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Microwave Digestion and Ultrasonic Nebulization for Determination of Boron in Animal Tissues by Inductively Coupled Plasma Atomic Emission Spectrometry With Internal Standardization and Addition of Mannitol

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Determinations of boron in animal tissues were performed by inductively coupled plasma atomic emission spectrometry (ICP-AES) with an ultrasonic nebulizer. The method is based on the microwave digestion of tissues with HNO_3–H_2O_2 and the use of mannitol as a modifier. It was found that mannitol can significantly enhance the analytical sensitivity, improve the precision and minimize the memory effect for the determination of boron. Acid effect and matrix effects were controlled by using the Be I 234.861 nm line as the internal standard. Six animal tissue samples, including two NIST standard reference materials (Oyster Tissue and Bovine Liver), were analyzed to test the reliability of the method. A limit of detection (3s) of 0.7 mg l^{-1} and recoveries of added boron from selected matrices between 95.0 and 100.2% were obtained.

Keywords: *Boron; ultrasonic nebulizer; microwave digestion; mannitol; inductively coupled plasma; internal standardization*

In the recent years, interest in the role of boron in human and animal nutrition has increased rapidly. It is considered that B, together with Ca and P, helps maintain strong bones and plays a key role in the production of estrogen and other hormones. It is also indicated that a deficiency of B may reduce stamina during exercise, increase the loss of Ca and Mg from bones and decrease the ability to produce vitamin D.[1] Therefore, the demand for reliable determinations of B in food and biological samples, including animal tissues, is growing.[2–5]

ICP-AES has been widely employed for multi-elemental analysis, including the determination of B in various materials.[4–6] However, ICP-AES with typical pneumatic nebulizers is often not sensitive enough to detect B when the amount of sample is limited or if the concentration of B is relatively low. It is known that an ultrasonic nebulizer (USN) has some significant advantages over a pneumatic nebulizer.[7] However, the USN has not been applied to the determination of some elements, such as B and Hg, owing to memory effects. The reasons for the memory effect of B are still not clear. Generally, it is considered that the memory effects in the USN originate from the re-nebulization of residual droplets of previously nebulized solution or the existence of the dead volume for most elements.[8] It seems that the memory effect of B has a different mechanism from that of other elements. For example, when compared with the blank value, the emission signal was still very high with a 25 min flush time after a 100 mg l^{-1} B solution was aspirated to the ICP by a USN.[9] Recently, we have found that the memory effect in USN-ICP-AES can be markedly reduced with the addition of mannitol,[10] thereby permitting ultrasonic nebulization to be an efficient sample introduction technique for the determination of trace levels of B by ICP-AES.

The accurate determination of B in animal tissues has proven difficult because of the very low concentrations found in some tissues and the possible contamination and loss during sample preparation. The dry-ashing method, which has been successfully applied for the determination of B in plants and plant-derived foods,[10,11] is not applicable for some animal tissues because the tissues cannot be completely oxidized under the normal ashing conditions. If the residue is moistened with H_2O or dilute HNO_3 and re-heated in the furnace, most of the B would be lost. Wet ashing of samples in open flasks with HNO_3–$HClO_4$ on a hot-plate is an easy and reliable preparation method for the determination of most mineral elements in tissues. Using this procedure, however, the samples can be readily contaminated with B owing to the use of borosilicate glassware. In the past several years, the microwave digestion technique has been extensively applied for the sample preparation of many materials.[12,13] For the recommended procedures of microwave digestion, however, substantial volumes of mineral acids have to be employed with most of the acids remaining in the vessels after digestion, which would result in serious acid effects for sample introduction by ultrasonic nebulization. Correction for acid effects may be carried out by either matching the acid concentration or internal standardization.[14,15] The preparation of exact acid matching solutions with high accuracy is very difficult. In contrast, internal standardization is readily performed and is advantageous in that it can simultaneously reduce the effects of both noise and drift.

In this paper, a method for the determination of B in animal tissues by ICP-AES is described. The samples were prepared by microwave digestion and the sample solutions were nebulized and introduced to the ICP by ultrasonic nebulization. Memory effects were minimized by the addition of mannitol. The acid and matrix effects were controlled by the use of internal standardization.

EXPERIMENTAL

Instrumentation and Working Conditions

The microwave system used for sample digestion was an MDS-81D microwave oven (CEM, Matthews, NC, USA). The sample solutions were nebulized by a U-5000AT ultrasonic nebulizer (CETAC, Omaha, NE, USA) with a Gilson (Worthington, OH, USA) peristaltic pump. Atomic spectrometric determinations were carried out with an ARL 3410+sequential ICP spectrometer (Fisons, Dearborn, MI,

USA) with a Minitorch. The main operating conditions are given in Table 1.

Reagents

The de-ionized (DI) water used for the preparation of all blank, standard and sample solutions was obtained directly from a Nanopure system (Barnstead, Dubuque, IA, USA; specific resistance 18 MV). Standard solutions of B were prepared from the certified atomic absorption standard solution (boric acid in H_2O, containing 1000 mg l^{-1} B; Fisher Scientific, Fair Lawn, NJ, USA) by serial dilution. A stock standard solution of Be (1000 mg l^{-1}) was purchased from Spex (Metuchen, NC, USA). Matrix stock standard solutions (2000 mg l^{-1} of Ca, K, Mg and Na) were prepared by dissolving the appropriate amount of the corresponding salts [Certified ACS grade $CaCl_2{\cdot}2H_2O$, KCl, $Mg(NO_3)_2{\cdot}6H_2O$ and NaCl, respectively; Fisher Scientific] in water. TraceMetal grade HNO_3 (Fisher Scientific) and 99+% mannitol (Aldrich, Milwaukee, WI, USA) were used for the preparation of standard solutions and samples. Nalgene polypropylene calibrated flasks (Nalge, Rochester, NY, USA) and Fisherbrand non-pyrogenic polystyrene pipets (Fisher Scientific) were employed throughout for solution preparation to avoid possible boron contamination. The calibration blank and two working standards of B contained 0.5% m/v mannitol, 0.1 mol l^{-1} HNO_3 and 0.2 mg l^{-1} Be. The low and high standard concentrations of B for the calibration curve were 0.1 and 0.5 mg l^{-1}, separately. All solutions were stored in clean Nalgene polypropylene bottles.

Sample Preparation

Four meat samples, which were bought from a local supermarket, were freeze-dried at a pressure of approximately 3 Pa in a Labconco (Kansas City, MO, USA) freeze-dry system. The dried samples were ground in a Retsch mill (Brinkmann Instruments, Westbury, NY, USA) equipped with a 0.5 mm stainless-steel sieve. Two NIST standard reference materials, SRM1566a (Oyster Tissues) and SRM1577b (Bovine Liver), were dried in a desiccator at room temperature for 120 h over fresh anhydrous magnesium perchlorate.

Table 1 Instrumentation and operating conditions

ICP spectrometer—	
Frequency/MHz	27.12
Forward power/kW	0.65
Reflected frequency/W	<4
Coolant gas/l min^{-1}	10.5

The sample (1 g) was accurately weighed into a 120 ml PTFE vessel to which 10 ml of HNO_3 (70%) were added. For spiking tests, 5 mg of B were also added. The safety valve was placed on the vessel and the cap was tightened in a capping station. Twelve vessels were placed in the turntable and the venting tubes were attached. If the number of vessels was less than 12, additional vessels containing 10 ml of HNO_3 were placed in the turntable so as to maintain a total number of 12 vessels. The samples were heated in the microwave oven following the programs listed in Table 1. After heating, the sample solutions were cooled to room temperature. The vessels were first vented manually, then opened using the capping station. The cap, relief valve and the inside walls of each vessel were washed down with a small volume of de-ionized (DI) water, then 5 ml of hydrogen peroxide (30%) were added to each vessel and the contents were swirled. The 12 uncapped vessels were placed back into the microwave oven and the samples were then digested at 630 W for approximately 30 min until each solution was evaporated to 2–3 ml. Towards the end of the digestion period, the vessels should be observed so as to prevent the solutions from evaporating dryness. Following cooling, the contents were transferred into individual 100 ml Nalgene calibrated flasks followed by the addition of 10 ml of 5% m/v mannitol solution containing 2 mg l^{-1} Be to each flask and dilution to volume with DI water. A reagent blank was also prepared by the same procedure.

Analytical Determination

To obtain accurate results, the background and possible spectral interferences must be corrected. For animal tissues, the most likely spectral interference on the B I 249.773 nm line is from the Fe II 249.782 nm line. To observe the magnitude of this interference, a pure Fe solution was scanned at the analytical wavelength of B (249.773 nm). It was found that 1000 mg l^{-1} of Fe resulted in an apparent B concentration of approximately 0.25 mg l^{-1}. Because the concentration ratio of Fe to B is possibly high (>100) in some tissues, the correction of the above spectral interference should be considered. In this work, a method based on interference coefficients[16] was employed to correct the interference from the Fe II 249.782 nm line. The correction of background interferences was performed *via* the 'off-peak background correction' procedure.[16] Only one point, which was set to the left side of the peak with a wavelength distance of 0.023 nm, was chosen for the background correction to avoid the influence of the Fe II 249.782 nm line. The samples were analyzed without further dilution.

Blanks, standards and samples were aspirated for 40 s before the emission signals were collected. A flush solution, including 0.5% m/v mannitol and 0.1 mol l^{-}

glass wall.[17] If the inference is reasonable, the memory effect should be eliminated or notably reduced when the reaction between B and the walls of glass is avoided.

Mannitol and B can form a very stable boron–mannitol complex,[18] which is the primary reason why mannitol is often used to extract B from soils and other materials or to suppress the volatilization of B from HF or HCl solutions.[18,19] It was expected that the memory effect of B could be significantly reduced in the presence of an appropriate amount of mannitol in the sample solution.

The influences of mannitol on the emission intensity of B under different conditions were observed and the results are shown in Figs. 1–3. The emission signals of B in Figs. 1–3 are expressed as relative intensity. When the desolvating and condensing temperatures are 140 and 5 °C, respectively, the emission intensity of 0.1 mg l^{-1} B in 0.1 mol l^{-1} HNO_3 is defined to be unity.

Fig. 1 displays the variation of emission signal of 0.1 mg l^{-1} B in 0.1 mol l^{-1} HNO_3 with different concentrations of mannitol (COM). It can be seen that the emission of B was markedly intensified with increasing COM and reached a plateau at all values of COM >0.25% m/v. For concentrations of B below 100 mg l^{-1}, this influence remained unchanged. A linear dynamic range of at least four orders of magnitude was obtained with a correlation coefficient of 0.9999 when 0.5% m/v mannitol was present in the solutions. This means that the enhancement is only related to COM, and not to the concentration of B for a given range. Although the emission of B was not further changed when the COM was more than 0.25% m/v, a high COM (>1% m/v) is not recommended, because the particles of mannitol have been observed to block

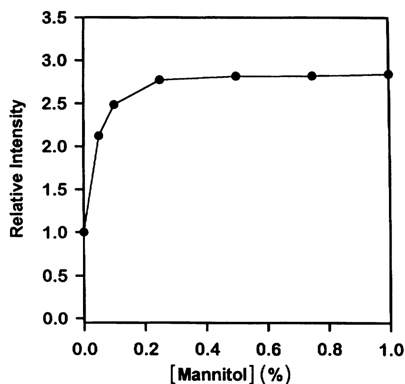

Fig. 1 Influence of the concentration of mannitol on the emission signal of boron.

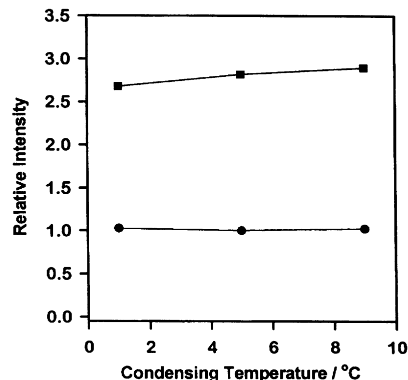

Fig. 3 Influence of mannitol on the emission signal of boron under different condensing temperatures. Desolvating temperature: 140 °C. &, Emission signal of 0.1 mg l^{-1} B with 0.5% m/v mannitol; and $, emission signal of 0.1 mg l^{-1} B without mannitol.

the plasma torch. In this work, 0.5% m/v was chosen as a compromise COM for all other studies and sample analyses.

A similar experiment conducted with a standard Meinhard pneumatic nebulizer showed no enhancement of mannitol on the emission of B. The influences of 0.5% m/v mannitol on 24 other lines, including 11 atomic lines, of 19 elements with the USN were observed and the results are given in Table 2. It can be seen that mannitol caused a depression (7–12%) on most of the atomic lines ('soft lines') and had no influence on the Cu I 324.754 nm, Zn I 213.856 nm and all ionic lines. This indicates that the enhancement effect of mannitol only occurs on B with the USN.

The results in Figs. 2 and Fig. 3 show the enhancement effects of mannitol on B with different desolvating and condensing temperatures. It can be seen that the enhancement was unaffected by different condensing temperatures. On the other hand, the enhancement was slightly intensified with increasing desolvating temperature.

The memory effects of 0.5 mg l^{-1} B in USN-ICP-AES were compared with and without the addition of mannitol and/or with different flush solutions. The experiment was conducted in the following order: first, aspirate the B solution for 2 min; then introduce the flush solution for 5 min, referred to as period I; and finally aspirate 0.1 mol l^{-1} HNO_3–0.5% m/v mannitol solution for at least 10 min, referred to as period II. The emission signal was continuously recorded. The flush solutions chosen for period I were H_2O, 0.1 mol l^{-1} HNO_3 and 0.1 mol l^{-1}–0.5% m/v mannitol, respectively. The results are depicted in Fig. 4, in which the relative intensity was calculated according the assumption that the average emission intensity of 0.1 mg l^{-1} B in 0.1 mol l^{-1} HNO_3 was unity. As

Table 2 Emission intensity ratio (EIR) of 24 lines with and without the addition of 0.5% m/v mannitol (medium: 0.1 mol l^{-1} HNO_3)

Analyte* and wavelength/nm	EIR	Analyte* and wavelength/nm	EIR
Al I 396.152	0.88	Mg I 285.213	0.93
Ba II 455.403	0.99	Mg II 279.553	1.00

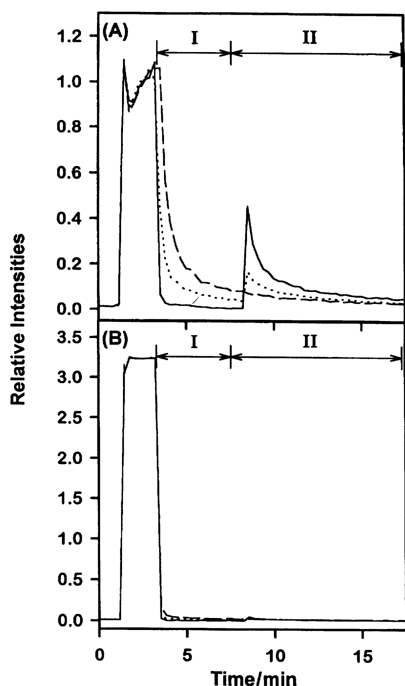

Fig. 4 Memory effects of 0.5 mg l^{-1} B in different media and with different flush solutions. Medium: (A) 0.1 mol l^{-1} HNO_3; and (B) 0.5% m/v mannitol–0.1 mol l^{-1} HNO_3. Flush solution: period I, H_2O (solid line), 0.1 mol l^{-1} HNO_3 (dotted line) and 0.5% m/v mannitol–0.1 mol l^{-1} HNO_3 (broken line); period II, 0.5% m/v mannitol–0.1 mol l^{-1} HNO_3 for all three lines.

can be seen in Fig. 4(A), a boron solution without mannitol resulted in a strong memory effect. Although the emission signal immediately returned to baseline with H_2O as the flush solution, an intense peak immediately appeared and descended slowly when a mannitol solution was introduced following the water flush. The signal did not return to the baseline even after a 5 min flush with dilute HNO_3 (period I) and a reduced peak was observed when the mannitol solution was introduced (period II). The emission signal dropped continuously and slowly when the mannitol solution was used as the flush solution for both periods I and II. In contrast, almost no memory effect was observed when the B solution containing 0.5% m/v mannitol was nebulized, regardless of which flush solution was utilized [Fig. 4(B)]. In addition, it can be seen from Fig. 4 that the precision of boron emission signal was strikingly improved in the presence of mannitol.

The above results indicate that our stated hypothesis, that the memory effect of B comes from the reaction of B with the walls of desolvating and condensing tubes, is reasonable. This reaction not only leads boron to remain in the tubes, but also results in an increase in the transportation resistance of B from the aerosol chamber to the plasma torch. When mannitol is added to the sample solution, a boron–mannitol complex is thought to be formed, which is stable enough to prevent B from binding to the glass walls. As a result, both the memory effect and the transportation resistance are reduced. The latter effect will result in an enhancement of boron sensitivity.

Control of Acid and Matrix Effects with the Use of Internal Standardization

Internal standardization (IS) has been extensively employed to improve analytical precision and accuracy in quantitative analytical emission spectroscopy.[14] Generally, the roles of IS are only considered to reduce the effects of noise and drift on analytical signals and to improve the nebulizer performance in ICP-AES. Usually, IS is not recommended for the correction of matrix effects since these effects are mostly related to the properties of the analytes and matrices.[20,21] However, it is possible to reduce or eliminate the effects of acids and matrices if the internal standard line has similar excitation characteristics to those of the analytical line. In this work, the Be I 234.861 nm line was selected to control the possible acid and matrix effects since Be is very rare in animal tissues, both analytical and internal standard lines are atomic lines, and the excitation potential of the internal standard line (5.28 eV) is close to that of the analytical line (4.96 eV).

The effects on the measurement of B of different concentrations of HNO_3, which was the only digesting acid employed in this work, and of four different concentrations of four matrix elements (Ca, K, Mg and Na at individual concentrations of 200, 400, 600 and 800 mg l^{-1}) were observed with and without IS. The results are presented in Fig. 5 and Table 3. The analytical signal is expressed as relative intensity with the intensity of B I 249.773 nm or the B I 249.773 nm/Be I 234.861 nm intensity ratio without acid (Fig. 5) or matrix (Table 3) being defined as unity.

Fig. 5 shows that a distinct depression of the emission intensity of B can be observed in the absence of IS when the concentration of HNO_3 is greater than 0.05 mol l^{-1}. The magnitude of the depression increases with increasing concentration of HNO_3. On the other hand, when the internal standard line is employed, the analytical signal (B I 249.773 nm/Be I 234.861 nm intensity ratio) remains constant until the concentration of HNO_3 is increased to 0.5 mol l^{-1}, thus, indicating that the analytical signal is more tolerant of HNO_3 with IS than without. At HNO_3 concentrations

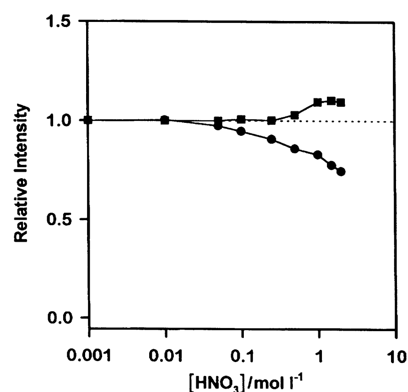

Fig. 5 Effect of HNO_3 on B (0.1 mol l^{-1}) with (&) and without ($) the use of internal standardization.

Table 3 Matrix effects of Ca, K, Mg and Na (200, 400, 600 and 800 mg l^{-1}) on B (0.1 mg l^{-1}) with and without the use of internal standardization (medium: 0.1 mol l^{-1} HNO_3 with 0.5% m/v mannitol)

	B I 249.773 nm			
Matrix/mg l^{-1}	200	400	600	800
—	1.00	1.00	1.00	1.00
Ca	0.93	0.87	0.82	0.76
K	0.93	0.91	0.88	0.83
Mg	0.93	0.88	0.83	0.80
Na	0.91	0.88	0.83	0.82
	B I 249.773 nm/Be I 234.861 nm			
Matrix/mg l^{-1}	200	400	600	800
—	1.00	1.00	1.00	1.00
Ca	1.02	1.02	1.02	0.99
K	1.02	1.01	1.03	1.00
Mg	1.00	0.99	0.98	0.96
Na	1.01	1.02	1.02	1.02

Table 4 Concentration of B in six tissues and recoveries of added B

Sample	Concentration of B without spike*/mg kg^{-1}	B added/	B recovered*/	Recovery*