

1959

Hydroxyazo dyes as metal ion indicators

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HYDROXYAZO DYES AS METAL ION INDICATORS

by

Frederick John Lindstrom

A Dissertaion Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Major Subject: Analytical Chemistry

Approved:

Signature was redacted for privacy.

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Iowa State University
Of Science and Technology
Ames, Iowa

1959

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ERIOCHROME BLACK T AS A METAL ION INDICATOR

Introduction

Discovery and early investigation

The o,o'-dihydroxyazo dye most used as an indicator in complexometric titrations for calcium and magnesium employing EDTA is Eriochrome Black T. Eriochrome Black T was first prepared by Hans Hagenbach in 1904 by coupling diazotized and nitrated 1-amino-2-naphthol-4-sulfonic acid, Figure 12(a), to 1-naphthol in a strongly basic solution (21). The resultant dye was found to have excellent fastness qualities as a black dye for wool using the "after-chroming process". In this process, the dye was applied to the fabric by heating in a hot dilute base solution which colored the wool a dull reddish-brown. The permanent black color was developed by heating the dyed fabric in an alkaline chromate solution. The color and fastness of dyes of this type are apparently due to the formation of a chelate complex of chromium. The dyeing action of this class of dyes has been reviewed by Venkataraman (25).

A later investigation of the constitution of Eriochrome Black T produced an unexpected result: The coupling had not taken place at the supposedly favored 4 position on the 1-naphthol but rather the dye resulted from exclusive

coupling in the 2 position (10). Since most diazonium salts couple to 1-naphthol almost exclusively in the 4 position, it was surprising that the nitrated diazonium compound should violate the rule and couple to 1-naphthol to produce only the *o,o'*-dihydroxyazo dye with no trace of the expected *o,p*-dihydroxyazo dye. Even if the 1-diazo-2-naphthol-4-sulfonic acid was not nitrated, it was found to couple preferentially but not exclusively in the 2 position forming the *o,o'*-dihydroxyazo dye known as Eriochrome Blue-Black B.

The position of the nitro group on the diazonium salt and on the resultant dye was not correctly known by Hagenbach and other early workers. At that time it was assumed to be in the 5 position on the naphthalene ring. The dye was listed as a 5 nitro derivative by no less authorities than the Colour Index which appeared in 1924 (7) and the seventh edition of the Farbstofftabellen which appeared in 1931 (21). In 1930, however, Paul Ruggli and co-workers conclusively proved that the nitro group entered the 6 position on the diazonium compound (18) and later proved that the nitro group was also in the same 6 position in Eriochrome Black T (19). The second edition of the Colour Index which appeared in 1956 has the correct formula (8). See Figure 12(b). Many recent references to the use of the dye use the older incorrect chemical name of the compound.

Use of Eriochrome Black T as a metal ion indicator

Eriochrome Black T and several other dyes were shown to form complexes with certain metal ions including calcium and magnesium by Brenner (3). The complexes were of different color than the dyes themselves.

Schwarzenbach and Biedermann (23), in the first paper on the use of azo dyes as indicators in complexometric titration, investigated four dyes, Eriochrome Blue-Black B, Eriochrome Blue-Black R, Eriochrome Black T and Eriochrome Black A. These dyes are designated 239, 240, 241 and 242 respectively in the Schultz-Lehmann Farbstofftabellen (21); 201, 202, 203 and 204 in the first edition of the Colour Index (7); and 14640, 15705, 14645 and 15710 in the second edition of the Colour Index (8). Each of these *o,o'*-dihydroxyazo dyes had the property of forming calcium and magnesium derivatives having colors different from those of the dyes themselves. All of the dyes were found to be useful as indicators in complexometric titrations with EDTA. Schwarzenbach and Biedermann found in all cases that the ratio of dye to calcium or magnesium was one to one in the complexes. They further measured the acid dissociation constants of the dyes and the apparent stability constants of the metal derivatives. From these constants they were able to show that Eriochrome Black T was the most sensitive of the four

dyes for the detection of small amounts of magnesium and therefore presumably the best of the dyes as an indicator for complexometric titrations.

Contrary to the findings of Schwarzenbach and Biedermann two American groups using the method of continuous variations have reported that Eriochrome Black T and magnesium combine in the ratio of two to one at pH 10.1 (12) and with calcium and magnesium in the ratio of one to one, two to one and three to one depending on pH (27).

A series of *o*-hydroxyazo and *o,o'*-dihydroxyazo dyes was investigated by Ellingboe (9) who found that two hydroxy groups ortho to the azo group were necessary for the union of calcium and magnesium with the dyestuff and that with all of the dyes studied the combining ratio was one to one, irrespective of pH.

There are two spectrophotometric methods which have been employed in the study of metal complexes. These two methods, both outlined by Paul Job, are known as the log-ratio method and the method of continuous variations. The log-ratio method permits one to obtain the combining ratios and the apparent stability constants of complexes. The method has the advantage that one does not need to know the concentration of the complexing material in solution; it has the disadvantage that the complex must be weak enough so that the concentration of metal ion added to the solution is only

slightly decreased by the formation of the complex. The method of continuous variations permits one to obtain the combining ratio and also it permits one, in certain cases, to obtain the apparent stability constant of the complex. The method has the advantage that it permits one to investigate very stable complexes; it has the disadvantage that the concentration of both metal ion and the complexing component in the solution must be equal and quite accurately known. These two methods complement each other nicely. A more detailed description of these methods will be given along with the experimental study of the calcium and magnesium compounds of Eriochrome Black T.

Diehl and Ellingboe used the method of continuous variations to study only those compounds that were prepared in crystalline form and of known composition. However, most of the dyes they studied formed complexes weak enough to permit them to use the more convenient log-ratio method.

Schwarzenbach and Biedermann (23) used a method which was equivalent to the log-ratio method in determining the stability constants and the combining ratio of the Eriochrome series of dyes. By working at pH values less than 9 they were able to work in a region in which the stability of the magnesium dye complex was low enough so that the log-ratio method could be successfully applied. The American workers on the other hand investigated both the calcium and the

magnesium derivatives at a pH of 10 or higher. They used the method of continuous variations to study the Eriochrome Black T system and were therefore required by the method to prepare solutions of metal ions and of the dye of equimolar concentration. While the metal ion solutions provided no difficulty, they committed a serious error in preparing the Eriochrome Black T solutions. Both of the American groups prepared their dye solutions by weighing out commercial samples of the dye and diluting to volume with the assumption that the dye was 100 percent pure.

Commercial sample of dyes are seldom 100 percent pure since the purity of dyes is not important in commercial uses and purification of dyes is quite difficult. As long as dyeing or coloring results are consistent, the usual commercial dye customer is quite satisfied. In order to insure this consistency manufacturers often dilute lots of dyes of higher than usual purity with salt so that each lot of dye will have the same coloring power.

Commercially available Eriochrome Black T was found to contain a large amount of salt; some of which may have been added to obtain consistent dyeing results and a large amount which remained from the preparation of the dye which was salted out of solution. When viewed under a microscope, commercial samples of Eriochrome Black T showed many large transparent crystals which appeared to be inorganic. When

a sample of the dye was ignited to destroy the organic and carbonaceous material, a large amount of a fusible ash remained which was completely water soluble.

Since it was desired to repeat the work of the American workers at pH 10 in order to establish the correct combining ratio of the calcium and magnesium derivatives of Eriochrome Black T, it was necessary to prepare a sample of Eriochrome Black T either 100 percent pure or of known purity. Of the various workers who investigated this dye in the past, none was found who had ever prepared a sample 100 percent pure or who had ever given an analysis of a sample of the dye. The physical properties of the pure material were still unknown. While a chemical analysis may have seemed attractive, there was no assurance that the impurities in the dye would not vitiate the analysis.

John L. Ellingboe in private communication told of his attempts to purify Eriochrome Black T. He tried to crystallize the dye from various organic solvents without success of any sort. He also tried to duplicate the work of Schwarzenbach and Biedermann (23) who reported that they recrystallized the dye from hot ammonia solution. After many attempts he was unable to obtain crystals large enough to see under a microscope. Ellingboe also tried the Craig counter-current distribution apparatus in order to separate the impurities. He was successful only in separating the

various protonated forms of the dye. He was able to remove one of the impurities from the dye by using paper chromatography. If the dye was placed on the paper with methanol and eluted with benzene and other hydrocarbon solvents, a yellow material was separated which was very soluble in the non-polar solvents. He did not pursue the problem further for lack of time.

At this point, the author set out to prepare a crystalline form of Eriochrome Black T.

Preparation of a Pure Form of Eriochrome Black T

Attempts to purify commercial samples of the dye

Various commercial samples of Eriochrome Black T were examined to determine which of them would be most suitable for further purification. Each dye was examined using a Beckman DU spectrophotometer at 620 m μ and with the dye solutions at pH 10. The values obtained for $E_{1\%}^{1\text{cm}}$ for various commercial dye samples is given as follows:

Mathieson Coleman and Bell 392, Hartman-Leddon 383, K & K Laboratories 404, Hach Chemical Co. 371 (material used by one American group as a primary standard (12)), W. H. & L. D. Betz 403 (material used by the other American group (27)), Eastman Kodak practical grade 443. It is easy to see from the above data that the material supplied by Eastman Kodak is the best and is in fact better than the material used by

the previous American investigators as a pure product. Eastman Kodak practical grade (only grade supplied) 1-(1-Hydroxy-2-naphthylazo)-5-nitro-2-naphthol-4-sulfonic Acid Sodium Salt was selected for further purification. It may be noted that the above name is incorrect in the placement of the nitro group in the 5 rather than the 6 position.

The progress of the purification was followed by preparing dilute solutions of known concentration that had been adjusted to pH 10 using ammonia-ammonium chloride buffer and observing the spectra which were obtained by using the Cary model 11 recording spectrophotometer. By this means it was hoped that the removal of colorless impurities could be noted by the change in the absorption spectra. The level of purity was indicated by the absorbancy of a 1% solution or its equivalent, 1 cm. thick, at 620 m μ ($E_{1\%}^{1\text{cm}}$).

Commercial Eriochrome Black T might be expected to contain sodium chloride or sulfate from the salting out process or as a diluent, perhaps some excess 1-naphthol and excess 1-diazo-6-nitro-2-naphthol-4-sulfonic acid and its decomposition and side reaction products. Little difficulty was anticipated in removing the inorganic salts and the 1-naphthol but the impurities caused by the diazonium salt were expected to be another matter since chemically they should

be very much like the dye itself. Also it was felt that there might be a very good reason why no one had successfully prepared and reported a pure form of the dye for analysis since its discovery in 1904.

Purification of the dye was started by attempting to recrystallize the Eastman Kodak material from several solvents. These included water, buffered solutions, methyl alcohol, ethyl alcohol, butyl alcohol, isoamyl alcohol, acetone, methyl isobutyl ketone, mesityl oxide, dioxane and also many others and their mixtures. In each case the material was dissolved at the boiling point of the solvent, the solution was filtered and a drop of the cooled mixture was examined under a microscope to observe crystallization. Of all the solvents and mixtures tried only butyl alcohol showed any promise of producing crystals. While the dye did separate as crystals from butyl alcohol, they were far too small to be successfully filtered and the yield was extremely small since the dye was not appreciably soluble in butyl alcohol. This method was without value.

Recrystallization from hot ammonia solution, the method used by Schwarzenbach and Biedermann (23) and unsuccessfully tried by Ellingboe, was also attempted with no success whatever.

On the assumption that the dye was too impure to crystallize from one of its solvents it was decided to remove what impurities could be readily removed before attempting to recrystallize the material again. From the work of Ellingboe it was evident that a yellowish material, soluble in benzene, could be removed by extraction. Therefore, a sample of the dye was treated for twenty-four hours in a Soxhlet extractor with benzene. A small amount of the yellowish impurity was removed. The waxy, semi-crystalline material remaining in the flask after the benzene had been evaporated smelled strongly phenolic so it was supposed that this step removed the 1-naphthol which was present in addition to the yellow impurity. No change in the spectra of the dye was noted.

It was expected that the removal of the salt from the dye would be simple since one should be able to find an organic solvent that would be suitable for the dye that would not dissolve the salt. Since sodium chloride and certain other inorganic salts are quite insoluble in acetone and the dye is soluble in this solvent, it would seem to be the solvent of choice. However, in actual practice the dye was not soluble enough in acetone to process more than small amounts of the dye and also acetone dye solutions had a serious tendency to bump if heat was applied during the removal of the solvent. Combined with this,

the acetone seemed to undergo some sort of condensation, probably catalyzed by the extremely acid nature of the solute, which left a higher boiling, oilish material on the dye which had an odor like a higher ketone. Ethanol was found to be more suitable in some respects but appreciable amounts of the salt dissolved in the solvent. Higher alcohols were found to be much poorer solvents for the dye.

It was noted that the dye was insoluble in dilute hydrochloric acid and that the dye could be precipitated from aqueous solution by the addition of acid. However, when the dye was dissolved in water, it had the properties of a thick, red soap solution. When acid was added to precipitate the dye, the dye separated as a slimy, amorphous precipitate with all the physical properties of hydrated ferric oxide. A few grams of the dry dye produced an enormous volume of precipitate that was all but impossible to filter.

It was found that if the dried dye was stirred up with 1:5 hydrochloric acid it showed no tendency to go into solution or to swell and disperse as a colloid. This permitted the desalting of large, 100 g. samples of the commercial dye with reasonable amounts of the solvent (about 2,000 ml. of the dilute acid). By extracting the dye with four portions of the solvent it was possible to remove essentially all of

the salt originally present. The dye was washed with ether to remove the excess acid. The presence of salt was tested using the usual flame test. The value $E_{1\%}^{1\text{cm}}$ on a sample of the dye which had been benzene extracted, desalted, ether extracted and dried at 110° was found to be 632 compared to 443 for the unpurified material. If the sample just prepared was considered to be pure, the original material contained about 30 percent salt.

The material was then dried at 120° for three hours under a vacuum. The value for $E_{1\%}^{1\text{cm}}$ rose to 665. Since sulfonic acids are often hydrated, it was expected that such drying would result in a loss in weight. The calculation of a hydrate, molecular weight ratio did not result in either a 1:1 or a 2:1 ratio but rather fell between the two.

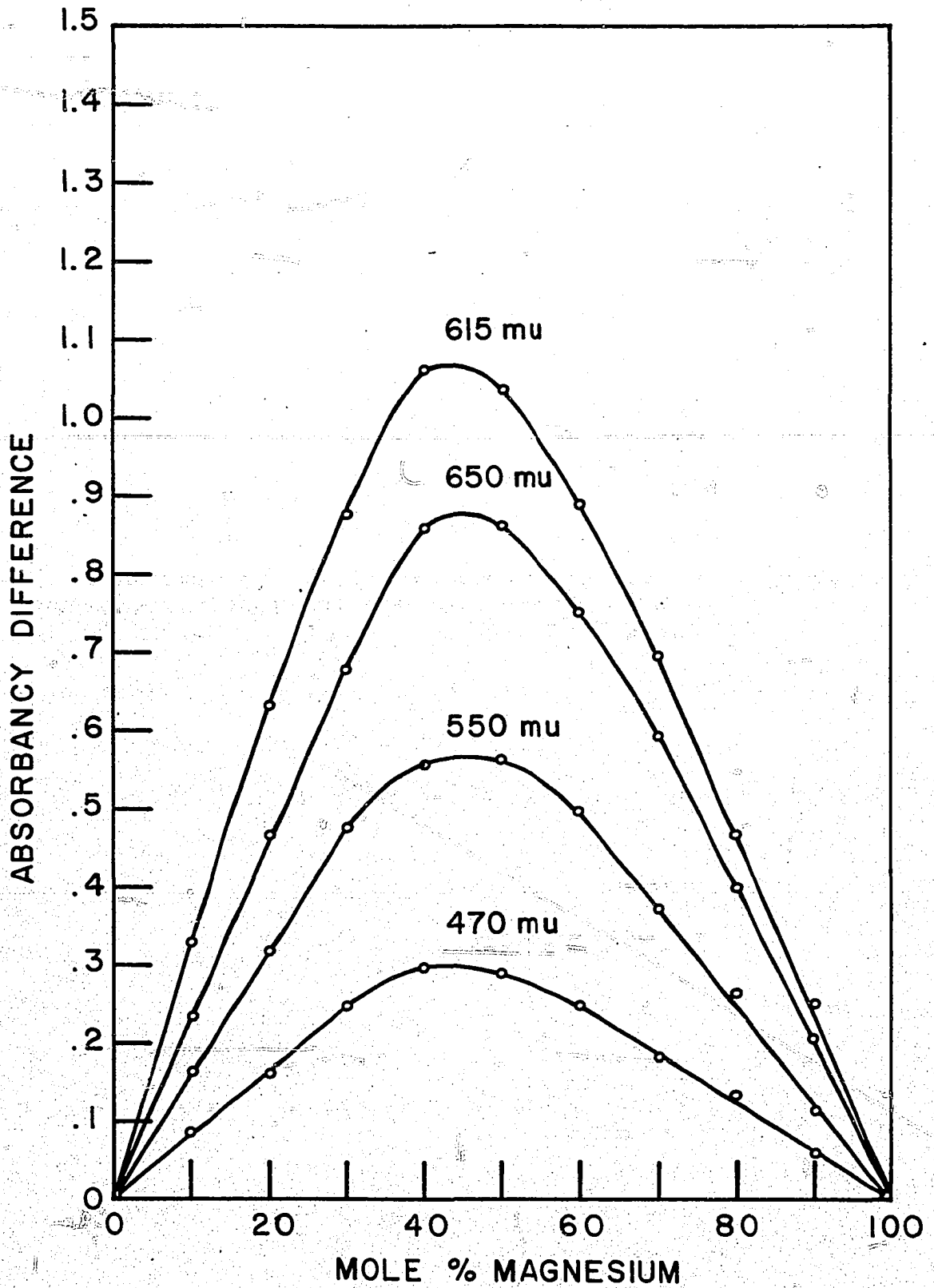
The dried, salt-free extracted dye was used in an attempt to determine the complexing ratio of magnesium and Eriochrome Black T at pH 10 by the method of continuous variations.

Assuming that the dye so treated was pure, equimolar dye and magnesium stock solutions were made up and the continuous variations mixtures were prepared, each solution being buffered to pH 10 with ammonia-ammonium chloride buffer. The absorption spectra were obtained using the Cary spectrophotometer. The plots obtained at various

wave lengths are shown in Figure 1. It is evident that the plots do not illustrate a one to one complex. However, the plots do indicate, according to Vosburg and Cooper (26), that only one complex is present in solution. It was postulated that the reason the plots did not show a one to one combination was that the dye was not pure and the experiment was invalid. The dye probably contained organic impurities which behaved chemically similar to the dye and a purification process must be found which would be able to separate chemicals of similar properties.

The first of such attempts was made using chromatography with an activated alumina column. This had been tried before in the purification of another dye and had worked quite well. The desalted and extracted Eriochrome Black T was dissolved in ethyl alcohol and placed on the column. The dye adhered quite tenaciously and organic solvents were without effect in an attempt to spread the dye out along the column except for a very small amount of a yellowish impurity which did move off the column with the alcohol. Finally it was found possible to remove the dye from the column with a 1:1 mixture of hydrochloric acid and ethyl alcohol. The dye thus treated was examined spectrophotometrically and found to contain appreciable amounts of what was presumed to be an aluminum complex. The value for $E_{1\%}^{1\text{cm}}$ was found to have decreased to 439.

Figure 1. Continuous variations plots for magnesium and salt-free, benzene extracted Eriochrome Black T at a pH of 9.95



This material after drying was treated with 1:5 hydrochloric acid, washed with ether and again dried. The $E_{1\%}^{1\text{cm}}$ value rose to 557 and the evidence of a supposed aluminum complex in the spectra had vanished. Chromatography on alumina was unsuccessful for the purification of F-241, but it was interesting to learn that the aluminum could be removed from the dye by the acid washing process.

An attempt was then made to separate the dye and the impurities on an ion exchange resin column (Amberlite IR 120). The dye was dissolved in water and placed on the column which was in the hydrogen form. Attempts to move the dye band or any impurities from the column were completely unsuccessful. The resin was discarded containing all the dye which had been placed on it originally.

Then it was hoped that the dye would precipitate from solution at pH 10 as the copper complex by the addition of a cuprammina solution leaving the impurities in solution. The dye readily precipitated from solution under the conditions mentioned and the material was filtered from solution and dried. Then the dye-copper compound was treated with 1:5 hydrochloric acid to break up this complex in the same manner as the aluminum complex. All of the copper was removed by the dilute acid. The recovered dye was dried for two hours under a vacuum at 110° and was examined.

spectrophotometrically at pH 10. The absorption spectra showed no trace of a complex but indicated that this process actually resulted in a more impure product for the $E_{1\%}^{1\text{cm}}$ value was now 577 while it had been 665. It was becoming evident that some of these chemical treatments might be decomposing the dye faster than they were effecting the purification. Since the author had exhausted his ideas for purification, this line of work was discontinued for the time being at least. It was thought that it might be worthwhile to investigate the magnesium-dye complex near pH 8 using the log-ratio method. Since this method would not require a pure product, it would permit a check on the work of Schwarzenbach and Biedermann (23) who used a similar method. If our results agreed, it was hoped that work could continue with at least some measure of confidence.

Preliminary study of the magnesium dye compound at pH 8 by the log-ratio method

The dye stock solution for the study was prepared by dissolving 0.2204 g. of the salt-free dye in water and diluting to 500 ml. to give a solution which was roughly 1×10^{-3} M. A standard magnesium acetate solution was prepared by dissolving 0.02 M of magnesium acetate (Baker's Analyzed reagent %Ca = 0.001) in 2,000 ml. of deionized water. The solution was analyzed for magnesium using an

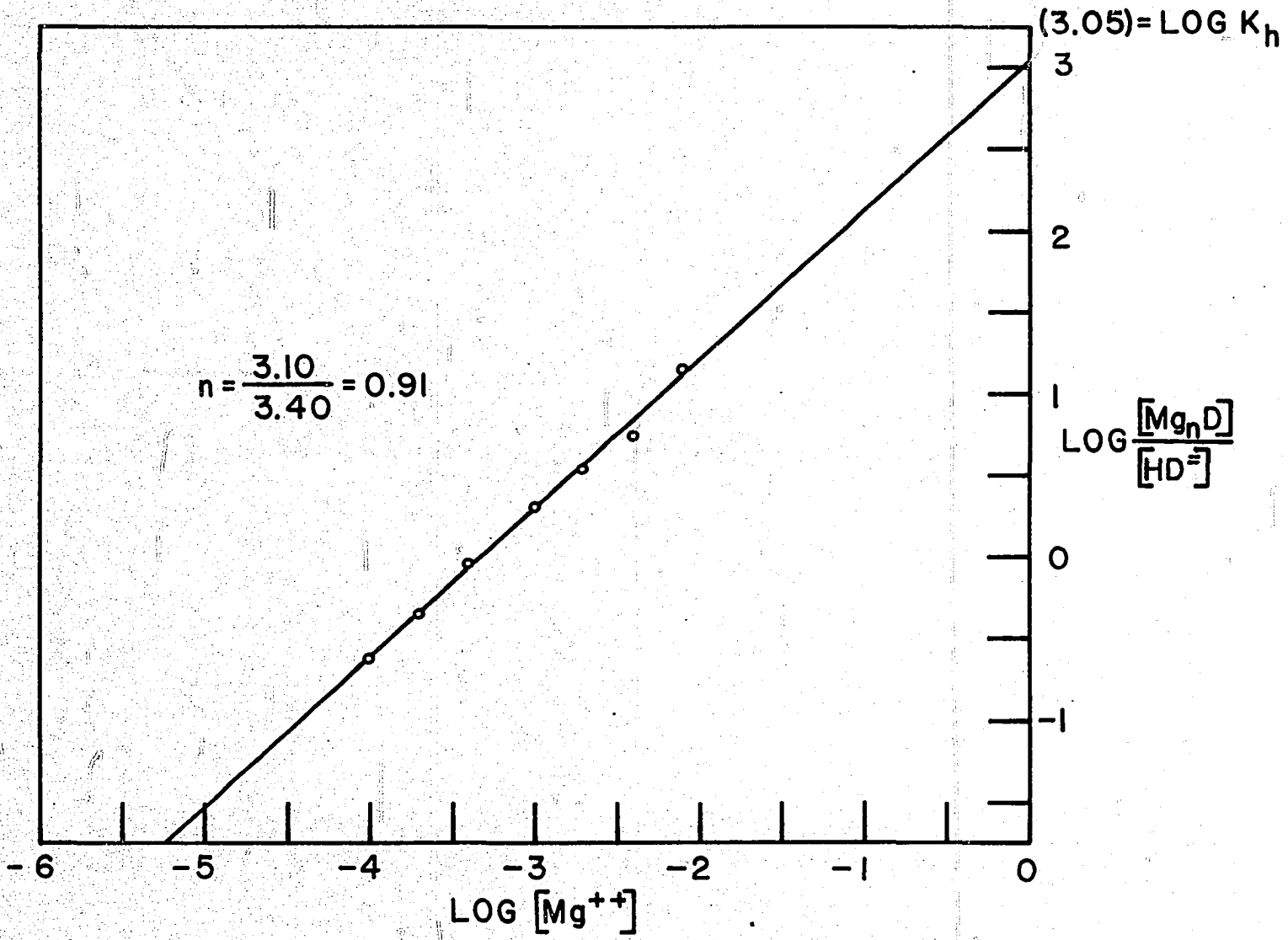
EDTA titration and found to be 0.01002 M. A buffer solution was prepared using the proportions of ammonium chloride and ammonium hydroxide necessary to produce a buffer with a pH near 8. The solutions which were used for the measurements were prepared in the following manner: 0, 1, 2, 4, 10, 20, 40, and 80 ml. of the magnesium solution was added to each of eight 100 ml. volumetric flasks. Then 3 ml. of the dye stock solution and 1 ml. of the buffer were added and each of the flasks was made up to 100 ml. with deionized water. No attempt was made to control the ionic strength. The spectra were obtained with the Cary spectrophotometer using 2 cm. cells and water as a reference. The pH of each of the solutions was measured using a Beckman model G pH meter with type E electrode which had been standardized against the NBS borax buffer. It was found to be 8.17 in each case.

Table 1 gives the concentration of magnesium added, the log of the magnesium concentration, the absorbancy of the solutions at 620 m μ , and the data which was used to compute the log of the ratio of the concentration of the complex and of the concentration of the dye. The log of the ratio was plotted versus the log of the magnesium ion concentration in Figure 2. The plot indicated that the complex at pH 8.17 was one magnesium to one dye molecule.

Table 1. Preliminary log-ratio method for magnesium and Eriochrome Black T at pH 8.17 and $\lambda = 620 \text{ m}\mu$

$[\text{Mg}] \times 10^4$	$\text{Log}[\text{Mg}]$	A	$A_{\text{HD}} - A$	$A - A_{\text{Mg}_n\text{D}}$	$\frac{A_{\text{HD}} - A}{A - A_{\text{Mg}_n\text{D}}}$	$\text{Log} \frac{A_{\text{HD}} - A}{A - A_{\text{Mg}_n\text{D}}} = \text{Log} \frac{[\text{Mg}_n\text{D}]}{[\text{HD}]}$
0	-	1.66	-	1.46	-	-
1	-4.00	1.39	0.27	1.19	0.227	-0.644
2	-3.70	1.21	0.45	1.01	0.445	-0.351
4	-3.40	0.96	0.70	0.76	0.922	-0.035
10	-3.00	0.68	0.98	0.48	2.04	0.310
20	-2.70	0.53	1.13	0.33	3.43	0.534
40	-2.40	0.41	1.25	0.21	5.95	0.774
80	-2.10	0.28	1.38	0.08	17.25	1.14
excess	-	0.20	1.46	-	-	-
		slope or n = 0.91		$\text{log } K_{8.17} = 3.05$		

Figure 2. Log-ratio plot for magnesium and salt-free Eriochrome
Black T at pH 8.17. $\text{Log } K_{8.17} = 3.05$



The extrapolation of the straight line gave the log of the apparent stability constant as 3.05 at pH 8.17. From this one would have 2.88 as the log of the stability constant at pH 8.0. Schwarzenbach's (22) value at pH 8 was 3.45. The agreement was not very good but it indicated that this work was in considerably greater agreement with the work of Schwarzenbach than it was with the work of the two American groups.

A more detailed presentation of the log-ratio method is given in the discussion of the study of the magnesium dye compound at pH 8.00 using the pure dye which was finally obtained.

Synthesis of Eriochrome Black T

Being at a loss for a method for the further purification of the dye and having been reassured by the log-ratio experiment that the work of Schwarzenbach was indeed correct, an attempt was made to synthesize Eriochrome Black T and compare it with the commercially available dyestuff.

The first step in the preparation of Eriochrome Black T is the preparation of 1-diazo-2-naphthol-4-sulfonic acid. This was prepared according to the method of Schmidt and Maier (20); 150 g. of 1-amino-2-naphthol-4-sulfonic acid was dissolved in 1,000 ml. of water in a 2,000 ml. beaker. The beaker was placed in an ice-bath and 12.5 g. of copper

sulfate was added as a concentrated solution. Then 45 g. of sodium nitrate also as a solution was added slowly over the period of about an hour. The mixture was kept below about 25°C. Foaming was limited by the addition of a drop or so of octyl alcohol. The resultant brownish-black solution was then filtered with a basket centrifuge to remove tar and oil. The filtrate was treated with concentrated hydrochloric acid which precipitated the diazo oxide. The precipitate was filtered on a Büchner funnel (Whatman #50 paper) and washed with dilute hydrochloric acid. The air dried product was a brownish-yellow powder which would turn reddish when exposed to bright light. Schmidt and Maier state that it is possible to purify the product by recrystallization from hot water but that some decomposition takes place. The recrystallization was tried but the loss due to decomposition was so great that this purification step was abandoned. Somewhat later it was found that this material could be obtained from the Aldrich Chemical Company of Milwaukee, Wisconsin.

The above material was used for the synthesis of the 1-diazo-2-naphthol-6-nitro-4-sulfonic acid. This was prepared according to the directions which accompany the description of Eriochrome Black T in the Farbstofftabellen (21). The nitration proceeded smoothly and easily. The

orange material from the nitration was found to be soluble in water and ethanol. It was washed with diethyl ether to remove the adherent nitration acids. The material could be easily recrystallized from hot ethanol giving rhombohedral yellow crystals about 1 mm. long. On standing in air to dry, the clear crystals became opaque and started to crumble. The resultant material was ground and air dried further. The compound was sensitive to light turning dark after a day's exposure to room light. It was not explosive but decomposed without melting going from orange to brown to black.

Since the material was strongly acidic, it was thought that a neutralization equivalent determination might be in order. Three samples of the nitro diazo oxide were titrated with 0.1 N sodium hydroxide using a Beckman H-2 pH meter for a potentiometric end point. The values obtained were 330.7, 330.9 and 333.0 for an average of 331.5. The molecular weight for $C_{10}H_5O_6N_3S$ is 295.23; however, the molecular weight for $C_{10}H_5O_6N_3S \cdot 2H_2O$ is 331.29. It was evident that the crystalline material was the dihydrate of 1-diazo-2-naphthol-6-nitro-4-sulfonic acid. It was believed that since sulfonic acids as a group and this one in particular are hydrated, there was a strong possibility that the free acid form of Eriochrome Black T was also hydrated.

The above material was then used in the preparation of Eriochrome Black T by the method described in the Colour Index (7). The resultant dyestuff was extracted with benzene and 1:5 hydrochloric acid until all traces of 1-naphthol and sodium chloride were absent. The dye was then dried in a 30% hygostat in the hopes of preparing an undecomposed form of the dihydrate of the dye if it in fact existed. The absorption spectra was taken at pH 10 and was found to be identical in shape with that obtained from the Eastman Kodak product. The value for $E_{1\%}^{1\text{cm}}$ was 629 compared to 632 for the air dried Eastman Kodak product.

At this point it seemed pointless to attempt to outdo the chemists at the Eastman works so further attempts to obtain a pure product were to be attempted using the desalted Eastman product.

Since the synthesis of Eriochrome Black T required the preparation of the intermediates, samples of the intermediates were available for study as possible impurities in the purified material. With this in mind, the infra-red spectrum of 1-diazo-2-naphthol-6-nitro-4-sulfonic acid and purified samples of Eriochrome Black T were obtained. The spectrum of the diazo compound had a very strong absorption band at 4.6 microns indicating the presence of triple bonding. This triple bonding was assumed to be due to the

diazonium group in the molecule. Since the absorption band was completely absent from the spectra of the dyestuff samples, it was felt that the dyestuff was free of any unreacted diazo material. This would not, however, rule out the presence of decomposition products of the 1-diazo-2-naphthol-6-nitro-4-sulfonic acid.

Returning to the problem of the further purification of the salt-free, benzene extracted dye, another attempt was made to recrystallize the material from dimethylformamide, a liquid being increasingly used because of its remarkable solvent properties. Some of the purified dye was added to a few milliliters of the liquid and it dissolved quite easily in sharp contrast to its low solubility in other solvents. More of the dye was added and it too went into solution. Finally a volume of the dye equal to the volume of the liquid was found to dissolve quite readily and to produce considerable heat on dissolving. A concentrated solution of the dye in dimethylformamide was filtered and allowed to stand during the supper hour. Upon returning it was found that the entire solution had congealed to a stiff mass. Some of the material was examined under a microscope and the entire field was a mass of long red needles. A photograph of these crystals is given as Figure 3. It began to appear that there was a road out of the forest.



Figure 3. Photomicrograph of Eriochrome Black T crystals obtained from dimethylformamide

Since the supply of the salt free, benzene extracted Eastman Kodak dye was almost exhausted, it was decided that a batch of crystalline dye would be made by starting with the Eastman Kodak material and carrying it through the entire purification process.

Procedure for the preparation of a pure form of Eriochrome Black T

Two hundred grams of practical grade Eastman Kodak 1-(1-Hydroxy-2-naphthylazo)-5-nitro-2-naphthol-4-sulfonic Acid Sodium Salt was desalted by stirring the dye with 500 ml. of 1:5 hydrochloric acid. The slurry was stirred for

several minutes and was then filtered by suction using a fritted glass funnel. This process was repeated with another 500 ml. of the dilute acid. The acid extracts were evaporated and yielded 48 g. of salt. The acid extracted dye was washed with ether to remove excess acid and was allowed to air dry.

The acid extracted dye was then extracted with benzene for 12 hours in a Soxhlet extractor. The benzene was evaporated, yielding a yellowish, semi-crystalline material of indefinite melting point having a phenolic odor. The extracted dye was again air dried.

The dye was desalted again with a liter of 1:5 hydrochloric acid. The extract was evaporated yielding a further 6.5 g. of salt. In all, 54.5 g. of salt were removed from the 200 g. of starting material; this represents 27 percent salt in the original material. If the starting material was the pure sodium salt of Eriochrome Black T, 25.4 g. of sodium chloride should have been obtained in converting the dye to the free acid.

All of the acid extracts were bright yellow. This yellow color was found to be due to iron present in the dye. The iron probably came from the action of the starting materials on iron reaction kettles.

Some of the desalted dye was then dissolved in about 50 ml. of dimethylformamide at room temperature. The

solution heated up very noticeably as the dye dissolved. When the solution appeared to dissolve no more dye, it was filtered and was allowed to stand for a few hours. The filtrate became quite stiff with crystals. These were filtered from solution and washed with benzene. They were labeled Lot I and weighed 12.5 g.

The mother liquor still contained a large amount of the dye, so it was used as the solvent for some more dye which was dissolved after the mother liquor had been heated to near the boiling point of dimethylformamide, 154°. The hot solution was filtered and on cooling deposited crystals. These crystals were washed with benzene, air dried, weighed 32 g. and were labeled Lot II. The remainder of the desalted dye was then dissolved in the heated mother liquor from Lot II, filtered, cooled, filtered again and the crystals obtained were washed with benzene, air dried and weighed, yielding 31 g. of dye which was labeled Lot III.

The mother liquor from the Lot III crystals still contained a large amount of dye and this was recovered by adding benzene to precipitate the dye. The precipitated dye was filtered off, washed with benzene, air dried and weighed. This was labeled Lot IV and weighed 50 g.

The 200 g. of starting material yielded 54.5 g. of salt, a small amount of benzene soluble material, 75.5 g. of crystalline dye and 50 g. of amorphous dye; therefore, 180 g. of

of the original impure dye was accounted for. Since the nature of hydration of the original dye and all of the products was not known, this amount of recovery was very pleasing.

The recovered crystalline and amorphous dye was examined spectrophotometrically to obtain an indication of the purity of the material. About 15 mg. of each lot of dye was dissolved in a liter of water buffered to pH 10 and was measured with the Beckman DU spectrophotometer at 620 m μ . Lot I had an $E_{1\%}^{1\text{cm}}$ of 656.6. Lot II had an $E_{1\%}^{1\text{cm}}$ of 654.4. Lot III had an $E_{1\%}^{1\text{cm}}$ of 653.0. Lot IV, the precipitated amorphous material, had an $E_{1\%}^{1\text{cm}}$ of 424.6. Since the $E_{1\%}^{1\text{cm}}$ values of Lots I through III were the same within experimental error, it was evident that each lot was a pure crystalline form of Eriochrome Black T possibly in the form of a dimethylformamide solvate. Since the $E_{1\%}^{1\text{cm}}$ of the Lot IV dye was so much lower, it was evident that it contained the bulk of the impurities. The crystals composing the crystalline fractions were a reddish brown and were of microscopic size.

Conclusions

After many unsuccessful attempts to purify Eriochrome Black T, a method was finally developed whereby the largest impurity, inorganic salt, could be completely and simply

removed from the dye. The salt-free product was not crystalline, however, no further attempts were made to find some means of preparing a crystalline form of the dye. After many unsuccessful attempts to prepare crystals it was found that the dye behaved in a very singular manner when dissolved in the solvent, dimethylformamide. Unlike all other solvents tested, very large amounts of dye dissolved readily in dimethylformamide and, very much unlike other solvents, the solutions when allowed to cool deposited crystals. This was the first time that this commercially important dyestuff and recently important analytical reagent was prepared in a crystalline form since its discovery by Hans Hagenbach in 1904 over half a century ago.

Analysis and Characterization of Eriochrome Black T Crystals

Elemental analysis

A sample of the Lot II dye was sent to the Huffman Microanalytical Laboratories for elemental analysis. The percentages reported were: carbon 53.37, hydrogen 4.28, nitrogen 10.99 and sulfur 6.30. The oxygen percentage was calculated by difference to be 24.73. From the above data the following empirical formula was computed: $C_{22.6}H_{21.7}N_{4.00}S_{1.00}O_{7.87}$. Since the empirical formula of Eriochrome Black T was $C_{20}H_{13}N_3S_1O_7$, these results were quite

disturbing. Since the nitrogen to sulfur ratio in the crystalline material was 4 to 1, it seemed plausible that one mole of dimethylformamide had crystallized with each mole of the dye. If this had happened, the empirical formula would be $C_{23}H_{20}N_4S_1O_8$. This agreed fairly well with the experimental results but the value for hydrogen was still considerably in error. In any event the next step was to be a check for dimethylformamide in the crystalline dye.

Detection and determination of dimethylamine from the crystalline dye

Knowing that dimethylformamide would hydrolyze very rapidly in basic solution to produce dimethylamine and the formate of the base employed, it was decided to treat a sample of the crystalline dye with strong sodium hydroxide solution in a semi-micro Kjeldahl steam distillation apparatus (17). Since dimethylamine had a boiling point of 7.4° and was very soluble in water, it was supposed that the amine could be distilled with steam and collected in water. About 1 g. of the Lot II crystals were treated in the above manner with about 25 ml. of 40 percent sodium hydroxide solution. After the steam distillation, the distillate was strongly basic and had an odor like ammonia.

The Hinsberg test was performed on a portion of the distillate. The properties of the benzenesulfonamide product

indicated that the amine was a secondary amine. Since the benzenesulfonamide of dimethylamine has a very low melting point (47°), it was not considered as a practical derivative. To prepare a useful derivative of dimethylamine the remainder of the distillate was treated with phenyl isocyanate to form the phenylthiourea of dimethylamine. A white crystalline material was obtained which was recrystallized from ethanol. It melted from $134-137^{\circ}$ on a melting point block. Shriner and Fuson (24) gave 135° as the melting point of the phenylthiourea of dimethylamine.

Since it had been demonstrated that the crystalline dye would release dimethylamine when treated with strong base, the next obvious step was to determine the actual amount of dimethylamine that would be released under these conditions. Since dimethylamine is an even stronger base than ammonia, the direct or back titration with acid of the organic base in the distillate was expected to be quite easy. The data and results of four determinations are given in Table 2. The first two determinations were by means of the back titration; the second two were by collecting the amine in saturated boric acid solution and titration of the amine directly. Chlorophenol red was selected as the most suitable indicator for the titration. The normality of the base used was 0.1146 N, the acid 0.0968 N. Both standard solutions were prepared in the usual manner.

Table 2. Determination of dimethylamine in the distillate from the basic hydrolysis of samples of Lot II Eriochrome Black T crystals

Weight of dye	Volume of acid	Volume of base	Percent of dimethylamine
0.5033 g.	25.00 ml.	12.00 ml.	9.36
0.5283	20.00	7.58	9.10
0.2002	4.14	-	9.04
0.4365	9.15	-	9.14
Average value			9.16

The theoretical amount of dimethylamine which should be released from the basic hydrolysis of a one to one combination of dimethylformamide and Eriochrome Black T was 8.80 percent so the experimental results were not greeted with any enthusiasm.

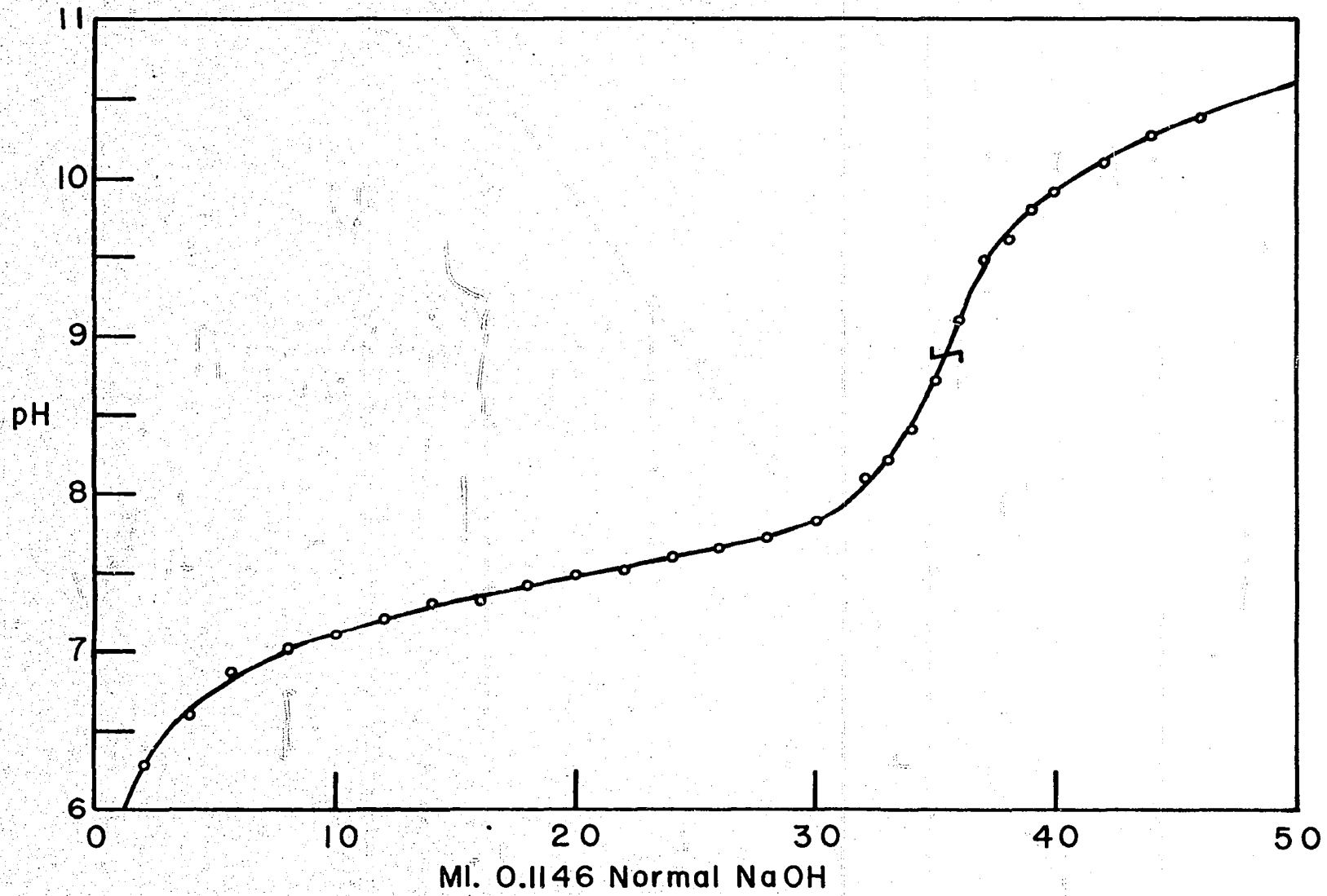
Determination of neutralization equivalent

In order to determine the molecular weight of the crystalline material it was decided to try a potentiometric titration to obtain the neutralization equivalent. The sulfonic acid group was expected to be titrated by the addition of the standard base solution. Therefore 2.0078 g. of the Lot II dye was dissolved in 100 ml. of a 20 percent dimethylformamide-water solution. It was necessary to add the dimethylformamide to the water in order to dissolve the dye. A blank run on the 20 percent solution showed that it would

not consume measurable amounts of base. The titration was followed using a Beckman model H-2 pH meter with the usual glass and calomel electrodes. The titrant was 0.1146 N sodium hydroxide which had been prepared and standardized in the usual manner. A 50 ml. Pyrex certified buret was used. The titration curve is presented as Figure 4. In an 80 percent water solution the activity of water is very roughly 0.80 and the pH is about 0.1 unit higher than it should be (2). The observed pH scale should be lowered 0.1 unit to give the values expected for pure water.

The titration yielded an astounding result. When the first few drops of base were added, the solution changed from dark red to blue. This indicated that the base was neutralizing one of the two hydroxyl groups on the molecule and not the sulfonic acid group which had been expected. Evidently the sulfonic acid had already been neutralized and the crystalline material was a salt and not the free acid. This was confirmed by the pH of the mid-point of the titration. If it were a sulfonic acid, the mid-point should lie near pH 2 or 3. However the pH at the mid-point was 7.4 (7.3 if corrected for the lowered activity of water) which is a reasonable value for a phenolic hydroxyl group and is in the same range as the first pK for Eriochrome Black T, 6.9, reported by Schwarzenbach and Biedermann (23). It was assumed that the crystalline material was the

Figure 4. Potentiometric titration of dimethylammonium salt of Eriochrome Black T in 20 percent dimethylformamide-water solution. 2.0078 g. of crystalline dye required 35.40 ml. of 0.1146 N sodium hydroxide for a neutralization equivalent of 494.9



dimethylamine salt of the dye, the dimethylamine presumably coming from the acid catalyzed decomposition of the dimethylformamide.

The neutralization equivalent was found to be 494.9. The molecular weight computed for the dimethylamine salt of Eriochrome Black T was 484.5. The results were ten units higher than the theoretical value but one might be able to account for this by a certain amount of hydration or surface moisture. If, for example, the dye contained a half-molecule of water, the results would be in very close agreement.

Determination of moisture content

Prior to an actual determination of the moisture content of the crystalline dye it was thought wise to run the pyrolysis curve of the material on the thermo-balance. When this was done, the material gradually lost weight from 110° to 280° at which point the material exploded. The actual percent lost in weight could not be determined with any accuracy by this method because it was so small.

A sample of the crystals was dried to constant weight for two days at room temperature in a vacuum over anhydrous magnesium perchlorate. The loss in weight was 1.73 percent. The theoretical loss in weight for a half hydrate of the dimethylamine salt of the dye was 1.83 percent. While the

agreement was not excellent it did complete the picture.

Summary of analytical results

A summary of the analytical results and a comparison with the theoretical percentages for the half hydrate of the dimethylammonium salt of Eriochrome Black T is given in Table 3.

Table 3. Summary and comparison of analytical results with theoretical values

Component	Experimental values	Theoretical values for the formula $C_{20}H_{12}O_7N_3S \cdot (CH_3)_2NH_2 \cdot \frac{1}{2}H_2O$
Carbon	53.37	53.54
Hydrogen	4.26	4.29
Oxygen	24.73 ^a	24.32
Nitrogen	10.99 ^b	11.35
Sulfur	6.30	6.50
Neutralization Equivalent	494.9	493.5
Dimethylamine	9.16	9.14
Water	1.73	1.83

^aThis value was obtained by subtracting the other elemental values from 100 percent

^bLow value for nitrogen possibly due to difficulty of using Dumas method for nitrogen on a sample containing low molecular weight amines

Conclusions

The crystalline Eriochrome Black T obtained from dimethylformamide solution was subjected to elemental analysis for carbon, hydrogen, sulfur and nitrogen. The results of the elemental analysis indicated that some of the solvent had crystallized with the dye. When the dye was heated in basic solution it released an amine which proved to be dimethylamine.

After performing a neutralization equivalent titration and several determinations of the dimethylamine content of the crystals it became evident that the crystals were actually the dimethylamine salt of the dye. A half mole of water was also found.

A comparison was made between the analytical results and the percentages calculated for the half hydrate of the dimethylamine salt of Eriochrome Black T. This was the first time analytical data on the compound was obtained which agreed with the accepted formula of the dye. The present formula of the dye (See Figure 12 (b)) is based on degradation studies by Ruggli and co-workers (19).

Having a supply of Eriochrome Black T of known composition and high purity, an investigation of the dye and its compounds could now be made with considerably more confidence than that enjoyed by the several other investigators

of the problem since the discovery of the dye at the turn of the century.

Free Acid Form of Eriochrome Black T

Preparation of the free sulfonic acid

In order to study some of the properties of the material which the American workers thought they were using, the free acid form of the dye was prepared in the following manner: A sample of the crystalline dimethylammonium salt was extracted repeatedly with dilute hydrochloric acid to form dimethylammonium chloride and remove this material from the dye. Since the dye itself was completely insoluble in the acid, the separation was quite effective. The material was air dried and dissolved in alcohol. The alcohol solution was filtered and allowed to evaporate. A semicrystalline mass was obtained which was also air dried. This material was qualitatively checked for the presence of dimethylamine by testing for the evolution of a basic gas when a small sample was heated with a strong base solution. The test was negative.

Study of the free dye acid

The acid form was dried to constant weight in a vacuum desiccator to remove surface and combined water. The loss in weight from the air dried basis was found to be 8.66

percent. Because of this large loss in weight the presence of a hydrate was suspected and an attempt was made to find the hydrate ratio. The anhydrous material was extremely hygroscopic and rapidly gained weight when weighed in an open container. The material was then stored in a desiccator over a saturated solution of calcium chloride six hydrate (relative humidity 31 percent) for several days until constant weight was obtained. The gain in weight was 6.96 percent.

Then the material was stored in a desiccator over a saturated solution of sodium bromide dihydrate (relative humidity 58 percent) for a month. The gain in weight relative to the anhydrous material was 13.60 percent. None of these percentages could be successfully compared with the calculated percentage of water present in an integral or half integral hydrate. The value calculated for a four hydrate was 14.09 percent. Possibly the dye would form a stable four hydrate if stored in a more humid atmosphere than 58 percent. This problem was not pursued further for lack of time and interest.

When heated in the thermobalance, the air dried material started to lose weight at 70°. The material continued to lose weight until it exploded at 223°.

It may be recalled that the diazonium component used in the synthesis of Eriochrome Black T, 1-diazo-2-naphthol-6-nitro-4-sulfonic acid, was proved to be a dihydrate by means of a neutralization equivalent determination.

Eriochrome Black T and Its Calcium and Magnesium Derivatives

Apparatus and materials

All of the pH measurements were made using a Beckman model G pH meter with a type E low sodium error glass electrode. Prior to and immediately after each series of measurements the meter was standardized against an appropriate National Bureau of Standards reference buffer (2), phthalate (4.01 at 25°), phosphate (6.86 at 25°) and borax (9.18 at 25°).

The absorption measurements were made by recording the complete spectrum in each case from 400 to 700 m μ using the Cary recording spectrophotometer, model 11. The cells had a path length of 1 cm. and the blank was 0.100 M potassium chloride.

All solutions were prepared in borosilicate glassware using distilled and deionized water and were stored in plastic containers. The deionized water had a specific conductance of less than 2×10^{-6} reciprocal ohms per centimeter. All of the inorganic chemicals used were of reagent grade quality. The organic reagents that were used for the

buffer systems were purified as needed by recrystallization or redistillation. In those cases where the organic acid or base salts were not commercially available for the buffers they were prepared. The magnesium standard solutions were prepared by using reagent grade magnesium acetate ($\%Ca=0.001$). The concentration of the magnesium solutions was checked by EDTA titrations. The EDTA solution was in turn standardized against calcium carbonate. The calcium carbonate was a product of special purity supplied by the Mallinckrodt Chemical Works on special order. The standard calcium solutions were prepared by dissolving weighed amounts of the pure calcium carbonate in dilute hydrochloric acid, evaporating to dryness, and diluting to volume. All of the dye solutions were prepared by using the crystalline dimethylamine salt (Lot II), prepared and analyzed as previously mentioned. All measurements involving Eriochrome Black T solutions were made within two hours of the preparation of the dye stock solution to limit errors caused by the decomposition of the dye in water solution.

Acid dissociation constants of Eriochrome Black T

As pointed out by Schwarzenbach, the sulfonic acid group on the Eriochrome Black T molecule is a strong acid and is not of direct interest in the functioning of the dye as a metal ion indicator. Rather, the phenolic hydrogen

atoms are of importance in the basic solutions in which the metal-dye interaction occurs. A new measurement of the acid dissociation constants of the two phenolic hydrogen atoms was now made on the purified dimethylamine salt of the dye using the spectrophotometric method.

A series of solutions of Eriochrome Black T of varying pH and constant ionic strength was prepared for spectrophotometric measurements. A stock solution of the dye was prepared by dissolving 0.1226 g. of the crystalline dimethylammonium salt in 500.0 ml. of 0.100 M potassium chloride solution. In order that the absorption and pH measurements could be made on solutions of constant ionic strength ($\mu=0.100$) the procedure described by Bates was employed in designing buffers for each pH range for the absorption measurements (2). For an ionic strength of $\mu=0.100$ a stock solution A was prepared containing 0.05 M of a salt of a monobasic weak acid or of a salt of a monoacidic weak base and 0.05 M potassium chloride. The pH was varied by adding various amounts of a stock solution B which contained either 0.2 M sodium hydroxide or hydrochloric acid and 0.1 M potassium chloride. For pH values near 7.5 sodium p-nitrophenolate and hydrochloric acid were used. This buffer did not absorb light in the wavelength region of interest, being transparent down to 500 m μ .

For pH values near 8.0 trishydroxymethylmethyammonium chloride and sodium hydroxide were used. For values near pH 10.0 ethanolanmonium chloride and sodium hydroxide were used. For values near pH 11.0 piperidinium chloride and sodium hydroxide was used. Solutions of higher pH values were prepared using 0.100 M potassium hydroxide and 0.100 potassium chloride. Each of the solutions for the absorption measurements was prepared in the following manner: To a 100 ml. volumetric flask 10.0 ml. of the dye stock solution, 20 ml. of constant ionic strength buffer stock solution A, a sufficient amount of constant ionic strength buffer solution B to give the desired pH value, and sufficient 0.100 M potassium chloride to meet the mark was added. After thorough mixing the absorption spectra were recorded. In order to prepare a solution of pH greater than 13, approximately 5.6 g. of potassium hydroxide (0.1 M) was added to 10 ml. of the dye stock solution and the mixture diluted to 100 ml. with water. This solution was obviously not of the same ionic strength as the others but above pH 13 an ionic strength of $\mu = 0.100$ was an impossibility. Since the scale of the model G pH meter stops at 13, this solution was measured using a Beckman model H-2 pH meter.

Since the blue form of the dye was the species $\text{HD}^=$ and both of the other forms of the dye were red, it was felt that it should be possible to make a plot of absorbancy at 615 $\text{m}\mu$ would be directly proportional to the species $\text{HD}^=$ because the other forms of the dye do not absorb appreciably at this wavelength. Such a plot given as Figure 5. By the study of an enlarged graph the first pK was found to be 6.91. From an examination of the graphs in the paper by Schwarzenbach and Biedermann (23) it was evident that they obtained a value of near 6.9 at an ionic strength of $\mu = 0.08$. Schwarzenbach and Biedermann reported 6.3 as the first pK which they obtained by measurements at an ionic strength of $\mu = 0.008$. The first pK of Eriochrome Black T is apparently strongly influenced by the presence of salts, probably due to the polymerization or precipitation of the dye.

For the second pK the large graph gave a value of 11.50 compared to 11.55, the value obtained by the Swiss workers at an ionic strength of $\mu = 0.08$. The agreement was very gratifying.

The absorption spectra of the dye showing the absorption of the species H_2D^- , $\text{HD}^=$ and $\text{D}^=$ as a function of wavelength from 400 to 700 $\text{m}\mu$ is given as Figure 6. Similarly the spectra of the species CaD^- , MgD^- and $\text{HD}^=$ are given in Figure 7. These spectra are the same as given by

Figure 5. Acid dissociation constants of Eriochrome Black T. The absorbancy of the dye at 615 m μ is plotted versus pH for a series of solutions all containing the same amount of dye and carefully adjusted to an ionic strength of $\mu = 0.100$. The mid-point of each vertical curve represents a pK value. The first, pK₁ = 6.91; the second, pK₂ = 11.50

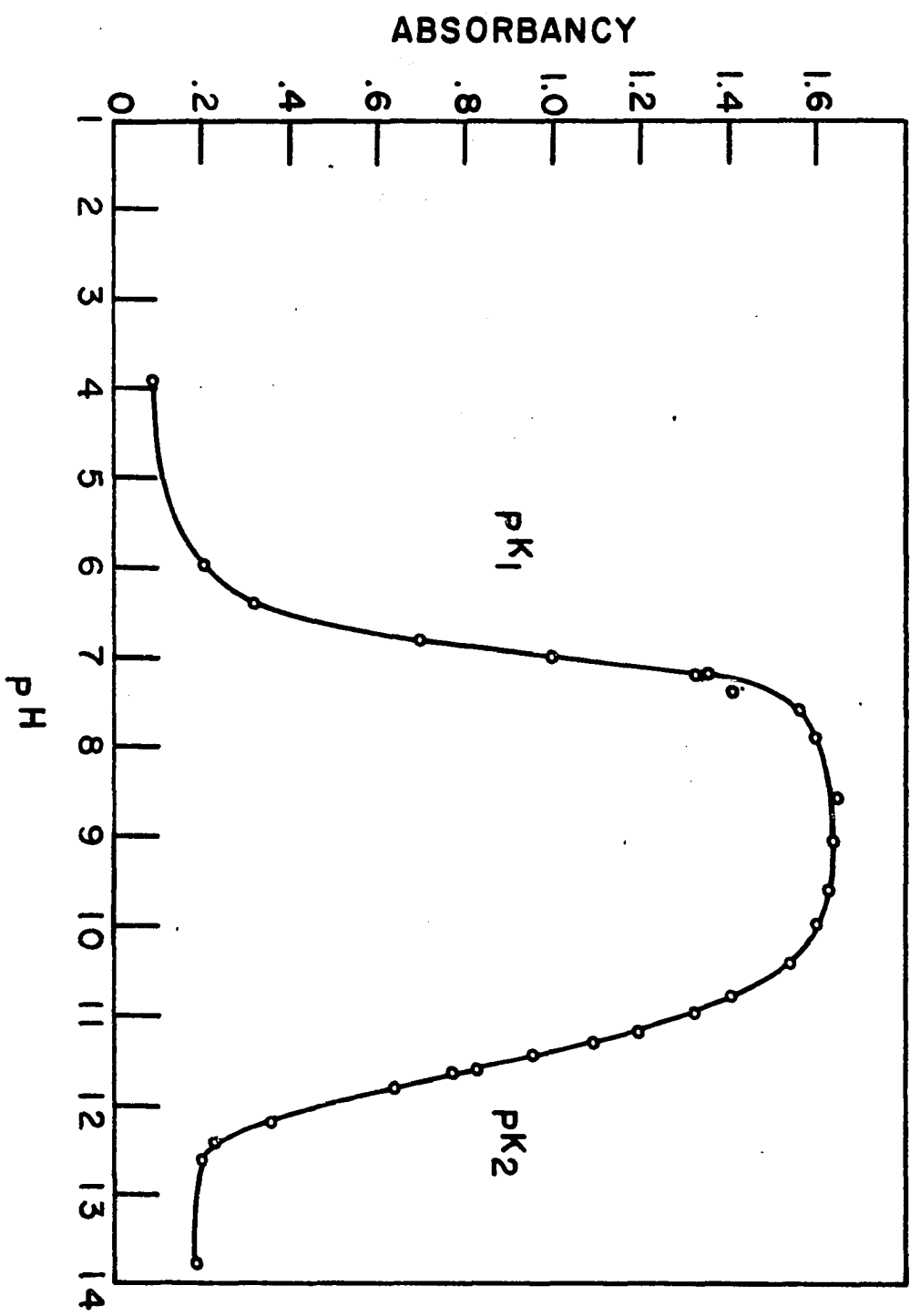


Figure 6. Absorption spectra of Eriochrome Black T at different pH values. Ionic strength: $\mu = 0.100$. pH of the solution for the different species: H_2D^- 3.91, HD^- 10.0, and D^{2-} 13.76.

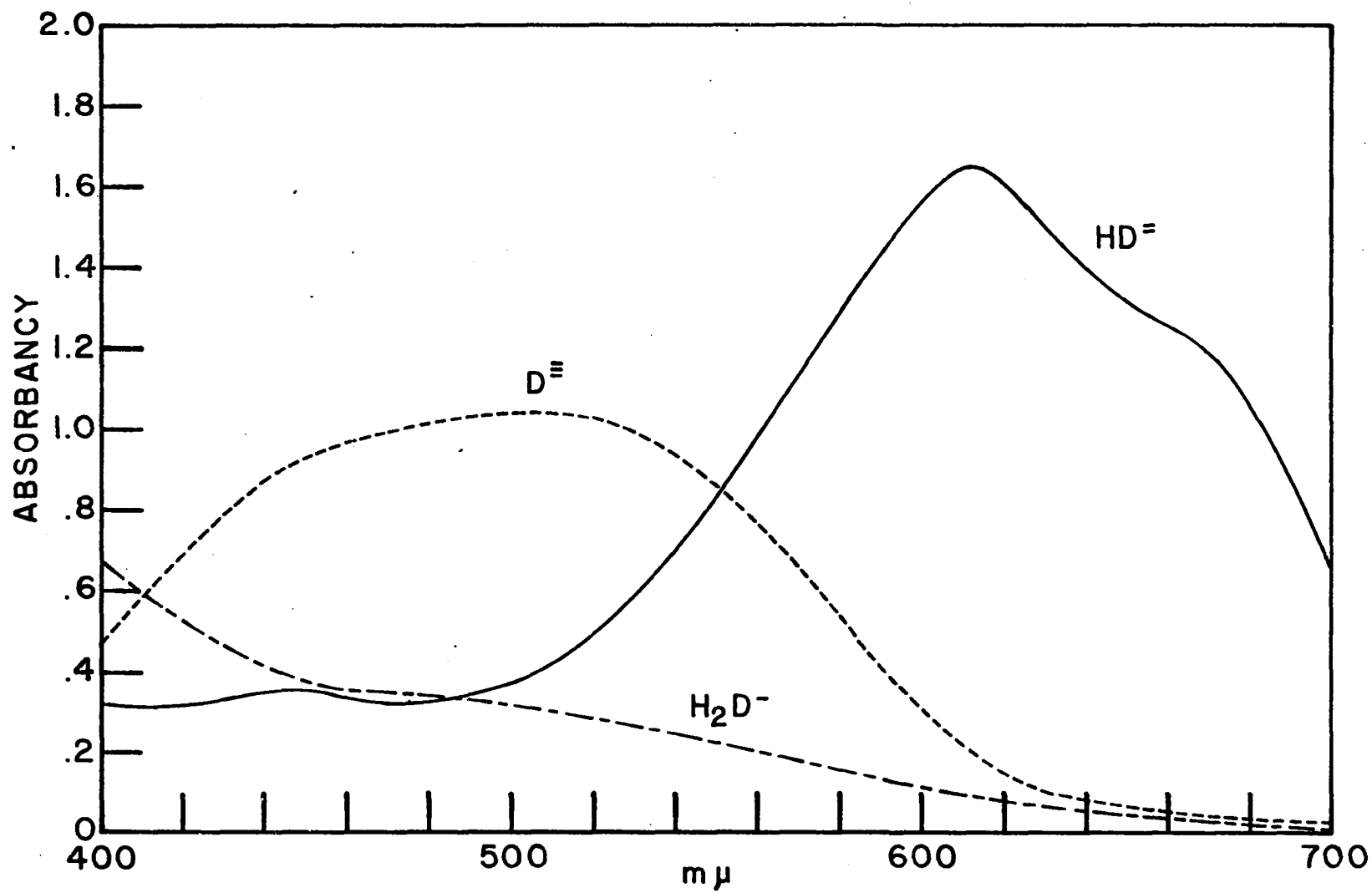
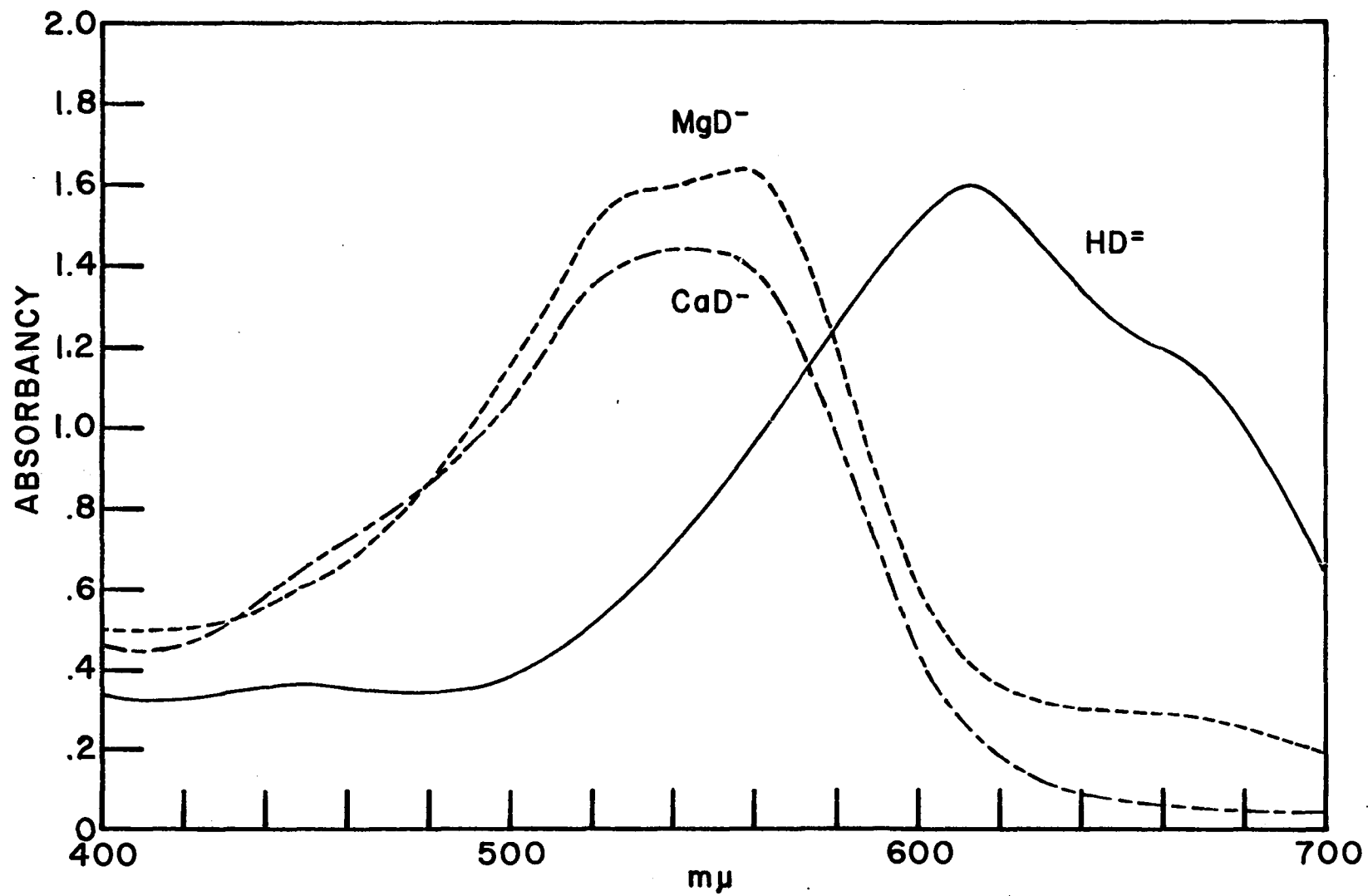


Figure 7. Absorption spectra of Eriochrome Black T and its calcium and magnesium derivatives at pH 10. Ionic strength;
 $\mu = 0.100$



Schwarzenbach in the second edition of the book "Die komplexometrische Titration" (22) but are markedly different from the spectra in the first edition of that book which were apparently made with a spectrophotometer of lower resolving power. These spectra are also greatly different from those reported by one group of American workers (12) who used a Beckman DU spectrophotometer at maximum sensitivity and consequently minimum resolution.

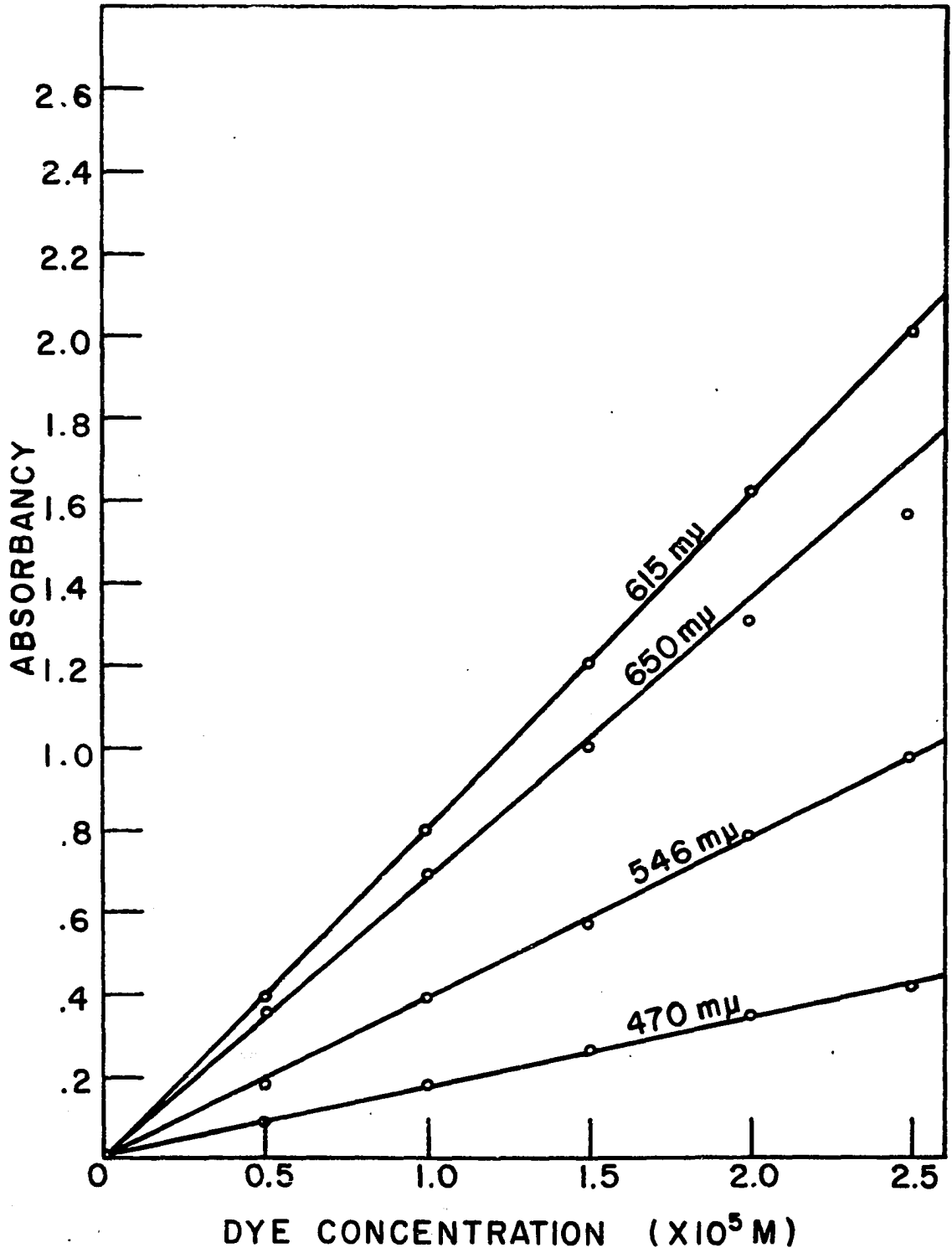
Beer's law study of the purified dye and the molar extinction coefficient

Since most methods of studying metal complexes by spectrophotometric means tacitly required the absorbancy of the colored species to be directly related to the concentration of that species in solution, an experiment was performed to be sure that Eriochrome Black T solutions obeyed Beer's law under the conditions to be employed in the study of the calcium and magnesium compounds. It would also have been desirable to know whether the metal-dye compounds also obeyed Beer's law. This would have been very difficult to determine, for the metal-dye compounds readily dissociated in solution. This experimental difficulty was to be limited by confirming the more serious studies to wave-length regions in which the absorption was primarily due to the dye itself.

The absorption spectra of the pure dye was obtained at different dye concentrations at pH 10, and ionic strength of $\mu = 0.100$ with a cell length of 1 cm. A plot of the absorbancy versus concentration was made from the spectra for 650 m μ , 615 m μ , 546 m μ and 470 m μ ; Figure 8. Since the relationship was linear with the exception of 650 m μ above a concentration of 2×10^{-5} M, little difficulty was anticipated from this source in the study of the complexes.

The Beer's law plots furnished an accurate and convenient means of obtaining the molar extinction coefficient of the dye. The slope of the line relating absorbancy to concentration at 615 m μ , $\mu = 0.100$, pH 10 and 1 cm. was found to give a molar extinction coefficient for the dye of 32,300. Schwarzenbach has given a plot of dye spectra at pH 8 in the form of molar extinction coefficient versus wavelength (22). The peak value on the curve was found to be about 29,600. Since the dye exhibited about the same absorption at 615 m μ at pH 8 as at pH 10 (see Figure 5), it was evident that he was not working with material of high purity. On the other hand, one of the American groups (27) found the absorbancy of a 1.299×10^{-5} M solution of the dye at pH 9.32 to be 0.78 using 5 mm. cells; using these data a molar extinction coefficient of 12,000 was computed.

Figure 8. Plots of absorbancy at different wavelengths for different concentrations of Eriochrome Black T. pH: 10.0, ionic strength: $\mu = 0.100$. The slope of the line for 615 m μ gave a molar extinction coefficient of 32,300. One cm. cells

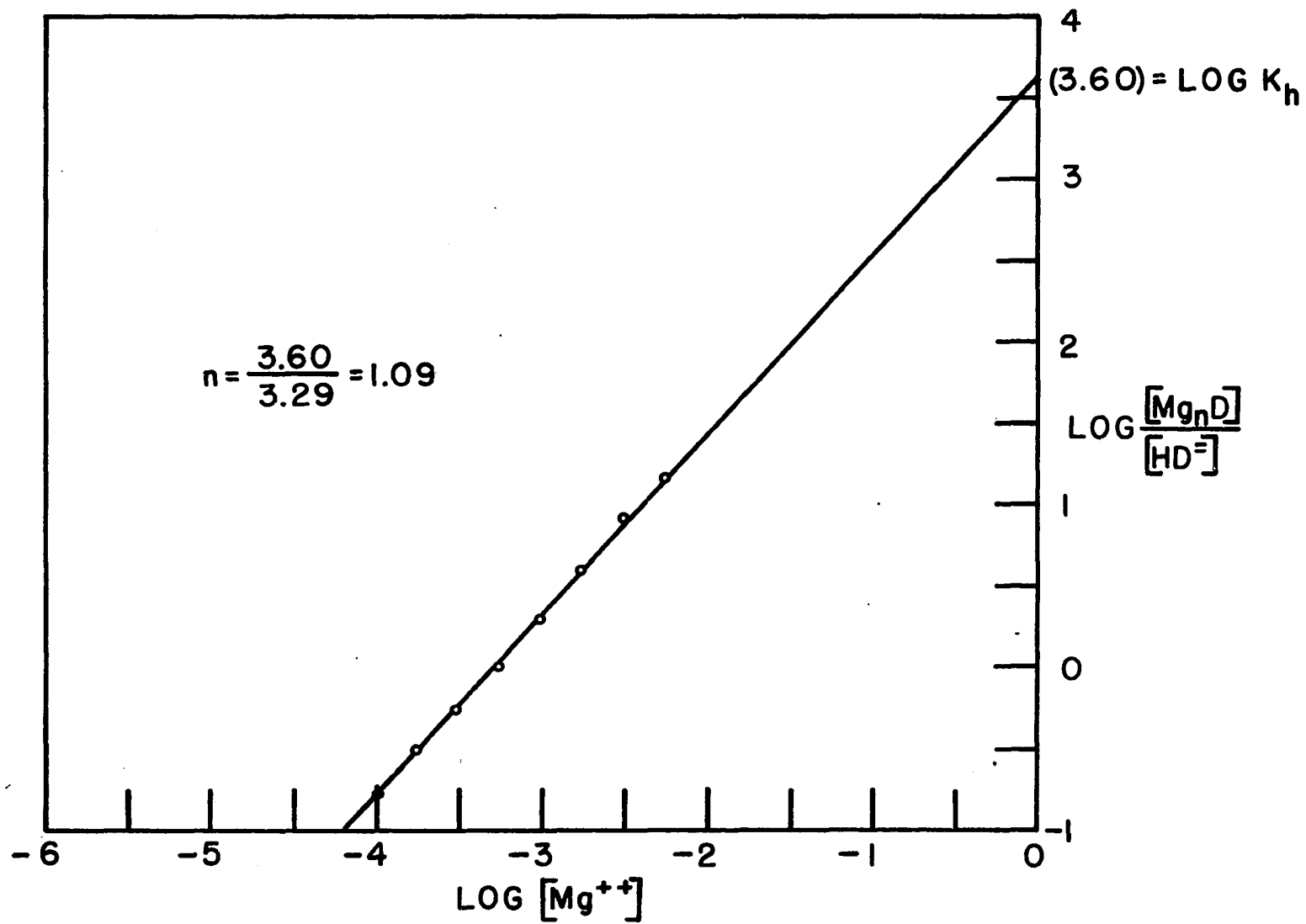


Log-ratio study of the calcium and magnesium compounds of Eriochrome Black T

For the reaction $HD + nMg = Mg_nD + H$, in which HD represents the uncomplexed dye in solution between pH 8 and 10 and in which the charges are neglected, the equilibrium expression is $K = \frac{[Mg_nD][H]}{[HD][Mg]^n}$. The apparent stability constant, $K_h = K/[H]$, may be substituted in the above expression, giving the expression used by Schwarzenbach (22) $K_h = \frac{[Mg_nD]}{[HD][Mg]^n}$. Casting the latter equation into logarithmic form and rearranging, the linear equation $\log [Mg_nD]/[HD] = n \log [Mg] + \log K_h$ may be obtained. The first term in this equation can be obtained by examining the absorption spectra of a series of solutions which have the same total dye concentration but different concentrations of magnesium. The second term contains the concentration of free magnesium in solution. The success of this method depends on the complexes having such a low stability that the concentration of free magnesium in solution is essentially the same as the total amount of magnesium added. The plot of $\log [Mg_nD]/[HD]$ versus $\log Mg$ is thus linear with slope n and intercept K as shown in Figure 9. This method has been used in a somewhat similar manner by a number of previous workers (4, 9, 14, 15).

The experimental work to determine n and $\log K_h$ was carried out as follows: In each 100 ml. volumetric flask

Figure 9. Log-ratio plot for magnesium and the dimethylammonium salt of Eriochrome Black T at pH 8.00. Data obtained from absorbancy readings at 615 m μ . Ionic strength: $\mu = 0.100$, 1 cm. cells. $\text{Log } K_{8.00} = 3.60$



in a series was placed 10.0 ml. of a solution containing 0.2422 g. of the dimethylammonium salt of Eriochrome Black T in 1000 ml. of 0.100 M potassium chloride solution, and 10.0 ml. of pH 8 buffer, $\mu = 0.100$. The pH 8.00 buffer was prepared by mixing the proper proportions of solution A (0.05 M trishydroxymethylmethyammonium chloride and 0.05 M potassium chloride) and solution B (0.2 M sodium hydroxide and 0.1 M potassium chloride). The first flask of the series was then diluted to the mark with 0.100 M potassium chloride. This solution was to give the spectra of the dye alone at pH 8.00. The remaining flasks were then treated with the following amounts of 0.01002 M magnesium acetate solution which contained sufficient potassium chloride to raise the ionic strength to $\mu = 0.100$, 1, 1.78, 3.16, 5.62, 10.0, 17.8, 31.6 and 56.2 ml. The resultant concentrations of magnesium were uniformly distributed along a logarithmic interval. One of the solutions was treated with a large excess of magnesium acetate to provide the spectra of the magnesium complex at pH 8.0. The solutions were then diluted to the mark with 0.100 M potassium chloride. After thorough mixing the absorption spectra were recorded. The actual pH of each solution was measured and found to be 8.00 ± 0.02 .

The ratio $[\text{Mg}_n\text{D}]/[\text{HD}]$ was obtained from the absorption spectra using the relationship developed as follows:

By the law of additive absorbancies the absorbancy of each solution is given by $A = \epsilon_{\text{HD}} [\text{HD}] + \epsilon_{\text{Mg}_n\text{D}} [\text{Mg}_n\text{D}]$ where ϵ represents the molar extinction coefficient.

For the solution containing no magnesium $A_{\text{HD}} = \epsilon_{\text{HD}} [\text{D}]$ and for the solution containing excess magnesium $A_{\text{Mg}_n\text{D}} =$

$\epsilon_{\text{Mg}_n\text{D}} [\text{D}]$. In these two expressions $[\text{D}]$ is the total dye concentration or $[\text{D}] = [\text{Mg}_n\text{D}] + [\text{HD}]$. Substitution of the two expressions in the additive absorbancy equation gives:

$A = A_{\text{HD}} \frac{[\text{HD}]}{[\text{D}]} + A_{\text{Mg}_n\text{D}} \frac{[\text{Mg}_n\text{D}]}{[\text{D}]}$. Multiplying the latter equation through by $[\text{D}]$ gives: $A [\text{D}] = A_{\text{HD}} [\text{HD}] + A_{\text{Mg}_n\text{D}} [\text{Mg}_n\text{D}]$. Substituting the expression for total dye concentration and collecting like terms furnishes:

$$[\text{Mg}_n\text{D}] (A - A_{\text{Mg}_n\text{D}}) = [\text{HD}] (A_{\text{HD}} - A). \text{ By division,}$$

the desired expression is obtained which has the concentration terms on the left and the absorbancy terms on the right; $[\text{Mg}_n\text{D}]/[\text{HD}] = (A_{\text{HD}} - A)/(A - A_{\text{Mg}_n\text{D}})$. A similar expression can be obtained even if only one of the species is colored as can be demonstrated by letting either ϵ_{HD} or $\epsilon_{\text{Mg}_n\text{D}}$ equal zero which has only the effect of simplifying the calculations and experimental details.

The computation of the ratio $[\text{Mg}_n\text{D}] / [\text{HD}]$ and its logarithm from the experimentally obtained absorbancies can be seen in Table 4. The log-ratio plot of the data of Table 4 is given in Figure 9. The dye obviously formed a one to one compound with the magnesium at this pH. The logarithm of the apparent stability constant was found to be 3.60. The value given by Schwarzenbach (22) at the same pH and ionic strength was 3.45.

The calcium compound was studied in precisely the same manner as the magnesium compound except that the study was made at pH 10 rather than at pH 8. A 0.0100 M standard calcium solution was prepared by dissolving the proper amount of the special grade calcium carbonate in dilute hydrochloric acid, evaporating to dryness and diluting to volume after adding sufficient potassium chloride to insure that the resultant solution would have an ionic strength of $\mu = 0.100$. The pH 10 buffer was prepared using the proper proportions of solution A (0.05 M ethanolammonium chloride and 0.05 M potassium chloride) and solution B (0.2 M sodium hydroxide and 0.1 M potassium chloride). The computation of the ratio $[\text{Ca}_n\text{D}] / [\text{HD}]$ and its logarithm from the experimentally determined absorbancies can be seen in Table 5. The log-ratio plot of the data of Table 5 is given in Figure 10. The dye formed a one to one compound with calcium at this pH. The logarithm of the apparent

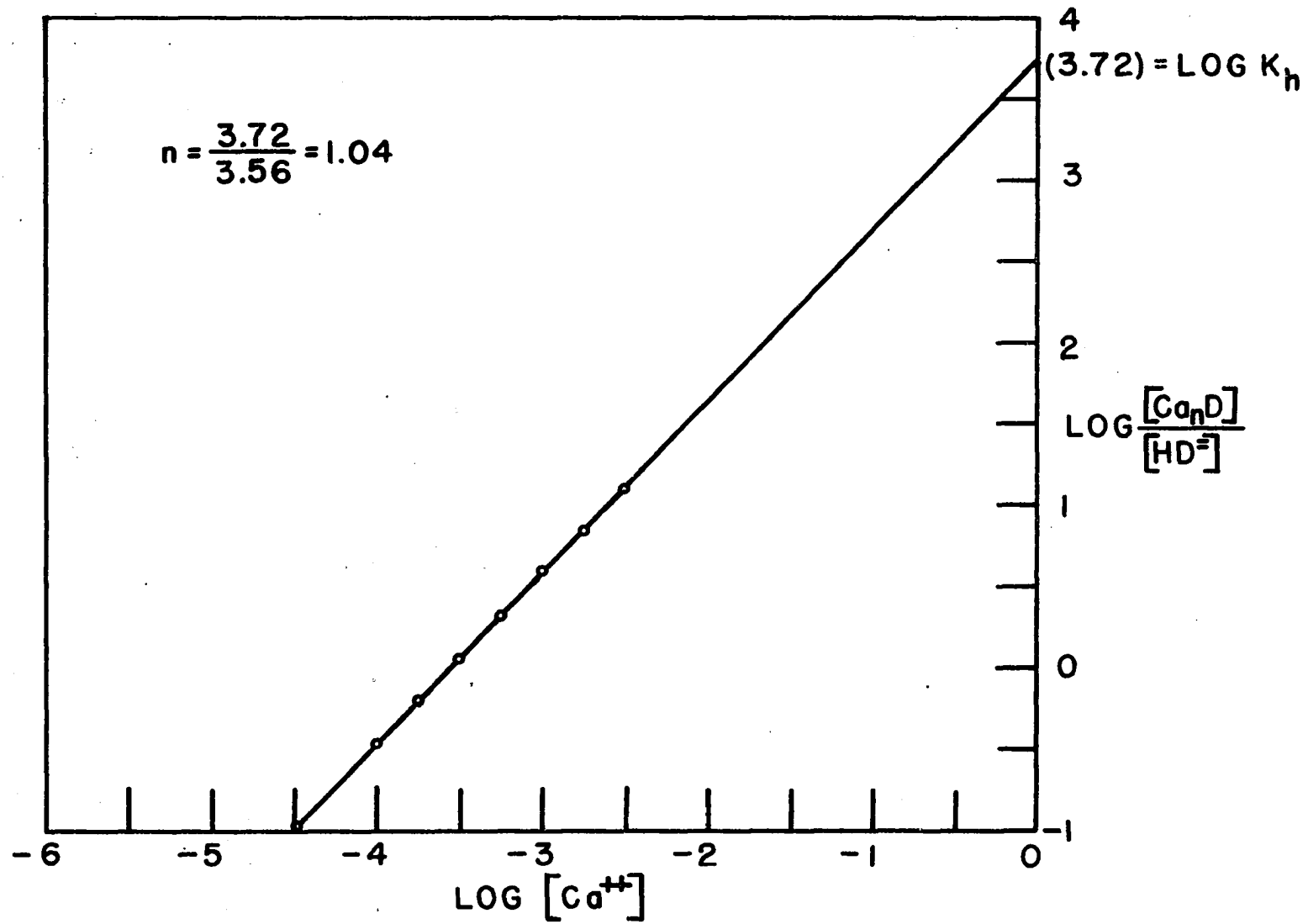
Table 4. Log-ratio method for magnesium and Eriochrome Black T at pH = 8.00, $\mu = 0.100$ and $\lambda = 615 \text{ m}\mu$

$[\text{Mg}] \times 10^4$	$\text{Log}[\text{Mg}]$	A	$A_{\text{HD}} - A$	$A - A_{\text{Mg}_n\text{D}}$	$\frac{A_{\text{HD}} - A}{A - A_{\text{Mg}_n\text{D}}}$	$\text{Log} \frac{A_{\text{HD}} - A}{A - A_{\text{Mg}_n\text{D}}} = \text{Log} \frac{[\text{Mg}_n\text{D}]}{[\text{HD}]}$
0	-	1.60	-	1.37	-	-
1.00	-4.00	1.40	0.20	1.17	0.171	-0.767
1.78	-3.75	1.28	0.32	1.05	0.305	-0.516
3.16	-3.50	1.12	0.48	0.89	0.539	-0.268
5.62	-3.25	0.92	0.68	0.69	0.985	-0.007
10.00	-3.00	0.69	0.91	0.46	1.98	0.296
17.80	-2.75	0.51	1.09	0.28	3.89	0.590
31.60	-2.50	0.38	1.22	0.15	8.14	0.910
56.20	-2.25	0.32	1.28	0.09	14.20	1.15
excess	-	0.225	1.37	-	-	-
slope or n = 1.09				log $K_{8.00} = 3.60$		

Table 5. Log-ratio method for calcium and Eriochrome Black T at pH = 10.00, $\mu = 0.100$ and $\lambda = 615 \text{ m}\mu$

$[\text{Ca}] \times 10^4$	$\text{Log} [\text{Ca}]$	A	$A_{\text{HD}} - A$	$A - A_{\text{Ca}_n\text{D}}$	$\frac{A_{\text{HD}} - A}{A - A_{\text{Ca}_n\text{D}}}$	$\text{Log} \frac{A_{\text{HD}} - A}{A - A_{\text{Ca}_n\text{D}}} = \text{Log} \frac{[\text{Ca}_n\text{D}]}{[\text{HD}]}$
0	-	1.60	-	1.37	-	-
0.316	-4.50	1.47	0.13	1.24	0.101	-0.990
1.00	-4.00	1.26	0.34	1.03	0.332	-0.479
1.78	-3.75	1.08	0.51	0.85	0.600	-0.222
3.16	-3.50	0.88	0.72	0.65	1.11	0.047
5.62	-3.25	0.67	0.93	0.44	2.12	0.326
10.00	-3.00	0.51	1.09	0.28	3.96	0.598
17.80	-2.75	0.40	1.20	0.17	7.03	0.846
31.60	-2.50	0.33	1.27	0.10	12.7	1.103
excess	-	0.23	1.37	-	-	-
		slope or n = 1.04		log $K_{10.00} = 3.72$		

Figure 10. Log-ratio plot for calcium and the dimethylammonium salt of Eriochrome Black T at pH 10.00. Data obtained from absorbancy readings at 615 m μ . Ionic strength: $\mu = 0.100$, 1 cm cells $\text{Log } K_{10.00} = 3.72$



stability constant was found to be 3.72. The value given by Schwarzenbach (22) at the same pH and ionic strength was 3.84.

An attempt to study the calcium compound at pH 8 was unsuccessful by this method because the compound was too unstable. When sufficient calcium ion was added to the dye solution to form the complex, the dye precipitated. There was no reason to believe that the compound at pH 8 was anything other than the one to one compound which was found in solution at pH 10.

Continuous variations study of the magnesium compound of Eriochrome Black T

Since the compound formed between magnesium and the dye at pH 10 was too stable to be able to assume that essentially all of the magnesium added to a solution of the dye would remain as free magnesium, the log-ratio method could not be employed. The method of continuous variations, on the other hand, was found to be well suited for this study. The method used was derived in the following manner which is similar to the development given by Job and later by Vosburg and Cooper (13, 26).

The complex that was studied, Mg_nD , was produced by the following reaction: $HD + nMg = Mg_nD + H$ in which HD represented the dye species present in solution at pH 10 and in which the charges were omitted. Two solutions were

prepared; one of concentration C in magnesium and the other of concentration C in the dye. Mixtures were prepared of x times a certain volume in ml. of the magnesium solution and $(1 - x)$ times the same certain volume in ml. of the dye solution which reacted to form the complex. A search was made for that value of x which resulted in the maximum production of the complex in the mixtures. When a plot was made corresponding to the concentration of the complex $[Mg_nD]$ versus x , a maximum value was observed at a particular value of x . The derivative of the concentration of the complex with respect to x , designated $[Mg_nD]'$, had to be equal to zero at this maximum value. Knowing this, it was possible to find the value of n in the formula of the complex as follows:

The equilibrium expression for the dissociation of the complex was written $K [Mg_nD] = [Mg]^n [HD]$ (I) in which the hydrogen ion concentration was absorbed in the equilibrium constant, it also being a constant in the buffered solutions used. The concentration of the other species in the mixtures was given by the relations, $Cx = [Mg] + n[Mg_nD]$ (II) and $C(1 - x) = [HD] + [Mg_nD]$ (III). The derivative of each of these equations was taken with respect to x yielding the following: $K[Mg_nD]' = n[Mg]^{n-1}[Mg]' [HD] + [Mg]^n [HD]'$ (I)', $C = [Mg]' + n[Mg_nD]'$ (II)', and $-C = [HD]' + [Mg_nD]'$ (III)'. When $[Mg_nD]'$ was set equal to zero, these equations

reduced to the following: $0 = n[Mg]^{n-1}[Mg]' [HD] + [Mg]^n [HD]'$ (IV), $C = [Mg]'$ (V), $-C = [HD]'$ (VI). Combining equations (IV) through (VI), division of the resultant expression by C and $[Mg]^n$, and rearranging yielded the expression $n [HD] = [Mg]$. Substitution of this equation for $[Mg]$ in equation (II), subtracting the result from equation (III), rearranging and simplifying yielded the desired expression, $n = x/(1 - x)$. Using this expression, if the maximum concentration of the complex was found in the mixture corresponding to $x = 0.5$, then $(1 - x)$ would also equal 0.5 and n would equal 1.0 indicating a one to one ratio of magnesium to dye in the complex.

It remained to demonstrate that in mixtures which contained the dye and the magnesium complex the maximum, or perhaps the minimum, in the difference between the observed absorbancy values and the absorbancy values expected if no reaction took place occurred in the same mixture as did the maximum concentration of the complex. That is to say that the derivative of $(A_m - A_0)$ with respect to x occurred at the same value of x as did $[Mg_n D]'$, A_m being the measured absorbancy of that mixture at any selected wave-length and A_0 being the corresponding absorption of the same mixture assuming that no reaction took place.

By the law of additive absorbancies the absorbancy of any mixture was given by $A = l (\epsilon_{HD} [HD] + \epsilon_{MgD} [Mg_n D])$.

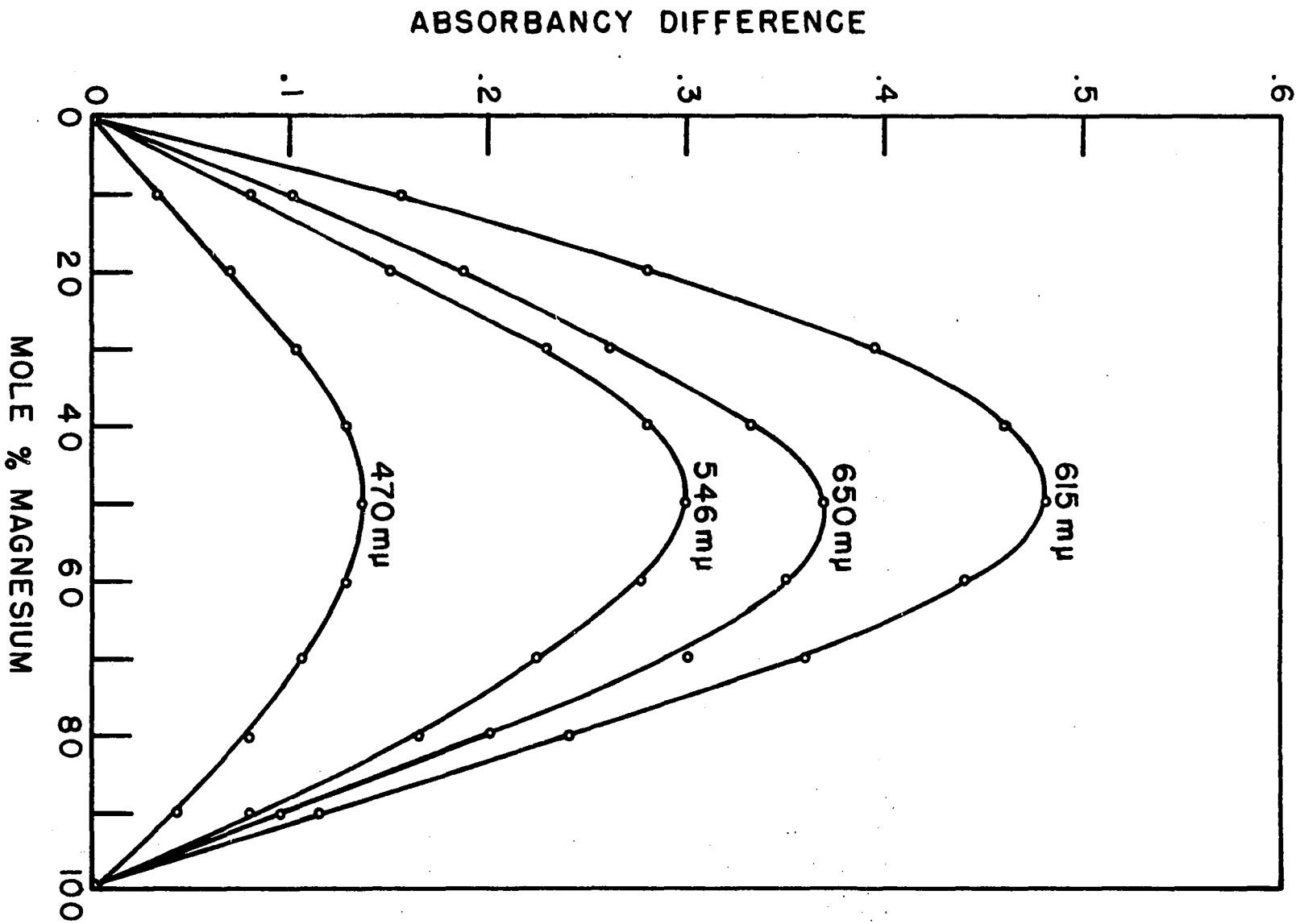
The absorbancy of any of the mixtures if the reaction did not take place was given by $A_0 = l \epsilon_{HD} C(1 - x)$ or better by $A_0 = l \epsilon_{HD} ([HD] + [Mg_nD])$ (see equation (III)). In these expressions l was the cell length and ϵ represented the molar extinction coefficient of the indicated species. By subtracting A_0 from A_m the expression $A_m - A_0 = l (\epsilon_{HD}[HD] + \epsilon_{MgD} [Mg_nD] - \epsilon_{HD}([HD] + [Mg_nD]))$ was obtained. After performing the obvious cancellation the expression was differentiated with respect to x yielding: $(A_m - A_0)' = l (\epsilon_{MgD} [Mg_nD]' - \epsilon_{HD} [Mg_nD]')$. It was obvious that when $[Mg_nD]' = 0$ $(A_m - A_0)' = 0$ and therefore both maximum values would occur at the same value of x .

Experimentally, the continuous variations study was made as follows: A 1×10^{-4} M magnesium solution was prepared by quantitatively diluting a standard 1×10^{-2} M solution prepared as described before. Sufficient potassium chloride was added to the magnesium solution so that it would have an ionic strength of $\mu = 0.100$. The standard dye solution of the same concentration was prepared by dissolving 0.2422 g. of the crystalline dimethylammonium salt (Lot II) and the appropriate amount of potassium chloride in 500 ml. of water and diluting to volume. Actually this solution was 0.982×10^{-4} M, for at the time this solution was prepared it was not known that the crystalline dimethylammonium salt contained about a half mole of water. To each of a

series of ten 100 ml. volumetric flasks ten ml. of pH 10 buffer was added. This buffer was of the constant ionic strength type mentioned previously and was composed of ethanolanmonium chloride, sodium hydroxide and potassium chloride. Then to each flask in order 0, 5, 10, 15, 20, 25, 30, 35, 40 and 45 ml. of the magnesium solution and 50, 45, 40, 35, 30, 25, 20, 15, 10, and 5 ml. of the dye solution was added. The flasks were diluted to volume with 0.100 M potassium chloride. All of the solutions were measured and found to be at pH 10.0. After thorough mixing the absorption spectra were obtained and continuous variations plots were made at the wavelengths 650 m μ , 615 m μ , 546 m μ and 470 m μ ; Figure 11. The first two wavelengths represented the maximum and subsidiary maximum respectively; the latter wavelengths represented isoabsorbive points in the system of spectra and were selected as recommended by Vosburg and Cooper as the most sensitive for the detection of other possible complexes in solution (26). It was obvious from the continuous variation plots that only a one to one compound was formed under these conditions.

The apparent stability constant was calculated after obtaining the fraction of the complex which was dissociated. This was found in the following manner from the continuous variations plots: The straight line portion of

Figure 11. Continuous variations plots for magnesium and the dimethylammonium salt of Eriochrome Black T at pH 10.0. Magnesium and dye solutions both 1×10^{-4} M. Ionic strength: $\mu = 0.100$, 1 cm. cells



the plots was extended until the lines intersected. This would be the plot obtained if the reaction were quantitative. The fraction dissociated was obtained by dividing the distance from the maximum of the plot to the intersection of the straight line segments by the distance from the intersection to the horizontal axis. The values of the fraction dissociated obtained were 0.245 (615 m μ), 0.233 (650 m μ), 0.219 (546 m μ) and 0.233 (470 m μ) for an average of 0.233. When the fraction dissociated into the simple ions was designated as d , the apparent stability constant $K_h = (1 - d)/d^2 C_0$, where C_0 was the concentration of the complex in solution assuming no dissociation; $C_0 = 2.5 \times 10^{-5}$. The value obtained was $K_h = 5.64 \times 10^5$ and the $\log K_h = 5.75 \pm 0.10$.

Results and Discussion

To demonstrate that the work of Schwarzenbach and Biedermann (23) on the properties of Eriochrome Black T and its calcium and magnesium derivatives was correct and the efforts of two American groups (12, 27) were incorrect, the author set out to prepare, for the first time since its discovery in 1904, a pure crystalline form of the dye of known composition.

Starting with commercial Eriochrome Black T a long series of unsuccessful attempts were made to separate the impurities from the dye so it could be crystallized from some solvent. The first significant positive results were obtained when it was found possible to remove all of the some 30 percent of inorganic salt from the dried commercial product by repeated extractions with dilute hydrochloric acid. Attempts to crystallize this salt-free dye from numerous solvents and by various techniques were unsuccessful, nor were similar efforts successful with material synthesized from purified intermediates.

Further attempts to obtain a crystalline form of the dye were finally rewarded when an attempt was made to recrystallize the salt-free material from dimethylformamide. Unlike any of the other solvents tried, dimethylformamide dissolved a very large amount of the dye and when the dye was recovered from the solvent it was found to be crystalline.

When the crystalline material was analyzed for carbon, hydrogen, nitrogen and sulfur, the ratio of nitrogen to sulfur was found to be four to one instead of the expected three to one ratio corresponding to the formula of the dye, Figure 12(b). By further study of the crystalline material, it was found that the dye had reacted with the solvent and

that the crystalline dye was the half hydrate of the dimethylammonium salt of the dye. The dimethylamine obviously came from the hydrolysis of the dimethylformamide.

The percentages calculated for the formula for the half hydrate of the dimethylammonium salt of Eriochrome Black T and the percentages found from analyses are compared in Table 3. This was the first elemental analysis reported for this commercially important dyestuff since its preparation in 1904. The percent of dimethylamine in the crystalline material was also determined as was the neutralization equivalent. These results also agreed closely with the calculated values.

Having finally prepared a sample of the dye of known composition, it became possible to authoritatively investigate the properties of the dye and its calcium and magnesium derivatives. In so doing it was possible to refute the erroneous work of the American groups (12, 27) and to duplicate the results of Schwarzenbach and Biedermann (23). Since the erroneous work of the American groups had resulted from the use of impure Eriochrome Black T as a primary standard to prepare solutions for continuous variations studies, the same method of investigation was used substituting a more careful technique and pure samples of the dye. The Swiss workers obtained correct results because they did not use the method of continuous variations

and so did not need dye of known purity.

As shown by the log-ratio plot in Figure 9 magnesium and Eriochrome Black T at pH 8 combine in the ratio of one to one. The value of the log apparent stability constant for the magnesium compound at pH 8 was found to be $\log K_{8.00} = 3.60$. At pH 10 the combining ratio of Eriochrome Black T and magnesium was found also to be one to one using the method of continuous variations; Figure 11. Data from the plots permitted the calculation of the log apparent stability constant for the magnesium compound at pH 10 as $\log K_{10.00} = 5.75$.

The calcium compound was studied at pH 10 using the log-ratio method; Figure 11. The ratio of calcium and dye in the compound formed at that pH was one to one. The log apparent stability constant for the calcium compound at pH 10 was found to be $\log K_{10.00} = 3.84$. An attempt to study the calcium compound at pH 8 by this method was unsuccessful, for the amount of calcium ion necessary to form a measureable amount of the compound was sufficient to precipitate the dye.

The logarithm of the acid dissociation constants for the two hydroxyl groups on the dye molecule were determined as shown in Figure 5; for the first, $pK_1 = 6.91$, for the second, $pK_2 = 11.50$. The results obtained are compared

with the results reported by Schwarzenbach and Biedermann in Table 6.

The molar extinction coefficient of Eriochrome Black T at pH 10 and 615 m μ , the absorbancy maximum, was measured and found to be 32,300 compared to 29,600, the value reported by Schwarzenbach (22). The free acid form of the dye was prepared from the crystalline dimethylamine salt and found to be very hygroscopic, the water content varying widely with the conditions of storage. This was the form of the dye used by earlier workers who ignored these properties of the dye.

It has been shown that calcium and magnesium form only one to one compounds with Eriochrome Black T under the conditions which are likely to be encountered in the use of the dye as an indicator. The chemical properties of the dye have been shown to be in good agreement with the values originally obtained by Schwarzenbach and Biedermann (23) who used a somewhat less refined technique. The properties of the dye as a complexing agent were found to be quite different from the properties given by the American workers (12, 27) in their attack on the work of Schwarzenbach.

The error of the American workers was anticipated by Paul Job in the paper in which he described the method of

Table 6. Summary of results on the combining ratios and formation constants of Eriochrome Black T with magnesium and with calcium

Item studied	Schwarzenbach and Biedermann (22, 23)	This work
Complex combining ratio M:dye		
Mg at pH 8	1:1	1:109
Mg at pH 10	1:1	1:1
Ca at pH 10	1:1	1:1.04
Log acid dissociation constants		
pK_1^a	6.3 ($\mu = 0.008$)	-
	6.9 ($\mu = 0.08$) ^b	6.91 ($\mu = 0.100$)
pK_2^a	11.55 ($\mu = 0.08$)	11.50 ($\mu = 0.100$)
Apparent stability constants for the complexes		
log K_8 for Mg	3.45 ($\mu = 0.10$)	3.60 ($\mu = 0.100$)
log K_{10} for Mg	5.44 ($\mu = 0.10$)	5.75 ($\mu = 0.100$)
log K_{10} for Ca	3.84 ($\mu = 0.10$)	3.72 ($\mu = 0.100$)

^aSecond and third replaceable hydrogen atoms of the molecule

^bValue obtained from inspection of graph in (23)

continuous variations (13), for the following passage, translated from that paper, was set in italics for emphasis:

The necessary and sufficient condition in order that the maximum composition will not vary if one changes the concentration of the two simple solutions is that the two solutions shall be equimolecular. In this case and in this case only, the maximum composition corresponds to the proportion of the two constituents which react to form the complex.

Paul Job stated further: "In all other cases, the maximum composition depends on both the concentration of the two fundamental solutions and the equilibrium constant". Since both American groups prepared their dye stock solutions from the grossly impure commercial dye and assumed it was 100 percent pure, the source of the difficulty they encountered has been found.

SOME NEW HYDROXYAZO DYES AS METAL ION INDICATORS

Introduction

The disadvantage of Eriochrome Black T as an indicator

Ever since its introduction by Schwarzenbach and Biedermann in 1948 (23), Eriochrome Black T has been used as the indicator for the direct titration of calcium and magnesium using EDTA. Even though never intended for this purpose this excellent wool dye has served well as an indicator. However, as an indicator it was found to have a serious disadvantage.

The difficulty with Eriochrome Black T as an indicator was that solutions of the dye in water and other solvents decomposed so rapidly that it was not practical or convenient to make up stock solutions of the indicator as a shelf reagent. Many attempts have been made to circumvent this difficulty. A recent review (1) listed seven different references describing methods of preparing solutions of the dye that were stable for extended periods of time. The most successful of these enabled one to keep an indicator solution for only about seven months (6). One way to avoid the problem has been to prepare a powdered mixture of the dye diluted with an inert inorganic salt (11). A small amount of this mixture was added to each titration flask before starting the titration.

Proposed solution to the instability problem

Since azo dyes are so easy to synthesize, it was difficult to see why no one had tried to solve the problem of the instability of Eriochrome Black T in solution by preparing another dye which would not decompose in solution rather than to try to find a crutch for the weakness of the commonly used indicator.

When the structure of Eriochrome Black T was examined, it was noted that the molecule possessed both a center of high oxidation in the form of the nitro group and a center of low oxidation in the form of the azo group. This seemed sufficient reason to expect the molecule to be unstable. Accordingly, an attempt was to be made to prepare suitable stable azo dyes not containing the nitro group or a similar oxidizing group anywhere in the molecule.

Preparation of New Indicators

Introduction

Since in most respects Eriochrome Black T was quite satisfactory as an indicator except for its instability in solution, thought to be due to the presence of the nitro group, an attempt was made to prepare suitable indicators using the diazonium compound used in the preparation of Eriochrome Black T except that it would not be nitrated. The preparation of the diazonium compound, 1-diazo-2-naphthol-4-

sulfonic acid, was described in the first part with the preparation of Eriochrome Black T. Remembering from the work of J. L. Ellingboe (9) that dyes had to have the o,o'-dihydroxyazo grouping in their structure to function as indicators for calcium and magnesium, several dyes were synthesized and tested as indicators. The dyes which had the shortest end point transitions in the EDTA titration for these alkaline earths were tested as indicators to replace Eriochrome Black T.

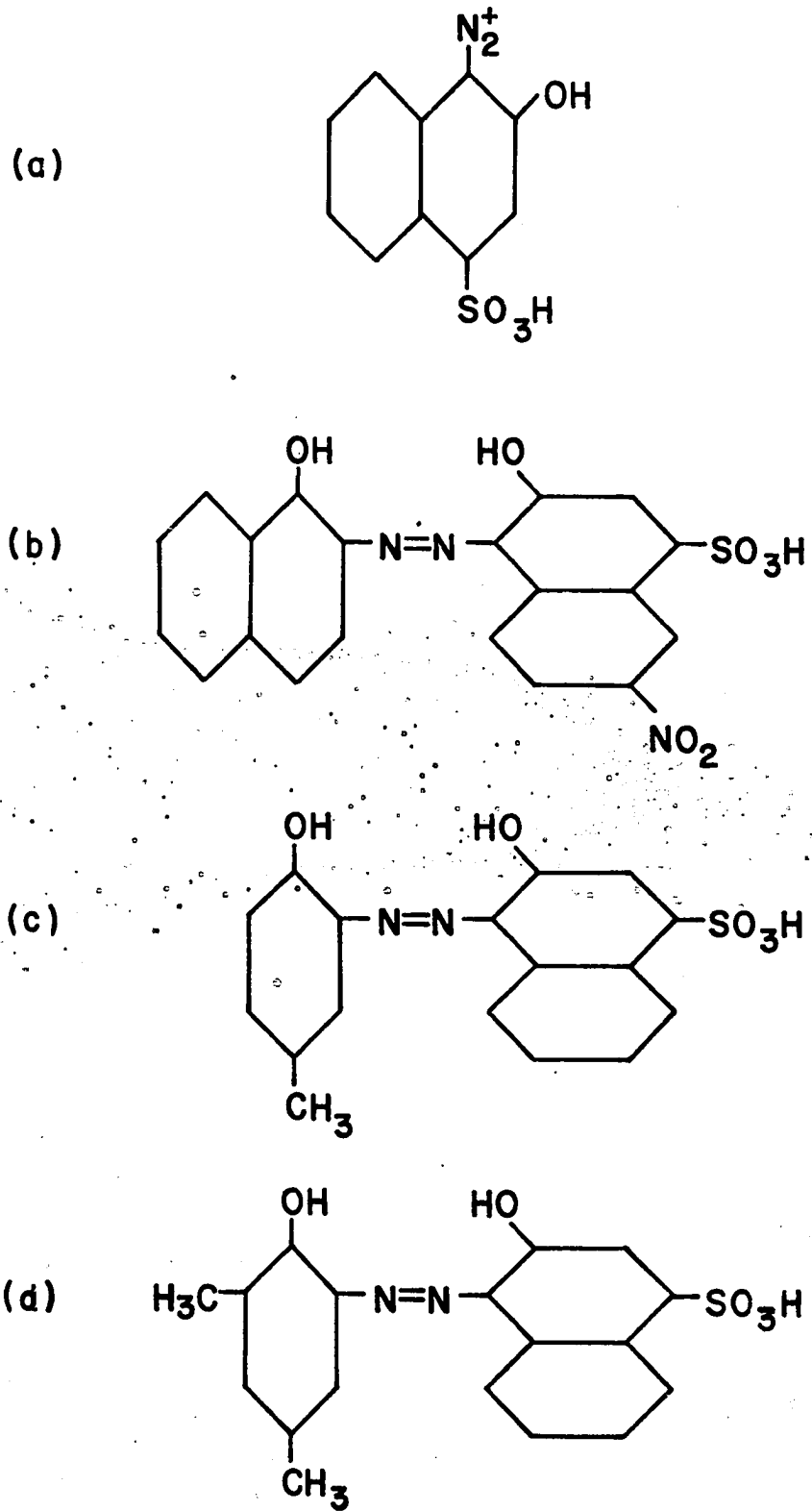
The structure of the diazonium compound, Eriochrome Black T, and the two dyes found to be the most valuable as replacements for Eriochrome Black T as indicators in the titration of calcium and magnesium with EDTA are illustrated in Figure 12. The new indicators resulted from the coupling of 1-diazo-2-naphthol-4-sulfonic acid to p-cresol and 2,4-dimethylphenol or xylenol. Their synthesis follows.

p-Cresol indicator

For the preparation of 1-(1-hydroxy-4-methyl-2-phenyl-azo)-2-naphthol-4-sulfonic acid, Figure 12 (c), Eastman Kodak practical grade p-cresol was purified by vacuum distillation. The material boiling from 95-100° at 15 mm. pressure was taken. This material solidified in the receiver. The solid was melted, cooled to 34°, and about half of the cresol crystallized. The liquid layer was decanted from the crystals. The crystalline product was in the form of water

Figure 12. Structural formulas of compounds discussed

- (a) 1-Diazo-2-naphthol-4-sulfonic acid. Diazonium compound used in the synthesis of indicators. It is nitrated in the 6 position to produce the diazonium compound used to prepare Eriochrome Black T
- (b) Eriochrome Black T 1-(1-hydroxy-2-naphthylazo)-6-nitro-2-naphthol-4-sulfonic acid
- (c) p-Cresol indicator: 1-(1-hydroxy-4-methyl-2-phenylazo)-2-naphthol-4-sulfonic acid
- (d) Xylenol indicator 1-(1-hydroxyl-4,6-dimethyl-2-phenylazo)-2-naphthol-4-sulfonic acid



white, hygroscopic crystals; mp. 31-35.5°. The handbook value was 36°. The low melting point was attributed to traces of moisture and the presence of a small amount of isomeric cresols which are extremely difficult to remove.

Twenty-two grams of the purified p-cresol dissolved in 100 ml. of 16 percent sodium hydroxide solution in a 1 liter beaker. Two hundred ml. of a saturated sodium hydroxide solution was added. Fifty grams of 1-diazo-2-naphthol-4-sulfonic acid was whipped up with 100 ml. of water until a creamy suspension was formed that was entirely free from lumps. This creamy suspension was added to the cresol solution slowly over an hour's time while vigorously stirring the mixture.

After all of the diazonium compound had been added, the mixture was heated to 60° for a half hour with continuous stirring. Then the temperature was raised to 90°. Completion of the coupling was indicated by the formation of a clear dark red solution having no apparent suspended matter. A drop of this solution when placed on filter paper was completely absorbed, leaving no residue. The solution was filtered through a sintered glass Buechner funnel, and cooled.

The dye was precipitated by adding concentrated hydrochloric acid until the solution was just acidic. After cooling again, the dye was filtered on Whatman No. 50 paper and washed twice with 100 ml. portions of a solution containing

150 g. of sodium chloride and 50 ml. of concentrated hydrochloric acid per liter. It was allowed to air dry. The product was suitable for use as an indicator but some of it was purified as follows:

The dried dye was extracted for 12 hours using petroleum ether in a Soxhlet extractor to remove unreacted p-cresol. Then it was allowed to air dry. The dye was extracted into acetone to leave impurities and salt behind. The acetone extracts were allowed to evaporate, depositing the dye. When the acetone solutions of the dye were heated to boil off the acetone, they bumped violently. Twenty grams of the dried dye were obtained for a yield of 27 percent. The product was found to be extremely soluble in water. Unfortunately, when the dye was assayed by the titanous chloride method it was found to be only 80.5 percent pure. After a search for a purification procedure the following rather pure derivative was prepared.

p-Toluidine salt of the p-cresol indicator

Seven grams of the acid form of the dye dissolved in 250 ml. of alcohol containing 2 g. of p-toluidine. The solution was filtered into a crystallizing dish and water was added with stirring until the dye salt just showed signs of precipitation. The solution was heated on a steam bath until all of the alcohol evaporated leaving fine crystals of the dye salt which were very insoluble in water. The

crystals were extracted twice with reagent grade ether (alcohol free) and once with water. Yield: 4 g. of fine, loose crystals having a metallic luster. This material was extracted into ethanol, in which it was partially soluble, yielding roughly two equal fractions. Both were air dried. By absorption measurements it was evident that the dye rich fraction was the one insoluble in alcohol.

The alcohol insoluble fraction was assayed by the titanous chloride method and was found to be 93.8, 95.0, 95.2 and 96.4 percent pure p-toluidine salt of 1-(1-hydroxy-4-methyl-2-phenylazo)-2-naphthol-4-sulfonic acid; average 95.1. This material was used in the study of the properties of the dye and its calcium and magnesium derivatives. It was quite unsuitable for use as an indicator for it was almost completely insoluble in water.

Xylenol indicator

To prepare 1-(1-hydroxy-4,6-dimethyl-2-phenylazo)-2-naphthol-4-sulfonic acid, Figure 12 (d), 19 g. of Eastman Kodak 2,4-dimethylphenol (xylenol) and 40 g. of sodium hydroxide were mixed with 200 ml. of water in a 2 liter beaker. One hundred ml. of saturated sodium hydroxide was then added and the mixture was heated to 80°. Using vigorous stirring, a smooth slurry of 1-diazo-2-naphthol-4-sulfonic acid in water was then slowly added over an hour's time. After

all of the diazonium compound had been added, the mixture was stirred until the coupling was complete as indicated by the complete absorption of a drop of the mixture placed on filter paper. After cooling, the mixture was filtered through a sintered glass Buechner funnel. Sufficient hydrochloric acid was then added to neutralize all the base. After cooling again, the precipitated dye was filtered from solution and air dried.

The dried dye was then extracted with repeated portions of butyl alcohol to remove the dye and leave the salt behind. The alcohol extracts were evaporated to dryness on a steam plate. Water was then added and the solution was boiled until the vapors no longer had the odor of the 2,4-dimethylphenol. The hot solution was treated with Norite, boiled and filtered. The water was removed on a steam plate and the dye was allowed to air dry. The material was assayed by the titanous chloride method and found to be 82.5, 83.1 and 82.4 percent pure; average 82.7.

Conclusions

The preparation of two new indicators has been described. The one prepared by coupling 1-diazo-2-naphthol-4-sulfonic acid to p-cresol proved to be quite stable in water solution and to have an end point transition shorter than that of Eriochrome Black T. Another indicator prepared by coupling

the diazonium compound to 2,4-dimethyl phenol, was interesting, for although it had a longer end point transition than the p-cresol dye it seemed to be somewhat selective for magnesium in the presence of calcium. This was attributed to the blocking of the much larger calcium ion by the presence of the methyl group adjacent to the hydroxyl group on the phenyl ring. The ionic radius (Pauling) of the calcium ion is 0.99 A while that of magnesium is 0.65 A.

Both of these indicators, one suggested as a replacement for Eriochrome Black T and the other apparently showing selectivity for magnesium, were carefully prepared as described in this section and analyzed and studied in some detail as outlined in the following sections.

Analysis of Indicators

Titanous chloride method

The new azo dye indicators prepared by the author had only one reducible group on the molecule so it was decided that a direct assay of their purity could be made using the titanous chloride reduction method of Knecht and Hibbert (16). Eriochrome Black T, on the other hand, also had a nitro group on the molecule. Since this nitro group was also reducible by this method and the probable impurities in Eriochrome Black T may have also had the nitro group, it was not thought wise to use this method to determine the

purity of Eriochrome Black T samples in the previous section. The dyes in this section were checked for the absence of unreacted and reducible diazonium material by means of the intense infrared absorption of the 4.6 micron band corresponding to the diazonium linkage in the 1-diazo-2-naphthol-4-sulfonic acid.

In the Knecht and Hibbert method used in this work the dye was treated with an excess of standard titanous chloride solution which reduced the azo linkage and split the molecule into two amines. Each azo group consumed four equivalents of titanous chloride. The excess unreacted titanous chloride was determined by a back titration using a standard ferric ion solution with thio-cyanate as an indicator. The titanous chloride standard solution as well as the reaction mixture was protected from atmospheric oxygen with hydrogen or carbon dioxide. It was found that the method was quite rapid and convenient once a little practice had been obtained. This method is commonly used industrially.

Reagents and detailed procedure

The standard ferric solution was made by dissolving 2.2356 g. of electrolytic iron in hot concentrated perchloric acid, cooling and diluting to 1,000 ml. This solution was 0.04003 N. The standard titanous solution was prepared by diluting about 25 ml. of a commercial 20 percent titanous chloride reagent solution to 1,000 ml. with dilute

hydrochloric acid. This solution was stored in a Machlett automatic buret which had been flushed with hydrogen. The solution was allowed to stand in the buret for a day to eliminate by reduction traces of oxygen which may have been present. The buret was fitted with the simple but very effective automatic hydrogen generator described by Knecht and Hibbert (16). This generator supplied hydrogen to take the place of solution withdrawn from the buret. The indicator used was a 20 percent potassium thiocyanate solution.

The titrations were carried out in a tall form beaker fitted with a rubber stopper having two holes, the larger of which was used for the introduction of the titrant. The smaller hole was fitted with a glass tube for the introduction of the carbon dioxide used for stirring and purging and protecting the solution from oxygen. For each titration about 0.1 g. of an accurately weighed sample of the dye, 100 ml. of water, 5 ml. of sulfuric acid, and 25 ml. of a 20 percent potassium thiocyanate solution were placed in the beaker. Alcohol was added to keep the sample in solution. The stopper was inserted and carbon dioxide was bubbled through the solution for about five minutes to remove dissolved oxygen. Then, while using the carbon dioxide stream to stir the solution, exactly 48.00 ml. of the titanous chloride solution was added. Then the beaker was placed on a hot plate and heated until the color of the dye was destroyed

plus a little longer for safety. The hot colorless solution was then titrated with the standard ferric perchlorate solution until the first color of the ferric thiocyanate complex appeared.

The titanous chloride solution was standardized exactly as described above except that a dye sample was not added. The concentration obtained for one such standard solution was 0.03284 N, 0.03288 N and 0.03294 N for an average of 0.03289 N.

The method was checked by titration of samples of p-nitroaniline which had been repeatedly recrystallized until the melting point was 146-148°. The literature (Handbook) value was 147.5°. This compound, which was employed by Knecht and Hibbert as a primary standard, was found to assay 99.41 percent and 99.48 percent pure p-nitroaniline. The results of the analysis of the individual dye samples was given with the discussion of the preparation of each dye.

The p-Cresol Indicator and Its Calcium and Magnesium Derivatives

Apparatus and materials

The same apparatus and materials that were used for the study of Eriochrome Black T and its calcium and magnesium derivatives described in Part I were used in the study of the p-cresol dye and its corresponding derivatives.

Acid dissociation constants of the p-cresol indicator

A measurement was made of the dissociation constants of the hydroxyl groups on the dye molecule which were of interest in the complex formation. A series of solutions each containing the same amount of 1-(1-hydroxy-4-methyl-2-phenylazo)-2-naphthol-4-sulfonic acid, of constant ionic strength, and of varying pH values were prepared for spectrophotometric examination in precisely the same manner employed in the study of the acid dissociation constants of Eriochrome Black T as described in Part I. The same buffer systems were employed also.

The spectra were examined and the absorbancy of the solutions at 610 m μ was selected as a measure of the concentration of the blue species HD⁼ in solution. A plot of the absorbancy at 610 m μ versus pH is given in Figure 13. From the plot it was evident that the first pK was 8.20 and the second pK was 12.35. It was noted that the curve was centered about pH 10, the pH value used in the titrations of calcium and magnesium with EDTA.

The absorption spectra of the dye showing the absorption of the species H₂D⁻, HD⁼ and D⁼ as a function of wavelength from 400 to 700 m μ is given in Figure 14. Similarly the spectra of the species CaD⁻, MgD⁻ and HD⁼ are given in Figure 15.

Figure 13. Acid dissociation constants of the p-cresol indicator. The absorbancy of the dye at 610 m μ is plotted versus pH for a series of solutions all containing the same amount of dye and carefully adjusted to an ionic strength of $\mu = 0.100$. The first $pK_1 = 8.20$; the second $pK_2 = 12.35$

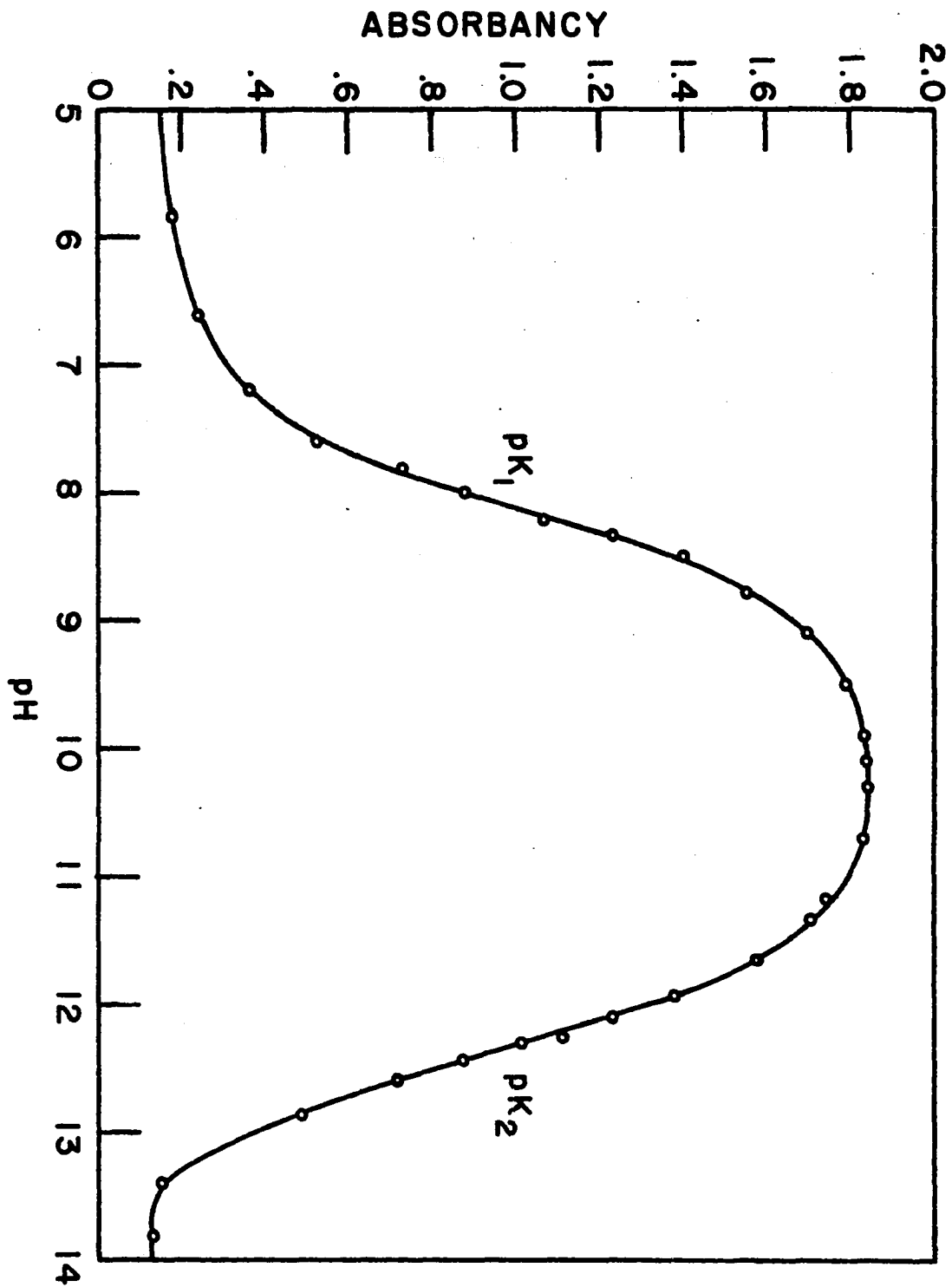


Figure 14. Absorption spectre of the p-cresol indicator at different pH values. Ionic strength: $\mu = 0.100$. pH of the solution for the different species: H_2D^- 2.14, HD^- 10.29, and D^{\equiv} 13.8

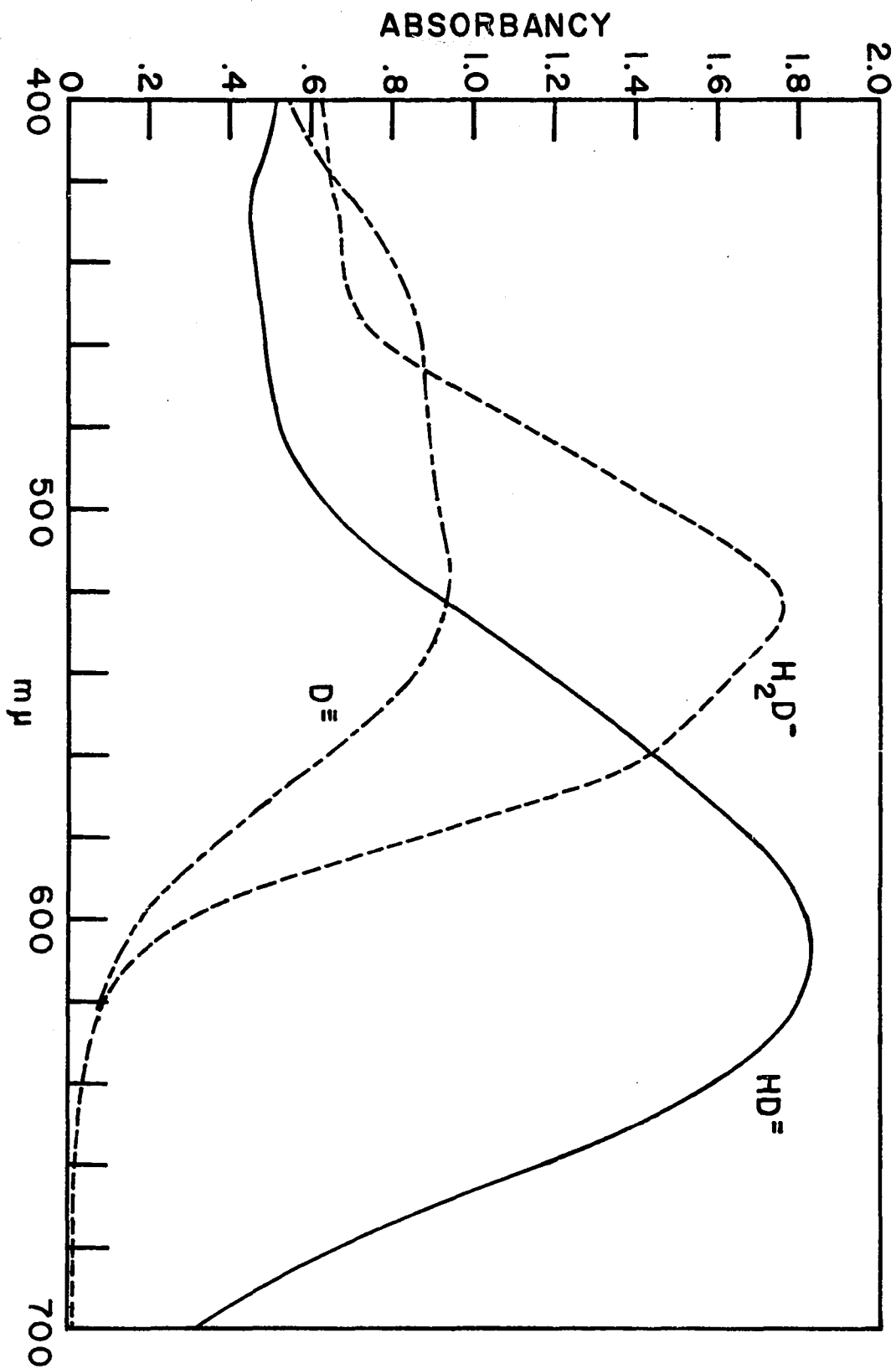
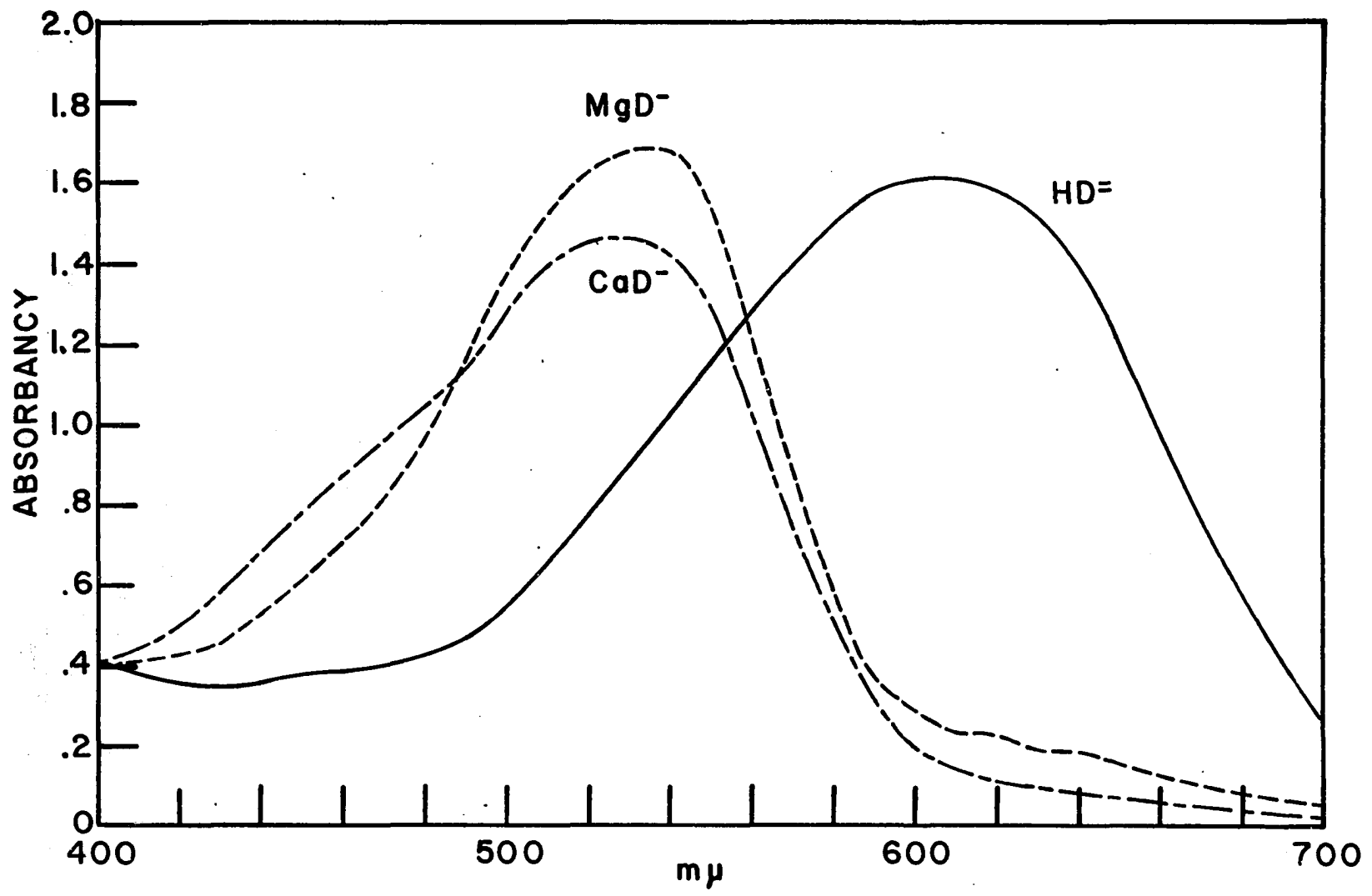


Figure 15. Absorption spectra of the p-cresol indicator and its calcium and magnesium derivatives at pH 10. Ionic strength: $\mu = 0.100$



Beer's law study of the p-cresol indicator and the molar extinction coefficient

Since the method which was to be used for investigating the calcium and magnesium compounds of the dye depended on the assumption that the absorbancy of the solution at a given wavelength was proportional to the concentration of the dye in solution, it was thought wise to make sure that solutions of the dye obeyed Beer's law under the conditions to be used in those investigations.

A dye stock solution was prepared by treating 0.4895 g. of the 95.09 percent pure p-toluidine salt of the dye with 20 ml. of 0.1 M potassium hydroxide solution and diluting to 1000 ml., giving a solution 1.00×10^{-3} M. One hundred ml. of this solution and 0.1 mole of potassium chloride was then diluted to 1000 ml. yielding a 1.00×10^{-4} M solution having an ionic strength of $\mu = 0.100$.

Using the 1.00×10^{-4} M stock solution a series of solutions was prepared that were 1, 2, 3, 4, and 5×10^{-5} M in dye. Each of the solutions was buffered to pH 10 with 2 ml. of ammonium hydroxide - ammonium chloride buffer and adjusted to an ionic strength of $\mu = 0.100$ by adding potassium chloride solution. The actual pH of each solution was found to be 10.10. The absorption spectra of each solution in a 2 cm. cell was recorded using the Cary spectrophotometer. Plots of the absorbancy at 450 m μ ,

530 m μ and at the maximum, 610 m μ , versus concentration were found to be exactly linear.

From the slope of the absorbancy vs. concentration line the molar extinction coefficient was computed as 20,300 at 610 m μ , pH 10.10 and $\mu = 0.100$.

Log-ratio study of the calcium compound of the p-cresol indicator

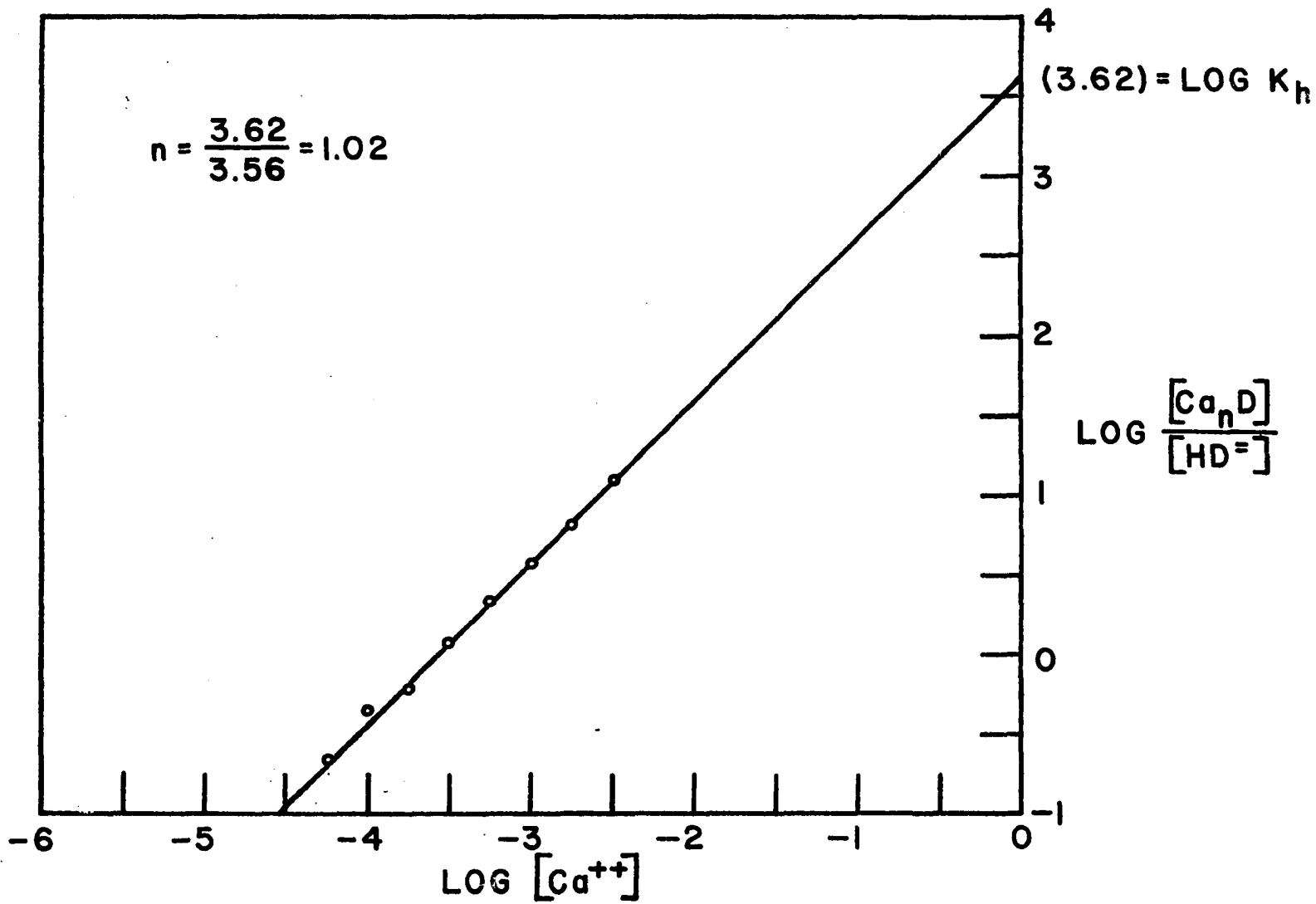
The compound formed between the p-cresol indicator and calcium was studied in exactly the same manner as the compound formed between calcium and Eriochrome Black T which was discussed previously. The same standard 0.00100 M calcium solution and the same buffer solutions employed in the earlier study were used in this investigation. The concentration of the dye in each flask was about 3.85×10^{-5} M. The computation of the ratio $[\text{Ca}_n\text{D}]/[\text{HD}]$ and its logarithm from the absorbancies obtained from the Cary spectrophotometer can be seen in Table 7. The log-ratio plot of the data of Table 7 is given in Figure 16.

It was evident that calcium formed a one to one compound with this indicator at pH 10 just as it did with Eriochrome Black T. The logarithm of the apparent stability constant was found to be 3.62. No attempt was made to study the calcium compound at any other pH value.

Table 7. Log-ratio method for calcium and p-cresol indicator at pH = 9.95, $\mu = 0.100$ and $\lambda = 610 \text{ m}\mu$

$[\text{Ca}] \times 10^5$	$\text{Log}[\text{Ca}]$	A	$A_{\text{HD}} - A$	$A - A_{\text{Ca}_n\text{D}}$	$\frac{A_{\text{HD}} - A}{A - A_{\text{Ca}_n\text{D}}}$	$\text{Log} \frac{A_{\text{HD}} - A}{A - A_{\text{Ca}_n\text{D}}} = \text{Log} \frac{[\text{Ca}_n\text{D}]}{[\text{HD}]}$
0	-	1.58	-	1.44	-	-
5.62	-4.25	1.32	0.28	1.18	0.220	-0.658
10.00	-4.00	1.14	0.44	1.00	0.440	-0.357
17.60	-3.75	1.05	0.53	0.91	0.583	-0.234
31.6	-3.50	0.81	0.77	0.67	1.15	-0.060
56.2	-3.25	0.61	0.97	0.47	2.06	0.314
100.0	-3.00	0.45	1.13	0.31	3.64	0.561
178.0	-2.75	0.33	1.25	0.19	6.58	0.818
316.0	-2.50	0.25	1.33	0.11	12.1	1.082
excess	-	0.14	1.44	-	-	-
slope or n = 1.01				$\text{log } K_{10.00} = 3.67$		

Figure 16. Log-ratio plot for calcium and the p-cresol indicator at pH 9.95. From absorbancy readings at 610 m μ . Ionic strength: $\mu = 0.100$, 2 cm. cells. $\text{Log } K_{9.95} = 3.62$; corrected log $K_{10.00} = 3.67$

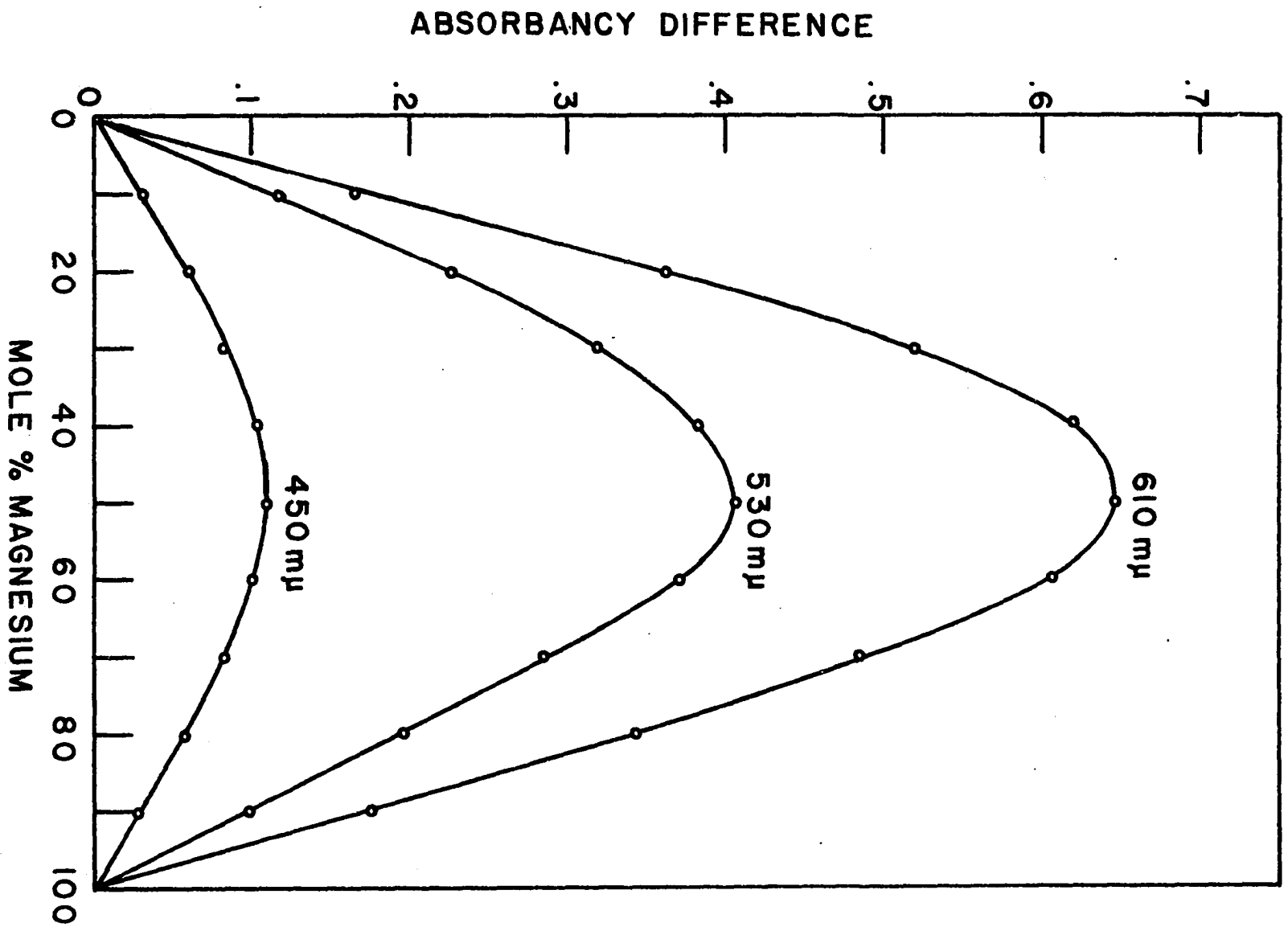


Continuous variations study of the magnesium compound of the p-cresol indicator

The magnesium compound of the new indicator was studied in exactly the same manner as the magnesium compound of Eriochrome Black T at pH 10. The magnesium stock solution, 1.00×10^{-4} M, and the buffer solutions were the same as those employed in the earlier study. The dye stock solution used was the 1.00×10^{-4} M one used in the Beer's law study and in the determination of the molar extinction coefficient of the p-cresol indicator. After the continuous variations solutions were prepared and the spectra obtained, plots were made for the wavelengths 610 m μ , 530 m μ and 450 m μ ; Figure 17. It is obvious from the plots that magnesium and this indicator formed only a one to one compound. The pH of each continuous variations solution was measured and found to be 10.00.

Also in the manner previously described, the amount of the dissociation of the compound formed between the metal and the dye indicated by the plot at each wavelength was found to be 0.267 (610 m μ), 0.236 (530 m μ) and 0.242 (450 m μ) for an average dissociation value of 0.248. Using this value and the concentration of complex if there were no dissociation, 2.5×10^{-5} M, the apparent stability constant was then computed as $\log K_{10.00} = 5.69 \pm 0.10$.

Figure 17. Continuous variations plots for magnesium and the p-toluidine salt of the p-cresol indicator at pH 10.00. Magnesium and dye solutions both 1×10^{-4} M. Ionic strength: $\mu = 0.100$, 2 cm. cells



Stability of the p-cresol indicator in aqueous solution

In order to determine the stability of the p-cresol indicator in aqueous solution, the 1.00×10^{-3} M stock solution that was used for the Beer's law study and the determination of the molar extinction coefficient was placed in a polyethylene bottle and stored for about eight months. At the end of that time a measure of the amount of dye still remaining in solution was made by measuring the absorbancy of a solution prepared from an aliquot of the stock solution in the same manner as was done for the determination of the molar extinction coefficient. Exactly 25.00 ml. of the stock solution, 0.100 mole of potassium chloride, a small amount of EDTA and 20 ml. of ammonium hydroxide - ammonium chloride buffer was diluted to 1,000 ml. with deionized water. The absorbancy was measured with a Beckman DH spectrophotometer at 610 m μ using 1 cm. cells versus water and was found to be 0.495. Since the molar extinction coefficient of the indicator was known to be 20,300 at 610 m μ under these conditions, the concentration of the dye stock solution was calculated to be 0.975×10^{-3} M. This represents a loss of 2.5 percent of the dye by decomposition or precipitation over a period of 259 days. At this rate only half the dye would decompose after about 16 years if the decomposition followed first order kinetics. However, it was not postulated that

the apparent loss of dye was only due to decomposition for other factors may have been involved. The important thing was that the solution of the dye was stable for an extensive period of time in aqueous solution near pH 5. Under similar conditions Eriochrome Black T would decompose completely in a matter of weeks.

Conclusions

The acid dissociation constants for the phenolic hydrogens of the p-cresol indicator were found to be $pK_1 = 8.20$ and $pK_2 = 12.35$ at an ionic strength of $\mu = 0.100$. It was noted that pH 10 was in the middle between these values.

The absorption spectra of the various protonated forms of the dye and of the dye and its calcium and magnesium complexes at pH 10 were recorded.

The calcium complex at pH 10 and an ionic strength of $\mu = 0.100$ was found to be a one to one combination of dye and metal. The log apparent stability constant was found to be $\log K_{10} = 3.67$. The magnesium complex at pH 10 at an ionic strength of $\mu = 0.100$ was also found to be a one to one compound. The log apparent stability constant found was $\log K_{10} = 5.69$.

The molar extinction coefficient was found to be 20,300 at pH = 10, $\mu = 0.10$ and $\lambda = 610$ m μ . Solutions of the dye under these conditions were found to obey Beer's law exactly.

Finally, the dye was found to be very much more stable in aqueous solution than Eriochrome Black T, over 97 percent of the dye remaining in solution after a storage period of eight months. This dye is a practical shelf reagent.

The Xylenol Indicator and Its Calcium and Magnesium Derivatives

Apparatus and materials

See the section on apparatus and materials accompanying the discussion of the p-cresol indicator.

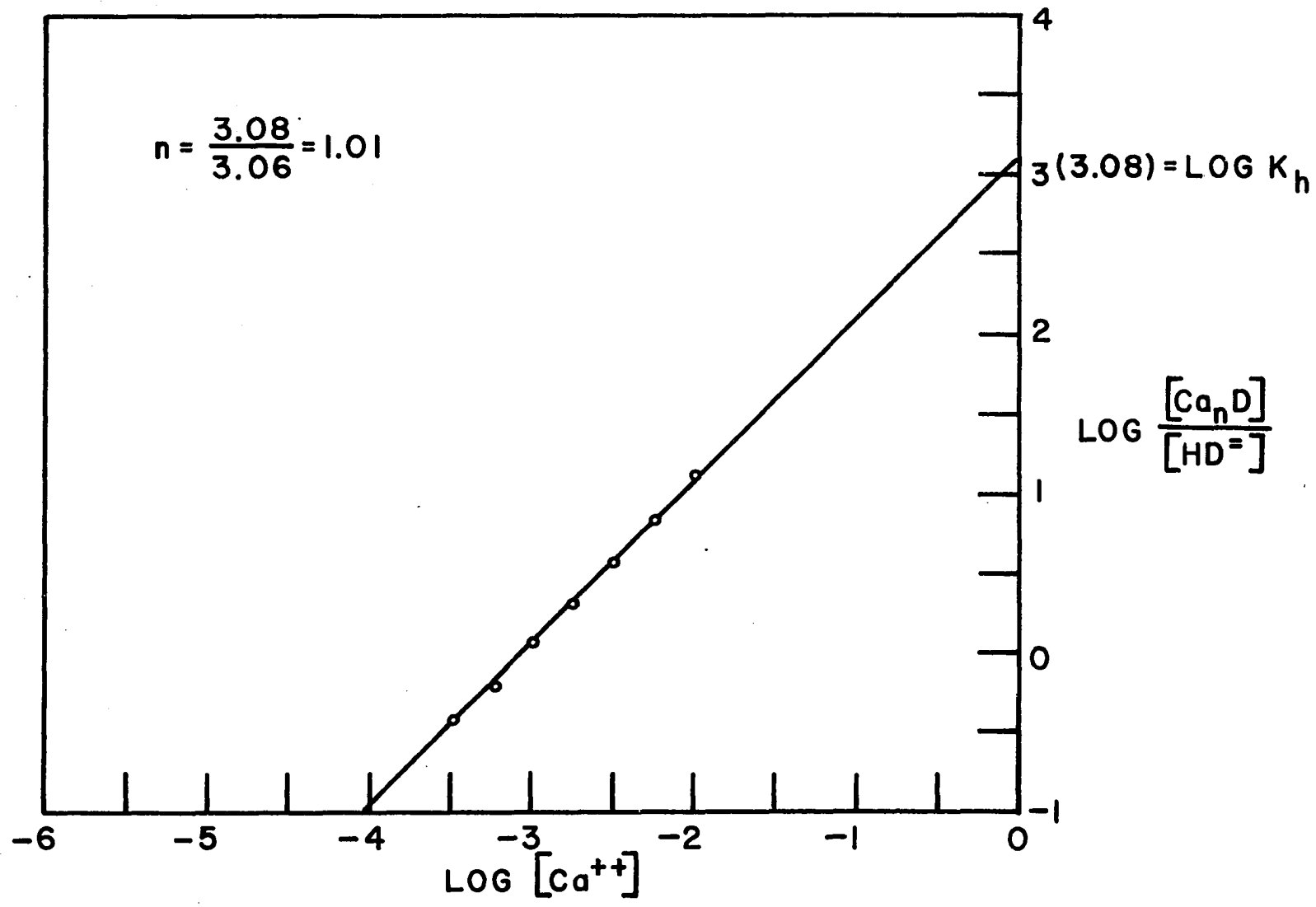
Log-ratio study of the calcium compound of the xylenol indicator

The calcium compound of the xylenol indicator at pH 10 was investigated in the same manner as the p-cresol indicator but the buffer was 2 ml. of the ammonium hydroxide - ammonium chloride buffer used for EDTA titrations. The computation of the ratio $[Ca_nD]/[HD]$ and its logarithm from the absorbancies obtained from the Cary spectrophotometer can be seen in Table 8. The log-ratio plot of the data of Table 8 is given in Figure 18.

Table 8. Log-ratio method for calcium and xylenol indicator at pH = 10.08, $\mu = 0.10$ and $\lambda = 625$ m μ

[Ca] x 10 ⁴	Log [Ca]	A	A _{HD} - A	A - A _{Ca_nD}	$\frac{A_{HD} - A}{A - A_{Ca_nD}}$	$\text{Log} \frac{A_{HD} - A}{A - A_{Ca_nD}} = \text{Log} \frac{[Ca_nD]}{[HD]}$
0	-	1.96	-	1.84	-	-
3.16	-3.50	1.46	0.50	1.34	0.373	-0.428
5.62	-3.25	1.24	0.72	1.12	0.643	-0.192
10.00	-3.00	0.97	0.99	0.85	1.16	0.064
17.8	-2.75	0.725	1.235	0.605	2.04	0.310
31.6	-2.50	0.510	1.450	0.39	3.72	0.570
56.2	-2.25	0.355	1.60	0.235	6.84	0.834
100.0	-2.00	0.250	1.71	0.13	13.2	1.120
excess	-	0.120	1.84	-	-	-
		slope or n = 1.00		log K _{10.00} = 3.00		

Figure 18. Log-ratio plot for calcium and the xylenol indicator at pH 10.08. Data obtained from absorbancy readings at 625 m μ . Ionic strength: $\mu = 0.10$, 2 cm. cells. $\text{Log } K_{10.08} = 3.08$; corrected $\text{log } K_{10.00} = 3.00$

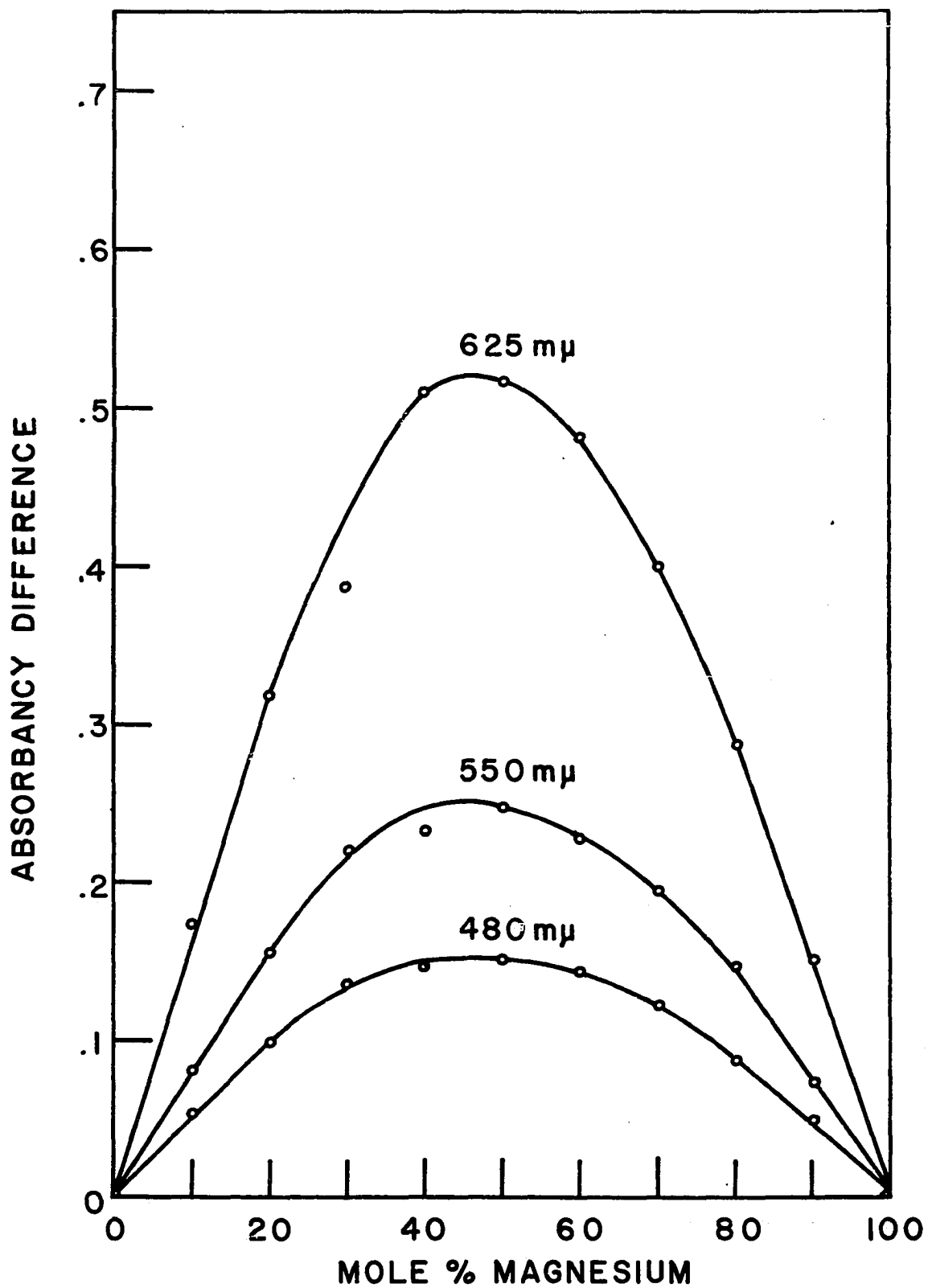


It was evident that calcium also formed a one to one compound with this indicator at pH 10. The logarithm of the apparent stability constant was found to be 3.05. It may be noted that this compound is weaker than the one formed between calcium and the p-cresol indicator.

Continuous variations study of the magnesium compound of the xylenol indicator

The magnesium compound of this indicator at pH 10 was studied in the same manner as the two dyes discussed previously except that the dye stock solution was prepared by dissolving 0.4510 g. of the 82.7 percent pure dye in de-ionized water and diluting to 1,000 ml. to yield a 1.00×10^{-3} M solution. One hundred milliliters of the solution was treated with 0.100 mole of potassium chloride and diluted to 1,000 ml., yielding a solution that was 1.00×10^{-4} M in dye and with an ionic strength of $\mu = 0.100$. This solution was used in the preparation of the continuous variations solutions. These solutions were buffered in the same way as the solutions used in the log-ratio study. The plots obtained from the absorbancy of the solutions at 625 m μ , 550 m μ , and 480 m μ are given in Figure 19. It is obvious from the plots that magnesium and this indicator formed only a one to one compound at this pH. The pH of each continuous variations solution was found to be 10.05.

Figure 19. Continuous variations plots for magnesium and the xylenol indicator at pH 10.05. Magnesium and dye solutions both 1×10^{-4} M. Ionic strength: $\mu = 0.10$, 2 cm. cells



The amount of the dissociation of the compound formed between the metal and the dye indicated by the plot at each wavelength was found to be 0.317 (625 m μ), 0.322 (550 m μ), and 0.342 (480 m μ) for an average dissociation value of 0.327. Using this value and the concentration of complex assuming no dissociation, 2.5×10^{-5} M, the apparent stability constant was computed as $K_{10.05} = 2.52 \times 10^5$. The log apparent stability constant was then computed as $\log K_{10.05} = 5.40 \pm 0.10$; corrected $\log K_{10.00} = 5.35 \pm 0.10$. It may be noted that this stability constant is also lower than the corresponding value for the p-cresol indicator.

Conclusions

The calcium complex of the xylenol indicator at pH 10 and an ionic strength of $\mu = 0.10$ was found to be a one to one compound. The log apparent stability constant was found to be $\log K_{10.00} = 3.00$. The magnesium complex at pH 10 at an ionic strength of $\mu = 0.10$ was also found to be a one to one compound. The log apparent stability constant of the magnesium compound was found to be $\log K_{10.00} = 5.35$.

The methyl group in the 6 position on the xylenol indicator (see Figure 12) decreased the log apparent stability constant of the magnesium complex at pH 10 from 5.69

for the p-cresol indicator to 5.35 for the xylenol indicator; the log apparent stability constant for the calcium complex at pH 10 decreased from 3.67 to 3.00. The decrease was 0.34 for the magnesium complex and 0.67 for the calcium complex. While the additional methyl group in the 6 position on the xylenol indicator made it somewhat selective against calcium in the presence of magnesium it obviously was not sufficient to permit it to be used as a practical reagent.

Another indicator was synthesized with an isopropyl group in the 6 position to increase the blocking effect. This indicator was indeed less sensitive for calcium but unfortunately the sensitivity of the reagent for magnesium was much less than the p-cresol dye too.

From an inspection of the continuous variations spectra of the xylenol indicator its molar extinction coefficient was found to be 19,600 at pH = 10, $\mu = 0.10$ and $\lambda = 625 \text{ m}\mu$.

The absorption spectra of the xylenol indicator and its calcium and magnesium complexes were, as expected, very similar to the spectra of the p-cresol indicator and its complexes. The maximum in the dye spectra at pH 10 was simply shifted from 610 m μ to 625 m μ , the maximum in the magnesium spectra was shifted from 540 m μ to 545 m μ , but the calcium spectra was not shifted at all. These spectra

have not been included.

Use of the New Indicators in Titrations
of Calcium and Magnesium with EDTA

Introduction

The value of the new indicators in EDTA titrations of calcium and magnesium at pH 10 was determined by counting the number of drops of 0.01 M EDTA consumed in the transition from the color of the magnesium derivative to the elimination of the last trace of the red shade in the blue color of the uncomplexed dye. This of course represented the end point of the titration.

Using the purified Eriochrome Black T, nine drops of titrant were required to go from a rose shade to a greenish blue. With the p-cresol indicator only five drops were needed to go from a rose shade to a clear blue. The xylenol indicator on the other hand required the same amount of titrant as did the Eriochrome Black T. The amount of indicator used for each titration was such that the level of the rose color before the end point was the same in each case. Several other workers in addition to the author repeated these simple tests with similar results. While these tests were very subjective in nature the choice of an indicator was thought to be a subjective matter in the end regardless of other considerations.

Because of its superior end point and vastly superior keeping qualities as a shelf reagent the p-cresol indicator was selected as the one to replace Eriochrome Black T. The xylenol indicator, while having keeping qualities at least as good as the p-cresol indicator was rejected because it gave an end point no better than the old indicator.

Apparatus and materials

All of the volumetric apparatus employed was made of borosilicate glass. Experience had shown that it was much more accurately calibrated than the usual soft glass volumetric apparatus.

Except as noted below all of the chemicals used were of reagent grade. The zinc employed as a primary standard was National Bureau of Standards melting point standard. This material was checked spectrographically and found to be free of any other metallic elements. The crystalline calcium carbonate, calcite, had been previously analyzed for magnesium spectrographically and found to contain less than 40 ppm. This was the same material employed by Diehl and Ellingboe in the testing of the indicator "Calcein" (5). The EDTA used was Eastman Kodak #6354 (Ethylenedinitrilo) tetraacetic acid disodium salt. The magnesium EDTA complex compound was prepared by mixing concentrated

equimolar solutions of magnesium sulfate and EDTA and filtering off the precipitated magnesium compound. The triethanolamine used was Matheson Coleman & Bell practical grade. Deionized distilled water was used throughout this study. All solutions were stored in polyethylene containers except for certain acidic solutions that were stored in glass.

Procedure (22)

Samples were dissolved in perchloric acid, neutralized to the methyl red end point with sodium hydroxide, and diluted to the mark in a volumetric flask. After the solution was well mixed, an aliquot equivalent to about 40 mg. of calcium carbonate was transferred to a 250 ml. Erlenmeyer flask. Then about 25 mg. of ascorbic acid, 5 ml. of five percent potassium cyanide solution, 5 ml. of twenty percent triethanolamine, 2 ml. of ammonium hydroxide - ammonium chloride buffer and finally 10 drops of 1×10^{-3} M p-cresol indicator was added in turn with careful mixing after each addition. Sufficient water was added to the flask so that the volume would be near 100 ml. at the end point. Standard 0.01 M EDTA was then added until all the red color of the magnesium indicator complex was destroyed which was the end point. It was not quite possible to split drops at the end point.

The buffer was prepared by dissolving 70 g. of ammonium chloride in 570 ml. of concentrated ammonium hydroxide and diluting to 1,000 ml.

The ascorbic acid was added to reduced any trivalent iron to the divalent state. The potassium cyanide was added to complex any iron, copper, zinc and similar metals which might be present so they would not interfere. The triethanolamine performed the same function for aluminum.

Standard solution

The 0.01 M EDTA solution was prepared by dissolving 22.3 g. of the Eastman Kodak disodium salt in 6,000 ml. of water. It was standardized against samples of calcite, natural crystalline calcium carbonate, by the above procedure. For the standardization, however, it was not necessary to use the ascorbic acid, cyanide, or triethanolamine; but, as with Erlochome Black T, a small amount of a precisely equimolar solution of the magnesium - EDTA complex was added to furnish the magnesium for the proper operation of the indicator. The molarities were found to be 0.009915 M, 0.009908 M, 0.009918 M, and 0.009922 M for an average of 0.009916 M.

To check this molarity value the N. B. S. melting point standard zinc was analyzed by the above procedure leaving out the cyanide and triethanolemine and was found to be

99.88 percent pure. If one assumed that the zinc was 100 percent pure, this amounted to a difference of 12 parts per 10,000. The end points in the zinc titrations were the sharpest ever observed by the author in a titrimetric procedure.

A study of the interfering metal ions on the titration of calcium and magnesium with EDTA and the new indicator

In order to make this study, a solution of known calcium and magnesium content was prepared by dissolving 1.6676 g. of N. B. S. sample number 88, Dolomite, in hydrochloric acid and diluting to 1,000 ml. Twenty-five milliliters of this solution was then pipetted into the titration flask and the appropriate interfering agent was added either as a solution or as a solid salt. The titration was performed using the procedure outlined earlier. Each aliquot of the dolomite solution was found by titration to be equivalent to 25.09 mg. of calcium oxide. By calculation, 25.17 mg. of calcium oxide would be equivalent to the calcium oxide, magnesium oxide and strontium oxide content reported for the amount of sample in each twenty-five milliliter aliquot.

The effect of the interference can be seen in Table 9. It may be noted that while apparently large amounts of potassium and the common anions can be tolerated, the addition of large amounts of sodium caused trouble. Small

Table 9. The effect of interfering ions on the EDTA titration of calcium and magnesium using the p-cresol indicator

Calcium oxide taken mg.	Calcium oxide found mg.	Interference added mg.		End point quality	Percent calcium oxide recovered
25.09	25.13	sodium sulfate	2,000	very poor	100.16
"	-	" "	10,000	none	-
"	25.19	sodium chloride	2,000	satisfactory	100.40
"	25.95	" "	10,000	poor	103.43
"	25.12	potassium chloride	10,000	excellent	100.12
"	25.12	potassium nitrate	10,000	excellent	100.12
"	25.06	potassium perchlorate ^a		excellent	99.88
"	25.25	potassium bromate	1,000	excellent	100.64
"	25.10	copper	10	excellent	100.03
"	25.08	"	25	"	99.96
"	-	"	50	none	-
"	24.87	iron	5	excellent	99.12
"	24.63	"	10	poor	98.17
"	25.07	aluminum	1	satisfactory	99.92
"	24.91	"	5	poor	99.28

^asaturated solution

amounts of the important interfering ions, copper, iron and aluminum can apparently be rendered harmless by the masking agents commonly used for the similar titration using Eriochrome Black T.

Analysis of standard samples N. B. S. No. 88

National Bureau of Standards Standard Sample Number 88, Dolomite, was analyzed using the procedure which has been outlined. The results are tabulated in Table 10. Results are expressed as percent calcium oxide. The computed value represents the percent of calcium oxide equivalent to the sum of the alkaline earth oxide content of the sample as reported by the Bureau.

Table 10. Analysis of Dolomite, N. B. S. 88

Sample weight grams ^a	Volume of 0.009916 M EDTA milliliters	Percent calcium oxide
0.5434	47.21	60.39
	47.22	60.40
	47.26	60.45
0.5199	45.30	60.57
	45.22	60.46
	45.26	60.51
0.5174	44.91	60.34
	44.85	60.26
	44.94	60.38
	Average	60.42
	Computed N.B.S. value	60.37
	Range	0.31
	Average deviation	0.071
	Standard deviation	0.087
	Standard deviation as parts per thousand	1.44

^aSample dissolved in dilute hydrochloric acid, evaporated to dryness, diluted to 250 ml. and 20 ml. aliquots taken.

Results and Discussion

p-cresol indicator

Several azo dyes possessing the necessary o,o'-dihydroxyazo configuration (9) were synthesized and tested as stable substitutes for Eriochrome Black T as indicators in titrations for calcium and magnesium using EDTA. The dye resulting from the coupling of 1-diazo-2-naphthol-4-sulfonic acid, Figure 12 (a), with p-cresol, 1-(1-hydroxy-4-methyl-2-phenylazo)-2-naphthol-4-sulfonic acid Figure 12 (c), gave end points superior to those obtained with Eriochrome Black T and seemed to be quite stable in aqueous solution. A quantity of this dye was prepared, purified and used as the p-cresol indicator. A p-toluidine salt derivative of the dye was prepared, purified, crystallized and assayed; this material was used in studying the properties of the pure material.

The log acid dissociation constant for the two hydroxyl groups were determined spectrophotometrically and found to be 8.20 for pK_1 and 12.35 for pK_2 ; Figure 13. The curve was symmetric about pH 10, the value to which solutions are buffered prior to the titration for calcium and magnesium with EDTA.

The compound formed between magnesium and the p-cresol indicator at pH 10 was studied using the method of

continuous variations; Figure 17. As with Eriochrome Black T a one to one compound was formed; the log apparent stability constant for the compound at pH 10 was 5.69. The compound formed between calcium and the p-cresol indicator at pH 10 was studied using the log-ratio method; Figure 16. A one to one compound was formed with a log apparent stability constant at pH 10 of 3.67. These stability constants are very similar to the stability constants for the corresponding Eriochrome Black T metal compounds. All of these studies were carried out at an ionic strength of $\mu = 0.100$.

The stability of aqueous solutions of the dye was tested by the reanalysis of an aqueous solution of the dye which had been stored for eight months. The decomposition was so slight that a shelf "half-life" of 16 years was estimated.

The indicator was used in EDTA titrations for calcium and magnesium in National Bureau of Standards standard sample No. 88, Dolomite; Table 9. The effects added interferences on the titration of a solution of calcium and magnesium using the usual procedure were determined as shown in Table 8. It was evident that the ordinary masking agents could be used to control the interferences. It was also noted that very large quantities of potassium salts could be present.

In summary, a new dye has been prepared and studied as a metal ion indicator for use in EDTA titrations for calcium and magnesium. It is superior to the old indicator, Eriochrome Black T, in that aqueous solutions of dye may be made up and stored almost indefinitely as a shelf reagent. In addition better end points are obtained. Also the new indicator is easier to synthesize, only one step being necessary employing commercially available starting materials. The dye and its metal ion compounds are water soluble, and at pH 10 a larger proportion of the dye is present in the desired blue form. Unfortunately, when the new indicator was compared to Eriochrome Black T as a dye for wool it proved to be very unsatisfactory.

This new indicator is being prepared and marketed by the G. F. Smith Chemical Company as "Calmagite". Professor Smith proposed this name from the combination calcium + magnesium + ite.

Xylenol indicator

Another indicator dye was prepared that was similar to the p-cresol indicator except that an additional methyl group was adjacent to the hydroxyl group on the phenyl ring; Figure 12 (d). 1-(1-Hydroxy-4,6-dimethyl-2-phenylazo)-2-naphthol-4-sulfonic acid was prepared by coupling 1-diazo-2-naphthol-4-sulfonic acid to 2, 4-dimethylphenol (xylenol).

This indicator was hoped to be selective for magnesium in the presence of calcium due to the blocking action of the additional methyl group upon the much larger calcium ion. A difference was noted in the action of this indicator toward solutions of the two ions when compared to the p-cresol indicator, but the effect was not large enough to be practical.

The log apparent stability constants for the compounds formed between the xylenol indicator and calcium and magnesium were determined using the log-ratio method and the method of continuous variations, respectively. The results are compared with similar results for the p-cresol indicator in Table 11.

Table 11. Comparison of log apparent stability constants of the calcium and magnesium compounds of the p-cresol and xylenol indicators

	p-Cresol indicator	Xylenol indicator	Difference
Log K_{10} for calcium	3.67	3.00	.67
Log K_{10} for magnesium	5.69	5.35	.34

Another indicator was prepared which had an isopropyl group in the same position as the additional methyl group in the xylenol indicator. Its magnesium complex was so weak it was not studied further.

SUMMARY

Eriochrome Black T

The discrepancies between the findings of Schwarzenbach and Biedermann and American workers on the properties of Eriochrome Black T and its calcium and magnesium derivatives has been resolved by the preparation of a highly pure crystalline form of the dye and careful measurements of its properties and reactions.

Commercial Eriochrome Black T was purified by the removal of some 30 percent inorganic salt from the dried commercial product by repeated extractions with dilute hydrochloric acid. Attempts to crystallize the salt-free dye from numerous solvents and by various techniques were unsuccessful nor were similar efforts successful on material synthesized from purified intermediates. Success was finally obtained by recrystallization from dimethylformamide. Ultimate analysis of the crystalline material showed it to be the dimethylammonium salt and this was confirmed by identifying and determining the dimethylemine present.

The logarithm of the acid dissociation constants for the two hydroxyl groups on the dye molecule were determined and the values $pK_1 = 6.91$ and $pK_2 = 11.50$ obtained. These values are about the same as those of Schwarzenbach and Biedermann.

The molar extinction coefficient of Eriochrome Black T at pH 10 and 615 m μ , the absorbancy maximum, was measured and found to be 32,300, a higher value than that reported by Schwarzenbach being due to the greater purity of the material being used. The free acid form of the dye was prepared from the crystalline dimethylamine salt and found to be very hygroscopic.

The combining ratio of Eriochrome Black T and magnesium at pH 8 was shown by the log-ratio method to be one to one. The value of the log apparent stability constant for the magnesium compound at pH 8 was found to be $\log K_{8.00} = 3.60$. At pH 10 the combining ratio of Eriochrome Black T and magnesium was found also to be one to one using the method of continuous variations; $\log K_{10.00} = 5.75$. The combining ratio of Eriochrome Black T and calcium was determined at pH 10 using the log-ratio method and found to be one to one; $\log K_{10.00} = 3.84$. An attempt to study the calcium compound at pH 8 by this method was unsuccessful for the amount of calcium ion necessary to form a measurable amount of the compound was sufficient to precipitate the dye.

Earlier American work, using preparations of the dye-stuff of doubtful purity has been shown to be wrong.

New Hydroxyazo Dyes as Metal Ion Indicators

Since Eriochrome Black T is very unstable in solution new indicator dyes were prepared as stable substitutes for the older indicator. One of these was prepared by coupling 1-diazo-2-naphthol-4-sulfonic acid to p-cresol. This dye was found to give somewhat better end-points than those produced by Eriochrome Black T and aqueous solutions of the dye could be kept for extended periods of time. A crystalline p-toluidine salt derivative was prepared, analyzed and used for an investigation of the properties of the dye and its calcium and magnesium compounds.

The logarithm of the acid dissociation constants for the two hydroxyl groups on the molecule were determined and the values $pK_1 = 8.20$ and $pK_2 = 12.35$ obtained. The molar extinction coefficient at pH 10 and 610 m μ was measured and found to be 20,300.

The combining ratio of the p-cresol indicator and magnesium at pH 10 was shown by the method of continuous variations to be one to one. The value of the log apparent stability constant for the magnesium compound at pH 10 was found to be $\log K_{10.00} = 5.69$. The combining ratio of the p-cresol indicator and calcium was determined at pH 10 using the log-ratio method and found to be one to one; $\log K_{10.00} = 3.67$.

The new dye was used successfully in the determination of the calcium and magnesium in dolomite with EDTA, and the effect of interferences was studied.

A new indicator was prepared which effectively serves as a stable substitute for Eriochrome Black T in titrations of calcium and magnesium using EDTA; it is available from the G. F. Smith Chemical Company under the name "Calmagite".

Another dye was prepared like the p-cresol dye with a methyl group adjacent to the hydroxyl group on the phenyl ring. It was hoped that the blocking action of this added methyl group would make this indicator more sensitive for magnesium ion than for the larger calcium ion. The calcium and magnesium compounds of the dye at pH 10 were studied and found to be formed in a one to one ratio. The log apparent stability constant for the calcium compound decreased to $\log K_{10.00} = 3.00$; compare $\log K_{10.00} = 3.67$ for the p-cresol dye. However, the log apparent stability constant for the magnesium compound decreased also to $\log K_{10.00} = 5.35$; compare $\log K_{10.00} = 5.69$ for the p-cresol dye. While the stability of the calcium compound was decreased more than the magnesium compound, the effect was too small to be of analytical interest.

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ACKNOWLEDGMENT

The author wishes to express his gratitude for the interest, encouragement and advice given in the course of this work by Professor Harvey Diehl. The author would also like to thank the Ames Laboratory of the Atomic Energy Commission for the valuable privilege of using the Cary recording spectrophotometer. He also wishes to thank the administration of Clemson College, Clemson, South Carolina for their kind indulgence during the preparation of this dissertation.