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A study of the curcumin method for boron determination

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A STUDY OF THE CURCUMIN METHOD
FOR BORON DETERMINATION

BY

JAMES EDWARD HARDCASTLE

A THESIS
SUBMITTED TO THE GRADUATE FACULTY
OF THE UNIVERSITY OF RICHMOND
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FOR THE DEGREE OF
MASTER OF SCIENCE IN CHEMISTRY

APPROVED:

W. Allan Powell

Jackson G. Taylor

H. W. Wofford

Stanton R. R. R.

H. Pearce Atkins

Wm E. Trout Jr.

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INTRODUCTION

The purpose of the research project, of which this thesis is a partial report, was twofold: first, to develop a method for determining small quantities of boron, and second, to develop a rapid field test for the determination of atmospheric boron. A literature survey revealed that the curcumin method is the most sensitive colorimetric method presently available for boron determination. Also, the curcumin paper test should be readily adapted to the determination of atmospheric boron.

The experimental work was divided into three major sections: first, a preliminary survey of several methods for boron determination; second, a study of the curcumin solution method, and third, a study of the curcumin paper method. The preliminary survey covered several colorimetric reagents commonly used for boron determination; these reagents were curcumin, quinalizarin, Alizarin Red-S, Alizarin Blue-S, Chromotrope 2B, carminic acid, and 1-amino-4-hydroxyanthraquinone. In the study of the curcumin solution method, the variables affecting the methods of Naftel (77) and of Hegedus (50) were investigated. Before a study of the curcumin paper test for

determining atmospheric boron could be made, a synthetic atmosphere sampling apparatus had to be constructed. The curcumin paper test was then evaluated using this apparatus.

This thesis reports a broad, preliminary survey of the curcumin method for boron determination and, as such, was intended to show the way for later more detailed studies. Thus, some of the work was necessarily incomplete. Much of the work was completed later by other members of the team working on the overall project and, where pertinent, a brief summary of this later work will be given.

HISTORICAL

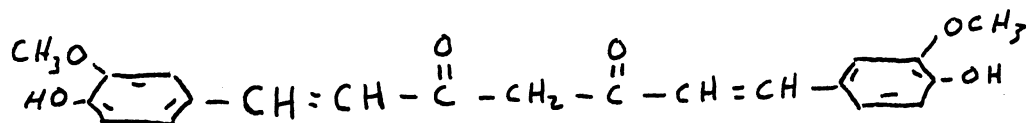
I Introduction

Boron and its compounds are of importance in several fields, metallurgy, agriculture, as mild antiseptics, as preservatives, and in the manufacture of certain glasses, enamels and glazes. Boron, because of its neutron absorbing ability, may be used in nuclear reactors. The use of boron and its compounds as fuel has received much attention. In these fields of research the determination of microquantities of boron is important and colorimetric methods are well suited for the determination of microquantities of boron.

Many colorimetric methods for the determination of boron are reported in the literature. De Ford, Lucchesi and Thoburn (26) have published an excellent survey, concerning the analytical chemistry of boron, in which the various methods used in the colorimetric determination of boron are critically reviewed. The two most widely used colorimetric methods employ either quinalizarin or curcumin (turmeric) as the color forming reagent. The quinalizarin method is less subject to interferences by other elements

and is more specific for boron; however, the curcumin method is more sensitive. Manuele (67) states that the turmeric method of Naftel (77) has greater sensitivity than the quinalizarin method. Powell and co-workers (83) found the curcumin method to have a much greater sensitivity than the methods employing reagents dissolved in concentrated sulfuric acid. Spicer and Strickland (96) state that the boron-curcumin reaction can be made the most sensitive absorptiometric method presently known for any element except beryllium. The main advantage of the curcumin method, according to Dible, Berger and Truog (27), is its great sensitivity for small amounts of boron. They state that the precision of the curcumin and quinalizarin colorimetric methods are about the same in the determination of boron in plants and soils. McHargue and Hodgkiss (70) compared the official AOAC titration (52) method, the Berger and Truog (4) quinalizarin method and the Naftel (77) turmeric method. They found that the turmeric method gives results that vary somewhat from the results of the titration method, but the results of the titration method and the quinalizarin method agree very well.

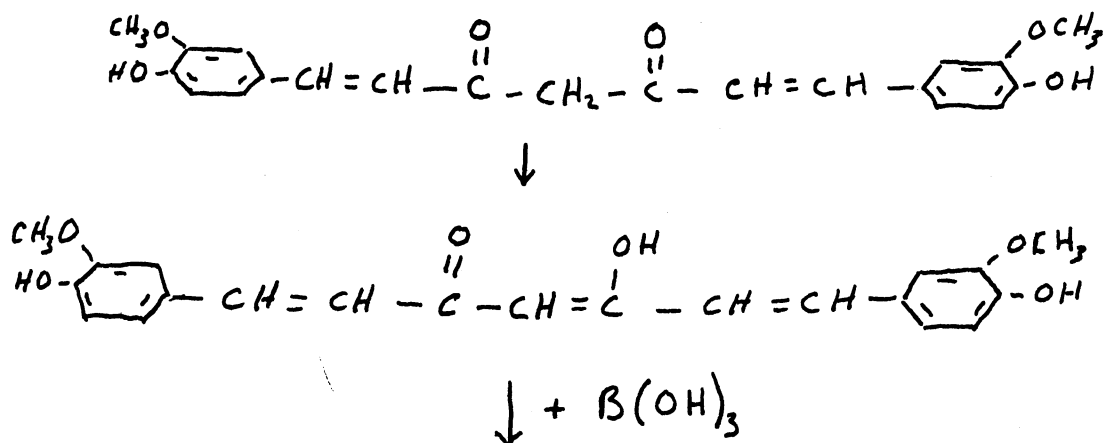
Turmeric is a mixture obtained by grinding the rhizomes of various species of curcuma. Pelletier and Vogel (101) state that turmeric contains cellulose, gum, starch, mineral matter, volatile oil, brown coloring matter, and a characteristic yellow coloring matter which they named curcumin. Curcumin is actually the substance which undergoes change in color in the presence of boron. Curcumin, according to Ghosh (39) has the chemical formula

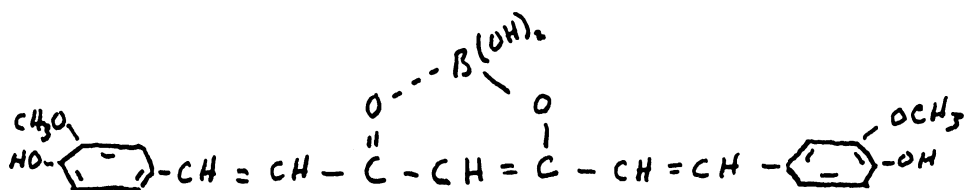


and its chemical name is 1,7 bis (4 hydroxy 3 methoxy phenyl) 1,6 heptadiene 3,5 dione. Spicer and Strickland (97) confirmed this structure by infrared studies.

II Mechanism of Boron-Curcumin Reaction

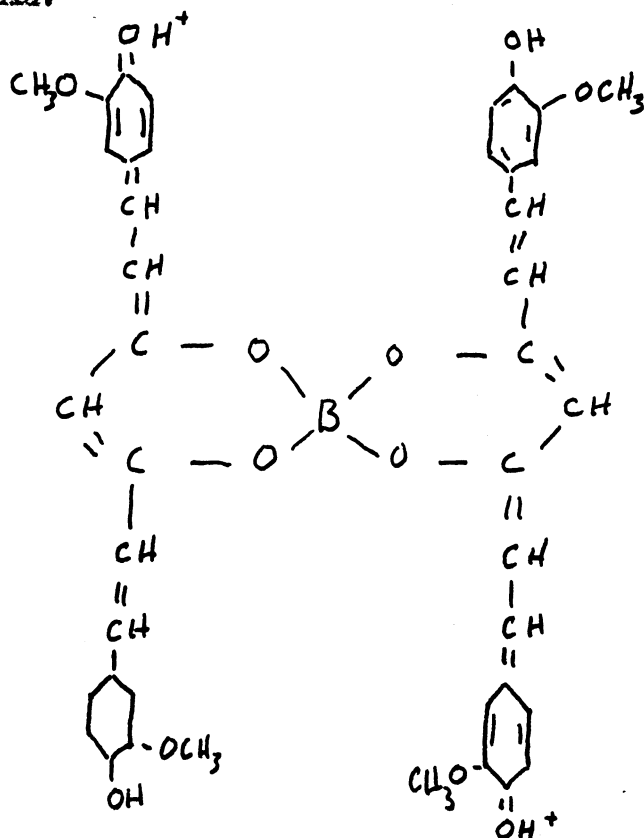
The reagent curcumin (or turmeric) for many years has been used for the detection and determination of small quantities of boron. Early in the nineteenth century several investigators noted that a red color forms in the reaction between boron and curcumin, and they suggested that the reaction be used to detect the presence of boron. Schlumberger (91), in 1866, named the red compound formed by the boron curcumin "rosocyanine" and stated that this compound contains no boron. In 1908 Clarke and Jackson (21) isolated two compounds, rosocyanine and rubrocurcumin, in the product of the boron-curcumin reaction. Clarke and Jackson found rosocyanine to be an isomer of curcumin (no boron present). According to Yoe and Sarver (110) boron is present in rosocyanine. They suggest that a possible mechanism is ring formation by coordination of boron with the methoxy and hydroxy groups of the curcumin molecule. Koreman (58) points out that practically all reagents that form color complexes with boron contain an enolic beta-diketone group and that the reaction product is a six membered ring closed by boron. This reaction may be represented as follows:



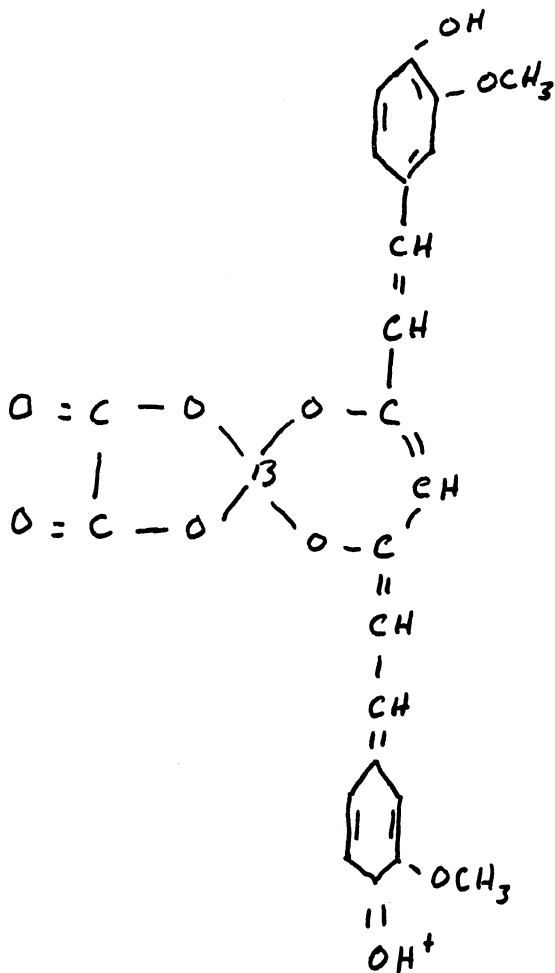


Dible, Berger and Truog (27) state that Hafford (PhD Thesis, University of Wisconsin, 1942) suggested rosocyanine is probably formed by a loose combination of borate with one of the hydroxyl groups of the curcumin molecule.

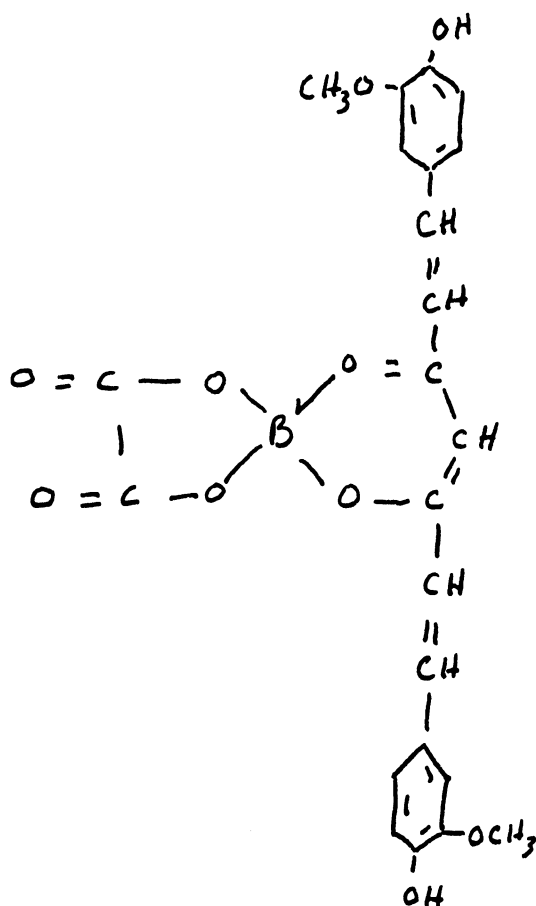
More recently, Spicer and Strickland (96) have found that the rosocyanine and rubrocurcumin are boron-curcumin complexes. They state that rosocyanine is a 1:2 boron-curcumin complex and the rubrocurcumin is a 1:1 boron-curcumin complex. They further state that rosocyanine is formed in the absence of oxalic acid and rubrocurcumin is formed in the presence of oxalic acid. Rosocyanine, according to Spicer and Strickland, has the following formula:



and rubrocurcumin has the formula:



Powell and co-workers (84) found boron, curcumin, and oxalic acid to react in a 1:1:1 ratio. By using Job's method of continuous variation, Pollio (80) found that even in the absence of oxalic acid the boron and curcumin react only in 1:1 ratio. Pollio concludes that the boron - curcumin - oxalic acid complex has the following structure:



III Curcumin Solution Methods

The first reported use of the boron-curcumin reaction for the quantitative determination of boron was by Hebebrand (49) in 1902. This method makes use of the red color developed when turmeric is added to a water - alcohol solution containing boron and hydrochloric acid. The color, after developing for a period of a half hour, varies from light brown for 0.1 mg of boron to red for 10 mg in a final volume of 35 ml. Also in 1902 Cassel and Gerrans (18) developed a method for determining boron with turmeric. They found that oxalic acid increased the sensitivity of the method. In this method a solution containing the boron sample, turmeric, and oxalic acid is evaporated to dryness, the residue dissolved in ethyl alcohol, and the red color compared with standards.

Gauvry (38) used the turmeric solution method for the detection of boric acid in butter and milk. This method involved evaporation (below 85°C) of an alcoholic solution of the boric acid sample, turmeric, and oxalic acid. The method is sensitive to 0.1 mg of boric acid.

Filippi (33) in 1914 reviewed the methods of Hebebrand and others, and pointed out that the main source of error was the varying composition and purity of curcumin used in the various methods. The method he suggested differed little from Cassel and Gerrans (18) method; however, Filippi's method is more sensitive, 0.00002 gm of boron may be estimated with rapidity and great precision.

It should also be pointed out that some investigators use the evaporation step to develop the color and others allow the color to develop in solution. To the boron sample Halphen (46) added HCl and an ethylacetate solution of turmeric, allowed the color to develop in solution for 50 minutes,

and then compared the resulting red color to standards prepared in the same manner. Hawley (47) used a similar method but allowed the solution to develop for $1\frac{1}{2}$ to 3 hours. He could estimate $0.5 \pm 0.1\%$ H_3BO_3 in a 20 gm sample.

Pope and Ross (31) compared the residue after the evaporation step to standards for the determination of 0.1% $\text{Na}_2\text{B}_4\text{O}_7$ in a 2 gm fertilizer sample. They found that nitrates interfered with the test.

Holmes (51) determined one part boron in 25,000,000 parts of aqueous solution by mixing one ml of sample, one ml of reagent (50 mg turmeric in 100 ml of 95% ethanol) and one ml of glacial acetic acid. This mixture was then evaporated to dryness, the residue taken up in a suitable solvent, and the intensity of this final solution was measured with a spectrophotometer at 495 - 510 mμ.

Foster (35), in 1929, compared samples to standards made up of 0.000 mg, 0.0025 mg, 0.005 mg, 0.0075 mg and 0.01 mg of BO_3 . She could estimate the BO_3 sample to 0.001 mg in the above stated range. For quantities of BO_3 above 0.01 mg, Foster found that a wider range of standards (i.e. 0.01, 0.03, 0.06, 0.10, 0.25, and 0.50 mg of BO_3) was necessary to show a distinct gradation in color. This method involves the evaporation on a steam bath of a mixture of one ml of 0.46 N HCl, one ml 1% turmeric, and 10 ml of sample. High salt content may cause difficulties in comparing the samples, and some boron may be dissolved from the glassware.

Curtman and Edmonds (25) found that HCl increases the color intensity of the boron - turmeric reaction, but also increases the color intensity of interference reactions. Traces of copper interfere with the boron curcumin reaction in strong acid solutions.

After a review of the turmeric methods available in 1935, Guterman (45) preferred the method of Hebebrand (49) to that of Bertrand and Agulhon (6). Guterman found Hebebrand's procedure accurate even in the presence of phosphates.

Laska and Rund (60) used the method of Cassel and Gerrans (18) to determine $\text{Na BO}_3 \cdot 4 \text{H}_2\text{O}$ in the range 0.25 to 0.5 mg. To the boron sample they added HCl, oxalic acid, and an alcoholic solution of curcumin, then evaporated this solution to dryness on a water bath, dissolved the residue in ethanol (methanol and ether were also used), and compared the color of the unknown to the color of standard solutions. The magenta residue of the evaporation step was permanent in air, turned blue in alkalies, but decomposed in water. The color did not develop in the presence of BrO_3 or ClO_3 , but persulfates did not interfere with the color formation.

Schafer (90) reported that the turmeric method is most sensitive in strong HCl reaction mixtures. As little as one microgram B_2O_3 can be detected, though fluorides tend to prevent the reaction. Any large quantities of titanium, zinc, molybdenum or tungsten give color reactions with turmeric solution, though there is no danger of mistaking their color reaction with that of boron. Oxidizing agents destroy the dye stuff (turmeric).

Robinson (86) used turmeric-coated sand to prevent loss of boron (as ethyl borate) during the evaporation step. One disadvantage of this method is that several evaporations of the reaction mixture are necessary to insure complete reaction. After evaporation the residue is dissolved in 70% ethanol and the color intensity measured. This method has excellent precision in the range of 0 to 50 mg B_2O_3 per 100 ml of final solution.

Robinson reported that phosphates, silicates, salts of the alkali metals interfere with the reaction. Sodium hydroxide used for the collection of the distillate, and for the evaporation interfere but this interference may be reduced by keeping the concentration of the sodium hydroxide at an optimum concentration.

In 1939 Naftel (77) reported a procedure that was similar to previous methods, particularly the method of Cassel and Gerrans, but perhaps more control of the numerous variables was achieved by Naftel. The boron sample is made alkaline with 0.1N $\text{Ca}(\text{OH})_2$ and this solution is evaporated to dryness. One milliliter of an oxalic acid-hydrochloric acid solution (80 ml of saturated oxalic acid and 20 ml HCl) and 2 ml of curcumin (0.1%) or turmeric (1.0%) are added to the cooled residue. This mixture is evaporated to dryness at $55 \pm 3^\circ\text{C}$ and heated an extra 30 minutes at this temperature. The residue is extracted with 95% ethanol, centrifuged, diluted to constant volume, and the color intensity compared with standards similarly prepared. The effect of different amounts of oxalic acid, hydrochloric acid and turmeric (or curcumin) were studied and optimum amounts of these reagents were determined. Apparently other substances do not interfere with the test. Boron in the range 0.5 to 8.0 micrograms can be determined by this method. Naftel used this procedure to determine boron in soils.

Bewick, Beamish, and Bartlet (11) obtained excellent accuracy when they used a turmeric method for the determination of boron (0.5 to 1.0 microgram) in magnesium and calcium. This method is a modification of the Naftel method that employs an interesting technique of adding oxalic acid-hydrochloric acid dropwise into the boron sample and turmeric reagent in a platinum dish cooled in dry ice. Each drop freezes as it is added to the dish; this prevents

any loss of boron as a volatile compound. The dish is warmed by hand and then evaporated to dryness on a water bath at 60°C. The residue is taken up in 70% ethanol and the color intensity read at 550 mμ with air as the 100% transmission point. Russel (89) reported a method for determining boron (0.5 to 1.5 micrograms) that differed little from that of Bewick, et. al., except that Russel used a higher evaporation temperature, 80° to 85°C.

In 1953 Silverman and Trego (93) reported a more detailed study of the condition for the boron-curcumin-oxalic acid reaction. In their method the curcumin is completely dissolved in a carbitol-acetone solution and the resulting solution is stable for several months. Phenolphthalein is used to control the acidity of the reaction mixture. The optimum amounts of the various reagents, oxalic acid, curcumin, and hydrochloric acid were determined. Stable curcumin-boron-acetone solutions were prepared. The method has a precision of 0.02 micrograms for the range 0 to 0.2 micrograms of boron in 10 ml of reaction mixture, 0.05 micrograms for the range 0.2 to 2.0 micrograms in 25 ml, and 95% precision for the range 2 to 25 micrograms in 25 ml, 25 to 50 micrograms in 50 ml, and 50 to 100 micrograms in 100 ml. The authors reported the colorimetric method is superior to the potentiometric method for the determination of boron in the lower concentration range (0 to 25 micrograms).

To the boron sample in a porcelain crucible add 0.1 gm Na_2CO_3 and evaporate the solution to dryness at 110°-130°C in an oven. Cool the solution, add one drop phenolphthalein indicator, titrate with 1:4 HCl to the disappearance of the red color, and add 0.5 ml excess HCl. Add 0.5 ml of 5% oxalic acid-acetone solution, 3 ml curcumin (0.625 gm curcumin in 5 ml Carbitol monoethyl ether of diethylene glycol, then dilute to 500 ml with acetone), and then evaporate to dryness at

$55^{\circ} \pm 3^{\circ} \text{C}$ in an oven or a water bath. Cool the residue, take up the residue in acetone, filter, and dilute to volume with acetone. The absorbance of the final solution is measured at 535 mu.

The authors report that the evaporation is the most critical step of the procedure, and further report that dryness is reached when square crystals appear in the crucible.

The same authors, Silverman and Trego (94), in 1956 used essentially this same method to determine boron in uranium metal and uranyl chloride. They reported, however, that the concentration of oxalic acid to be used depends on the amount of boron to be determined. Chloride ions does not interfere with the reaction if excess oxalic acid is present. As little as 0.3 micrograms of boron in 100 mg of uranium may be determined by this procedure.

Valenti, Conti, and LoMoro (102) used the method of Silverman and Trego to determine boron in nuclear reaction materials. However, they found that dioxan, as well as acetone, can be used as a solvent. They further state that the water content of the reaction mixture, as well as other variables already mentioned, must be standardized.

Luke (65) states that the turmeric method is difficult because close control of many variables is necessary. The concentration and volume of reagents, temperature of evaporation, method of dissolving the colored residue, even the shape and smoothness of the evaporating dish all must be closely controlled. All the factors that influence the rate of evaporation must be controlled. Luke carried out the evaporation step avoiding direct sunlight, in a draft free area, until there was no HCl odor. An outline of his procedure is as follows:

Distill the boron sample as the methyl borate and collect the distillate in a platinum evaporating dish, add 1 ml of 1:1 HCl, 2.00 ml of the curcumin reagent (0.005 gm curcumin, 0.5 gm oxalic acid in 50 ml of ethanol), and then evaporate this solution at $55^{\circ}\pm 1^{\circ}\text{C}$. The color intensity is measured at 540 mμ with ethanol as the reference.

This method was used for the determination of boron in the range 0 to 0.50 micrograms.

Luke and Flaschen (66) determined traces of boron in silicon. They reported that humidity is another factor that should be controlled to obtain best results with the turmeric method. The method used here was essentially the same as previously reported by Luke (65). The one difference was that rather than employing a draft free evaporation they used a controlled air flow across the evaporating mixture. They could determine as little as 0.02 ppm of boron in silicon. Luke (67) also used this procedure to determine 0.2 ppm of boron in nickel.

Spicer and Strickland (96) state that the red product in the Naftel type turmeric method is mainly rubrocurcumin, a boron-curcumin-oxalic acid complex. Boron analyses based on this reaction are only half as sensitive as analyses based on the boron curcumin reaction in which the only product is rosocyanine, a 2:1 curcumin-boron complex. They report three methods for the determination of boron, two (for the ranges 2 to 15 micrograms and 0.5 to 4 micrograms) of which are based on the boron-curcumin-oxalic acid reaction. These methods are of the Naftel type but modified to give higher precision and sensitivity. These methods require the use of pure curcumin and the reaction mixture is evaporated at 55° to 60°C . The third method, a more sensitive method for the range 0.01 to 0.2 micrograms boron, is based on the boron-curcumin reaction in the absence of oxalic acid. In this third pro-

cedure, a method that is quite different from the turmeric methods that have been reported, the reaction yields only rosocyanine. The evaporation is carried out at 50°C and requires exact timing. The intensity of the colored solution is measured at two wave lengths, 550 mμ and 630 mμ, to take advantage of the different spectral characteristics of curcumin and rosocyanine. From the molar absorbancy at the two wave lengths the amount of boron present is calculated. High concentrations of salts reduce the amount of colored complex formed by a given amount of boron. Many other substances may interfere with the reaction, but those are eliminated by distilling the boron sample as the methyl ester.

The same authors, Spicer and Strickland (98), in another report emphasized the importance of controlling the many variables of the boron-curcumin reaction to achieve the best precision and accuracy. They pointed out that there are many sources of contamination during the determination of boron by the curcumin method and these contaminations should be minimized. The reagents used in the analysis may have too high a boron content and should be further purified. All storage containers and reaction vessels should be boron free. As much as 200 to 1000 ppm of boron may be present in the atmosphere and could contaminate the reaction. Spicer and Strickland gave procedures for minimizing these sources of contamination.

In 1941 Michel (72) reported a more sensitive and more specific method for the detection of boron that requires the use of salicylic acid rather than oxalic acid. The product of this reaction usually shows a green fluorescence and dissolves in ammonia to give a blue colored solution.

To a solution containing the boron sample and a little HCl add 1 or 2 drops of 0.1% alcoholic curcumin, then a few drops of pure ethanol, a few

drops of salicylic acid, and a drop of 6N HCl. This solution is then evaporated to dryness on a water bath and the residue dissolved in ethanol to give the usual red boron curcumin complex solution. To test specifically for boron the solution is again evaporated to dryness, the remaining residue usually shows green fluorescence, and the residue is dissolved in 6N ammonia to get the blue color.

Apparently, either the blue color or the usual red color may be measured to give a quantitative determination of boron, though Michel did not report a quantitative procedure. Michel tested 50 organic acids in place of the salicylic acid and found only beta naphthoic acid as satisfactory as salicylic acid.

Zaletel (111) studied the optimum conditions for the boron-curcumin reaction in the presence of salicylic acid and hydrochloric acid. He reported that the color intensity increases as the amount of turmeric is increased and for precise work the amount of turmeric used depends on the amount of boron to be determined. A procedure for the determination of 3 micrograms of boron was outlined. To the boron sample in one ml of solution add 0.15 ml of turmeric solution (1.29 gm/100 ml methanol), 0.18 ml of 1% salicylic acid, and 0.03 ml of 2:1 hydrochloric acid. The mixture is allowed to stand for one hour in a porcelain crucible, then evaporated to dryness on a water bath, and heated for an extra 30 minutes. The cooled residue is washed into 50 ml of distilled water with 6 ml of ethanol. After the addition of 1.5 ml of 2N sodium carbonate the intensity of the solution is determined at 530 mμ. Boron in the concentration range 0.015 to 0.50 micrograms per ml was determined with an experimental error of 2 to 3%.

Dudins (29) found the color reaction between boron and curcumin takes place in the presence of salicylic acid, succinic acid, para-amino salicylic acid, and phosphoric, as well as oxalic acid. He determined as little as

0.08 micrograms per ml of boron and stated that a more concentrated curcumin reagent must be used for larger boron concentrations. For the higher concentrations of boron succinic and para-amino salicylic acids may be used in place of the hydrochloric acid. However, high concentrations of phosphoric acid interfere with the boron-curcumin reaction and an optimum amount of phosphoric acid was recommended. Iron above 548 micrograms per ml, nitric acid above 0.26 mg, and fluorides above the B:F ratio 1:1000 interfered.

Philipson (79) used trichloroacetic acid rather than oxalic acid in the turmeric method and was able to determine boron within 3% in the range 0.2 to 10.0 micrograms. The boron sample is ashed with calcium oxide, and the alkaline distillate is evaporated. After addition of one ml of one molar trichloroacetic acid and one ml of turmeric (0.125% in ethanol) solution the mixture is evaporated to dryness in an oven at 106°C. The residue is dissolved in 10 ml of ethanol containing a trace of trichloroacetic acid, and the color intensity of the solution is measured immediately.

Coursier, Hure, and Platzer (22,23) reported a turmeric method determining boron in the range 0 to 0.4 micrograms with a maximum error of ± 0.04 micrograms. To the prepared boron sample add one ml of one normal trichloroacetic acid, one ml turmeric solution (0.125% in ethanol) and evaporate in an oven at $106 \pm 1^\circ\text{C}$ for 60 ± 3 minutes. The residue is dissolved in ethanol and the color intensity measured at 540 mμ.

In determining boron in silicon Ducret and Seguin (28) used a method similar to the method of Philipson (79). They used trichloroacetic acid with the curcumin reagent in ethanol and reported a precision of 0.5 micrograms in determining boron in silicon. The reaction mixture is evaporated in an oven at 105°C , the residue taken up in alcohol, and the absorbancy measured at

540 mu. Ducret and Seguin state that curcumin, even as the dry solid, is unstable in the presence of air, light and heat. They also reported that special care should be given polyethylene ware, if it is to be used in boron analysis, since the oxidation products of polyethylene interfere with the boron-curcumin reaction.

Hegedus (50) reviewed the literature, through 1951, concerning the turmeric solution method and proposed a procedure that involved the use of glacial acetic acid as the solvent. To the boron sample (not more than 8 micrograms boron) diluted to 2 ml in a quartz crucible, add 2 ml of curcumin reagent (0.1 gm curcumin in 100 ml glacial acetic acid saturated with oxalic acid), and evaporate the mixture to dryness on a water bath. The residue is cooled, dissolved in 20 ml of 95% ethanol, illuminated for three minutes, and the color intensity measured. Hegedus found that large amounts of fluorides, titanium, zirconium, molybdenum, beryllium, and tungsten interfere. The boron may be separated from these interferences by distillation as the methyl borate, prior to the turmeric analysis procedure. Oxidizing agents, such as nitrates, chlorates, bromates, iodates, and nitrites destroy the curcumin reagent.

After reviewing the reported methods for determining boron, Bottini (14,15) proposed a procedure in which the amount of boron is first estimated and later determined exactly. After an elaborate preparation the boron sample is acidified with acetic acid, two ml of 0.1% curcumin is added, and the solution is evaporated to dryness on a steam bath. From the color of the residue the amount of boron is estimated. The excess curcumin is removed with ether, then the residue is treated with a methyl acetate - potassium hydroxide solution, and the color is compared with standards. The blue color

reported by Michel (72) must be formed, since the residue is treated with a basic solution. The sensitivity of the method is one microgram. Guevara (44) used the boron curcumin reaction in a basic media to determine boron in fresh fruits. He mixed the reagents and sample in a test tube, and allowed the mixture to stand for 30 minutes. The product varied from blue violet to blue green and was compared to standards prepared in the same manner.

The turmeric method has been used to determine boron in liquor antiseptics (53), soils (48), plants (40, 108), rocks (59), metals and alloys (2,13,24,37,42,54,56,82,87,88,106) nuclear reactor coolant gases (107) and other substances (20,43,99,100,109). McMullen (71) and Wilson (107) determined boron in wood by applying the turmeric reagent (saturated with salicylic acid) directly to the surface of the wood, allowing it to dry, and comparing the color of the spots that developed to standards.

There are many variables in the boron-curcumin reaction that need to be controlled to give better accuracy and precision in determining boron. Chrinside, Cluley and Profitt (20) state that the reaction depends on the rate of heating and evaporating, which in turn are effected by the temperature, size of dish, salts present, and the alcohol-water ratio. Some of these variables can be detected in the data interpretation, others are more difficult to detect. Lima, Pagano and Schneiderman (63) applied modern statistical methods to the study of the effect of the variables in the boron-curcumin reaction on the quantitative determination of boron. The significant variables affecting the boron determination can easily be detected.

Feigl (31) and others (29,50,90) report that in acid solutions ferric ions, molybdenum, titanium, columbium, tantalum, and zirconium interfere with the boron-curcumin reaction if they are present in large quantities. Oxidizing

agents (15,29,31,60,81,90) such as peroxides, chromates, permanganates, nitrates, and chlorates interfere with the reaction. Muraki and Huro (74) report that alkalis and alkaline earths interfere. Gol'tman and Gurevich (40) report interference from sodium, potassium, calcium, magnesium, sulfates, chlorides, nitrates and oxalates. Yoe and Sarver (110) state that aluminum, beryllium, zirconium, magnesium and iron form color lakes with curcumin and this can interfere with the boron-curcumin reaction. Kolthoff (57) and Dible, Berger, and Truog (27) found that excess oxalic acid prevents these lakes from forming. Several investigators (20,35,86,96) have reported that a high salt content in the reaction mixture interferes with the color formation. Most of these interferences may be eliminated by distilling the boron as methyl borate.

Fluorides have been reported (29,50) to interfere with the boron-curcumin reaction. Gaestel and Hure (36) were able to determine boron in the presence of fluorides by first complexing the fluorides with aluminum chloride. Boron is distilled as methyl borate from a solution of methanol, hydrochloric acid and aluminum chloride. The aluminum chloride acts as a complexing agent, preventing the volatilization of hydrogen fluoride.

IV Curcumin Paper Tests

Concurrent with the development of the turmeric solution method for boron determination, a turmeric paper method was developed. Around the turn of the century a number of workers (6,32,41,61,62,85,103,105), reported the use of turmeric paper to detect boron, though no one had made the test quantitative. Emrich (30) used turmeric coated linen fibers to detect .001% boron. Chamot and Cole (19) also investigated the use of various fibers impregnated with turmeric to detect boron (.025 micrograms). Low (64) reported a semi-

quantitative method for boron using turmeric paper. However, it was Bertrand and Agulhon (7) who first used turmeric paper to determine quantitatively small amounts of boron. They later used it to determine boron in organic matter, fruits, vegetables and sea water (8,9). In their method boron sample is distilled as methyl borate, the distillate evaporated to dryness, and four drops of 10N hydrochloric acid and 0.5 ml of distilled water are added. This solution is washed into a vial to a volume of 1.5 ml. A strip of turmeric paper 45x3mm is immersed into the solution to a depth of 15mm and the vial is heated for three hours at 35°C or kept at room temperature for 10 to 24 hours. The height of red coloration on the paper strip is a measure of the amount of boron present. The unknown boron sample is compared to known amounts of boron prepared in the same manner. They could determine 0.1 to 100 micrograms of boron by this method.

Scott and Webb (92) used the method of Bertrand and Agulhon to determine 0.005 to 1.0 mg of B_2O_3 in a 100 gm soil sample. For boron oxide in the range 0.1 to 1.0 mg longer strips of turmeric paper and larger vials are used. Badger (4), using Bertrand and Agulhon's method, determined 0.01 to 5.0 mg of boron in water, brine and salt. Mix (73) modified the method of Bertrand and Agulhon in that the intensity and lengths of coloration on the turmeric paper are compared after one hour development. Mix reported that large amounts of sodium and potassium sulfates do not interfere.

Camus (17) reported a method for the determination of boron that depends upon the shade and intensity of coloration on the turmeric paper rather than the height of coloration. To the sample in 3 ml of dilute hydrochloric acid, add a strip of turmeric paper, and evaporate to dryness. He reported that the intensity of coloration is dependent upon the size of the paper strip and con-

centration of turmeric on the paper strip. Camus determined from .02 to 1 mg of boric acid, and reported that salts interfere with test. He also stated that the red color on the turmeric paper (after reaction with boron) changes to sky blue in the presence of ammonium hydroxide.

Nemejc (73) put a turmeric paper in the interference free boron sample and evaporated as long as the paper continued to change color. When the reaction on the paper is completed, the paper is dried in an oven at 80°C. By this method 0.5 to 5.0 micrograms of boric acid in waters can be determined with a sensitivity of 0.3 micrograms. Nemejc reported the turmeric reagent is most stable in phosphoric acid, and NO_3^- , NO_2^- , Br^- , I^- , K^+ , NH_4^+ , SO_4^{--} , Ca^{++} , and Mg^{++} interfere with the reaction. Bordas and Touplain (12) reported that fluorides, iodides and formates interfere with the turmeric paper test for boron.

Flood and Risberg (34) reported that K_2CrO_4 , KMnO_4 , NaOCl , and other strong oxidizing agents will not interfere if the solution is allowed to rise on the paper by capillary action. Muto (75,76) used paper chromatography to separate boron from acids, alkalies, salts and certain metals. The boron spot is identified by spraying with 0.02% turmeric solution and 1N sodium hydroxide solution. The area of the spot and the amount of boron present are nearly proportional in the range 5 to 50 micrograms of boron. Muto apparently made use of the blue color of the boron turmeric reaction product in a basic media. Arzberger (3) and Brinsmaid (16) reported that a blue color develops on turmeric paper in the presence of boron and ammonium hydroxide.

Allen and Zies (1) used the turmeric paper test to determine boric acid in glass. Bertrand and Silberstein (10) used the turmeric paper test to determine 7 to 50 mg of boron per kilogram of soil sample. Sokolova and

Meckharashvili (95) determined one micrograms of boron per gram of silicon on filter paper by means of curcumin.

As well as the interferences already mentioned, some commercially purchased turmeric paper may contain enough boron to give a positive reaction (69).

V Summary

Many investigators report the use of curcumin, both in solution and on paper, to determine small quantities of boron. To obtain best results with the curcumin reagent, considered the most sensitive color reagent presently known for boron, many variables need to be controlled. The time and temperature of evaporations are two most important factors that influence the rate of reaction. Though there is a great difference of opinion in the literature concerning the mode, the time, and the temperature of evaporation, investigators agree that the factors that influence the rate of evaporation must be controlled. Other variables are concentration and volume of reagents, water content of the reaction mixture, humidity, size and shape of the evaporation dish, and the many sources of contamination. Boron may be separated from most substances that interfere with the boron-curcumin reaction by distilling as methyl borate.

EXPERIMENTAL

I Apparatus and Reagents

Beckman Model B Spectrophotometer - This instrument was used for all the spectrophotometric measurements that were made. Pyrex absorption cells were used in the instrument, and dissolution of boron from the cells during the time of measurement, if it occurred, caused no interference.

Miscellaneous Apparatus - Soft glass (low boron content) was used in all cases in which glassware was needed. All solutions, other than those prepared with sulfuric acid, were stored in polyethylene bottles. When such accuracy was needed, the burets and volumetric flasks were calibrated. Porcelain dishes were used for the evaporation of the test solutions.

Water Bath - The test solutions, in porcelain dishes, were evaporated on ordinary water baths. Bunsen burners were used to maintain the required temperature in the water bath. It was not difficult to maintain the proper temperature with this set-up.

Reagents - Reagent grade chemicals were used. The colorimetric reagents (curcumin, quinalizarin, carminic acid, alizarin Red-S, alizarin Blue-S, Chromotrope 2B, 1-amino - 4-hydroxy anthraquinone) were the best grade obtainable from Eastman Kodak Co.

II Preliminary Study of Some of the Reagents Available for the Colorimetric Determination of Boron

A. Introduction

The purpose of this preliminary survey was to find a sensitive colorimetric reagent for the determination of microquantities of boron. Also it was desired that the reagent be suitable for rapid field test of boron. To this end seven reagents were chosen for the preliminary study. The seven reagents chosen were quinalizarin, carminic acid, Chromotrope - 2B, Alizarin Red-S, Alizarin Blue-S, 1-amino-4-hydroxyanthraquinone, and curcumin. Curcumin forms a colored complex with boron in organic solvents (usually ethanol, acetone, or glacial acetic acid). However, the other six reagents listed form colored complexes with boron in concentrated sulfuric acid. The intensity of coloration of these complex solutions depends on the amount of boron present (provided there is enough reagent present to complex all the boron) and this forms a basis for quantitative determination of boron. This preliminary survey was a two part study; the first, a study of a method using the curcumin reagent, and the second, a study of the reagents that require concentrated sulfuric acid as a solvent.

B. Curcumin Method

From the literature reports, the Hegedus (50) method appeared to be one of the best methods employing the curcumin reagent for the determination of boron. Thus this method was chosen as a basis for a preliminary study of the turmeric reagent. Hegedus' procedure is as follows:

To the sample which has been diluted to 2 ml with water, add 2 ml of reagent solution and evaporate to dryness at 55°C. Take up the residue with 20 ml of 95% ethanol and measure the absorbance. The

reagent solution is prepared by dissolving 0.1 gm of curcumin powder in 100 ml of glacial acetic acid which has been saturated with oxalic acid.

For this preliminary survey the Hegedus procedure was used with one modification, the solutions were evaporated at steam bath temperature (100°C) rather than at 55°C.

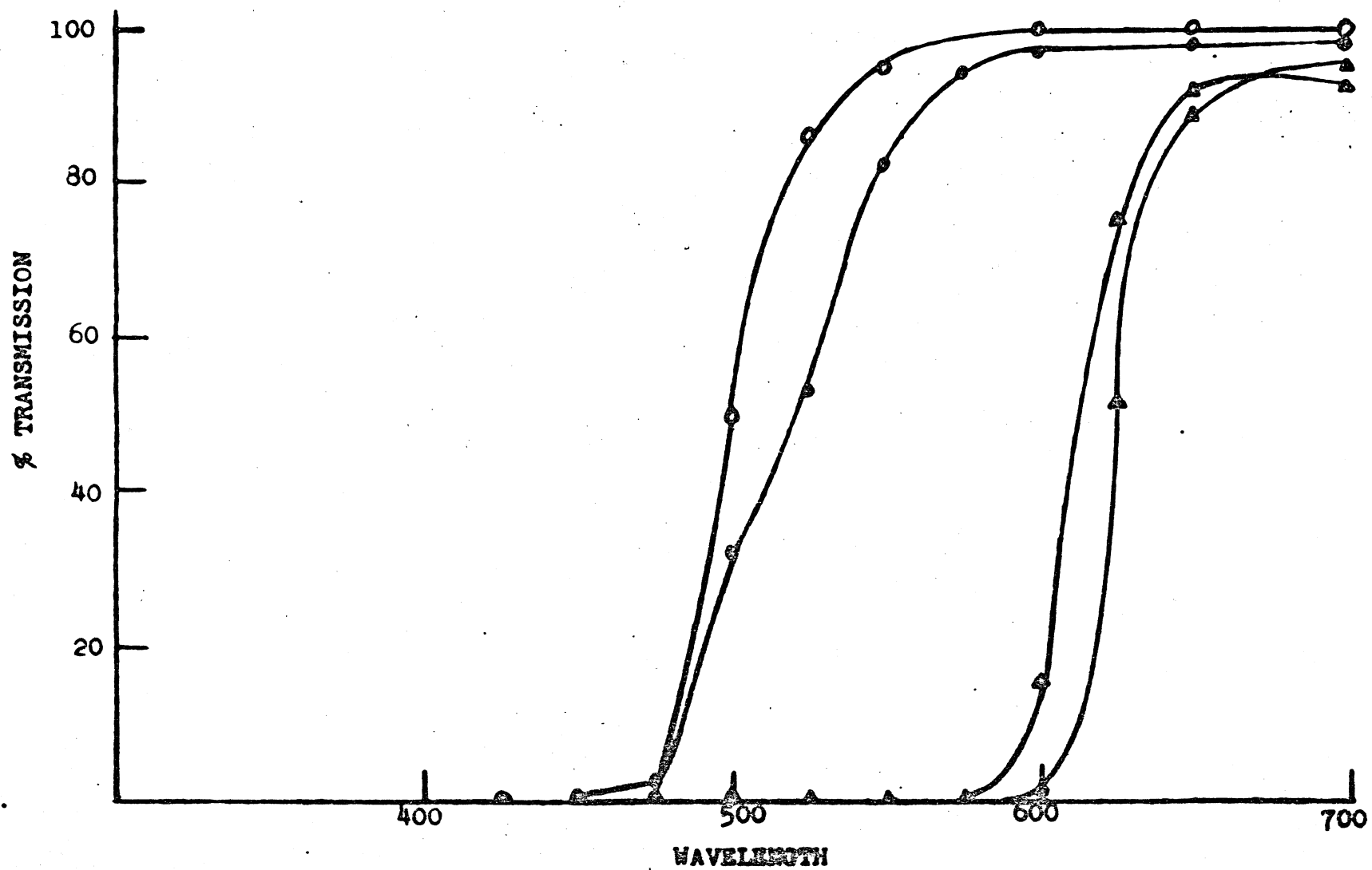
A study of the absorption spectra (Figure 1) of the reagent and the reagent-boron complex showed 575 mu to be the best wave length for future color intensity measurements. At this wave length the boron-reagent complex solution was found to have maximum absorbance and the reagent solution nearly minimum absorbance. To attain maximum color intensity the boron reagent was prepared from a 1000 microgram boron sample. The reagent solution was prepared by adding two ml of distilled water to two ml of curcumin reagent, evaporating this mixture to dryness, and then taking up the residue in 20 ml of 95% ethanol. The percent transmission of the complex solution and of the reagent solution was measured over the range 325 mu to 700 mu with a sepctrophotometer, and this data plotted as shown in Figure 1.

The absorption spectra study was repeated using curcumin reagent without oxalic acid. As shown in Figure 1, oxalic acid had only a small effect on the absorption spectra. However, if the reagent without oxalic acid is to be used for boron analysis, 550 mu is a better wave length at which to measure color intensity. At 550 mu the boron reagent complex has maximum absorbance and the reagent has nearly minimum absorbance. Another reagent solution with a different amoijnt of oxalic acid was prepared and used for another absorption spectra study. This curcumin reagent solution was a 1:1 mixture of the curcumin solutions with and without oxalic acid. The spectra of

Figure 1

**Absorption Spectra of Boron-Curcumin Complex
and of Curcumin Reagent
(Modified Hegedus Method)**

- Curcumin Reagent - with oxalic acid**
- Curcumin Reagent - without oxalic acid**
- △ Boron Curcumin Complex - with oxalic acid**
- ▲ Boron Curcumin Complex - without oxalic acid**



boron complex and reagent solutions prepared from this reagent (1:1 mixture) coincided with the spectra of boron complex and reagent solutions prepared from the reagent with saturated oxalic acid.

To obtain a standard calibration curve a series of solutions with varying amounts of boron were carried through the modified Hegedus procedure. The absorbance of these solutions were determined at 575 mμ and the absorbance plotted against micrograms of boron per 20 ml of solution (Figure 2). It was found that the boron-curcumin reaction (in acetic acid saturated with oxalic acid) followed Beer's Law over the range 0 to 8 micrograms of boron per milliliter. The sensitivity of the method is 0.0044 microgram of boron per 20 ml per 0.001 absorbance unit. Above eight micrograms of boron the sensitivity decreases and the standard curve deviates from Beer's Law.

Many reports in the literature show that oxalic acid increases the sensitivity of the turmeric method for boron determination. To determine how great this increase in sensitivity is, a standard calibration curve (see Figure 2) obtained in the same manner as described above except that the curcumin reagent did not have oxalic acid added, and the absorbance was measured at 540 mμ. Without oxalic acid the sensitivity of this method is 0.1 micrograms of boron per 20 ml per 0.001 absorbance unit, thus the method with oxalic acid is about 25 times as sensitive as the method without oxalic acid.

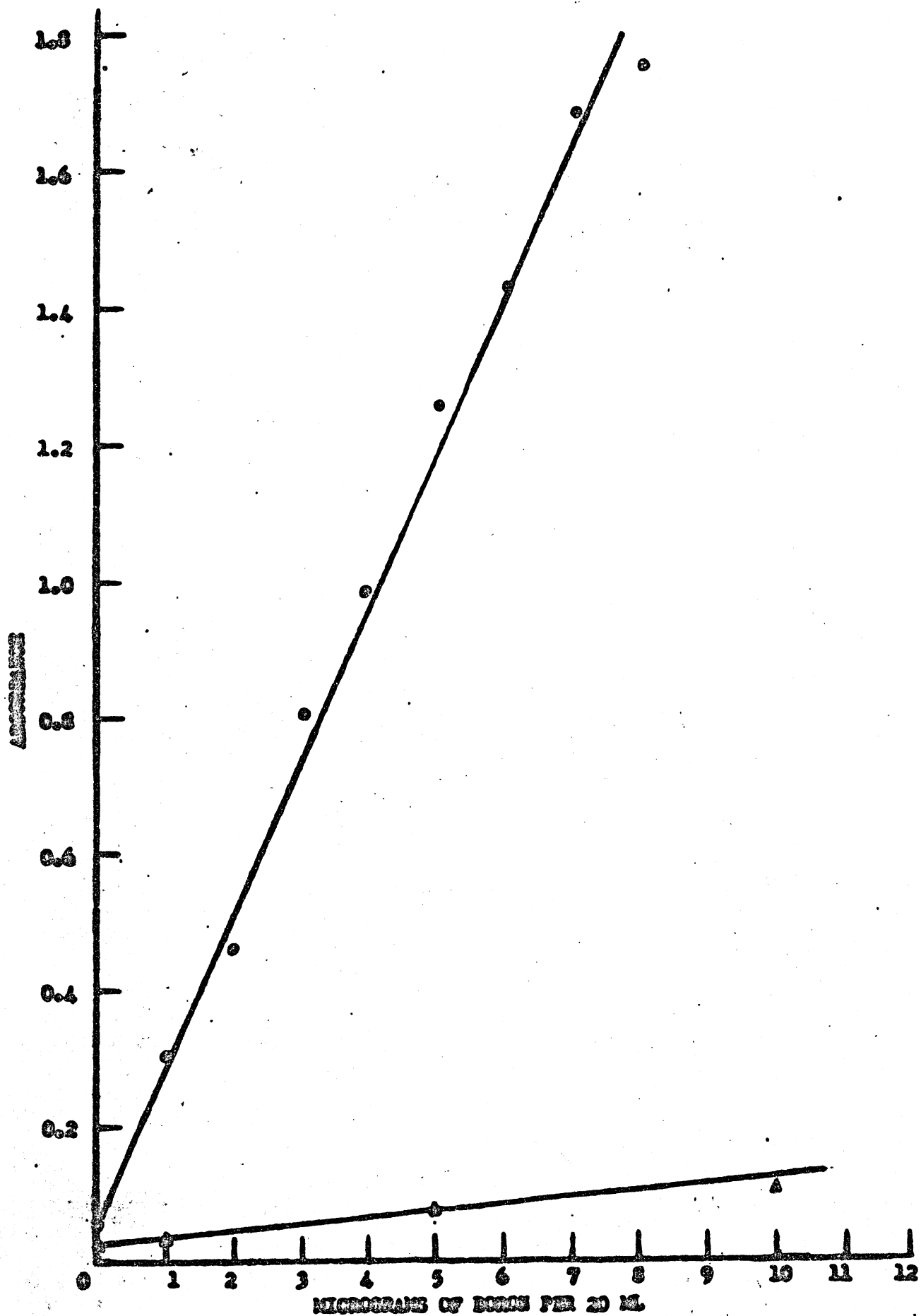
The reproducibility of the method with oxalic acid was ± 0.1 micrograms of boron for solutions containing 2 micrograms of boron, and ± 0.2 for solutions containing six micrograms of boron. To determine the reproducibility four solutions containing two micrograms of boron and four containing

Figure 2

Standard Curve for Modified Hegedus Procedure

Absorbance Versus Micrograms of Boron

- Oxalic Acid Present
- ▲ No Oxalic Acid Present



six micrograms of boron were analyzed according to the modified Hegedus procedure. The results of this test are given in Table I.

TABLE I

Reproducibility of Method and Stability of Boron - Curcumin Complex

Micrograms Boron Present	Micrograms Boron Found		
	$\frac{1}{2}$ hr	1 $\frac{1}{2}$ hr	2 $\frac{1}{2}$ hr
2.0	2.0	1.9	1.9
2.0	1.6	1.6	1.6
2.0	1.7	1.7	1.7
2.0	1.8	1.9	1.8
Avg	1.8 \pm 0.1	1.8 \pm 0.1	1.8 \pm 0.1
6.0	6.2	6.1	6.0
6.0	5.5	5.4	5.4
6.0	5.5	5.4	5.4
6.0	5.5	5.2	5.2
Avg	5.6 \pm 0.2	5.5 \pm 0.3	5.5 \pm 0.2

The absorbance of the eight solutions described above were measured $\frac{1}{2}$, 1 $\frac{1}{2}$, and 2 $\frac{1}{2}$ hours after the ethanol was added to the evaporation residue and, as shown in Table I, the complex solutions were found to be stable over this period.

At 50°C the evaporation of the test solution was found to take approximately three hours. This evaporation time seemed unnecessarily long, thus higher temperatures were used to reduce the time of evaporation. Also at 50°C the reaction apparently was not complete and the precision was poor. At 75°C the evaporation took one to two hours but the precision was poor. At 100°C (steam bath temperature) the evaporation took 15 minutes and the precision was good. Ten minutes extra heating at 100°C, after the solution reached dryness, resulted in loss of boron, though the precision was still good. The most efficient evaporation temperature was found to be 100°C. (Table II). The solutions used for this test prepared according to the

modified Hegedus procedure, except that the temperature of evaporation was varied.

TABLE II

Effect of Temperature of Evaporation on Absorbance

<u>Temperature</u>	<u>Time of Evaporation</u>	<u>Absorbance</u>	
		Set #1	Set #2
50°C	3 hrs	0.51	1.02
75°C	1-2 hrs	1.12	1.50
100°C	15 minutes	1.28	1.33
100°C & 10 minutes	15 minutes plus	1.19	1.24
extra heating at 100°C	10 minutes		

C. Methods Using Sulfuric Acid as the Solvent

The second part of this preliminary survey was a study of the six reagents (quinalizarin, Alizarin Red S, Alizarin Blue S, carminic acid, Chromotrope 2B, 1-amino-4-hydroxyanthraquinone) that employ concentrated sulfuric acid as the solvent. Since this study of the sulfuric acid methods was conducted cooperatively by two graduate students (this author and Emmett H. Poindexter) only a summary of this work will be given here. Only the results of this study and not the study itself are important to this thesis. (For the details of this work see Reference

First the absorption spectra of the reagent and the boron-reagent complex were determined for the range 325 mμ to 700 mμ. For future color intensity measurements, the wave length at which the greatest separation of reagent spectra and complex spectra occurred was taken as most suitable. The effect of reagent concentration and H₂SO₄ concentration on the intensity of the color and on the sensitivity on the method were studied as were the time required for development of color and the stability of the color.

When these studies were completed a standard calibration curve was drawn. Then the reproducibility of the methods were determined. The experimental procedures followed in each of these studies were very similar for all the reagents and were modifications of procedures reported in the literature.

The results of this preliminary study of the sulfuric acid methods and the turmeric method are summarized in Table III. Each reagent has been rated on a 1 to 5 scale based upon the information obtained in this survey. From this study carminic acid appears to be the best reagent. The sensitivity, reproducibility and adherence to Beer's Law are good for the carminic acid method. The main disadvantage of this reagent is the time required for the development of color. Though not as good overall as the carminic acid method, the other reagents do offer certain advantages. Quinalizarin is as sensitive as carminic acid and requires a shorter time for color development, but is affected more by the sulfuric acid concentration than the carminic acid. Alizarin Red-S and Alizarin Blue-S follow Beer's Law nicely over the range 0 to 25 micrograms per 10 ml of solution; this is the best adherence to Beer's Law of all the reagents studied in this survey. However, only 1-amino-4-hydroxyanthraquinone is less sensitive than Alizarin Red-S and Alizarin Blue-S. The reagent Chromotrope 2B requires only 5 minutes for color development and is not greatly affected by the sulfuric acid concentration.

D. Summary

Of all the methods studied in this survey the curcumin method is the most sensitive (Figure 3 and Table III). The curcumin reagent compares very favorably in all respects to the methods that require sulfuric acid. The reproducibility and adherence to Beer's Law are good over a short range.

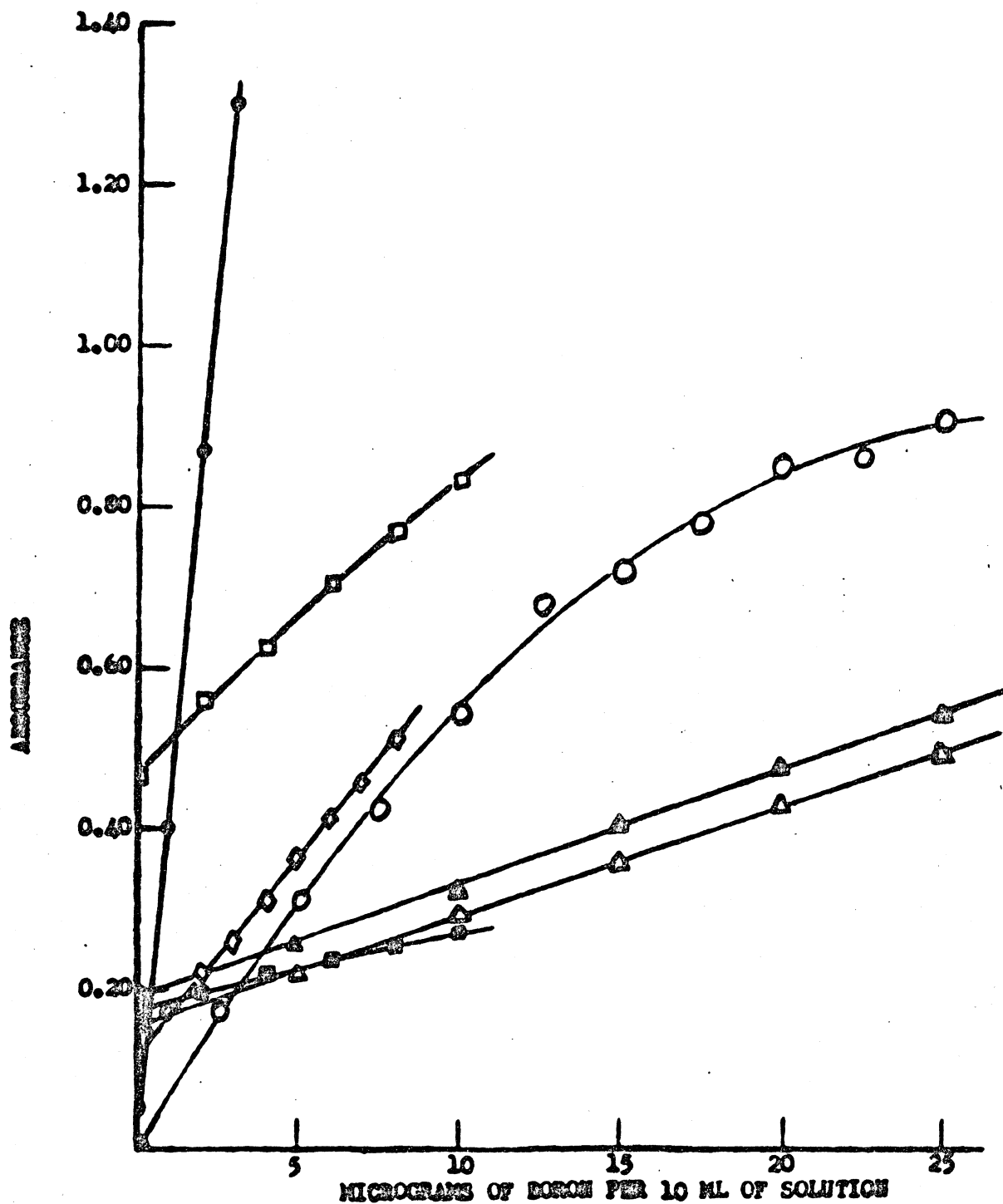
Figure 3

Standard Curves for Various Reagents

Used to Determine Boron

Absorbance Versus Micrograms of Boron

- Turmeric
- ◻ Chromotrope - 2B
- ◊ Carminic Acid
- Quinalizarin
- △ Alizarin Red S
- ▲ Alizarin Blue S
- 1-Amino-4-hydroxy-anthraquinone



However, if one wishes to determine quantities of boron in excess of 5 micrograms per 10 milliliters a sulfuric method should be used. The main disadvantage of the curcumin method is that the test solution must be evaporated to dryness. This is a disadvantage only as concerns ease and time of sample preparation.

This preliminary study has shown that the curcumin method is worthy of further study because of its great sensitivity and because it may be adaptable to a rapid field test. The methods requiring sulfuric acid would not be suitable for rapid field test. The methods requiring sulfuric acid would not be suitable for rapid field tests such as a test in which the color is developed on paper or some other absorbant. The next section of this thesis concerns a study of the various modifications of the curcumin method for boron determination.

TABLE III (***)

Reagent	Best Wave-length	Spectra Separation	Sensitivity			Reproducibility	Time for Development		Adherence to Beer's Law	Effect of Change in the Sulfuric Acid Concentration						
			*R	B/10ml per 0.001 Absorbance	R		R	Min.		R	R	Range of Water per 10 ml	**Boron per 10 ml per 1% change			Ave.
	N	Comments				/10ml	R						0 B	5 B	10 B	B
Quinalizarin	620	Poor	3	0.018	2	± 0.1 to ± 0.6	2	0-5	1	Deviates slightly over entire range	2	0.2- 0.5 0.5- 1.0 1.0- 2.0	1.26 0.88 0.48	0.69 1.21 0.96	0.68 0.95 1.43	Ave. 0.95 2
Carminic Acid	610	Good	1	0.020	2	± 0.1	1	50	3	Follows Beer's Law	1	0.2- 0.5 0.5- 1.0	0.04 0.04	0.30 0.04	1.16 0.03	Ave. 0.27 1
Alizarin Blue S	650	Fair	2	0.067	4	± 0.1	1	20	2	Follows Beer's Law	1	0.2- 0.5 0.5-1.0 1.0- 2.0	1.24 0.87 0.60	1.20 1.33 0.85	1.60 1.20 1.31	Ave. 1.13 2
Alizarin Red S	575	Fair	2	0.071	4	± 0.3	2	100	4	Follows Beer's Law	1	0.2- 0.5 0.5- 1.0 1.0- 2.0	0.89 0.60 0.59	1.19 1.37 1.00	1.19 1.10 1.06	Ave. 1.00 12
1-Amino-4-hydroxy-anthraquinone	550	Good	1	0.125	5	$\pm .1$ to ± 0.7	2	150 or larger	5	Follows Beer's Law	1	0.2- 0.5 0.5- 1.0 1.0- 2.0	0.88 0.70 1.52	1.04 0.78 1.74	1.25 0.83 1.97	Ave. 1.19 2
Chromotrope-2B	650	Poor	3	0.035	3	$\pm .3$	2	0-5	1	Deviates slightly from Beer's Law	2	0.2- 0.5 0.5- 1.0 1.0- 2.0	0.28 0.33 0.25	0.48 0.23 0.14	0.76 0.09 0.00	Ave. 0.28 1
Turmeric	575	Good	1	0.0026	1	± 0.08 to ± 0.10	1	15 Min for evap.	2	Follows for short range	1					

*R - Rating

** Error in micrograms caused by a change in sulfuric of 1% for the amount of boron indicated.

*** Reproduced from Report by W. A. Powell - Tech. Rept Subcontract N-4960 Sept. 1953 Callery Chemical Co.

III A Study of the Curcumin Solution Method for Boron Determination

Though the curcumin method for boron determination has been widely used and reported in the literature much can be gained from a study of the variables affecting this method. The Naftel (77) method, a refinement of the method of Cassel and Gerrans (18), is probably the most popular method employing curcumin for the determination of boron. The Hegedus (50) method may be considered a modification of the Naftel method. Since the preliminary study showed the Hegedus method to be quite sensitive and deserving more attention, this method and the Naftel method were investigated more thoroughly.

In each case it was first necessary to find the best wave length at which to measure the color intensity of the boron-curcumin complex. The best wave length was found from a study of the absorption spectra of the curcumin-boron complex. Also it was necessary to know the effect of the concentrations of the various reagents (curcumin, oxalic acid and, when used, HCl) on the sensitivity of the method. The evaporation step is probably the most important in the entire determination. Thus it was necessary to determine the optimum temperature for evaporation. For each method a standard curve was drawn and the reproducibility of the method determined.

A. Hegedus Method

The exact experimental procedure of the Hegedus method has been given earlier in this thesis. Here, as in the preliminary study, one modification of the Hegedus procedure was made; the solutions were evaporated to dryness on a steam bath (unless otherwise stated) rather than at 55°C. The 20 ml of 95% ethanol was added after the residue had cooled 10 minutes, and the absorbance was measured 20 minutes after the addition of ethanol. In all of the colorimetric measurements water was used as the zero absorbance reference.

Absorption Spectra

In the preliminary study 575 mu was chosen as the best wave length at which to measure the color intensity of boron-curcumin complex solutions. The boron-curcumin complex, in this case, was prepared from a solution containing 1000 micrograms of boron. However the resulting complex may yield a different absorption spectra than a complex formed from a solution containing only a few micrograms of boron. Thus, using the modified Hegedus procedure, complex solutions were prepared from a solution containing 10 micrograms of boron and from a saturated boron solution. The absorption spectra of these complexes were determined. Also the absorption spectra of the curcumin reagent, carried through the evaporation procedure using 2 ml of distilled water as the sample, was determined. The absorption spectra were determined by reading the absorbance of the test solution at 25 minute intervals between 400 mu and 700 mu.

The absorption spectra, obtained by plotting absorption versus wave length, of the reagent-boron complex are shown in Figure 4. The curcumin reagent has maximum absorbance below 475 mu and minimum absorbance above 600 mu. The complexes formed from the 10 microgram boron solution and from the saturated boron solution had nearly the same absorption spectra; they had maximum absorption below 575 mu and minimum absorption above 650 mu. That the complex formed with 10 micrograms of boron and the complex formed from the saturated boron solution have similar absorption spectra should be expected since it was found that maximum absorption was reached above 8 micrograms of boron. Indeed for this reason the method is not useful for quantiles of boron greater than 8 micrograms.

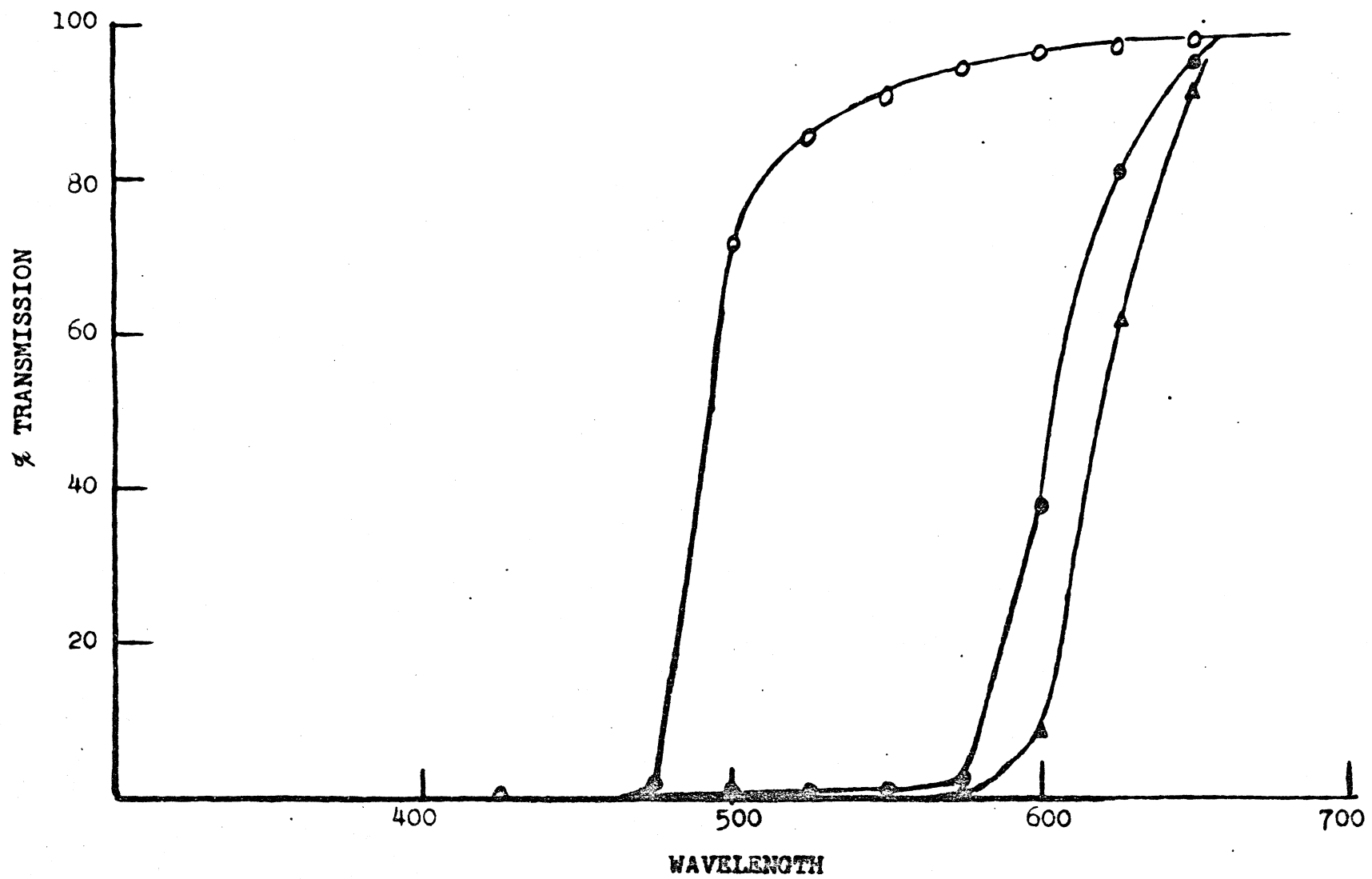
In colorimetric analysis the best wave length to read the absorbance of the test solution is that wave length where the reagent and test solution

Figure 4

Absorption Spectra of Boron-Curcumin Complex
and of Curcumin Reagent

(Modified Hegedus Method, Evaporation at 100°C)

- Curcumin Reagent
- Boron-Curcumin Complex (10 microgram boron sample)
- △ Boron-Curcumin Complex (saturated boron sample)



have the greatest spectra separation. Also it is important that the slope of the absorption spectra curve be zero (small change in absorbance per change in wave length). Usually this wave length occurs where the test solution shows maximum absorbance and the reagent shows minimum absorbance. For the boron-curcumin system the best wave length was found to be 550 mμ; here the complex and reagent curves are relatively flat and have maximum separation. In larger amounts of boron a wave length of 575 mμ may be used, as was suggested in the preliminary work, though beyond 575 mμ the absorbance of the complex begins to decrease sharply.

As would be expected the absorption spectra was affected by a change of boron concentration below 10 micrograms of boron. Samples containing 0, 2, 5 and 10 micrograms of boron and a sample saturated with boron (made up to two ml with water) were carried through the Hegedus procedure. However, in this case, the solutions were evaporated to dryness at 55 to 60°C. The absorption spectra of the saturated boron complex and the 10 microgram boron complex almost coincided and were quite similar to the absorption spectra of these same complexes prepared at steam bath temperatures (see preliminary study). The absorbance of the 2 microgram boron complex begin to decrease rather sharply above 525 mμ whereas the higher boron complexes did not begin to decrease until 550 mμ or 560 mμ was reached. At the lower boron concentrations the greatest spectra separation was still at 550 mμ. The absorption spectra of the reagent solution (i.e. the zero microgram boron sample) carried through the evaporation did not coincide with the spectra of the pure reagent, indicating that the evaporation had some effect on the absorption spectra of the reagent. Based upon this study 550 mμ is probably the best wave length for small amounts of boron. The absorption spectra referred to here are shown in Figure 5.

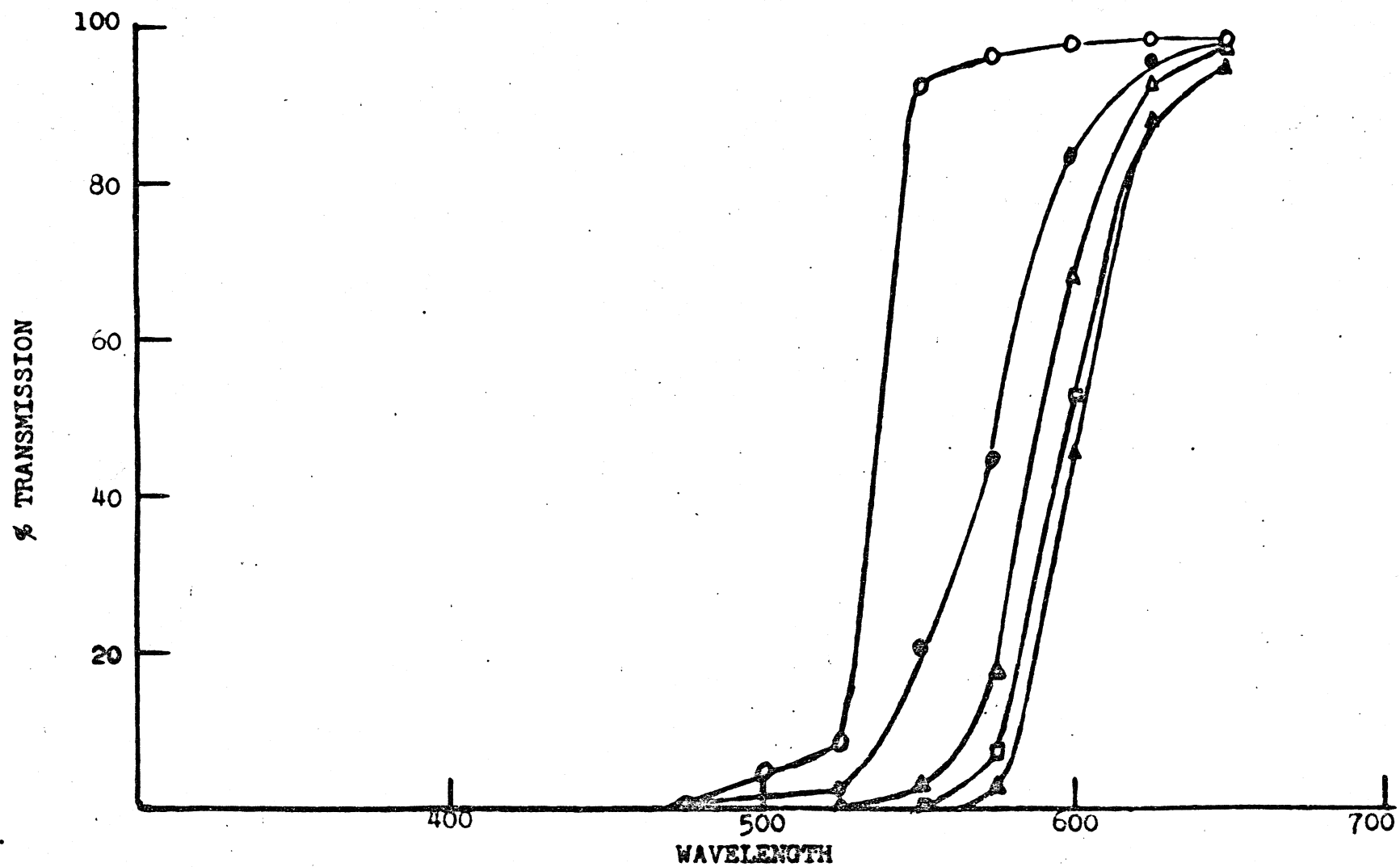
Figure 5

Absorption Spectra of Boron-Curcumin Complex

and of Curcumin Reagent

(Modified Hegedus Method, Evaporation at 55 to 60°C)

- Curcumin Reagent
- Boron-Curcumin Complex (2 microgram boron sample)
- △ Boron-Curcumin Complex (5 microgram boron sample)
- ▲ Boron-Curcumin Complex (10 microgram boron sample)
- ▣ Boron-Curcumin Complex (saturated boron sample)



Evaporation Temperature and Time of Extra Heating

One of the most important parts of this study was the investigation of the evaporation step. Naftel (77) recommended an evaporation temperature of $55^{\circ}\pm 3^{\circ}\text{C}$, plus 30 minutes extra heating at this temperature after dryness was reached. Spicer and Strickland (96) recommended 50°C with exact timing of the evaporation step. Coursier and co-workers (22,23) evaporated to dryness at $106^{\circ}\pm 1^{\circ}\text{C}$ for 60 ± 3 minutes. The important point here is to obtain a complete reaction without loss of boron. If the rate of evaporation is too fast, the solvent may be lost before the reaction is complete and the complex solution will not reach maximum color development. If the evaporating, i.e. the heating, is carried too far loss of boron results and again the complex solution does not reach maximum color development. It is apparent that a study of the evaporation temperature and time of extra heating is important.

Though Hegedus recommended an evaporation temperature of $55\pm 3^{\circ}\text{C}$, the preliminary study indicated that at this temperature the reaction was not complete. Also at this temperature the precision was poorer than at higher temperatures. In an attempt to obtain complete reaction at 55°C the following experiment was conducted. Six samples containing 5 micrograms of boron were prepared according to the Hegedus procedure. Three of the samples were evaporated to dryness at 50 to 60°C and heated an extra 30 minutes at this temperature. The other three samples were evaporated to dryness at steam bath temperature (100°C) and no extra heating was used. In this experiment the absorbance of the final solution was measured at 575 mu. The results of this experiment are given in Table IV.

TABLE IV

Reproducibility at Different Evaporation Temperatures

Absorbance of solutions evaporated at 50 to 60°C plus 30 minutes extra heating:	Absorbance of solutions evaporated at steam bath temperature:
1.09	1.10
1.11	1.13
1.11	1.05
Avg 1.10±0.01	1.09±0.03

As shown by the average absorbance values, the reaction reached the same degree of completeness at each temperature. The reproducibility at 50 to 60°C was greatly improved by the 30 minutes extra heating, in fact the reproducibility was better at 50 to 60°C than at steam bath temperature. The reproducibility at steam bath temperature was still the same and the overall results were about the same at the two temperatures, indicating that little boron was lost at the higher temperature. After seven hours of standing, open to the atmosphere, the solutions prepared at 50 to 60°C with 30 minutes extra heating had the following absorbance, 1.04, 1.06, and 1.06. Based on the standard curve this represented a decrease in amount of boron found of 0.2 micrograms. This indicates good stability of the complex solutions.

There is a chance, however, that boron may be lost if the reaction solutions are heated too long at steam bath temperature. A temperature intermediate between 50 to 60°C and steam bath temperature may be desirable. Thus the following experiment was performed. Six solutions containing 2 micrograms of boron were prepared according to the Hegedus procedure. Two of these solutions were evaporated to dryness at 55 to 60°C, two at 75 to 80°C, and two at 100°C (steam bath temperature). The effect of extra heating after dryness at these temperatures was also studied. Six more solutions containing

2 micrograms of boron were prepared as described above but each of these solutions were heated an extra 30 minutes after dryness was reached. The absorbance of the boron complex solutions was measured at 550 mu and these measurements are given in Table V.

TABLE V

Effect of Extra Heating at Different Evaporation Temperatures

<u>Temperature</u>	<u>Absorbance</u>	
	<u>No Extra Heating</u>	<u>30 Minutes Extra Heating</u>
55 to 60°C	0.75	0.86
	0.65	0.81
75 to 80°C	0.89	0.99
	0.74	0.99
100°C	0.77	0.45
	0.79	0.56

With no extra heating the evaporation at 100°C gave the best precision, but with 30 minutes extra heating the best precision was at 75 to 80°C. The precision was relatively good at 55 to 60°C with extra heating. The reaction was more complete at 75 to 80°C with 30 minutes extra heating. Apparently at the lower temperature, even with extra heating, the reaction was not quite complete. At 100°C with no extra heating the absorbance values were low indicating either incomplete reaction or loss of boron. However, extra heating at 100°C definitely resulted in loss of boron.

To obtain a more complete understanding of the effect of extra heating after dryness twelve solutions containing 2 micrograms of boron were prepared according to the Hegedus procedure. Six of these solutions were evaporated to dryness at 55 to 60°C and six at 75 to 80°C. Each solution was then heated an extra length of time, 0, 10, 20, 30, 40 or 50 minutes. The absorbance of the boron complex solutions, measured at 550 mu, are given in Table VI:

TABLE VI

Effect of Temperature and Extra Heating on the Absorbance

Time of Extra Heating at 55 to 60°C	Absorbance				
	Set #1	Set #2	Set #3	Set #4	Avg.
0 min.	0.83	0.71	0.66	0.81	0.75±0.17
10 min.	0.77	0.81	0.71	0.82	0.78±0.04
20 min.	0.78	0.75	0.72	0.85	0.78±0.04
30 min.	0.87	0.82	0.79	0.88	0.84±0.04
40 min.	1.01	0.86	0.82	1.06	0.94±0.10
50 min.	0.91	0.85	0.74	0.94	0.86±0.06

Time of Extra Heating
at 75 to 80°C

0 min.	0.76	—	0.90	0.83±0.07
10 min.	0.82	0.85	0.94	0.87±0.05
20 min.	0.92	0.87	0.94	0.91±0.02
30 min.	0.92	0.92	0.91	0.92±0.00
40 min.	0.90	0.96	0.97	0.94±0.03
50 min.	1.02	—	0.91	0.96±0.06

There was an increase in absorbance with increase in extra heating time at 55 to 60°C, the absorbance leveled off with 30 to 40 minutes extra heating and decreased beyond this point. Above 40 minutes extra heating loss of boron occurred. The precision was better with 30 minutes extra heating than with 40 minutes extra heating. At 75 to 80°C a similar increase in absorbance with increase in extra heating time was noted though this increase was not as great. This should be expected since the reaction was found to be more complete at this temperature even without extra heating. The reaction was essentially complete with 20 to 30 minutes extra heating at 75 to 80°C.

Based upon the preceeding temperature studies either 55 to 60°C with 30 to 40 minutes extra heating or 75 to 80°C with 20 to 30 minutes extra could be used. Since the evaporation time was shorter at 75 to 80°C, this would

seem to be the more desirable evaporation temperature. To reduce the evaporation time even more the steam bath temperature, carrying the solution just to dryness, could be used; though with less dependability than the lower temperatures.

To be able to judge when the solution has reached dryness is a most important part of the evaporation procedure. One can readily understand that poor control of this step leads directly to poor precision and accuracy in the final analysis. Silverman and Trego (93) state that the residue is dry when square crystals appear in the evaporation residue. During this study the appearance of square crystals in the evaporation residue was noted, though it was felt that this would be a poor way to ascertain dryness. Other investigators (79,96) have stated that the evaporation step must be exactly timed. It was noted during this investigation that the evaporation to dryness at 55 to 60°C usually took 65 ± 5 minutes, at 75 to 80 C 31 ± 1 minute, and at 100 C 17 ± 1 minute. Exact timing does not appear to be an entirely satisfactory method of determining when the solution has reached dryness, though it could probably be used in the case of evaporations at 75 to 80°C and 100°C. At the risk of being too vague it can be said that familiarity with the system and the evaporation step will enable one to judge when dryness has been reached.

Concentration of Oxalic Acid and Curcumin "eagent"

An experiment was conducted to determine whether or not there is an optimum concentration of oxalic acid necessary for the reaction. To the boron sample, held constant at two micrograms of boron in 2 ml of water, 2 ml of the curcumin reagent (0.1% curcumin in glacial-acetic acid with no oxalic acid) was added. Then varying amounts of an oxalic acid solution (5 gm oxalic acid in 100 ml of glacialacetic acid) were added to the boron-reagent mixtures. The reaction solutions were made up to a constant volume

by adding appropriate volume of glacialacetic acid. The solutions were then evaporated to dryness at 75 to 80°C and heated an extra 20 minutes. The results are given in Table VII.

TABLE VII

Effect of Oxalic Acid on Absorbance

<u>Oxalic Acid Solution ml</u>	<u>Acetic Acid ml</u>	<u>Absorbance at 550 mu</u>
0.0	3.0	0.09
0.5	2.5	0.55
1.0	2.0	0.46
1.5	1.5	0.45
2.0	1.0	0.48
3.0	0.0	0.48

The data show that oxalic acid does increase the absorbance of the boron curcumin complex solution. There was a sharp increase in absorbance with the initial addition of oxalic acid in this test, there after with increased amounts of oxalic acid the absorbance decreased slightly and then became nearly constant. Since these results represent only one set of determinations the apparent absorbance maximum obtained with 0.5 ml of the oxalic acid solution cannot be regarded too highly. However, it can be said that essentially maximum absorbance was reached with the larger amounts of oxalic acid. Surely this was the case when the oxalic acid saturated reagent was used in the work thus far reported.

For all studies thus far reported two ml of a 0.1% curcumin solution was used. This was considered to be in excess of the amount actually needed for complete reaction with all the boron present. To determine the optimum volume of 0.1% curcumin needed for the reaction the volume of curcumin reagent added to the boron sample was varied from 0 to 4.0 ml. The curcumin reagent was prepared by dissolving 0.1 gm of curcumin in 100 ml of glacial acetic acid that had been saturated with oxalic acid. The total volume of the reaction mixture was kept constant by adding an appropriate volume of glacial acetic

acid that had been saturated with oxalic acid. For this study the sample contained two micrograms of boron in two ml of aqueous solution. The solutions were evaporated to dryness at 75 to 80°C and heated an extra 20 minutes at this temperature.

The results of this experiment (Table VIII) show that the absorbance began to level off after 2 ml of reagent had been added but did not reach a maximum until 3.5 ml of reagent had been added. Actually the absorbance values of the solutions with two or more ml of curcumin reagent were very close to one another, the average of all these absorbance values being 0.86 ± 0.03 . The average deviation of ± 0.03 represents ± 0.1 micrograms of boron and this is within the reproducibility limits of the method. Thus essentially maximum absorbance was reached with 2 ml of the curcumin reagent.

TABLE VIII

Effect of Curcumin Concentration on Absorbance

<u>Volume of Curcumin</u>	<u>Volume of Acetic Acid</u>	<u>Absorbance at 550 mμ</u>	
		<u>Set #1</u>	<u>Set #2</u>
0.0 ml	4.0 ml	0.00	--
0.5	3.5	0.34	--
1.0	3.0	0.53	--
1.5	2.5	0.62	0.71
2.0	2.0	0.79	0.85
2.5	1.5	0.84	0.81
3.0	1.0	--	0.89
3.5	0.5	0.87	0.93
4.0	0.0	0.86	0.91

Sensitivity, Reproducibility, and Stability

Next it was necessary to construct a standard calibration curve to check the adherence of the color reaction to Beer's Law. From the standard curve the sensitivity may be determined. The modified Hegedus procedure was used to obtain the necessary data for a standard curve. To the sample solutions, 0.0 to 5.0 micrograms of boron in 2 ml of water was added 2 ml of curcumin reagent. The reagent was prepared by dissolving 0.1 gm of curcumin in 100

ml of glacial acetic acid that previously had been saturated with oxalic acid. The solutions were evaporated to dryness at 55 to 60°C and heated an extra 30 minutes at this temperature. Each residue was taken up in 20 ml of 95% ethanol and the absorbance measured at 550 mu (Table IX).

TABLE IX
Data for Standard Curve
(Evaporation Temperature - 55 to 60°C)

<u>Micrograms of Boron</u>	<u>Absorbance at 550 mu</u>		<u>Average Absorbance</u>
	<u>Set #1</u>	<u>Set #2</u>	
0.0	0.08	0.09	0.08±0.00
1.0	0.42	0.40	0.41±0.01
2.0	0.72	0.77	0.74±0.02
3.0	1.07	1.13	1.10±0.03
4.0	1.47	1.52	1.50±0.02
5.0	1.65	1.84	1.74±.10

The standard curve (Figure 6) was obtained by plotting average absorbance versus micrograms of boron. That this plot resulted in a straight line showed that the boron-curcumin reaction adheres to Beer's Law over the range 0 to 5 micrograms of boron. The sensitivity of this procedure was found to be 0.029 micrograms of boron per 20 ml per 0.01 absorbance unit.

A standard curve was also drawn from data collected using an evaporation temperature of 75 to 80°C and 20 minutes extra heating at this temperature. The sample solutions were prepared in the same way as described above. Under these conditions the boron curcumin reaction also adheres to Beer's Law over the range 0.0 to 5.0 micrograms of boron. The sensitivity was found to be 0.033 micrograms of boron per 20 ml per 0.01 absorbance unit. The data for this curve is given in Table X and the curve is drawn in Figure 6.

Figure 6

Standard Curves for Modified Hegedus Method
at Different Evaporation Temperatures
Absorbance Versus Micrograms of Boron

- Evaporation Temperature 75 to 80°C
- Evaporation Temperature 55 to 60°C

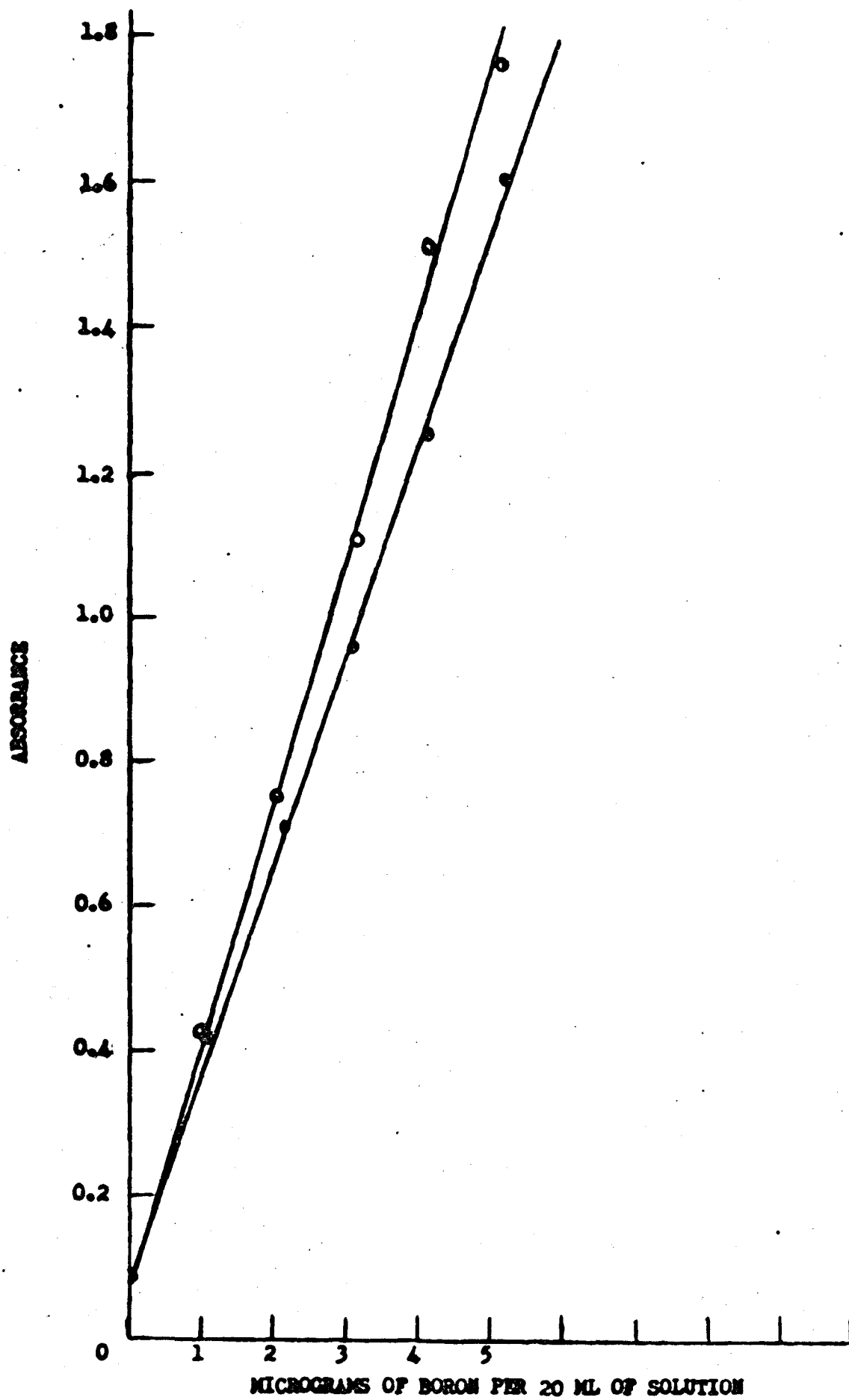


TABLE X

Data for Standard Curve
(Evaporation Temperature - 75 to 80°C)

<u>Micrograms of Boron</u>	<u>Absorbance at 550 mu</u>		<u>Average Absorbance</u>
	<u>Set #1</u>	<u>Set #2</u>	
0.0	0.10	0.07	0.08±0.02
1.0	0.47	0.37	0.42±0.05
2.0	0.71	0.69	0.70±0.01
3.0	0.97	0.95	0.96±0.01
4.0	1.24	1.27	1.26±0.02
5.0	1.52	1.66	1.59±0.07

The data used to plot these standard curves showed a reproducibility of 0.1 to 0.2 micrograms of boron. The sensitivity of this procedure, at each evaporation temperature, was better than previously reported (in the preliminary survey) for the Hegedus procedure. In the earlier work, however, the absorbance was measured at 575 mu and 575 mu has been shown to be a poor wavelength to use for small amounts of boron.

The final alcoholic extracts were found to be very stable over a period of 3½ hours. Sample solutions containing 1.0 and 2.0 micrograms of boron were prepared according to the modified Hegedus procedure, evaporated to dryness at 55 to 60°C with 30 minutes extra heating, and were taken up in 20 ml of 95% ethanol. The absorbance of these solutions was measured at 550 mu over a period of 3½ hours. The solutions were left in the absorption cells during this 3½ hour period. The results are given in Table XI. A portion of these final solutions were left in soft glass test tubes open to the atmosphere. The absorbance of these solutions was measured after 3½ hours and under these conditions the solutions were found to be quite stable (Table XI). In Table XI the column entitled "Time Elapsed" refers to the period of time that had elapsed since alcohol was added to the evaporation residue.

TABLE XI

Stability of Final Alcoholic Extract
of Boron-Curcumin Complex

<u>Time Elapsed</u> <u>Minutes</u>	<u>Micrograms of Boron</u> <u>Present</u>	<u>Micrograms of Boron</u> <u>Found</u>	<u>Time Elapsed</u> <u>Minutes</u>	<u>Micrograms of Boron</u> <u>Present</u>	<u>Micrograms of Boron</u> <u>Found</u>
15	1.00	0.96	4	2.00	2.03
40	1.00	0.95	29	2.00	2.03
70	1.00	0.92	59	2.00	1.97
100	1.00	0.90	89	2.00	1.93
130	1.00	0.90	119	2.00	1.91
160	1.00	0.88	149	2.00	1.90
190	1.00	0.87	179	2.00	1.88
220	1.00	0.86	209	2.00	1.85
*220	1.00	0.88	*209	2.00	1.93

*Solutions left in soft glass test tubes for 3½ hours

B. Naftel Method

Since the Hegedus procedure is only a modification of the Naftel (77) procedure and since there may be some advantage to using ethanol rather than glacial acetic acid as the solvent, a study of the variables affecting the Naftel procedure was made. The exact experimental procedure of the Naftel method is as follows:

To the boron sample add 1 ml of an oxalic acid - HCl solution (prepared by mixing 80 ml of 20% oxalic acid solution and 20 ml of concentrated HCl), and to this solution add 2 ml of 0.1% curcumin (or 1.0% turmeric) in ethanol. Evaporate the test solution to dryness at 55±3°C and continue heating for 30 minutes at this temperature. Extract the residue with 25 ml of 95% ethanol and measure the absorbance at 540 mμ.

For this study the Naftel procedure was modified. Earlier studies showed that maximum absorbance was obtained in saturated oxalic acid solutions. Thus, to assure maximum color development, the curcumin reagent was prepared by dissolving 0.1 gm of curcumin in 95% ethanol that had previously been saturated

with oxalic acid. To the boron sample made up to 1.0 with water was added 2.0 ml of the curcumin reagent and 5 drops (or 0.2 ml) of 1:1 HCl. The amount of HCl used was somewhat less than the amount suggested by Naftel, but a study reported later in this thesis showed that this quantity was sufficient for the reaction. The test solutions were then evaporated to dryness at 55 to 60°C and heated an extra 30 minutes at this temperature (except during the temperature studies when other temperatures and periods of extra heating were used). The residue was taken up in 20 ml of 95% ethanol and the absorbance was measured at 550 mμ or 575 mμ.

Absorption Spectra

Since the final boron-curcumin complex should be the same whether prepared by the Hegedus or the Naftel procedure no study of the absorption spectra was made in this investigation of the Naftel method. From the previous study of the Hegedus method it was found that for small quantities of boron 550 mμ was the best wavelength; for larger quantities 575 mμ was best. This will become apparent in the following investigation as many of the absorbance measurements were made at both wavelengths. Naftel studied the absorption spectra of the boron-curcumin complex and found 540 mμ to be a good wavelength to measure the absorbance of the complex for quantities of boron in the range 0 to 8 micrograms.

Evaporation Temperature and Time of Extra Heating

Earlier studies have shown the evaporation step to be one of the most critical steps in a procedure of the Naftel type. It is during the evaporation and extra heating after dryness that boron-curcumin reaction takes place. Obviously, poor control of these steps results in poor precision and accuracy in the analysis. To find the optimum evaporation temperature and period of extra heating the effect of higher evaporation temperatures and various periods of extra heating on the absorbance were studied.

In the first study temperatures of 55 to 60°C, 75 to 80°C, and 100°C with no extra heating were used and in the second study the same temperatures with 30 minutes extra heating were used. The test solutions were prepared by adding to the boron samples (2 micrograms) 0.2 ml of 1:1 HCl and enough water to make the total volume 2.0 ml. Then 2.0 ml of the curcumin reagent was added, and the solutions were evaporated to dryness at the specified temperature. One set of solutions was heated an extra 30 minutes at the specified temperature, while another set of solutions received no extra heating. The residues were taken up in 20 ml of 95% ethanol and the absorbance measured at 575 mu. The results are given in Table XII.

TABLE XII

Effect of Temperature and Extra Heating on Absorbance

Temperature °C	Sample	Absorbance at 757 mu	
		No Extra Heating	30 Min. Extra Heating
55 to 60°C	#1	0.34	0.66
	#2	0.26	0.62
75 to 80°C	#1	0.47	0.38
	#2	0.41	0.33
100°C	#1	0.10	0.05
	#2	0.10	0.10

Complete reaction was not obtained at 55 to 60°C with no extra heating. The highest absorbance value was reached at 55 to 60°C with 30 minutes extra heating. At 100°C, even without extra heating, and at 75 to 80°C with extra heating boron was lost. Apparently at 75 to 80°C with no extra heating boron was also lost, though the data could also indicate incomplete reaction.

A further study of the effect of extra heating was made. Solutions containing 5 micrograms of boron were prepared according to the modified Naftel procedure, evaporated to dryness at 55 to 60°C, and then heated for different lengths of time after dryness. The absorbance was measured both

at 575 mu and at 550 mu. The results are given in Table XIII.

TABLE XIII

Effect of Extra Heating on Absorbance

<u>Extra Heating Period</u>	<u>Absorbance</u>	
	<u>At 550 mu</u>	<u>At 575 mu</u>
0 Minutes	1.26 (0.59)	0.69 (0.35)
10 Minutes	2.09 (2.04)	1.22 (1.19)
20 Minutes	2.04 (1.98)	1.19 (1.17)
30 Minutes	2.32 (2.24)	1.36 (1.32)
40 Minutes	2.40 (2.32)	1.48 (1.44)
50 Minutes	2.40 (2.31)	1.49 (1.46)

The numbers in parenthesis indicate absorbance values measured one hour after original absorbance values were measured.

These results show that maximum absorbance was not developed with 30 minutes extra heating. Maximum absorbance was developed, and thus the reaction was complete, with 40 minutes extra heating. Powell (84) found that with 2.5 micrograms of boron the reaction was complete after 30 minutes extra heating. For larger amounts of boron then, it may be necessary to increase the period of extra heating to 40 minutes. However, good reproducibility was obtained with only 30 minutes extra heating.

When the total volume of test solution was approximately 3 ml (2 ml curcumin, 1 ml boron sample and 5 drops of HCl) the evaporation to dryness at 55 to 60°C took 30±3 minutes. When the total volume of test solution was 4 ml (2 ml curcumin 2 ml aqueous solution of boron sample and HCl) the evaporation to dryness at 55 to 60°C took 42±4 minutes. At 75 to 80°C with a volume of 4 ml the evaporation took 22±3 minutes. In these cases exact timing could probably be used to ascertain when the solution has reached dryness though the author feels that familiarity with the system and the evaporation step will enable one to judge when the solution has reached dryness.

In Table XIII the numbers in parenthesis are the absorbance values of the same solutions (described in the third paragraph preceeding) measured one hour after the original measurements were made. All that had received some extra heating were stable, as the absorbance of these solutions decreased only a small amount (an amount equivalent to 0.2 micrograms of boron). However, the solution that had not been heated for an extra period beyond dryness had lost 50% of its absorbance. Though this is based on only one run, it would seem that the more complete the reaction the more stable is the final complex solution.

Concentration of Reagents

Though little has been said about the part HCl takes in the reaction it does have an effect on the color intensity of the boron-curcumin complex. Therefore, it was necessary to find the optimum amount of HCl necessary for the reaction. To solutions containing 3, 5 and 7 micrograms of boron, volumes of 1:1 HCl from 0.0 to 1.0 ml were added. Enough water was added to make the total volume 2.0 ml, then 2.0 ml of curcumin reagent was added, the solutions were evaporated to dryness at 55 to 66°C and were heated an extra 30 minutes at this temperature. The residues were taken up in 20 ml of 95% ethanol and the absorbance was measured at 575 mμ. The results are given in Table XIV. All values represent the average of duplicate runs.

TABLE XIV

Effect of HCl on The Sensitivity

<u>Volume of HCl</u>	<u>3 Micrograms</u>	<u>5 Micrograms</u>	<u>7 Micrograms</u>
0.0 ml	0.81	1.13	1.68
0.1 ml	0.81	1.38	1.72
0.2 ml	0.90	1.37	1.79
0.5 ml	0.96	1.44	1.90
1.0 ml	0.78	1.19	1.22

These results show that the absorbance and the sensitivity increased as the volume of 1:1 HCl was increased up to 0.5 ml and then dropped sharply for 1.0 ml of 1:1 HCl. The absorbance and sensitivity for 0.2 ml of HCl was nearly the same as that for 0.5 ml of HCl. Any volume of 1:1 HCl between 0.2 ml and 0.5 ml should be satisfactory. Naftel suggested an amount equal to 0.4 ml of 1:1 HCl and this checks closely with the results of this test. Silverman and Trego (93) suggested an amount approximately equal to 0.2 ml of 1:1 HCl. Actually the sensitivity was relatively good with no HCl present. In a later study Powell (84) substantiated these results, and further found that in the absence of both water and HCl the sensitivity was just as good as that obtained with the optimum amount of HCl. Apparently HCl only helps the reaction to completion and, in small amounts, has little effect on the sensitivity of the method. Excess HCl was found to retard the reaction. Muraki and Huro (74) report that HCl interferes with the reaction, though the abstract of their work did not mention the amount of HCl that causes interference.

No specific study was made of the optimum concentration of curcumin reagent and oxalic acid necessary for complete reaction. It is well established that oxalic acid does increase the sensitivity of the boron-curcumin reaction. Previous work with the Hegedus method indicated that the boron curcumin reaction in saturated oxalic acid solutions reached maximum absorbance and sensitivity. Based on the solubility of oxalic acid in ethanol at 15°C the weight of oxalic acid in 2 ml of the curcumin reagent used in this modified Naftel procedure would be approximately 0.4 gm. Powell (84) reported that maximum absorbance was reached with 0.5 gm of oxalic acid. Naftel suggested an amount equal to 0.4 gm of added oxalic

acid and stated that excess oxalic acid had little effect. The boron-curcumin reaction does take place in the absence of oxalic acid and, though less sensitive, may be used for boron analysis.

One test was carried out according to the Naftel procedure except oxalic acid and HCl both were left out of the reaction. Solutions containing 5 micrograms of boron were evaporated to dryness at 55 to 60°C and heated an extra 30 minutes. No color developed in these solutions. Pollio (80) also reported no color development in the absence of both oxalic and HCl. Apparently the reaction requires an acid medium since the color did develop when only the oxalic acid or only the HCl was present. The oxalic acid sensitizes the reaction and the HCl catalyzes the reaction.

In the work with the Hegedus method essentially maximum absorbance (for the range 0 to 5 micrograms of boron) was reached with 2 ml of 0.1% curcumin reagent. This was the quantity of curcumin suggested by Naftel. Gol'tman and Gurevich (40) state that it is necessary to have 100 micrograms of curcumin for 0.8 micrograms of boron. The essential point is to have enough curcumin to complex all the boron present and enough excess curcumin to press the reaction to completion. It was felt that the amount of reagent used in this work fulfilled this requirement.

Sensitivity, Reproducibility and Stability

Using the modified Naftel procedure, sufficient data were collected to construct a standard calibration curve. Solutions containing 0 to 10 micrograms of boron made up to 1 ml with water, 2 ml of 0.1% curcumin reagent (in 95% ethanol saturated with oxalic acid), and 5 drops of 1:1 HCl were evaporated to dryness on a water bath at 55 to 60°C and heated an extra 30 minutes at this temperature. The residue was taken up in 20 ml of 95% ethanol and the absorbance measured at 575 mμ or at 550 mμ. Standard calibration curves were drawn for each wavelength by plotting absorbance versus micrograms of boron

(Figure 7). The data for these curves are given in Table XV.

TABLE XV

Data for Standard Curve

Micrograms of Boron	Set #1	Absorbance at 575 mu					Avg. Dev.	Set #1	Absorbance at 550 mu			Avg. Dev.
		#2	#3	#4	#5	Avg			#3	#4	Avg	
0	0.05	0.04	0.04	0.04	---	0.04±0.00	0.09	0.07	0.06	0.07±0.01		
1	0.32	0.27	---	---	---	0.30±0.02	0.55	---	---	---	---	---
2	0.54	---	---	---	---	0.54	0.92	---	---	---	---	---
3	0.78	0.74	---	---	0.68	0.73±0.04	1.36	---	---	---	---	---
4	---	1.02	---	---	0.96	0.99±0.03	---	---	---	---	---	---
5	1.24	1.19	1.19	1.25	1.28	1.23±0.05	2.15	2.21	2.25	2.20±0.04		
6	1.48	---	---	---	---	1.48	*	---	---	---	---	---
10	---	---	2.30	2.37	---	2.34±0.04	---	*	*	---	---	---

*Absorbance was too great to be measured at this wavelength

The sensitivity of the method was greater when the absorbance was measured at 550 mu, but a wider range of boron could be determined at 575 mu. At 550 mu the sensitivity of the method was 0.024 micrograms of boron per 20 ml per 0.01 absorbance unit, and at 575 mu 0.041 micrograms of boron per 20 ml per 0.01 absorbance unit. However, when the absorbance was measured at 550 mu the reaction adhered to Beer's Law only over the range 0 to 5 micrograms of boron, while at 575 mu the reaction followed Beer's Law over the range 0 to 8 micrograms of boron. At 575 mu the range of boron determined could possibly be extended to 10 micrograms though at this level of boron the curve begins to deviate from Beer's Law. The reproducibility at 575 mu was ±0.2 micrograms of boron per 20 ml, and at 550 mu ±0.1 micrograms of boron per 20 ml.

Using the same experimental procedure as described above, a standard curve for the boron-curcumin reaction in the absence of oxalic acid was constructed. The reaction in the absence of oxalic acid proved (See Figure 7) and Table XVI) to be less sensitive than in the presence of oxalic acid.

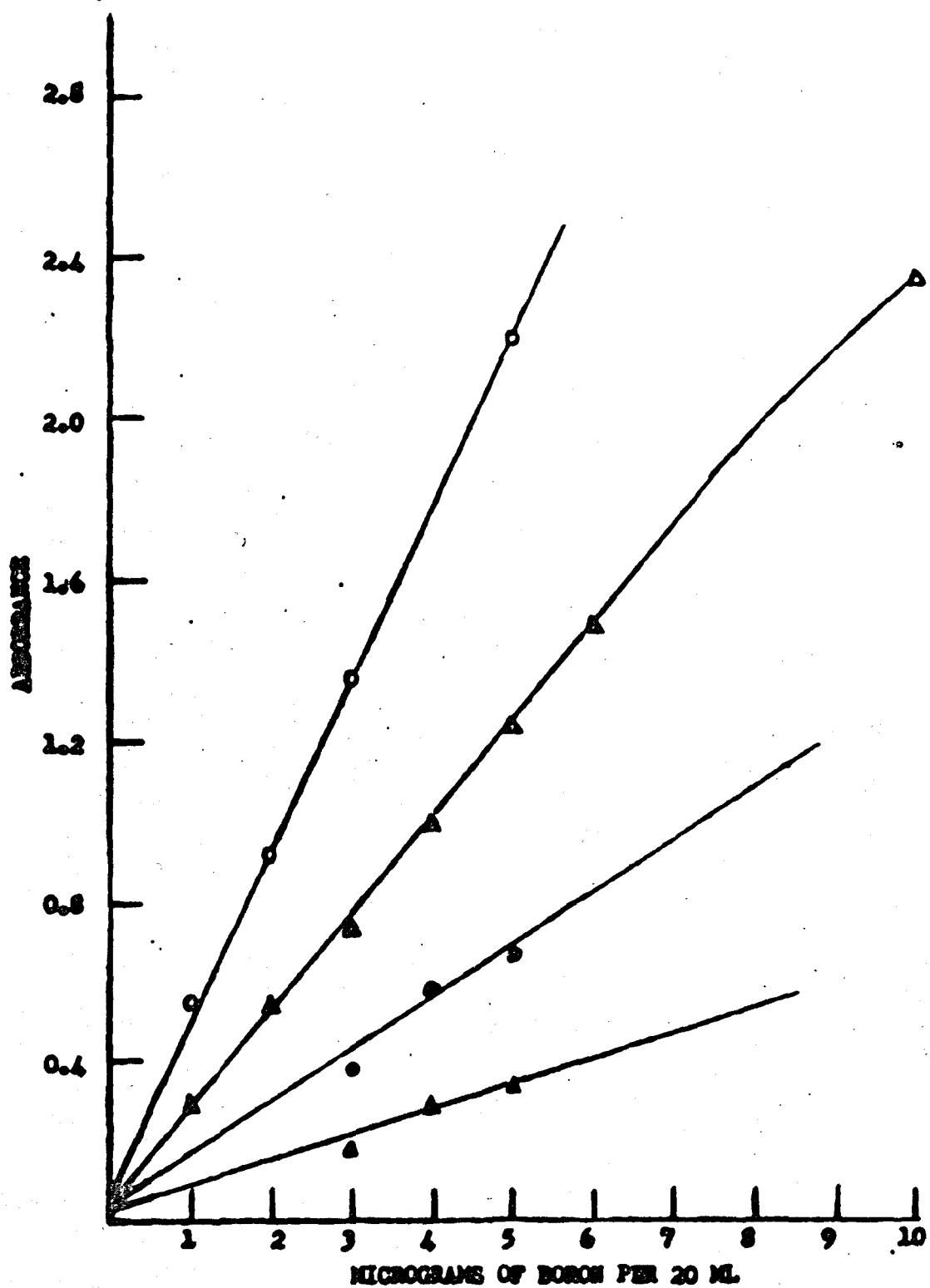
Figure 7

Standard Curve for Modified Naftel Method

With and Without Oxalic Acid

Absorbance Versus Micrograms of Boron

- With Oxalic Acid (absorbance measured at 550 mμ)
- Without Oxalic Acid (absorbance measured at 550 mμ)
- △ With Oxalic Acid (absorbance measured at 575 mμ)
- ▲ Without Oxalic Acid (absorbance measured at 575 mμ)



In the absence of oxalic acid the sensitivity of the reaction was 0.077 micrograms of boron per 0.01 absorbance unit per 20 ml when the absorbance was measured at 550 mu. When the absorbance was measured at 575 mu the sensitivity was even less, being 0.167 micrograms of boron per 0.01 absorbance unit per 20 ml. The oxalic acid increases the sensitivity of the method three fold.

TABLE XVI

Standard Curve in Absence of Oxalic Acid

<u>Micrograms of Boron</u>	<u>Absorbance</u>	
	<u>At 550 mu</u>	<u>At 575 mu</u>
3.0	0.38	0.18
4.0	0.58	0.29
5.0	0.66	0.33

To check the stability of the boron-curcumin complex, solutions containing 2.0 micrograms of boron were prepared according to the modified Naftel procedure. The absorbance of these solutions were measured at intervals over a $3\frac{1}{2}$ hour period (the solutions were left in the absorption cells during this $3\frac{1}{2}$ hour period). The absorbance values were within the limits of reproducibility of the method for the first 70 minutes after the addition of the ethanol to the residue. The remaining portions of these solutions had been left standing, open to the atmosphere in soft glass test tubes. These portions were measured 160 minutes and 220 minutes after the addition of ethanol to the residue, and were found to have absorbance values within the limits of reproducibility of the method. The data, converted to micrograms of boron, is given in Table XVII.

TABLE XVII

Stability of Final Alcoholic Extract
of Boron-Curcumin Complex

<u>Time Elapsed from Addition of Ethanol</u>	<u>Micrograms of Boron Present</u>	<u>Micrograms of Boron Found Sample 1</u>	<u>Sample 2</u>
15 Min.	2.0	2.0	2.0
40 Min.	2.0	1.8	1.9
70 Min.	2.0	1.8	1.8
100 Min.	2.0	1.7	1.7
160 Min.	2.0	1.7	1.7
220 Min.	2.0	1.7	1.7
*160 Min.	2.0	1.8	1.8
*220 Min.	2.0	1.8	1.8

*Solutions left in soft glass test tubes for the period of time indicated.

C. Conclusions

This study has shown the Naftel and the Hegedus methods to be closely comparable to one another. The sensitivity of the Hegedus method is 0.029 micrograms of boron per 0.01 absorbance units and of the Naftel method 0.024 micrograms of boron per 0.01 absorbance units, the absorbance being measured at 550 mμ for both methods. Both methods have a precision of about 5% (or 0.1 to 0.2 micrograms) in the range 0 to 5 micrograms and this is the range over which each method follow Beer's Law. Due to the use of different solvents in each method, the Hegedus procedure has a longer evaporation period than the Naftel procedure at an evaporation temperature of 55 to 60°C. However, the evaporation temperature of the Hegedus method may be raised to 75 to 80°C, thereby decreasing the evaporation time but not affecting the reliability of the method.

It has been pointed out in the literature that, in addition to the usual sources of contamination (such as from the glassware, apparatus, and possible reagent impurity), the dust in the air contains enough boron to cause errors. However, one of the major causes of error seems to be aging of the curcumin

reagent, and this causes poor day to day reproducibility. For better precision when daily analyses are to be made, a new standard curve should be prepared each day.

Many workers have emphasized the need for rigid control of the many variables of the curcumin solution method for boron determination. The work reported in this thesis has shown this to be true if great precision is needed. However, for results within 5% one need only control the evaporation step while using optimum quantities of reagents. Such factors as size and shape of evaporating dish, type of evaporating dish, humidity, air currents and drafts, and other minor factors need to be controlled only for the greatest precision.

IV A Study of the Curcumin Paper Method of Boron Determination

One of the objects of this research project was the development of a method that could be adapted to a rapid field test for atmospheric boron. Those solution methods that require sulfuric acid as a solvent would probably not be easily adapted to a rapid field test; whereas the curcumin method, particularly the curcumin paper test for boron, should be readily adapted for this purpose. This portion of the thesis concerns a study of the curcumin paper test and its adaptation to the determination of atmospheric boron.

A. Preliminary Study of the Curcumin Paper Test

Bertrand and Agulhon (6) introduced the curcumin paper test for boron about the same time that Cassel and Gerrans (18) introduced the curcumin solution method. The experimental procedure of the Bertrand and Agulhon method is as follows.

The boron samples are evaporated to dryness, cooled, and then 4 drops of 10N HCl and 0.5 ml of distilled water are added. The solution is then washed into a glass vial 30 mm high until the volume is 1.5 ml. A strip of turmeric paper (45 x 3 mm) is immersed in the solution to a depth of 15 mm and the vial is heated for 3 hours at 35°C.

The height of color on the strip, compared to standards, is a measure of the amount of boron present. An alternate method is to allow samples to develop at room temperature for 10 to 24 hours before comparing. Bertrand and Agulhon determined 1 to 100 micrograms of boron with this method. The author of this thesis, following the Bertrand and Agulhon procedure exactly, could not reproduce their results. One of the main reasons for this was thought to be the insensitivity of the turmeric paper as purchased.

Based on the study of the Naftel and Hegedus methods one major modification of the Bertrand and Agulhon method was made; i.e. oxalic acid was added to the test solutions. Other modifications were used of ethanol and glacial acetic acid as well as water, for solvents; the use of different temperatures for the development of color; and the preparation of more sensitive turmeric paper. These modifications will be described more fully in connection with the specific tests in which they are used.

To determine the effect of oxalic acid on the turmeric paper test of Bertrand and Agulhon, sample solutions containing 0, 50, and 100 micrograms of boron were made up to one ml with distilled water, 4 drops of 1:5 HCl, 1.5 ml of water saturated with oxalic acid, and a strip of turmeric paper were added. The test solutions were allowed to stand at room temperature (24°C Avg.) and even after 54 hours no color developed on the turmeric paper. Apparently the paper as purchased contained only a small amount of turmeric and was too insensitive for this test. The above test was repeated with specially prepared turmeric paper. Turmeric paper as purchased was soaked for 20 hours in an ethanol solution of curcumin (0.1 gm per 100 ml of ethanol). Also some turmeric paper as purchased was soaked for 20 hours in a glacial acetic acid solution of curcumin (0.1 gm of curcumin per 100 ml acetic acid saturated with oxalic acid). Some color developed on these specially prepared

turmeric papers when they were used in the modified Bertrand and Agulhon procedure, though the color developed was still not enough to differentiate the various boron solutions. The oxalic acid did not increase the sensitivity of the method.

In the curcumin solution methods the solutions must be evaporated to dryness at elevated temperatures to insure good color development. Also Bertrand and Agulhon suggested that the test be carried out at 35°C, a temperature somewhat higher than the temperature used in the above tests. To determine whether heat would increase the effect of oxalic acid and improve the color development, solutions, prepared as above, containing 0, 50 and 100 micrograms of boron, both with and without oxalic acid were heated at steam bath temperature for 80 minutes. There was, at the end of this heating period, only a small amount of color developed on the turmeric paper in the 50 and 100 micrograms boron samples. Fourteen hours later there was good color development, though indistinguishable between 50 and 100 micrograms, and the solutions containing oxalic acid did not show increased sensitivity. Thus, heating did help the color formation somewhat though the method was still insensitive to small amounts of boron.

Similar tests, as described above, were made using solutions prepared by mixing 2 ml of aqueous boron sample with 2 ml of glacial acetic acid saturated with oxalic acid. Only turmeric paper as purchased was used in this test and only slight color development resulted after 24 hours of standing at room temperature. The different amounts of boron could not be differentiated after this time. Heating at steam bath temperature seemed to prevent color development. The glacial acetic acid apparently was detrimental to the reaction on paper at higher temperatures. However, for those tests

in which no acetic acid was present the heating helped color development and it was concluded that evaporation to dryness was necessary before oxalic acid would affect the sensitivity of the method.

The turmeric paper method of Camus (17) involved evaporation to dryness of the test solution with the turmeric paper in the solution. Camus determined the amount of boron from the intensity of coloration rather than the height of coloration. Thus using a modification of the Camus procedure, test solutions were prepared, strips of turmeric paper were added, and the solutions were evaporated to dryness. The test solutions were prepared by adding 2 ml of a saturated aqueous solution of oxalic acid to the boron samples (0, 10, 20, 30, 40, 50, and 100 micrograms of boron made up to 2 ml with distilled water.) Turmeric paper as purchased was used. Though there was good color development on the paper strip, the color was not uniform throughout the paper strip and, thus, did not allow good differentiation of the various amounts of boron. Generally speaking the paper in those solutions containing larger amounts of boron had more intense coloration than the papers in those solutions containing smaller amounts of boron. This test was repeated except that the test solutions were heated an extra 10 minutes after dryness. The result of extra heating was good differentiation in the color intensity on the paper, the color changing from yellow to pink to red for increasing amounts of boron. Similar tests in which no oxalic acid was present showed much less color development. Evaporation to dryness and extra heating improved the color development and, thus, made easier the differentiation of the various amounts of boron.

A study was made using the same procedure except that 2 ml of glacial acetic acid saturated with oxalic acid was used rather than an aqueous solution saturated with oxalic acid. There was good differentiation in

the color developed on the turmeric paper in 0, 10, 20 and 30 micrograms boron solutions; however, no difference in color could be detected for those amounts of boron above 30 micrograms. Since 30 micrograms seemed to be the upper limit of this method solutions containing 0, 4, 8, 12, 16 and 20 micrograms of boron were prepared according to the above procedure, strips of turmeric paper were added, and the solutions were evaporated to dryness on a steam bath. All these solutions could be differentiated. The colors on the turmeric paper did not fade very much up to two hours. Again turmeric paper in solutions containing no oxalic acid showed little or no color development.

In another series of experiments the turmeric paper was dipped in the boron test solution, then removed and allowed to dry at room temperature or at elevated temperatures in an oven. This, in effect, was an evaporation to dryness procedure whereby the boron-curcumin reaction took place and the typical red color of the boron-curcumin complex developed on the paper. Three types of turmeric paper were used for this test: (1) turmeric paper as purchased, (2) turmeric paper as purchased that had been soaked for 20 hours in an ethanol solution of curcumin, and (3) turmeric paper as purchased that had been soaked for 20 hours in a glacial acetic acid solution of curcumin. One set of test solutions contained 0, 50, and 100 micrograms of boron made up to 1.5 ml with distilled water, and another set contained 0, 50, and 100 micrograms of boron made up to 1.0 ml with water plus an additional 0.5 ml of saturated oxalic acid solution. The three types of turmeric paper were soaked for 5 minutes in the test solutions, then removed, and allowed to dry at room temperature (22° to 25°C). The papers dried in

in 30 minutes and began to develop color after 45 minutes. After one hour the various amounts of boron could not be differentiated. At end of $1\frac{1}{2}$ hours the various quantities of boron could be differentiated on the paper which had been dipped into the test solutions containing oxalic acid. This was true for all three types of turmeric paper, though the specially prepared turmeric paper (types (2) and (3) above) developed more intense colors. The colors for the various boron test solutions not containing oxalic acid could be differentiated only on the specially prepared turmeric paper. There was no further color development after $2\frac{1}{2}$ hours and the colors developed on the papers dipped into the test solutions containing oxalic acid could be differentiated one from the other. At the end of 24 hours there was considerable fading of the colors on the papers which had been dipped in the test solution containing no oxalic acid, while those papers dipped in the test solutions containing oxalic acid showed little or no fading.

This paper test, at least in the presence of oxalic acid, appeared to be quite sensitive. Thus another experiment was conducted in which the test solutions contained 0, 5, 10, 20, 30, and 50 micrograms of boron made up to 1.0 ml plus 0.5 ml of a saturated oxalic acid solution. Turmeric paper (types (1) and (2) described previously) were soaked in the test solutions for ten minutes, removed and allowed to dry at room temperature. At the end of $1\frac{1}{2}$ hours only the colors developed on the specially prepared turmeric paper for 0, 5, and 10 micrograms of boron could be differentiated, for larger amounts of boron the colors were more intense but were of approximately equal color. After four hours both types of paper gave good differentiation for all boron concentrations, though the colors developed on the specially prepared turmeric paper were more intense than on the

turmeric paper as purchased.

The above test was repeated; however, this time the papers were dried in an oven at 85°C; the papers remained in the oven for 6 minutes. There was good color development on both types of paper and though the papers had a tendency to char, all amounts of boron used could be differentiated. The rate of color development was increased by drying the papers in the oven, but perhaps a lower temperature could be used to eliminate charring.

The next experiment employed a volatile boron compound in an attempt to test the paper method on an air sample. This represented a preliminary step in developing a rapid field test for atmospheric samples. A solution was prepared in a test tube by mixing 0.1 ml of boric acid solution, 1.0 ml of 1:1 HCl and 5.0 ml of methanol. Strips of turmeric paper prepared as described previously (types (1), (2) and (3) above) were moistened and placed over the mouth of the test tube. On heating (the reaction should yield volatile methyl borate) tubes containing 0, 10 and 20 micrograms of boron a color change proportional to the amount of boron present occurred. However, the flushing effect of the methanol was so great that uniform development did not occur and thus, it was difficult to differentiate the various quantities of boron. Several modifications of this test gave the same results.

The various tests performed in this preliminary survey showed that the turmeric paper test could be made quite sensitive and could be adapted to a rapid field test for atmospheric boron samples. It has been further concluded that oxalic acid increases the sensitivity of the turmeric paper only if the reaction is carried to dryness. Also oxalic acid seemed to prevent fading of the color on the paper. The special treatment of the turmeric paper as purchased improved its sensitivity.

B. Development of Apparatus for Study of Curcumin Paper Test Using Synthetic Atmospheres

To determine whether the turmeric paper test could be used to determine atmospheric boron, a device to produce and deliver a sample of boron from a synthetic atmosphere was needed. This section of this thesis describes the method used to produce the synthetic atmosphere of boron and the apparatus used to deliver a sample of boron from this synthetic atmosphere. To prepare the synthetic atmosphere a small volume of methyl borate dissolved in methanol was volatilized in a five gallon carboy. One milliliter of such a solution volatilized spontaneously in the five gallon carboy and large corks were used as agitators. The amount of methyl borate introduced into the carboy depends on the size of sample desired for a given test. The five gallon carboy (19.3 liters by accurate measure) was equipped with a three-holed rubber stopper through which passed a plug (to be removed for sample addition), an air intake tube, and a sample outlet tube. In the outlet line was the paper holder, and the device for with drawing and measuring the volume of the air sample. The paper holder, though various modifications were tried, consisted of two tubes pressed together with the test paper between them. Either a rubber suction bulb or a 50 ml syringe was used to withdraw the sample. Bulbs containing CaCl_2 and P_2O_5 were placed in the air intake line to keep the synthetic atmosphere in the carboy as dry as possible.

During the course of the investigation several modifications of the synthetic atmosphere sampling device were found to be necessary. Thus a study was made of the effect of various modifications of the apparatus and the testing procedure on the uniformity of color developed and on the efficiency of sampling. The factors studied were as follows:

1. Preparation of the curcumin paper
2. Type of filter paper used
3. Diameter of the testing device (equal to the diameter of the exposed portion of the turmeric paper)
4. Device used to pull through and measure the volume of synthetic atmosphere
5. Modifications to prevent leakage

The general procedure used involved flushing the carboy with dry nitrogen, adding the methyl borate sample, agitating, and then with drawing a measured volume of the atmosphere through the turmeric test paper. In all tests made to test the apparatus, sufficient methyl borate was added to the carboy so that each 50 ml of air removed contained 2.5 micrograms of boron. The test papers were allowed to dry in air at room temperature and were compared at the end of one hour. All observations were visual since no instrument was available for reading the intensity of color on paper.

Preparation of the Curcumin Test Paper

In the literature it was reported that some commercially available turmeric test paper contained enough boron to give a positive test. Also most filter papers contain a certain amount of boron. To remove the boron present in the test papers they were first soaked, successively, for 5 minutes each in 5 different saturated solutions of oxalic acid in water, and were then washed with distilled water. Papers treated in this manner gave very low blanks. After the test had dried they were soaked in an alcohol solution saturated with both curcumin, and oxalic acid. The period of soaking was varied from one to eleven days, but the period of soaking seemed to have little effect, as far as the eye could tell, on the sensitivity of the test paper. Apparently the filter paper becomes saturated with curcumin in a relatively short time and even soaking for one day seems more than is necessary.

More efficient sampling resulted when the test papers were moistened just prior to sampling. The test papers were moistened with either ethanol, glacial acid, or water, then were inserted in the testing device and the air sample was then drawn through the test paper. The methyl borate dissolved in the solvent on paper and thus was held for reaction with the curcumin (dissolved also to some extent in the solvent). However the methyl borate apparently passes through the dry test paper causing little or no reaction. The effect of the three solvents (glacial acetic acid, ethanol, water) used for moistening the test paper will be described in a later section of this thesis.

Type of Filter Paper Used

Commercial turmeric paper (purchased from Matheson, Coleman, and Bell), No. 619 qualitative filter paper, and Whatman No. 1 filter paper were tried as the test papers. Since the turmeric paper as purchased did not contain much turmeric it was soaked in curcumin according to the procedure given in the preceding section. Of course, the other two types of filter papers also had to be impregnated with curcumin by the same procedure. Uniformity of the spot developed and low blanks were the criteria used to determine good test papers.

The spots developed on the commercial turmeric paper (after the special treatment) were fairly uniform; however, the strips were not wide enough for the larger diameter sampling tubes that were later found to be necessary. The qualitative No. 619 filter paper gave low blanks after the leaching process but the spots developed when this paper was used for a test were not very uniform. As concerns low blanks and uniformity of the spot developed the Whatman No. 1 paper gave the best results of the three types of paper used for the curcumin paper test. Thus Whatman No. 1 paper was used

for all work after this preliminary examination of the types of paper.

Diameter of the Testing Device (Sampling Device)

The diameter of the portion of the testing device that holds (see Figure 8) the curcumin test paper was of importance since it affected the size, intensity, and uniformity of the spot developed. Two different sizes of glass tubing and filtering funnels were used for this portion of the testing device. The paper was placed between the tubings (or funnels) and the tubings were then held together by an arrangement of clamps.

The two funnels used had diameters at the top of 37 mm and 26 mm respectively. When the funnels were used, relatively large areas of the test paper were exposed to the air sample stream and the spots developed were neither very intense nor very uniform. The dynamic conditions of sampling caused "channeling", which, in part, affected the uniformity of the spot. Also, as will be described later, leakage was a major problem when the funnels were used. In general the funnels were unsatisfactory for this purpose.

A smaller exposed area of test paper should give more intense and more uniform spots thus 6 mm and 12 mm glass tubing was tried for this purpose. The glass tubing was inserted in a onehole cork stopper so as to make it flush with the large end of the stopper. The test paper was placed between two such glass tube arrangements and the stoppers were clamped tightly together to act as a gasket to seal the opening. The air sample was then drawn through the test paper in this sampling device. This type of paper holder was found to give very uniform and intense spots; the 6 mm tubing gave better results for the range 0 to 2.5 micrograms of boron and the 12 mm tubing was best for the range 2.5 to 15 micrograms of boron.

Device for Pulling Air Through and For Measuring the Volume of Air

In the initial stages of this investigation a rubber bulb was used to draw the air through the testing device and to measure the air volume coming through the device. The volume withdrawn at one intake of the bulb was found to vary by approximately 10% from the average volume of 50 ml, and this was satisfactory for qualitative work. To obtain a more accurate measure of the volume of air sample withdrawn from the carboy, a 50 ml syringe was substituted for the bulb and was found to give quite satisfactory results.

Modifications to Prevent Leakage

When funnels were used as the paper holder of the sampling device, it was difficult to prevent leakage at the connection between the two funnels, and any type of sealing agent made the changing of the test papers tedious, if not impractical.

The cork stoppers, or even better rubber stoppers, used with the 6 mm and 12 mm tubing described above provided sufficient sealing to prevent leakage. To maintain the needed pressure "C" clamps were used to hold the stoppers firmly together. Metal plates were placed behind each stopper to reinforce the device (the "C" clamps were positioned on the metal plates) and to give more uniform pressure, thus assuring a good seal at all points.

Figure 8 is a diagram of the synthetic atmosphere sampling apparatus and its component parts.

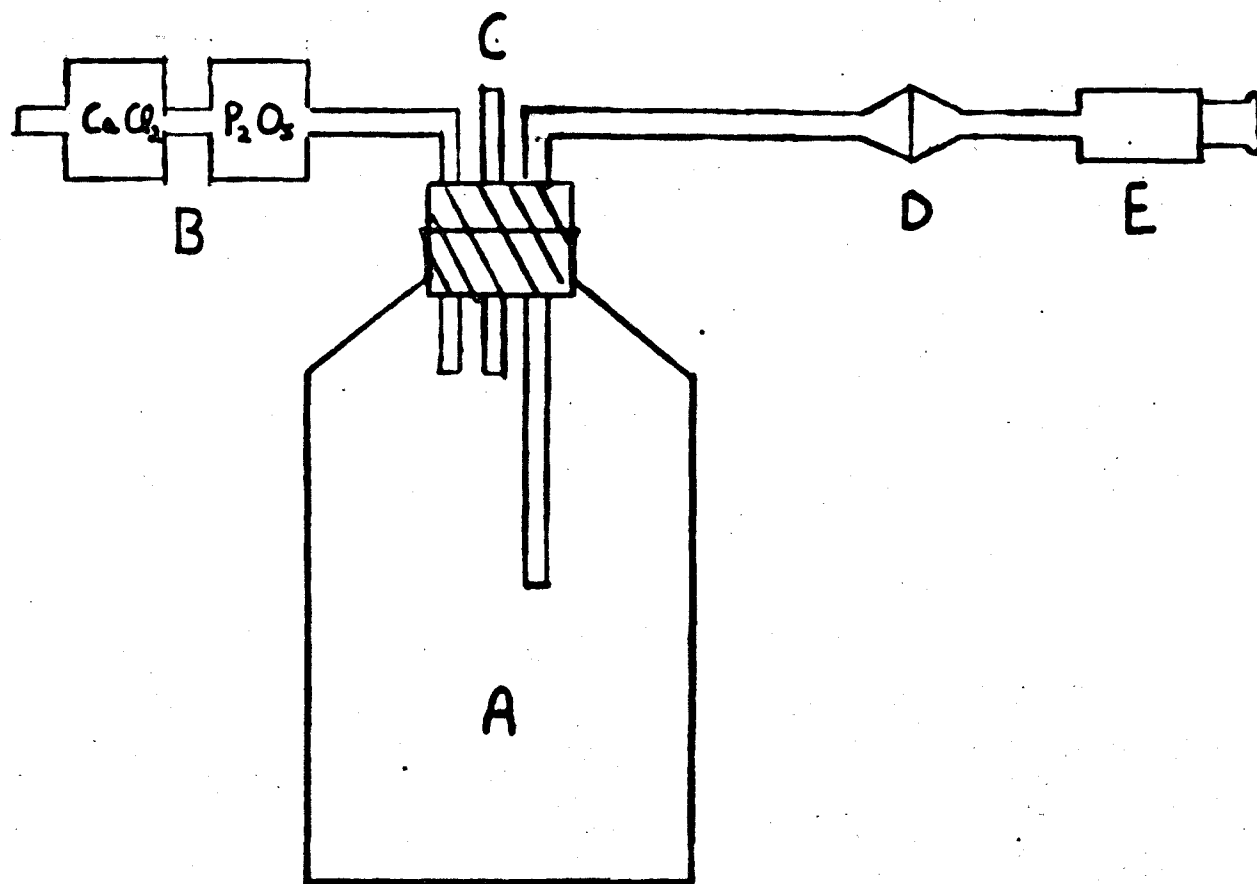
C. A Study of the Curcumin Paper Test Using the Apparatus Developed

From the preceding study, the best of the modifications were used in assembling the apparatus to be used to study the curcumin paper test. The final apparatus (Figure 8) consisted of the five gallon carboy (equipped with a three hole stopper for air intake and outlet, and for sample

Figure 8

Diagram of Synthetic Atmosphere Sampling Apparatus

- A. Carboy
- B. Intake Line
- C. Plug for Sample Injection
- D. Paper Holder
- E. Sample Withdrawing Device



introduction), P_2O_5 and $CaCl_2$ drying tubes, a 50 ml syringe for withdrawing the sample, and the paper holder (also called testing device). Both the 6 mm and the 12 mm glass tubing paper holders were used, depending on the range of boron to be determined. Leached Whatman No. 1 filter paper impregnated with curcumin as described above was used as the test paper. The testing procedure was the same as that described in the preceding section of this thesis (Experimental, Part IV, Section B).

It was previously mentioned that moistening of the test paper just prior to sampling was necessary to insure efficient sampling. Since this previous work was done as a preliminary study, a more thorough study of the moistening reagents and procedure was made using the improved apparatus. The various solvents used for this purpose were sprayed on the test paper with an atomizer, since dipping the paper in the solvent gave too much excess solvent which, in turn, increased the flushing effect.

When the test papers were moistened with 95% ethanol spots of good color and uniformity developed after 15 minutes for samples of air containing 7.5 micrograms of boron. Papers moistened with water, and others moistened with a 50-50 ethanol water mixture showed no color after 15 minutes. Papers moistened with glacial acetic acid gave good color after 15 minutes, and very little flushing was observed. The comparison time of 15 minutes was used here as a criterion for good color development though maximum intensity was not reached with any of the solvents in this time. The time required for maximum development of color was shortest when 95% ethanol was used for moistening and thus it seemed better suited for this purpose than glacial acetic acid. Water and the 50-50 water alcohol mixture were unsatisfactory since the color developed was not as intense as with the 95% ethanol or

or glacial acetic acid. Thus, 95% ethanol was found to be the most satisfactory moistening reagent for this test procedure.

In the preceding work, the test papers were allowed to develop at room temperature, and it took several hours to reach maximum color development. To reduce the development time, tests were made in which the curcumin test papers were dried in an oven at 55°C (avg) after sampling. The test papers were heated at this temperature for one hour, and the spots developed were of good intensity, uniformity, and reproducibility. The spots developed in the oven were even somewhat better, as concerns intensity, uniformity, and reproducibility, than those developed at room temperature. This temperature was used since it was found to be the most satisfactory evaporation temperature in the curcumin solution, and note that the one hour heating time is approximately equal to the total evaporation period and extra heating period of the solution method.

The sensitivity of the method was determined by passing air samples, from the synthetic atmosphere, through the curcumin test paper and comparing the spots developed from known concentrations of boron. In the first such test the concentration was such that each 50 ml volume withdrawn from the synthetic atmosphere contained 2.5 micrograms of boron. Comparisons were made in duplicate for 50, 100, 150, 200 and 250 ml samples, these volumes contained 2.5, 5, 7.5, 10 and 12.5 micrograms of boron. All of these amounts were easily differentiated, and the colors were quite intense, indicating that the test could be used for amounts of boron much smaller than the 2.5 micrograms minimum used in these tests. The colors were reproducible as far as the eye could tell.

Similar tests were made with concentrations of boron in the synthetic atmosphere such that 50 ml contained 1.0, 0.5, 0.2, 0.1 and 0.05 micrograms of boron. At 1.0 and 0.5 micrograms per 50 ml differentiation was very good, the color was quite intense, and reproducibility seemed to be good. With increments of 0.2 micrograms the spots were still easily differentiated. With 0.1 micrograms and 0.05 micrograms increments, all spots were visible and differentiation was good in most cases.

This study shows that a difference of 0.2 micrograms can be distinguished quite well and possibly with some improvement of reproducibility 0.1 or 0.05 micrograms increments could be determined. Calculations for 0.2 micrograms show that for a volume of 50 ml this is equivalent to approximately 10 ppm; thus increments of 10 ppm, and possibly even 5 or 2 ppm, can be differentiated by this method.

SUMMARY

A broad experimental survey of the curcumin method for the determination of boron has been made. It is felt that the twofold objective of this research project has been accomplished: first, a study of a sensitive method for the determination of small quantities of boron, and second, a study of a curcumin paper test that could be adapted to a rapid field test for atmospheric boron.

Though it is the most sensitive method presently available for the determination of boron, many variables need to be controlled to obtain precision with the curcumin method. Some of the variables affecting the Naftel (77) and the Hegedus (50) curcumin methods have been studied. The temperature of evaporation and the period of extra heating were found to be two of the most important variables. Oxalic acid increases the sensitivity of both the Hegedus and the Naftel methods, and there is an optimum concentration of the curcumin reagent beyond which it has little effect. In the Naftel method there is an optimum concentration of HCl necessary for maximum color development.

This work suggested other studies that should have been made; such as the use of other solvents for the reaction, and the optimum amount of water present in the reaction. Later work, by other members of the team that worked on this research project, indicates that there was an optimum amount of water necessary for the reaction, and that other solvents could be used for the reaction. Also more detailed studies of the optimum concentrations of reagents, evaporation temperature, and time of extra heating were made by other members of the team. These later studies substantiated the work reported in this thesis.

A study of the existing curcumin paper tests and the effects of various modifications was made. A synthetic atmosphere sampling apparatus was constructed and used to evaluate the curcumin paper test for determination of atmospheric boron. From this study a sensitive semi-quantitative test for atmospheric samples was developed. Later work, by other members of the research team, fully quantitized this procedure by varying the technique and using a reflectance densitometer to measure the intensity of color developed.

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AUTOBIOGRAPHICAL SKETCH

Name: James Edward Hardcastle

Place of Birth: San Diego, California

Date of Birth: August 14, 1932

Education: Diploma from Portlock High School, Norfolk County,
Virginia, 1949.

B. S. College of William and Mary, Williamsburg,
Virginia, 1953. Candidate for M. S. degree, University
of Richmond, Richmond, Virginia, 1960.

Military Service: U. S. Army, Engineers, 1954-1957. Overseas duty,
LaRochelle, France, 1955-1957.

Industrial Experience: Research Chemist (Physical Section 1958-1959,
Radiochemical Section 1959 to present) Research and
Development Division of Philip Morris, Inc., Richmond,
Virginia.

Honor Societies: High School; Honor Society.

College; Sigma Pi Sigma, Physics Honor Society;
Gamma Sigma Epsilon, Chemistry Fraternity.