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On-line generation and hydrolysis of methyl borate for the spectrophotometric determination of boron in soil and plants with azomethine-H

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Abstract

A continuous-flow system for boron determination in soils and plants with spectrophotometric detection using the azometihine-H-boron complex method was developed. In order to avoid the interferences of concomitants present in samples and to increase the sensitivity, the element was separated on-line from the matrix by methyl borate generation. For this purpose, a concentrated sulfuric acid sample solution was combined with methanol in 1:3 ratio which produce enough heating for the esterification reaction without external source. Subsequently, the methyl borate produced was stripped by the addition of a nitrogen flow and separated from the bulk solution in a gas–liquid separator to be then hydrolyzed in an ammonium-phosphate buffer solution (pH 6.8). Finally, the new bulk of phases were separated in a second gas–liquid separator and the liquid phase was combined with azomethine-H to form a boron complex for its detection at 420 nm. The effects of a number of possible interferents, both anionic and cationic were evaluated. The most severe depressions were caused by fluoride and potassium for which a concentration of 100 $\mu g\,ml^{-1}$ caused a 5% depression on the signal. A linear response was obtained between the detection limit of 0.05 $\mu g\,ml^{-1}$ (3σ of the blank) and 50 $\mu g\,ml^{-1}$ of boron. The precision (R.S.D.%) for 10 consecutive readings of the same solution (5.0 $\mu g\,ml^{-1}$ of boron) was 2.6%. Recoveries of boron added to the samples before the extraction process were 94, 97, and 101% for soil, fruit tissue, and leaf tissue, respectively. The developed system was applied to the determination of boron in soil, fruits tissue, and leaves tissue of coffee plantations from different towns of Mérida State, Venezuela.

Keywords: Boron determination; Spectrophotometry; Azomethine-H; Methyl borate vapor generation; Soil and plants analysis

1. Introduction

Boron is a naturally occurring element that has been recognized as an essential element for higher plants early in the present century. It is one of the seven essential micronutrients, or trace elements and is; therefore, extremely important in the production of commercial crop plants [1]. Boron is an inevitable component of all animal tissues though there is no conclusive evidence that it performs any essential function in human and animal nutrition [2]. However, during the last two decades it has been accumulating a large circumstantial evidence which strongly indicates that boron is probably an essential micro-nutrient for higher animals and human [3,4]. Deficiency of this element in plants causes the growth center to die, the roots develop slowly and others symptoms depending on the severity of deficiency [5]; while its excess is toxic for both plants [6] and animals [7]. Boron toxicity symptoms may vary from necrosis of some plants organs to death of the whole plant depending on the extent and severity of the toxicity. Therefore, the establishing of the status of boron in plant and plant-available boron in soil it is of high interest for predicting deficiencies as well as toxicities in a wide selection of crops worldwide.

The determination of boron in soil and plants may be realized by a diversity of analytical methods which have improved with advances in analytical instrumentation [8–43]. Boron concentration has been determined utilizing flame atomic absorption and emission spectroscopy (AAS and AES) [8–10], atomic absorption spectrometry with electrothermal atomization (ETAAS) [8,11,12], neutron activation analysis (NAA) [13–16], inductively coupled plasma (ICP) AES [17–19]

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and mass spectrometry (MS) [20–25], and spectrophotometry [28–43].

The element determination by AAS and AES methods generally require separation of boron from the sample matrix for getting adequate results [8], have serious memory effects, interferences, calibration drift, background noise [9] and their sensitivity is often poor for many applications [10]. The ETAAS method is one of the most difficult assignments for boron determinations because this element forms compounds of high thermal stability (oxides, nitrides and carbides) during the analysis [11]. For that reason this method has severe memory effects, poor detection limit and sensitivity. One way of overcoming these difficulties and improving the performance of ETAAS is using chemical modifiers [8,11,12].

NAA involves various methods such as neutron activation mass spectrometry (NA-MS) [13], neutron capture radiography (also called α-track etching) [14], neutron depth profiling (NDP) [15] and prompt gamma spectroscopy [14,16] being this last the most extensively used of all. In spite of fact that NAA methods are nondestructive with ability of managing solid samples and multielemental detection; they demand access to a nuclear reactor and are time-consuming what make them not practical with a mere academic significance due to their non sensitive detectability for the determination of boron.

Many existent limitations about boron determinations were ameliorated with the introduction of plasma sources (e.g. inductively coupled plasma, ICP) coupled with a very sensitive detection technique such as MS and AES which result in powerful methodologies such as: ICP-MS and ICP-AES. Some methodological developments for boron determinations employing ICP-AES have been applied in plants [17] and in soil analysis [18]. The major limitation for the boron determination by ICP-AES in plants and particularly in soils matrix with high content of iron, is the fact that iron has emission lines at 249.77, 249.65, and 249.70 nm, which produce spectral interferences for the more sensitive lines of born at 249.77 and 249.67 nm [19]. In the course of the last years ICP-MS has showed an increasing interest of many researchers on boron determination in a diversity of materials with special interest in biological samples [20–22]. The advantages of ICP-MS over other methods are higher sensitivity, lower detection limits and simultaneous measurements of boron concentrations and boron isotope ratios (^{11}B to ^{10}B). However, one requirement for the application of these methods is that the samples must be in a disintegrated state because even small particles clog the capillary tube going to the nebulizer [22]. Also, a cautious pretreatment of samples is necessary for the removal of most matrix components; these include the electrothermal vaporization for the analyte introduction to the plasma [23], the evaporation of some of the matrix components by treatment with a mixture of HCl and KF [24] or the use of less sensitive boron lines, which obviously will deteriorate the sensitivity [22]. Measuring boron at ultratrace levels by ICP-MS also is plagued by serious memory effects which could be minimized by diluting the samples with mannitol + ammonia [20], the introduction of ammonia solution simultaneously with the sample just before the nebulizer [21] or by injection of ammonia gas into the spray chamber during analysis [25]. Internal standardization is also necessary in ICP-MS to control drift and signal fluctuations [20]. Additionally, these methods are disadvantageous since the instrumentation is expensive and dedicating such instrumentation to routine boron analysis in soil and plants is not economically feasible.

Spectrophotometry is essentially a trace analytical technique and is one of the more powerful tools in chemical analysis with an instrumentation of very low cost of acquisition and operation, as well as worldwide availability. A wide variety of reagents which form colored or fluorescent complex with boron have been proposed for the spectrophotometric determination of the element [26–44]. Some of the most utilized reagents include curcumin [28], methylene blue [29], quinalizarine [30], crystal violet [31], chromotropic acid [32], carminic acid [33,46] and azomethine-H [34–43]. Among them, the last one is the most commonly used spectrophotometric method for boron determination. This method is more reliable, fast, simple, sensitive and convenient than other colorimetric methods used for boron determination in soil and plants. Additionally, this method does not require concentrated acids, which make it desirable for automation. The main drawback of the azomethine-H method for the determination of boron in soil and plants is the interferences due to the presence of several species including Al, Cu, Fe, Zn, Ni, and Co [26,27]. Color of the sample (especially in soil extracts) and high Fe levels may cause severe interference and a wide variability in absorbance readings [34]. The presence of iron increases the analytical signal of the azomethine-H. Iron and other species interferences may be suppressed by the addition of ADTA [35–38], EDTA + thioglycolic acid [39,40], EDTA + mannitol [41], EDTA + nitriloacetic acid [42] and polyphosphate ion + thiourea + ascorbic acid [43]; however, the use of these chelating agents reduce the sensitivity of the azomethine-H method. These interferences and loss of sensitivity limit the application of the azomethine-H method to soil and plants samples with low boron concentrations and complex matrices. In these cases, the use of a prior boron separation appears to be necessary in order to isolate it from the remainder of the sample and to obtain reliable values.

Generation of gaseous methyl borate has been used for the separation of boron from various sample matrixes [27,39,44–51]. Boron is converted into the volatile methyl borate, $B(OCH_3)_3$, by reaction of borate with methanol in concentrated sulfuric acid. Methyl borate generation conditions were first established by Sthal [45], who obtained the optimum methanol/sulfuric acid volume ratio and best heating temperature; this author also indicated the necessity of limiting the amount of water present in the process in order to avoid methyl borate hydrolysis. However, the exothermic reaction between methanol and sulfuric acid generates enough heat to rise the temperature of the reaction mixture allowing distillation of methyl borated without using additional heating [39,44,46,48,50,51]. The process may be performed as a manual distillation [39,46–49] or an on-line continuous-flow technique [44,50,51]. The generation of volatile methyl borate has been applied to the determination of boron in combination with AAS [48], AES [46], ICP-AES [44,50,51], ICP-MS [47] and spectrophotometric methods [39,49]. But, to our best knowledge,

only one work on spectrophotometric determination of born with azomethine-H, involving the prior distillation and hydrolysis of methyl borate has been published [39]. In this case, a "batch" procedure was developed for the determination of boron in water samples. However, to perform this, a considerable manipulation is necessary with risk of contamination and loss of analyte.

In the present study, a continuous-flow system for boron determination in soil and plants by spectrophotometric analysis using azomethine-H, after on-line generation, distillation and hydrolysis of methyl borate, was developed.

2. Experimental

2.1. Instrumentation

Determinations were performed with a Varian 634 spectrometer with a quartz flow cell (Starna Cells Inc., USA) of 3 mm i.d., 10 mm length and 70 μL capacity. Besides, it was employed an analytical mill (Tekmar, model A-10) and an 80 mesh sieve during the pretreatment process of samples. The manifold, shown schematically in Fig. 1 was constructed from 0.8 mm i.d. PTFE tubing, and different pump tubing (Cole-Parmer) including: Viton orange/yellow tubing for the delivery of H_2SO_4-containing samples, silicon orange/orange tubing for the delivery of methanol, tygon green/yellow tubing for azomethine-H and tygon orange/white tubing for delivery of buffer, and hydrolyzed solutions. Two home-made gas–liquid separators showed schematically in Fig. 3 were used. Two programmable peristaltic pumps (GILSON Minipuls-3, 8-roller) with the option of remote control were used for the propulsion of the reagents. The flows of reagents were regulated varying the pump head rotation speed and different internal diameter tygon pump tubing (Cole-Parmer). Home-made software (Windows platform, PC compatible) was developed to control the pumps and valves and other necessary devices. An R232/RS485 card, incorporated in a Pentium I processor PC, was used to interface the periphery devices.

2.2. Reagents

Unless stated otherwise, all solutions were prepared from analytical-reagents grade chemicals in doubly deionized water of 18 MΩ cm specific resistivity obtained in a Milli-Q system (Millipore, Bedford, MA, USA) and kept in polyethylene flasks. A stock boron solution (1000 μg/ml) was prepared by dissolving 0.5760 g of boric acid (Merck) in 100 ml of concentrated H_2SO_4 (Riedel-de Haën). Working solutions were prepared by suitable dilution from it with concentrated H_2SO_4. The derivatizing reagent was prepared by dissolving 0.70 g of azomethine-H, monosodium salt (Sigma) and 2.00 g of ascorbic acid (Mallinckodt) in 100 ml of water. A buffer solution was prepared by dissolving 57.50 g of $NH_4H_2PO_4$ (Fisher Scientific) and 132.00 g of $(NH_4)_2HPO_4$ (Fisher Scientific) in water to give 750 ml of solution. Also, there was used methanol, HPLC grade (J.T. Baker) and nitric acid (Riedel-de Haën).

All containers were thoroughly rinsed with tap water before being soaked for 24 h in a 2% (v/v) Extran MA 03 cleaner from Merck, rinsed with water, kept overnight in 1.6 mol l^{-1} nitric acid and finally rinsed several times with Milli-Q water before using.

2.3. Sample preparation

The coffee plant tissue samples (fruits and leaves) were dried in an oven at 70 °C and then powdered with an analytical mill. Plant tissue samples were treated by a hot 1 mol l^{-1} nitric acid extraction procedure of boron according to Al-Ammar et al. [52]. A 2 g sample aliquot was heated with 15 ml of 1 mol l^{-1} nitric acid in a sealed Teflon PFA microwave vessel (CEM, 120 ml) at 80 °C in a drying oven for 1 h. After the sample-acid mixture was cooled to room temperature; it was filtered, then evaporated over a hot plate at around 50 °C to dryness, and finally diluted to 25 ml with concentrated sulfuric acid before measurement.

The soils were selected from the root of each coffee tree. These samples were air dried for 5 days and crushed to pass through an 80 mesh sieve. Boron was extracted from soil samples with 0.05 mol l^{-1} HCl, which works well for predicting boron availability to plants in soil [53]. For that, 10 g of soil samples were placed in 200 ml polyethylene centrifuge tubes and shaken for 30 min with 25 ml of 0.05 mol l^{-1} HCl. The sample–acid mixture was filtered, then evaporated over a hot plate at around 50 °C to dryness, and finally diluted to 25 ml with concentrated sulfuric acid before measurement.

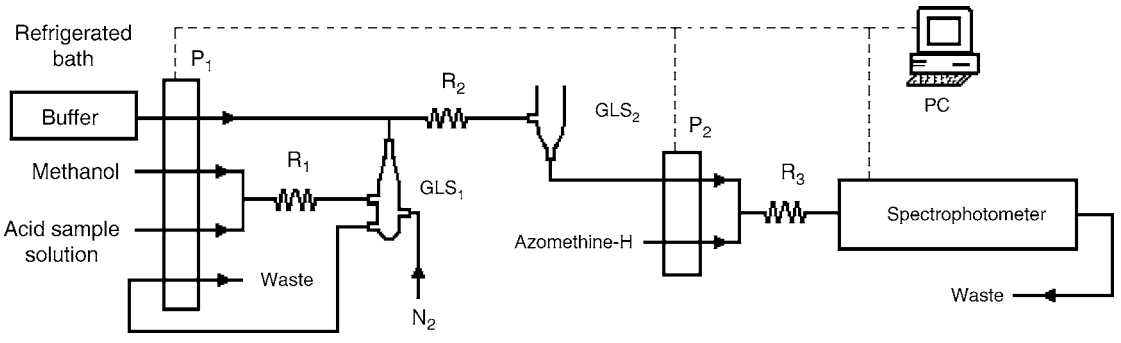

Fig. 1. Schematic diagram of the continuous-flow system and the instrumentation set up for the spectrophotometric determination of boron with on-line generation, distillation and hydrolysis of methyl borate. $P_{1,2}$, peristaltic pumps; $R_{1–3}$, reaction coils; $GLS_{1,2}$, gas–liquid separators; PC, computer; N_2, nitrogen.

Table 1
Optimal operation conditions

Spectrophotometer	
Wavelength (nm)	240
Signal measurement	Absorbance
On line methyl borate generation and hydrolysis	
H_2SO_4 concentration in sample (mol l^{-1})	18
Sample flow rate (ml min^{-1})	1
Methanol concentration	Absolute
Methanol flow rate (ml min^{-1})	3
Carrier gas flow (nitrogen) (ml min^{-1})	20
Ammonium-phosphate buffer concentration (mol l^{-1})	2 (pH 6.8)
Ammonium-phosphate buffer flow rate (ml min^{-1})	1.3
Ammonium-phosphate buffer temperature (°C)	3
Esterification reaction coil, R_1 (cm)	20
Hydrolysis reaction coil, R_2 (cm)	110
Spectrophotometric boron determination	
Azomethine-H concentration (%)	0.75 (m/v)
Azomethine-H flow rate (ml min^{-1})	0.5
Color developing reaction coil, R_3 (cm)	400
Drains from GLS_2 (analyte solution) flow rate (ml min^{-1})	1.5

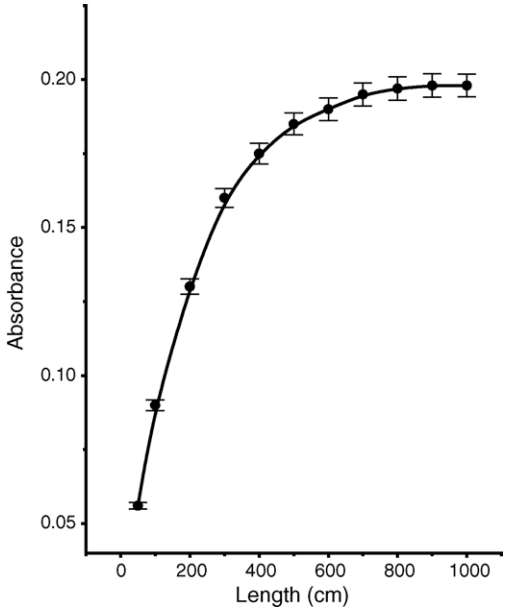

Fig. 2. Effect of the reaction coil length (R_3, Fig. 1) on the absorbance signal for 5.0 μg ml^{-1} of boron. The error bars are the standard deviations for five replicate measurements.

2.4. Procedure

All of the experiments were carried out using the manifold shown in Fig. 1 under the optimal operation conditions shown in Table 1. Initially, ammonium-phosphate buffer solution (pH 6.8) and azomethine-H solutions were pumped continuously throughout the process to ensure a stable baseline. The concentrated sulfuric acid sample solution and methanol were pumped and combined in the reaction coil 1 (R_1) for ester methyl borate generation. The exothermic reaction between methanol and sulfuric acid generates enough heat to rise the temperature of the reaction mixture approximately up to 65 °C, allowing distillation of methyl borated without using additional heating. The bulk phases were separated in a first gas–liquid separator (GLS_1), and methyl borated was transported to the buffer solution stream with the aid of a nitrogen flow. Gaseous methyl borate was hydrolyzed in the reaction coil 2 (R_2) and the new bulk phases were separated in a second gas–liquid separator (GLS_2). The eluent solution was combined with azomethine-H in a third reaction coil (R_3) and the absorbance signals of the resulting complex were monitored at 420 nm.

3. Results and discussion

3.1. Preliminary studies

Preliminary studies were necessary in order to establish the optimal conditions for azomethine-H-boron complex formation. In a previous work [35], we have proved that the color of the azomethine-H-boron complex is highly pH sensitive, especially in the 6.4–7.0 range. Therefore, a 2 mol l^{-1} ammonium-phosphate buffer solution (pH 6.8) was selected in further experiments. This buffer has a high buffering capacity, it is not corrosive and the preparation is simple, without creating offensive fumes. In order to study the kinetics of the color-formation reaction a simple two lines flow system was constructed to simulate the final part of the manifold shown in Fig. 1. For that, the length of the reaction coil, R_3, was varied within the range 50–1000 cm, while 5 μg ml^{-1} boron standard solution containing 2 mol l^{-1} ammonium-phosphate buffer (pH 6.8) was pumped at 1.5 ml min^{-1} and combined with 0.75% (m/v) azomethine-H solution at 0.5 ml min^{-1}. As shown in Fig. 2, the signal rapidly increased as the length of the coil increased to 400 cm (about 60 s), thereafter, a slightly and stable increase in the signal was observed as the length of the reaction coil increased up to 700 cm (about 105 s). Then, the signal keeps approximately constant as the length of the reaction coil increased up to 1000 cm (about 150 s). In flow systems the time is a very critical factor, because reagents consumption, however, these systems offer high reproducibility avoiding the need of reaching the equilibrium for most reactions. Therefore, the reaction coil length (R_3, Fig. 1) was then defined as 400 cm long, which corresponds to 85% of the reaction completion.

The influence of the azomethine-H concentration ranging from 0.25 to 1.50% (m/v) in color formation was also investigated. The absorbance values increase with an increase in the concentration of azomethine-H. However, concentrations of it higher that 0.75% cause larger reagents blanks without significant increase in net absorbance values. Thus, a 0.75% (m/v) azomethine-H concentration was chosen, meaning a consumption of about 3.75 mg of the reagent per determination. This represents a significant increase in the reagent consumption compared with our previous work (about 0.3 mg) [35], but it is comparable with reagent consumption in other automatic methods [37,38].

3.2. Generation and hydrolysis of methyl borate

Since the optimum ratio of methanol-to-sulfuric acid for esterification reaction is reported to be around 3:1 [39,44,46,48]; the delivery tubes in the system were chosen accordingly. The flow of the acid containing sample solution was fixed in 1 ml min^{-1} and thus the flow of methanol was fixed 3 ml min^{-1}. The amount of water present in the process must be limited in order to avoid losses of methyl borate by hydrolysis of it [39,46]. Therefore, the samples and calibration standards solutions were prepared in concentrated sulfuric acid.

In order to achieve the on line generation and hydrolysis of methyl borate the following parameters were optimized: The length of the esterification reaction coil, R_1, the gas–liquid separator 1 (GLS_1) volume, the carrier gas flow rate, the flow rate and type of hydrolysis solution, the length of the hydrolysis reaction coil, R_2, and, the gas–liquid separator 2 (GLS_2) volume. The figures of merit for the optimization process ware maximum net absorbance (i.e. blank subtracted) and reproducibility. A 5 μg ml^{-1} boron standard solution was used for the optimization process.

The length of the esterification reaction coil, R_1, was varied between 5 and 50 cm. The results shown that the signal increased constantly as the length increased to 15 cm, it keeps approximately constant between 15 and 25 cm, and finally slightly decreased for longer reaction coils. The optimum length for R_1, was fixed in 20 cm long, which is a relatively short length and represents a reaction time of approximately 0.015 s. This confirms that the esterification reaction it is very fast and that the ester methyl borate is produced almost instantaneously.

The gas–liquid separator, GLS_1, (Fig. 3a) was a device designed and constructed in-house, four different sizes of it, namely, 3.0, 6.0, 9.0, and 12.0 ml, were tasted for methyl borated separation. The drains from all these devices were pumped. Lower and irreproducible signals (30% of maximum absorbance and R.S.D.% of 40%) were obtained with the smaller one. This is due to an inefficient separation of the phases and the carry-over of sulfuric acid, which produces an appreciable diminution in the pH of the buffer solution and even the total loss of the buffering capacity. The 6.0 ml device, produce better results in terms of sensitivity (65% of maximum absorbance) but low reproducibility was still observed (R.S.D.% of 15%). The 9.0 ml device produced the highest sensitivity and a very good precision (R.S.D.% of 3%). Excellent reproducibility (R.S.D.% of 2.5%) with a small reduction in the sensitivity (90% of maximum absorbance) was observed for the bigger device, probably due to the dilution of methyl borate by nitrogen used as carrier. The 9.0 ml capacity device produced the best results and was therefore used throughout this work.

The effect of the carrier gas flow rate is shown in Fig. 4. When no carrier gas was used, the buffer solution goes inside of the GLS_1, indicating that the pressure of the vapor phase was not sufficient for transport it into the buffer stream. A positive flow of vapor phase into the buffer stream only was possible when nitrogen flow rates were higher than 10 ml min^{-1}. Therefore, the optimum value was obtained by varying the nitrogen flow rate between 10 and 50 ml min^{-1}. When the nitrogen flow was increased from 10 to 20 ml min^{-1}, an increase in the signal was observed. The signal reached a plateau within the range 20–30 ml min^{-1}. Thereafter, the signal notably decreased as the flow rate increased, which could be due to excessive dilution. Additionally, very pour reproducibility (R.S.D.% of 20%) was observed at higher flow rates, as consequence of inefficient separation of the new bulk of phases in the GLS_2. A flow rate of 25 ml min^{-1} was chosen as optimal.

The effect of the flow rate and type of hydrolysis solution was investigated. Preliminary experiments using water as simple hydrolysis media were under taken. Water proved to be an

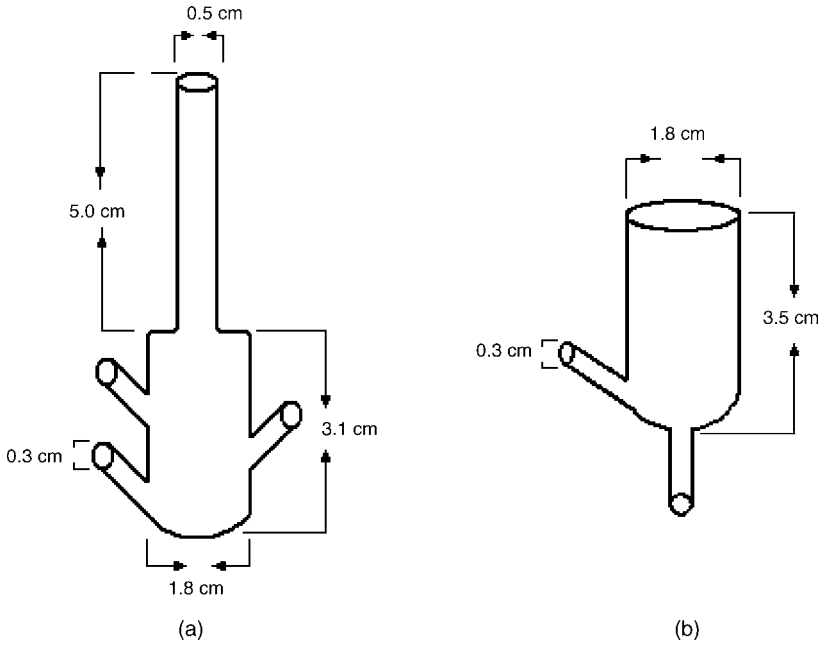

Fig. 3. Dimensions and shape of the gas–liquid separators used: (a) GLS_1 and (b) GLS_2.

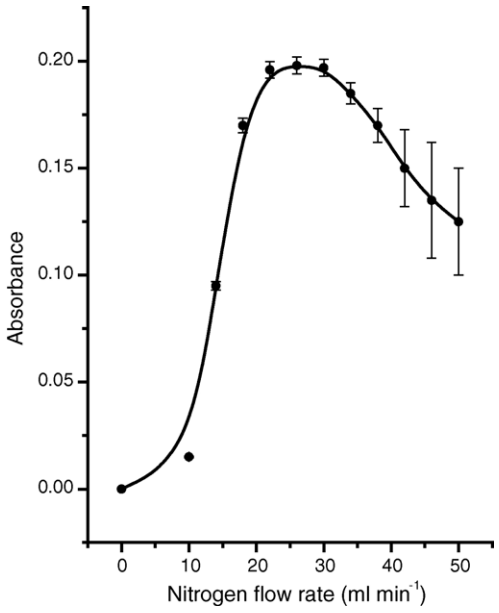

Fig. 4. Effect of the carrier gas (nitrogen) on the absorbance signal for 5.0 μg ml^{-1} of boron. The error bars are the standard deviations for five replicate measurements.

appropriated media to carryout the methyl borate hydrolysis, however, an additional channel for the buffer solution has to be added to the flow system. In order to avoid the analyte dilution and to achieve the maximum sensitivity, the buffer solution was tested as hydrolysis media. The 2 mol l^{-1} ammonium-phosphate buffer solution (pH 6.8) also proved to be an excellent media for methyl borate hydrolysis. The optimum value of the buffer flow rate was obtained by varying it between 0.5 and 2.5 ml min^{-1}. Buffer flow rates lower than 0.8 ml min^{-1} resulted to be inappropriate in terms of sensitivity and reproducibility (less than 80% of maximum absorbance and R.S.D.% higher than 30%). The best signal was obtained when the buffer flow rate was increased up to 1.3 ml min^{-1}, thereafter, the signal constantly decreased (about 65% of maximum absorbance) as the flow rate increased up to 2.5 ml min^{-1}. Additionally, the temperature of the buffer solution must be controlled. The methyl borate is distilled at 55 °C in a constant boiling mixture with methanol, containing 1 molecule of ester to 7.6 molecules of alcohol [44]. When the buffer solution was used at room temperature, the incomplete liquefaction of the methanol resulted in appreciable losses of analyte. Using the buffer solution at 3 °C during the analysis solved this difficulty. However, the liquefaction of the methanol increases the volume of the liquid phase that reaches the GLS_2. In order to avoid accumulation of analyte solution in the GLS_2 the flow rate of the drains of it was fixed somewhat higher than buffer flow rate. Finally, the optimum flow rates values for buffer and drains were fixed at 1.3 and 1.5 ml min^{-1}, respectively.

The length of the hydrolysis reaction coil, R_2, was varied between 20 and 200 cm. Reaction coils shorter than 80 cm produced unsatisfactory results, low signal with bad precision were obtained (less than 85% of maximum absorbance and R.S.D.% higher than 25%). Maximum and stable signal was obtained for coil lengths larger than 100 cm. In order to achieve reliable results and to minimize the time of the analysis a 110 cm long hydrolysis reaction coil was selected for further experiments. Finally, the liquid phase which contain the analyte was separated from the bulk phases in the gas–liquid separator, GLS_2, (Fig. 3b). The GLS_2 was a device designed and constructed in-house; the dimensions and shape of it were chosen accordingly to guarantee the appropriated separation without losses of analyte.

3.3. Effect of interferences

The tolerance of the system to interferences was evaluated by investigating the effect of a number of possible interferents, both anionic and cationic: Li^+, Na^+, K^+, NH_4^+, Cu^{2+}, Co^{2+}, Ni^{2+}, Cd^{2+}, Pb^{2+}, Hg^{2+}, Zn^{2+}, Mg^{2+}, Ba^{2+}, Sr^{2+}, Ca^{2+}, Fe^{3+}, Al^{3+}, V^{5+}, NO_3^-, Cl^-, and F^-. The tolerance limits to the interferences, expressed as the maximum concentration of the interfering element added to a boron solution which differed less than 5% to the signal of a solution of boron alone (5 μg ml^{-1}) were determined. The results of this study are shown in Table 2. The most severe depressions were caused by fluoride and potassium for which a concentration of 100 mg l^{-1} caused a 5% depression on the signal. For the other cations concentrations between 200 and 1000 mg l^{-1} could be tolerated. For chloride, and nitrate, a concentration of 1000 mg l^{-1} could be tolerated. In the case of F^- this is due to the formation of BF_4^- stable complex, which drastically reduce the boron available for methyl borate generation [39,46,48]. The mechanism of action of K^+ still it is not clear, Castillo et al. [46] reported a positive deviation on the signal between 18 and 21%, while other authors [39,48] observed that K^+ produced a suppressing effect on the signal. The concentrations of the interfering species in

Table 2
Interferences from diverse species

Interferent	Added as	Boron to interferent ratio	Tolerance limit[a]
K^+	K_2SO_4	20	100
Li^+	Li_2SO_4	40	200
Na^+	Na_2SO_4	40	200
NH_4^+	NH_4Cl	100	500
Cu^{2+}	$CuSO_4$	80	400
Co^{2+}	$CoSO_4$	80	400
Ni^{2+}	$NiSO_4$	80	400
Zn^{2+}	$ZnSO_4$	80	400
Ba^{2+}	$BaCl_2$	80	400
Sr^{2+}	$SrCO_3$	80	400
Ca^{2+}	$CaCO_3$	80	400
Cd^{2+}	$CdSO_4$	140	700
Mg^{2+}	$MgSO_4$	160	800
Pb^{2+}	$PbCl_2$	200	1000
Hg^{2+}	$HgCl_2$	200	1000
Fe^{3+}	$Fe_2(SO_4)_3$	100	500
Al^{3+}	$Al_2(SO_4)_3$	100	500
V^{5+}	V_2O_5	80	400
Cl^-	NH_4Cl	200	1000
NO_3^-	NH_4NO_3	200	1000
F^-	NaF	20	100

[a] Maximum concentration (mg l^{-1}) causing ±5% signal deviation with that for boron (5 μg ml^{-1}) alone.

the sample solution tolerated in present work are very similar to those of other investigations. However, our ratios of boron to interferent elements in most cases were appreciably higher than other works, i.e. 1:40 for Li^+ and Na^+ compared to 1:3 [46] and 1:25 [48]; 1:80 for Cu^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Ba^{2+}, Sr^{2+}, and Ca^{2+}; 1:100 for NH_4^+, Fe^{3+}, and Al^{3+}; 1:140 for Cd^{2+}; 1:160 for Mg^{2+}; 1:200 for Pb^{2+}, Hg^{2+} compared to 1:30 [46] and 1:25 [48]; 1:200 for NO_3^-, Cl^- compared to 1:30 and 1:125, respectively [46] and 1:50 [48]. In the particular cases of F^- our ratio of 1:20 were comparable to 1:15 [48], 1:30 [46], and 1:10 [39]. Finally, our ratio 1:20 for K^+ was very close to 1:25 [39,48], but higher than 1:3 [46]. The high tolerance to interfering species observed here may be explained by quick and continuous removal of the methyl borate formed from the reaction mixture. The precipitates that arise from sulfates of Ba^{2+}, Sr^{2+}, Pb^{2+}, and Ca^{2+} do not interfere.

3.4. Analytical performance

The system responded linearly from the detection limit up to 50 μg ml^{-1}. The precision of the procedure, calculated as the %R.S.D. of 10 determinations of 1.0 and 5.0 μg ml^{-1} of boron solutions, was 3.5 and 2.6%, respectively. The limit of detection, LOD, defined as the concentration giving a signal equal to three times the standard deviation of the blank signal, was 0.05 μg ml^{-1}. The proposed method takes 110 s per determination, giving the method a sampling frequency of 33/h. It is possible to increase the frequency further by shortening the length of color developing reaction coil (R_3), at a cost of some sensitivity.

The percentage recoveries of spikes added to the soil, coffee leaves and coffee fruit samples prior sample preparation are shown in Table 3. The values range from 92 to 96, from 95 to 99 and from 98 to 103 for soil, coffee fruit, and coffee leaves, respectively. Indicating that boron can be quantitatively recovered from soil and plant tissue using the developed procedure. To further confirm the accuracy and check the reliability of the analytical procedure boron was determined in two standard reference materials (SRM 1570a, spinach leaves and SRM 1547, peach leaves from NIST). The concentrations found of 38.4 ± 1.5 and 29.8 ± 0.9 μg g^{-1} were in good agreement with the certified values of 37.6 ± 1.0 and 29 ± 2.0 μg g^{-1} for SRM 1570a and SRM 1515, respectively. None soil with appropriated certified concentration values of boron (boron available to plant) was found.

Table 3
Recovery of boron spiked in soil and plant tissue prior to samples preparation

Sample	Boron added (μg)	Boron found (μg)[a]	Recovery (%)
Soil	0	161 ± 6	–
	25	184 ± 7	92
	75	233 ± 9	96
Coffee fruit	0	62 ± 3	–
	25	86 ± 3	95
	75	136 ± 5	99
Coffee leaves	0	185 ± 3	–
	25	209 ± 5	98
	75	262 ± 8	103

[a] Average of triplicate analysis of the same sample.

Table 4
Analytical results for boron determination in soil and plant tissue of coffee crops from different towns of Mérida State, Venezuela

Location and sample type	Boron found (μg/g)[a]
Chiguara	
Soil	6 ± 2
Coffee fruits	23 ± 5
Coffee leaves	55 ± 4
Tovar	
Soil	9 ± 6
Coffee fruits	27 ± 5
Coffee leaves	65 ± 5
Santa Cruz de Mora	
Soil	15 ± 7
Coffee fruits	35 ± 4
Coffee leaves	115 ± 8

[a] Average of the concentration found in 10 different samples.

3.5. Determination of boron in soil and plants samples

The developed system was applied to the determination of boron in soil, fruit tissue, and leaves tissue of coffee plantations from different towns of Mérida State, Venezuela. The results of the determinations are shown in Table 4. The amount of boron found varied within the ranges 6–15, 23–35, and 55–115 μg g^{-1} for soil, fruit tissue, and leave tissue, respectively.

4. Conclusions

The continuous-flow system developed, allowed the on-line generation, distillation and hydrolysis of methyl borate with the subsequent spectrophotometric determination of boron using azomethine-H as colorimetric reagent. In spite of the use of a complicated manifold, complex chemistry and home-made separators, the developed procedure is reliable, fast, sensitive and convenient for the determination of boron in extracts of soil and plants tissue in a concentration range up to 50 μg ml^{-1}. The quick and continuous removal of the methyl borate formed from the reaction mixture permitted high tolerance to interfering species. The procedure is noticeably fast (33 determinations/h) which is an important factor in routine analysis of soil and plants samples.

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