A NEW FLUORESCENCE REACTION FOR THE DETECTION OF BORIC ACID

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The detection of boric acid and borates in admixture with inorganic substances has always been a problem of considerable difficulty. New reagents and procedures have been suggested from time to time but all of them suffer from one or other defect so that no satisfactory reagent is available at present.

The turmeric test described by Trömmsdorff[1] as early as 1815 is highly sensitive, but it is interfered with by the oxidising agents,[2–6] such as hydrogen peroxide, chromate, chlorate, nitrite, arsenate and ferricyanide ; iodides and fluorides also interfere but only to a lesser degree. Consequently elaborate procedures[2,3,5,11] have been described to eliminate or reduce these interfering radicles so that the test might be employed successfully.

The commonly employed alkyl borate test is also rendered uncertain in presence of chlorides and copper[7,10] for the former leads to the formation of alkyl halides which burn with a green edged flame and the latter itself colours the flame green. Further Krauss[8] has shown that barium also interferes with this test on account of the volatility of the $BaSO_4$, H_2SO_4 compound. Even phosphoric acid has been shown to interfere with this test. It is interesting to note in this connection that the use of glycerol[10] in place of sulphuric acid minimises the interference by copper and chloride considerably.

The boron trifluoride test[11] also suffers from similar disadvantages ; it cannot be carried out in test-tubes made of boro-silicate glass, and copper and barium interfere with the test in the same way as they do with the alkyl borate test.

Recently many hydroxy-anthraquinones and their derivatives have been introduced by Feigl and others as reagents for the detection of boric acid. These give intensely coloured solutions in concentrated sulphuric acid which show characteristic colour changes in presence of boric acid. From this group of compounds, alizarin–S, purpurin and quinalizarin have been suggested by

Feigl and Krumholz[12] and rufianic acid by Rosenthaler.[13] The naphthalene derivative, chromotrope-2B, has been suggested by Komarovsky and Poluektov.[14] Though all these are highly sensitive, fluorides and oxidising agents interfere so that their removal is essential for the success of the test. The elimination of fluorides as recommended by Komarovsky and Poluektov (*loc. cit.*) and adopted by Feigl, consists in heating the substance with silica and concentrated sulphuric acid till fumes of sulphur trioxide appear; a procedure which obviously involves loss of boric acid.[15]

Various other tests described in literature are of limited applicability. Mention may be made of the tests employing Congo-red,[16] Methyl red or Sofnol-indicator No. 1,[17] Carob seed gum[18] and Carmine red.[19] An interesting reaction[20] is the rose-pink to blood red colour given by a tincture of mimosa flowers by means of which boric acid can be detected in presence of chloride, iodide, nitrate and sulphate. The test is, however, interfered with by organic acids and sodium phosphate.

Phosphorescence and fluorescence effects obtained with boric acid have also been employed for its detection. The phosphorescence with fluorescein as an activator has been suggested by Zhirov.[21] A fluorescence reaction with tincture cochineal has been described by Szebellady and Gaal[22]; this, however, is interfered with by several metals.

In a previous communication,[23] it has been reported that boric acid yields with resacetophenone dissolved in concentrated sulphuric acid a brilliant blue fluorescence visible under Cenco Black Light Source and this fact can be utilised for the detection of boric acid on a semi-micro scale. This reaction has now been investigated further to determine its applicability in qualitative analysis and the results obtained are reported below.

Experimental

A. With Concentrated Sulphuric Acid.—

Interference by Basic and Acidic Radicles.—A preliminary investigation has been carried out with all the basic radicles and several of the acidic radicles included by Caven in *Systematic Qualitative Analysis* (1934).

The method of testing adopted was as follows.—

Resacetophenone (10 mg.) was dissolved in concentrated sulphuric acid (10 c.c.) and the solution divided into two equal parts. To one part placed in a quartz test-tube was added 1·0 mg. of boric acid and 100 mg. of the sodium or potassium salt of the acidic radicle under investigation. For the basic radicle the metallic borates were used; where these were not available, an easily soluble salt of the metal along with 1·0 mg. of boric acid was

employed. If the solution could not be effected in the cold, the salt was at first brought into solution in concentrated sulphuric acid with warming and the solution cooled before use. The second part of the resacetophenone solution was placed in another quartz test-tube of approximately the same dimensions and was used for preparing the appropriate blank in each case. After thorough mixing the test-tubes were placed under the Cenco Black Light Source inside a darkened cabinet and observations made. All the tests were carried out in the cold.

Basic Radicles.—None of the basic radicles examined gave any fluorescence in the absence of boric acid. In testing for boric acid also there was no interference and in all cases a brilliant blue fluorescence was obtained. Under the conditions of testing, the interference which might be expected due to the colour of the metal ion in the case of copper, nickel, cobalt and chromium was also found to be absent.

Acidic Radicles.—None of the sodium and potassium salts of the acid radicles examined gave any fluorescence in the blank test. In testing for boric acid, however, there was complete interference from nitrate and fluoride and no fluorescence was noticeable. With thiosulphate there was precipitation of sulphur but this did not interfere with the observation of fluorescence. Chlorate, chromate and ferricyanide oxidised the reagent producing coloured solutions in which, however, the blue fluorescence was visible. In the case of bromide and iodide, the separation of the halogen besides bromination of the reagent in the former case made it difficult to observe the fluorescence. Tartrate interfered to some extent by undergoing carbonisation; this difficulty was reduced by diluting the solution with sulphuric acid.

In view of the fact that several of the common acidic radicles interfered in this method, the following were not examined:—nitrite, hypochlorite, cyanide, sulphocyanide, cyanate, bromate, iodate, the per-acids, peroxide, manganate, phosphite, hypophosphite and silicofluoride. In all other cases a brilliant blue fluorescence was observed with boric acid.

Limits of Identification and Sensitiveness.—

Reagents: (1) *Boric Acid.*—A solution containing 10 mg. of boric acid per c.c. was prepared from an A.R. quality sample by dissoliving in concentrated sulphuric acid. A series of solutions containing 1·0, 0·1, 0·01.... mg. per c.c. was prepared by diluting the above solution with concentrated sulphuric acid.

(2) *Resacetophenone.*—Pure resacetophenone (0·25 g.) was dissolved in 100 c.c. of concentrated sulphuric acid.

Method.—1 c.c. of the boric acid solution was placed in a quartz test-tube, treated with 3 c.c. of the reascetophenone solution and the volume made up to 10 c.c. with concentrated sulphuric acid. To facilitate this operation the test-tube was roughly graduated at the 10 c.c. mark. After mixing the tube was placed under the lamp and the following observations made:—

TABLE I

No.	Boric acid mg. per c.c.	Observations
1	10·0	Brilliant blue fluorescence
2	1·0	Blue fluorescence—easily visible
3	0·1	Very pale blue fluorescence—visible
4	0·01	Not easily visible

Limit of Identification = 100 γ

Limit of Sensitiveness = 1 : 10,000.

B. With Phosphoric Acid.—

Since in the above experiments using concentrated sulphuric acid interference occurred with several acidic radicles, experiments were performed with phosphoric acid in the place of this acid. In the first place it was found that the boric acid—resacetophenone reaction occurred in the presence of syrupy phosphoric acid yielding a blue fluorescence visible under the lamp and identical with that obtained with sulphuric acid. Secondly, it was found that the number of interfering acidic radicles was considerably less when phosphoric acid was used. Consequently a detailed investigation was carried out using this acid.

Interference by Basic and Acidic Radicles.—In addition to the basic radicles which were examined with concentrated suphuric acid the following were also included:—Beryllium, Lanthanum, Thorium, Zirconium, Tungsten, Vanadium, Cerium, Uranium, Titanium and Molybdenum. All the acidic radicles previously examined were investigated and the list was extended to include nitrite, bromate, iodate, perchlorate, periodate, persulphate, sulphocyanide, cyanate, and peroxide (hydrogen peroxide).

The method of testing adopted was similar to that previously described except that the salt was dissolved in dilute acetic acid whenever possible or a dilute nitric or hydrochloric acid solution of the salt was treated with acetic acid and used. Solid resacetophenone (10 mg.) was dissolved in this solution and to it was added an equal volume of syrupy phosphoric acid.

Basic Radicles.—None of the basic radicles examined interfered with the blue fluorescence obtained with boric acid. Though precipitation of phosphates occurred in many cases, the precipitates dissolved in excess of phosphoric acid. The colours of the solutions in the case of copper (pale blue), nickel (pale green) and chromium (green) were not visible under the lamp so that there was no interference from them and any fluorescence present could be readily detected. In a few cases, however, though there was no interference with the test, certain peculiarities were observed and these are given in the table below:—

TABLE II

Solution	Observation	Remarks
1. *Vanadium*— Vanadium pentoxide was dissolved in hot dilute nitric acid.	A pale yellow solution resulted ; with phosphoric acid a deep blue ring was obtained ; on shaking the soultion became dirty greenish blue. Blue fluorescence easily observed.	The colour of the solution did not interfere with the test.
2. *Cerium*— (*a*) Cerous sulphate dissolved in warm dilute nitric acid.	At first clear colourless solution with bright blue fluorescence. It turned gradually pink to deep red solution masking the fluorescence.	There is no interference immediately after mixing the solution. Interference occurs on standing probably due to ceric ions.
(*b*) Ceric sulphate dissolved in dilute nitric acid.	Deep yellow solution with bright blue fluorescence ; gradually turns deep red the fluorescence being masked.	Interference occurs only on standing.
3. *Uranyl acetate*— Dissolved in water.	Pale yellow solution. Bright blue fluorescence.	Very dilute aqueous solutions of uranyl acetate exhibit green fluorescence in day light, which, however, disappears on addition of acetic acid.
4. *Uranium trioxide*— Dissolved in dilute nitric acid	Bright blue fluorescence. Slight reddish colour developed on standing.	No fluorescence in the blank.

Acidic Radicles.—Among the acidic radicles which interfered in the sulphuric acid method, it was found, that bromide, iodide, nitrate and tartrate no longer interfered. With fluoride, however, there was complete interfence as in the method using sulphuric acid. With perchlorate, persulphate, periodate and cyanate the solution was colourless and there was no interference. Thiosulphate yielded a turbid solution due to separation of sulphur, but this did not interfere with the fluorescence. With other acid radicles, however, certain peculiarities were observed and these are reported below:

TABLE III

Radicle in solution	Observation	Remarks
1. Chromate	Pale yellow solution at first ; turned reddish and finally deep red. Blue fluorescence.	Carbonisation of the reagent occurred and this rendered observation of fluorescence difficult.
2. Nitrite	The solution, on addition of phosphoric acid, quickly turned deep cherry red ; fluorescence could not be observed. Repeated with a small amount of nitrite.—Blue fluorescence.	Development of colour is probably due to the interaction of nitrous acid with the resacetophenone.
3. Chlorate	At first colourless solution exhibiting blue fluorescence ; solution gradually developed yellow colour.	Except for the colour developed there is no interference.
4. Bromate	Solution at first deep yellow ; decolourised on shaking. No fluorescence.	The deep yellow colour is due to the liberation of bromine. The negative reaction is probably due to bromination of resacetophenone.
5. Iodate	Yellow colour developed. Blue fluorescence.	No interference.
6. Ferricyanide	Solution yellow. Blue fluorescence.	Do.
7. Sulphocyanide ..	Do.	Do.

Limits of Identification and Sensitiveness.—

Reagents (1) *Boric Acid Solution.*—A series of aqueous solutions containing 1·0, 0·1, 0·01.....mg. of boric acid per c.c. was prepared.

(2) *Resacetophenone Solution.*—Pure resacetophenone (0·25 g.) was dissolved in 100 c.c. of dilute acetic acid.

Method.—The method adopted was similar to that used previously with sulphuric acid. In making up the solution to 10 c.c., however, syrupy phosphoric acid was used.

TABLE IV

Boric acid (mg. per c.c.)	Observation
1·0	Deep blue fluorescence
0·1	Blue fluorescence—easily visible
0·01	Pale blue fluorescence
0·001	Very pale blue fluorescence—just visible
0·0001	No fluorescence

Limit of Identification = 1 γ

Limit of Sensitiveness = 1 : 1,000,000.

Limit Proportions.—In order to throw light on the scope of the reaction, experiments were performed to determine the approximate quantities of the various acidic and basic radicles in the presence of which a definite amount of boric acid (0.1 mg.) can be detected by the phosphoric acid method.

Solutions.—Solutions of various acidic and basic radicles containing about 10 mg. of the radicle per c.c. were prepared using soluble salts. These solutions were then diluted with water suitably for getting the lower concentrations.

(2) *A solution containing* 0·1 mg. of boric acid per c.c. was used.

(3) A 0·25 per cent. solution of resacetophenone in dilute acetic acid was used.

Method.—1 c.c. of the boric acid solution was placed in a quartz test-tube, treated with 1 c. c. of the test solution followed by 3 c.c. of the resacetophenone solution and finally the volume was made up to 10 c.c. with syrupy phosphoric aicid. After mixing, observations were made under the lamp. If the fluorescence was not easily visible, the next lower concentration of the test solution was employed until it could be detected without difficulty. The results obtained were approximate and no attempt was made to determine the exact magnitudes of the "limit proportions" in view of the limitations imposed by the lamp.

TABLE V

No.	Radicle	Limit proportions in mg. with (0·1 mg. of boric acid)	No.	Radicle	Limit proportions in mg. (with 0·1 mg. of boric acid)
1	Copper	60·0	7	Chromate ..	2·0
2	Cobalt	10·0	8	Chlorate	10·0
3	Chromium ..	10·0	9	Nitrite	4·0
4	Nickel	30·0	10	Iodate	20·0
5	Ferricyanide ..	10·0	11	Fluoride (KHF_2) ..	4·0
6	Bromate	2·0			

From the above data it is evident that bromate and chromate interfere most with the test while nitrite and fluoride come next in order. It is interesting to note that the interference by fluoride which was found to be complete in the previous experiments is only partial, it being possible to detect easily as little as 0·1 mg. of boric acid in presence of 40 times its weight of the fluoride ion. The interference by nitrite is of no serious consequence as the radicle could readily be eliminated by means of dilute acids.

A noteworthy feature of the above data is that relatively large amounts of coloured cations do not interfere with the test.

Discussion

The resacetophenone—sulphuric acid method for the detection of boric acid suffers from several defects. Sulphuric acid being an oxidising agent liberates the halogens from bromides and iodides and these interfere with the test on account of their colour. Further free bromine interferes with the reaction by brominating the resacetophenone. In this connection it may be pointed out that the introduction of bromine into a hydroxy-ketone has a considerable bathofloric effect as has been shown in a previous communication.[23] The complete interference by nitrate in this method is evidently due to nitration of the reagent (*loc. cit*). In the case of fluoride the complete interference observed could be readily explained as due to the formation of the complex fluoborate ions. Oxidising agents such as chromate, chlorate and ferricyanide obviously interfere by oxidising the reagent itself producing deeply coloured solutions which render observation of the fluorescence difficult. In the case of tartrate carbonisation is the cause of interference. In addition the viscosity of the solutions renders the test less sensitive.

The substitution of sulphuric acid by syrupy phosphoric acid, however, obviates several of the defects observed in the above case. The acid radicles which interfere are chromate, bromate, nitrite and fluoride. With bromate, the interference can be explained as due to bromination of the reagent. To explain the formation of bromine under the experimental conditions it is suggested that the bromate either contained a trace of bromide or a small amount of the latter was produced by reduction and that the mixture of bromate and bromide in presence of the strong acid yielded bromine. Chromate interfered by oxidising the reagent and producing coloured solutions. The interference of nitrite, however, is due to the formation of a deep red colour by the interaction of nitrous acid with the reagent. Attention may also be drawn to the fact that the interference by fluoride is only partial and that the fluorescence with boric acid is observed in presence of as much as forty times its weight of the fluoride ion.

A comparison of the limits of identification and sensitiveness of the reactions carried out with the above acids shows that the method using phosphoric acid is one hundred times more sensitive than that using sulphuric acid. While with sulphuric acid 100γ is the limit of identification, with phosphoric acid the limit is 1γ so that the latter method is serviceable for the detection of micro quantities of boric acid. It may be also pointed out

that the number of acid radicles which interfere in the phosphoric acid method is comparatively small and these with the exception of the fluoride can be readily eliminated.

The sensitiveness of the resacetophenone—phosphoric acid method compares favourably with various reactions which have been suggested in the literature as shown in the table below:—

TABLE VI

Test	Limit of Identification (Boric Acid)	Method of Testing
1. Resacetophenone—Phosphoric acid ..	1·0 γ	Macro-method
2. Turmeric[6,11]	1·0 γ	Do.
	0·1 γ	Do.
3. Tincture of Mimosa Flowers[20]	0·4 γ	Do.
4. Phosphorescence[21]	20·0 γ	Do.
5. Boron Trifluoride[11]	0·1 γ	Do.
6. Alkyl Borate[24,25]	200·0 γ	Do.
	2·0 to 7·0 γ	Micro-method
7. Tincture cochineal[22]	12·0 γ	Macro-method
	0·5 γ	Micro-method
8. Alizarin-S[2]	5·5 γ	Spot test
9. Purpurin[2]	3·3 γ	Do.
10. Quinalizarin[2]	0·33 γ	Do.
11. Chromotrope—2B[2]	0·44 γ	Do.

Further several of these tests suffer from interference by many acidic and basic radicles (*vide infra*) while in the resacetophenone-phosphoric acid method the interference is much less; none of the basic radicles interferes and the acid radicles that do so are few in number and these with the exception of fluoride can be easily eliminated.

The results obtained for limit proportions indicate that the reaction is quite serviceable for the detection of boric acid in qualitative analysis. It may also be pointed out that resacetophenone is readily prepared and easily purified.

Summary

A new fluorescence reaction for the detection of boric acid with resacetophenone and phosphoric acid has been described. None of the metallic radicles examined interferes while among the acid radicles chromate, bromate, chlorate, nitrite and fluoride do so to a limited extent. The reaction compares favourably with those described in the literature both as regards limit of identification and also interference. It has been shown that the test is applicable for the detection of micro-quantities of boric acid.

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