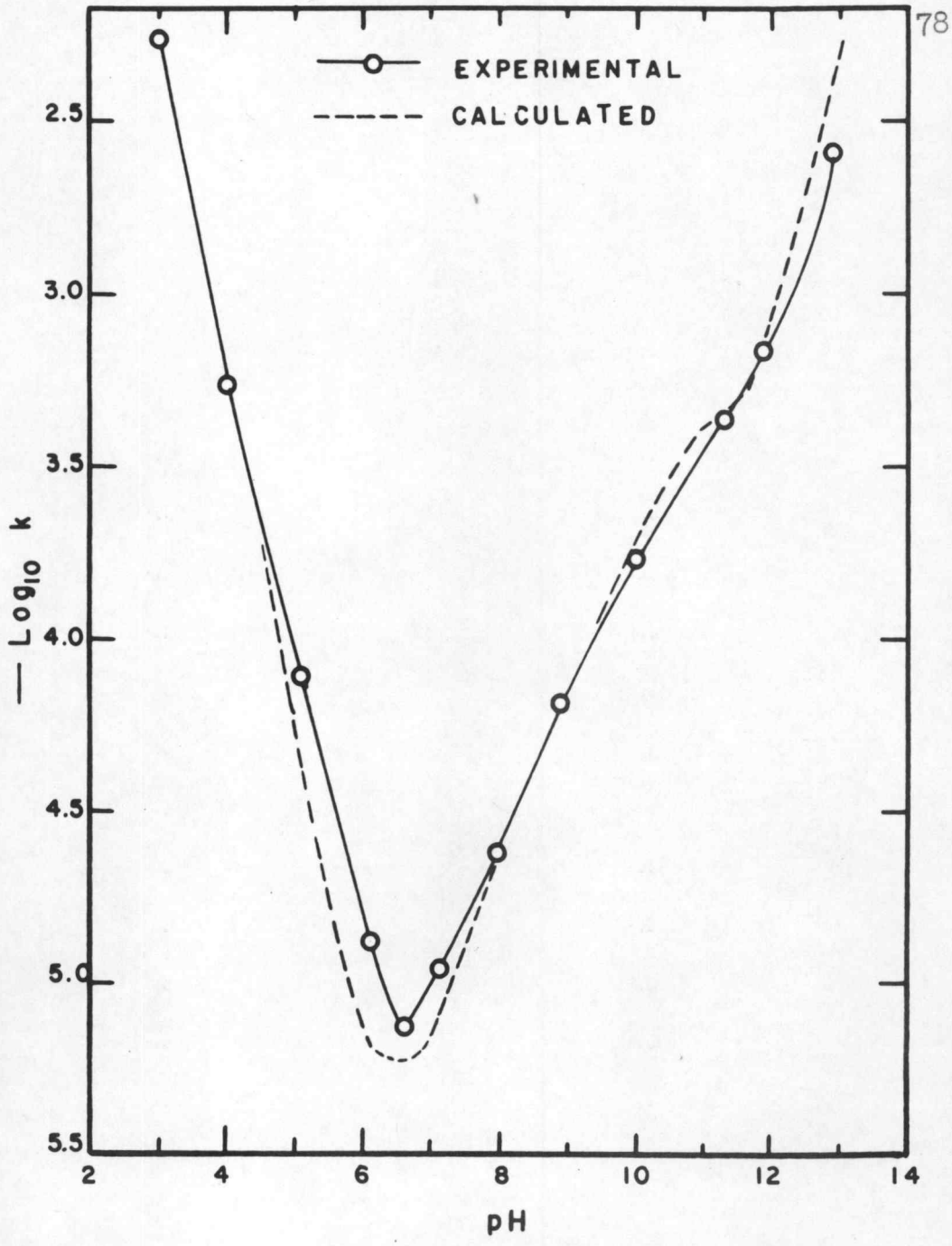


FIGURE 9

VARIATION OF k WITH pH

2.7°C. IONIC STRENGTH 0.10

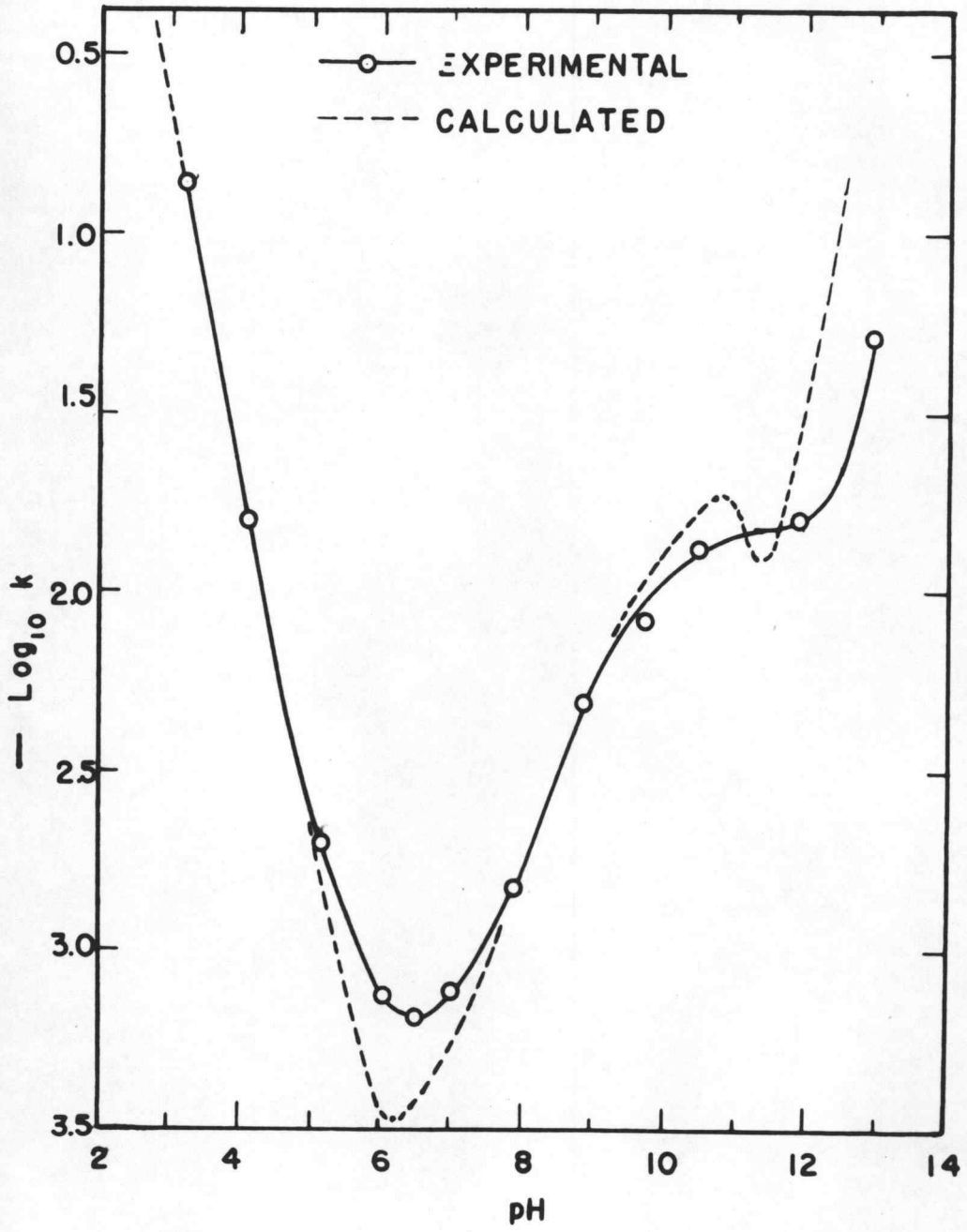


FIGURE 10

VARIATION OF k WITH pH

40° C. IONIC STRENGTH 0.10

first order rate constants (k) at these respective temperatures were 2.91×10^{-2}, 7.95×10^{-2} and 3.21×10^{-2} ($min.^{-1}$) at measured pH values of 3.03, 3.13 and 4.15, in the order given. The conventional plot of the specific rate constant k_1 calculated from the observed rate constant k for pH values of about 3 and 4 is shown in figure 11, and it can be seen that the data apparently fit the Arrhenius equation, although the scattering is greater than in the case of the basic solutions. This larger error is mainly attributed to the larger rate of change of the observed rate constant with pH in acid solution compared with that in basic solution, for an error in determination of pH in the acid region would cause a greater error in the specific rate than it would in alkaline solution (see figures 6, 9 and 10).

Effect of Glass Surface Area on Rate: In order to determine if heterogeneous catalysis due to glass could have been present, the rate of decomposition of murexide in the presence of glass beads was obtained in acid and basic solution as follows: Buffered solutions of murexide at about pH 3 (phthalate buffer) and about 11 (piperidine buffer plus 10 mg. disodium dihydrogen ethylenediaminetetracetate to remove effects of foreign ions resulting from solution of the glass) were made up in the usual manner. 63 g. soft glass beads of diameter about 3 mm.

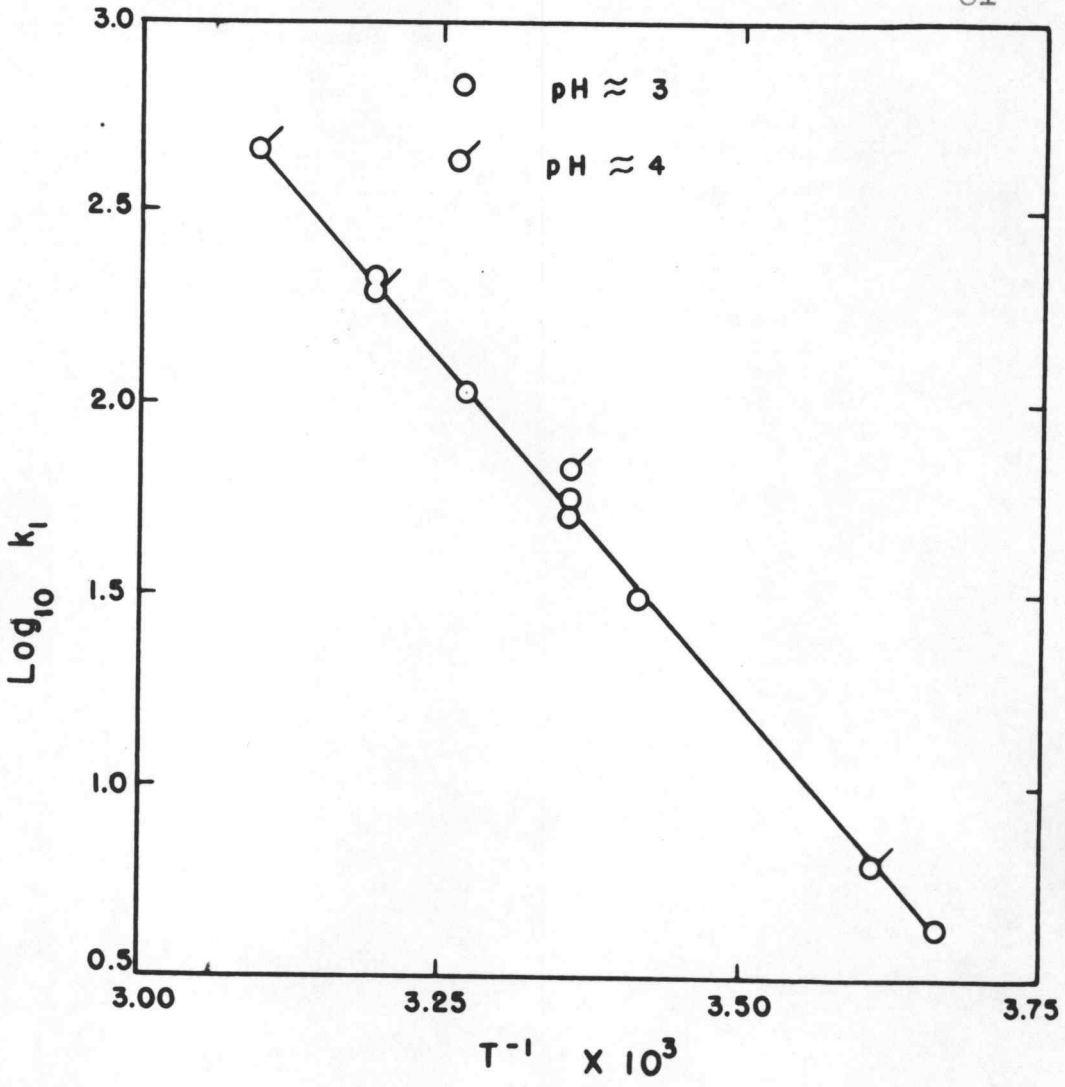


FIGURE 11

DEPENDENCE OF k_1 ON TEMPERATURE

IONIC STRENGTH 0.10

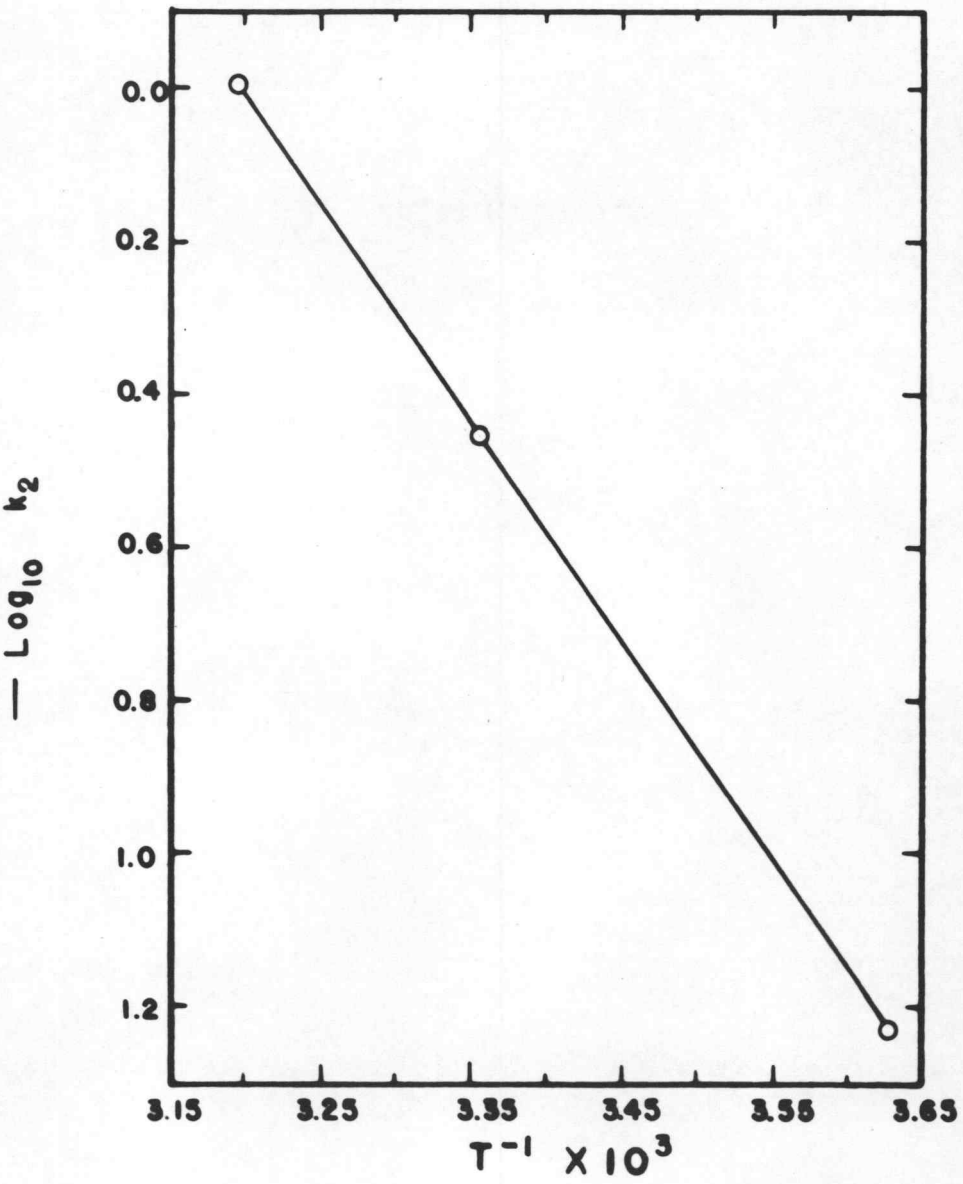


FIGURE 12

DEPENDENCE OF k_2 ON TEMPERATURE

pH ≈8.4 IONIC STRENGTH 0.10

were placed in each of two 250 ml. glass stoppered Erlenmeyer flasks and these flasks as well as the 100 ml. volumetric flasks were placed in the water bath to attain temperature equilibrium. After this time, the solutions were poured into the flasks containing the beads and the absorbances determined in the usual manner. The flasks were well shaken between each absorbance determination. For a measured pH of 3.03, the observed rate constant (k) obtained was 3.72×10^{-2} min.$^{-1}$. It was concluded that the glass did not catalyze the reaction in acid solution, for if the pH determination had been 0.05 unit in error, k would have agreed with the value listed in table 9, 4.20×10^{-2} min.$^{-1}$. The value of k obtained for a measured pH of 11.0 was 3.75×10^{-3} min.$^{-1}$, which fell on the smooth curve drawn through the experimental points in figure 6. Therefore it was concluded that the added surface area of the glass had no significant effect on the rate of decomposition in basic solution. Thus, it is probably reasonable to assume that glass does not catalyze the reaction over the whole pH range studied.

Effect of Light: The absence of significant photosensitivity for the decomposition of murexide in an acid and basic solution was verified in the following manner: Two 100 ml. volumetric flasks were filled with the proper amount of ca. pH 3 phthalate buffer mixture, KCl, and

water to the 95 ml. mark, and two flasks were filled similarly with ca. pH 11 piperidine buffer mixture, KCl and water. Then one of the flasks containing the acid solution and one containing the basic solution were dip-coated twice with black Tygon paint to about 1 cm. below the 100 ml. mark. At the same time, two 8 inch test tubes were given two dip coats of the black paint. The two blackened flasks were placed in the water bath (25°C.) to attain temperature equilibrium, 5 ml. of stock murexide solution was added, then distilled water to the 100 ml. mark, and each flask was covered with the blackened test tubes in order that light would be excluded. The absorbances of these two solutions were then determined in the usual manner as a function of time, with the solutions being exposed to light only at the times of sample withdrawal. The rate of decomposition of "illuminated" solutions was observed by making the runs in the usual manner with the two remaining solutions contained in the unpainted flasks immersed in the water bath, which were illuminated by a 500 watt incandescent lamp contained in a reflector unit suspended about 12 inches above the bulbs of the volumetric flasks. The observed rate constants (obtained graphically) are presented in table 21. Since the values of k at each pH do not differ significantly within the experimental error, and since the observed rates for the

Table 21

EFFECT OF LIGHT ON THE RATE OF DECOMPOSITION OF MUREXIDE

25° C. Ionic Strength 0.10

Average pH	k, min.$^{-1}$ "Illuminated"	k, min.$^{-1}$ Dark
3.05	4.11×10^{-2}	4.25×10^{-2}
11.10	3.90×10^{-3}	4.04×10^{-3}

dark solutions are slightly higher than those for the "illuminated" solutions, it was concluded that the reaction is not significantly photosensitive.

Effect of Calcium on the Rate of Decomposition: Due to a previous interest, the rate of decomposition of the calcium complex existing in the narrow region of pH 10.5 to 11.7 was investigated. The dye was essentially completely converted to the calcium complexed form by making the ionic strength up to 0.10 with 1 M calcium nitrate rather than with KCl. The observed rate constants are given in table 22 and are plotted in figure 6. It can be seen that the calcium purpurate decomposes at a different rate than does murexide, so one must take care that no calcium (and probably no other ion that combines with murexide) is present in the solutions in which the decomposition of the dye is being studied.

Table 22

RATES OF DECOMPOSITION OF CALCIUM PURPURATE IN ALKALINE SOLUTION

25° C. Ionic Strength 0.10

pH	moles/l. Ca.	k min.$^{-1}$ x 10^3	s_k min.$^{-1}$ x 10^6	Degrees of Freedom
10.53*	0.033	1.059	6.55	16
10.55**	0.020	1.046	5.57	7
10.90	0.223	1.383	10.78	5
11.23	0.025	2.035	4.26	7
11.70	0.031	4.522	43.5	14

* Unbuffered.
**Piperidine buffer.

Evaluation of Data: Except for the study involving the variation of ionic strength at pH 11.88, all data taken at 25° C. were fitted to the integrated form of the first order rate equation by the method of least squares (23, chapter II). The rate constants for the decomposition of murexide under the other conditions were evaluated graphically, for the work at 25 degrees showed that the estimated standard error of the rate constants was about one percent, and it was found that the graphical results agreed well with the computed values to this number of

significant figures.

The $\log_{10}$ of the absorbance was plotted against the time for <u>every</u> run in order that any tendency toward deviation from linearity might be observed; none was.

<u>Discussion</u>.

It was shown on pages 61 and 62 that the observed rate constant, k, is a function of pH. Thus the equation

$$\frac{-d(M)}{dt} = k_1 (H^+)(M) + k_2(OH^-)^{1/2}(M) - \frac{k_3(OH^-)(M)}{k_4 + (OH^-)} \qquad (I)$$

was evolved, where

t is the time in minutes,
(H^+) is the hydrogen ion activity,
(OH^-) is the hydroxyl ion activity,
(M) represents the concentration of murexide, and
k_1, k_2, k_3 and k_4 are constants at any one temperature.

It can be seen that (M) may be factored out of the terms on the right side of this equation, and thus the equation can be integrated at constant pH by separation of variables. The sum of the constant terms remaining after (M) is factored out is equal to the calculated observed rate constant, k. The calculated values of k are represented by the dotted curves at 2.7°, 25° and 40° in figures 6, 9 and 10, respectively. The ion product of water used for the calculation of hydroxyl ion activities at temperatures other than 25° C. was computed using an enthalpy of dissociation of water of 13,560 calories per mole. The

values of the constants in the above equation and their activation energies (obtained graphically) are given in table 23, with the logarithms of k_1 and k_2 plotted versus the reciprocal of the absolute temperature in figures 11 and 12. The temperature plots of k_3 and k_4 apparently deviated less from linearity than does that of k_2.

Table 23

SPECIFIC RATES AND APPARENT ACTIVATION ENERGIES FOR EQUATION I

	Constant			
	k_1	k_2	k_3	k_4
Temperature degrees C.	$min.^{-1}$	$min.^{-1}$ $(moles/l.)^{-1/2}$	$min.^{-1}$	moles/l.
2.7	5.50	5.87×10^{-2}	2.28×10^{-3}	9.25×10^{-4}
25.0	58.0	0.351	4.04×10^{-2}	4.83×10^{-3}
40.0	204	1.018	2.05×10^{-1}	1.32×10^{-2}
Apparent Activation Energy, kcal./mole...	16.51	13.10	20.70	12.24

It is apparent that the above equation does not fit the data to the degree desired; however, this was the best fit obtainable by the author. One must keep in mind that much of the difficulty of fitting such a curve can be ascribed to the fact that one has to deal with a hydrogen

ion (or hydroxyl ion) activity range of 10^{10}. It was realized that an equation in the form of an extended polynomial would empirically represent the data more precisely, but such an equation would have little theoretical significance. If knowledge of the rate of decomposition of the dye is required for practical reasons, it would be more accurate and less time-consuming to refer to graphs and tables presented.

The discrepancy between the observed curves and the calculated ones near the point of minimum rate of decomposition may be due to another reaction corresponding to another term of equation I and/or the effect of specific ions on the activity coefficient quotients of the rate constants, for phthalate buffers were used for pH 3 to 5, phosphate buffers for pH 6 to 7, and borate buffers for pH 8 to 10. The difference between the observed and calculated curves in the very alkaline solutions may be due to the foregoing two reasons, or perhaps the third term of equation I is not <u>apropos</u>.

As mentioned before, the decomposition of murexide in the mixed solvents was studied for two reasons: 1) perhaps to gain an insight into the reaction mechanism and 2) to find a solvent system in which the minimum rate of decomposition of the dye is much less than for water. The first standpoint will be discussed in the next chapter.

As was pointed out in the introductory chapter, some difficulty has been experienced with the preparation of a stable solution of murexide. It was thought that the relative minimum rates of decomposition of the dye in various solvents could be calculated with a knowledge of the specific rates corresponding to the first two terms of equation I. Since the pH of the various solutions composed of water-organic liquid mixtures was determinable with the techniques used (glass electrode), but not the overall dissociation constant of the mixed solvent nor the hydroxyl ion activity, equation I was modified by substitution of $K_s^{1/2}(H^+)^{-1/2}$ for $(OH^-)^{1/2}$ to give

$$\frac{-d\ (M)}{d\ t} = k_1(H^+)(M) + k_2'(H^+)^{-1/2}(M) \qquad (II)$$

where $k_2' = k_2K_s^{1/2}$ and K_s is the "overall" dissociation constant for the solvent. The third term of equation I was dropped, for it is negligible up to a pH value of about 9, and the present region of interest is below this value. From this equation, it can be seen that the pseudo-first order rate constant with respect to murexide is

$$k = k_1(H^+) + k_2'(H^+)^{-1/2}. \qquad (IIa)$$

Therefore, by utilizing the average values of k_1 and k_2' obtained for ethanol-, dioxane-, and glycerol-water mixtures (tables 14, 15, 16, 17 and 23), equation IIa was

differentiated with respect to hydrogen ion activity and the result set equal to zero, in order to determine the pH (calculated) of minimum rate of decomposition. The pseudo-first order rate constants at these pH values were then calculated in order to determine the maximum half-life of murexide in the various systems. As discussed above, these calculations cannot be expected to give the true rate of minimum decomposition, but would only give the relative order. The resultant calculated half-lives at 25° C. at the minima were 146, 950, 605 and 249 hours for water, dioxane, ethanol and glycerol, respectively (the latter three organic solvents were present at 50 volume percent). Although it appears that the use of a dioxane-water solvent would lead to the least rate of murexide decomposition, the possibility of using this solvent was removed from further consideration for it was thought that the peroxides often present in dioxane would render its use impractical. In view of this, more data were obtained for ethanol-water mixtures, as described in a previous section. It would appear that a buffered ethanol-water mixture would be the best solvent for preparation of stock murexide solution in case it is desired to store such a solution with the occurrence of minimum decomposition, and for room temperature this is recommended. In a previous report (41) the use of 70%

(unbuffered) ethanol was recommended. It was not possible to use this alcohol concentration in the present experiments, due to the limited solubilities of the buffer salts in this media. Thus a high alcohol concentration is desirable but is limited by the solubility of murexide (and other compounds, if used). Murexide forms a supersaturated solution in 50% ethanol at refrigerator temperatures with the subsequent precipitation of very small crystals at unpredictable times, and would therefore be expected to be a source of trouble if such a solution stored in the cold was utilized for an analytical procedure. It was thus concluded that an aqueous solution buffered at pH 6.7 stored near zero degrees centigrade would be the most practical for use in an analytical method, for the half-life of the murexide at these conditions would be about 65 days. It seems quite apparent that one cannot completely inhibit the decomposition of murexide in solution.

It is believed that the largest source of error in these studies is due to pH measurement, and that the temperature variation contributed a relatively negligible amount of error. Since one cannot generally expect an accuracy of absorbance determination of better than about one percent with the apparatus used, the relative standard errors of the rate constants reported appear quite acceptable.

VI. A POSSIBLE MECHANISM

The author was unable to explain either of the last two terms of equation I by a possible mechanism of reaction. It is probable that one will not be able to evolve a path of decomposition of murexide in basic solution until a thorough study of all the reactions involved is made. It is also possible that the last term of the equation is not applicable.

However, one can make certain qualitative statements about the behavior of the system in basic solution. Referring to figures 6, 8 and 10, one will notice that if the segments of the curves corresponding to the second term of equation I (half order with respect to hydroxyl ion) are extended linearly into the basic region, the experimental curve exhibits a maximum deviation and then reapproaches the straight line as the pH becomes very high. Therefore, if the assumption is made that only one decomposition reaction takes place in basic solution, it is probable that the observed rate is the net effect of a decomposition and a formation reaction with the maximum relative rate of formation occurring near pH 12 in aqueous solution. The broadening of the "step" in figures 6, 9 and 10 as the temperature increases from 2.7° to 25° and then to 40° can therefore be attributed to a relatively large difference

in activation energies of the forward and reverse reactions. The hypothesis of existence of a formation reaction is qualitatively supported by the fact that murexide is synthesized under basic conditions. In the method of Hartley, anhydrous ethanol and ammonia are used, and in the method of Davidson, ammonium acetate is dissolved in glacial acetic acid, and of course, ammonium acetate is a base in this solvent. As an additional point of interest, it is shown in tables 14, 16 and 17 that the rate of reaction decreases in basic solution as the concentration of water decreases, and so it is most probable that water enters into the rate equation.

It has been shown that the decomposition of murexide in aqueous solution is second order, first order with respect to murexide and first order with respect to hydrogen ion (the first term of equation I). The additional facts reported in various parts of this paper enable one to suggest a possible mechanism for the decomposition of the dye.

The nature of the reacting species is of importance. It may be safely assumed that one of the reactants is hydrogen ion and its degree of solvation is a moot point. Purpuric acid has five ionizable protons, but since two of them have very large pK values, only the ionization of the remaining three protons need be considered here. The

dissociation constant for the first hydrogen is probably about or greater than one, as shown by Schwarzenbach, and by the fact that the titration curve of murexide shows a point of inflection at about pH 7 (see chapter II). Schwarzenbach also reports values of 9.2 and 10.9 for pK_2 and pK_3, respectively. Thus it must be concluded that the predominant species present in solutions of pH values of about 3 to 5 have a unit negative charge. However, deductions based upon the relation of k_1 to ionic strength seem to be at variance with this.

A relationship between the rate constant and ionic strength under the conditions involved in this investigation may be derived for the limiting case of very dilute solutions in the following manner (cf. 19,pp.117-118): If the rate of reaction is proportional to the concentration of the activated complex, then, according to the transition state theory,

$$\text{rate} = (RT/Nh)c_{C*} \qquad \text{(III)}$$

where R is the gas constant, T denotes the absolute temperature, N is Avogadro's number, h represents Planck's constant and c_{C*} is the concentration of the activated complex. If, for the special case in question

$$H + M = C* ,$$

where the reactants H and M represent hydrogen ion and

some species of purpurate ion, respectively, then

$$c_{C*} = K^* \, a_H \, a_M / f_{C*} \qquad \text{(IV)}$$

in which K* is the thermodynamic equilibrium constant for the above reaction, a denotes the activity of the subscripted species and f_{C*} is the activity coefficient of the activated complex C*. Substituting equation IV into equation III there results

$$\text{rate} = (RT/Nh)K^* \, a_H \, a_M / f_{C*} = (RT/Nh)K^*(f_M/f_{C*})c_M a_H \qquad \text{(V)}$$

where f_M represents the activity coefficient of the purpurate species and c_M denotes the concentration of this species. Now, in an infinitely dilute solution

$$f_M/f_{C*} = 1 \, ,$$

so it can be seen from equation V that

$$k_o = (RT/Nh)K^* \qquad \text{(VI)}$$

where k_o represents the specific rate in an infinitely dilute solution. In the present case, the hydrogen ion activity (a_H) and the murexide concentration (c_M) were experimentally determined, therefore when equation VI is substituted into equation V,

$$\text{rate} = k_o \, (f_M/f_{C*}) \, c_M \, a_H \qquad \text{(VII)}$$

and

$$k = k_o \, (f_M/f_{C*}) \qquad \text{(VIII)}$$

or

$$\log k = \log k_o + \log (f_M/f_{C*}) \qquad \text{(VIIIa)}$$

where k is the specific rate for a real solution.

According to the Debye-Hückel theory, the relation between activity coefficient and ionic strength for a species i in a very dilute solution is

$$- \log_{10} f_i = Z_i^2 \, a' \, I^{1/2} \qquad \text{(IX)}$$

in which Z_i indicates the charge on the ith ion species, a' is a constant of value 0.509 for aqueous solutions at 25° C., and I is the ionic strength. By substituting the appropriate equations obtained from equation IX for M and C* into equation VIIIa, assuming that Z_{C*} equals Z_H plus Z_M, and making the appropriate algebraic manipulations, the resulting equation is

$$\log_{10} k = \log_{10} k_o + (2Z_H \cdot Z_M + Z_H^2) \, a' \, I^{1/2} \qquad \text{(X)}$$

and

$$\frac{d \, (\log_{10} k)}{d \, (I^{1/2})} = a' \, (2Z_H \cdot Z_M + Z_H^2) = m. \qquad \text{(XI)}$$

Since Z_H equals +1, lines of slope m corresponding to values of Z_M of -1, -2 and -3 were drawn as dotted lines on figure 7, page 66, for k_1 (which corresponds to k in equation XI above). This plot therefore seems to indicate that the charge on the purpurate species taking part in the rate determining step may be -2, but not -1.

However, it was pointed out before that investigations could not be practically carried out at ionic strengths less than 0.04 due to the necessity of working with buffer solutions, so some doubt remains as to the applicability of equation XI and the conclusions drawn therefrom. Despite the contradictory views presented, it will be assumed that a negative monovalent purpurate ion takes part in the rate determining step of the mechanism to be proposed below, since the evidence for this assumption is the stronger.

It was of interest to determine the role of the solvent in the reaction path for the decomposition of murexide in acid solution. As was discussed earlier, it was impossible to work with completely nonaqueous solvents due to the insolubility of the dye in such liquids. It was found that the values of k_1 obtained for the various solvent mixtures studied were apparently best related by the equation

$$k_1 N_W = k_1' \qquad \text{(XII)}$$

or, for acid solutions

$$-\frac{d(M)}{dt} = k_1' \frac{(M)(H^+)}{N_W} \qquad \text{(XIII)}$$

where N_W represents the mole fraction of water and k_1' is a constant. The values of k_1' calculated from equation XII are presented for the various solvent systems in

table 24. The values for dioxane-water mixtures were not computed, for the results obtained would be of doubtful significance, due to the inability to adjust, even approximately, the ionic strength by use of the Debye-Hückel equation to a value comparable to 0.1 for water. The mole fractions of water shown in this table were calculated using the approximation that there was no volume change for mixing of the liquids. It is believed that the calculations of mole fractions in this manner are of sufficient accuracy, for the use of the Debye-Hückel limiting law in the adjustment of ionic strengths represents an even greater approximation. Inspection of table 24 shows that equation XII seems to hold fairly well although it is realized that other effects, such as the basicity of the alcohols, may have lesser influences on the reaction.

In view of the above discussion, the mechanism shown in plate I is suggested. It is understood that the formula for the purpurate ion written is only one of several mesomeric and tautomeric forms. "$M \cdot H_2O^-$" designates an adduct of water and purpurate ion of which no suggestions as to structure are offered. The question of existence of such a species has been referred to before.

Thus by assuming that "non-hydrated" purpurate ion M^- is the species involved in the rate determining reaction b, that the concentration of the analytically

Table 24

RELATION OF SPECIFIC RATE TO MOLE FRACTION OF WATER IN VARIOUS SOLVENT MIXTURES

25° C.

Solvent	Volume %	Mole Fraction Water, N_w	k_1 min.$^{-1}$	k_1' min.$^{-1}$
Methanol	25	0.8586	74.0	65.2
	50	0.693	124.2	55.7
Ethanol	20	0.930	60.8	57.1
	40	0.840	72.2	60.6
	50	0.778	86.8	67.5
n-Propanol	25	0.9254	60.0	55.5
	50	0.8058	70.6	57.0
Isopropyl Alcohol	25	0.9272	53.3	49.4
	50	0.8095	63.4	51.2
Acetone	25	0.9243	48.7	45.0
	50	0.8029	58.2	46.7
Ethylene Glycol	25	0.9026	60.1	54.4
	50	0.7559	77.7	58.7
Glycerol	25	0.9238	54.8	50.6
	50	0.8017	61.8	49.6
Water	100	1.0000	---	49.4*
				58.0**

* Average value taken from table 11.
**Obtained from table 23.

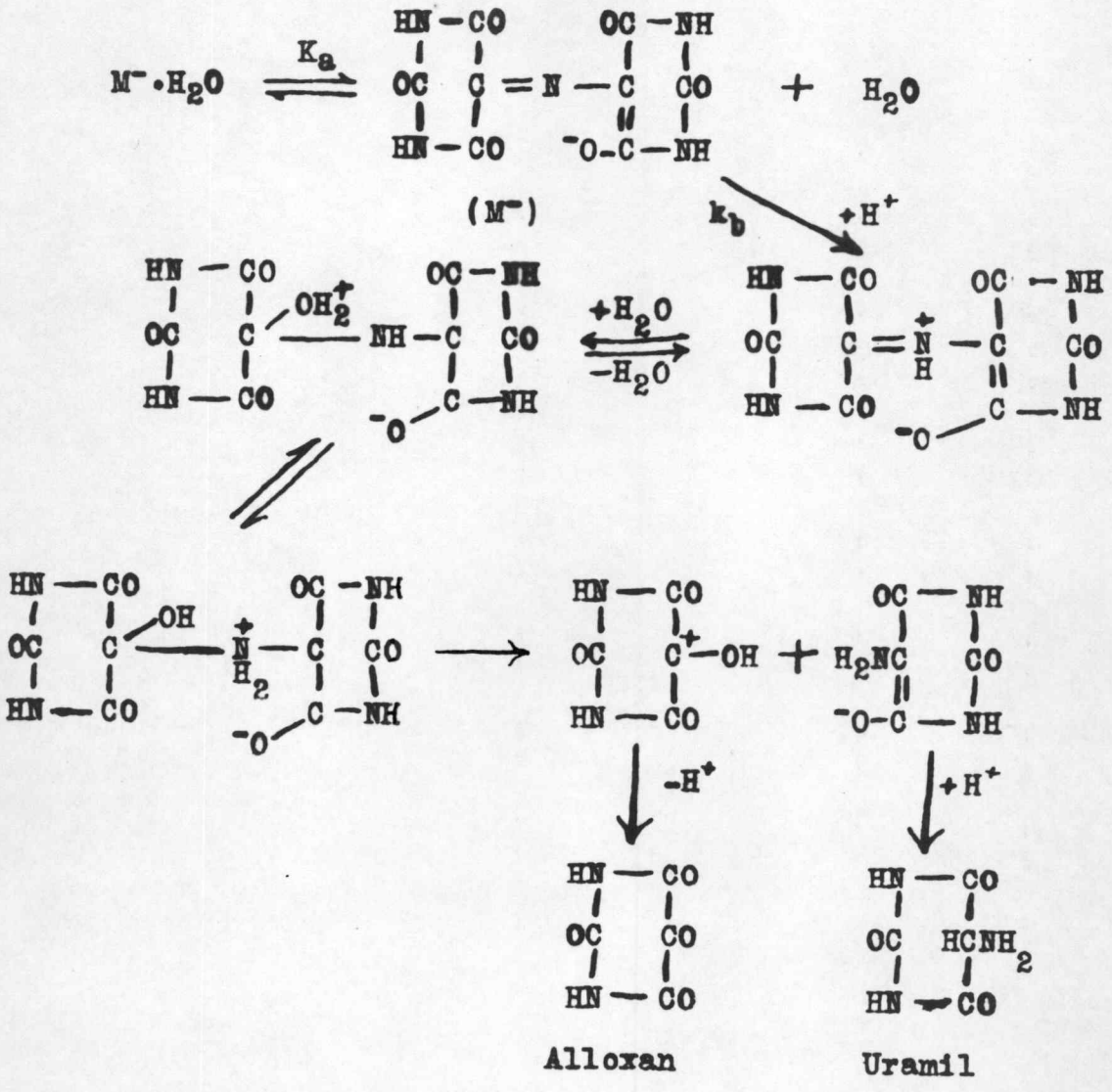


Plate 1. POSSIBLE MECHANISM FOR MUREXIDE DECOMPOSITION IN ACID SOLUTION

determinable "hydrated" form is very large compared to the "non-hydrated" form, and that all other steps are relatively fast, the rate expression

$$-\frac{d\,(M\cdot H_2O^-)}{d\,t} = k_b K_a \frac{(M\cdot H_2O^-)(H)}{(H_2O)}$$

may be evolved. If (H_2O) is expressed as mole fraction, the product $k_b K_a$ is experimentally identical with k_1' discussed above. The individual values of k_b and K_a cannot be determined with the information available.

Of many mechanisms tried, the suggested one was the only one found by the author that seemed to agree at all well with the evidence. However, there are several objections to the proposed reaction path. It seems strange that a proton transfer would be the rate determining step, for this type of reaction generally is expected to occur very rapidly, and one would expect a C-N cleavage to be more difficult and therefore slower. The concurrent existence of purpurate ions of different degrees of hydration is somewhat held in doubt, as is the supposition that the susceptibility of the purpurate ion to attack by a proton depends on the extent of hydration in reaction a whereas it does not in the third reaction.

It might be argued that the type of proton solvation is an important factor in the mechanism. Thus one would expect that, for similar concentrations of alcohols,

the rate constant would regularly change over that of strictly aqueous solution. On the basis of this line of reasoning, table 25 was prepared with the values of k_1 corresponding to certain alcohol-water mixtures listed in order of increasing relative basicity of the solvents. It can be seen that the constant for water is unexplainably opposite to the trend of k_1 for the alcohols. Therefore it is possible that the type of proton solvation can play an unknown part in the decomposition of murexide, but it appears that the dilution effect predominates. One would hesitate to make further theoretical conclusions on the basis of the information available at the present time.

Table 25

COMPARISON OF k_1 FOR CERTAIN SOLVENT MIXTURES

Solvent*	Volume %	Mole Fraction Water	k_1 $min.^{-1}$
Water	100	1	54**
Methanol	50	0.69	124
Ethanol	50	0.78	87
n-Propyl Alcohol	50	0.81	71
Isopropyl Alcohol	50	0.81	63

* Listed in increasing order of relative basicity.
** Average value taken from table 23.

VII. SUMMARY

A gravimetric method based on the precipitation of calcium purpurate monohydrate, $CaC_8N_5O_6H_3 \cdot H_2O$, has been developed for the assay of murexide with an estimated standard deviation of 0.52%. This procedure provides a previously unavailable means of standardization for the colorimetric determination of the dye, so that subsequent samples of murexide can be assayed colorimetrically or spectrophotometrically.

A cursory investigation of the decomposition products of murexide in basic solutions has indicated that uramil and alloxan are possible primary reaction products, which subsequently break down to form various other species. According to other investigators, these two compounds are apparently the initial products of the decomposition reaction in acid solution.

The effects of foreign materials (contained in an impure commercial sample) and of compounds probably present as impurities on the decomposition rate of murexide in buffered solutions have been studied. No redox catalysis was found, but a commercial sample contained acidic impurities, which in unbuffered solutions would increase the rate of decomposition. These results answer

some of the questions regarding erratic behavior of stock solutions of different samples of the dye.

The rates of decomposition of murexide in aqueous solutions of ionic strength 0.10 and at pH values ranging from 3 to 13 have been determined at temperatures varying from about 0° to 40° C. The decomposition rate reaches a minimum near pH 6.5. The reaction rate is first order with respect to the concentration of murexide under all the conditions studied. A rate expression that approximately fits the experimental data has been presented for the above mentioned conditions. In addition, the variation of rates with ionic strength for certain pH regions has been investigated. At a given pH, the addition of water-miscible organic solvents resulted in an increase in the rate of reaction in acid solutions, and a decrease in weakly alkaline solutions. These investigations should therefore enable one to critically evaluate the applicability of murexide for use in development of analytical procedures.

A possible mechanism for the decomposition of murexide in acid solution has been developed which is apparently consistent with the experimental evidence.

BIBLIOGRAPHY

1. Andreasch, Rudolf. Über Methylviolursäure und Methyldilitusäure. Monatschefte für Chemie 21: 281-301. 1900.

2. Beilstein, Fritz. Ueber das Murexide. Justus Liebig's Annalen der Chemie 107:176-191. 1858.

3. Beilstein's Handbuch der Organischen Chemie 3:772. Berlin, Julius Springer, 1922.

4. Ibid., 3:776.

5. Ibid., 4:469.

6. Ibid., 24:449.

7. Ibid., 24:500.

8. Ibid., 24:506.

9. Ibid., 25:85.

10. Ibid., 25:492.

11. Ibid., 26:556.

12. Betz, J. D. and C. A. Noll. Total hardness determination direct colorimetric titration. Journal of the American water works association 42:49-56. Jan. 1950.

13. Blitz, Heinrich, Meyron Heyn and Margarete Bergius. Alloxansäure. Justus Liebig's Annalen der Chemie 413:68-77. 1916.

14. Blitz, Heinrich and Guenther Schiemann. Darstellung von Parabansäure. Berichte der Deutschen Chemischen Gesellschaft 59:721. 1926.

15. Davidson, David. Murexide and leucomurexide. Journal of the American chemical society 58:1821-1822. 1936.

16. Davidson, David and Elias Epstein. The murexide question. Journal of organic chemistry 1:305-314. 1936.

17. Davidson, David and Harold Solaway. The formation of uramil from dialuric acid. Journal of organic chemistry 3:365-367. 1938.

18. Dixon, W. J. and F. J. Massey. Introduction to statistical analysis. Eugene, Oregon, 1949. 217p.

19. Frost, Arthur A. and Ralph G. Pearson. Kinetics and mechanism. New York, John Wiley and Sons, 1953. 343p.

20. Hartley, Walter Noel. Journal of the chemical society of London 87:1791. 1905.

21. Heilbron, I. M. Dictionary of organic compounds. 1:46. London, Eyre and Spottiswoode, 1943. 1072p.

22. Ibid., 1:63.

23. Ibid., 2:905.

24. Hill, Edgar S. The spontaneous oxidation of dialuric acid. Journal of biological chemistry 85:713-725. 1930.

25. Hughes, H. K., et al. Suggested nomenclature in applied spectroscopy. Analytical chemistry 24:1349-1354. 1952.

26. Knott, E. B. Color reactions of reducing pyrimidines. Journal of the society of chemistry and industry 60:313-314. 1941.

27. Kortüm, G. and J. O'M. Bockris. Textbook of electrochemistry. Vol. II. Amsterdam, Elsevier, 1951. 882p.

28. Kuhn, Richard and John C. Lyman. Über das Redoxpotential des Murexids. Berichte der deutschen chemischen Gesellschaft 69:1547. 1936.

29. Labes, R. and J. Freisburger. Alloxan as an oxidizing agent for thio groups, as a capillary poison and as a convulsive poison. Naunyn-Schmiedeberg's Archiv für experimentelle Pathologie und Pharmakologie unter Mitwirkung der deutschen pharmakologischen Gesellschaft 156:226. 1930. (Abstracted in Chemical Abstracts 25:4046. 1931).

30. Latimer, Wendell M. The oxidation states of the elements and their potentials in aqueous solutions. New York, Prentice-Hall, 1938. 352p.

31. Moser, James Howard. Colorimetric determination of calcium with ammonium purpurate. Master's thesis. Corvallis, Oregon state college, 1952. 81 numb. leaves.

32. Noller, Carl R. Chemistry of organic compounds. Philadelphia, W. B. Saunders Co., 1951. 885p.

33. Ostertag, Helene, and Emile Rinck. Le dosage colorimetrique du calcium a l'aide de la murexide. Chimie analytique 34; No. 5:108-109. 1952.

34. Piloty, Oscar and Carl Finckh. Ueber das Uramil. Justus Liebig's Annalen der Chemie 333:77-80. 1904.

35. Schwarzenbach, G. and H. Gysling. Metallindikatoren I. Murexide als Indicator auf Calcium- und andere Metall-Ionen. Helvetica Chemica Acta 32:1314-1325. 1949.

36. Schwarzenbach, G. and H. Gysling. Das Redoxgleichgewicht Purpureat = Dibarbiturylamin. Helvetica Chemica Acta 32:1108-1113. 1949.

37. Slimmer, M. and J. Steiglitz. The constitution of purpuric acid and murexide. American chemical journal 31:661. 1904.

38. Sweeney, Keith Holcomb. Ferromagnetic compounds of manganese with group V-A elements. Ph.D. thesis. Corvallis, Oregon state college, 1951. 122 numb. leaves.

39. Tukey, J. W. Comparing individual means in analysis of variance. Biometrics 5:99-114. 1949.

40. Willard, H. H., L. L. Merritt and J. A. Dean. Instrumental methods of analysis. New York, D. Van Nostrand, 1949. 247p.

41. Williams, M. B. and James H. Moser. Colorimetric determination of calcium with ammonium purpurate. Analytical chemistry 25:1414-1417. 1953.

APPENDIX

APPENDIX

EVALUATION OF FIRST ORDER RATE CONSTANTS FROM ABSORBANCE DATA

Suppose that the rate law for the decomposition of murexide under constant solvent conditions is

$$\frac{-d(M)}{dt} = k(M) \qquad (1)$$

where (M) equals the concentration of the dye determined colorimetrically, t is the time and k is a constant. Since (1) is a differential equation with separable variables, the integrated form is

$$\log_e (M)_o/(M)_t = kt \qquad (2)$$

for a change of murexide concentration from $(M)_o$ at zero time to a value of $(M)_t$ at time t.

If Beer's law is obeyed by the dye under these constant solvent conditions, then

$$A = ab(M) \qquad (3)$$

where A is the absorbance and b is the length of the light path through the solution*. Now, if the rate of reaction is followed by measuring the absorbance at various times in cuvettes of equal length at a given wavelength, equation

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* According to reference 25, c is recommended for concentration, but (M) will be used here in order to lend continuity to the discussion.

3 may be substituted into equation 2 (with the use of the appropriate subscripts) to give

$$\log_e A_o/A_t = kt \qquad (4)$$

or

$$\log_e A_o - \log_e A_t = k^t. \qquad (5)$$

Since $\log_e A_o$ is a constant for any one run, $\underline{k}$ may be numerically evaluated by the method of least squares (18, pp.117-121), or by plotting $\log_e A_t$ or $\log_{10} A_t$ against the time with subsequent graphical determination of the slopes $\underline{k}$ or $\underline{k}/2.3026$, respectively.