COLORIMETRIC ESTIMATION OF BORIC ACID WITH PENTAMETHYLQUERCETIN

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Several methods for the determination of boric acid are known and of these the alkalimetric method which is largely employed depends on the titration of boric acid with standard alkali in the presence of glycerol, mannitol or invert sugar. Gravimetric methods depending on the absorption of boric acid in a suitable reagent and determining the increase in weight are also known.[1] Both types of methods yield satisfactory results only with appreciable amounts of boric acid. Colorimetric methods which have been suggested in the literature for the estimation of small amounts of boron depend on the reaction of boric acid with (*a*) turmeric, (*b*) curcumin in presence of oxalic acid, and (*c*) quinalizarin dissolved in sulphuric acid. To these may be added the unusual colorimetric procedure of Stahl[2] which depends on the comparison of the intensities of the green flame colour given by methyl borate from the test and standard samples.

Hebebrand[3] showed that 0·1 mg. of boric acid gives a feeble brown tint whilst 10·0 mg. of the acid gives a bright rose-red colour with a 1% solution of turmeric and a colorimetric method was developed on this basis. The colouration is somewhat fugitive, and is destroyed by prolonged exposure to light and also by boiling. Bertrand and Agulhon[4] employing strips of turmeric paper carried out successfully the estimation of 0·1 to 0·0001 mg. of boric acid. This method depends on the comparison of the lengths of the colourations produced on the turmeric strips with the test and standard solutions of boric acid in presence of dilute hydrochloric acid. Schäfer[5] pointed out that concentrated hydrochloric acid itself gives a red colour with turmeric and this changes progressively to yellow on dilution with water. Hence the concentration of hydrochloric acid employed is of importance in this method.

The second colorimetric method depends on the red colour given by boric acid with curcumin in presence of oxalic acid. Using this method as much as 2·0 mg. of boron trioxide can be estimated (Snell[6]). Neither of the above two methods, however, is rapid.

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The third colorimetric method depends on the quinalizarin-boric acid reaction in presence of concentrated sulphuric acid. The solution of the reagent in concentrated sulphuric acid is itself violet in colour and this changes to blue with increasing additions of boric acid, the actual colour produced being composed of varying amounts of red and blue. Scharrer and Gottschall[7] employed the method for the estimation of 120 to 12γ of boric acid. Smith[8] found that it was necessary to work with concentrations of boric acid less than 0·04 mg. per c.c. and that the lower limit was 0·002 mg. per c.c.

Using the photo-electric colorimeter Olson and De Turk[9] estimated amounts up to 0·03 mg. of boron. These authors found that maintenance of constant temperature during the estimation was important for obtaining good duplicates and that decrease in the concentration of the sulphuric acid decreased the sensitivity, so that a definite high concentration of acid had to be employed. Patricia W. Mansell[10] using the Lovibond tintometer found that about 2γ of boron was the most satisfactory quantity to measure. It is evident from the above that this method is available only for the estimation of very small quantities of boric acid.

Stahl (*loc. cit.*) found that quantities of the order of 0·3 mg. or more of boron trioxide in the upper limit and smaller quantities of the order of 0·0022 to 0·007 mg. could be determined with an accuracy of 0 to 15% by his method.

Several other colour reactions for boric acid of great sensitivity have been described in the literature, but no attempt seems to have been made to adapt them for colorimetric work. Rangaswami and Seshadri[11] have recently pointed out that the boric acid reaction characteristic of 5-hydroxy- and 5-methoxyflavones and -flavonols and 2-hydroxy- and 2-methoxy-chalkones first described by Wilson[12] and subsequently developed by them is capable of application for the detection of boric acid itself. These compounds in anhydrous acetone solution containing anhydrous citric acid undergo a change of colour on the addition of traces of boric acid. Compounds that are themselves coloured yellow become deeper yellow on the addition of boric acid while those that give only colourless solutions in acetone containing citric acid develop a yellow colour on the addition of boric acid. Using pentamethylquercetin, which gives a practically colourless solution in acetone containing citric acid only, as the reagent (Rangaswami and Seshadri, *loc. cit.*) showed that 1 part of boric acid in 30,000 parts of solution could be easily detected—a reaction of a fairly high degree of sensitivity. This reaction has now been investigated in detail with a view to develop a method for the colorimetric estimation of boric acid.

Experimental

Solutions:

(1) *Reagent.*—Dry pentamethylquercetin (225 mg.) was dissolved in anhydrous acetone and the solution made up to 100 c.c. with same solvent. For this purpose acetone (extra pure) was dehydrated by standing over fused calcium chloride for 72 hours and then distilled under anhydrous conditions.

(2) *Boric Acid.*—A pure sample of boric acid was finely powdered and dried in a steam oven for 6 hours. 200 mg. of the acid was accurately weighed out, dissolved in anhydrous acetone and the solution made up to 100 c.c. with the same solvent. 5 c.c. of this solution was diluted to 25 c.c. with the same solvent and used as the standard.

(3) *Citric Acid.*—Citric acid (pure) crystals were finely powdered and dehydrated by drying in a steam oven for 30 hours. 50 g. of the acid were dissolved in 500 c.c. of anhydrous acetone and the solution was filtered rapidly through a plug of cotton wool, to remove some finely divided insoluble material.

In preparing the above solutions carefully dried apparatus was used and the solutions were kept in well-stoppered dry bottles.

(4) *Potassium chromate.*—1·0 g. of potassium chromate (A.R) was weighed accurately, dissolved in water and the solution made up to 100 c.c. with water. 20 c.c. of the solution was pipetted out and diluted to 100 c.c. with water. The latter solution was employed in the experiments.

Preliminary Investigation

The colour (yellow) was developed by adding to a measured volume of the boric acid solution an excess of the reagent solution—more than one molecular proportion—and then diluting to volume with the 10% citric acid solution.

As the various aspects of this colour reaction had not been studied before, they were investigated now and the results are reported below :—

1. No colour was developed until the citric acid solution was added.

2. The yellow colour developed was progressively reduced in intensity and it finally disappeared altogether on dilution with anhydrous acetone free from citric acid. Experiments showed that this could not be a mere dilution effect.

3. Attempts to use the Lovibond tintometer for the measurement of colour and investigation of its variation, if any, with time were not successful on account of the volatility of the solvent at the temperatures obtaining in the

tintometer and also due to the rapid absorption of moisture of the citric acid solution.

4. Experiments carried out with the Duboscq type of colorimeter (balancing method) gave satisfactory results with identical solutions. With solutions which differed slightly in concentration of boric acid, however, erratic results were obtained showing thereby that Beer's Law does not hold good.

5. The intensity of colour in a given case depended on the actual amount of reagent solution added and possibly also on the amount of citric acid solution employed so that for good reproducibility definite conditions had to be prescribed.

6. The duplication method was more successful than the balancing method but here again the same factors as in (3) above precluded satisfactory results. Consequently it was necessary to work with closed vessels and with the minimum of exposure to air.

7. With quantities of boric acid less than 0·4 mg. the yellow colour developed was considerably reduced in intensity on dilution to 25 c.c. with the citric acid solution. Experiments showed that the magnitude of this reduction was quite out of proportion to what may be expected on the basis of dilution. With 0·4 mg. and above of boric acid apparently this did not occur. From this it appears that different final dilutions must be adopted depending on the amount of boric acid present in the test.

8. The yellow colours obtained resembled closely, though not perfectly, the pure yellow of potassium chromate solutions so that the former could be matched with the latter. In this manner permanent standards for use in routine work could be easily prepared.

Based on the above results the following empirical method has been worked out for the approximate estimation of small quantities of boric acid ranging from 0·4 to 1·8 mg.

Procedure

An aliquot part of the standard boric acid solution was pipetted out into a dry 50 c.c. Nessler cylinder having a graduation at 25 c.c., 5 c.c. of the reagent solution added and the volume made up to the 25 c.c. mark with the citric acid solution. After stirring, the cylinder was tightly corked. 15–20 c.c. of water were placed in another cylinder of the same dimensions and the matching was carried out by adding the standard chromate solution from the burette in the usual manner for the duplication method. Except during actual matching the cylinder containing the test solution was kept corked. The matching was repeated three or four times in each case. With quantities of

boric acid of the order of 1·2 mg. and above, the intensities of the yellow colour, when the whole of the solution (25 c.c.) was employed, were felt to be too deep for satisfactory matching. In these cases, therefore, after making up to 25 c.c. with the citric acid solution, the volume was halved using a measuring cylinder and the matching carried out. The total intensity (for 25 c.c.) was obtained in terms of the chromate solution by doubling the value obtained.
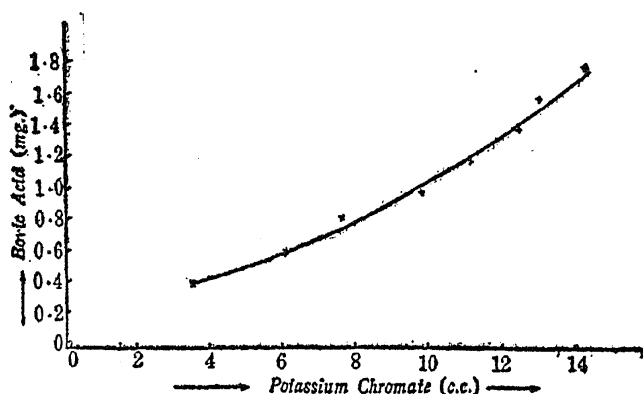
Results

The results obtained are recorded in Table I. The amounts of boric acid were plotted against the mean volumes of the chromate solution and a mean curve was drawn (*cf.* Graph). To evaluate the magnitude of the

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Boric acid mg.	Potassium chromate c.c.	Mean volume c.c.	Pot. chromate c.c. (interpolated)	Deviation %
1	2	3	4	5
0·4	3·2 3·3 3·7	3·4	3·4	nil
0·6	6·1 5·9 6·3	6·1	6·1	nil
0·8	7·9 7·4 7·6	7·6	8·0	−5·0
1·0	9·8 9·8 9·9	9·8	9·5	+3·2
1·2*	11·0 11·4 10·9	11·1	10·8	+2·8
1·4*	11·8 12·4 12·6	12·3	12·1	+1·7
1·6*	13·2 12·4 13·2	12·9	13·2	−2·3
1·8*	14·0 14·0 14·4	14·1	14·3	−1·4

* After developing colour only half the test solution was employed for matching.

errors in matching, the mean volumes of the chromate solution corresponding to the boric acid taken were obtained by interpolation on the graph

and are recorded in column 4. The percentage deviation of the mean volumes (experimental) from the interpolated figures was then calculated and the results are recorded in column 5.

In order to test the relationship thus established, the colour was developed with other known quantities of boric acid and then matched with the chromate solution as before. From the mean volume of the chromate solution required, the amount of boric acid was read out from the graph. The results obtained are given in Table II.

TABLE II

Boric acid taken mg.	Potassium chromate c.c.	Mean volume c.c.	Boric acid found mg.	Error %
1	2	3	4	5
0·48	5·5 5·6 5·7	5·6	0·55	+14·6
0·68	6·5 6·8 6·8	6·7	0·65	− 4·4
1·48*	13·9 13·6 13·9	13·8	1·7	+14·9

* After developing colour only half the test solution was employed for matching.

Discussion

The figures given in Table I, column 2, show a variation of 0·5 c.c. in matching except in two cases where it reaches 0·8 c.c. This increase obviously arises from the fact that the errors in matching are multiplied by two (*vide*

supra) according to the procedure adopted. The maximum deviation from the mean volumes (column 3) is only 0·5 c.c. Thus the figures in column 2 when compared with the mean volumes represent maximum errors in matching of about 15 and 3·5% on the lowest and highest limits of boric acid respectively. However, the deviations of the mean volumes from the mean curve range from 0 to 5·0% (*cf.* columns 4 and 5) so that if the mean volumes are employed for interpolation on the graph the deviation from the truth due to errors in matching does not exceed 5·0%.

The results given in Table II, however, show a maximum error of about 15% in the estimation of boric acid by interpolation of the mean volumes (column 3) on the graph. It appears probable that the higher error is partly due to the difficulty in measuring small volumes of boric acid solution in the highly volatile solvent, acetone. It may, however, be pointed out in this connection that errors as high as 10% are not unusual in visual colorimetric methods.

Regarding the mechanism of the reaction the following may be mentioned:—

In aqueous solutions boric acid reacts with polyhydroxy compounds such as glycerine, mannitol, etc., to form simple esters; with the *o*-hydroxy carbonyl compounds and in presence of the dehydrating agent, sulphuric acid, however, it yields inner complex (chelate) esters. The interaction of boric acid with the O-Hydroxy-carbonyl compounds, *viz.*, 5-hydroxy-flavones and -flavonols and the 2-hydroxy-chalkones, in presence of anhydrous citric acid may perhaps be explained on the assumption that the latter serves as a dehydrant, though the possibility of interaction between the hydroxy acid (citric acid) and boric acid as a preliminary to the former reaction could not be altogether ignored. The mechanism of the interaction of boric acid with the 5-methyl ethers of the above hydroxy-flavones and-flavonols and 2-methyl ethers of the above hydroxy-chalkones is obviously more complicated as no free hydroxyl, ortho to the carbonyl group, is available in any one of these compounds. The part played by the anhydrous citric acid is itself not quite clear and consequently the disappearance of colour on dilution with acetone, free from citric acid, could not be explained. The available evidence is meagre and hence the reaction must be regarded as empirical at present.

Regarding the method itself the following points may be considered:—

1. The use of the highly volatile solvent, acetone is apparently a serious disadvantage but it is of interest to note in this connection that even the more volatile ether is used for the extraction of nickel dimethyl glyoxime complex

in colorimetric work.[6a] The use of a less volatile solvent is an obvious solution of the difficulty but the choice is limited by the solubilities of the substances involved and also by the fact that hydroxylic solvents could not be employed.

The present method possesses the advantage that the blank is practically colourless whereas in the three colorimetric methods referred to already the blanks are coloured. Its chief advantage, however, is in its rapidity as compared with the turmeric and curcumin methods (*loc. cit.*), although the results obtained are only approximate.

3. The need for the rigorous exclusion of moisture is apparently a serious drawback of the method, but it may be pointed out that the drying of the reagents can be readily accomplished and the time taken in drying the few pieces of apparatus could not be regarded as of serious consequence. Further the need for stoppering the Nessler cylinder containing the test solution except during matching is not a great inconvenience in practice.

Summary

A new method for the approximate colorimetric estimation of boric acid depending on the yellow colouration yielded by boric acid with pentamethyl-quercetin in presence of citric acid in anhydrous acetone medium has been described. The method is empirical in character and gives results with a maximum error of about 15% with quantities of boric acid ranging from $0 \cdot 4$ to $1 \cdot 8$ mg.

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