Spectrophotometric Determination of Boron in Plants Using Monosegmented Continuous Flow Analysis

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A monosegmented continuous flow analysis (MCFA) procedure for the spectrophotometric determination of boron using azomethine-H as colour reagent is presented. Under the experimental conditions described the calibration graph is linear up to 4.00 μg ml^{-1}. The detection limit found is 0.02 μg ml^{-1}, calculated from three times the standard deviation of the blank signal. The common interfering elements present in plants were investigated under dynamic MCFA conditions. As expected, iron and copper present the most severe interferences. This MCFA method for the determination of boron was used with test samples from the Plant Sample Exchange Programme (PSEP) of the Wageningen Agricultural University. The results compared favourably with those obtained by the conventional static procedure and those published by the PSEP.

Keywords: *Monosegmented continuous flow analysis; boron determination; plant analysis*

Boron and its compounds are widely used in industry,[1,2] but its major importance is in relation to agriculture owing to its role in food production. Boron is an essential element to plants.[3] Its function is closely related to the activity of the meristems, the development of cell walls, fruit development and the translocation of sugars.[3–5] As either a deficiency or an excess of boron can cause severe damage to plants, routine control of the boron content in plants (and soil) is highly desirable in order to maintain this element at an adequate level for healthy plant growth.

From the various analytical procedures for the determination of boron, those with spectrophotometric detection are the most commonly used.[2,6] Among the spectrophotometric methods, those reporting the use of azomethine-H[7] as the colour reagent are now more widely accepted than the older methods,[2] either for conventional static procedures[6–11] or under the dynamic conditions of continuous flow analysis,[12–16] such as segmented continuous flow analysis (SCFA, auto-analyser)[12–15] and flow injection analysis (FIA).[16]

As the rate of interaction between boron (as boric acid) and azomethine-H is fairly slow,[7] a higher sensitivity is obtained using the SCFA analyser[12–15] than with FIA[16] because the air segmentation reduces the longitudinal dispersion of the sample along the flow path, favouring sensitivity when slow reactions are employed.

In this paper we report work carried out in our laboratory which demonstrates that the sensitivity of the azomethine-H method for boron can be improved by making use of the recently introduced[17] monosegmented continuous flow analysis (MCFA) approach, which maintains the important characteristics of FIA, such as simplicity, reproducibility and high sample throughput[18,19] while reducing the longitudinal dispersion of slowly developing reactions.

Experimental

Reagents

All chemicals were of analytical-reagent grade and distilled, de-ionised water was used throughout. The water should be distilled from an apparatus made from quartz or other non-boron containing material in order to avoid high blank values. The solutions, including samples and standards, were prepared and stored in high-density polyethylene flasks. The ambient temperature during the experiments was kept at 22 ± 2 °C.

A boron stock solution (1000 μg ml^{-1}) was prepared by dissolving 5.7178 g of boric acid (Carlo Erba) in water and diluting to 1 l. The working solutions of boron and the standards for the calibration graphs were prepared daily by suitable dilution of aliquots taken from the stock solution with 0.10 M HCl.

The azomethine-H reagent was synthesised and purified as previously described.[7,12] Except when stated otherwise the working solution of the reagent was also prepared daily by dissolving 0.60 g of azomethine-H and 2.0 g of ascorbic acid (Merck) in about 40 ml of water with stirring, then diluting to 100 ml.

The buffer-masking reagent was prepared by dissolving 14.0 g of diammonium hydrogen phosphate and 5.0 g of EDTA (as the disodium salt) in about 90 ml of water, adjusting the pH to 8.1 ± 0.2 with a 1 + 2 *V/V* ammonia solution and then diluting to 100 ml.

Stock solutions (1000 or 2000 μg ml^{-1}) of Fe^{3+}, Cu^{2+}, Al^{3+}, Ca^{2+}, Mg^{2+}, Zn^{2+}, Mn^{2+}, Na^{+} and K^{+}, as chlorides, and of NO_3^- and SO_4^{2-}, as potassium salts, were prepared for on-line interference studies. These tests were performed by adding, separately, appropriate aliquots of each of these solutions to 0.50, 1.00 and 2.00 μg ml^{-1} boron standard solutions.

The plant samples used to test the MCFA method for the determination of boron were prepared by ashing 250-mg portions of oven-dried, ground plant tissue in porcelain crucibles for 2 h at 500 °C and then dissolving the residue with 10.0 ml of 0.10 M HCl.[8,16]

Experimental Conditions

The determination of boron was carried out using the MCFA manifold shown in Fig. 1. As can be seen from Fig. 1, the colour reagent, the buffer-masking solution and the sample are brought to a confluence point for mixing in a micro-chamber, M (detailed in M′). This results in a more homogeneous mixture than is obtained by using conventional tee-connectors and/or mixing coils.

The mixture passes into the sampling loop, L_s. On injection, the reaction mixture is inserted between two air bubbles[17] (of variable sizes; in this work $L_1 = L_2 = 30$ μl) and then carried to the delay coil DC (detailed in DC′) by the carrier solution (0.10 M HCl) where the reaction is completed. The air bubbles, introduced during the injection of the sample into the carrier stream, separate the reaction mixture from the carrier solution. They are removed by permeation through a piece of

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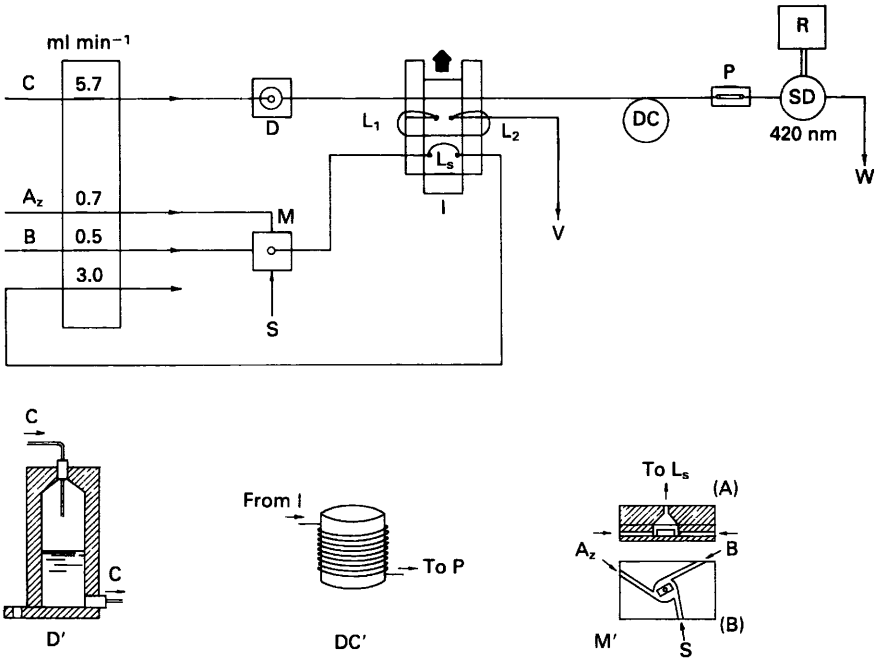

Fig. 1. MCFA arrangement used for the spectrophotometric determination of boron with azomethine-H. C, Carrier (0.10 M HCl); Az, azomethine-H (see text); B, buffer-masking solution (see text); D, pulse damper; M, mixer; S, sample; L_1 and L_2, loops for introduction of air bubbles (30 µl); L_s, injection loop (volume variable, see text); I, injection valve; V, water aspirator; DC, delay (incubation) coil; P, permeation cell; SD, spectrophotometric detector; R, recorder and W, waste. Flow-rates: as indicated. Note that the sample is aspirated into the mixer by suction. Details: D', pulse damper cross-section; for a carrier flow-rate up to 7.0 ml min^{-1} the dimensions are ϕ_i = 7 mm; $h \approx 25$ mm; DC', spatial view of the delay coil (see text); M', mixer cross-section (A) and top view (B); the top and bottom parts were made of acrylic and sealed together with a silicone-based glue; the reagents were mixed with a micro, PTFE coated magnetic stirrer. Internal net volume: 175 µl

about 68 µm thick commercial PTFE tape (Vedarosca, Inconflon Ind. Com. Ltda., São Paulo, Brazil), which acts as a gas - liquid membrane separator.[17,20] When using a carrier flow-rate of 5.7 ml min^{-1} (Fig. 1), as in this work, it is not necessary to evacuate the permeation cell, as is necessary at lower flow-rates.[17] The PTFE membrane should be changed after 6–8 h of use.[21]

Air bubble removal can also be carried out mechanically by sampling the centre of the reaction zone by means of one or two valves operated by a microcomputer[21] or by sequence timers. Such a procedure permits a lower carrier flow-rate and a shorter delay coil, without affecting such parameters as wash time and, by implication, the rate of analysis. In addition, mechanical separation also permits easier adjustment of the sample residence time determined by the delay coil length and carrier flow-rate. However, despite these advantages, we recommend the use of the permeation cell in routine work, owing to its operational simplicity and efficiency under the experimental conditions described here.

It is also desirable to use a pulse damper, D, in the carrier line, described in Fig. 1 (D'). This will decrease the amplitude of the sample pulse in the delay coil, caused by the pumping process and enhanced by the higher compressibility of air compared with liquids. Less pulsing facilitates the de-bubbling procedure using either permeation or mechanical separation.

A high carrier flow-rate may fragment the aliquot of reaction mixture introduced into the delay coil, allowing sample intercontamination and lowering the sensitivity. In this work a carrier flow-rate of 5.7 ml min^{-1} gave reproducible results with a negligible carry-over, even for higher boron concentrations (up to 4.00 µg ml^{-1}). The other liquid flow-rates given in Fig. 1 gave the best results under our MCFA experimental conditions.

All solutions were pumped using a Micronal B 332 peristaltic pump and Tygon pumping tubing (Technicon). The absorption measurements were made using a Zeiss PM2A spectrophotometer at 420 nm[7,16] with a 80-µl Zeiss flow cell (optical path length 10 mm). Polyethylene tubing was used for the loops, as transmission lines and as mixing (i.d. 0.8 mm) and delay (i.d. 2.0 mm) coils. The delay coil (variable length) was supported on a 15-cm diameter cylinder.

The injection valve, a laboratory-made acrylic proportional injector,[22] was operated by means of two solenoids controlled by an electronic sequence timer, programmed to unload the sample loop (L_s) in 10 s after a loading period of 20 s.

As liquids and gases have different compressibilities, it is necessary at the beginning of the working day to fill the delay coil with the monosegmented stream. This is accomplished by injecting blank samples until the delay coil is equilibrated. After equilibrium, reproducible blank signals are obtained.

The sample changes are carried out just after the injection valve is brought to the unloading position. This procedure gives the operator sufficient time to wash the sampler (L_s) with the new sample solution, and to eliminate undesirable small air bubbles which are introduced into the L_s feed line during the sample changes if a switching device[23] is not used during this operation.

It is also desirable to couple a numerical counter to the MCFA injection valve in order to avoid confusion about the number of injections performed per sample, as this tends to be a tedious task in routine work. A very simple and inexpensive digital counter, which makes use of the display and logic board of a low-cost pocket calculator can be used for this purpose.[24]

Results and Discussion

The best MCFA working conditions for boron determination were ascertained by a series of screening experiments. All results are an average of at least three injections, corrected against the blank.

As the MCFA approach differs from FIA in many respects,[17] the influence of the diammonium hydrogen phosphate buffer concentration on the spectrophotometric signal

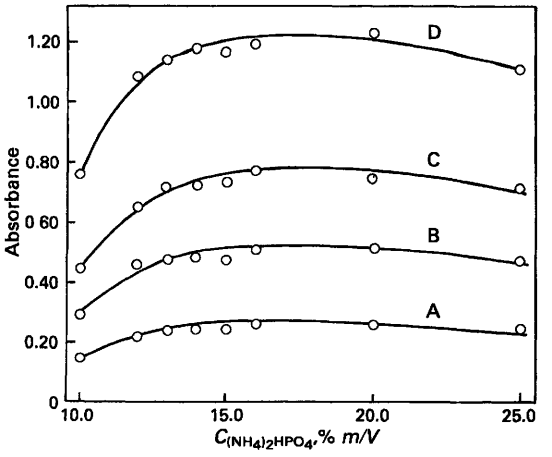

Fig. 2. Effect of the diammonium hydrogen phosphate analytical concentration on the MCFA signal. Boron concentrations: A, 1.00; B, 2.00; C, 3.00; and D, 5.00 μg ml^{-1}. Azomethine-H, 0.60% *m/V* in 2.0% ascorbic acid; residence time; 280 s; L_s = 300 μl. No EDTA added to the buffer. The results are corrected against the blank values

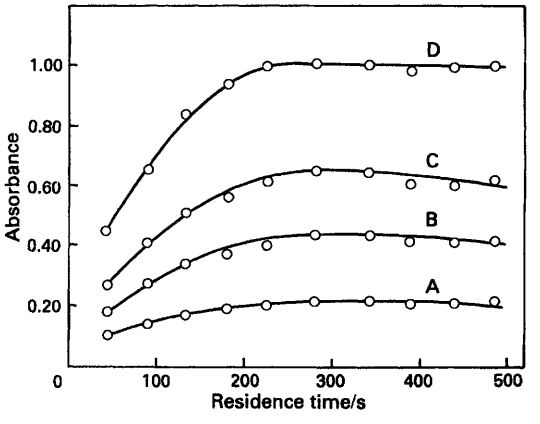

Fig. 3. Variation of absorbance with the sample residence time. Buffer-masking solution, 14.0% *m/V* $(NH_4)_2HPO_4$ in 5% EDTA; buffer pH = 8.1 ± 0.2. Other experimental conditions as in Fig. 2

was established under the MCFA conditions. As shown in Fig. 2, the maximum MCFA signal is observed for $(NH_4)_2HPO_4$ concentrations between 14.0 and 15.0% *m/V*, much lower than the 26.4% *m/V* reported for FIA work.[16] The buffer concentration used throughout this work was 14.0% *m/V*.

As the buffer pH varies from 7.9 to 8.1 only by changing the amount of hydrogen phosphate in solution, the influence of the buffer pH on the MCFA signal was also tested by changing the pH of a 14.0% *m/V* buffer solution from 7.7 to 8.5 using hydrochloric acid or ammonia solution. The MCFA signal is only slightly altered in this pH range, indicating that small variations in the buffer pH are not critical. The buffer pH was fixed at 8.1 ± 0.2 for further experiments. This results in a pH of 6.5 ± 0.2 at the end of the waste line.

Although both buffer concentration and pH are different for MCFA from those reported for the FIA approach,[16] the use of the diammonium hydrogen phosphate buffer proved to be better for the boron - azomethine-H colour development in both MCFA and FIA than the acetate buffer (pH *ca.* 4.8), suggested by Capelle[7] and widely used in SCFA systems. At a fixed pH, variation (to 6% *m/V*) of the EDTA concentration, added to the buffer as masking agent, does not change the MCFA signal.

As shown in Fig. 3, the residence time needed to complete the reaction between boron and azomethine-H under the dynamic MCFA experimental conditions described above was found to be around 280 s. This is consistent with FIA stopped-flow studies,[16] which state that maximum colour

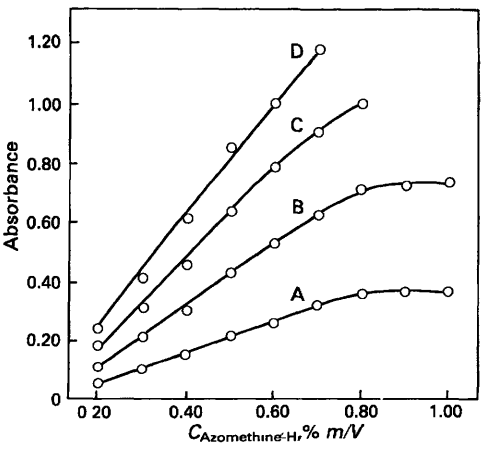

Fig. 4. Variation of the absorbance for various concentrations of azomethine-H in 2.0% ascorbic acid. Buffer-masking solution, 14.0% *m/V* $(NH_4)_2HPO_4$ in 5% EDTA; buffer pH = 8.1 ± 0.2; residence time, 280 s; L_s = 300 μl. Boron concentrations: A, 1.00; B, 2.00; C, 3.00; and D, 4.00 μg ml^{-1}. The results are corrected against the blank values

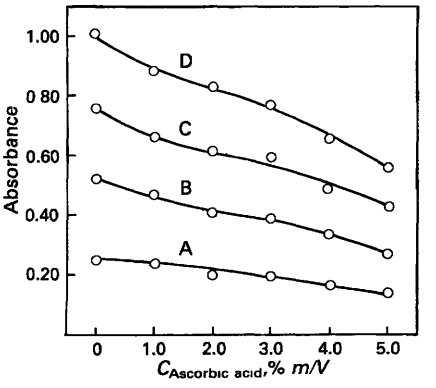

Fig. 5. Influence of the amount of ascorbic acid added to the azomethine-H on the absorbance. Azomethine-H: 0.60% *m/V*. Other experimental conditions as in Fig. 4

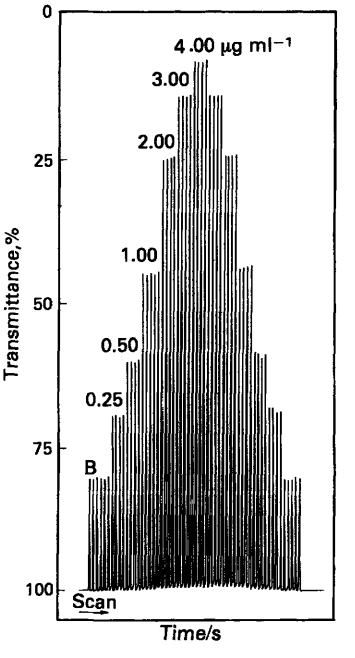

Fig. 6. Typical MCFA calibration graph for boron. Injected volume, L_s = 350 μl; colour reagent, 0.60% *m/V* azomethine-H in 2.0% ascorbic acid. Other experimental conditions as in Fig. 4

Table 1. Interferences in the determination of boron with azomethine-H using monosegmented continuous flow analysis. Results given are the interference factors, where a factor of 1.00 means no interference within ±2%, a factor greater than 1.00 means an enhancement and a factor of less than 1.00 means a depression of the expected value. The results are averages of at least ten injections. The experimental conditions are the same as those used to obtain the calibration run of Fig. 6 The values were corrected against the blank

		Boron/μg ml^{-1}											
		0.50				1.00				2.00			
		EDTA,%											
Interferent	Concentration/μg ml^{-1}	0	1	3	5	0	1	3	5	0	1	3	5
Fe^{3+}	10	6.64	1.30	1.22	1.15	3.43	1.07	1.04	1.00	2.89	1.00	1.00	1.00
	50	8.87	1.46	1.32	1.28	5.01	1.11	1.09	1.07	3.09	1.00	1.00	1.00
	100	10.21	1.62	1.48	1.44	5.61	1.18	1.14	1.12	3.29	1.00	1.00	1.00
Cu^{2+}	5	2.70	1.07	1.00	1.00	1.84	1.03	1.00	1.00	1.40	1.04	1.00	1.00
	15	6.93	1.12	1.00	1.00	4.01	1.04	1.00	1.00	2.37	1.04	1.00	1.00
	30	10.99	1.12	1.00	1.00	5.90	1.04	1.00	1.00	2.54	1.04	1.00	1.00
Al^{3+}	10	1.06	1.00	1.00	1.00	1.06	1.00	1.00	1.00	1.07	1.00	1.00	1.00
	50	1.42	1.00	1.00	1.00	1.40	1.00	1.00	1.00	1.23	1.00	1.00	1.00
	100	1.78	1.00	1.00	1.00	1.77	1.00	1.00	1.00	1.35	1.00	1.00	1.00
Ca^{2+}	100	7.73	1.11	1.00	1.00	(a)	1.10	1.00	1.00	(a)	1.00	1.00	1.00
	500	(a)	(a)	0.92	0.89	(a)	(a)	0.93	0.90	(a)	(a)	0.96	0.95
	1000	(a)	(a)	0.86	0.73	(a)	(a)	0.91	0.80	(a)	(a)	0.89	0.85
Mg^{2+}	100	1.00	1.00	1.00	0.97	1.06	1.00	1.00	1.00	1.23	1.21	1.00	1.00
	250	2.07	1.65	0.94	0.88	(a)	(a)	1.00	0.92	(a)	(a)	0.96	0.95
	500	(a)	(a)	0.92	0.74	(a)	(a)	0.94	0.81	(a)	(a)	0.93	0.88

[a] Signals not recorded owing to precipitate formation.

Table 2. Determination of boron in plant extracts using the MCFA approach Comparative study using plant samples from the Plant Sample Exchange Programme (PSEP)[26]

	This study		Conventional†	PSEP reported data for boron/mg kg^{-1}		
Sample*	B/μg ml^{-1}	B/mg kg^{-1}	B/mg kg^{-1}	Median	Accepted range‡	Observed range¶
569 – Wheat grain	0.05 ± 0.01	2.0 ± 0.4	2.2 ± 0.4	2	0–5	0–10
757 – Spinach	1.00 ± 0.01	40.0 ± 0.4	39.8 ± 0.4	34	27–41	14–42
810 – *Basela alba*	0.87 ± 0.01	34.8 ± 0.4	34.9 ± 0.4	32	22–42	11–42
822 – Carrots	0.64 ± 0.01	25.6 ± 0.4	25.5 ± 0.4	21	14–29	9–29
833 – Mixed pasture	0.25 ± 0.01	10.0 ± 0.4	9.8 ± 0.4	9	6–12	4–24

* Samples of September–October, 1986.
† Azomethine-H conventional static procedure used in routine plant analyses.[8,9] Samples run in triplicate.
‡ Lowest and highest values accepted by PSEP.
¶ Lowest and highest values reported by PSEP.

development is achieved in about 5 min and remains stable for at least 90 min. An 8.5-m delay coil proved to be sufficient to achieve the required colour development with our carrier flow-rate of 5.7 ml min^{-1}.

The relationship between MCFA signal and azomethine-H concentration is shown in Fig. 4. Above 0.80% *m/V*, the variation in absorbance is not significantly affected, at least for lower boron contents. For higher boron contents (above 3.00 μg ml^{-1}) the absorbance values are off-scale.

In the experiments described by Capelle,[7] the azomethine-H solution was prepared with ascorbic acid added as a preservative against oxidation. Under the MCFA conditions, the absorbance values fall when ascorbic acid is present in the azomethine-H solution, as shown in Fig. 5. In addition, no significant variation in the absorbance was observed in the absence of ascorbic acid during 8 h of determinations, using boron standards. However, we have used the conventional 2.0% *m/V* ascorbic acid in the reagent solution as this permits a more reliable comparison between the MCFA and FIA approaches.

Variation of the injection volume between 200 and 500 μl indicates that this parameter does not appreciably influence the measured absorbance, in contrast to FIA determinations, as the reaction mixture and not the analyte sample is injected into the MCFA delay coil. Variation of the injection volume will mainly affect the sampling rate.

A typical graph taken from the calibration run shown in Fig. 6 is linear up to 4.00 μg ml^{-1} ($A = 1.19 \times 10^{-2} + 2.50 \times 10^{-1}$ [B] μg ml^{-1}, where A = absorbance and the correlation coefficient $r = 0.9998$). Variations of the sensitivity may be observed depending on the azomethine-H purity, which may vary from batch to batch, and on reagent storage conditions. The relative standard deviation (RSD) is almost constant over the range tested. From ten replicate determinations the RSD varied from 1.0% at the 0.50 μg ml^{-1} level to 1.4% at the 3.00 μg ml^{-1} level (minimum RSD = 0.9% at 1.00 μg ml^{-1}). The detection limit, calculated from three times the standard deviation of the blank signal,[25] was 0.02 μg ml^{-1}. As the injection cycle is completed in 30 s, the actual sampling rate is 120 h^{-1}, with negligible carry-over.

Based on the early experiments by Capelle,[7] the behaviour of some elements usually present in plants was tested under the MCFA conditions described. These results are summarised in Table 1.

As expected,[7] the most severe interferences are caused by iron (either Fe^{II} or Fe^{III}) and copper. The level of interference from these elements is a function not only of the relative concentration ratios of interferent to boron but also of the actual boron and interferent levels. For example, a concentration ratio of Fe : B of 100 gives an I.F. value of 8.87 for boron and iron levels of 0.50 and 50 μg ml^{-1}, respectively, but an I.F. value of 5.61 for boron and iron levels of 1.00 and 100 μg ml^{-1}, respectively (see Table 1 for definition of I.F. values). This relationship was not observed for aluminium (see Table 1). A similar behaviour can be deduced for the FIA work.[16]

This unusual behaviour could be explained by a reaction mechanism in which these elements act as a catalyst for an azomethine-H condensation reaction in a similar way to that

proposed by Capelle for boron.[7] Hence, the over-all rate of the azomethine-H equilibrium displacement would be a function of the concentration of each chemical species involved in the reaction in addition to a function of the individual catalysing efficiency of each interferent. Further investigation is necessary to confirm this hypothesis, as the actual chemical system is rather complex.

On the other hand, Na^+ (2000), K^+ (1000), Mn^{2+} (50), Zn^{2+} (30), NO_3^- (1000) and SO_4^{2-} (1000) do not interfere within ±2% for any of the boron concentrations tested. The numbers in parentheses indicate the maximum interferent concentration tested in μg ml^{-1}. Ca^{2+} and Mg^{2+} do not interfere up to 100 μg ml^{-1} if the buffer contains at least 3% *m/V* of EDTA. Higher Ca^{2+} and Mg^{2+} concentrations may interfere with the boron determination either by precipitate formation, when the EDTA concentration is lower than 3% *m/V* (noisy signal), or by complex formation, when the EDTA concentration is equal to or higher than 3% *m/V* (I.F. <1.00).

Considering the results and limits shown in Table 1, the addition of 5% *m/V* of EDTA in the buffer is sufficient to eliminate the most common interfering elements for boron determination in plant tissues.

The MCFA procedure for the determination of boron was also tested using real plant samples from the Plant Sample Exchange Programme (PSEP) of Wageningen Agricultural University. These samples were used for intercalibration studies among 56 laboratories.

Each sample was divided into three 250-mg portions prior to the treatment described under Experimental. The resulting solutions were injected into the MCFA system in quintuplicate, performing a total of 15 injections per sample. The final results are presented in Table 2 as the average value obtained from conventional data treatment of these measurements. These averages are compared with the results from the conventional static azomethine-H procedure[8,9] and with the data reported from the PSEP.

The results obtained from our MCFA dynamic conditions are in excellent agreement with those found using the static azomethine-H method. By direct comparison, our results are equal to or better than the PSEP reported median, but all of them are in the accepted range, according to the marking procedure.[26] Sample 757, which has the highest absolute deviation from the median, also has a high content of other elements (1078, 12 and 1390 mg kg^{-1} for iron, copper and aluminium, respectively). Hence, in samples with known high contents of interfering elements, especially iron, it is necessary to decrease the interferent concentrations to acceptable levels prior to the determination of boron. Alternatively, a higher EDTA concentration in the buffer could be tested as the MCFA signal appears to be constant over a large EDTA range.

This MCFA procedure for the determination of boron is now in use for routine plant analyses at the Instituto Agronômico de Campinas, using the experimental conditions cited in the legends of Figs. 1 and 6. Studies are presently underway to extend this procedure to soil analysis.

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References

1. Braman, R. S., "Boron Compounds," *in* Snell, F. D., and Hilton, C. L., *Editors*, "Encyclopedia of Industrial Chemical Analysis," Wiley-Interscience, New York, 1968, p. 329.
2. Braman, R. S., "Boron," *in* Kolthoff, I. M., and Elving, P. J., *Editors*, "Treatise on Analytical Chemistry," Part 2, Volume 10, Wiley, New York, 1978, p. 1.
3. Bradford, G. R., "Boron," *in* Chapman, H. D., *Editor*, "Diagnostic Criteria for Plants and Soils," Quality Printing, Abilene, TX, 1965, p. 33.
4. Cohen, M. S., and Lepper, R., Jr., *Plant Physiol.*, 1977, **59**, 884.
5. Shorrocks, V. M., "Boron Deficiency: Its Prevention and Cure," Technical Service and Development Department, Borax Consolidated Limited.
6. Binghan, F. T., "Boron," *in* Page, A. L., Miller, R. H., and Keeney, D. R., *Editors*, "Methods for Soil Analysis: Chemical and Microbiological Properties," Part 2, Agronomy Monograph No. 9, Second Edition, 1982, p. 431.
7. Capelle, R., *Anal. Chim. Acta*, 1961, **24**, 555.
8. Bataglia, O. C., Furlani, A. M. C., Teixeira, J. P. F., Furlani, P. R., and Gallo, J. R., "Métodos de Análise Química de Plantas," IAC Technical Bulletin No. 78, Serviço de Divulgação Tĕcnico-Crentífica do Instituto Agronômico de Campinas, Campinas, 1983, p. 30.
9. John, M. K., Chuak, H. H., and Neufeld, J. H., *Anal. Lett.*, 1975, **8**, 559.
10. Lohse, G., *Commun. Soil Sci. Plant Anal.*, 1982, **13**, 127.
11. Gaines, T. P., and Mitchell, G. A., *Commun. Soil Sci. Plant Anal.*, 1979, **10**, 1099.
12. Basson, W. D., Böhmer, R. G., and Stanton, D. A., *Analyst*, 1969, **94**, 1135.
13. Basson, W. D., Pille, P. P., and du Preez, A. L., *Analyst*, 1974, **99**, 168.
14. Edwards, R. A., *Analyst*, 1980, **105**, 139.
15. Porter, S. R., Spindler, S. C., and Widdowson, A. E., *Commun. Soil Sci. Plant Anal.*, 1981, **12**, 461.
16. Krug, F. J., Mortatti, J., Pessenda, L. C. R., Zagatto, E. A. G., and Bergamin, F. H., *Anal. Chim. Acta*, 1981, **125**, 29.
17. Pasquini, C., and Oliveira, W. A., *Anal. Chem.*, 1985, **57**, 2575.
18. Růžička, J., and Hansen, E. H., "Flow Injection Analysis," Wiley, New York, 1981.
19. Valcarcel Cases, M., and Luque de Castro, M. D., "Analisis por Inyeccion en Flujo," Universidad de Cordoba, Cordoba, 1984.
20. de Andrade, J. C., Pasquini, C., Baccan, N., and Van Loon, J. C., *Spectrochim. Acta, Part B*, 1983, **38**, 1329.
21. Pasquini, C., *Anal. Chem.*, 1986, **58**, 2346.
22. Reis, B. F., Zagatto, E. A. G., Jacintho, A. O., Krug, F. J., and Bergamin, F. H., *Anal. Chim. Acta*, 1980, **119**, 305.
23. de Andrade, J. C., Rocha, J. C., and Baccan, N., *Analyst*, 1985, **110**, 197.
24. de Andrade, J. C., and Loboschi, E. M., *Analyst*, 1988, **113**, 295.
25. Nomenclature, Symbols, Units and Their Usage in Spectrochemical Analysis—II. Data Interpretation, *Spectrochim. Acta, Part B*, 1978, **33**, 245.
26. van Montfort, M. A. J., and Houba, V. J. G., "A Statistical Contribution to the Plant Sample Exchange Programme: Marking Data," Plant Sampling Exchange Programme, Wageningen Agricultural University, 1986.

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