A New Method for the Spectrophotometric Determination of Boron with Quercetin

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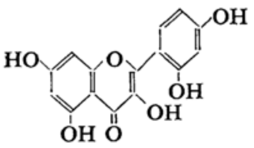
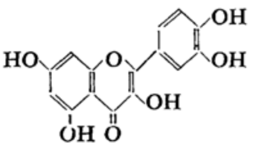
(Received January 12, 1961)

In previous publications it was reported that morin, a kind of oxyflavone, was used as a reagent for the fluorimeric and spectrophotometric determination of boron[1,2]. By an analogous consideration on the basis of the chemical constitution of the reagent, it may be said that there is a possibility of using quercetin instead of morin for the photometric determination of boron.

The formula for both compounds are:



Morin



Quercetin

In the field of photometry, quercetin was already used as a coloring reagent for germanium[3,4], zirconium[5] and molybdenum[6] etc. in previous publications. Wilson formerly reported the reactivity of boron with several kinds of flavone compounds including only a brief description on quercetin[7].

On the result of preliminary experiments the present author was confident that quercetin could be applicable to the determination of boron. In this paper, therefore, the application of the reagent to a new photometric method for boron has been described. The effects of reagent concentration, oxalic acid, and hydrogen ion concentration are examined. In addition, various conditions including temperature, duration of heating, and vessel material for evaporation of sample solution, and stability of the color, solvent of dried residue and diverse salts and the stability of the reagent solution have been investigated. It was found that the colored complex of boron-quercetin has a mole ratio of 1:1 under the proposed condition, and that Beer's law was obeyed over the range 0.00 to 0.50 p. p. m. of boron at 445 mμ.

The new photometric method for determination of boron was applied to graphite samples, and satisfactory results were obtained.

Experimental

Apparatus. — Spectrophotometric measurements were made with a Shimadzu quartz spectrophotometer, Model QB-50, using 1 cm. glass transmission cells. For the operation of color development a water bath of 50×35×35 cm. and an air bath of ordinary type were used.

Materials. — Dissolve 0.5716 g. of reagent grade boric acid in 1 l. of water to make a solution corresponding to 100.0 μg. of boron per 1.0 ml. Dilute an aliquot of the solution with water, and prepare standard solutions containing 5.0 p.p.m. and 2.0 p.p.m. of boron. Prepare a 0.2% ethanolic quercetin solution. Dissolve oxalic acid of analytical reagent grade in 95% ethanol to make a 20% solution. Prepare 1 N hydrochloric acid solution. All other salt solutions are prepared from reagents of analytical grade.

Standard Procedure.—Pipet an aliquot of the boron solution containing about 5.0 μg. of boron into a 100 ml. quartz beaker, add a suitable amount of water up to 2.0 ml. and add successively 1.0 ml. of 1 N hydrochloric acid, 3.0 ml. of 20% oxalic acid solution and 1.0 ml. of quercetin solution. Mix thoroughly and evaporate the contents to dryness on a water bath at 55±3°C. Subsequent to complete dryness, place the beaker in an air bath at 60∼90°C, and keep it there for about 1 hr. to make sure of complete color development. After cooling to room temperature, dissolve the residue in 10 ml. of acetone and measure the absorbance at 445 mμ with acetone as reference.

To ascertain the stability of the color, read absorbance of the solution at proper intervals of time after color development. In order to study the effect of diverse salts, color developments were made using 2.0 μg. of boron in the presence of various amounts of 0.5 or 2.0% salt solutions.

Measurements of absorption spectra were made in respect to the solution obtained by the abovementioned procedure at every 5 mμ intervals of wavelength.

Determination of Boron in Graphite Samples.— Determination of boron in graphite samples was

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1) A. Murata and F. Yamauchi, *J. Chem. Soc. Japan, Pure Chem. Sec.* (*Nippon Kagaku Zasshi*), **79**, 231 (1958).

2) A. Murata and F. Yamauchi, ibid., **79**, 1454 (1958).

3) Y. Oka and S. Matsuo, ibid., **74**, 931 (1953).

4) Y. Oka and S. Matsuo, ibid., **76**, 610 (1955).

5) F. S. Grimaldi and C. E. White, *Anal. Chem.*, **25**, 1886 (1953).

6) G. Goldstein, D. L. Manning and O. Menis, ibid., **30**, 539 (1958).

7) C. W. Wilson, *J. Am. Chem. Soc.*, **61**, 2303 (1939).

made as follows[8]. Weigh powdered sample into a platinum boat, add 2.0 ml. of 3% calcium hydroxide aqueous suspension and mix well. Evaporate the graphite to dryness on a small heater, introduce the boat into a combustion tube in an electric furnace heated at 900°C and ash the sample in a current of oxygen. After cooling, remove carefully the ash into a distillation apparatus and distil off boron as methyl borate into a 100 ml. quartz beaker containing 1.4 ml. of 0.2 N sodium hydroxide solution and 9.0 ml. of water. Evaporate the contents in a quartz beaker to dryness on a water bath warmed at 40~50°C, and add 1.4 ml. of 0.2 N sulfuric acid to the dried residue and then treat as mentioned above.

Results

Absorption Spectra of the Complex.—The absorption curves of colored solutions obtained by standard procedure are shown in Fig. 1. There is a maximum at 445 mμ, which suits for the spectrophotometric measurements.

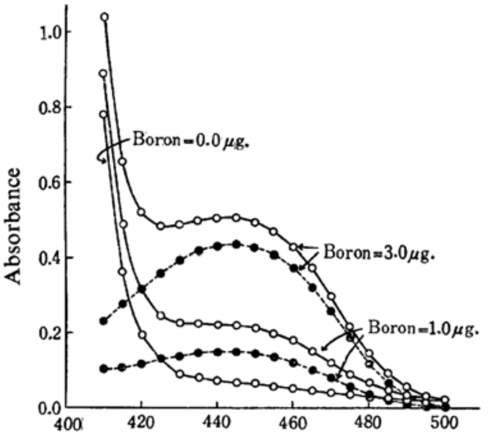

Fig. 1. Absorption spectra of colored complex of boron-quercetin.

Boron ; 3.0 μg., 1.0 μg. and 0.0 μg.
Quercetin ; 0.2% soln., 1.0 ml. (A-lot)
Oxalic acid ; 20% soln., 3.0 ml.
HCl ; 1 N soln., 1.0 ml. Water ; 3.0 ml.
—○— Obtained against acetone as reference
--●-- Obtained against blank as reference

Reagent Concentration.—Absorbances of a series of solutions contained 1.0 and 3.0 μg. of boron were measured in the presence of various amounts of the reagent. As shown in Fig. 2, 1.0 ml. of 0.2% quercetin solution is the optimum amount.

Oxalic Acid Concentration.—A test was made using 1.0 and 3.0 μg. of boron in the presence of varying amounts of 20% oxalic acid solution. The results indicate that 3.0 ml. of oxalic acid is recommended as shown in Fig. 3.

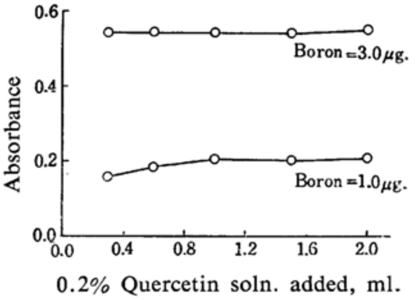

Fig. 2. Effect of quercetin added.

Boron ; 3.0 μg. and 1.0 μg.
HCl ; 1 N soln., 1.0 ml.
Oxalic acid ; 20% soln., 3.0 ml.
Water ; 3.0 ml. Quercetin ; A-lot

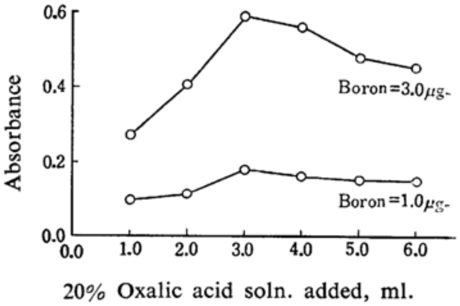

Fig. 3. Effect of oxalic acid added

Boron ; 3.0 μg. and 1.0 μg.
Quercetin ; 0.2% soln., 1.0 ml. (A-lot)
HCl ; 1 N soln., 1.0 ml. Water ; 3.0 ml.

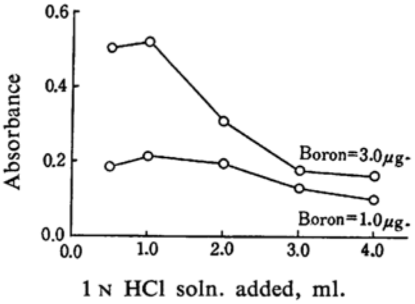

Fig. 4. Effect of hydrochloric acid added.

Boron ; 3.0 μg. and 1.0 μg.
Quercetin ; 0.2% soln., 1.0 ml. (A-lot)
Oxalic acid ; 20% soln., 3.0 ml.
Water ; 3.0 ml.

Hydrochloric Acid Concentration.—As shown in Fig. 4, it appears that the maximum absorption is obtained when 1.0 ml. of 1 N hydrochloric acid is used.

Effect of Water.—The absorbances were affected by the amount of water. As shown in Fig. 5, 3.0~4.0 ml. of water is always needed for complete color development in this procedure.

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[8] I. Muraki and K. Hiiro, *J. Chem. Soc. Japan. Pure Chem. Sec.* (*Nippon Kagaku Zusshi*), **78**, 850 (1957).

Effect of Drying Conditions on Color Formation.—As shown in Table I, the absorbance of the resulting solution containing 2.0 μg. of boron is affected by drying conditions for color development. Most satisfactory results were obtained when evaporation was made first on a water bath at 55±3°C for 4 hr. and then in an air bath at 60 to 90°C for 1 hr.

The effect of the kind of vessel on evaporation was tested. It was desired to use a quartz beaker or a platinum basin as shown in Table II. In the cases of a glass or polyethylene beaker some interferences were observed.

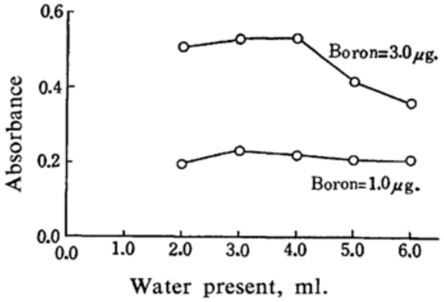

Fig. 5. Effect of water present.
Boron; 3.0 μg. and 1.0 μg.
Quercetin; 0.2% soln., 1.0 ml. (A-lot)
Oxalic acid; 20% soln., 3.0 ml.
HCl; 1 N soln., 1.0 ml.

Table I. Effect of drying conditions on color formation

Boron; 2.0 μg. Quercetin; 0.2% soln., 1.0 ml. (B-lot) Oxalic acid; 20% soln., 3.0 ml. HCl; 1 N soln., 1.0 ml. Water; 3.0 ml.

Drying conditions				
On water bath (55±3°C) hr.	In air bath °C, hr.	In desiccator, hr.	In air bath °C, hr.	Absorbance
5.0				0.435
6.0				0.451
4.0	60, 1.0			0.466
4.0	60, 2.0			0.453
4.0	75, 0.5			0.388
4.0	75, 1.0			0.455
4.0	75, 2.0			0.466
4.0	90, 0.5			0.462
4.0	90, 1.0			0.455
4.0	90, 2.0			0.450
4.0	75, 1.0	40	75, 0.5	0.461
4.0	75, 1.0	40	75, 1.0	0.465

Table II. Effect of kinds of vessel

Boron; 2.0 μg. Quercetin; 0.2% soln., 1.0 ml. (B-lot) Oxalic acid; 20% soln., 3.0 ml. HCl; 1 N soln., 1.0 ml. Water; 3.0 ml.

Kinds of vessel	Absorbance	Boron found μg.
100 ml. Quartz beaker	0.465	1.9_8
50 ml. Quartz beaker	0.470	2.0_0
100 ml. Glass beaker	0.503	2.1_6
50 ml. Glass beaker	0.484	2.0_7
Platinum basin, deep type	0.468	1.9_9
Platinum basin, shallow type	0.470	2.0_0
100 ml. Polyethylene beaker	0.558	2.4_3

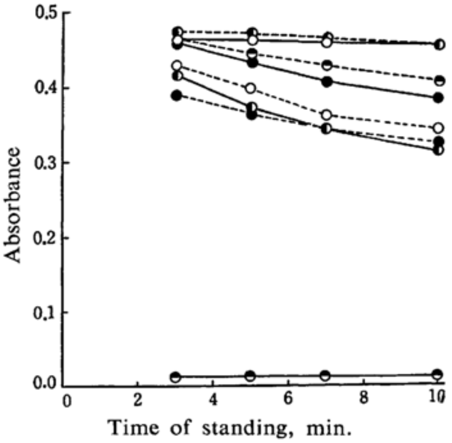

Fig. 6. Effect of kind of solvent.
Boron; 2.0 μg.
Quercetin; 0.2% soln., 1.0 ml. (B-lot)
Oxalic acid; 20% soln., 3.0 ml.
HCl; 1 N soln., 1.0 ml.
Water; 3.0 ml.
—○— Pure acetone
--○-- Acetone: Water=1:1
--●-- Ethyl alcohol
--◐-- Methyl isobutyl ketone
—◓— Toluene
—●— Acetone: Water=4:1
—◐— Methyl alcohol
--◓-- Butyl alchol
—◑— Carbon tetrachloride

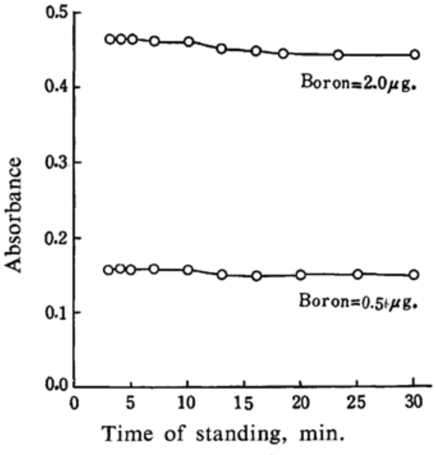

Fig. 7. Stability of the color.
Boron; 2.0 μg. and 0.5 μg.
Quercetin; 0.2% soln., 1.0 ml. (B-lot)
Oxalic acid; 20% soln., 3.0 ml.
HCl; 1 N soln., 1.0 ml.
Water; 3.0 ml.

Effect of the Kind of Solvent on Colored Complex.—Various kinds of solvent for extraction of boron-quercetin colored complex are tested with 2.0 μg. of boron. Fig. 6 shows that the best results are obtained in the case of pure acetone as a solvent.

Color Stability.—The absorbance of colored solutions is almost constant within 10 min., and then decreases slightly as shown in Fig. 7. It is thus desired to measure the absorbances within 10 min. after colored complex is dissolved with acetone.

Stability of Quercetin Solution during Storage.—It is found that the absorbance is increased as the duration of storage increases after preparation of the quercetin solution, and becomes constant after about 10 days as shown in Fig. 8. Hence, it is advisable to use the reagent solution after standing for about 10 days. It may be used at least 20 days after preparation.

Effect of Diverse Salts.—The effect of diverse ions on the spectrophotometry was examined. These salts cause interference seriously as shown in Fig. 9.

Mole Ratio of Boron to Quercetin in Colored Complex.—As shown in Fig. 10, maximum

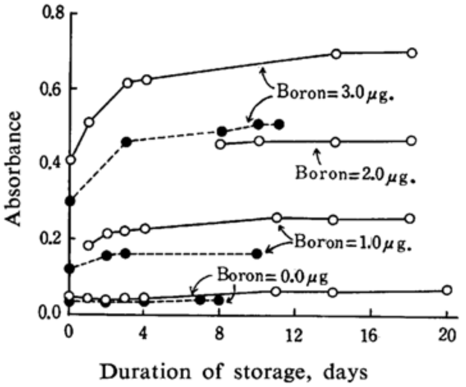

Fig. 8. Effect of duration of storage of quercetin solution.
Boron; 3.0 μg., 2.0 μg., 1.0 μg., and 0.0 μg.
Quercetin; 0.2% soln., 1.0 ml. (A- and B-lot)
Oxalic acid; 20% soln., 3.0 ml.
HCl; 1 N soln., 1.0 ml. Water; 3.0 ml.
—○— In the case of quercetin of B-lot
--●-- In the case of quercetin of A-lot

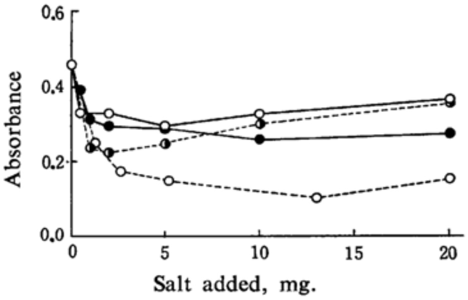

Fig. 9. Effect of diverse salts.
Boron; 2.0 μg.
Quercetin; 0.2% soln., 1.0 ml. (B-lot)
Oxalic acid; 20% soln., 3.0 ml.
HCl; 1 N soln., 1.0 ml. Water; 3.0 ml.
—○— NaCl —●— Na_2SO_4
--◑-- $CH_3COONa\cdot 3H_2O$
--○-- $Na_2HPO_4\cdot 12H_2O$

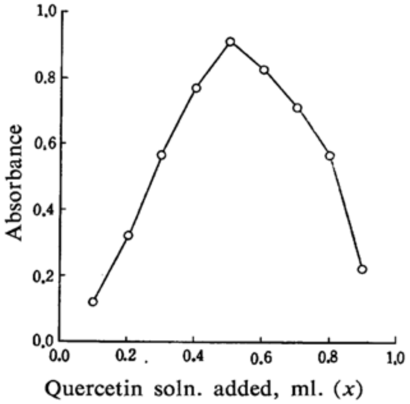

Fig. 10. Determination of ratio of boron to quercetin
Boron; 10^{-3} M soln., $(1.0-x)$ml.
Quercetin; 10^{-3} M soln., x ml. (B-lot)
Oxalic acid; 20% soln., 3.0 ml.
HCl; 1 N soln., 1.0 ml. Water; 3.0 ml.

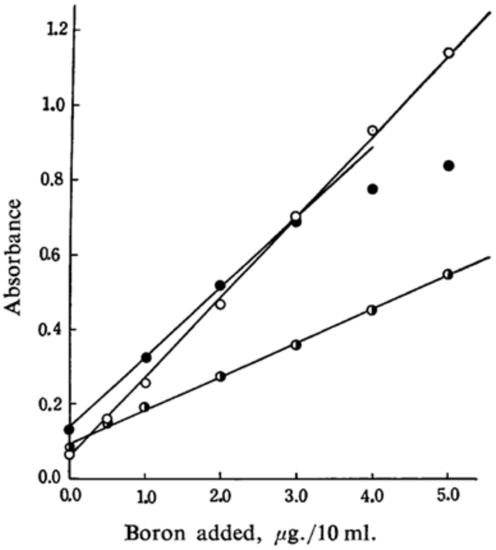

Fig. 11. Calibration curves.
Quercetin; 0.2% soln., 1.0 ml. (B-lot)
Oxalic acid; 20% soln., 3.0 ml.
HCl; 1 N soln., 1.0 ml. Water; 3.0 ml.
—○— Obtained by standard procedure
—◑— Obtained in the presence of 20 mg. of sodium sulfate
—●— Obtained after separation of boron by distillation

absorbance of the complex is obtained when the mole ratio of boron to quercetin has a value of 1 to 1[9].

Calibration Curves.—Measurements of absorbance were made with a number of the colored solutions containing varying amounts of boron. The results show that Beer's law is obeyed over the range 0.00 to 0.50 p. p. m. of boron as shown in Fig. 11. The sensitivity of this method, mean error and molar extinction coefficient were 0.204 μg., 4.90% and 22100 respectively. Even in the presence of 20 mg. of sodium sulfate, Beer's law is also obeyed over the range 0.00 to 0.50 p. p. m. of boron, but the sensitivity is decreased to 0.101 μg. in this case. After distillation of varying amounts of boron and evaporation of the distillate containing alkali and water to dryness, absorbances of the solutions of the dried residues were measured. As shown in Fig. 11, Beer's law is obeyed over the range 0.00 to 0.30 p. p. m. of boron.

Determination of Boron in Graphite Samples.—The results obtained by the above-mentioned experiments are presented in Table III.

TABLE III. RESULTS OF DETERMINATION OF BORON IN GRAPHITE SAMPLES

Sample	Taken g.	Absorbance	Boron found μg.	Boron determined p.p.m.	Results obtained by curcumin method p.p.m.
Graphite-A	0.5	0.660	2.8	5.6	5.53
Graphite-B	3.0	0.380	1.3	0.43	0.47
Graphite-C	2.0	0.450	1.7	0.85	0.90

Discussion

A sensitive new photometric method for the determination of boron by using quercetin as color reagent is proposed. The constitution of the boron-quercetin colored complex is considered to have the mole ratio of 1 : 1. In this procedure, if a different lot of quercetin, even though from the same maker, is used for color development, the absorbance is also varied, hence the determination of boron must be made using the calibration curve obtained by the same lot of quercetin as is used in the working operation. In this procedure, the color intensity of the solution is considerably affected by diverse salts, but the calibration curves are quite linear in the presence of a definite amount of salts. For the determination of boron in many kinds of practical samples, the proper separation of boron is desired. From the results of determination of boron in graphite samples, it was found that there is satisfactory agreement of the results obtained by the proposed method with those by the curcumin method in the limit of experimental error. This method may thus also be utilized for the determination of boron in other samples after preliminary separation of boron by distillation.

The author is indebted to Dr. Yuroku Yamamoto, Kyoto University, for his guidance and to Dr. Isao Muraki, Chief of the 5th Department of our Institute, for his useful suggestion.

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[9] W. C. Vosburgh and G. R. Cooper, *J. Am. Chem. Soc.*, 63, 437 (1941).