Instrumental Achievements

# Fluorometric Determination of Boric Acid by High Performance Liquid Chromatography after Derivatization with Chromotropic Acid

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Boric acid reacts with chromotropic acid in an aqueous solution to form an anionic complex. By using reversed phase HPLC, we developed a sensitive spectrophotometric method for boric acid.[1] The formation of the complex anion was enhanced by adding an quaternary ammonium salt; this addition resulted in lowering the concentration of chromotropic acid. Recently, especially in the field of semiconductors, determination of sub ppb of boron has become necessary. In this paper, fluorometric detector was introduced to enhance the sensitivity; a stoichiometric study was also made. The sensitivity of detection increased 4 times compared with our spectrophotometrical detection.[1] Boric acid reacted with chromotropic acid at 1:1 molar ratio; the apparent formation constant of the complex was estimated to be $2.3\times10^3$.

## Experimental

*Apparatus*

The HPLC system used is almost the same as previously reported[1], except for the detector: a Hitachi 650-10s spectrofluorometer with 18 µl volume flow cell was used.

*Reagents*

Reagent solution (A) contained $2.0\times10^{-2}$ M chromotropic acid (disodium salt) and 0.1 M EDTA. Reagent solution (B) contained 2 M octyltrimethylammonium chloride (OTMA-Cl) and 1 M acetate buffer solution (pH 4.8). The eluent consisted of 0.2 M sodium perchlorate and $1\times10^{-3}$ M ammonium buffer (pH 9.3); it was used after filtering through a 0.2 µm pore size membrane filter and degassing. Distilled water prepared with non-boiling type quartz apparatus was used throughout. All solutions were stored in polyethylene bottles, and test tubes made of polypropylene were used for reactions of boric acid with chromotropic acid. All chemicals used were of analytical reagent grade.

*Recommended procedure*

Transfer 10 ml or less of the sample solution (up to 0.4 µg of boron) to a polypropylene test tube and dilute it to 10 ml, if necessary. Add 0.5 ml each of the reagent solutions (A) and (B). Mix and allow to stand for 40 min at room temperature. Inject 10 µl of the solution with the injection valve. Detection was performed at 355 nm (excitation wavelength, 328 nm), and peak heights were recorded.

## Results and Discussion

*Reaction with chromotropic acid*

In our previous paper[1], quarternary ammonium salt (OTMA-Cl) was added to enhance the reactivity of the complex formation. In this work, OTMA-Cl was added similarly, and the effect of OTMA-Cl concentration was examined by varying the concentration from 0 to $1.2\times10^{-1}$ M. The highest peak heights were obtained above $8\times10^{-2}$ M; $9.0\times10^{-2}$ M was adopted. As a method to enhance the complex formation, addition of Triton X-100 solution was also examined. The peak height became higher by 10% when 0.1% of Triton X-100 was added. The reaction time, however, could not be shortened. In later experiments, Triton X-100 was not added. In Fig. 1, the relationship between chromotropic acid concentrations and the reaction time is shown. The higher the concentration, the shorter the reaction time, but the worse the separation of peaks is. The concentration of chromotropic acid was adjusted to $1.0\times10^{-3}$ M.

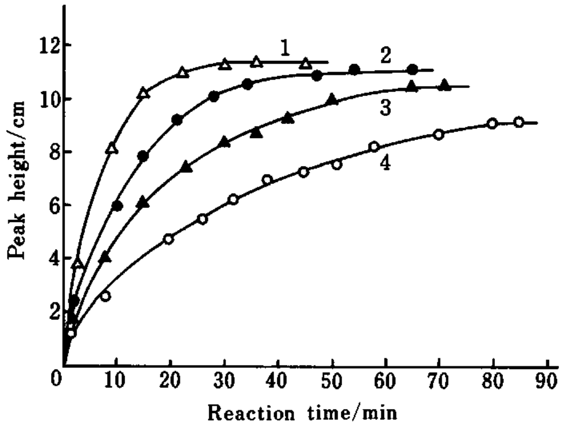


Fig. 1 Effect of the chromotropic acid concentration on the reaction time. Chromotropic acid concentration ($\times10^{-3}$ M): △, 1.5; ●, 1.0; ▲, 0.75; ○, 0.50. Boric acid: $2.0\times10^{-6}$ M.

### *Effect of reaction temperature*

The completion of the reaction between boric acid and chromotropic acid takes 40 min at room temperature. To shorten the reaction time, the effect of reaction temperature against time was examined at room temperature, 50°C and 90°C. The higher the temperature, the shorter the reaction time was. But the peak height at 90°C was diminished by 45% in comparison with that at room temperature and the tailing of the chromotropic acid peak became larger. This is because of decomposition products of chromotropic acid. So the reaction was carried out at room temperature, after the solution was kept standing for 40 min.

### *Effect of eluent concentration and pH*

Sodium perchlorate solution was used as eluent. The effects of the concentration and pH were examined. The effect of perchlorate concentration was almost the same as reported in a previous paper;[1] 0.20 M sodium perchlorate solution was adopted to achieve both sensitivity and peak separation. The effect of pH of eluent was tested as shown in Fig. 2. The separation of the peaks was the best at the range from pH 7 to 9.5. Therefore ammonium buffer solution (pH 9.2) was used.

### *Calibration curve*

Emission wavelengths of chromotropic acid and the complex with boron were nearly the same.[2] In this work, excitation wavelength was 328 nm and emission wavelength was 355 nm; these were chosen after considering the sensitivity and peak separation. The calibration curve was rectilinear from 0 to 0.4 μg of boron, and the detection limit was 1 ppb of boron (0.01 μg/10 ml). The sensitivity was 4 times better than that of previous spectrophotometry[1] which was somewhat lower than we expected. We think that quenching occurred due to the larger amounts of the excess chromotropic acid.

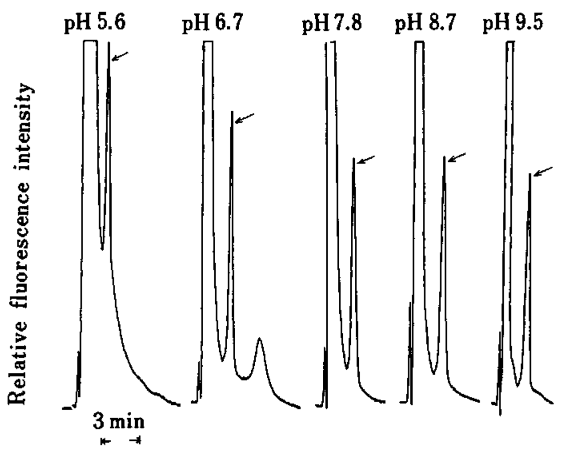
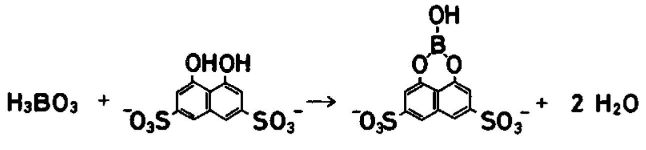


Fig. 2 Effect of eluent pH on chromatograms. Boric acid, $2\times10^{-6}$ M. The arrows indicate the peaks of the chromotropic acid-borate complex.

### *Composition of the complex*

Under the recommended condition, logarithms of capacity factors ($\kappa'$) for the boron complex and chromotropic acid were plotted against logarithms of the eluent concentrations. The graphs show good linearity and the ratio of the slopes of boron complex anion against chromotropic acid is 2 to 3. The ratio of these slopes corresponds to the ratio of the charges of the solute anions. At pH 9.2, chromotropic acid is present as a monoprotonated form because acid dissociation constants of chromotropic acid are $pK_{a3}=5.37\pm0.03$[3] and $pK_{a4}=15.6$.[4] At the complex formation pH, 4.8, the reaction proceeds as follows:



$H_2R^{2-}$ $B(OH)R^{2-}$ (1)

where $H_2R^{2-}$ and R represent a diprotonated and a nonprotonated form of chromotropic acid, respectively.

### *The apparent formation constant*

The formation constant of the complex of boric acid and chromotropic acid, *K*, is given by Eq. (2).

$$K=[B(OH)R^{2-}]/[H_3BO_3][H_2R^{2-}] \qquad (2)$$

Now $C_0$ is the total concentration of boron, $C$ and $C'$ are the concentrations of boron complex and chromotropic acid, and $\alpha=1+K_{a3}/[H^+]$ for chromotropic acid at pH 4.8, respectively. Then, $K$ is written as

$$K=C\alpha/(C_0-C)C' \qquad (3)$$

or

$$1/C=1/C_0+(\alpha/KC_0)(1/C'). \qquad (4)$$

From the plots of $1/C$ against $1/C'$, the apparent formation constant, $K'$, can be calculated. At pH 4.8, this graph shows a linear relationship. The slope was $1.53\times10^2$ and the intercept was $2.73\times10^5$. Therefore, $K'$ is calculated to be $2.3\times10^3$ from Eq. (4).

## References

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(Received April 28, 1990)
(Accepted June 16, 1990)