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1-(2,3,4-Trihydroxybenzylideneamino)-8-hydroxynaphthalene-3,6-disulfonic acid as reagent for spectrophotometric determination of boron in plants

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Abstract

A highly sensitive and selective method has been developed for spectrophotometric determination of boron in plants, the method based on the color reaction of new reagent 1-(2,3,4-trihydroxybenzylideneamino)-8-hydroxynaphthalene-3,6-disulfonic acid (THBA) with boron (III). In an ammonium acetate solution of pH 8.0, boron(III) reacts with THBA to form a 1:2 yellow complex which has a maximum absorption peak at 430 nm. The reaction can complete within 90 min and the absorbance of the complex remains maximum and almost constant at least for 24 h under a temperature range from 0 to 35 °C. The apparent molar absorptivity and Sandell's sensitivity are $2.95 \times 10^4\,\mathrm{l\,mol^{-1}\,cm^{-1}}$ and $0.00036\,\mathrm{ng\,cm^{-2}}$, respectively. The limit of quantification, limit of detection and relative standard deviations were found to be 5.1, $1.5\,\mathrm{ng\,ml^{-1}}$ and 1.12%, respectively. Under the optimum conditions, the absorbency of the complex ($\lambda_{max} = 430$ nm) increases linearly with concentration up to $0.8\,\mu\mathrm{g\,ml^{-1}}$ of boron(III). The influences of foreign ions on the determination of boron were investigated in detail. Most of foreign ions can be tolerated in considerable amounts. Experiments have indicated that THBA as chromogenic reagent for spectrophotometric determination of boron has excellent analytical characteristics. Its sensitivity is more than 4.2-fold that of azomethine-H, and stability is advantage over other derivatives of azomehine-H remarkably. Moreover, the synthesis of THBA and its physicochemical properties of THBA were also investigated in detail. Proposed method has been applied to the determination of boron in plants with satisfactory results.

Keywords: 1-(2,3,4-Trihydroxylbenzylideneamino)-8-hydroxynaphthalene-3,6-disulfonic acid; Boron; Plants; Spectrophotometry

1. Introduction

Boron is an essential element for plants. Boron deficiency in plant may result in reduced growth yield loss, and even death, depending on the severity of deficiency. Excess boron is toxic to plants. Boron toxicity symptoms may range from necrosis of some plant organs to death of the whole plant depending on the extent and severity of the toxicity. The tendency of boron to accumulate in vegetable tissues constitutes a potential hazard to the health of those consuming food and water with a high boron content [1]. Because effective methods have been discovered which can control the concentration of boron at a suitable level, if we know the boron concentration in plant tissue at a particular time, the above adverse effects can easily be eliminated. Thus, the determination of boron in plants is becoming increasingly important, especially in agriculture. In literatures, a number of the methods have been reported to determination of boron such as atomic absorption spectrometry (AAS) [2], inductively coupled plasma atomic emission spectrometry (ICP-AES) [3–6], inductively coupled plasma optical emission spectrometry

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(ICP-OES) [7], the prompt-γ method [8], inductively coupled plasma mass spectrometry (ICP-MS) [9], electrochemical method [10], X-ray fluorescence spectrometry (XFS) [11] and spectrophotometry [12]. Among these, AAS has remained limited because these methods have poor sensitivity and suffer from serious memory effects and interferences. ICP-AES, ICP-OES, the prompt-γ method, ICP-MS and XFS were complex to use and expensive. The most common methods for the determination of boron concentration are spectrophotometric methods [13]. At present, there are a number of organic reagents were used to determination of boron such as curcumin [14], D-sorbitol [15] and methylene blue [16]. Although each chromogenic system has its advantages and disadvantages with respect to sensitivity, selectivity and rapidity due to using different chromogenic reagents, most of them required using concentration H_2SO_4 as reaction medium, this bring inconvenient to routine boron analysis.

Azomethine-H as a reagent for the determination of boron [17] can be directly applied in aqueous acidic medium and has been widely used [18–20]. However, its sensitivity, stability and selectivity are very poor. In the recent years, some new derivatives of azomethine-H have been synthesized and applied to spectrophotometric determination of boron. Although analytical characteristics of the reagents have been improved obviously in sensitivity and selectivity, color systems are also sensitive to pH of medium, reaction temperature or reagent concentration (see Table 1). In order to continue, the search for new, highly sensitive and selective chromogenic reagents for the determination of trace boron and to study the effect of substituent groups on the analytical characteristics of reagents, 1-(2,3,4-trihydroxybenzylideneamino)-8-hydroxynaphthalene-3,6-disulfonic acid (THBA), a new derivative of azomethine-H (Fig. 1), designed and synthesized in our laboratory. It was found that THBA gave a very sensitive reaction with boron(III) in an ammonium acetate solution of pH 8.0. In this paper, a spectrophotometric method was developed for determination of boron and applied to the determination of boron in some plant samples.

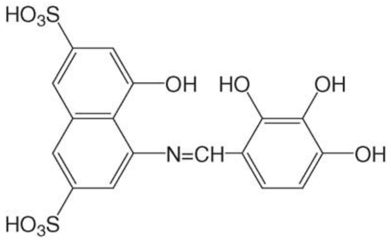

Fig. 1. Reagent's molecular structure.

2. Experimental

2.1. Synthesis of THBA

Transfer 18 g of 1-amino-8-naphthol-3,6-disulphonic acid monosodium salt (H-acid) into a 2000 ml of beaker, add 1000 ml of water and heat to dissolve it on a magnetic stirrer. A pH meter was used to show the pH of the solution and 10% NaOH was employed to adjust the pH to about 7.0. A portion of 20 g of 2,3,4-trihydroxybenzaldehyde was added to the above solution slowly. Then use concentrated HCI to adjust the pH value to 1.4 and continue stirring for 10 min. When the solution turn brownish-red, it was then divided into 10 equal quantities and each placed in a 200 ml plastic screw capped test tube, then put it in a constant temperature shaker at a rotation speed of 25 rotation min^{-1}. Continue shaking for 72 h. Shake the test tube heavily once a day. After 72 h, the mixture was centrifuged. The yellow precipitate formed was filtered and washed with 100 ml 95% ethanol for five times. The product was placed in 80 °C drying oven for 3 h, then cooled, ground and reserved in a brown bottle and placed in refrigerator. THBA was obtained with a yield of 48%. Thermogravimetry analysis showed that THBA contained two molecules of water and the result of elemental analysis corresponded with the composition of THBA. Moreover, ultraviolet spectrum, infrared spectrum, ^1H NMR, ^{13}C NMR, mass spectrometry methods were also applied to study on the

Table 1
Review of the derivatives of azomethine-H as reagent for spectrophotometric determination of boron

Reagent	Medium	λ_{max} (nm)	ε ($l\,mol^{-1}\,cm^{-1}$)	Remarks	Ref.
Azomethine-H	NH_4Ac–HAc (pH 6.5)	420	8.1×10^3	1. Narrow range of acidity. 2. Reagent concentration and temperature influence on the sensitivity and reaction rate seriously. 3. Fe, Al, Cu, Ti and Zr interfere seriously	[21]
5-Cl-azomethine-H	NH_4Ac–HAc (pH 5.5)	430	5.95×10^3	1. Poor water-solubility and required using ethanol solution to improve water-solubility. 2. Low sensitivity. 3. Temperature influence on the sensitivity. 4. Narrow range of acidity	[22]
4-Methoxyazomethine-H	NH_4Ac–HAc (pH 5.7)	420	1.05×10^4	1. Highly sensitive. 2. Temperature influence on the sensitivity seriously. 3. Narrow acidity range. 4. Color reaction rate is rapid	[23]
3-Methoxyazomethine-H	NH_4Ac–HAc (pH 5.5)	423	7.19×10^3	1. Temperature influence on the sensitivity seriously. 2. Narrow acidity range. 3. Color reaction rate is rapid	[24]
Azomethine-HR	NH_4Ac (pH 7.0)	425	2.45×10^4	1. Highly sensitive. 2. Narrow acidity range. 3. Reagent concentration influence on the sensitivity seriously. 4. Color reaction is slow very much	[25]

structure of the reagent, the results confirmed the proposed structure of THBA molecule.

2.2. Apparatus

A Beckman DU-7HS spectrophotometer was used to record absorption spectra and a model pH-29A pH meter (Shanghai Second Analytical Instrument Factory, Shanghai, China) was used to measure the pH solutions.

2.3. Reagents

Unless otherwise stated, all reagents used were of analytical grade and all solutions were prepared with distilled water.

Standard boron solution (1.0 mg ml^{-1}) was prepared by dissolving 0.8820 g of sodium borate in an appropriate volume of water and diluting to 100 ml with water. Solutions of 10 μg ml^{-1} of boron(III) were prepared freshly by diluting 1.0 ml of 1.0 mg ml^{-1} boron(III) to 100 ml with water.

1-(2,3,4-Trihydroxylbenzylideneamino)-8-hydroxynaphthalene-3,6-disulfonic acid (THBA) (0.3%) was prepared by dissolving 0.3 g of THBA and 1.0 g of ascorbic acid in 100 ml water and stored in a polyethylene bottle.

Buffer solution (pH 8.0) was prepared by dissolving 500 g of ammonium acetate in about 700 ml of water, and the pH was adjusted with diluted ammonia solution on a pH-meter.

2.4. Procedure for determination of boron

Boron standard solution or samples solution containing no more than 20 μg of boron(III) was transferred into a 25 ml calibrated flask. An aliquot of 5.0 ml of pH 8.0 buffer solution and 6.0 ml of 0.3% THBA solution was added successively. The solution was diluted to the mark with water and mixed thoroughly. After 90 min, the absorbance was measured at 430 nm with a 1 cm cell against the reagent blank.

2.5. Procedure for decomposition of plant materials

All samples are very delicate tree leaves and collected from Wuxi, China. Sample was dried in an oven at 70 °C, ground and ash in covered porcelain crucibles at 500 °C for 2 h. Ashes were extracted with 10 ml of 0.01 mol l^{-1} HCI. The extracted solution was filtered through Whatman no. 40 filter paper, and finally diluted to 25 ml with water [24].

3. Results and discussion

3.1. Physicochemical properties of THBA

THBA is a yellow solid, it dissolves in water and is difficult to dissolve in organic solvents such as ethanol and acetone. THBA solution shows different colors in different acidities due to its dissociation, its progressive dissociation constants were calculated by the following formula [26]:

$$K_n = \frac{(A_i h_i - A_p h_p)(h_i - h_m) - (A_i h_i - A_m h_m)(h_i - h_p)}{(A_i - A_m)(h_i - h_p) - (A_i - A_p)(h_i - h_m)}$$

in which K_n is the stepwise dissociation constant of the reagent, A_i, A_p, A_m and h_i, h_p, h_m are the absorbance and hydrogen ion concentration at various times, respectively. The results from above calculated are pK_1 = 1.4, pK_2 = 2.5, pK_3 = 3.3, pK_4 = 4.8, pK_5 = 5.4 and pK_6 = 6.5. Based on the results, the stepwise dissociation procedure was suggested in Fig. 2.

3.2. Absorption spectra

In an ammonium acetate solution of pH 8.0, boron(III) reacts with THBA to form a yellow complex. The absorption spectrum of the complex and reagent are shown in Fig. 3. It can be seen that THBA has no maximum absorption peak between 400 and 450 nm, whereas the complex has an absorption peak at 430 nm. In order to obtain high sensitivity, a wavelength of 430 nm was chosen for the spectrophotometric determination of the boron.

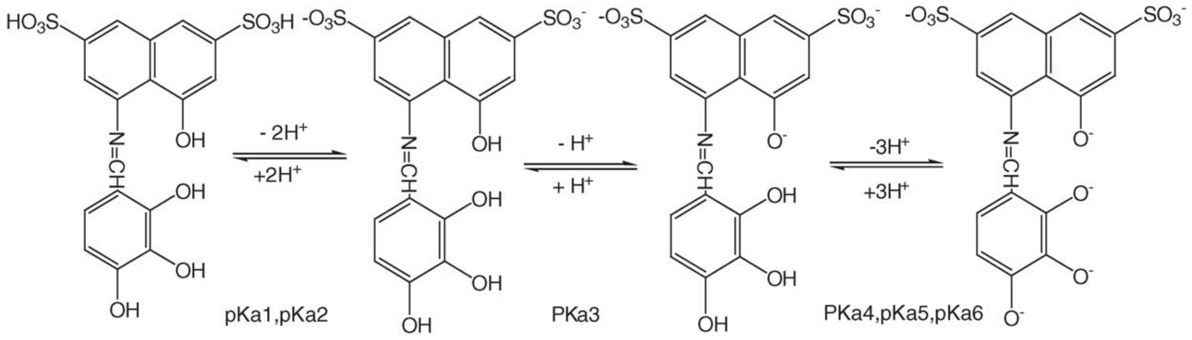

Fig. 2. The equilibrium of the dissociation of THBA in water.

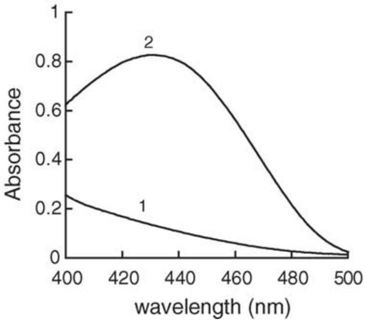

Fig. 3. The absorption spectrum of the reagent and the complex: (1) the reagent against water; (2) the complex against reagent blank, boron(III): 8.0 μg.

3.3. Selection of the color reaction medium

The effect of various medium including ammonium chloride, sodium acetate, sodium citrate, sodium phosphate and ammonium acetate on the reaction of boron(III) with THBA were examined closely. It was found that ammonium acetate solution give the highest reaction rate and sensitivity and was selected in the following experiments, in which ammonium ion may associate with THBA to form ions-association and improve the water-solubility of the reagent. The effect of acidity on the sensitivity of the reaction of boron(III) with THBA was listed in Fig. 4. Fig. 4 indicates that the acidity has obviously influences on the sensitivity. When pH value is between 7.5 and 9.0, color system gives an almost constant absorbance unlike other derivatives of azomethine-H, a buffer solution of pH 8 is employed for the following experiments to control the pH. When the volume of buffer solution varies from 4 to 10 ml, the absorbance remains constant and maximum (Fig. 5). Thus, an addition of 5.0 ml of the buffer solution of pH 8 is recommended.

3.4. Effect of concentration of THBA

With the increase of the concentration of THBA, the absorbance of the complex increases rapidly, and reaches a plateau. After that, it dropped slowly. In 25 ml of solution, the optimum volume of 0.3% THBA was between 5.0 and 8.0 ml unlike azomethine-HR and azomethine-H. Thus, 6.0 ml of the reagent was employed for this work.

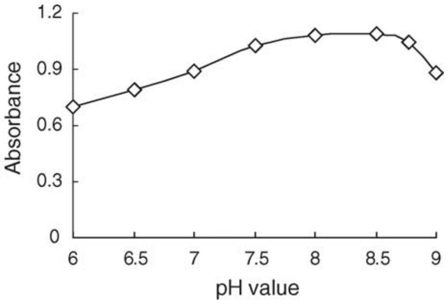

Fig. 4. The effect of the acidity on the sensitivity, boron(III), 10 μg.

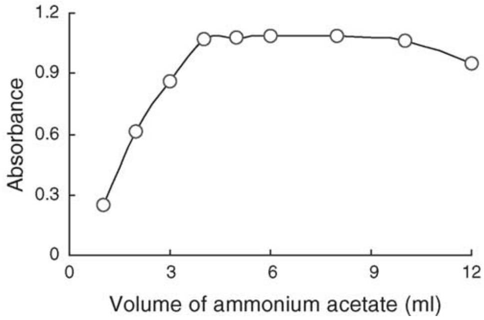

Fig. 5. The effect of volume of ammonium acetate of pH 8.0 on sensitivity.

3.5. The effect of ascorbic acid and EDTA

THBA solution is easily oxidized by oxygen in air and influence its use. In order to overcome this drawback, ascorbic acid was introduced into the system for the eliminating effect of oxygen. Experiment show that ascorbic acid can improve the stability of the reagent solution. After the addition of ascorbic acid, the reagent solution can stable for at least 4 months under room temperature. When ascorbic acid amounts is lower than 50 mg in 25 ml solution, it has no effect on the sensitivity and an addition of 50 mg ascorbic acid in 25 ml solution was therefore selected for subsequent experiment. Apart from increase the stability of the complex, the addition of ascorbic acid was also found to be able to increase the selectivity of the system for iron(III) obviously.

Although THBA as reagent for spectrophotometric determination of boron is very selective, the tolerance for Ca, Mg and Fe, which are present in great amounts in plants may interfere when boron is determination directly in some samples. We found that the addition of EDTA to the reaction system was very effective in masking these ions and greatly improved the selectivity. When the volume of 2% EDTA varied from 0.0 to 8 ml, the absorbance remains constant and maximum. Thus, addition of 6.0 ml of 2% EDTA is recommended.

3.6. Effect of temperature

In literatures, the derivatives of azomethine-H including azomethine-H,5-chloroazomethine-H, 4-methoxyazomethine-H and 3-methoxyazomethine-H as reagent for spectrophotometric determination of boron are very sensitive to reaction temperature. When the temperature is higher than 0 °C, the complex were rapidly decomposed with increasing temperature and required strong control a constant reaction temperature in order to obtain reliable results. However, THBA–B(III) complex is very stable. When temperature is no more than 35 °C, color reaction of boron(III) with THBA can be completed within 90 min and the absorbance of the complex remains maximum and almost unchanged for at least 24 h. Thus, the stability of the color system is advantage

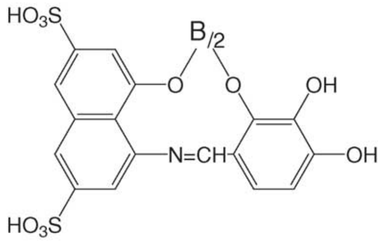

Fig. 6. Proposed structure of the complex.

over other derivatives of azomethine-H remarkably, which is very important to routine analysis.

3.7. Composition of the complex

The complex composition was determined by the Job's method of continuous variation and the slope-ratio method. The results showed that the composition ratio of THBA–boron complex was 1:2. The molecule structure was suggested in Fig. 6.

3.8. Effect of foreign ions

Under the optimum conditions, the effects of various foreign ions on the determination of 10 μg boron(III) were examined separately. With a relative error being ±5%, the tolerance limits for various foreign ions were listed in Table 2. It is found that all foreign ions studied can be tolerated in considerable amounts, so that the proposed method can be used to determine boron(III) in plants directly.

3.9. Analytical characteristics

A calibration graph was constructed in the usual way according to the procedure in Section 2.4. Beer's law was obeyed over the range of 0–20 μg of boron(III) in 25 ml of solution at 430 nm (see Fig. 7). The molar absorptivity was calculated from the slope of the calibration graph to be $2.95 \times 10^4\ \mathrm{L\,mol^{-1}\,cm^{-1}}$, the limit of quantification [27,28] and the limit of detection, as defined by IUPAC [29], were found to be 5.1, $1.5\ \mathrm{ng\,ml^{-1}}$, respectively. Twenty replicate analysis of a test solution containing 2.0 μg of boron(III) by the general procedure in Section 2.4 gave a relative standard deviation of 1.12%.

Table 2
Tolerated limit of various foreign ions

Foreign ion	Tolerated limit (mg)	Foreign ion	Tolerated limit (mg)
Al(III)	1.0	La(III)	5.0
Ca(II)	25	Zr(IV)	0.1
Co(III)	5.0	Mo(VI)	0.7
Cu(II)	5.0	Mg(II)	5.0
Fe(III)	0.1	K(I)	100
W(VI)	1.0	Cl^-	2000
Pb(II)	1.0	Br^-	200
Ni(II)	1.0	I^-	100
Mn(II)	1.0	$SO_4{}^{2-}$	100
Ti(IV)	0.1	$PO_4{}^{3-}$	100
Zn(II)	5.0	$CO_3{}^{2-}$	100
NH_4(I)	100	$SiO_3{}^{2-}$	50
Ba(II)	2.5	$C_2O_4{}^{2-}$	100

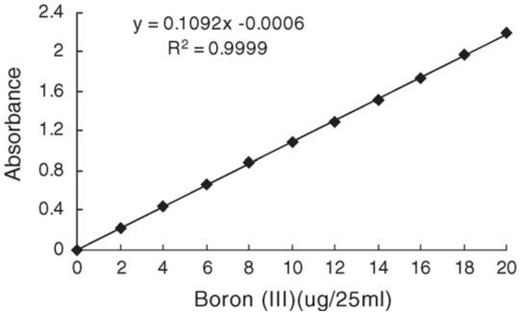

Fig. 7. Calibration graph.

3.10. Determination of boron in plants

In order to confirm the usefulness of the proposed method, it has been applied to the determination of boron in plant samples. The results observed are compared with those obtained using the inductively coupled plasma atomic emission spectrometry (ICP-AES) (Table 3). The results were also examined statistically by using the *t*-test for accuracy and *F*-value for the assessment of precision for 5° of freedom and a 95% confidence level. The calculated values did not exceed the corresponding theoretical values, indicating insignificant differences between the results (Table 3).

3.11. Conclusions

1-(2,3,4-Trihydroxylbenzylideneamino)-8-hydroxynaphthalene-3,6-disulfonic acid (THBA) as a new organic reagent for spectrophotometric determination of boron can be easily synthesized and purified in ordinary laboratory. It has very stable physicochemical properties and can be used to determine trace boron conveniently. Compared to the reported spectrophotometric methods, especially other derivatives of azomethine-H, the proposed method offers several noticeable advantages: (1) high sensitivity—the molar absorptivity of the complex is $2.95 \times 10^4\ \mathrm{l\,mol^{-1}\,cm^{-1}}$, that is 4.2-fold higher than with azomethine-H, mainly due to introducing three hydroxyl groups into 2,3,4-postions at benzene ring and increases obviously electron cloud density of functional groups of the reagent molecule. Thus, the proposed method can directly determine ppb level of boron without prior pre-concentration procedure. (2) The reaction of the derivatives of azomethine-H with boron is commonly sensitive to the temperature and the reagent concentration very much, and their reaction condition such as temperature and amounts of reagent concentration required seriously control in order to obtained a precise and accuracy results (see Table 1). However, the reaction of THBA with boron(III) is very stable. The absorbance of

Table 3
The results of determination of boron in plant samples[a]

Sample	Boron found by proposed method ($\mu g\,g^{-1}$)	Boron found by ICP-AES method ($\mu g\,g^{-1}$) [3]
Paddy	0.085 ± 0.002, $F = 4.0$, $t = 1.96$	0.083 ± 0.004
Chilli	0.123 ± 0.004, $F = 3.06$, $t = 1.47$	0.120 ± 0.007
Soybean	3.21 ± 0.11, $F = 1.86$, $t = 0.36$	3.23 ± 0.15
Potato	1.17 ± 0.05, $F = 1.56$, $t = 1.48$	1.20 ± 0.04
Chinese chestnut	0.99 ± 0.04, $F = 1.56$, $t = 0.49$	0.98 ± 0.05
Sugar beet	1.97 ± 0.07, $F = 2.04$, $t = 1.4$	2.02 ± 0.10

[a] $X \pm St/n^{1/2}$ ($n = 5$); the t- and F-values refer to comparison of the proposed method with ICP-AES. Theoretical values at 95% confidence limits: $F = 6.39$, $t = 2.78$.

the complex can remain maximum and almost constant for at least 24 h under temperature between 0 and 35 °C, this is very important to routine analysis. Moreover, proposed method is also selective. Therefore, proposed method can be used routinely for the determination of trace boron in plants.

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