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Spectrophotometric study of the Boron-Curcumin system

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Spectrophotometric Study Of The Boron-Curcumin System

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INTRODUCTION

Prior to about a half century ago, existing methods for quanitative detection of boron were sensitive enough only for the macrorange of boron concentration. With the introduction of the curcumin colorimetric method at the turn of the century, a potential tool for microrange determination of boron was in the hands of the analytical chemist.

Throughout the last fifty years or so after the introduction of the method, much work has been done in modifying it for various different situations, but very little study has actually been done on better understanding the mechanism of the method itself.

With mechanism and structure in mind, the aim of this research project was to investigate the role played by the various reagents used in the curcumin spectrophotometric method. The reagents were used in varied concentrations and at times some eliminated entirely, in the hope that a greater understanding be gained in the overall mechanism followed by the reaction.

One of the major objectives was to try to determine the structure of the colored substance formed in the reaction. A step towards this goal was gained by obtaining molar ratios of the entities suspected to be an intricate part of the colored substance.

Most of the studies made were studies of absorption using the visible range of the spectrum (550 millimicrons) where the colored substance was found to absorb at its maximum. A photoelectric colorimeter could have been used instead of a spectrophotometer, however the resolution and precision would not have been as great.

A potentiometric titration study was also carried out so as to gain greater insight into the preliminary reaction occurring between boric acid and oxalic acid prior to the addition of curcumin in the final color forming step of the method.

HISTORICAL

The practical importance of boron in relation to agriculture is one of the recent findings of science. The reason why this fact remained obscure to science and mankind was in part due to the lack of detection of the minute quantities of boron to be found in the soils and plants of the universe. Up until the turn of the century, methods existing for quantitative detection of boron were sensitive enough only for the macrorange of boron concentration. Since the concentration of boron in plans and soils are confined to a narrow range and in general, the concentration of boron in the soil should not exceed a few parts per million, a micromethod for the determination of boron was desirable.

It will suffice to mention at this point that the early methods for quantitative analysis of boron were chiefly gravimetric or volumetric, the latter being the more reliable and accurate of the two. Gravimetric methods were not reliable because a sparingly soluble stoichiometric compound of boron which could serve as a basis for gravimetric detection was not available. (11) All volumetric methods in general follow a similar pattern in which the pH of a boron solution is adjusted with subsequent addition of a polyol and titration with a standard strong base (4).

With a need for a method for microdetermination of boron, the colorimetric methods were investigated. Of the colorimetric methods the two most widely employed and investigated were those in which quinalizarin and curcumin served as the color forming reagent.

The quinalizarin method was found to be the more rapid and less subject to interference by other trace elements, while the curcumin method the more sensitive of the two. In the quinalizarin method (17) quinalizarin reacts with boric acid in concentrated sulfuric acid giving a colored substance which is used as the basis for analysis.

Since the work which will be presented in this research project is mainly concerned with the curcumin method, a brief historical review of the origin of the use of curcumin as a boron detection tool and the subsequent important modifications and applications of the method shall be presented.

Curcumin, 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene, has as empirical formula $C_{21}H_{20}O_6$. It is an unsaturated hydroxy-ketone and is the coloring principle of curcuma (turmeric) i.e. the dye from the rhizones of curcuma tincturia, which fluorishes in the East. Commercially curcumin is extracted from this dye by a series of organic extractions. (7) Curcumin still finds a limited use as a dye for silk and is also used as an indicator (gives a brown color with caustic alkali and red coloration with boric acid).

By means of infrared studies, Spicer and Strickland (24) confirmed the enol structure of curcumin given by Ghosh (9)
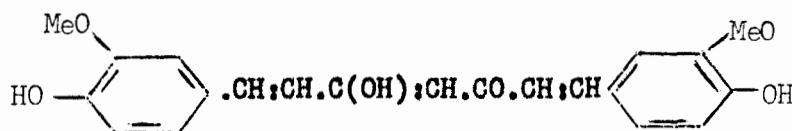
The accepted structure of curcumin (keto form) given by Yoe and Sarver
(28) and most of the recent investigators is given as:

$$MeO—HO—\langle\rangle.CH{:}CH.CO.CH_2.CO.CH{:}CH.\langle\rangle—MeO,OH$$
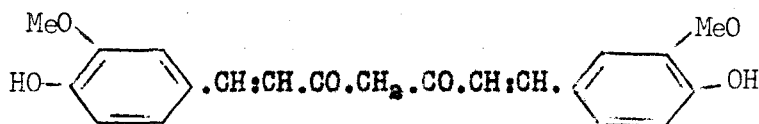
Curcumin in the keto form resembles acetyl acetone of which it most
certainly appears to be a derivative.

Curcumin was first used in a colorimetric method for boron
determination at the beginning of the century. In 1902, Hebebrand (11)
devised a colorimetric method depending upon the formation of a red color
upon the addition of turmeric to a water-alcohol solution containing boron
and hydrochloric acid. In the same year Cassal and Gerrans (4) revised
the method somewhat introducing oxalic acid which was found to increase
the sensitivity of the method considerably.

Another modifier of Hebebrand's method at about the year 1915
was Filippi (8). He modified the method so as to eliminate sources of
error due mainly to the varying composition of the curcumin used in the
tests. Filippi modified the method as follows: to better establish the
different intensity of colors he took up the residue containing curcumin
and boric acid with 2 ml. of 90% ethyl alcohol pipetting it into very narrow
test tubes of the same capacity and then observed the color which he claimed
persisted unmodified for 12 hours.

The turmeric or curcumin method actually became popular when the
method was revised further by Bertrand and Agulhon (2). Their method was
essentially a paper method in which the concentration of boron present in

a sample was proportional to the length which the colored compound formed between boron and curcumin in a strip of turmeric paper would travel by capillary action along the length of the paper.

Halphen (10) came out with his method a short while after Bertrand and Agulhon introduced their paper method in 1913. His method consisted in collecting boric acid as methyl borate in 0.2 ml. of 1N sodium hydroxide solution. The solution was then evaporated to dryness in a small test tube and the residue dissolved in 1 ml. of water plus 2 ml. of hydrochloric acid. Standards containing known amounts of boric acid were similarly treated. To each sample was then added 1 ml. of an ethyl acetate solution of turmeric and the resulting red colors were compared after 50 minutes.

Numerous other investigators studied the Bertrand and Agulhon method extensively in the years to follow adding slight modifications of their own but the method remained essentially the same.

It was not until 1939 that Naftel (16) came out with modifications of the Cassal and Gerrans method (4). This modification is probably the most popular curcumin procedure used to this date. This is the method with some modification which is followed in this research project. Essentially it involves making the boron sample basic with calcium hydroxide followed by evaporation of the sample to dryness. An alcoholic solution of oxalic acid and curcumin along with hydrochloric acid is then added to the cooled sample and evaporated further to dryness on a water bath. The residue is then extracted with ethyl alcohol and compared with known standards.

Naftel's method has been used quite extensively and has received some modifications from later investigators. Silverman and Trego (22) dissolved the curcumin dye in carbitol-acetone, which solution is stable for months, and used phenolphalein to control the acidity. They used sodium carbonate instead of calcium hydroxide in the preliminary drying step.

Winsor (27) extracted boron from fresh plant tissue and analyzed the boron present in microquantities by another modification of Naftel's method.

Hegedus (12), after a critical review of various methods based on the formation of rosocyanin from yellow curcumin under the influence of free boric acid, presented his modified method. It consisted of adding 2 ml. curcumin reagent (prepared by dissolving 0.1 g. curcumin in 100 ml. glacial acetic acid saturated with oxalic acid.) The solution was then evaporated to dryness on a water bath and the residue dissolved in 20 ml. of ethyl alcohol. The amount of boron present was then determined from the color intensity after illumination for 3 minutes. He found that F, Ti, Zr, Mo, Be, W, in large amounts interfered with the test. Oxidizing agents such as nitrate, chlorate, bromate, iodate, or nitrite should be reduced if present, to prevent the oxidation of curcumin. The method is most suitable for establishing the boron content in mineral waters of low boron content. The method is sensitive for a range of boron from 0.5 to 8 micrograms.

Bottini (3) used still another modification of the curcumin method using a basic instead of acidic media. He acidified a boron solution with acetic acid and added 2 ml. of a 0.1% curcumin solution. After evaporation on a steam bath he separated the excess curcumin by washing with ethyl ether until the washings were colorless. The residue was then treated with methyl acetate (100 ml. methyl acetate + 1 ml. 0.25N potassium hydroxide). The solution obtained was then compared with standards. The method gave good results in the microrange with a sensitivity of 1 microgram.

Rynascewicz, Sleeper and Ryan (20) extracted boron from sodium chloride with ethyl alcohol and estimated the boron present colorimetrically using curcumin. Although 0.5 micrograms of boron can be measured by their method, an increment of only 1 microgram of boron can be detected in concentrated sodium hydroxide by their method. Their procedure is also a modified version of the Naftel method but is much longer and drawn out.

In 1954 Troug and Berger (26) presented a simplified curcumin procedure of that used by Naftel for plant and soil analysis which r equired only one evaporation and filtration.

Martin and Maes (14) replaced the concentrated acid used by Naftel with a 1:1 mixture of glacial acetic acid and acetic anhydride.

With the realization of the powerful deoxidizing ability of elemental boron, its strong affinity for many gases, potential as a fuel for special applications, excellent insulating possibilities, neutron absorbing ability and that it could possibly replace weightly lead and concrete as protection

around nuclear reactors, boron research intensified and numerous methods of determining boron appear in recent literature, many for boron and curcumin. compounds. Among these some may be mentioned at this point dealing with modifications of the curcumin method applied to particular cases.

Staple, Marshall, Nelson, Simon (25) developed a method for determining boron in TF_6-UF_6. Their method consists of evaporation, dissolution of the boron containing substance in an alcohol-oxalic acid-hydrochloric acid solution and then addition of curcumin with subsequent spectrophotometric analysis.

De Abinati and Pacques (17) emphasize that it is critically important that the drying time be constant in the colorimetric analysis of boron in uranium compounds and in graphite by means of the red color formed by the action of boric acid on curcumin in the presence of oxalic acid. They state that color intensity is higher with shorter drying periods.

Silverman and Trego (22) determined boron colorimetrically (curcumin-oxalic acid method) in uranyl chloride solution without prior removal of uranium. They claimed that the interference ascribed to the presence of uranium lies in the fact that uranium preferentially combines with the available oxalic acid. Therefore if a suitable excess of oxalic acid is added, the uranium forms a soluble uranium-oxalate complex which need not be removed.

Rynasiewicz and Consalvo (19) describe a procedure for the analysis of boron in 7% U-Zr alloys containing approximately 0.015% boron. They fused fine metal chips from the alloy with sodium carbonate. The melt was dissolved in water and then made slightly alkaline, filtered and evaporated to dryness. The boron was then leached from a slurry of the salt using 95% ethyl alcohol and then determined colorimetrically by the curcumin method.

Not much has been done on a study of the curcumin method itself, that is in determining the optimum conditions necessary for maximum sensitivity and the effects of varying the conditions of the reaction. Also very little is known about the actual mechanism that the reaction follows. (This is the major aim of the work presented in this thesis.)

Among the first investigators of the curcumin method were Clarke and Jackson (6) back in 1908. They made a study of rosocyanin, the red substance formed by the action of boric acid on turmeric paper (curcumin). They claimed that rosocyanin and curcumin were merely isomers ($C_{14}H_{14}O_4$), only that one of the two hydroxyls of curcumin was modified, probably to an oxide form in rosocyanin. They found that aqueous alkali in the cold converted the curcumin into rosocyanin. They found that white wool was dyed blue by the rosocyanin reaction and alkali. They heated curcumin with boric acid and sulfuric acid in alcohol, and when this was poured into water rosocyanin was obtained. This was then purified by washing with ether and reprecipitated from alcohol by dilute sodium chloride solution. No boron was found in rosocyanin and analysis and molecular weight determination in-

dicated the formula to be $C_{14}H_{14}O_4$. It formed a purple powder with metallic yellowish-green reflex. They found further that curcumin and boric acid in the cold poured into ether yielded an addition product of the two, decomposed by water forming rubrocurcumin $C_{14}H_{14}O_4$, a vermilion powder, unstable on standing at 240 degrees centigrade or upon heating with sulfuric acid, forming rosocyanin.

Silverman and Trego (23) made a study of the effect of oxalic acid by varying its concentration and holding everything else constant. They found that 2 ml. of a 5% solution to be the best working concentration of oxalic acid for a study of boron in the range of 0.0 to 50 micrograms of boron. They also made a study of the hydrochloric acid concentration and found poor results were to be obtained if a great excess of acid were used. They used sodium carbonate to neutralize the acidity and give a solution of residual alkalinity. They claimed that if the solution were not alkaline then significant amounts of boric acid (probably metaboric acid) would be lost by volatilization in the primary evaporation step of the curcumin method. Instead of extracting the residue with 25 ml. ether alcohol they used acetone.

Troug and Berger (26) stated that curcumin dissolved in ethyl alcohol gave a solution that is strongly yellow in color while the product of the curcumin-borate reaction, rosocyanin, is of a deep red color, giving the test solution a reddish hue. To show that curcumin and rosocyanin are spectrally separable, the absorption curve of each of the two pure substances dissolved in ethyl alcohol was determined using a Beckman DU spectrophotometer. They noticed that the absorption peak for rosocyanin falls at approximately 480 mu. Curcumin did not absorb light over the range of

maximum absorption of rosocyanin thus eliminating an absorption effect due to excess of reagent in the determination of boron.

Troug and Berger (26) state that Hafford (Ph.D. Thesis, University of Wisconsin 1942) suggests that the rosocyanin is probably formed by a loose combination of the borate with one of the hydroxyl groups of the curcumin molecule.

Yoe and Sarver (28) point out that beryllium, aluminum, iron, zirconium and magnesium form colored lakes with curcumin. Kolthoff (13) showed that these lake formations take place in alkali solutions. The excess of oxalic acid present in the regular curcumin procedure for boron prevents by solution the formation of these interfering lakes, according to Kolthoff.

Yoe and Sarver (28) postulate that curcumin probably acts in one of two ways with boron: (a) the hydrogens of the end hydroxyls may be replaced by an equivalent of metal, where upon a ring is closed by coordination with the oxygen of the o-methoxy group (b) it may act like an enolizable acyclic diketone since it also resembles acetyl acetone in structure.

Troug and Berger (26) point out that rosocyanin slowly hydrolyzes to curcumin, hence all colorimetric readings should be made within two hours after solution of the colored residue in alcohol.

Spicer and Strickland (24) working on a macro scale did some work in elucidating the structure of the compounds formed in the reaction between: (a) boron and curcumin, (b) boron, curcumin and oxalic acid, discussing the probable mechanisms involved in the two reactions.

Rosocyanin, the compound formed when curcumin and boric acid react in the presence of a mineral acid, has been shown by the two investigators to contain two curcumin molecules coordinated around a boron atom associated with one equivalent of an anion. The probable structural formula and electronic configuration of the molecule have been suggested by them on the basis of spectrophotometric and ion exchange studies. They also claim to have isolated the red compound formed when curcumin reacts with boric acid in the presence of oxalic acid and have called this compound rubrocurcumin. The substance is shown to be a compound of curcumin, boron and oxalate in a 1:1:1 ratio. Using analytical results, absorption spectra etc. they suggested a structure similar to that of rosocyanin but containing the curcumin molecules joined by a boro-oxalate bridge. They claimed further that if both oxalic acid and a mineral acid are present then a mixture of rubrocurcumin and rosocyanin results, although the yield of the former greatly exceeds that of the latter when the two acids are present in equimolar quantities. Observed also was the effect of water in inhibiting the formation of both rubrocurcumin and rosocyanin but its effect was most marked on the yield of the former.

EXPERIMENTAL

I. Apparatus

Beckman Model B Spectrophotometer - Absorbance readings were obtained at a wavelength of 550 millimicrons (in mostcases) and pyrex cells were used. Ethyl alcohol was used as the standard in absorbance measurements but at times a blank containing all of the experimental reagents minus boron was substituted.

Beckman Model H2 Glass Electrode pH Meter - All pH measurements and potentiometric titrations were carried out using this meter exclusively. A Beckman pH solution (pH = 7.0 ± 0.1) was used to standardize the instrument prior to each series of measurements.

Water Bath - For all work requiring dryings at $55° \pm 3°C$. a water bath designed to maintain a constant temperature was used. This bath was designed so as to hold a dozen porcelain evaporation dishes and to keep water vapor away from samples being evaporated.

Hot Plate - A three temperature range control hot plate was used for all preliminary dryings using the low heat position so as to minimize splattering.

Centrifuge - A motor driven centrifuge was used in separating any residue in the alcoholic extractions before spectrophotometric measurements.

Miscellaneous Apparatus - Soft glass was used in all cases in which glassware was required. Porcelain dishes were used for the reaction containers. Polyethylene bottles were used for storage of boron solutions. Volumetric flasks were used for preparation of the curcumin solutions and other reagents required. A 50 ml. burette was used in conjunction with the pH meter in the potentiometric titrations.

II. Reagents

Reagents - Reagent grade chemicals were used when necessary or possible.

Standard Boron Solution - A stock solution was prepared containing 100 micrograms of boron per ml. by dissolving 0.5716 gms. of boric acid in water and diluting to one liter. Dilute solutions of boron were prepared from this stock solution.

Calcium Hydroxide Solution - A .1N calcium hydroxide suspension was prepared by dissolving 1.853 g. of solute in water and diluting to 500 ml.

Curcumin Solutions - Solutions were prepared by dissolving 0.1 gram of Eastman curcumin in 100 ml. of either acetone or 95% ethyl alcohol depending on which modification was being studied. Solutions were prepared fresh before use.

Oxalic Acid Solution - An acetone or .95% ethyl alcohol solution containing .2 g. oxalic acid per ml. acetone was prepared by dissolving 20 g. of di-hydrated oxalic acid in 100 ml. acetone or 95% ethyl alcohol depending on the solvent being studied.

Hydrochloric Acid - A 1:1 solution of hydrochloric acid was prepared by mixing equal volumes of concentrated hydrochloric acid and water.

Ethyl Alcohol - The alcohol used throughout was 95%.

Acetone - Reagent grade.

Organic Acids - Eastman organic chemicals were used throughout when a study was made of various organic acids substituted in place of oxalic acid.

Alcoholic Sodium Hydroxide - A .1N solution was prepared by dissolving 2 g. of sodium hydroxide pellets in 95% ethyl alcohol and making it up to 500 ml. in a volumetric flask with the 95% alcohol.

Acid Potassium Phthalate - Primary Standard used to standardize the alcoholic sodium hydroxide. It was previously dried in an oven before use.

III. Preliminary Investigation

Powell and coworkers (18), did a great deal of work to
elucidate the mechanism of the reaction involved between boron-curcumin
and oxalic acid. Evidence obtained by them indicates that the red color
of the curcumin method is that of a 1-1-1 boron-curcumin-oxalic complex.
They also found that oxalic acid sensitizes the method, mainly because
oxalic acid increases the rate of reaction and permits equilibrium to be
reached before the reaction is frozen by loss of reaction medium. They
postulated a complexation between boron and oxalic prior to the main
reaction with curcumin. Also studied by them was the ability of other
materials, substituted in oxalic acid's place, to sensitize the reaction.

Because of the importance of adherence to Beer's Law and wave-
length of maximum absorbance it was decided to check these points as part
of the first phase of this project.

The modified Naftel procedure used was as follows: to a boron
sample (present as boric acid) in a small porcelain evaporation dish was
added 1 ml. of a 0.1N $Ca(OH)_2$ suspension. This mixture was then evaporated
to dryness on a hot plate using the lowest heat possible so as to avoid
splattering and consequent loss of some of the boron. To the cooled residue
was then added 1 ml. acetone containing 0.2 g. oxalic acid, 0.2 ml. 1-1 HCl
and finally 2 ml. of a 0.1% curcumin solution (.1 g. curcumin/100 ml. acetone).
The solution was then evaporated to dryness on a water bath maintained at
$55° \pm 3°C$. and heated at this temperature for an additional 30 minutes
after complete dryness was observed. The colored residue was then

extracted with 25 ml. of 95% ethyl alcohol, the resulting solution
centrifuged and the absorbance measured at a wavelength of 550 milli-
microns using ethyl alcohol as reference solution to set the zero of
the instrument.

A. Determination of Wavelength of Maximum Absorbance

To determine the wavelength of maximum absorbance a 2 micro-
gram sample of boron was placed in a porcelain dish and run according to the
procedure on page 17. Using a sensitivity of 4 and ethyl alcohol as the
standard the absorbance was measured in the wavelength range of 475 to 675
millimicrons, taking readings at increments of 25 millimicrons. The re-
sults are shown in the following table.

TABLE I

Maximum Wavelength Determination

Wavelength (millimicrons)	Absorbance
475	Inf.
500	0.78
525	1.00
550	1.02
575	0.42
600	0.10
625	0.05
650	0.05
675	0.05

A maximum absorbance was recorded at 550 millimicrons in agreement with
previous investigators.

In subsequent work a wavelength of 550 millimicrons was used
exclusively for absorbance measurements.

B. Verification of Adherence to Beer's Law

Solutions containing 0.0, 0.5, 1.0, 1.5, 2.0, 2.5 micrograms of boron were treated according to the modified Naftel procedure discussed on page 17. Duplicate samples were used throughout.

TABLE II

Adherence to Beer's Law

Reference solution - Ethyl Alcohol

Sensitivity - 4

Wavelength - 550 millimicrons

Boron (micrograms)	Absorbance		Average Absorbance
0.0	0.365	0.335	0.350
0.5	0.495	0.485	0.490
1.0	0.625	0.615	0.620
1.5	0.760	0.760	0.760
2.0	0.890	0.900	0.895
2.5	0.960	1.110	1.040

Results:

When micrograms boron vs. average absorbance are plotted, a straight line is obtained as shown in Figure 1. This linear relationship between absorbance and micrograms of boron in the range 0.0-2.5 micrograms of boron is proof that Beer's Law is followed in that low boron concentration range.

It should be pointed out here that the method described here gives very good reproducibility for a series of samples run at a particular time, but the absorbance values for solutions containing the same amount of boron have been found to vary when run at different times. Therefore for work where greater than average precision is required in quantitative work,

ADHERENCE TO BEER,'S LAW

ABSORBANCE

MICROGRAMS OF BORON
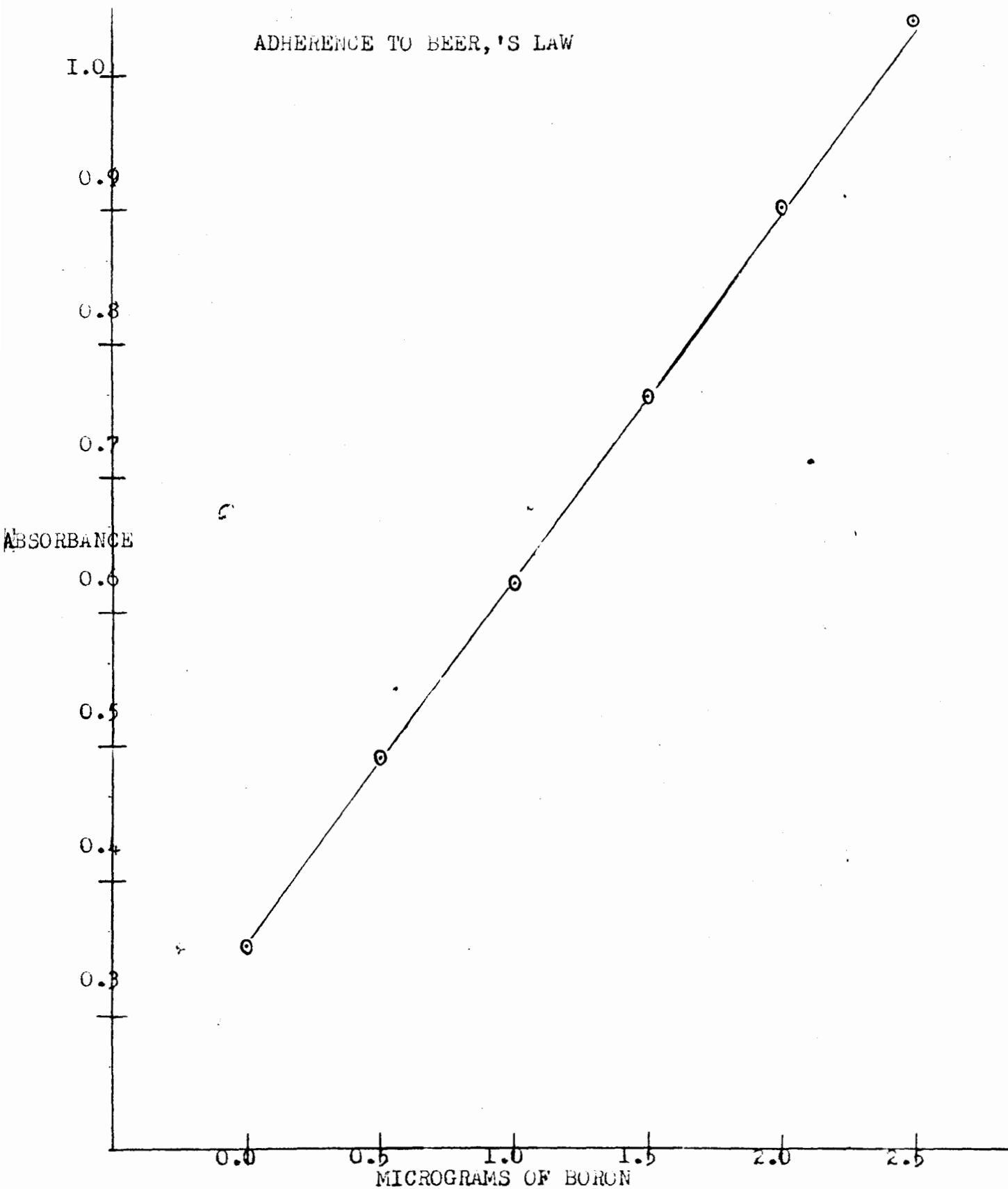
Figure I

a standard curve should be prepared at the time and under the same conditions as the unknown samples whose boron content is to be determined.

The cause of this variation in absorbance cannot be justly explained by one variable alone, but is caused by several variables. To mention a few the atmospheric conditions of the day can be one of the variables influencing the rate of evaporation. It has been found in much of the preliminary experimentation with the modified Naftel Method that shorter evaporation periods at 55°C. gives slightly greater absorbance readings. Consequently a hot dry day should favor quicker drying periods and correspondingly slightly greater absorbance readings. Curcumin itself is another variable to be considered. If the curcumin reagent is not freshly prepared but used after several days, it will cause the absorbance to be decreased somewhat, probably because there is some decomposition occurring when the curcumin is in solution.

It also appears that oxalic acid's activity decreases with the age of the solution.

IV. Determination of Function of the Various Reagents

Because of the great advantage to be gained in the interpretation of a possible mechanism of the boron-curcumin reaction and also of elucidating of the structure of the colored substance formed, a study was made of the function of the various reagents used in the curcumin reaction.

By greater understanding the function and effects of varying or totally eliminating some of the reagents, a keener knowledge of the mode of reaction was hoped to be gained.

Also included in this section of the project is a kinetic investigation of the reaction. Though the kinetic study was not as successful as hoped in elucidating the mechanism of the reaction, it did, however, give important generalizations regarding effects on the overall reaction when reagent quantities were varied or held constant depending on the situation being investigated. Since the kinetic study wasmore successful as a reagent study than on actual mechanism study, it is incorporated more appropriately under this heading.

A. Action of Oxalic Acid and Organic Acids

Oxalic acid is quite effective in increasing the sensitivity of the curcumin method, but little is actually known about the mechanism of its action. A possibility is that oxalic acid forms an intermediate complex with boric acid prior to the color forming reaction with curcumin. The complex formed between boric acid and oxalic acid should have little strain due to the planar structure of boric acid in which the 3 hydroxyl groups are separated by angles of 120°. Diol compounds, such as oxalic acid with hydroxyl groups on adjacent carbons have been found to react readily with boric acid even in aqueous solutions whereas heto compounds react to an appreciable extent in non aqueous solvents. A five membered ring should thus result from the interaction of boric acid and oxalic acid. The oxalic-borate complex resulting is probably more susceptible to complexation (or chelation) with curcumin than boric acid would be alone.

To gain greater insight into the effect of oxalic acid and to investigate other acids as substitutes for oxalic acid, a study of their effect on the sensitivity of the reaction was first started by Powell and coworkers (18) and continued in this study. The acids used were selected on a basis of similarity to oxalic acid or because of various substituted groups which might show effects of slight changes in structure.

Since the main advantage in using oxalic acid in the Naftel type method used in this project is to increase the sensitivity of the method, a comparison of individual runs using several different organic acids was made. The comparison was made against a run in which no organic acids was introduced at all. Absorbance readings were obtained from the final alcoholic extracts using the procedure on page 17. Absorbance readings were then plotted (y coordinate) vs. micrograms of boron (x coordinate) and the slopes of the resulting curves obtained from the relationship slope $= \frac{y}{x}$. By subtracting the slope of the standard curve, in which no organic acid was used, from the slope of a particular organic acid run, a "D" value (D $=$ tendency to sensitize) for the particular organic acid run was obtained. This "D" value could have a positive value or negative value, depending whether the organic acid sensitized or desensitized the method. Essentially the same procedure was used for all the organic acids investigated. Solutions containing 0.0, 0.5, 1.0, 1.5, 2.0, 2.5 micrograms of boron were prepared and treated in porcelain dishes with 1.0 ml. of 0.1N $Ca(OH)_2$ and evaporated to dryness. To the dry, cooled residues was added 1 ml. of

acetone or ethyl alcohol containing 1.59 millimoles of the particular
organic acid being investigated. The value 1.59 millimoles of oxalic
acid was arrived at by Powell (18) as the quantity of oxalic acid which
gave the optimum absorbance reading for a particular lquantity of boron.
Since it was desirable to compare the organic acids to oxalic acid it-
self, the same molar quantities of organic acids were used in each
particular run.

Next were added 0.2 ml. 1:1 HCl and 2 ml. of 0.1% curcumin
solution. (The curcumin solution used was made by dissolving .1 g.
curcumin powder in 100 ml. acetone or 95% ethyl alcohol, depending on
where the organic acid was dissolved in acetone or ethyl alcohol. The
resulting solution was then evaporated to dryness on a water bath at
$55° \pm 3°C.$ with an additional 30 minute standing period after the solu-
tions reached complete dryness. The residues were then extracted with
25 ml. of 95% ethyl alcohol, the extracts centrifuged and subsequent
absorbance readings obtained at a wavelength of 550 millimicrons using
ethyl alcohol as a standard.

Since most of the acids were not too soluble in acetone, ethyl
alcohol was used as the solvent in most cases. When the organic acids
were found to be not too soluble in either acetone or ethyl alcohol, a
solution for this problem was to dissolve the 1.59 millimoles of organic
acid and the required amount of curcumin together in 3 ml. of either
acetone or ethyl alcohol. This solution was successful in all the
difficult cases encountered. This 3 ml. method was used mostly in the
cases of aromatic acids with relatively high molecular weights.

TABLE III

Effect of Various Acids on Slope of the Curcumin Standard Curve

Acid	"D" Values	Acid[2]	"D" Values
Oxalic Acid	+ 0.237	Formic Acid	+ 0.131
Tartaric Acid	+ 0.004	Acetic Acid	+ 0.099
o-Phthalic Acid	+ 0.004	Propionic	+ 0.046
Phthalic Anhydride	- 0.033	Butyric	- 0.050
Salicylic Acid	+ 0.017	Caproic	- 0.112
Acetyl-Salicylic Acid	+ 0.012	Oxalic Acid	+ 0.251
2,5-Dihydroxy benzoic	+ 0.052	Malonic	+ 0.040
2,4-Dihydroxy benzoic	+ 0.014	Succinic	- 0.101
p-Hydroxy benzoic acid	+ 0.012	Adipic	- 0.094
Gallic Acid[1]	+ 0.177	Trichloroacetic	+ 0.206
		Dichloroacetic	+ 0.102
		Monochloroacetic	+ 0.099
		-Chloropropionic	+ 0.033
		-Chloropropionic	+ 0.008
		Maleic	+ 0.040
		Fumaric	- 0.092
		Benzoic	+ 0.180
		Phenylacetic	+ 0.047
		1,2,3-Tricarboxylic propane	+ 0.122
Acetone standard curve slope = 0.0450		Citric	+ 0.022
		Mandelic	+ 0.008
Ethyl alcohol " " " = 0.0478		Aconitic	- 0.086
		Aminoacetic	- 0.003
		Glyoxal	- 0.045
		Mercaptoacetic	- 0.122

[1] Gallic acid when extracted with 25 ml. EtOH gave a yellow-green solution instead of the usual yellow to orange-red solution.

[2] Results in this column from W. A. Powell's Tech. Quarterly Report for Callery Co., Callery, Pa. 1956.

From the above results it is readily seen that the best acids from the standpoint of increasing the sensitivity of the modified Naftel method are oxalic, trichloroacetic, benzoic, formic and 1,2,3-tricarboxylic propane. Gallic acid is not here included since when the dried residue of the 55° ± 3°C. drying was extracted with ethyl alcohol it gave a yellow-green solution, unlike the other acids extracted. Thus a direct comparison with the other acids cannot be made.

It must be remembered that the comparisons were made using conditions optimum for oxalic acid; possibly some of the other acids could be better under other conditions.

Going through the above list of acids, it can readily be seen that some are mono-acids, di-acids and tri-acids.

In the mono-acid group it can readily be seen that with an increase in acidic strength of the acid, complexation is favored more with boric acid. Thus trichloroacetic has a greater "D" value than dichloroacetic acid and monochloroacetic acid respectively.

In the di-acid group, chelation with boric acid rather than mere complexation seemed to be favored. Thus any group of acids that form relatively strain free rings with boric acid should sensitize to a greater extent. Of all the di-acids oxalic acid has the highest "D" value which is easily understood when ring formation is considered. The action between oxalic acid and boric acid gives rise to a 5 membered ring. Theoretically any acid which would in a similar way give rise to a 5 membered ring as oxalic acid does, should be ideal as a sensitizer. Malonic acid, which has an additional carbon more

than oxalic, upon chelation with boric acid should give rise to a 6 membered ring which is less strain free than a 5 membered heterocyclic ring. This explains certainly why its "D" value is less than that for oxalic acid.

Size seems to be quite important also regarding the organic acid. Most of the acids that were investigated in this project were much bulkier than those investigated by Powell (18). Thus almost all of the "D" values obtained in my work were much smaller than those obtained by Powell due to steric hinderance which would result. The intermediate chelate formed between the di-acids and boric acid would be less susceptible to direct attack from curcumin than would be the case for a less bulky organic acid. The extra two coordination positions on the boron would probably be covered up or shielded away from the attacking curcumin by the bulky part of the organic acids chelated with boric acid.

The importance of steric hinderance can also be observed in the case of maleic and fumaric acid. The carboxylic groups of maleic are cis to each other and therefore chelate more readily with boric acid than does fumaric acid, in which case the carboxylic groups are trans to each other.

B. Variation of Reagents in the Color Forming Reaction

After the first drying carried out on the hot plate in the modi- fied Naftel procedure for boron determination, a white precipitate, of calcium borate is formed. Boron is thus stabilized and less subject to loss on heating in this state. In the following step of the reaction, namely during the water bath evaporation at $55°C$., the color forming step of the reaction occurs.

Entering into this color formation reaction with the calcium borate are four reagents which play an intricate role in some manner in the formation of the colored substance. Acetone, oxalic acid, HCl, and curcumin together or alone actupon the calcium borate to produce the colored substance. All seem to have a definite role, though of not necessary equal importance in the overall picture. Five different runs, all aimed at solving a certain phase of the boron-curcumin reaction mechanism were carried out. Two of the color forming step reagents, namely oxalic acid and HCl were eliminated entirely in some of the runs. HCl in one particular run was varied in concentration while the remaining reagents were held constant. Still in another run HCl was added to the final alcoholic extract instead of in its customary spot, namely in the color forming step.

For all the runs, 2 micrograms of boron were previously treated with 1 ml. 0.1N $Ca(OH)_2$ and evaporated to dryness on a hot plate using low heat.

The color developing reagents were then added as indicated below and evaporation continued at $55° \pm 3°C$. followed as usual with subsequent extraction with 25 ml. of 95% ethyl alcohol. The pH was also measured for each alcoholic solution, to note any radical change in hydrogen ion concentration, and absorbance readings obtained using ethyl alcohol as the reference solution.

To the hot plate residues the following reagents were added:

Run 1

    1 ml. acetone

    no oxalic acid

    no 1:1 HCl

    2 ml. curcumin solution (.1 g. curcumin/100 ml. acetone)

Run 2

    1 ml. acetone

    no oxalic acid

    0.2 ml. 1:1 HCl

    2 ml. curcumin solution (.1 g. curcumin/100 ml. acetone)

Run 3

    1 ml. acetone (containing .2 g. oxalic acid)

    0.2 ml. 1:1 HCl

    2 ml. curcumin solution (.1 g. curcumin/100 ml. acetone)

Run 4

    1 ml. acetone (containing .2 g. oxalic acid)

    vary quantity of HCl from 0.2 to 2.4 m.e. (milli-equivalents)

    2 ml. curcumin solution (.1 g. curcumin/100 ml. acetone)

Run 5

    1 ml. acetone (containing .2 g. oxalic acid)

    add 0.2 to 2.4 m.e. HCl to the final alcoholic extract

    2 ml. curcumin solution (1 g. curcumin/100 ml. acetone)

Absorbance measurements were carried out at wavelengths of 450 through 700 millimicrons. Ethyl alcohol was used as the standard.

TABLE IV

Wavelength	Run 1	Run 2	Run 3
450	Infinity	Inf.	Inf.
475	Inf.	Inf.	Inf.
500	0.156	0.381	0.930
525	0.046	0.335	1.190
550	0.025	0.328	1.200
575	0.017	0.157	0.470
600	0.014	0.041	0.103
625	0.014	0.022	0.045
650	0.014	0.019	0.040
675	0.014	0.017	0.044
pH	9.5	4.0	1.8

TABLE V

Run 4 - Variation of HCl Added in Color Forming Step

Acid added (milli eq.)	0.2	0.4	0.6	0.8	1.0	1.5	1.8	2.4
Wavelength (millimicron)				Absorbance				
450	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.
475	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.
500	1.05	0.95	0.94	0.86	0.62	1.18	1.22	1.02
525	1.30	1.17	1.10	1.00	0.58	1.38	1.42	1.28
550	1.22	1.05	1.02	0.95	0.56	1.38	1.40	1.22
575	0.48	0.40	0.40	0.36	0.30	0.55	0.60	0.49
600	0.11	0.20	0.12	0.10	0.17	0.14	0.19	0.12
625	0.05	0.05	0.07	0.06	0.14	0.07	0.12	0.06
650	0.04	0.04	0.66	0.05	0.14	0.06	0.11	0.06
675	0.04	0.04	0.06	0.05	0.13	0.06	0.10	0.05
pH	2.4	2.3	2.4	2.3	2.1	2.2	2.3	2.3

TABLE VI

Run 5 - Variation of HCl Added to Final Alcoholic Extract

Acid added (milli eq.)	0.0	0.2	0.4	0.6	0.8	1.0	1.5	1.8	2.4
Wavelength (millimicron)					Absorbance				
450	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.
475	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.
500	0.32	0.32	0.35	0.34	0.39	0.42	0.39	0.40	0.35
525	0.24	0.24	0.26	0.25	0.31	0.34	0.32	0.31	0.26
550	0.22	0.26	0.26	0.23	0.27	0.31	0.30	0.29	0.24
575	0.08	0.09	0.10	0.09	0.11	0.14	0.13	0.15	0.11
600	0.02	0.2	0.04	0.03	0.04	0.05	0.05	0.08	0.04
625	0.01	0.01	0.02	0.02	0.03	0.04	0.03	0.07	0.03
650	0.01	0.01	0.02	0.02	0.03	0.03	0.03	0.06	0.03
675	0.01	0.01	0.02	0.02	0.03	0.03	0.03	0.06	0.03
pH	2.3	2.7	1.6	1.4	1.3	1.3	1.2	1.0	0.9

In run 1 with no oxalic acid and HCl added no maximum is reached and also no appreciable increase in absorbance is obtained at a wavelength of 525 to 575 millimicron indicating that no chelation seemed to occur when these two reactants are both absent.

In run 2 with HCl but no oxalic no maximum is reached either. There is however an increase in absorbance indicating that there is some complexation occurring between the boric acid- and curcumin in the presence of the mineral acid. In run 3 with HCl and oxalic acid both present chelation definitely occurs with the chelate absorbing at its maximum at 525 to 550 millimicrons.

In run 4 the water effect is evident from the addition of 0.4 m.e. to 1.0 m.e. of acid. The quantity of acid in the range of 0.2 to 1.0 m.e. was pipetted from a 1N HCl stock bottle and therefore as the quantity of acid added was increased from 0.4 m.e. to 1.0 m.e. the amount of water containing this acid also increased.

TABLE VII

Boron Samples with Addition of Ca(OH)$_2$

Milliequivalents of 1-1 HCl Added	Absorbance	pH
0.001	0.80	2.20
0.01	0.85	2.15
0.02	0.97	2.15
0.10	1.00	2.10

TABLE VIII

Boron Sample without Addition of Ca(OH)$_2$

Milliequivalents of 1-1 HCl Added	Absorbance	pH
0.001	0.055	2.30
0.01	0.061	2.20
0.02	0.056	2.15
0.10	0.058	2.10

From the results of Tables VII and VIII, it is evident from the absorbance readings that Ca(OH)$_2$ is definitely necessary in order to prevent loss of boron for the final alcoholic extracts of the colored substance formed in the modified Naftel reaction. When Ca(OH)$_2$ is not added, a great deal of the boron present as boric acid is probably lost during the hot plate evaporation. Contrary, with addition of Ca(OH)$_2$, boric acid is stabilized with Ca(OH)$_2$ to form a calcium borate precipitate which is stable during the evaporation process.

D. The Effect of Hydrochloric Acid

In the preceding study of the effect of $Ca(OH)_2$ addition in the modified Naftel reaction, there was evidence of an increase in absorbance as 1-1 HCl additions were increased from .001 to .1 m.e. In order to determine what quantity of HCl would give maximum absorbance when used in conjunction with 1.59 millimoles of oxalic acid (.2 g./ml. acetone), the quantity of HCl added to various runs was varied from .001 to 3.6 m.e. (in .2 ml. H_2O).

Runs were made with and without addition of 1.59 millimoles of oxalic. When oxalic was not used, 1 ml. of acetone was added nevertheless. Samples of 2.5 microgram boron were used throughout and the modified Naftel procedure followed as usual. Absorbances were measured at 550 millimicrons with ethyl alcohol as reference.

determined. Although this kinetic investigation did not prove to be as successful as hoped, several important generalizations were deduced from the various rate studies made.

The rate studies were carried out during the water bath evaporation stage of the reaction when the actual complexation seems to occur.

As a preliminary step several samples containing 2 micrograms of boron were placed in individual porcelain dishes and treated in the normal way, that is 1 ml. of $Ca(OH)_2$ was first added to the individual dishes and evaporated to dryness on a hot plate at low heat. To the cooled individual residues was then added 1 ml. acetone containing .2 g. oxalic acid, 0.2 ml. 1-1 HCl and 2.ml. curcumin solution (.1 g./100 ml. acetone). The resulting solutions were then placed on a water bath ($55° \pm 3°C.$). The samples were then removed from the bath at 5 minute intervals and cooled on ice far below the water bath temperature so as to stop any further reaction from occurring to any appreciable extent. The cooled samples were then extracted with 25 ml. of acetone instead of 95% ethyl alcohol so as not to complicate things with another solvent. Absorbance of each sample was then measured as soon as possible at a wavelength of 550 millimicrons using acetone as reference.

TABLE X

Variation of Absorbance vs. Time During Water Bath Evaporation

Time (min.)	Absorbance
5	0.17
10	0.20
15	0.36
20	0.62
25	0.58
30	0.60
35	0.61
40	0.60
45	-
50	0.60
60	0.62

From the above absorbance data it is seen that the major portion of the color forming reaction is complete after 20 minutes. This time also corresponds approximately to the time required for the solutions to reach dryness on the water bath at 55°. It can be observed from the above results that the additional 30 minutes standing period at this temperature is not strictly essential since the absorbance readings do not change appreciably during this additional stand-by period. From a standpoint of speed, the drying periods could be shortened by at least 20 minutes with the loss of very little sensitivity. Where accuracy is more desirable than speed, the additional standing period is desirable.

As a further step a kinetic study was carried out using the same reagents except for oxalic acid which was eliminated completely. In the place of oxalic acid 1 ml. of acetone containing no oxalic acid was added to the residue of the hot plate drying. Absorbances were found to be much lower than in the case where oxalic acid sensitizes the method considerably. Additional standing of the residues before extraction seems advisable when

oxalic acid is not used. Absorbance readings increased from a value of 0.157 after 20 minutes to 0.200 after approximately 50 minutes.

Next a study was made on the rate of the reaction for 3 different boron concentrations, 0.5, 1.0, 2.0 micrograms. The reagents used were the same and oxalic acid was again added to the procedure.

No correlation between concentration and time of reaction could be deduced from the absorbance readings obtained.

The effect of increasing the amount of oxalic acid added to the dry residue of the preliminary drying (hot plate) was also studied. Quantities of 50 micromoles, 100 micromoles and 1.59 millimoles of oxalic acid were added to the residues in three separate runs respectively. From absorbance values obtained, evidence again pointed to the fact that the sensitivity was greatly increased by addition of larger and larger quantities of oxalic acid. The optimum value of oxalic acid was not determined having previously been found to be 1.59 millimoles by Powell and coworkers (18). As a final step to the kinetic studies, the effect of curcumin on the re- action was studied. Three runs were again made using 2 micrograms of boron and varying the curcumin added (100 micrograms, 500 micrograms, 2 milligrams). The other reagents were used in their usual quantities. Absorbance readings were noticed to increase with the use of larger amounts of curcumin but not in the same order of magnitude as in the case of larger additions of oxalic acid. Unfortunately a simple relationship between concentration and time was not evident in any of the rate studies made. All evidence, however, seems to indicate that the reaction is not a simple order reaction but is complicated due to several independent variables.

V. Determination of Mechanism of Reaction

As the final stage of this project, the molar ratios of boron to curcumin and boron to oxalic acid were investigated in order to supplement the 1-1-1 boron-curcumin-oxalic acid data obtained by Powell and coworkers (18). Finally along with data obtained from a pH study involving boron and oxalic acid in conjunction with a non-aqueous potentiometric titration investigation of the boron-oxalic acid system, a possible explanation of the mechanism of the modified Naftel reaction was attempted.

An explanation regarding the structure of the colored substance formed during the reaction was also sought.

A. Molar Ratio of Boron to Curcumin

A study was made to determine the molar ratio in which boron and curcumin combined (in the absence of oxalic acid) using Job's method of continuous variation (5). Job's method is a most important investigation tool in order to gain a greater insight of the molar ratio of the color substance formed in the boron-curcumin reaction. Powell and coworkers (18) using this method, found that boron and curcumin in the presence of oxalic acid combined in a 1:1:1 molar ratio.

It was recognized early that a characteristic color is one of the most important and distinguishing features of chelate compounds. In recent years a simple colorimetric method has been used to determine the composition of chelate compounds in solution. This technique is called Job's method of continuous variation (5).

Consider the formation of a chelate compound AB_n where A is a metallic ion and B is a chelating agent.
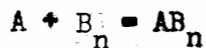
$$A + B_n = AB_n$$

$$K = \frac{(AB_n)}{(A)\ (B)^n}$$ where n = number of chelating groups

where the quantities in brackets represent molar activities which may be taken approximately equal to molar concentrations. If the restrictions $(A) + (B) = C$ is imposed where C is a constant, it can be shown readily that when concentration AB_n is a maximum then

$$\frac{(B)}{(A)} = n$$

or the first derivative of AB_n with respect to A is equal to zero.

$$\frac{d\ (AB_n)}{d\ (A)} = o$$

In other words, for a constant total concentration of metal A and chelating agent, the concentration of chelate is greatest when the metal and chelating agent are brought together in the same molar ratios in which they exist in the chelate. If the chelate is the only colored substance present, the optical density of absorbance of the solution is proportional to the chelate concentration. Hence a plot of absorbance vs. composition of the solution

under the restrictions imposed would give a curve with a maximum at the composition corresponding to the formula of the chelate. The method is not generally applicable to systems in which more than one compound is formed.

In Powell's work the total number of moles was held constant at 0.926 micromoles land the mole percent boron varied from 20 to 80%. Since the absorbance of the colored species in the absence of oxalic acid is much less than when oxalic acid ispresent, it was decided to use a larger total molar quantity. A total molar quantity of 4.63 micromoles was chosen after several trials as an ideal quantity to work with.

Twelve samples were then run in duplicate with the boron content ranging from 20 to 80% of the total moles. To the required amount of boron in porcelain dishes was added 1 ml. of 0.1N $Ca(OH)_2$. The resulting solution was then evaporated to dryness on a hot plate. To the cooled residues was then added 1 ml. acetone, 0.2 ml. 1:1 HCl and the required curcumin made up in 2 ml. of acetone. The resulting solutions were then evaporated to dryness in a water bath at $55° \pm 3°C$. letting the dried residues stand an additional 30 minutes after complete dryness was obtained. The residues were then extracted with 25 ml. ethyl alcohol, the solutions centrifuged and absorbance readings obtained at 550 millimicrons using ethyl alcohol as a reference solution. The data are shown in Table XI and Figure 2.

TABLE XI

Job's Method of Continuous Variation

% Boron	Micrograms Boron	% Curcumin	Curcumin Solution					Absorbance
20	10.0	80	1.6 ml.	diluted	to 2	ml.		0.62
25	12.5	75	1.5 "	"	"	"		0.94
30	15.0	70	1.4 "	"	"	"		1.00
35	17.5	65	1.3 "	"	"	"		1.22
40	20.0	60	1.2 "	"	"	"		1.62
45	22.5	55	1.1 "	"	"	"		1.92
50	25.0	50	1.0 "	"	"	"		2.00
60	30.0	40	0.8 "	"	"	"		1.02
65	32.5	35	0.7 "	"	"	"		0.90
70	35.0	30	0.6 "	"	"	"		0.60
75	37.5	25	0.5 "	"	"	"		0.44
80	40.0	20	0.4 "	"	"	"		0.24

A stock curcumin solution was prepared by dissolving 0.1853 g. curcumin in 50 ml. of acetone. Each 2 ml. of solution contained 4.63 micromoles of curcumin. The curcumin required for the above runs was pipetted from the stock bottle and diluted with acetone so as to use a total of 2ml. curcumin solution.

When total moles boron was plotted vs. absorbance it was readily seen that a maximum occurred at an absorbance reading of 2.00 which corresponded to a 1:1 boron-curcumin ratio.

Thus we concluded that as in the case of boron-curcumin-oxalic system so also in the boronecurcumin system in which no oxalic acid was used a 1:1 molar ratio is obtained for boron and curcumin.

B. Determination of the Boron-Oxalic Acid Molar Ratio

The boron-oxalic acid molar ratio was next determined by varying the total quantity of oxalic acid while maintaining boron constant in several runs. As a preliminary step the total volume of acetone giving

JOB'S METHOD OF CONTINUOUS VARIATION
(BORON-CURCUMIN MOLAR RATIO)
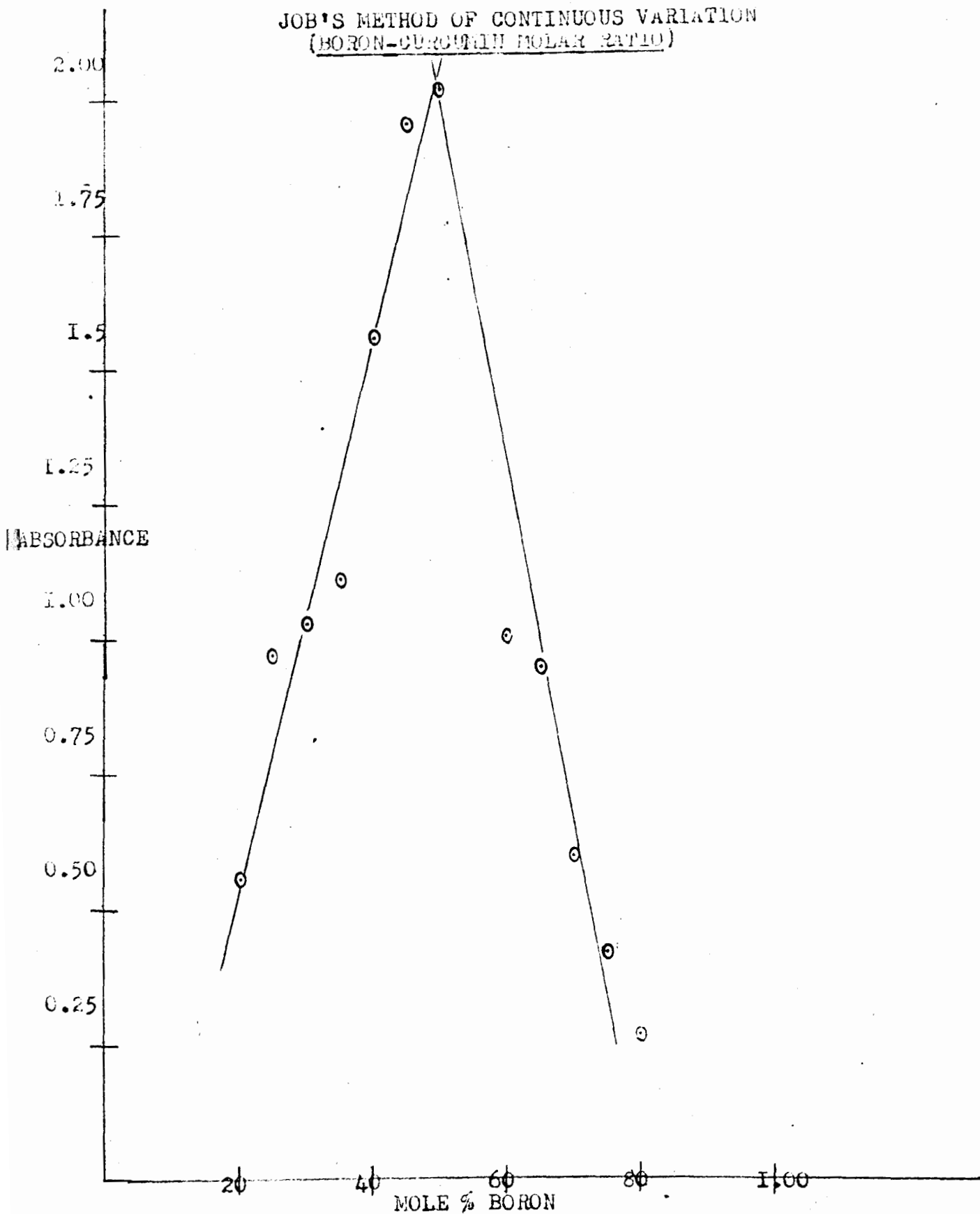
MOLE % BORON
Figure 2

maximum absorbance without oxalic acid was determined. This amount of acetone was then used to contain the amount of oxalic acid being investigated. The following scheme was followed: To 2 micrograms of boron in porcelain dishes was added 1 ml. 0.1N $Ca(OH)_2$ and evaporated to dryness on a hot plate. To the dried and cooled residues was added acetone in varying amounts from 1 ml. to 12 ml. to each of the individual dishes. This was done in order to determine what volume of acetone would give maximum absorbance. Next were added 0.2 ml. 1:1 HCl and 2 ml. of 0.1% curcumin solution in acetone and the resulting solutions were evaporated to dryness on a water bath held at a constant temperature ($55 \pm 3°C.$). The residues were then left on the water bath an additional 30 minutes after complete dryness was reached and then extracted with 25 ml. of ethyl alcohol. After the resulting solutions were centrifuged, absorbance readings were measured. When the volume at which maximum absorbance was determined, it was then used in subsequent runs to dissolve oxalic acid which was then varied while the other reagents were all held constant. Absorbance readings were again taken and maximum absorbance indicated at what molar ratio boron and oxalic acid combined.

DETERMINATION OF BORON-OXALIC MOLAR RATIO
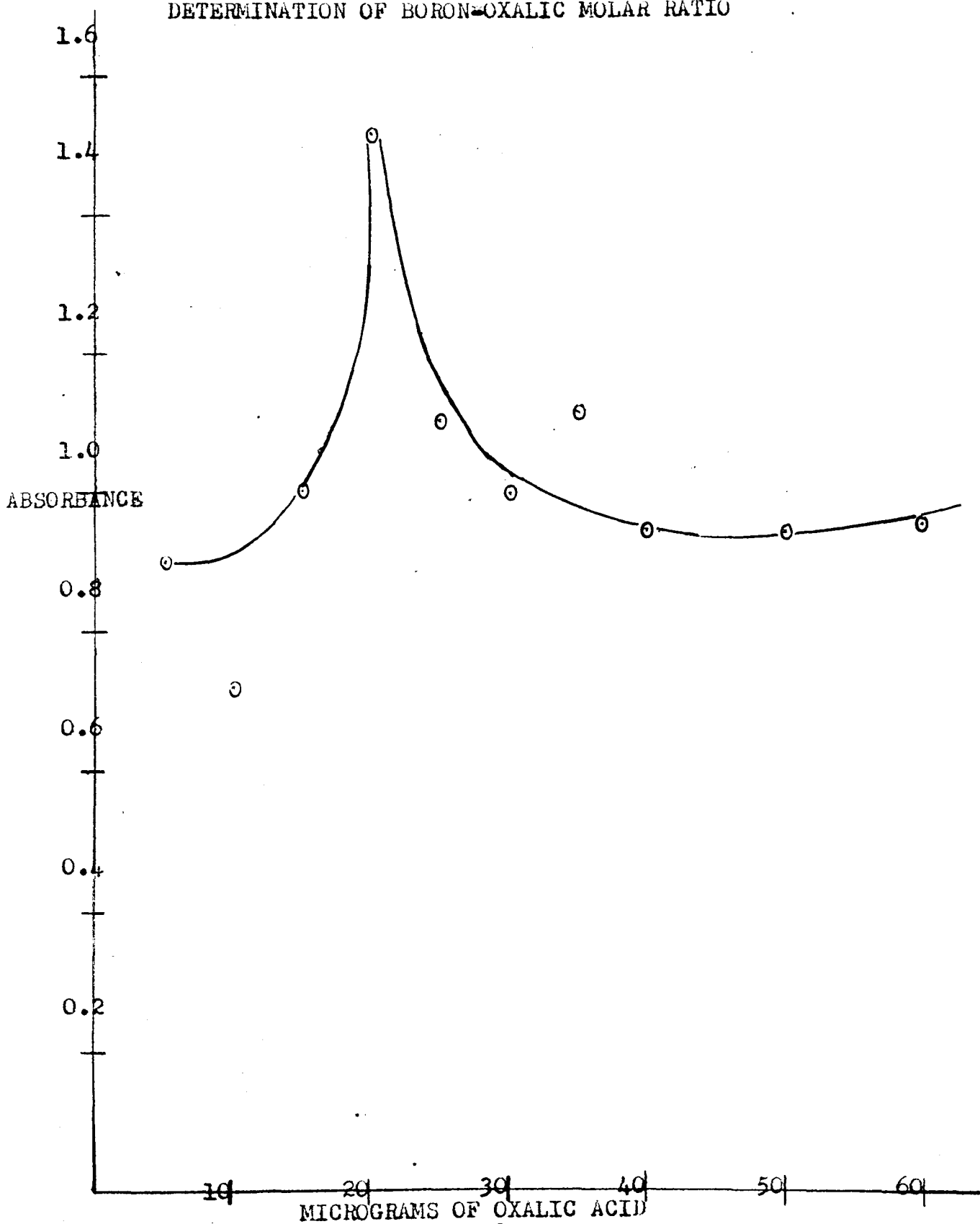
MICROGRAMS OF OXALIC ACID

Figure 3

$$\frac{2.0 \times 10^{-6} \text{ g. Boron}}{10.8 \text{ g. /mole Boron}} = 0.18 \text{ micromoles boron}$$

$$\frac{20 \times 10^{-6} \text{ g. Oxalic}}{90 \text{ g./mole Oxalic}} = 0.22 \text{ micromoles oxalic}$$

C. Potentiometric Titration Study of the Boric Acid-Oxalic Acid Reaction
   in a Non-Aqueous Medium

A potentiometric titration study of the reaction between boric

acid and oxalic acid was next carried out. A 0.0981N NaOH alcoholic

solution was prepared by dissolving 2 grams of NaOH pellets in 50 ml.

ethyl alcohol. The resulting alkaline solution was then standardized

against 0.8976 g. of acid potassium phthalate using phenolphthalein as

indicator. The standardized base was then used as titrant in the

potentiometric titration of solutions of oxalic acid and mixtures of

oxalic and boric acids. From the titration it was hoped that the extent

of reaction, if any, between oxalic and boric acid could be determined. Also

from pH measurements during the titrations a knowledge of the type of com-

pound formed between oxalic and boric acids was hoped to be gained.

Initially 50 ml. of ethyl alcohol containing 0.5 millimoles of

oxalic acid was placed in a porcelain casserole and titrated potentiometrically

with the 0.0981N NaOH. pH readings were then recorded after additions of

increments of 0.5 or 1 ml. of the standard base. A plot was then made of the

pH vs. ml. of NaOH added. To another casserole was added the same amount of

oxalic acid as above plus 5 millimoles of boron in ethyl alcohol. The initial

volume was held constant as above at 50 ml. with the addition of ethyl alcohol. This solution was then titrated in a similar manner and again pH measurements were plotted vs. ml. NaOH added.

Finally 1 millimole of oxalic acid and then a solution of 1 millimole oxalic acid plus 0.5 millimoles of boron were titrated in an analogous manner as above with NaOH.

TABLE XIV

Potentiometric Titration Data

0.5 Millimoles Oxalic Acid vs. 0.0981N NaOH		0.5 Millimoles Oxalic & 5 millimoles boron vs. 0.0981N NaOH	
ml. NaOH Added	pH	ml. NaOH Added	pH
0.0	3.70	0.0	4.00
1.0	4.00	1.0	4.35
2.0	4.12	2.0	4.48
3.0	4.40	3.0	4.80
4.0	4.88	4.0	8.22
4.5	5.59	4.5	9.10
5.0	6.90	5.0	9.30
5.5	7.88	5.5	9.40
6.0	9.00	6.0	9.42
7.0	10.80	7.0	9.50
8,0	11.29	8.0	9.51
9.0	11.48	9.0	9.53
10.0	11.58	13.0	9.62
11.0	11.71	18.0	9.81
12.0	11.72		

TABLE XV

Potentiometric Titration Data

1 Millimole Oxalic Acid vs. 0.0981N NaOH		1 Millimole Oxalic + 5 Millimoles boron vs. 0.0981N NaOH	
ml. NaOH	pH	ml. NaOH	pH
0.0	3.05	0.0	3.30
1.0	3.31	1.0	3.35
2.0	3.48	2.0	3.57
3.0	3.55	3.0	3.70
4.0	3.69	4.0	3.82
5.0	3.88	5.0	4.02
6.0	4.05	5.5	4.20
7.0	4.31	6.0	4.32
7.5	4.48	7.0	5.09
8.0	4.61	7.5	8.30
8.5	4.89	8.0	9.02
9.0	5.28	8.5	9.30
9.5	7.60	9.0	9.35
10.0	9.70	10.0	9.39
11.0	11.02	11.0	9.41
12.0	11.29		
13.0	11.31		
18.0	11.33		

From the preceding results and from a study of figures 4 and 5 we may postulate that the compound formed between boric acid and oxalic acid is a neutral species and not a positively charged compound.

A)
```
    O = C - O
            \
    |        B-OH      and not
            /
    O = C - O
```

B)
```
    ⎡ O = C - O     O - C = O ⎤
    ⎢          \   /          ⎥
    ⎢ |         B            | ⎥  H+
    ⎢          /   \          ⎥
    ⎣ O = C - O     O - C = O ⎦
```

is formed since from an observation of figures 4 and 5 we can see that initially upon addition of boric acid to oxalic acid the pH is greater than when oxalic acid is present alone at the start of the titrations. If compound B) were formed the initial pH of the solution containing the boron and oxalic acid mixture should be much lower (more acidic) than the pH of the oxalic acid solution alone since compound B) is a stronger acid than oxalic acid alone.

TABLE XV

Potentiometric Titration Data

1 Millimole Oxalic Acid vs. 0.0981NNaOH		1 Millimole Oxalic + 5 Millimoles boron vs. 0.0981N NaOH	
ml. NaOH	pH	ml. NaOH	pH
0.0	3.05	0.0	3.30
1.0	3.31	1.0	3.35
2.0	3.48	2.0	3.57
3.0	3.55	3.0	3.70
4.0	3.69	4.0	3.82
5.0	3.88	5.0	4.02
6.0	4.05	5.5	4.20
7.0	4.31	6.0	4.32
7.5	4.48	7.0	5.09
8.0	4.61	7.5	8.30
8.5	4.89	8.0	9.02
9.0	5.28	8.5	9.30
9.5	7.60	9.0	9.35
10.0	9.70	10.0	9.39
11.0	11.02	11.0	9.41
12.0	11.29		
13.0	11.31		
18.0	11.33		

From the preceding results and from a study of figures 4 and 5 we may postulate that the compound formed between boric acid and oxalic acid is a neutral species and not a positively charged compound.

A)
```
O = C - O
          \
           B-OH          and not
          /
O = C - O
```
B)
```
[ O = C - O       O - C = O ]
          \      /
           B
          /      \
  O = C - O       O - C = O  ] H+
```
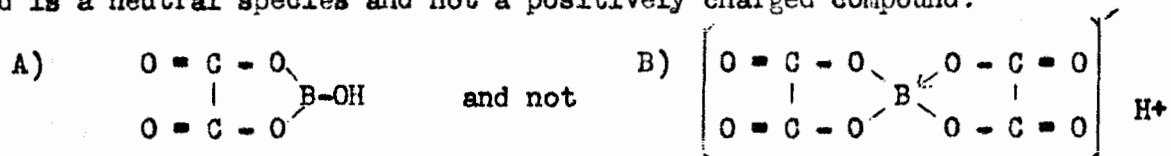
is formed since from an observation of figures 4 and 5 we can see that initially upon addition of boric acid to oxalic acid the pH is greater than when oxalic acid is present alone at the start of the titrations. If compound B) were formed the initial pH of the solution containing the boron and oxalic acid mixture should be much lower (more acidic) than the pH of the oxalic acid solution alone since compound B) is a stronger acid than oxalic acid alone.

POTENTIOMETRIC TITRATION DATA

⊙ 0.5MILLIMOLES OXALIC ACID vs.0.0981N NaOH
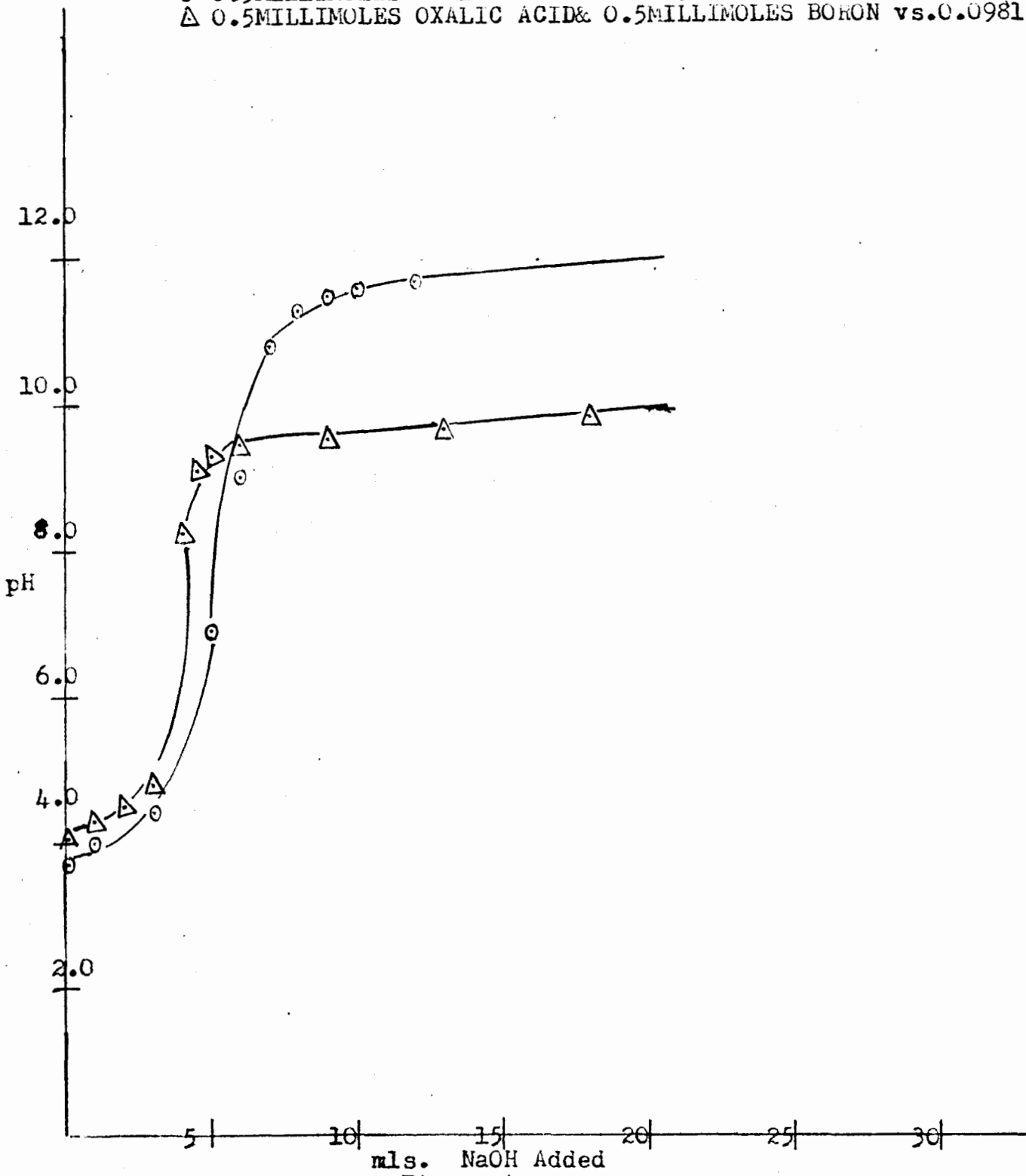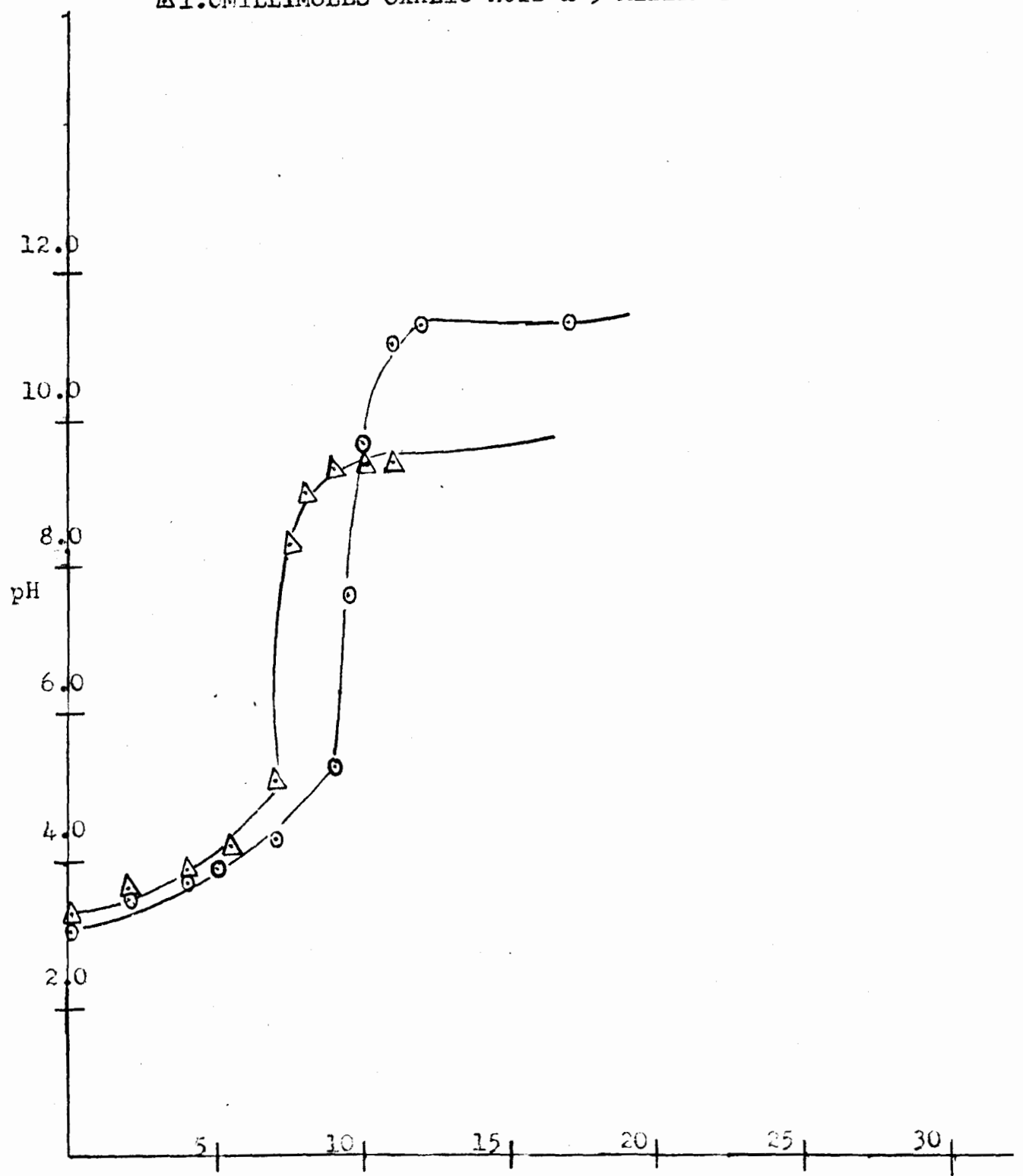△ 0.5MILLIMOLES OXALIC ACID& 0.5MILLIMOLES BORON vs.0.0981N NaOH

pH

mls.   NaOH Added
Figure 4

POTENTIOMETRIC TITRATION DATA

⊙ 1.0MILLIMOLES OXALIC ACID vs. 0.0981N NaOH
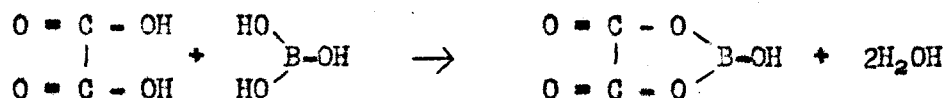△1.0MILLIMOLES OXALIC ACID & 5 MILLIMOLES BORON vs. 0.0981NNaOH

mls. NaOH Added
Figure 5

This is also another proof that boron and oxalic acid combine
in a 1-1 ratio before complexation with curcumin. If compound B) were
formed this would involve a 1-2 boron-oxalic complexation prior to the
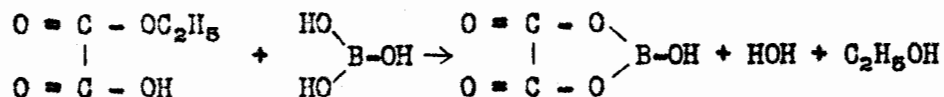main reaction with curcumin.

From the two curves of oxalic acid titrated with NaOH there is
evidence that the monoester of oxalic acid is formed when oxalic acid
is dissolved in ethyl alcohol. Esterification between oxalic acid and
ethyl alcohol is not complete however and therefore the titration with
NaOH is actually a titration of the monoester. This explains why there
is only one break in the titrating curves when we would normally have
expected two if we were titrating oxalic acid as such.

Certainly evident from the titration curves involving the mixture
of boric and oxalic acids, is a definite shift in curve breaks with respect
to the titration curves for the solutions containing oxalic acid alone.
This is conclusive evidence of a definite reaction occuring between boric
and oxalic acid (or ester). The following are the possible mechanisms that
could occur in the reaction between boric and oxalic acid (or monoester of
oxalic acid).

1. Molecule of Oxalic Acid + Molecule of Boric Acid

$$O = C - OH \quad HO \diagdown \qquad\qquad O = C - O \diagdown$$
$$\qquad | \qquad + \qquad B\text{-}OH \qquad \rightarrow \qquad | \qquad\qquad B\text{-}OH \; + \; 2H_2OH$$
$$O = C - OH \quad HO \diagup \qquad\qquad O = C - O \diagup$$

2. Monoester of Oxalic Acid + One Molecule of Boric Acid

$$
\begin{array}{l}
O = C - OC_2H_5 \\
\quad | \\
O = C - OH
\end{array}
+
\begin{array}{l}
HO \\
\quad \diagdown B-OH \\
HO \diagup
\end{array}
\rightarrow
\begin{array}{l}
O = C - O \\
\quad | \qquad \diagdown B-OH \\
O = C - O \diagup
\end{array}
+ HOH + C_2H_5OH
$$

3. Two Molecules of Oxalic Acid + One Molecule of Boric Acid

$$
\begin{array}{l}
O = C - OH \\
\quad | \\
O = C - OH
\end{array}
+
\begin{array}{l}
HO \\
\quad \diagdown B-OH \\
HO \diagup
\end{array}
+
\begin{array}{l}
HO-C=O \\
\quad | \\
HO-C=O
\end{array}
\rightarrow
\left[
\begin{array}{l}
O=C-O \diagdown \quad \diagup O-C=O \\
\qquad \quad B \\
O=C-O \diagup \quad \diagdown O-C=O
\end{array}
\right] H^+
+ HOH
$$
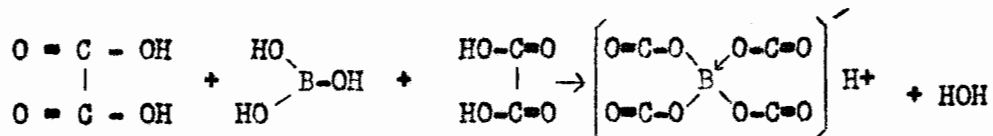
3. has been ruled out as improbable from the potentiometric study carried out in this section.

The following is the probable mechanism occurring during the modified Naftel reaction for Boron determination which can now be deduced.

$$
\begin{array}{l}
O = C - O \\
\quad | \qquad \diagdown B-OH \\
O = C - O \diagup
\end{array}
+
\begin{array}{l}
\quad C \\
O=C \\
HC \quad H \nearrow \\
O=C \\
H^+ \nearrow \quad C
\end{array}
\longrightarrow
\begin{array}{l}
O = C - O \\
\quad | \qquad \diagdown B \\
O = C - O \diagup \quad O
\end{array}
\begin{array}{l}
\quad C \\
O=C \\
HC \\
C \\
C \\
C
\end{array}
+ H^+ \\
\qquad \qquad \qquad \qquad \qquad \qquad \qquad + HOH
$$
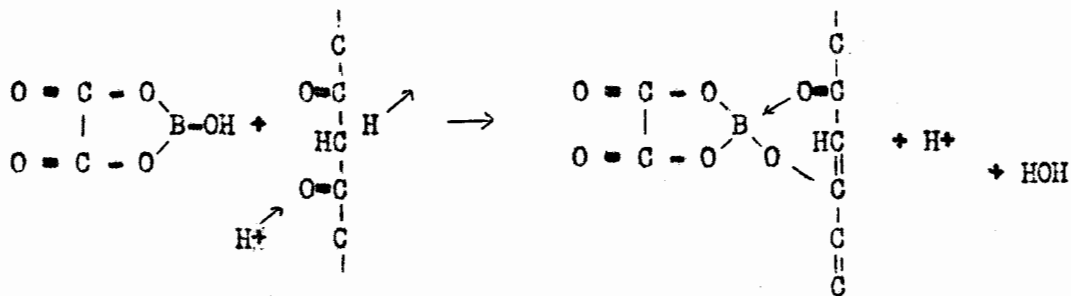
The hydrogen ion from the 1-1 HCl acts as catalyst serving to initiate the chelation process. The hydrogen ion goes to one of the oxygens of the carbonyl groups of curcumin. This gives the carbonyl carbon a residual plus charge. In order to recompensate for this electron unbalance, a hydrogen from the carbon between the two carbonyls of curcumin is lost leaving its pair of electrons to aid in rebalancing the electron unbalance created by the catalytic action of the hydrogen ion initiating the reaction. A double bond is thus formed between the carbon and the electron deficient carbon of the carbonyl.

SUMMARY

In the present research project an attempt has been made to determine the mechanism of the reaction followed in the curcumin spectrophotometric method for boron determination using a modification of Naftel's method.

The reagents taking part in the reaction were studied by varying their concentrations and often omitting some entirely.

The use of $Ca(OH)_2$ was proved to be essential in the method. Without it some of the boron as boric acid escapes during the preliminary hot plate drying. The $Ca(OH)_2$ tends to form a non-volatile calcium borate residue during the hot plate drying.

Several other organic acids were studied as possible substitutes for oxalic, but none was found to be more suitable than oxalic acid as a sensitizer. Either with or without oxalic acid Beer's Law seems to be obeyed in the microrange of boron concentration studied (0 to 3 micrograms boron).

The use of different solvents for curcumin does not seem to have any noticeable effect on the sensitivity of the method. Acetone and ethyl alcohol were both used as solvents, both proving satisfactory. A possibility in the use of ethyl alcohol is the possible esterification with oxalic acid. That this esterification does occur, was evident when a potentiometric titration study was carried out for a solution containing oxalic acid dissolved in ethyl alcohol. Upon titration of this solution with an alcoholic sodium hydroxide solution only one break in the titration curve of oxalic acid could be observed when two breaks would have been expected since oxalic is a dicarboxylic acid.

A potentiometric titration study was also carried out on a solution containing boric acid and oxalic acid in ethyl alcohol. It was at this point postulated that the compound formed between boric and oxalic acids is a neutral compound and not an anionic complex, since no hydrogen ions were found to be released in the process. It was further proved from this study that boric and oxalic acids combined in a 1-1 ratio. If a 1-2 boric-oxalic acid reaction occured, then release of hydrogen ions would have been expected.

From a kinetic study of the modified Naftel method, evidence obtained from absorbance data seemed to indicate that not much colored complex seems to form after the main reaction reaches dryness at $55 \pm 3°C$. on the water bath. If the additional 30 minute drying period is eliminated after complete dryness is reached, no great error seems to be introduced. This is quite an advantage since time of analysis may be shortened somewhat, making the method more appealing when time is a major factor in choice of a method.

The actual structure of the chelate formed between boron and curcumin was investigated. It was found that when boron and curcumin com bined under normal experimental conditions, a 1:1 complex formed in the absence of oxalic acid. Powell and coworkers (18), using oxalic acid, found that boron and curcumin combined in a 1-1 ratio also. A 1-1 boron-oxalic compound was also found to be formed in another study carried out in this project. Maintaining the boron concentration constant, oxalic acid was varied until a maximum of absorbance was obtained. This was found to correspond to a 1-1 boron-oxalic compound.

Summarizing, boron-oxalic and curcumin definitely seem to form a 1-1-1 chelate type compound in the presence of a mineral acid. The mineral acid (HCl) seems to initiate the final complexation process between a 1-1 boro-oxalate compound and curcumin by catalytic action.

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AUTOBIOGRAPHY

I, Frank Xavier Pollio, was born on June 4, 1928 in Weehawken, New Jersey. I attended elementary school at Sarah H. Gilmore School, Union City, N.J.

From September 1939 to October 1945, I resided in Sorrento, Italy. There I graduated from Nino Bixio Technical High School and later attended the Nino Bixio Merchant Marine Academy for two years.

In June 1947 I was graduated from Emerson High School, Union City, N.J. which I attended from October 1945. In September 1948 I enrolled at St. Peter's College (N.J.) obtaining a B.S. in Chemistry in June 1952. From June 1952 to October 1957 I was employed by Nitrogen Division, Allied Chemical & Dye, Hopewell, Va. as a control chemist.

In November 1957, I accepted employment on the research staff of the Rohm & Haas Co., Philadelphia, Pa. where I am presently employed.