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The determination of boron in food and seed by spectrophotometry using a new reagent 3,4-dihydroxyazomethine-H

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Abstract

A new sensitive and selective reagent, 3,4-dihydroxyazomethine-H, was studied for spectrophotometric determination of boron in food and seed. Boron (III) reacts with 3,4-dihydroxyazomethine-H to form a 1:2 yellow complex in an ammonium acetate solution of pH 8.0, which has a maximum absorption peak at 430 nm. Under the optimal conditions, Beer's law was obeyed over the range $0 \sim 20$ μg of boron (III) in 25 ml of solution. The apparent molar absorptivity is 2.95×10^4 l mol^{-1} cm^{-1}, in which the sensitivity is higher than 4.2-fold that of azomethine-H. The limit of quantification, limit of detection and relative standard deviations were found to be 5.1, 1.5 ng g^{-1} and 1.08 %, respectively. The influences of foreign ions on the determination of boron were also investigated in detail, most of the ions studied can be tolerated in considerable amounts. The reaction can be completed within 90 min and the absorbance of the complex remains maximum and almost constant for 24 h under room temperature from 0 to 35 °C, which is advantagous over other derivatives of azomethine-H reported remarkably. The proposed method has been successfully applied to the determination of boron in food and seed.

Keywords: Boron; 3,4-Dihydroxyazomethine-H; Spectrophotometry; Food and seed

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1. Introduction

Boron is a beneficial element for plant and animal (Nielsen, 1993), but excess boron is toxic to human's health and seed developing, its analysis in food and seed is becoming increasingly important. At present, the most common methods for the determination of boron are spectrophotometric methods (Sah & Brown, 1997). However, the number of reagents available for the spectrophotometric determination boron is relatively small. The main reagents are curcumin (Thangavel, Dhavile, Dash, & Chaurasia, 2004), carmimic acid (Pan & Zheng, 2000), quinalizarine (Pan, Chen, & Yan, 981) and azomethine-H (Van Staden & Van der Merwe, 2000). Among these, azomethine-H method is perhaps the most commonly used spectrophotometric method of determining boron. The method is fast, simple, and sensitive and does not require concentrated acids. In a comparative evaluation of azomethine-H, carminic acid, and curcumin methods for determination of boron in water, the azomethine-H method suffered the least interferences and was the most sensitive. In recent years, several new derivatives of azomethine-H were synthesized and studied for spectrophotometric determination of boron. These methods have better analytical characteristics in sensitivity and selectivity than using azomethine-H obviously, but these are also sensitive to temperature and

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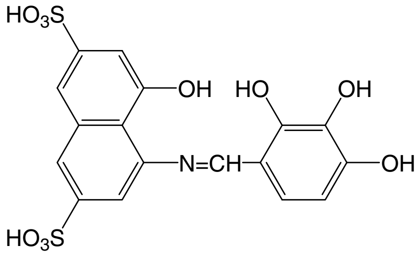

Fig. 1. Reagent's molecular structure.

pH, thus a strict reaction condition is required strongly in order to achieve accuracy and reliable results (Li, Zhu, & Pan, 1999, 2001; Pan, Wang, & Xu, 1994).

3,4-Dihydroxyazomethine-H, a new derivative of azomethine-H (Fig. 1), was designed and synthesized in the laboratory. It was found that 3,4-dihydroxyazomethine-H gave a very sensitive reaction with boron (III) in an ammonium acetate buffer solution of pH 8.0. In this work, we developed a spectrophotometric method for determination of boron in food and seed. The experiment has indicated that this method is one of the most convenient and efficient method for the determination of boron at the present time.

2. Material and methods

2.1. Reagents and materials

The standard boron solution was prepared by dissolving sodium borate (Shanghai Chemical Third Factory) in an appropriate volume of water and diluting to 100 ml with water. A 10 μg g^{-1} of boron working solution was prepared freshly by suitably diluting the standard boron solution with water. 3,4-Dihydroxyazomethine-H solution (0.3%) was prepared by dissolving 0.3 g of 3,4-dihydroxyazomethine-H and 1.0 g of ascorbic acid in 100 ml water and stored in a polyethylene bottle, which was synthesized in a similar manner as described by Edwards (Edwards, 1980), only 2,3,4-trihydroxybenzaldehyde was used instead of salicylaldehyde. Buffer solution of pH 8.0 was prepared by dissolving 500 g of ammonium acetate (Shanghai Chemical Third Factory) in about 700 ml of water, adjusting the pH with diluted ammonia solution by using a model pH-29A pH meter (Shanghai Second Analytical Instrument Factory, Shanghai, China). Unless otherwise stated, all reagents used was of analytical grade and all solutions were prepared with distilled water.

Foods including chicken, coffee bean, dried shrimp and rice, seeds including soybean, maize and mung bean, and reference material GBW07605 Tea available in the wuxi city in China were analysed.

2.2. Sample treatment

Prior to analysis, the sample (including various food, seed and reference material GBW07605 Tea) was ground well and dried in an oven at 105 °C for 2 h, about 0.3–1.0 g of the sample was accurately weighed into an acid washed Teflon digestion tube. Seven milliliters of concentrated nitric acid (65%) was added and the tube was heated in a microwave oven. A model MSW-2 microwave digestion unit (Berghof-laborprodukte Company) at the power setting of 80% was for 10 min and at 100% for 10 min. The maximum total output of the microwave generator was 750 W. The digest was transferred into a 25 ml acid washed volumetric flask, neutralized with 1.0 mol l^{-1} sodium hydroxide solution to pH about 7.0 (using pH meter), filled up with distilled water and stored in polypropylene flasks, two water blanks were run with each batch of samples.

2.3. Analytical method

A boron(III) solution containing less than 20 μg of boron (III) was transferred into a 25 ml calibrated flask, 5.0 ml of the buffer solution of pH 8.0 and 6.0 ml of 0.3% 3,4-dihydroxyazomethine-H solution were added successively, the solution was diluted to the mark with water and mixed well. Wait for 90 min and measure the absorbance at 430 nm in a 1 cm cell against the reagent blank. All absorbance measurements were carried out with a model Beckman DU-7HS spectrophotometer from Beckman America.

2.4. Reference method (ICP-AES method)

The measurements were carried out with an ICP atomic emission spectrometer "Palasma 400" from P-E America. The used emission lines of boron were B I 249.773, B I 249.678, and B I 208.959 nm. The line of internal standard indium (In I 325.609 nm) and copper (Cu I 324.754 nm) was used for matrix effect correction. The operating condition was as follows: plasma power supply 1.0 kW, observation height 6 mm, plasma gas flow 10.0 l min^{-1}, auxiliary gas flow 0.5 l min^{-1}, nebuliser gas flow 0.6 l min^{-1}, photomultiplier voltage 600 V, sample uptake rate 1.7 ml l^{-1}, integration time 1 s, and sample time delay 30 s (Anna & Tomas, 2003).

2.5. Statistical methods

Paired comparison of F and t-test was used to evaluate the effect of using different methods. Considering the rather low number of replicates ($n = 5$), the possibility of a non-Gaussian distribution was considered. The data sets were subjected to a non-parametric test; the same results as compared to parametric test were obtained. We preferred to give the results of parametric paired *t*-test (Chen, Zhang, Huang, & Qian, 1987). The limit of quantification (10 σ of blank) and the limit of detection (3 σ of blank) of the proposed method, as defined by IUPAC (Long & Wineforder, 1980, 1983; Medinilla, Ales, & Sanchez, 1986), were calculated.

3. Results and discussion

3.1. Absorption spectra

The absorption spectra of the boron complex and corresponding reagent blank were shown in Fig. 2. It can be seen that the complex has an absorption peak at 430 nm. Corresponding to the blank reagent solution, there was no maximum absorption peak between 400 and 450 nm. In order to obtain highest sensitivity, a wavelength of 430 nm was selected by all following experiments.

3.2. Optimization of the reaction medium

Various media including borax, citric acid, ammonia buffer solution, ammonium acetate and acetate buffer solution were introduced to examine the effect of the medium on the reaction. The results indicated that ammonium acetate solution gives a higher reaction rate and sensitivity and was selected in the following experiments. At the same time, ammonium ion in the system may associate with 3,4-dihydroxy–azomethine-H to form ions-association and improve the water-solubility of the reagent. The experimental indicated that the absorbance at 430 nm increases with increasing pH value up to 7.5 and then remained almost constant in the range of ammonium acetate solution from pH 7.5 to 9.0. The absorbance decreased considerably, a buffer solution of pH 8 is employed for the following experiments to control the pH value. When the volume of buffer solution varied from 4 to 10 ml, the absorbance remains constant and maximum. Thus, an addition of 5.0 ml of the buffer solution of pH 8 is recommended.

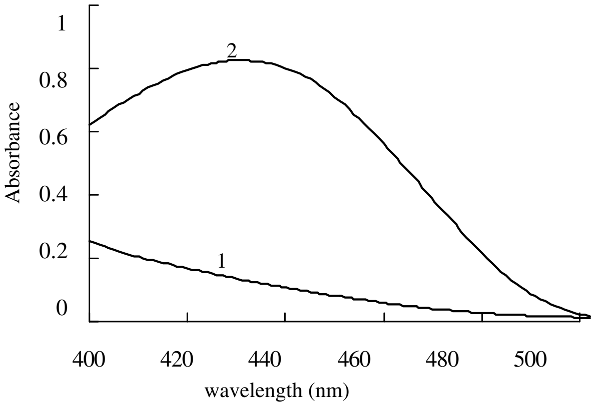

Fig. 2. The absorption spectrum of the reagent and the complex.

3.3. Reagent concentration

With the increase of the concentration of 3,4-dihydroxyazomethine-H, 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% 3,4-dihydroxyazomethine-H was between 5.0 and 8.0 ml. Thus, 6.0 ml of the reagent was employed for this work.

3.4. Effect of ascorbic acid and EDTA

3,4-Dihydroxyazomethine-H solution is easily oxidized by oxygen in air and influences its use. In order to overcome the above drawback, ascorbic acid was introduced into the system for the eliminating effect of oxygen. The experiment shows that ascorbic acid can improve the stability of the reagent solution. After that, the reagent solution can remain stable for four months at least under room temperature. When ascorbic acid amount are lower than 50 mg in 25 ml solution, it cannot effect on the sensitivity and an addition of 50 mg ascorbic acid in 25 ml solution was selected. Moreover, ascorbic acid also increases the selectivity of the system for iron (III) obviously. Although 3,4-dihydroxyazomethine-H, as reagent for spectrophotometric determination of boron, is very selective, the tolerance for Ca, Mg and Fe, which are present in great amounts in plant, may interfere when boron is determined directly in some samples. We found that EDTA addition to the reaction system can be very effective in masking these ions and greatly improves 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.

3.5. Analytical characteristics

At ambient temperature from 0 to 35 °C, the color reaction of boron with 3,4-dihydroxyazomethine-H can complete within 90 min. In order to obtain good accuracy and precision, a 90 min reaction time was adopted in all following experiments. After the color reaction completed, the absorbance of the complex can remain almost constant for 24 h at least, this exhibited that the complex is very stable, its stability is advantagous over other derivatives of azomethine-H obviously. The complex composition was also determined by the Job's method of continuous variation and the slope-ratio method. The results show that the composition ratio of 3,4-dihydroxyazomethine-H–boron complex was 1:2.

Calibration graph, obtained by the proposed procedure in Section 2.3 under the optimum conditions, was found to be linear up to 20 μg of boron (III) in 25 ml solution. The equation ($n = 10$, $r = 0.9999$) of relative absorbance at 430 nm (A) against concentrations (C μg per 25 ml) was: $A = 0.1092^{*}\mathrm{C}—0.0009$. 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 and the limit of detection were found to be 5.1 and 1.5 ng g^{-1}, respectively.

3.6. *Effect of foreign ions*

Under the optimum conditions, the effects of various foreign ions on the determination of 10 μg boron (III) in 25 ml solution were examined separately. With a relative error being ± 5%, the tolerance limits for various foreign ions were listed in Table 1. It is found that all of the foreign ions studied can be tolerated in considerable amounts, so that the proposed method can be used to determine boron (III) in various biological samples directly.

3.7. *Precision and accuracy*

Boron in Chinese reference material GBW07605 was determined for verifying validation of the method, in which standard value of boron is 15 μg g^{-1}, an average value of 14.95 μg g^{-1} was obtained by using the proposed method for six time analyses with a relative standard deviation of 1.08%. Moreover, the method was also tested by analyzing several synthetic samples containing different amounts of metal ions and boron (III) (Table 2), the analytically determined boron concentration agreed well in the known level of boron addition. The above results indicated that the proposed method has high precision and accuracy.

Table 1
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	$\mathrm{C}_2\mathrm{O}_4^{2-}$	100

Table 2
Determination of boron in some synthetic mixtures

Sample	Composition of mixture (μg in 25 ml solution)	Boron III (μg/25 ml)		Recovery (%)
		Added	Found	
1	Boron (III)	5.0	5.0	100
2	Boron (III) + Ca (1000)	5.0	5.01	100.2
3	Boron (III) + Mg (1000)	5.0	4.96	99.2
4	Boron (III) + Ca(1000) + Mg (1000)	5.0	5.11	102.2
5	Boron (III) + Ca(1000) + Mg (1000) + Fe (100)	5.0	4.98	99.6
6	Boron (III) 1 + Ca(1000) + Mg(1000) + Zn (100)	5.0	5.03	100.6

Table 3
The results of determination of boron in food and seed samples[a]

Sample	Boron found by proposed method (μg/g)	Boron founded by ICP-AES method (μg/g)
Food		
Chicken	0.077 ± 0.0031 $F = 1.58$, $t = 1.90$	0.074 ± 0.0039
Coffee bean	15.23 ± 0.12 $F = 2.51$, $t = 1.15$	15.30 ± 0.19
Dried shrimp	0.84 ± 0.032 $F = 1.64$, $t = 1.21$	0.82 ± 0.041
Rice	0.54 ± 0.020 $F = 1.10$, $t = 0.98$	0.55 ± 0.021
Seed		
Soybean	11.91 ± 0.17 $F = 1.83$, $t = 0.81$	11.84 ± 0.23
Maize	3.50 ± 0.14 $F = 1.65$, $t = 0.56$	3.46 ± 0.18
Mung bean	0.170 ± 0.0047 $F = 1.91$, $t = 0.33$	0.178 ± 0.0065

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

4. Analysis of food and seed samples

The food and seed samples were converted, according to the procedure described in Section 2.2 into samples solution. Then, the boron content was determined as described in Sections 2.3 and 2.4 and results are listed in Table 3. The analytical data for various samples given in Table 3 indicate a high degree of correlation between the results of ICP-AES and proposed procedure.

5. Conclusion

The experiment demonstrated that the proposed method has adequate sensitivity and accuracy for determination of boron in various foods and seeds, its analytical characteristics such as sensitivity, selectivity and stability excelled other azomethine-H reagents obviously. Although ICP-AES is presently one of the most common and powerful techniques for boron determination, the results show that the proposed method can provide similar results with considerably low cost, it is easily available in most laboratories for routine determination of boron, especially in developing countries.

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