A CRITICAL STUDY OF THE CURCUMIN METHOD FOR THE DETERMINATION OF BORON IN PLANT MATERIAL

- by -

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S U M M A R Y.

1. Methods for the determination of boron are reviewed.

2. A thorough investigation of the curcumin method was undertaken. Factors such as spectral absorption, boron volatilisation and the effects of pH, the stability of reagents and colour solutions, the drying of reaction products and the interference from certain elements, were investigated.

3. A study of the efficiency of extracting boron from plant material using an ashing procedure is presented.

4. The modified curcumin method for the determination of boron in plant material is described.

5. The statistical accuracy and precision of the modified method is presented.

6. The modified method was used to determine the boron content in citrus and pineapple leaf samples obtained from various parts of the Eastern Cape.

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C O N T E N T S.

3. 4. / .....

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1. INTRODUCTION.

Although experiments carried out as early as 1910 indicated the nutritional value of boron to the healthy growth of certain higher plant species, practical interest in boron as a beneficial fertilizer element did not develop until some twenty years later. This failure to appreciate the important role played by boron in stimulating plant growth is due, in part at least, to the lack of a method of analysis sufficiently sensitive and accurate to determine the small amounts of boron usually present in soils and plants.

The exact physiological roles which boron plays in promoting plant growth are, as yet, not specifically known. It is postulated that boron enters the plant as boric acid and combines with polyhydroxy compounds in the plant cells (1), and it is thought that the primary function of this boric acid is closely associated with plant cell division and the consequent metabolism in areas of the plant where new growth is most active. Subsequent functions are also thought to include the stimulation of photosynthesis, the ripening of fruit, and the promotion of root growth. Consequently a boron deficiency may have serious effects on the healthy growth of higher plant species. Such a deficiency appears to affect the differentiation

of / .....

of conducting tissues; this in turn affects mineral absorption and the distribution of other soil nutrients (2). These deficiencies produce stunted shoot tips, chlorotic patterns on leaf margins and inhibit fruit setting and even flowering. The presence of decayed brown spots on some fruits and the heart rot of root systems can be in some cases attributed to a boron deficiency. Such deficiency symptoms are always most marked in the stage of fruition or reproduction (2).

Alternatively, an excess of boron in the plant cells tends to stimulate the abnormal growth of cell tissue resulting from an increase in cell division shown in the yellowing of leaf margins and the dying back of shoot tips (3). Such exaggerated growth may be harmful to the plant and may even injure normal growth to such an extent that progressive degeneration sets in (3).

The tolerance of different plant species to a deficiency on the one hand, and to toxic amounts of boron on the other, differs considerably. In addition, the actual boron requirements of plants vary from species to species, but are nevertheless confined to a critical range of

concentration/.....

concentration for each particular species. For crop plants in general the range of concentration of boron in the dry leaf material is usually from 50 to 100 parts per million. Correspondingly, the concentration of soluble available boron in soils should not exceed approximately one part per million (4).

While the soluble available boron content of soils in general is less than 5% of the total content and is related to numerous variables, such as pH and organic matter content (5), the boron content of plant tissue is confined to some critical limit. For this reason the analysis of the plant material is of far greater significance, and the scope of the following discussion is limited accordingly.

Recent increased interest in these boron deficiencies has resulted in the development of several new methods for the determination of boron as boric acid in soils and plants. The rapid evolution of these methods has resulted in the development of several somewhat inferior procedures.

This work was undertaken in order to study the available methods for the determination of boron in plant material and to select, on the basis of available evidence,

the most / .....

the most promising method with a view to its thorough investigation and possible improvement.

The selection of the most suitable method was based on such analytical properties as accuracy, sensitivity and reproducibility, together with such practical considerations as speed, ease of manipulation, and the funds and apparatus necessary for such work to be carried out.

2. A REVIEW OF THE AVAILABLE METHODS FOR THE DETERMINATION OF BORON.

2.1. GENERAL METHODS.

Small amounts of boron may be determined spectrographically, volumetrically, electrometrically or colorimetrically.

The earlier methods involving a volumetric, electrometric (6, 7) or spectrographic (8, 9) procedure, are all either too elaborate for routine analysis or, more frequently, not sensitive enough for determining micro-quantities of boron. Among the more elaborate procedures is the separation of boron as the volatile methyl borate from an acid solution. The first quantitative methods based on this principle were those of Gooch (10) and Rosenbladt (11), carried out individually in 1887. Later methods, based on this methyl borate distillation, were developed by Chapin and Wherry (12), and by Allen and Zeiss (13).

Another elaborate procedure, involving a series of separations by fusion, acid digestion and precipitation, with the final removal of carbon dioxide by boiling, was developed by Ross and Deemer (14) and Dodd (15).

It was / .....

It was concluded that none of these older methods compared at all favourably with some of the more recent colorimetric procedures with respect to precision, accuracy and application to routine analysis.

2. 2. COLORIMETRIC METHODS.

Although the literature covering this subject is by no means extensive, only a brief critical review of the more recent methods will be given.

The colorimetric methods for the determination of micro-quantities of boron may be classed under two groups, viz., those in which the colour is developed in a strong sulphuric acid medium, and those making use of ethyl alcohol for this purpose.

First and foremost in the strong sulphuric acid group is the Quinalizarin Method. This method involves the addition of boric acid to quinalizarin (1, 2, 5, 8 - tetrahydroxy-anthraquinone) forming a blue colour-complex in a medium of 98% sulphuric acid.

The method, as developed by Berger and Truog(16), and De Turk and Olson (17) involves the use of 98% (by weight)

sulphuric acid. Factors favouring the method are:-

(i) The procedure is short and well-suited to routine analysis.

(ii) The method is one of the most sensitive; as little as 0.2 p.p.m. of boron can be satisfactorily determined.

(iii) Very little accuracy is forfeited in the presence of complex ionic solutions.

Some of the disadvantages of the method include:-

(i) The use of such a strong corrosive acid may cause serious damage to delicate instruments. In the case of the Cenco Sheard spectrophotelometer, used by the author, the small 1 cm. optical cells are of such a design as to make the method impracticable.

(ii) The acid reagent, prepared by using fuming sulphuric acid, is very prone to

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the absorption of moisture. It must be kept within 97.5 - 98.5% in order to prevent serious errors and very careful dispensing of the reagent is thus necessary.

(iii) The colour reagent is not particularly suited for use in a filter photometer, because there is a considerable over-lapping of the absorption bands of the pure reagent and the developed colour.

(iv) The high sensitivity of the method results in a limited range (0 - 2 of boron). This small range is further limited by the use of a specific 1 ml. test aliquot.

(v) Very little data as to the precision, accuracy and reproducibility of the method is available.

Kruger (18) modified the method considerably by using acetylated quinalizarin, whereby the concentration of the sulphuric acid was lowered to a tolerance of 70 - 80% in the

presence / .....

presence of 20 - 30% acetic acid. No specific reference is made as to the sensitivity and accuracy of the modified method.

MacDougall and Biggs (19) found that the concentration of the sulphuric acid could be diminished to 96% by increasing the concentration of the quinalizarin reagent fourfold; this reagent was not nearly as sensitive to changes in moisture content. The sensitivity of this modified method was not appreciably altered, quote :

> "the accuracy evaluated from a calibration curve varied between 90 and 30% transmittance from 2.4 to 0.6% relative error for a 0.2% absolute photometric error."

Another reagent which is used for determining boron in a strong sulphuric acid medium is chromotrop - 2B (p - nitrobenzene - azo - acid). In the presence of boric acid in a concentrated sulphuric acid medium, it gives a colour change from violet through violet-blue to blue with a greenish tinge.

The method as developed by Stettbacher (20), although even more sensitive than the quinalizarin method, has the

disadvantage / .....

disadvantage of involving a 12-hour colour development. Moreover, because of the interference of oxidizing agents, the procedure is rather tedious.

Austin and Mc Hargue (21) modified the method using a mixture of 60% sulphuric acid (by volume) with 40% of a 70% eolution of acetic acid. This shortened the colour development to only 30 minutes, but involved stringent control of the sulphuric acid concentration to within 1%.

The method was further improved and made more specific by Martin (22). He replaced concentrated sulphuric acid by a mixture of 1 volume sulphuric acid + 6.2 volumes acetic acid + 3.5 volumes of acetic anhydride.

Although Martin's modification appears to be a definite improvement, the chromotrop method in general suffers from a number of disadvantages:-

(i) The use of strong acid media for the colour development.

(ii) Complex reagents and different stages in tne colour development made the procedure rather tedious for routine

analysis / .....

analysis.

(iii) Certain elements interfere. Phosphate (a constituent of all plants) interferes when the ratio of PO_4/B exceeds 1000. Healy (23) has also shown that plant material rich in manganese may give rise to very serious errors when determining boron by this method.

(iv) Data on the accuracy and sensitivity of the method could not be traced in the literature.

Perhaps not as important as the above procedures, is the 1, 1′ Dianthrinide method. Developed by Ellis et al (24), the method involves the blue coloration, by small amounts of boric acid, of a solution of 1, 1′ dianthrinide (1, 1′ dianthraquinoylamine) in concentrated sulphuric acid.

The method has the advantage of obeying Beer's law over a wide range of boron content, as well as tolerating greater fluctuations in acid strength. However, the reaction producing the blue colour is dependant on the surface nature of the reaction vessels used, and on the concentration

of/ .....

of both the colour reagent and the boric acid. Moreover, the method is time-consuming and not as sensitive as the methods already reviewed.

Garfinkiel and Pollard (25), and Baron (26), have made some important modifications to the original method.

Other reagents which have been used in a strong sulphuric acid medium for determining small amounts of boric acid are Alizarin - S (27) and Carmine (28).

Of the methods employing ethyl alcohol as a medium, the Curcumin method is the most important. Developed by Naftel (29), the method depends on the red colour produced by an alcoholic solution of curcumin in the presence of boric and oxalic acids. Since this method was finally selected by the author for further study, it will be fully discussed in the following section.

Not as sensitive as any of the methods so far discussed, is the Benzoin fluorometric method developed by White et al (30). The method is based on the intensity of the greenish white fluorescence produced upon the addition of benzoin to boric acid in a slightly alkaline 85% ethanol solution.

The / .....

The sensitivity of the method is rather poor for determining the small quantities of boron normally found in plants; the accuracy being 1 - 2% in the range of 0 - 10 micrograms of boron in 50 ml. of solution. Moreover, many interferences necessitate a highly specific separation of the methyl borate. Apart from these considerable disadvantages, the actual determination is very short and well suited for routine analysis.

Strictly not in any of the above groups is the Chromotropic Acid ultraviolet method. Developed by Keummel and Mellon (31), this method involves the change in the ultraviolet absorption spectrum of chromotropic acid in a neutral aqueous solution in the presence of boric acid. The method is short and suitable for routine work but is very sensitive to light and is subject to many interferences. The accuracy is only mediocre, with a 3-4% average deviation over the range of 0.1 - 2.4 p.p.m. of boron.

2. 3. THE CURCUMIN METHOD:

2.31. The Properties of Curcumin. / .....

2.31. THE PROPERTIES OF CURCUMIN.

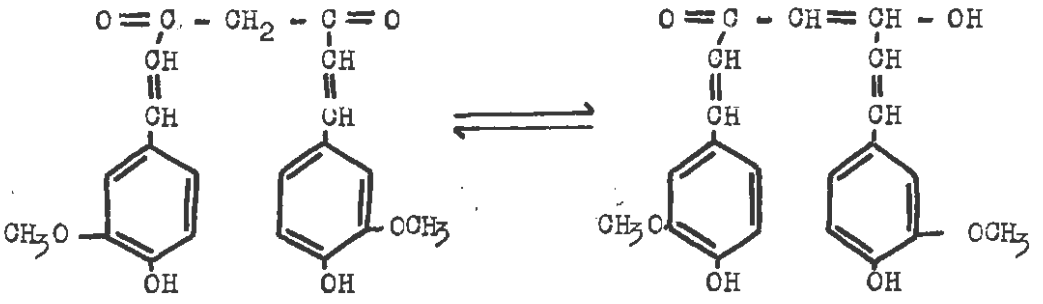
Curcumin is a natural plant pigment occurring in the rhizomes of the shrubs "curcuma tinctoria, longa, rotunda and viridiflora", which are native to tropical Asia. The dye extracted from these shrubs is termed turmeric and possesses an odour of ginger. It has a burning taste and apart from an oil and some brown colouring matter, curcumin is the principal constituent.

Perkin (32) described a method of preparing curcumin from curcuma. The process entails the precipitation of the lead salt from an alcoholic extract with some further purification of the pigment. The yield is only 0.65% and the dye is best prepared synthetically.

v. Kostanecki (33) first determined the constitution of curcumin, the synthesis being later carried out by Lampe (34).

Curcumin has the formula $C_{21}H_{20}O_6$, forming orange prisms (m.pt. 180-183°C). It is a diferuloyl-methane believed to exist in two tantomeric forms possessing the structures:-

O = C/.....



i.e. 1,7 - bis (4 - hydroxy - 3 - methoxy - phenyl)

- 1,6 - heptadiene - 3, 5 - dione.

Curcumin dissolves in concentrated sulphuric acid to give an orange-coloured solution. On making this solution alkaline, it changes to a reddish-brown with a yellow neutral colour, and on this account it is useful in the form of "turmeric paper" as a reagent for alkalis.

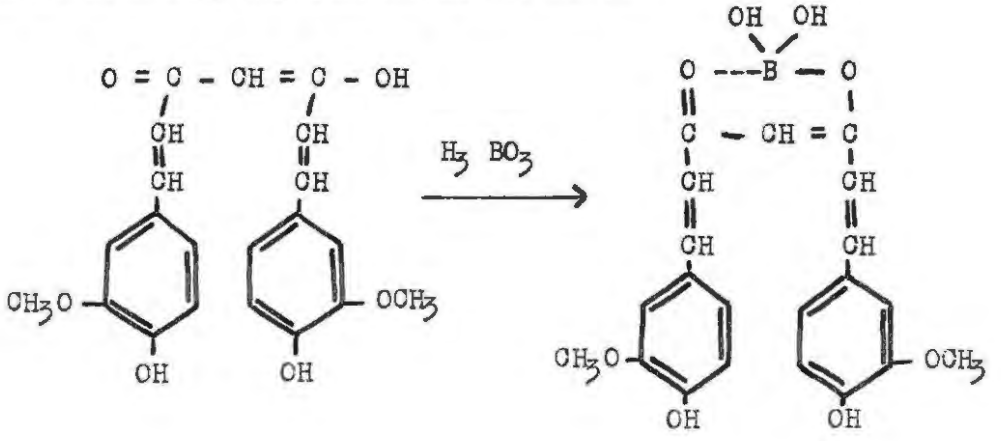
Not very soluble in water, curcumin is readily soluble in ethyl alcohol, acetone, carbon tetrachloride and ether. A solution in ether exhibits a weak greenish fluorescence under ultra-violet light.

On treating the orange alcoholic solution of curcumin with boric acid, rubrocurcumin $C_{21}H_{20}O_6$ is obtained, and when an acidified solution of this isomer of curcumin is evaporated

to/.....

to dryness, the red complex, rosocyanin, is formed. Rosocyanin is soluble in alcohol and is formed in amounts proportional to the amount of boric acid present. The acid form gives a rose-coloured solution, whilst the metallic salt is blue.

This reaction between boric acid and curcumin in an acidic medium, such as an alcoholic solution of oxalic acid, may be structurally represented as follows:-



The reaction is thought to involve only the enolic form of curcunim. Evidence in favour of this enolic reaction is tentatively presented in Section 3.51.

Korenman (35) maintained that the reaction is characteristic of the quinone type structure, having hydroxyl groups in peri-position with the carbonyl groups of the quinone.

The / .....

The above reaction is best carried out in the presence of a weak acid. For colorimetric work, the acid must be colourless and readily soluble in both water and alcohol. Both oxalic acid and acetic acid have been used for this purpose.

2.32. REVIEW OF THE RELEVANT LITERATURE.

In 1903 Cassel and Gerrans (36) first outlined a colorimetric method in which a solution of curcumin, boric acid and oxalic acid was evaporated to dryness and the orange-red reaction products then taken up in ethyl alcohol. The procedure was tedious, entailing numerous evaporations to dryness, the recovery of volatilized boron in potash bulbs, and other steps not suited to routine analysis.

Modifying the method, Naftel (29) eliminated many of the time-consuming steps. Adopting proposals by Gooch (10), solutions containing boron were concentrated by evaporation to dryness in the presence of excess calcium hydroxide in order to prevent the volatilization of boric acid from hot acidic solutions.

In 1954 Dible, Truog and Berger (37), published a refined curcumin method for the determination of boron in soils and plants. The method involves only one evaporation and

filtration/.....

filtration of the aqueous test solution, the entire procedure being possibly a little longer than the quinalizarin method. The method is more sensitive to small amounts of boron than the quinalizarin method and is carried out without the use of such a strong corrosive reagent as concentrated sulphuric acid.

Although the concentration range of the method is rather limited because of high sensitivity, Beer's law is obeyed between the limits 0 - 1.2 micrograms of boron.

The authors also showed that the precision of the modified method is equal to that of the quinalizarin procedure.

Russell (38), after making an interesting study of the various methods for the determination of boron, favoured the curcumin method, as did Winsor (39).

Thus, because of the apparently satisfactory nature of the modified method, it was selected for detailed study.

2.33. THE METHOD OF DIBLE, TRUOG AND BERGER.

Reagents:

Standard Boron Stock Solutions.

2.8578 g. of boric acid (reagent grade) was dissolved in 1000 ml. of distilled water giving a primary stock solution containing

0.5 mg. / .....

0.5 mg. of boron per ml. By subsequent dilution two standard solutions containing 0.01 mg. and 0.001 mg. of boron were made up.

Curcumin - Oxalic Acid Solution:

0.04 g. of finely-ground curcumin (Eastman Kodak No.1179) and 5 g. of oxalic acid were dissolved in 100 ml. of 95% ethyl alcohol. The reagent was stored in a cool dark place, thus being stable for several days. If stored in a refrigerator, the reagent will keep for a week if not unduly exposed to room temperature.

Ethyl Alcohol.

A good grade 95% strength was used.

THE COLOUR DEVELOPMENT PROCEDURE:

Having obtained from the sample under test a water solution of the boron to be determined, a 1 ml. aliquot, containing from 0.0 to 2.0 µg. of boron, was placed in a 250 ml. beaker (boron-free glass). Curcumin-oxalic acid reagent (4 ml.) was then added and the solution mixed thoroughly by rotating the beaker. This was evaporated on a waterbath at 55 ± 3°C and the residue baked at the same temperature for a minimum of 15 minutes to ensure complete dryness.

After / .....

After cooling, the reaction products were treated with 25 ml. of 95% ethyl alcohol and filtered directly into a comparison tube through a close-textured filter (Whatman No.2 was found to be satisfactory). The colorimeter transmittancy readings were recorded, using the appropriate filter (usually 540 mμ.), and the boron concentration of the unknown was determined by reference to a standard curve which had been previously prepared using appropriate amounts of the boron standard solutions.

THE DETERMINATION OF BORON IN PLANT MATERIAL.

A 0.25 to 0.50 g. sample of plant material, oven-dried and ground, was placed in a porcelain (or quartz) crucible (or dish) and ashed in a muffle furnace at 550°C , or over an open flame, to a grey-white ash. This was dissolved in 5 ml. of 0.1ON hydrochloric acid and diluted with distilled water to a definite volume, usually 10 to 20 ml., so as to come within the range of the standard reference curve used. A 1 ml. aliquot of the clear solution was then treated as indicated under the section "Colour Development Procedure. "

PRECAUTIONS.

Great care was exercised in preventing boron contamination of the test sample via the chemicals, filter

paper / .....

paper and glassware used, or via dust, boron-containing fumes or the operator's hands.

Glassware made of Corning alkali-resistant glass No. 728 was shown to be satisfactory. Storage of reagents in vessels of ordinary soft glass produced no contamination. Ordinary C.P. and A.R. grade chemicals were found satisfactory, but should be tested by means of a blank determination.

The curcumin-oxalic acid reagent decomposes rapidly upon standing in direct light, but if stored as suggested under "Reagents", it will keep for a considerable period. Rosocyanin slowly hydrolyses to curcumin, hence all colorimetric readings should be recorded within 2 hours after the solution of the colour residue in alcohol.

None of the elements normally present in plant tissue were found to interfere with the method.

3. EXPERIMENTAL STUDIES ON THE CURCUMIN METHOD.

Considering such factors as have already been dealt with, together with minor conflicting reports appearing in the literature, the present study embraces all the more important aspects of the method. The factors selected for further study will be presented as follows:-

3.1. The apparatus and reagents used.

3.2. The most suitable wavelength for colorimetric measurement.

3.3. The influence of pH on the colour development.

3.4. The influence of the concentration of curcumin on the colour development.

3.5. The influence of temperature on the colour development.

3.6. The influence of the drying period on the colour development.

3.7. The stability of the final colour solutions.

3.8. / .....

3.8. The range of the modified method.

3.9. Interference from foreign ions.

3.10. The extraction of boron from plant material.

3.1. THE APPARATUS AND REAGENTS USED.

3.11. INSTRUMENTS.

All colorimetric readings were recorded using a Cenco Sheard Spectrophotelometer (Cat. No. 29450). As the existing light source to the instrument was found to be defective, a constant light source unit was made.

Using a 1:5 stepdown transformer (A) and a selenium plate rectifier (B) in series with an adjustable 22 ohm, 10 amp. resistance (R), the 220 Volt, 60 cycle A.C. main power supply was adjusted to light a 6 Volt, 36 Watt filament bulb. This current supply was further stabilized using two 6 Volt, 21 plate lead accumulators (C) in parallel with the bulb. The bulb placed in a lamp housing (D) clamped on an optical bench, was focussed 35 cms. from the entrance slit of the spectrophotelometer (E), adjustments being made on the optical bench and the adjustable

convex / .....

convex lens.

This served as a satisfactory light source to the spectrophotelometer. Stability measurements showed that a weak potassium permanganate solution remained completely constant, after allowing an half hour warming-up period.

The spectrophotelometer (E) was of the diffraction grating type, housing twin 1 cm. optical glass cells on a sliding optical carriage. % Transmission readings were recorded on a Rubicon galvanometer (G), with a very high resistance placed across its terminals to act as a stabilizer.

The final set-up is diagramatically represented in Fig. 1.

Before using the instrument, the diffraction grating was set in conjunction with the wavelength adjustment knob. Using a sodium lamp as a light source, and with the wavelength set on 589.6 mμ , the 589.6 mμ line of sodium was brought to focus on the cross-wires of the eye-piece and the adjusting screws locked. This then ensured the accurate reading of wavelength.

A final check on the optical and photometric systems

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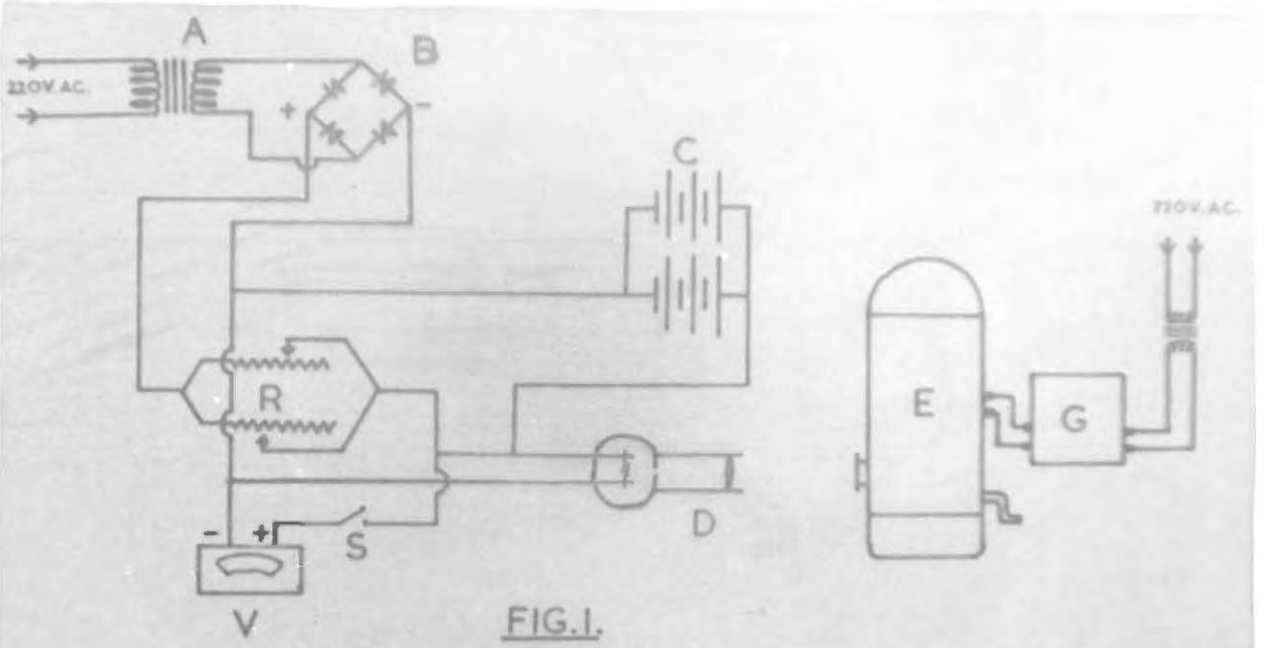

FIG.1.

A SCHEMATIC DIAGRAM OF THE LIGHT SOURCE TO THE SPECTROPHOTELOMETER.

of the instrument was then carried out, using a set of accurately diluted solutions of potassium permanganate. Straight Beer's law lines were obtained with virtually no instrumental error. The instrument was then thought to be in a satisfactory condition for the work that was to follow.

3.12. REAGENTS:

WATER. Deionised water was used throughout this work. This was obtained by passing freshly-distilled water through an ion exchange column containing Amberlite MB3 (Mixed-bed) resin. Tests indicated that the water so obtained was of exceptional purity.

STANDARD BORIC ACID SOLUTIONS. A primary stock solution was prepared by dissolving 2.8578g. of boric acid ("Specpure" Johnson Matthey & Co., Ltd.) in a 1000 ml. of deionised water. This gave a solution containing 500 µg. boron per ml. i.e. 500 p.p.m. with respect to boron.

By subsequent dilution of solution A, standard solutions B, C, D, E were prepared with concentrations 100, 10, 0.5 and 0.1 µg. boron per ml. respectively.

These solutions were immediately stored in polythene

bottles / .....

bottles to prevent any possible leaching of boron from glass containers.

SOLVENTS. Initially, the curcumin-acetone method developed by Silverman and Trego (40) was favoured. The method entailed the use of acetone in developing the colour as well as taking up the reaction products and making up to volume. Although a slight increase in sensitivity resulted from the use of acetone, alcohol was found to be far superior to acetone as regards volatility in volumetric work, liquid "creep" on vessel walls, offensive odours and expense. The method was discarded in favour of the Dible, Truog and Berger method using alcohol.

As reproducible results in the case of both methods were not attainable in the preliminary investigation, no comparable data are presented.

A good grade 95% ethyl alcohol was eventually used. Recovery of the alcohol was both easy and effective, using a distillation column connected to a water-cooled condenser. Distillation was carried out in the presence of excess calcium hydroxide in order to eliminate any possible boron contamination of the distillate as well as possible boron pollution of the

atmosphere / .....

atmosphere within the laboratory.

CURCUMIN-OXALIC ACID SOLUTION. The solution was prepared by dissolving 0.04g. finely ground curcumin (B.D.H. "Laboratory Reagent") and 5 g. of oxalic acid (B.D.H. "Analar") in 100 ml. 95% ethyl alcohol. The reagent was stored in a polythene container in a refrigerator, in which it remained stable for a week if not unduly exposed to light.

3.13. GLASSWARE AND STORAGE OF SOLUTIONS.

As pyrex glasses contain up to 11% boron oxide as a borosilicate, and soda glass up to 2% B_2O_3, the possible leaching of boron from these glasses was investigated, as well as the use of porcelain or quartz ware as an alternative. Boron-free glassware e. g. Corning No. 728 was not available.

Initially, open 9 cm. quartz basins were used in the colour developing stage. This resulted in irregular rates of evaporation and as this step was thought to be critical at the time, cover-slips, and finally, 200 ml. quartz beakers were used. The use of both involved a much longer evaporation at 55°C, and as the evaporation rates were later shown not to be critical, open quartz basins were again used.

Owing / .....

Owing to the annoyance of adherent salts when using quartz-ware, the possible use of porcelain was investigated. Standard determinations using quartz, porcelain, pyrex and soda reaction vessels showed no detectable leaching of boron from either quartz or porcelain, but very large errors were introduced in the case of the pyrex and soda-glass vessels.

Out of interest, the effect of siliconed glasses was investigated. Determinations using siliconed "pyrex" and "Hysil" reaction vessels were found satisfactory for perhaps one analysis, but subsequent evaporations destroyed the non-wetting surface-film resulting in errors as contaminating boron leached from the glass. However, the silicone provided a non-wetting surface for the alcoholic solution which was inclined to "creep" rather badly during evaporation. In overcoming this solution "creep", the reaction vessels were siliconed around the entire rim of the vessel.. This siliconing was effected by inverting each dish into a $\frac{1}{4}$" pool of a 5% solution of Dow Corning 200 in carbon tetrachloride and then baking the vessel at 200°C for 12 hours

9 cm. porcelain evaporating dishes with siliconed

rims / .....

rims were eventually found to be quite satisfactory for the evaporation stage.

STORAGE OF SOLUTIONS.

As few workers in this particular field have reported that the storage of reagents in soft glass vessels is satisfactory, it was thought advisable to investigate the leaching of boron from several different grades of glass containers. 300 ml. of deionised water were stored in each of several different grade glass containers at room temperature for a total period of 6 months. Readings were taken every month on 25 ml. samples from each individual container. Each sample was evaporated to dryness in the presence of 0.1 gm. of sodium carbonate and the boron determined as described in Section 4. The results are recorded in Table 1.

TABLE 1.

THE LEACHING OF BORON FROM GLASSWARE.

Grade of Glass.	µg. of Boron per 25ml. after a period of ..... 1 month	2 months	3 months	4 months	5 months	6 months
Soda	0.50	0.57	0.54	0.60	-	-
Soda	-	-	-	-	-	0.46
"Pyrex"	0.15	0.18	0.22	0.26	-	-
"Pyrex"	-	-	-	-	-	0.80
"Exax"	0.00	0.00	0.02	0.04	-	-
"Technico"	0.00	0.00	0.00	0.00	-	-
- no determinations were carried out.						

From / .....

From Table 1, it was concluded that even though the rate of leaching is greatest from pyrex glasses, the actual age and prior usage of the vessels is extremely critical.

Both of the soda glass containers were new reagent bottles and extremely high leaching occurred within the first month, remaining virtually constant thereafter. On the other hand no measurable leaching was recorded from the solution stored in a much-used soft glass "Technico" flask.

In the case of the pyrex glasses, much the same conclusion was reached, though the rate of leaching was considerably higher.

All reagents and stock solutions were consequently stored in polythene bottles.

Where glass burettes, pipettes and volumetric flasks had to be used, solutions were kept in contact with the glass for as short a period as possible.

3.14. FILTERPAPERS.

Work carried out by Winson (39) on numerous grades of Whatman filter papers indicated that the minimum content of easily extractable boron was found in Whatman No.4 paper

(1.8 µg. B per 9 cm. paper) / .....

(1.8 µg. B per 9 cm. paper). However, the author found Whatman No.4 filter paper to be too coarse for filtering off the very fine precipitates resulting after the colour development. Moreover it was found that any contaminating boron introduced after the colour had been developed at 55°C, had no effect on the final results : See section 3.6.

Since the filtering follows only after the colour has been developed, 9 cm. Whatman No.40 papers (4.5 µg. B per 9 cm. paper) were used and found to be satisfactory.

3.2. THE MOST SUITABLE WAVELENGTH FOR COLORIMETRIC MEASUREMENT.

Although Dible et al (37) recorded the spectral separation of the two colour components (dissolved in ethyl alcohol) by means of absorption curves, they did not present the effects of the one component in the presence of the other, or in the presence of oxalic acid. It was considered necessary, therefore, to plot the transmittancy curves of curcumin and of rosocyanin in 95% alcohol, and in the presence of oxalic acid, in order to determine the wavelength at which maximum separation occurs.

PROCEDURE. The freshly prepared curcumin reagent (4 ml.) was added to each of three quartz evaporating dishes containing

0, / .....

0, 1 and 10 µg. B. This was evaporated to dryness at 55 ± 1°C, and the residue baked for a further 15 minutes at the same temperature. After cooling for 15 minutes the reaction products were taken up in 95% ethyl alcohol and made up to 100 ml. in a volumetric flask. The % transmission was then recorded at wavelengths ranging between 520-570 mµ. The results are recorded in Tables 2 and 3, while Figs. 2 and 3 show a graphical representation.

TABLE 2.

THE SPECTRAL SEPARATION OF CURCUMIN AND ROSOCYANIN IN THE PRESENCE OF 1 µg. OF BORON

Wavelength (mu)	% Trans. of Curcumin. T_1.	% Trans. of Curc. + 1 µg. of B. T_2.	Separation $T_1 - T_2$
520	52.0	29.5	22.5
5	56.8	31.4	25.4
530	60.0	33.0	27.0
5	63.8	34.2	29.6
540	67.5	35.0	32.5
5	71.0	36.4	35.6
550	74.2	37.0	37.2
5	77.0	39.0	38.0
560	79.0	41.3	37.7
5	81.8	45.0	36.8
570	85.5	50.0	35.5

TABLE 3. / .....

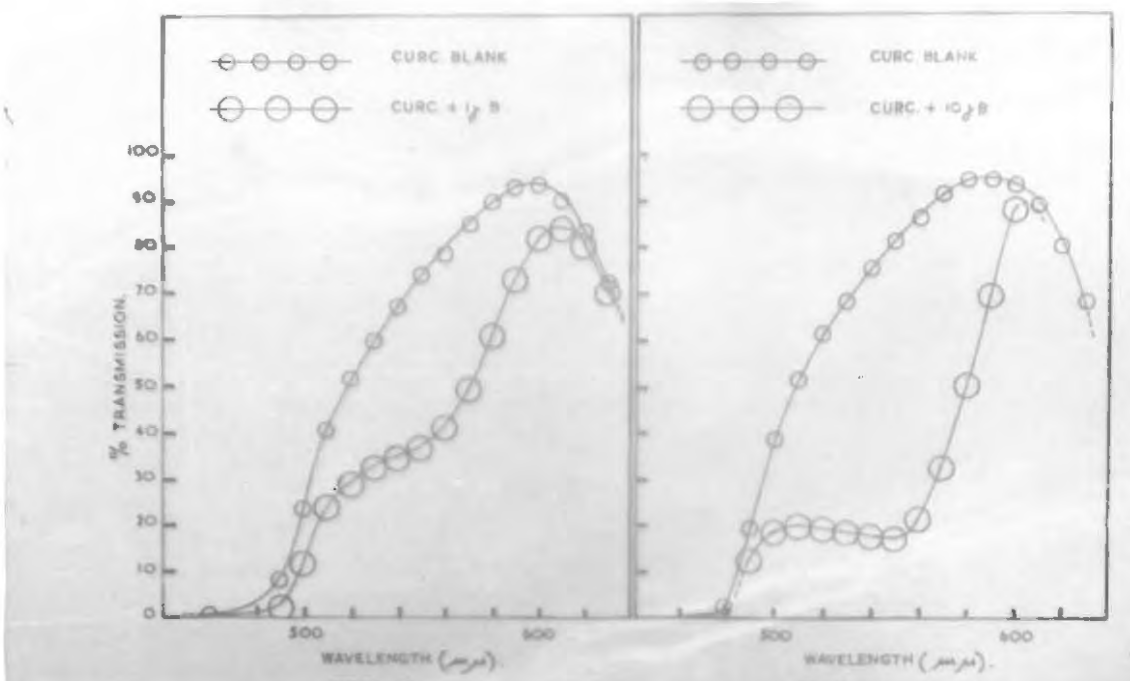

FIG. 2.

FIG. 3.

THE TRANSMISSION CURVES OF CURCUMIN & ROSOCYANIN.

TABLE 3.

THE SPECTRAL SEPARATION OF CURCUMIN AND ROSOCYANIN IN THE PRESENCE OF 10 µg. OF BORON

Wavelength (mµ)	% Trans. of Curcumin. T_1.	% Trans. of Curc. + 10 µg. of B. T_2.	Separation $T_1 - T_2$
530	69	19	50
540	76	18	58
550	82	18	64
560	87	22	65
570	92	33	59
580	95	51	44

Figs. 2 and 3, together with the separation data from Tables 2 and 3, illustrate that at both high and low concentrations of boron, a maximum spectral separation of the two colour components occurs at 555 mµ. In the method of Dible, Berger and Truog, 540 mµ was quoted as the optimum wavelength for maximum separation whilst 540 mµ was quoted as also being the wavelength of maximum absorption of pure curcumin dissolved in alcohol. The present author found a maximum absorption wavelength of 600 mµ for pure curcumin in the presence of oxalic acid.

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This discrepancy must be due, in part at least, to the influence of the oxalic acid on the spectral properties of the curcumin - rosocyanin equilibrium in ethyl alcohol. As the wavelength finally selected must conform as closely as possible with the actual experimental procedure, this influence of the oxalic acid could not be overlooked.

In true mixed-colour methods, it is the recommended procedure to take measurements at that specific wavelength where the separation between the transmission curves for the two components is greatest. A wavelength of 555 mμ was therefore used in all subsequent measurements.

3.3. THE INFLUENCE OF pH ON THE COLOUR DEVELOPMENT.

The use of oxalic acid in the method suggests that the colour development is sensitive to pH.

In the method of Dible, Berger and Truog, a 1.00 ml. aliquot of the test solution, containing 0.0 - 2.0 μg B, is used. The accuracy by which this aliquot can be dispensed and its effect on pH, has a profound influence on the ultimate accuracy of the whole determination. In the first place, the dispensing of 1.00 ml. (say to the nearest 0.02 ml.) introduces an unavoidable error of 2%. Secondly, the use

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of a test aliquot of unknown pH results in a colour-development stage carried out at a variable pH. Thirdly, the very limited range of the method is accentuated by using such a finite test aliquot. Consequently, the use of a larger test aliquot of up to 10 ml. was introduced, as well as an extra step involving the adjustment of the pH before developing the colour.

In the method of Silverman and Trego (41), in which acetone is used as a developing solvent throughout, the adjustment of the pH was effected by using sodium carbonate and hydrochloric acid. The procedure involved the addition of 0.1g. of sodium carbonate to the test aliquot, followed by an evaporation to dryness at 110°- 130°C. The pH was then adjusted by neutralising with 1:4 hydrochloric acid, using phenolphthalein as an indicator, and then adding 0.5 ml. hydrochloric acid in excess.

It was decided to investigate the possibilities of adopting this procedure when developing the colour in an alcoholic medium. A description of the experimental studies follows:

3.31. THE SODIUM CARBONATE CONCENTRATION.

If the aqueous test solution containing boric acid is not sufficiently alkaline, significant amounts of volatile metaboric acid are lost on evaporating the solution to dryness. No information as to the critical pH at which this volatilisation takes place or to any related effects of evaporation at different temperatures, could be traced in the literature. Because it was decided to carry out the initial evaporation on a waterbath, the optimum concentration of sodium carbonate to be used per determination in preventing any volatilisation at 100°C, was investigated.

PROCEDURE 1.

Separate amounts of A.R. sodium carbonate, ranging from 0.00 - 0.14 g. were weighed out into ten porcelain evaporating dishes, to each of which was added a 4 ml. aqueous aliquot containing 2.0 µg. B. These solutions were then evaporated to dryness on a waterbath at 100°C.

After cooling, the dry residues were treated with two drops of 1% phenolphthalein followed by a careful titration with 1:8 hydrochloric acid, adding 1 ml. in excess as suggested by Silverman and Trego (1:8 HCl was used because the neutralisation proceeded too vigorously when using 1:4 HCl).

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In order to eliminate any effect on pH due to volume changes, the volume of each sample was adjusted to the same value with deionised water. The % transmission of each sample was measured after the addition of the curcumin reagent and the development of the colour as outlined in section 2.3. The results are recorded in Table 4A and Fig. 4A.

TABLE 4A.

THE USE OF SODIUM CARBONATE IN PREVENTING BORON VOLATILISATION - ADJUSTING THE VOLUME BEFORE DEVELOPING THE COLOUR.

Sample No.	1:8 HCl (ml)	H_2O (ml.)	Total Vol.(ml.)	Na_2CO_3 (g.)	Initial pH.	% Trans.
Blank	2.0	0.5	2.5	0.100		100.0
1	1.0	1.5	2.5	0.000	5.3	100.0
2	1.1	1.4	2.5	0.010	9.2	77.4
3	1.2	1.3	2.5	0.020	9.4	75.5
4	1.3	1.2	2.5	0.030	9.5	76.0
5	1.4	1.1	2.5	0.040	9.6	75.8
6	1.6	0.9	2.5	0.060	9.7	75.0
7	1.8	0.7	2.5	0.080	9.6	74.0
8	2.0	0.5	2.5	0.100	9.6	75.3
9	2.2	0.3	2.5	0.120	9.5	77.6
10	2.4	0.1	2.5	0.140	9.5	78.5

From / .....

From Table 4A and Fig 4A, it is clear that the volatilisation of boron at 100°C is appreciable when less than 0.02 g. of sodium carbonate is present, corresponding to a critical pH of 9.4. Between the concentration range of 0.04 - 0.10 g. of sodium carbonate per sample, stable transmission readings were recorded, indicating no volatilisation of boron. The increase in % transmission when the sodium carbonate concentration exceeds 0.08 g. per sample, may be due to insufficient washing of the large sodium chloride precipitate which is formed at the higher sodium carbonate concentrations; an increase in boron volatility with increase in pH is certainly most unlikely.

PROCEDURE 2.

In order to observe the effect of the ratio, water : alcohol, on the colour development, procedure 1 was repeated, omitting the adjustment of the final volumes. The results are recorded in Table 4B and Fig. 4B.

The results show that the colour development stage is not sensitive to the change in the ratio alcohol : water within the limits 4 : 1 to 4 : 2. It was assumed, therefore, that the small change in pH caused by a change in the ratio alcohol : water, was buffered sufficiently by the oxalic acid added with the curcumin before developing the colour. This

buffering / .....

buffering effect of the oxalic acid is illustrated in Section 3.33.

TABLE 4 B.

THE USE OF SODIUM CARBONATE IN PREVENTING BORON VOLATILISATION - NO ADJUSTMENT OF VOLUME.

Sample No.	1:8 HCl (ml.)	Ratio alc./water	Na_2CO_3 (g.)	% Transmission
blank	2.0	2.0	0.100	100.0
1	1.0	4.0	0.000	99.2
2	1.1	3.7	0.010	78.2
3	1.2	3.3	0.020	77.0
4	1.3	3.0	0.030	76.1
5	1.4	2.9	0.040	75.9
6	1.6	2.5	0.060	75.3
7	1.8	2.2	0.080	75.3
8	2.0	2.0	0.100	75.7

PROCEDURE 3.

Two additional runs, similar to procedure 2, were carried out, using the final modified method as outlined in Section 4. The runs were carried out on two 4 ml. citrus leaf solutions, the one test solution having a high boron content.

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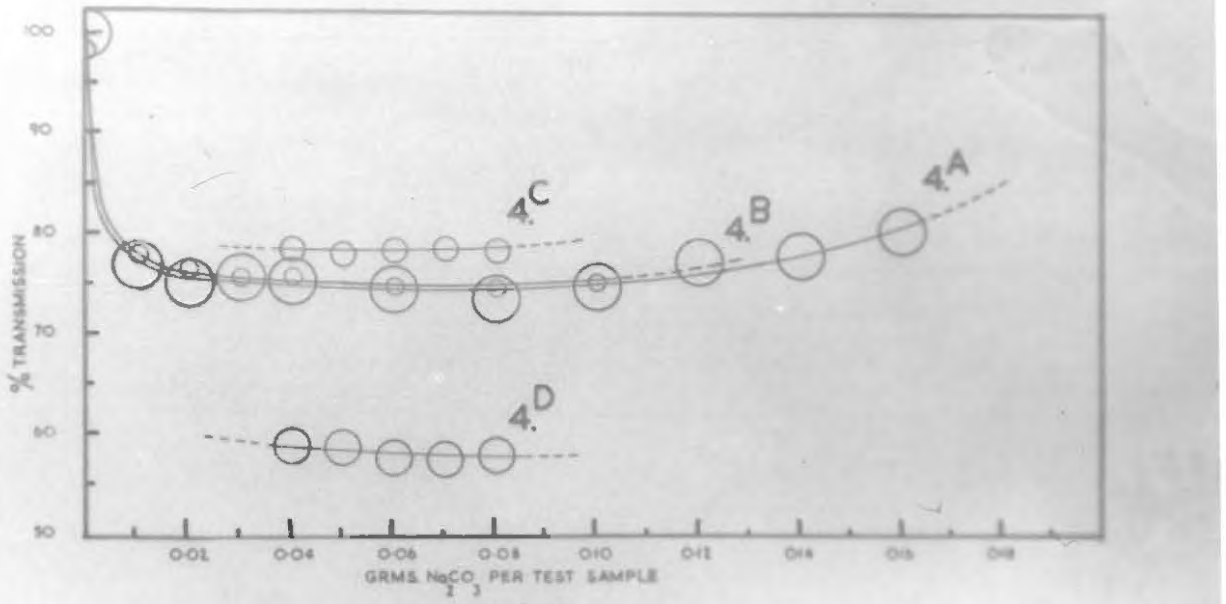

FIG. 4.

THE USE OF SODIUM CARBONATE IN PREVENTING BORON VOLATILIZATION.

The results are recorded in Table 4^C and Fig. 4^C.

TABLE 4^C.

THE USE OF SODIUM CARBONATE IN PREVENTING BORON VOLATILISATION FROM PLANT SOLUTIONS.

Sample No.	Na_2CO_3 (g.)	% Transmission Soln. C. 75p.p.m. of B.	% Transmission Soln. D. 165p.p.m. of B.
blank	0.06	100.0	100.0
1	0.04	78.8	59.3
2	0.05	78.5	59.1
3	0.06	78.9	58.2
4	0.07	79.0	57.8
5	0.08	79.0	58.4

The results again illustrate transmission stability, with no volatilisation of boron, between the concentration 0.04 - 0.08 g. of sodium carbonate per test sample.

As a result it was decided to add 0.06 ± 0.01 g. of sodium carbonate to each test sample in order to eliminate boron volatilisation before adjusting the pH.

NOTE / .....

NOTE:

It was found that if test aliquots were left standing after the addition of excess 1:8 hydrochloric acid they lost boron, even at room temperature. For example, 4 ml. aliquots of solution C (procedure 3) left to stand after adding 1.2 ml. excess hydrochloric acid, dropped in boron content from 1.50 µg. of B to 1.16 µg. after standing for 1 day, and then to 0.45 µ.g. after standing for two days before developing the colour. Similarly 4 ml. aliquots of solution D dropped from 3.30 µg. of B to 2.73 µ.g. on the first day, and then to 1.25 µg. on the second.

This would indicate that acidic solutions of boron are not stable, but will gradually lose boron on standing, even at room temperatures. However, a more detailed investigation of the different aspects of pH, temperature and time is necessary in order to verify this statement.

3.32. THE HYDROCHLORIC ACID CONCENTRATION.

In the method developed by Naftel (29), hydrochloric acid was used to promote colour development in an alcohol medium. Silverman and Trego (40), using an acetone medium, also used hydrochloric acid for the same purpose. However, Dible, Truog

and/ .....

and Berger (37) eliminated the use of such an excess of hydrochloric acid.

Having already incorporated the necessary pH adjustment step using hydrochloric acid to neutralise the sodium carbonate, the addition of an excess of hydrochloric acid, and its effects on transmission measurements, was thought essential. The following experiment was carried out:-

The contents of ten porcelain evaporating dishes, each containing 2 µg. B and 0.06 g. sodium carbonate, were evaporated to dryness at 100°C, cooled and then neutralised with 1 : 8 HCl, using 2 drops of phenolphthalein as indicator.

Excess 1:8 hydrochloric acid (0.0-3.0 ml.) were added to each of ten evaporating dishes from a microburette. After adding 4 ml. of the 5% oxalic -0.04% curcumin reagent to each sample, the determination was carried out as before

The results are recorded in Table 5A and Fig. 5A.

It can be seen from Fig. 5A, that the colour development stage is sensitive to changes in pH. Since the concentration of oxalic acid was later increased (Section 3.33)

/ the .....

TABLE 5A.

THE ADJUSTMENT OF pH IN THE PRESENCE OF 5% OXALIC ACID.

Sample No.	Excess 1:8 HCl per Sample (ml.)	% Transmission.
blank	1.0	100.0
1	0.0	79.9
2	0.2	78.3
3	0.4	76.7
4	0.6	76.5
5	0.8	76.1
6	1.0	75.5
7	1.5	76.7
8	2.0	78.7
9	2.5	80.5
10	3.0	84.7

the procedure described above was repeated, using a 10% oxalic - 0.10% curcumin reagent, as recommended in Section 4. Again 4 ml. test aliquots of two plant solutions were used as in Section 3.31. The results are recorded in Table 5B and Fig. 5B & C.

TABLE 5 B / .....

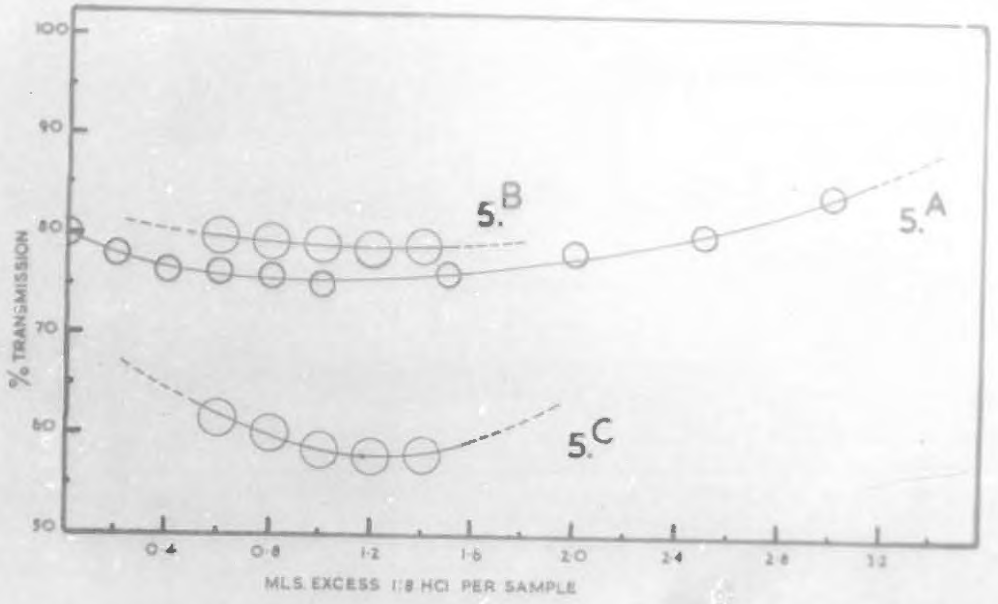

FIG. 5.

THE INFLUENCE OF EXCESS HYDRCHLORIC ACID ON THE COLOUR DEVELOPMENT.

TABLE 5^B.

THE ADJUSTMENT OF pH IN THE PRESENCE OF 10% OXALIC ACID.

Sample No.	Excess 1:8 HCl per sample (ml.)	% Transmission Soln.B 75p.p.m. of B.	% Transmission Soln.C 165p.p.m. of B.
blank	1.0	100.0	100.0
1	0.6	80.0	62.0
2	0.8	79.6	60.5
3	1.0	79.4	58.8
4	1.2	78.8	58.1
5	1.4	79.3	58.4

The results from Table and Fig. 5^A show that maximum absorption occurred in the presence of 1.0 ml. of excess 1:8 hydrochloric acid per sample, whilst in the presence of the extra oxalic acid (Table 5^B), maximum absorption occurred with an excess of 1.2 ml. of 1:8 hydrochloric acid. The difference was probably due to the extra buffering influence of the additional oxalic acid, requiring a greater excess of 1:8 hydrochloric acid to reach the same optimum pH. It is also clear that the use of the 10% oxalic acid had a greater

stabilizing / .....

stabilizing effect on the transmission than the 5% acid.

As a result of this study, the addition of an excess of 1.2 ml. of the 1:8 hydrochloric acid per test sample, was incorporated into the subsequent method. The reagent was prepared by diluting 25 ml. of concentrated C.P. hydrochloric acid with 200 ml. of deionised water.

3.33. THE OXALIC ACID CONCENTRATION.

The curcumin reagent as used by Dible, Berger and Truog contained 5g. of oxalic acid per 100 ml. of alcohol. To each test sample 4 ml. of this reagent were added, corresponding to 0.2g. of oxalic acid per sample. In the method of Silverman and Trego, 0.5 ml. of a 5% solution of oxalic acid in acetone was added to each sample, corresponding to only 0.025 g. of oxalic acid per sample. Since the author had already recommended the addition of an excess of hydrochloric acid (3.32), a procedure which differs from the Dible - Truog method, the influence of the buffering effect of the combined hydrochloric - oxalic acids was then investigated :-

Ten samples were prepared as before, each containing 2 µg. of boron, 0.6 gram of sodium carbonate and 1.0 ml. excess

1:8 hydrochloric acid. Oxalic acid (0.00 - 0.80 g.) was then added to each sample, followed by 4 ml. of a 0.04% solution of curcumin in alcohol. The blank was prepared using 0.04% curcumin - 5% oxalic acid reagent, while the colour development and transmission readings were carried out in the normal way.

The results are recorded in Table 6 and Fig. 6.

TABLE 6.

THE BUFFERING EFFECT OF OXALIC ACID.

Sample No.	Oxalic Acid (gram)	% Transmission.
blank	0.20	100.0
1	0.00	98.2
2	0.05	81.0
3	0.10	80.4
4	0.20	78.2
5	0.30	77.1
6	0.40	75.8
7	0.50	76.5
8	0.60	76.0
9	0.70	76.0
10	0.80	76.5

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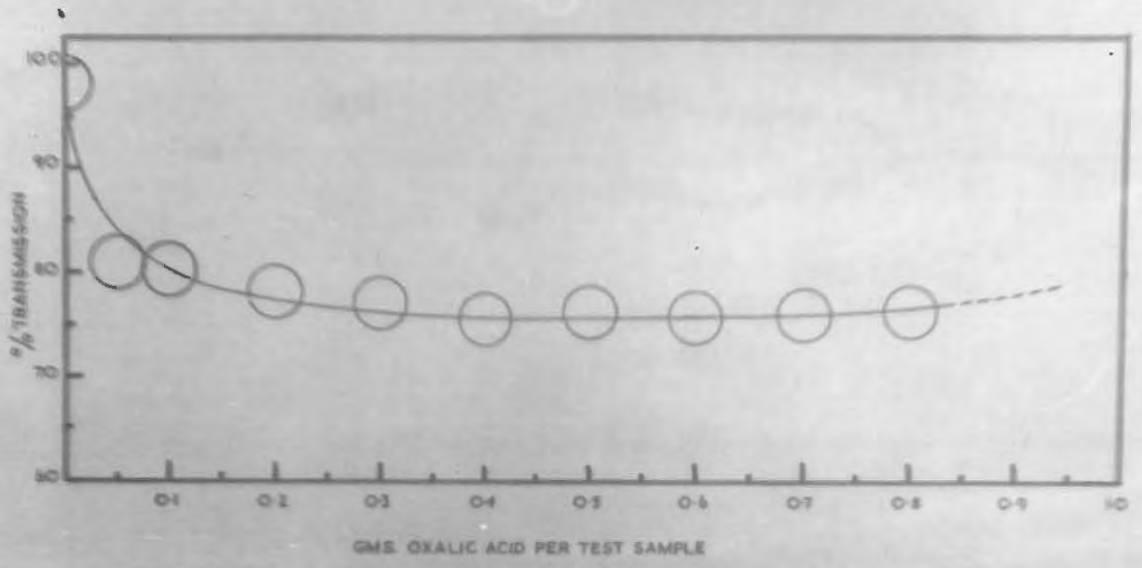

FIG. 6.

THE BUFFERING EFFECT OF OXALIC ACID.

The results show that transmission stability occurred between 0.3 g. and 0.8 g. of oxalic acid per sample. As the use of the 5% oxalic reagent corresponds to only 0.20 g. per sample, it is quite clear that the incorporation of the excess of 1:8 hydrochloric acid in order to attain higher sensitivity and greater stability, requires, in turn, a higher concentration of oxalic acid. As the solubility of the oxalic acid in alcohol limits its concentration, the use of 0.4 g. per sample was thought to be sufficient for bringing about the required buffering action. This corresponds to the use of a 0.04% curcumin - 10% oxalic acid reagent. The results quoted above, as well as the results obtained from repeated runs carried out in sections 3.31 and 3.32, confirmed the advantages of using a 10% oxalic reagent.

In addition, it was suspected at this stage, that the increase in the oxalic acid concentration had a stabilizing effect on the keeping quality of the curcumin solution (see sections 3.71. and 3.41.).

3.4. / .....

3.4. THE INFLUENCE OF THE CURCUMIN CONCENTRATION ON THE COLOUR DEVELOPMENT.

The work to date had involved the use of 4 ml. per sample of a 0.04% solution of curcumin in ethyl alcohol. As Dible, Berger and Truog (37) presented no data in favour of this concentration of curcumin, and since Naftel (29) in his original method, used as much as 2 ml. of a 0.1% solution per sample, whilst Trego and Silverman (40) used as little as 3 ml. of a 0.01% solution per sample, an investigation was carried out as follows:-

Twelve individual colour reagents were made up, each containing 10 g. of oxalic acid in 100 ml. alcohol with the curcumin concentration ranging from 0.01 g. up to 0.16 g. per 100 ml. alcohol.

Fifteen samples were then prepared, each containing 2.0 µg. of boron, 0.6 g. of sodium carbonate and 1.0 ml. excess of 1:8 hydrochloric acid. These samples were treated, up to the colour-development stage, as described in the previous section, a blank being run simultaneously with each individual test sample.

For developing the colour, a 4 ml. aliquot of each individual colour reagent was added to both the test sample

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and its corresponding blank. As the waterbath used in evaporating at 55°C could only take eleven dishes at a time, the colour development was carried out in three steps, the % transmission of each test sample being measured using its own corresponding blank.

The results are recorded in Table 7 and Fig. 7.

From the results it can be seen that the minimum change in % transmission with change in curcumin concentration only occurred at concentrations exceeding 0.08 % curcumin (i.e. using 4 ml. of a 0.08% curcumin reagent). It was, therefore, decided to use a 0.1% solution of curcumin, a concentration which is considerably higher than those used by previous workers.

It may be argued that sensitivity would surely be lost in using the higher concentration, since the transmission of the blank will decrease to such an extent that test samples would be measured from relatively dark solutions. However, since the % transmission is measured at 555 mμ (rosocyanin shows maximum absorption at 555 mμ) and not at 600 mμ (curcumin shows maximum absorption at 600 mμ), the decrease in % transmission with increase in curcumin

concentration/ .....

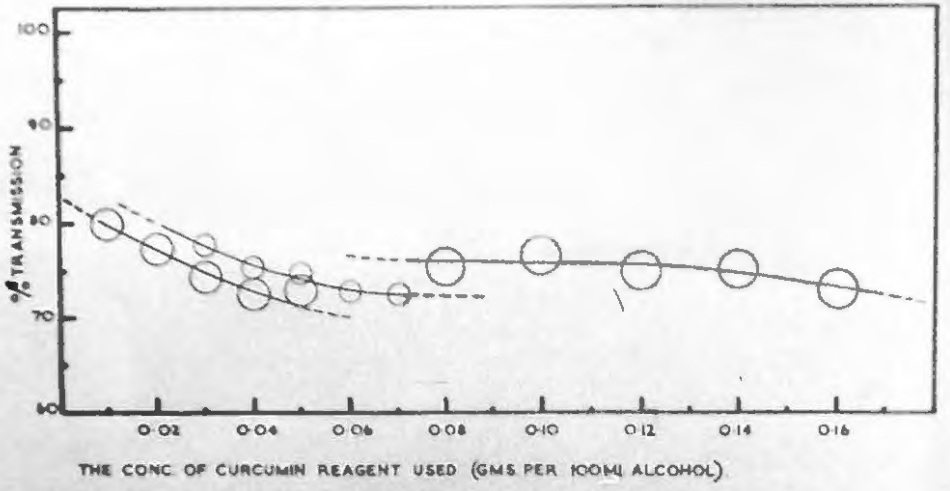

FIG.7.

THE INFLUENCE OF THE CURCUMIN CONCENTRATION ON THE COLOUR DEVELOPMENT.

TABLE 7.

THE INFLUENCE OF THE CURCUMIN CONCENTRATION ON THE COLOUR DEVELOPMENT.

Sample No.	Conc.of Curcumin Reagent (g./100ml.)	µg.boron per sample.	% Transmission.
blank	0.01	0.0	100.0
1	0.01	2.0	80.0
blank	0.02	0.0	100.0
2	0.02	2.0	77.4
blank	0.03	0.0	100.0
3	0.03	2.0	74.5
blank	0.04	0.0	100.0
4	0.04	2.0	72.9
blank	0.05	0.0	100.0
5	0.05	2.0	73.0
blank	0.03	0.0	100.0
6	0.03	2.0	77.8
blank	0.04	0.0	100.0
7	0.04	2.0	75.4
blank	0.05	0.0	100.0
8	0.05	2.0	74.0
blank	0.06	0.0	100.0
9	0.06	2.0	73.0
blank	0.07	0.0	100.0
10	0.07	2.0	72.5
blank	0.08	0.0	100.0
11	0.08	2.0	75.4
blank	0.10	0.0	100.0
12.	0.10	2.0	76.7
blank	0.12	0.0	100.0
13	0.12	2.0	75.0
blank	0.14	0.0	100.0
14	0.14	2.0	75.1
blank	0.16	0.0	100.0
15	0.16	2.0	73.0

concentration / ....

concentration is negligible. A decrease in transmission of only 1.5% for every increase of 0.02 g. curcumin per 100 ml. alcohol was measured.

3.41. THE STABILITY OF THE CURCUMIN REAGENT.

It was noticed that in dissolving the curcumin in alcohol, the solution was never quite complete. Even though the solubility of curcumin in alcohol is far greater than 0.1g. per 100 ml., a small quantity of the curcumin remained insoluble. These insoluble particles were thought to be due either to an impurity or to a slightly soluble oxidation product of the curcumin reagent used (B.D.H. "Laboratory Reagent"), or to the formation of an amorphous suspension. As the accuracy and reproducibility of the method (See section 5) was as yet far from satisfactory at this stage, the presencs of even such small particles was viewed with suspicion. These particles were removed by preparing the reagent as follows:-

The solution of 0.10 g. of curcumin and 10 g. of oxalic acid in 100 ml. alcohol was shaken up at ten-minute intervals, warming each time to about 60°C using a large beaker as a waterbath. After half an hour, the solution was cooled to -5°C in a refrigerator and then filtered

under / .....

under vacuum through a Whatman No. 42 filter paper. This resulted in a clear homogeneous solution.

Dible, Truog and Berger stated that the curcumin reagent, as prepared by them, was only stable for "several" days when stored in the dark, and only for a week when stored in a refrigerator. As little evidence in support of this instability was recorded, it was thought necessary to investigate the stability of the reagent as prepared above.

In order to measure the optical stability of the reagent, a stable inorganic blank was prepared. Using different amounts of potassium chromate (yellow) and potassium dichromate (orange-red), a blank solution was made to match the curcumin reagent. It was possible to match the colours to within 1% transmission. Readings taken over a period of a week on a curcumin reagent stored in the dark at room temperature showed absolute optical stability within this time limit. A variation of 0.2% was recorded, but this was regarded as an instrumental error.

Optical stability, however, does not necessarily ensure chemical stability, a factor which is of equal importance. The investigation of the chemical stability of the reagent was far more difficult to carry out.

Up / .....

Up to this stage the results have always lacked both accuracy and reproducibility, resulting in the repetition of many of the experiments. On a closer examination of these replicated results, it was noticed that the precision or conformity of the runs using a 2 - or 3 - day old curcumin reagent was always far better than that of the original run using a freshly-prepared reagent, although good reproducibility was still lacking.

Although experiments carried out at a later stage (see section 3.6) explained the lack in reproducibility of the method, this improvement in precision with ageing of the reagent could only be explained by an unstable colour reagent. The explanation for this initial instability appears to be related to the tautomeric equilibrium of the curcumin in solution. Thus the initial heating of the reagent during preparation, together with the use of a greater concentration of oxalic acid (the reaction is sensitive to pH) would have a stabilizing effect on this equilibrium.

It was only after reproducible results were eventually obtained, that the stability of the curcumin reagent, prepared in this way, could be adequately demonstrated.

Apart / .....

Apart from the first day (the day of preparation), the reagent proved to be chemically stable for at least 10 days when stored in the dark at room temperature. The results recorded in section 3.9., representing 30 consecutive determinations carried out over a period of 10 days using the same curcumin reagent, verify the stability of the reagent when stored in the dark at room temperature.

When the reagent was kept in a refrigerator at $-5^{\circ}C$, it was found to be chemically stable for at least 5 weeks. 20 replicate determinations carried out using a week-old reagent showed a % standard deviation of 2.3%, whilst a similar run carried out, using a 5 week-old reagent, showed a % standard deviation of 1.9%

However, the reagent was found to become slightly more sensitive to small amounts of boron - a decrease of 1.2% transmission (the mean difference of 6 duplicate standard determinations) at a boron concentration of 2.0 µg. B per sample was measured over a period of 4 weeks. For this reason standardisation curves are best plotted at least once every 2 weeks whilst using the same curcumin reagent.

3.5. / .....

3.5. THE INFLUENCE OF TEMPERATURE ON THE COLOUR DEVELOPMENT.

The choice of an evaporation temperature of 55 ± 3°C in developing the colour could not be credited to any one particular investigator, but was used in the earlier work carried out by Naftel (29). Later data, presented by both Hafford (42) and Russell (39), confirmed the use of this evaporation temperature.

At this stage the results obtained by the author were still not reproducible, and because the control of the temperature during the evaporation procedure was suspected, a closer investigation into the temperature effects was undertaken.

Prior to this investigation, analyses had been carried out using a waterbath fitted with a "Simmerstat" heating unit. This was found quite satisfactory in controlling the temperature to within ± 2° at 55°C, but for the present investigation a more specific temperature control was thought necessary.

Thus, besides the "Simmerstat" control, a "Sunvic" thermoregulator, in conjunction with a 100 Watt heating unit, together with an electric stirrer, was fitted to the waterbath.

The / .....

The thermoregulator was adjusted to take over the heating control at the maximum stabilizing temperature of the Simmerstat control, which was first adjusted to heat the water to just below the specific temperature desired. For further temperature stability and economy, the waterbath was insulated in a box, using cork-chip fillings. This setup enabled a control to within 0.5°C even at the higher temperatures.

Using the method as developed up to this stage, several series of determinations at a constant boron content of 2.0 µg.B per sample, were carried out using a wide range of evaporating temperatures. Each determination was carried out in duplicate, together with an individual blank.

Initially, determinations were carried out at 5° intervals between 35°- 70°C . At the lower temperatures this involved a tedious 8-hour evaporation, which shortened to half an hour only at the higher temperatures. It was also noted that development of the colour at the lower temperatures produced a pink precipitate of sodium chloride, which was virtually impossible to wash free of colour. This colouration diminished noticeably with temperature, becoming negligeable when evaporating above 50°C .

The results are recorded in Table 8 and Fig. 8 ., and from these it is seen that a stable maximum colour development

occurred / .....

occurred between 48° - 55°C.

TABLE 8 A.

THE EFFECT OF TEMPERATURE ON THE COLOUR DEVELOPMENT BETWEEN 35° - 70°C.

Temperature °C.	Approximate Time of Evaporation (hrs.)	% Transmission	Mean % Trans.
35	8.0	100.0 (blank)	
35	8.0	80.5	80.0
35	8.0	79.5	
40	6.0	100.0 (blank)	
40	6.0	74.6	74.3
40	6.0	74.0	
45	4.0	100.0 (blank)	
45	4.0	73.0	73.2
45	4.0	73.4	
50	2.5	100.0 (blank)	
50	2.5	68.5 x	69.0
50	2.5	69.5 x	
55	2.0	100.0 (blank)	
55	2.0	71.8	71.8
55	2.0	73.0 x	
60	1.5	100.0 (blank)	
60	1.5	72.3 x	74.3
60	1.5	74.3	
65	1.0	100.0 (blank)	
65	1.0	76.0	75.2
65	1.0	74.3	
70	0.5	100.0 (blank)	
70	0.5	79.0	79.8
70	0.5	80.6	

A Second / .....

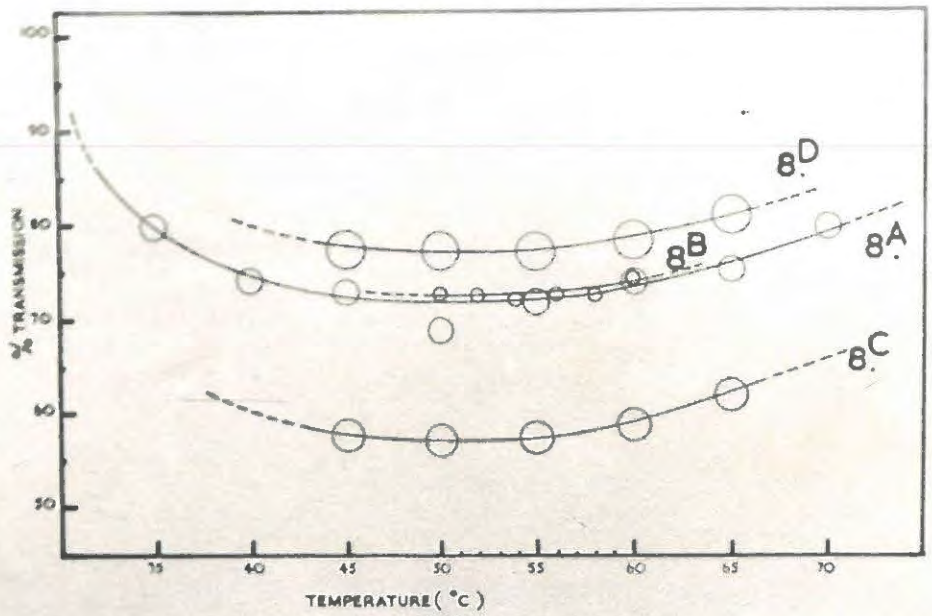

FIG. 8.

THE INFLUENCE OF TEMPERATURE ON THE COLOUR DEVELOPMENT.

TABLE 8^B.

THE EFFECT OF TEMPERATURE ON THE COLOUR DEVELOPMENT BETWEEN 50° - 60°C.

Temperature °C	Approximate Time of Evaporation (hrs.)	% Transmission	Mean % Transmission.
50	2.5	100.0	
50	2.5	72.5	72.7
50	2.5	72.9	
52	2.25	100.0	
52	2.25	72.6	72.5
52	2.25	72.4	
54	2.0	100.0	
54	2.0	72.0	72.0
54	2.0	70.5 x	
56	2.0	100.0	
56	2.0	72.8	72.8
56	2.0	72.8	
58	1.75	100.0	
58	1.75	72.8	72.4
58	1.75	72.0	
60	1.5	100.0	
60	1.5	73.8	74.0
60	1.5	74.2	

A second run was then carried out between 50° - 60°C, with 2° intervals between determinations. Again the

results / .....

results (Table 8^B and Fig. 8^B) indicated that a stable transmission occurred between 48^o - 55^oC. (the results marked with an asterisk indicate poor conformity and these are explained later in Section 3.6.)

Two additional runs, employing two plant solutions, were carried out, employing the method as finally modified (Section 4). The results (see Table 8^C and Fig. 8^C) again agreed with those recorded in Table 8^A.

If all the results and curves obtained to date are examined as regards accuracy and reproducibility, four interesting conclusions can be drawn:-

TABLE 8^C.

THE EFFECT OF TEMPERATURE ON THE COLOUR DEVELOPMENT OF PLANT SOLUTIONS.

Temp. ^oC.	Soln. C. 157p.p.m. of B. % Transmission	Soln.D. 60p.p.m. of B. % Transmission.
45	57.7	77.8
50	57.4	77.3
55	57.4	77.4
60	59.0	78.5
65	62.2	81.0

(i) / .....

(i) The agreement between the duplicate determinations was quite satisfactory, but the reproducibility was still poor. This lack of reproducibility could only be accounted for by considering the remaining stage not yet investigated, viz. the time allowed for the drying or baking of the reaction products after evaporation at 55°C. This conclusion was supported by the fact that the duplicate determinations carried out at 50°C in run 8^{A}, although agreeing to within 1% transmission, did not fit the resulting graph 8^{A} ; as it happened this particular duplicate determination was allowed to dry for over an hour instead of the required 30 minutes.

(ii) The reproducibility of each individual duplicate determination, although not particularly good, at least allowed for the final drawing of reasonably smooth curves. The curves 8^{A} and 8^{B} show up the rather erratic results obtained initially, whereas the

results / .....

results from 8^{0} are good, using the method as finally modified.

(iii) Fig. 8 in general indicates that little, if any, colour development occurred at temperatures below $30^{o}C$. It can be concluded, therefore, that no interference will result from any small contaminations of boron introduced after the colour development stage. Filtering of solutions after colour development is thus quite in order, but should be avoided beforehand.

(iv) Evaporations (in developing colour) carried out at $55^{o} \pm 3^{o}C$ would introduce an error of 4 - 6 % at concentrations of 2 - 3.5 µg. B per test sample. On the other hand, when the evaporation was carried out at $53^{o} \pm 2^{o}C$ virtually no error was introduced within these limits. The latter temperature range was, therefore, adopted in place of the former.

3.6. THE INFLUENCE OF THE DRYING PERIOD ON THE COLOUR DEVELOPMENT.

In the method of Dible, Berger and Truog, the concentration of the hydrochloric acid was not known as there was no adjustment of pH. On evaporating a test sample (prepared by using hydrochloric acid) at 55°C and baking for a further 15 minutes, the evaporation of the hydrochloric acid was in no way controlled. As the final pH of the residue is important as will be shown below, this drying period is of considerable importance.

Employing the method as modified up to this stage, and including the adjustment of the pH, the period of drying after evaporating at 53°C, was studied. For this investigation, deep 250 ml. porcelain fusion crucibles were used. These crucibles produced a slower, but more uniform, rate of drying, which allowed for the more accurate study of the drying effects.

Eight samples, each containing 2.0 µg. of boron, and each coupled with a blank, were evaporated at 53°C, just to dryness, and then removed at successive intervals of 30 minutes. After cooling each sample for 10 minutes they were filtered, made up to 100 ml. with alcohol, and their respective % transmissions measured. The results are recorded

in / .....

in Table 9^A and Fig. 9^A.

TABLE 9^A.

THE INFLUECNE OF DRYING THE REACTION PRODUCTS IN FUSION CRUCIBLES.

2.0 ug. B per Sample. Using fusion crubibles. No draught.	
Time (mins) (After evap. to complete dryness.)	% Transmission.
0	79.8
30	71.0
60	71.2
90	68.2
120	69.4
150	69.2
180	68.8
210	68.8

Since the deep fusion crucibles did not allow for efficient circulation of air in order to aid in the evaporation of excess hydrochloric acid, an unnecessarily long drying time was involved. Consequently a second run at a higher boron concentration was carried out, and removing the samples at 15 minute intervals. The results are recorded in Table 9^B

and / .....

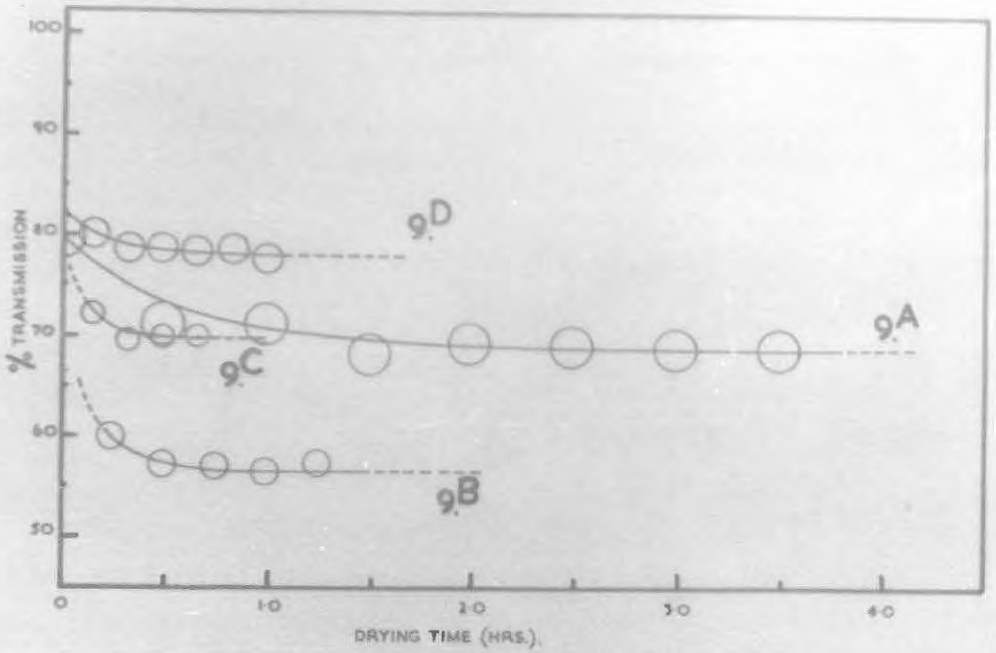

FIG.9.

THE DRYING OF REACTION PRODUCTS AT 54°C.

and Fig 9^B.

TABLE 9^B.

THE INFLUENCE OF DRYING THE REACTION PRODUCTS IN EVAPORATING DISHES.

3.0 µg. B per sample. Using Evaporating Dishes. No draught.	
Time (mins.) (After evap. to complete dryness.)	% Transmission.
0	-
15	59.8
30	57.1
45	57.0
60	56.4
75	57.5

Again, two extra series of determinations were carried out on two plant solutions, employing the modified method as outlined in Section 4. These results are recorded in Table 9^C and Fig. 9^C.

The results (9^A, B and C) clearly illustrate that the 15 minute drying time (used up to this stage), was not

sufficient / .....

TABLE 9^C.

THE USE OF A FORCED DRAUGHT IN DRYING THE REACTION PRODUCTS.

1.9 µg.B per Sample (C). Using Evap.Dishes. Forced draught used.		1.2 µg.B per sample (D). Using Evap. Dishes. No draught used.	
Time (Mins.) (after evap. to complete dryness)	% Trans-mission	Time (mins.) (after evap. to complete dryness)	% Trans-mission
0	-	0	-
10	72.2	10	80.0
20	69.5	20	78.8
30	70.0	30	78.6
40	70.1	40	78.3
50	spoilt	50	78.8
		60	77.8

sufficient, this short drying period being responsible for the non-reproducibility of the results obtained by the method as modified up to this stage. Whilst series 9^A (using deep fusion-crucibles) took 3 hours baking time before stable colour development was reached, series 9^C (using open evaporating dishes) took only $\frac{1}{2}$ hour, a forced draught being used to aid the evaporation. Series 9^B and 9^D were carried out using open dishes with no forced draught, and stable conditions were reached within $\frac{3}{4}$ hour of baking.

As / .....

As only 15 minutes were saved using a forced draught it was decided that samples should rather be baked for 45 minutes in open porcelain evaporating dishes, using no forced draught.

3.7. THE STABILITY OF THE FINAL COLOUR SOLUTIONS.

It was noted during the investigation on drying effects (section 3.6.), that solutions made up after only a short period of baking at 53°were far from stable, whilst those which were baked for a longer period appeared to be relatively stable. This was proved to be the case by carrying out the following experiments:-

Two test solutions were prepared:
Solution A, dried for 5 minutes at 53°, and Solution B, dried for 1 hour under a forced draught. Both solutions contained 2.0 µg. B, the colour being developed as before, using a 5% oxalic acid - curcumin reagent.

After filtering and taking up the reaction products in 100 ml. of alcohol (the procedure taking 30 mins. in all), the change in % transmission with time was measured in the case of each individual solution. The results are recorded

in / .....

in Table 10^{A}.

TABLE 10^{A}.

THE EFFECT OF NON-VOLATILISED HYDROCHLORIC ACID ON THE STABILITY OF THE FINAL COLOUR SOLUTIONS.

Time (hrs. & mins.)	% Trans. A.	Time (hrs. & mins.)	% Trans. B.
0.0	-	0.0	-
0.30	78.8	0.30	68.2
1.15	79.2	1.00	69.4
2.15	79.8	1.30	69.8
4.45	80.2	2.30	70.0
6.00	80.9	3.30	70.0
9.00	81.4	4.30	70.0
12.00	90.0	12.00	72.0

From these results it is clear, that Solution A, high in non-volatilized hydrochloric acid, is optically unstable; in fact after standing for two days the % transmission of this solution returned to almost 100% with respect to the blank. On the other hand, Solution B (low in hydrochloric acid content) reached optical stability within an hour and only increased by 2% over a period of 2 hours.

These / .....

These results again emphasised the need for a pH adjustment before carrying out the colour development step in order to obtain reproducible results - special note should be taken of the initial difference of 10% transmission between solution A and solution B.

As the experiment was carried out using a 5% oxalic acid - curcumin reagent, the optical stability of solutions developed from a 10% oxalic acid reagent was next observed. Two plant solutions were used, and their optical stability with time measured as before.

The results are recorded in Table 10^B.

TABLE 10^B.

THE STABILITY OF THE FINAL COLOUR SOLUTIONS.

Time (hrs.)	% Trans. C. Soln C. 157ppm.B.	% Trans Soln D. 60p.p.m. B.
0.0	-	-
1.0	59.0	79.0
2.0	59.0	79.0
3.0	59.2	79.3
4.0	59.2	79.8
18.0	61.1	81.6

The / .....

The experiments using a 10% oxalic acid reagent and a drying period of one hour (108), showed that the solutions were completely stable up to three hours after developing the colour, whereas in the previous run, using a 5% oxalic reagent (10^{A}, Solution B), the solution only attained stability after standing for one hour.

Thus all transmission measurements of solutions (following this later procedure) should be taken within 3 hours of being made up.

3.8. THE RANGE OF THE MODIFIED METHOD.

Owing to the extreme sensitivity of the curcumin - boric acid reaction used in the analytical procedure, the determination of boron is limited to a very small range. In the method of Dible, Berger and Truog, a 1 ml. test aliquot containing 0 - 2 µg. of boron was used in developing a 25 ml. final colour solution, the range of the method being from 0.0 to 1.2 µg. of B, with the % transmission dropping below 30% at the higher concentration.

As the boron content in plant tissue fluctuates greatly from species to species, and even sometimes from plant to plant of the same species (e.g. citrus leaves in this area were found to range between 50 - 20 p.p.m. of B), this limited

range / .....

range of the method is a distinct disadvantage.

With a view to increasing the effective range of the method, as well as for reasons outlined in section 3.3., several changes were instituted :

(a) The critical "1.00 ml." test aliquot was replaced by a more variable 4 to 8 ml.

(b) The final solution was made up to 100 ml. instead of 25 ml.

(c) The % transmission was limited to values lying between 50 and 100%, avoiding low instrumental sensitivity at high concentrations of boron.

Using a 5% oxalic - curcumin reagent and incorporating the above modifications, the range was extended to 3 µg. of boron, replicate determinations carried out at higher concentrations of boron became erratic with the eventual break-down of Beer's law (See Table 11 and Fig. 10^{A}) .

Using a 10% oxalic - curcumin reagent, the range of the method was increased to at least 5 µg. of B ; that is Beer's law was followed from 0 - 5 µg. of B (See Table 12 and Fig. 10^{B}). The limiting factor is actually not the break-down of Beer's law, but the intense colour development

at / .....

at the higher B concentration, the % transmission dropping down to 44% at 5 µg. of B per sample.

TABLE 11.

THE RANGE OF THE MODIFIED METHOD USING 5% OXALIC ACID.

ug. B per sample (A)	% Transmission.
0.0	100.0
0.5	93.0
0.5	94.0
1.0	87.3
1.0	86.3
1.5	81.9
1.5	80.3
2.0	75.1
2.0	75.2
2.5	70.8
2.5	70.0

NOTE: / .....

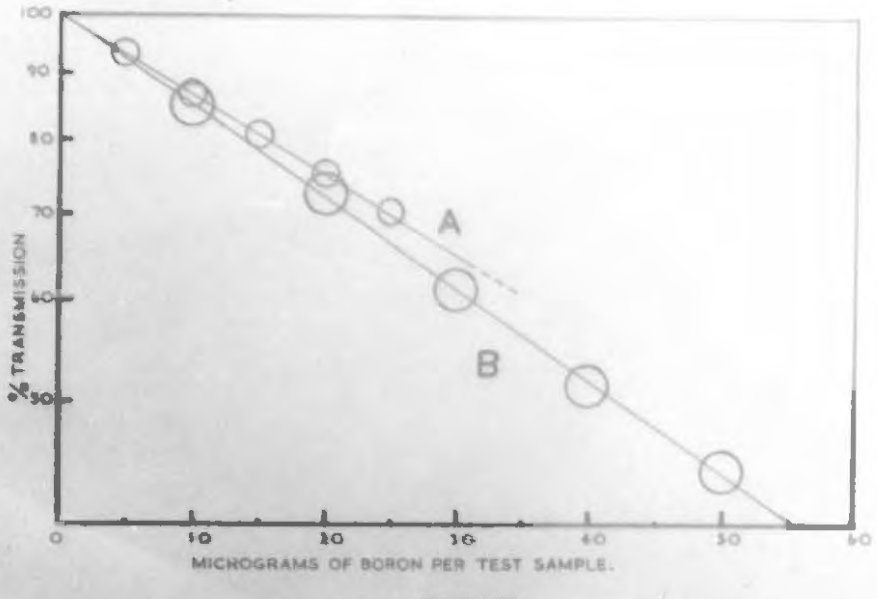

FIG.10.

STANDARD REFERENCE CURVES

FOR THE DETERMINATION OF BORON.

TABLE 12.

THE RANGE OF THE MODIFIED METHOD USING 10% OXALIC ACID.

µg. B per sample (B)	% Transmission.
0.0	100.0
1.0	85.0
2.0	72.2
3.0	62.2
4.0	51.4
5.0	44.0

NOTE: In the method of Dible, Berger and Truog, the use of a 1 ml. test aliquot, without subsequently adjusting the pH prior to the colour development, a simulated standard determination using 1 ml. aliquots of standard boron solutions, is not possible (standard solutions are prepared using distilled water, whilst hydrochloric acid is used in preparing the plant test solutions).

3.9. INTERFERENCE FROM FOREIGN IONS.

Having developed a somewhat modified curcumin procedure for the determination of small quantities of boron, it now remained to investigate interferences, if any, from

the/.....

the ions commonly found in plant ash.

Those ions known to interfere with the curcumin colour reaction will first be briefly reviewed:

Feigl (42) reported that ferric iron, molybdenum, titanium, colombium, tantalum and zirconium, when present in sufficient quantities, gave a red-brown colour in an acid solution of curcumin and thus these ions interfere. He also showed that oxidizing agents such as peroxides, chromates, permanganates, nitrates and chlorates prevent or retard the reaction between curcumin and boric acid.

Yoe and Sarver (43) stated that beryllium aluminium, iron and magnesium formed colour lakes with curcumin. However, Kolthoff (44) has shown that this lake is only formed in an alkaline medium. Hence the excess hydrochloric acid, together with the oxalic acid used in the method, would prevent any such lake-formation.

Schäfer (45), indicated that the presence of fluorides decreased the sensitivity of the reaction. However, the occurrence of fluorides in plant material in more than trace quantities is very rare.

Nitrates / .....

Nitrates are known to interfere with the curcumin - boric acid colour reaction, but as was shown by Berger, Truog and Dible, they only interfered at concentrations exceeding 20 p.p.m., a concentration which is seldom exceeded in plant ash treated as below.

In order to investigate the effects of various concentrations of the elements commonly found in plant material, the following experiments were carried out:-

Synthetic solutions were prepared so as to contain the elements Ca, Mg, Fe, Mn, Zn, K, S and P in the maximum proportions normally present in dried citrus leaf material. "Specpure" chemicals were used to prepare these solutions, the proportions of each being indicated in Table 13.

NOTE: Sulphur and Potassium are included in the different synthetic solutions, whilst sodium is present at high concentration in the actual method. Nitrogen is not considered as it is virtually all lost during the ashing procedure.

In the case of each specific element, five test samples containing 2.0 µ.g. of boron and 0.8 g. of sodium carbonate were first prepared (2 µg. B per test sample

corresponds / .....

corresponds roughly to the normal boron content found in a 4 ml. aliquot of a 200 ml. plant solution prepared from 1 g. of dried citrus leaf material). To each of the 5 test samples, different aliquots of the previously prepared synthetic solution were then added.

After evaporating these solutions to dryness at a 100°C on a waterbath, the boron content of each sample was determined as outlined in Section 4. (The results are recorded in Table 14).

From the transmission data presented in Table 14, the author concluded that none of these elements interfered with the curcumin reaction at these concentrations.

In concluding this section on interferences, final runs were carried out on synthetic plant solutions at different concentration levels. This involved the addition of up to 4 ml. of each of the six individual synthetic solutions to each test solution, resulting in an initial evaporation of up to 30 ml. before adjusting the pH. As before, 2.0 µg. of boron and 0.1 g. of sodium carbonate were added to each sample at this initial stage. The % transmission,

following/ .....

TABLE 14.

A STUDY OF INTERFERENCES FROM ANY ONE PARTICULAR ELEMENT.

Phosphorus		Calcium	
µg. P present	% Trans-mission.	µg. Ca present	% Trans-mission
0.0	73.7	0.0	72.3
2.5	73.7	12.5	73.1
5.0	73.7	25.0	74.0
7.5	75.0	37.5	74.0
10.0	73.5	50.0	73.3

Magnesium		Iron	
µg.Mg.present	% Trans-mission.	µg.Fe present	% Trans-mission.
0.0	73.7	0.0	73.8
10.0	74.0	0.5	74.3
20.0	73.3	1.0	73.4
30.0	73.7	1.5	73.7
40.0	73.2	2.0	73.2

Manganese		Zinc	
µg.Mn present	% Trans-mission	µg. Zn present	% Trans-mission.
0.0	73.7	0.0	73.1
1.75	73.4	0.5	73.3
3.5	73.5	1.0	73.4
5.25	74.0	1.5	73.0
7.0	73.0	2.0	73.3

following / .....

following the colour development, was then measured as before. For the compositions of the different synthetic plant samples, and their eventual % transmissions, see Table 15.

TABLE 15.

A STUDY OF POSSIBLE INTERFERENCE FROM A COMBINATION OF ELEMENTS.

Sample No.	µg. P	µg. Ca.	µg. Mg.	µg. Fe.	µg. Mn.	µg. Zn.	µg. K.	µg. S	% Trans.
blank	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0
1	1.2	6.2	5.0	0.2	0.8^{8}	0.2^{5}	1.5	7.2	74.0
2	2.5	12.5	10.0	0.5	1.7	0.5	3.0	14.4	73.2
3	3.7	18.7	15.0	0.7	2.6^{3}	0.7^{5}	4.5	21.6	74.0
4	5.0	25.0	20.0	1.0	3.5	1.0	6.0	28.8	74.0
5	6.2	31.2	25.0	1.2	4.3^{8}	1.2^{5}	7.5	36.0	73.5
6	7.5	37.5	30.0	1.5	5.2^{5}	1.5	9.0	43.2	74.0
7	8.7	43.7	35.0	1.7	6.0^{3}	1.7^{5}	10.5	50.4	74.2
8	10.0	50.0	40.0	2.0	7.0	2.0	12.0	57.6	74.0

From these results, it was concluded that no combination of the common elements investigated, interfered with the determination of boron by the modified curcumin method.

3.10 THE EXTRACTION OF BORON FROM/.....

3.10. THE EXTRACTION OF BORON FROM PLANT MATERIAL.

As yet no accurate method has been developed which can be applied to a variety of sample types without the prior separation of boron from those substances which interfere in its determination. Separation techniques which have been employed include ion exchange, precipitation, solvent extraction, distillation, paper chromatography and electrolytical procedures. Of these, only the distillation of boric acid as the methyl ester, and the ion exchange procedure (46), have shown any analytical applicability in the presence of interferences. Both procedures involve the use of additional apparatus and time-consuming techniques.

As the determination of boron in plant material, using the curcumin procedure, has been shown to be virtually free from inorganic interferences (section 3.9.), only a general separation of the boric acid from the organic plant material is necessary.

Due to the volatility of boric acid in a warm acid solution, the nitric-perchloric acid decomposition technique normally used in trace element work, is not practical. Consequently the dry ashing of the leaf

material/.....

material, followed by a mild acid leach, was investigated.

3.101. THE DRY ASHING OF PLANT MATERIAL.

Experiments carried out by McHargue and Hodgkiss (47), Hafford (41) and Berger and Truog (48), have shown that plant tissue does not lose boron when ashed at 550°C. These authors explain this phenomenon as being due to the high content of natural bases in the plant material preventing any such loss of boron.

Admittedly the final plant ash residue is very alkaline, all the basic metals having been converted to their respective oxides or carbonates, but it must be remembered that initially the plant tissue is virtually neutral with respect to pH. This latter point is important, as the work carried out in Section 3.31 indicated that boron is lost by volatilisation below a pH of 9.5 when heated at only 100°C. As the initial stage in plant ashing involves the heating to at least 450°C, the exact explanation for the quantitative recovery of boron from plant material after ashing at 550°C was not clear.

In order to clarify this problem, the relationship of boron volatilisation with the time and temperature of ashing, as well as the quantitative nature of the solvent extraction,

were / .....

were investigated.

The Time of Ashing at 550°C.

If any volatilisation of boron does take place during the ashing stage, the loss is most surely a time factor. Moreover the higher the boron content of the plant material, the greater the measureable loss. In investigating this possible source of error, the following procedure was adopted:-

About 50 g. of freshly-dried citrus leaf material, with a high boron content (184 p.p.m.) was first ground to a uniform fine powder in an agate ball mill. This large sample was then allowed to stand in an oven, set at 50°C, for 3 days to ensure an overall constant moisture content before further use.

Ten individual 1.000° g. samples of this dried leaf material were then ashed at 550°C for different lengths of time, the ashing being carried out in 6 cm. porcelain casserole dishes. In each case the temperature of the muffle furnace used was quickly raised to about 250°C, and kept at this temperature for one hour so as to burn off most of the carbon, then taken up to 550°C for different lengths of time.

After / .....

After allowing each sample to cool down, the alkaline ash residue was neutralised with 1:8 hydrochloric acid, finally adding 1 ml. in excess. Indicators proved to be unsatisfactory for obtaining this neutralisation due to the high adsorption of the indicator by the fine precipitate of silica present in the ash. Since the exact neutralisation was in no way a critical factor, the end point was best judged to have been reached when effervescence of the ashed residue ceased on addition of HCl. Each sample was then made up to 200 ml. in a volumetric flask using deionised water and transferred to a polythene bottle.

Using a 4 ml. test aliquot in each case, the boron content was determined as outlined in section 4. The results are recorded in Table 16.

From these results it is clear that no loss of boron occurred when ashing for up to 12 hours at 550°C. Slight losses were suspected when ashing for longer periods, but the small increases in the % transmission of samples 7 - 10 are within the limits of experimental error.

TABLE 16. / .....

TABLE 16.

THE EFFECT OF TIME OF ASHING AT 550°C ON THE VOLATILISATION OF BORON FROM CITRUS LEAF POWDER.

Sample No.	Time of Ashing at 550°C (hrs.) x	% Transmission.	p.p.m. of B per sample.
1.	2.5	spoilt	-
2	5	54.2	186.0
3	6	54.8	183.0
4	8	54.8	183.0
5	11	54.2	186.0
6	12	54.6	184.0
7	21	53.8	188.0
8	23	55.2	181.0
9	27	55.2	181.0
10	29	55.4	180.0
	x zero time taken after burning off at 250°C.		

The Temperature of Ashing.

In attempting to define the possible range of temperature which may safely be used in the ashing of plant material, a run similar to that carried out on the time of ashing, was undertaken using temperature as the variable factor.

0.500 ° g./ .....

0.500°g. samples, prepared as above, were ashed at different temperatures for 6-8 hours, followed by the same extraction procedure. As only 0.5 g. samples were weighed out, 5 ml. test aliquots were used in the final determination of boron. The results are recorded in Table 17.

TABLE 17.

THE EFFECT OF THE TEMPERATURE OF ASHING ON THE VOLATILISATION OF BORON FROM CITRUS LEAF POWDER.

Sample No.	Temperature°C.	% Transmission.	p.p.m. of B.
1	350	66.2	200.4
2	450	67.0	194.0
3	550	65.0	210.0
4	600	65.5	206.0
5	600	65.2	208.4
6	700	64.7	212.4
7	700	64.6	213.2
8	800	64.8	211.6
9	800	64.8	211.6

The results indicated that no loss of boron occurred, even when ashing at temperatures of up to 800°C. The rather

low/ .....

low results obtained when ashing at 350° and 450°C were credited to the presence of unburnt carbon still present in the sample after ashing.

NOTE:

Since the test plant samples were very finely ground and powdery, moistening of the sample before ashing was employed at one stage. This moistening had the desired effect of preventing a possible loss of sample due to draughts, but it caused a large error when the sample was ashed. Losses of up to 16% of the total boron present in the plant material were recorded from 20 replicate samples ashed in this way.

It would appear that the addition of any water to the sample before ashing would result in the extraction of the very soluble boron at a pH which was still virtually nautral, resulting in the volatilisation of some boron in the early stages of ashing.

The Efficiency of the Extraction.

Past workers in this field have generally favoured an extraction with a strong acid solution, following the ashing of the plant sample. Although the strong acid extraction might be quite quantitative with respect to boric acid, a very

mild / .....

mild acid extraction would be far more advantageous, because:

(a) Acidic solutions of boric acid are not very stable even at room temperatures (See Section 3.31).

(b) Boric acid is very soluble in both acid and alkaline aqueous media.

(c) A mild acid leach would ensure an easier adjustment of the pH in the initial stage of the actual determination of boron.

Since there was no apparent reason for the use of such a strong acid extraction, apart from the increase in solubility of some of the trace elements, the quantitative nature of a very mild acid leach was first investigated:

Following the ashing of 0.5 - 1.0 g. of plant material, the alkaline ash was first neutralised with 1:8 hydrochloric acid, adding only 1 ml. in excess - this ensured the solution of the heavier trace metals. Decanting with deionised water, the solution plus the fine precipitate of silica was washed into a 200 ml. volumetric flask and made up to the mark. After shaking thoroughly, the solution was transferred to a polythene container previously rinsed with the same solution.

This procedure, was followed in both the time

and/ .....

and temperature investigations (Section 2.101). % Standard Deviations of 1.8% (10 individual determinations) and 3.3% (8 individual determinations) respectively were obtained, which verify the quantitative nature of the extraction procedure.

Taking these factors into consideration, future citrus leaf samples were ashed at 550°- 600°C for 6 - 8 hours in completely dry porcelain casserole dishes, followed by a mild acid extraction as already outlined.

4. THE MODIFIED CURCUMIN METHOD FOR THE DETERMINATION OF BORON IN PLANT MATERIAL.

4.1. REAGENTS:

(i) Water.

Pure water should be used throughout for the preparation of standard solutions, test solutions and stock reagents. Exceptionally pure water may be prepared by passing freshly distilled water through an ion exchange column containing a "mixed-bed" resin (Amberlite MB_3). The water must be tested frequently and the resins regenerated when necessary, since boron contamination takes place when the resins become even slightly exhausted.

(ii) Ethyl Alcohol.

Use a good grade of 95% strength. Recovery for re-use may be satisfactorily carried out by an alkaline distillation in the presence of calcium hydroxide.

(iii) Standard Boron Stock Solutions.

Dissolve 1.4289 g. of boric acid ("Specpure" grade) in 1000 ml. of deionised water. This solution

contains / .....

contains 250 p.p.m. of boron and serves as the primary stock solution A. Dilute 40 ml. of A to 1000 ml. with deionised water, giving a standard solution B, containing 10 p.p.m. of boron. Likewise dilute 100 ml. of B to 1000 ml. giving standard solution C containing 1.0 p.p.m. of boron and then 50 ml. of B to 1000 ml. giving standard solution D containing 0.5 p.p.m. of boron.

Store all these standard solutions in polythene containers immediately after preparation.

(iv) Curcumin-Oxalic Acid Solution.

Dissolve 0.500 g. of finely ground curcumin (B.D.H. laboratory reagent) and 50.0 g. of oxalic acid (B.D.H."Analar") in 500 ml. of 95% ethyl alcohol in a polythene container. Warm to about 50 - 60°C in a waterbath, to assist complete solution, shaking the solution at regular intervals. After an hour, cool the solution down to about -5°C and then filter at the pump through a Whatman No.42 using a porcelain Buchner filter funnel.

This solution is chemically unstable for at least / .....

least 24 hours after preparation, but may be satisfactorily used 48 hours after preparation. If stored in a cool dark place, the reagent will keep for up to two weeks; this time limit may be lengthened to up to at least 5 weeks if the reagent is stored in a refrigerator without undue exposure to room temperature.

(v) 1:8 Hydrochloric Acid.

Dilute 100 ml. of C.P. concentrated hydrochloric acid to 900 ml., using deionised water, and store in a polythene container.

(vi) Sodium Carbonate.

Use an A.R. or C.P. grade reagent free of any detectable traces of boron. The B.D.H. "Analar" reagent has been found to be quite satisfactory.

(vii) 1% Phenolphthalein.

Dissolve 1 g. of the analytical reagent in 80 ml. of ethyl alcohol and dilute to 100 ml. with water.

4.2. APPARATUS:- / .....

4.2. APPARATUS:-

(a) Glassware:-

Ordinary soda or "pyrex" glassware has been found to be quite satisfactory for the temporary measurement of volumes (do not use new glassware, as the leaching of boron from these glasses is relatively high).

(b) Porcelain Ware:

Porcelain ware may be used during the ashing and evaporation stages of the determination. The following porcelain dishes were used:-

6 cm. porcelain casserole dishes (for ashing at 550°C).

9 cm. porcelain evaporating dishes with siliconed rims; in order to avoid any solution creep during the colour development stage, the lips of the dishes are siliconed by inverting each vessel into a $\frac{1}{4}$" pool of a solution of 1% Dowex Corning 200 in carbon tetrachloride and then baking for 12 hours at 200°C.

(c) Cleaning of Apparatus: -

Ensure the initial cleanliness of

all/ .....

all apparatus by cleaning with chromic acid, followed by a thorough rinsing with deionised water.

Between actual determinations, the rewashing of the apparatus was kept to a minimum as follows:-

(i) The casserole dishes, already thoroughly clean following the completion of the previous ashing stage, were rinsed with alcohol and allowed to dry.

(ii) The evaporating dishes containing adhering remains of the salt residue (the Na_2CO_3 and HCl used in the procedure produce an insoluble precipitate of NaCl in the alcoholic medium) was effectively removed by rubbing with a wad of filter paper, followed by a thorough rinse with alcohol.

(iii) The volumetric flasks were washed out using 3 - 4 small rinses of alcohol.

In / .....

In all cases washing was most conveniently carried out using two polythene washbottles, one containing deionised water, the other ethyl alcohol.

NOTE: Avoid dust contamination wherever possible.

Only the minimum of the purest analytical grease should be used for stopcocks of measuring apparatus.

4.3. PROCEDURE:

4.31. THE PREPARATION OF CITRUS LEAVES, FOR ANALYSIS.

Immediately after picking, transfer the leaf samples to polythene bags and store in a refrigerator. Removing one sample at a time from the refrigerator, wash off any surface contamination as follows:-

Take five polythene dishes; place about 750 ml. 0.1% teepol solution in the first 750 ml. distilled water in the second and third, and 750 ml. deionised water in the fourth. The fifth dish is used to collect the wet leaves. Sponge each leaf with cotton wool in the teepol solution and then rinse consecutively in the three sets of water. Using a pair of stainless-steel scissors, cut out the midrib of each leaf and place the leaf-halves in a suitably-sized

muslin / .....

muslin bag. Closing the muslin bag with a rubber band, suspend the sample inside a forced-draught oven set at 65°C. Dry in this manner for 48 hours and then grind the sample in an agate ball mill. After grinding, place the fine powder in a clean screw-capped bottle and dry in the forced draught oven for a further 24 hours. Seal the bottle tightly whilst still warm and store in a cool dark cupboard. Carry out the analysis as soon as possible, at any rate within 2 months after sampling.

NOTE: The above method of sample preparation was outlined by Steyn (49).

4.32. THE ASHING OF THE LEAF POWDER.

Accurately weigh out about 1 g. of the leaf powder (dried for 24 hours at 65°C) into a small dry porcelain casserole dish and ash for from 6 - 8 hours at 500 - 550°C in a muffle furnace.

Neutralise the ashed residue with 1:8 hydrochloric acid, adding the acid from a dispensing microburette until effervescence ceases. Add 1 ml. excess of acid in order to ensure a mild acid extraction of the very soluble boric acid as well as the solution of the heavier trace metals.

Using deionised water, decant the contents of the

casserole / .....

casserole into a standard 200 ml. volumetric flask and make up to the mark. After thorough shaking, transfer the solution to a polythene bottle which has previously been rinsed with 2-3 small quantities of the test solution. Allow the solution to stand for at least an hour before taking a clear aliquot for the actual determination of the boron content.

NOTE: (i) The weighing of the leaf powder should be carried out rapidly, as it is very hygroscopic.

(ii) The fine residue (mainly silica) present in the test solution must not be filtered off, as the use of filter paper at this stage would introduce considerable contaminating quantities of boron.

(iii) Ashing temperatures of up to 800°C do not appear to induce any volatilisation of boron from citrus leaf material. Similarly ashing at 550°C may be extended to 12 hours without apparent loss.

(iv) / .....

(iv) The use of wet casserole dishes during the ashing stage would introduce a considerable error, since boric acid is extracted by the water present, and would be lost by volatilisation on ashing.

(v) If appropriate test aliquots of from 2 - 8 ml. of the test solution (1g. plant material in 200Ø ml. solution) are used, virtually any concentration of boron likely to be found in different plant species, would be covered.

4.33. THE COLOUR DEVELOPMENT PROCEDURE.

Into a 9 cm. porcelain evaporating dish, containing 0.06 g. of sodium carbonate, burette out 4.00 ml. of the clear test solution (containing from 1 - 5 µg. of boron). After allowing the sodium carbonate to dissolve in the test aliquot, evaporate to dryness on a boiling waterbath and cool for 5 minutes.

Neutralise the dry residue with 1:8 hydrochloric acid, using 2 drops of phenolphthalein as indicator, and add 1.2 ml. of the acid in excess. Add 4.0 ml. of the curcumin -

oxalic / .....

oxalic acid reagent and mix the contents thoroughly by swirling. Evaporate the solution to dryness on a waterbath, controlled at 53 ± 2°C , and continue to bake the residue at the same temperature for a further 45 minutes.

After cooling for 10 minutes, dissolve the reaction products in a small volume of 95% ethanol, filter through a 9 cm. Whatman No.40 filter paper into a 100 ml. volumetric flask, wash well with alcohol and finally make up to the mark, also with alcohol. Record the % transmission of the final solution at 555 mμ and determine the boron concentration by reference to a standard curve prepared by using different aliquots of standard boron solutions. Carry out a blank determination with each series of analyses.

NOTE: (i) Transmission readings must be made within 3 hours of making up to volume with alcohol.

(ii) Adhere strictly to the time specifications as they are of significant importance.

(iii) None of the elements commonly found in citrus leaves interfere with the determination of boron as described above.

(iv) A 4 ml. test aliquot, prepared as described

in/ .....

in section 4.32, and containing 1-5 µg. B, covers a range of 50 - 250 p.p.m. of B in plant material. Since the sensitivity of the method falls off below about 1 µg. B per sample, a larger test aliquot is best used (up to 8 ml.) if the boron content is suspected of being less than 50 p.p.m. If exceptionally low concentrations of boron are expected (less than 20 p.p.m.) the specified 1 g. of dried leaf material is best made up to 50 or 100 ml.

(v) Once the test solutions have been prepared, 10 determinations can be conveniently carried out in 4 - 5 hours. 30 leaf samples can comfortably be analysed for boron during a week.

5. THE STATISTICAL ACCURACY AND PRECISION OF THE MODIFIED METHOD.

Since an analytical method is of little value for practical application unless some idea as to its reliability is available, the modified method, as outlined in section 4, was subjected to a rigorous statistical treatment.

5.1. THE ACCURACY OF THE MODIFIED METHOD.

The absolute accuracy of the method was tested by the addition of boron to citrus leaf powder as follows:-

A large sample (about 10 g.) of dry powdered citrus leaf material, containing about 100 p.p.m. B, was prepared. Four 1.000°g. samples were weighed out, and the boron content determined accurately as described above.

50 µg. of B were then added to each of 5 casserole dishes, and evaporated to dryness in the presence of 0.08 g. of sodium carbonate. After cooling the dishes, 0.500°g. of the above citrus leaf sample was weighed out into each, and the boron content again determined. The results are recorded in Table 18.

TABLE 18. / .....

TABLE 18.

THE RECOVERY OF BORON ADDED TO DRY CITRUS LEAF POWDER.

No.	% Trans. 1.000°g. samples.	Mean B. content (µg.)	% Trans. 0.500°g. sample +1.00 ug.B	Mean B content (µg.)	Mean % Recovery
1	70.6	2.00	71.4	1.96	98
2	70.3		70.4		
3	70.7		71.0		
4	70.3		71.0		
5			71.3		

From these results it was concluded that the values obtained on analysing citrus leaf powder by this method, were a true reflection of the total boron content of the material.

5.2. THE PRECISION OF THE MODIFIED METHOD.

Having ascertained the accuracy of the modified method (Section 5.1.), the precision was tested at different concentrations of boron:-

(a) Twenty-one replicate determinations were carried out on a citrus leaf sample high in boron content. Test aliquots of

4 ml. / .....

4 ml. (1g. leaf powder made up to 200 ml.) were used. The results are recorded in Table 19. A standard deviation of 2.3% was obtained, which is quite within the limits of experimental error.

(b) Twenty replicate determinations were carried out on a citrus leaf sample with an intermediate boron content, using exactly the same procedure. The results are recorded in Table 20. At this optimum concentration (embracing the average boron content of citrus leaf material), a standard deviation of 1.9% was obtained.

(c) Similarly, twenty replicate determinations were carried out on a citrus leaf sample low in boron content. The results are recorded in Table 21. The rather high standard deviation of 5.6% which was recorded, was mainly due to instrumental errors at this low boron concentration. A larger test aliquot of up to 10 ml. should be used when analysing leaf powder with a boron content between 20 - 50 p.p.m. When the content is suspected of being lower than 20 p.p.m., the initial 1 g. sample is best made up to 50 or 100 ml. instead of the specified 200 ml.

(d) The above results represent replicate determinations

carried / .....

TABLE 19.

THE PRECISION OF THE MODIFIED METHOD AT HIGH CONCENTRATIONS OF BORON.

No. (n)	% Trans.	p.p.m. B.	(y - m)	$(y - m)^2$
1	58.3	168	5	25
2	56.3	179	6	36
3	58.0	170	3	9
4	58.1	169	4	16
5	58.0	170	3	9
6	58.3	168	5	25
7	56.8	176	3	9
8	57.1	175	2	4
9	56.9	176	3	9
10	57.7	171	2	4
11	58.0	170	3	9
12	57.5	172	1	1
13	57.5	172	1	1
14	57.4	173	0	0
15	58.2	169	4	16
16	57.4	173	0	0
17	56.2	180	7	49
18	56.4	178	5	25
19	56.0	181	8	64
20	56.8	176	3	9
21	57.0	175	2	4
	TOTAL	3641		324
	MEAN	173		

$$\text{Standard Deviation} = \sqrt{\frac{(y - m)^2}{n - 1}}$$

$$= 4.0 \text{ p.p.m.}$$

$$\% \text{ Standard Deviation} = 2.3\%$$

TABLE 20.

THE PRECISION OF THE MODIFIED METHOD AT OPTIMUM CONCENTRATIONS OF BORON.

No.(n)	% Trans.	p.p.m. B.	(y - m)	$(y - m)^2$
1	67.3	109	0	0
2	68.0	106	3	9
3	67.3	109	0	0
4	67.7	107	2	4
5	67.4	108	1	1
6	67.1	110	1	1
7	67.0	110	1	1
8	66.6	112	3	9
9	68.1	105	4	16
10	67.6	108	1	1
11	66.2	113	4	16
12	67.0	110	1	1
13	66.9	111	2	4
14	67.2	109	0	0
15	66.9	111	2	4
16	67.6	108	1	1
17	66.8	111	2	4
18	67.7	107	2	4
19	67.1	110	1	1
20	67.8	107	2	4
	TOTAL	2181		81
	MEAN	109		

Standard Deviation = 2.1 p.p.m.

% Standard Deviation = 1.9%

TABLE 21.

THE PRECISION OF THE MODIFIED METHOD AT LOW CONCENTRATIONS OF BORON.

No.(n)	% Trans.	p.p.m. B	(y - m)	$(y - m)^2$
1	90.6	35	0	0
2	90.5	35	0	0
3	90.1	37	2	4
4	89.9	38	3	9
5	90.2	37	2	4
6	90.9	33	2	4
7	90.1	37	2	4
8	90.2	37	2	4
9	91.0	33	2	4
10	90.8	34	1	1
11	90.6	35	0	0
12	91.1	32	3	9
13	89.8	39	4	16
14	90.7	34	1	1
15	90.8	34	1	1
16	90.0	38	3	9
17	90.8	34	1	1
18	90.8	34	1	1
19	90.7	34	1	1
20	90.4	36	1	1
	TOTAL	706		74
	MEAN	35		

Standard Deviation = 1.97 p.p.m

% Standard Deviation = 5.6 %

carried out on a single test solution. Since this did not take into account the efficiency of the ashing or the extraction technique, determinations were next carried out on 10 separate 1.000°g. samples, obtained from a citrus sample high in boron content. The results are recorded in Table 22. A standard deviation of 1.8% was obtained

TABLE 22.
THE PRECISION OF THE MODIFIED METHOD.

No.(n)	Wt.of Sample (g.)	% Trans.	p.p.m. B	(y-m)	$(y-m)^2$
1	1.000°	54.2	186	3	9
2	1.000°	54.8	182	1	1
3	1.000°	54.8	182	1	1
4	1.000°	54.2	186	3	9
5	1.000°	54.6	183	0	0
6	1.000°	53.8	188	5	25
7	1.000°	55.2	180	3	9
8	1.000°	55.2	180	3	9
9	1.000°	55.4	178	5	25
10	1.000°	54.7	183	0	0
	TOTAL		1828		88
	MEAN		183		

Standard Deviation = 3.3 p.p.m.

%Standard Deviation = 1.8 %

It is clear from the results recorded above, that the

modified / .....

modified curcumin method is both precise as well as accurate.

6. THE APPLICATION OF THE METHOD.

In order to test the applicability of the method, the boron content in two widely-differing plant species, namely citrus and pineapples, was determined. Citrus and pineapple leaf samples were picked according to the standard technique (Steyn's thesis, (49)), from various areas in the Eastern Cape. After preparing the leaf samples for analysis (Steyn (49)), the boron content was determined in duplicate 1 g. samples. The duplicates agreed very well in all cases. The means of the results are recorded in Tables 23 and 24.

From the literature it appears that a boron deficiency is associated with boron contents ranging from 12 - 20 p.p.m. in citrus leaves. On this basis none of the above samples can be considered to be deficient in boron content, in fact they all appear to be remarkably well supplied with this element.

It is interesting to note that low performing

trees / .....

TABLE 23.

CITRUS LEAF SAMPLES FROM DIFFERENT PARTS OF THE EASTERN CAPE.

Sample No.	Area.	Description of Orchard	Soil pH.	B p.p.m.
1.	Kat river, Fort Beaufort	High-yielding trees	8.1	98
2	Kat river, Fort Beaufort	Low-yielding trees	8.0	64
3	Belmont Valley, Grahamstown	High-yielding trees	7.2	110
4	Belmont Valley, Grahamstown	Low-yielding trees	6.3	65
5a.	Kat river, Fort Beaufort	Old trees (43 years)	8.2	199
5b	"	Young trees, full-bearing (16 years)	8.0	160
6a	Kat river, Fort Beaufort. (Citrus Exchange Exp.plots).	NPK treatment since 1946	7.4	100
6b		Control (No treatment since 1946)	8.2	67
7a	Belmont Valley, Grahamstown (Citrus Exchange Exp. plots).	NPK treatment since 1946	6.4	73
7b		Control (No treatment since 1946)	7.3	109

trees usually contained less boron than high-performing trees on comparable soil types.

Soil reaction appeared to play no important role in

the /.....

the uptake of boron by the tree, since low and high boron figures were obtained both on highly alkaline soils as well as on slightly acid soils.

TABLE 24.

PINEAPPLE LEAF SAMPLES.x

Sample No.	Area.	Treatment of Plants	Soil pH	B p.p.m.
1	Bathurst	Zinc spray	5.0	6
2	"	Zinc-Manganese Spray	5.0	8
3	"	Zinc-Copper mixture to soil	4.9	6
4	"	Phosphorus	5.5	9
5	"	Control	5.7	8

x These samples were obtained from the experimental plots of the Rhodes University Research Team on plant nutrition.

From Table 24 it is clear that all the pineapple samples were very low in boron content, if compared with other plant species. Whether these figures represent an actual deficiency is difficult to say, since no data about the boron requirements of pineapples could be found in the literature. It is quite possible, too, that some of the boron may have been volatilised during the drying of the

leaf/ .....

leaf portions - these meristematic basal portions contain about 90% water! This matter needs further investigation.

7. CONCLUSION.

It is realised that this investigation into the factors influencing the curcumin - boric acid reaction, as well as to the applicability of the modified method to different plant materials, is not complete. Only an intensive statistical study, far beyond the scope of this thesis, would yield the complete answer. However, a method for the determination of boron in plant material has been developed, which, to the knowledge of the author, is superior to other alternative procedures. Considering the more critical aspects of an analytical procedure, a comparison with other methods can briefly be summarised as follows:-

(i) The accuracy and precision of the modified method are superior to that of any method published to date.

(ii) The modified procedure is perhaps a little more time-consuming and tedious than some of the alternative methods.

(iii) The method does not involve the use of a

strongly / .....

strongly corrosive reagent. This is important, as most of the alternative procedures make use of concentrated sulphuric acid as a colour developing medium.

(iv) The range of the modified method is easily adjusted to include any concentrations of boron likely to be found in plant material, whereas the alternative methods, which make use of concentrated sulphuric acid, are inclined to have a limited range owing to the use of very critical test aliquots.

(v) The modified method, besides being very economical as regards the use of reagents, is easily carried out using standard laboratory apparatus. Many of the alternative methods involve the use of boron-free glassware.

In conclusion, it must be pointed out that, although the procedure involved in the determination of boron by this modified method is perhaps longer than some of the alternative methods, the remaining analytical attributes are superior.

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