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A monosegmented flow-batch system for slow reaction kinetics: Spectrophotometric determination of boron in plants

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ABSTRACT

This work introduces the monosegmented flow-batch (MSFB) analysis concept. This system combines favourable characteristics of both flow-batch and the monosegmented analysers, allowing use of the flow-batch system for slow reaction kinetics without impairing sensitivity or sampling throughput. The MSFB was evaluated during spectrophotometric determination of boron in plant extracts, which is a method that involves a slow reaction between boron and azomethine-H. All calibration solutions were prepared in-line, and all analytical processes completed by simply changing the operational parameters in the MSFB control software. The limit of detection was estimated at 0.008 mg L^{-1}. The measurements could be performed at a rate of 120 samples per hour with satisfactory precision. The proposed MSFB was successfully applied to analyse 10 plant samples and the results are in agreement with the reference method at a 95% level of confidence.

1. Introduction

In 1999 Honorato et al. [1] introduced the concept of the flow-batch analyser (FBA). Flow-batch analysers are automated systems that use an instantaneous stop chamber and integrate batch and flow methods by using programmed multi-commutation [2]. The main component is the mixing chamber (MC) where the whole analytical process including; fluids addition, sample pretreatment, homogenization, precipitation, extraction, preparation of calibration solutions, and detection, takes place under the total control of the software [1–4]. The sample is processed with less: manipulation, consumption of reagents and samples, waste and chance for human error. Classical (discrete) methods can be performed with precision, accuracy and speed similar to other flow analysis methods.

Flexible and versatile flow-batch systems have been developed for differing applications such as: screening analysis [5], titration [6], analyte addition [7], internal standard solution addition [8]. Flow-batch has been used for prior assays [9]; for developing titration concentration gradients [10], and concentration gradients for nonlinear calibrations [11]. Other applications include enzymatic reactions [12], chemiluminescence [2], nephelometrics [13], in-line coulometric generation of standards and titrants [14]. Liquid–liquid extractions [15] as well as in-line uni- [12] and multivariate [11] calibrations have also been accomplished. Flow-batch systems have also been miniaturised [16,17].

A determination involving slow reaction kinetics has been implemented using an FBA [11], without impairing the sampling throughput. However, the system presented poor sensitivity when compared to the traditional batch method. Other automatic systems have been proposed for slow reaction kinetics in flow injection analysis (FIA) [18,19], stopped-flow FIA [20], sequential injection analysis (SIA) [21,22], multicommutation in flow analysis (MFA) [23], monosegmented continuous flow analysis (MCFA) [24] and monosegmented flow analysis (MSFA) [25,26]. Among them stands out the MSFA, which allows a longer residence time for the sample (minimal dispersion), without affecting the sampling throughput. However, MSFAs are automated systems with less flexibility and versatility compared to FBA.

In this study we proposed a monosegmented flow-batch (MSFB) which combines the favourable characteristics of the FBA and MSFA and allows implementation of methods involving slow reaction kinetics without impairing the sensitivity or sampling throughput. The proposed MSFB was evaluated using the determination of boron in plant extracts using the azomethine-H spectrophotometric method [27].

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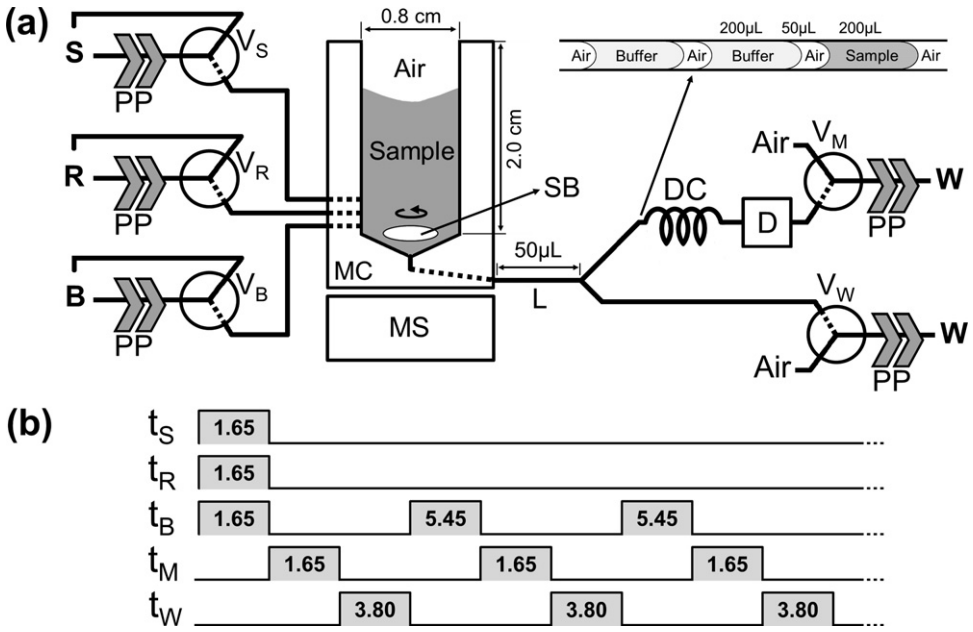

Fig. 1. (a) The MSFB diagram, (b) timing diagram for sample analysis. Mixing chamber (MC), peristaltic pump (PP), magnetic stirrer (MS), "loop" for introduction of air (L), delay coil (DC), spectrophotometric detector (D), solenoid valves (V_S, V_R, V_B, V_M and V_W), standard working solution or sample (S), reagent (R), buffer (B) and waste (W). t_S, t_R, t_B, t_M and t_W are the time intervals (in seconds) for switching valves V_S, V_R, V_B, V_M and V_W.

2. Experimental

2.1. Apparatus

A model 8453 Hewlett-Packard (HP) diode array UV–vis spectrophotometer, equipped with cuvette (Hellma QS 1000 quartz flow cell with 10 mm optical path) was used for the absorbance measurements at 420 nm employing both proposed and reference methods.

2.2. Reagents and solutions

All reagents were of analytical grade and freshly distilled and deionised water (18 MΩ cm^{-1}) was used to prepare all solutions. The solutions, including samples and standards, were prepared and stored in high-density polyethylene flasks.

Stock solution of 10.0 mg L^{-1} boron was prepared by dissolving the appropriate mass of boric acid (Synth) in water. Solutions of boron standards with lower concentrations were obtained by accurate dilutions of the stock solution with deionised water.

Azomethine H (Merck) stock solution was prepared from the pure product by dissolving 0.9000 g of salt and 2.0000 g ascorbic acid using 100.0 mL of water. The buffer-masking reagent was prepared by dissolving 14.00 g of ammonium acetate (Vetec), 10.00 g of potassium acetate (Vetec), 4.00 g of nitrilotriacetic acid (Synth) and 10.00 g EDTA (Vetec) in water. The pH 8.2 used was obtained by addition of glacial acetic acid (Synth).

2.3. Sample preparation

The plant samples were heated in an oven at 500 °C for 3 h. Afterwards they were cooled in a desiccator, and 1.0000 g from each sample was weighed and dissolved in 50 mL of nitric acid 10% (v/v). The mixture was filtered, transferred to a 100.0 mL polyethylene volumetric flask and completed with distilled-deionised water.

2.4. Monosegmented flow-batch system

A schematic diagram of the MSFB used for spectrophotometric determination of boron is shown in Fig. 1a.

The MSFB consists of five three-way solenoid valves (V_S, V_R, V_B, V_M and V_W) model EW-01540-13 (Cole Parmer); polyethylene tubing connectors with 0.8 mm id; a peristaltic pump (PP) model 78002-00 (Ismatec). A flow rate of 151.5 ± 1.6 µL s^{-1} (n = 20) was always employed.

The labmade mixing chamber (MC) was built in Teflon with about 1.0 mL of total volume. The mixture/homogenization of solutions was performed by a stirring bar (SB) located inside the MC driven by a magnetic stirrer (MS). A 750 µL total volume was used for each analysis. Before spectrophotometric detection (D), a 240 cm Teflon tube (2.0 mm internal diameter) was used as a delay coil (DC) in order to incubate the monosegments and to allow a slow kinetic reaction to occur.

A microcomputer connected with an interface (USB6009, National Instruments) was used to control the MSFB. The controlling software was developed in LabVIEW 7.1 (National Instruments). The MSFB software controls additions of solutions in MC, the monosegmented formation and incubation.

2.5. Monosegmented flow-batch procedure

Before starting the analytical procedure, working solutions for each channel are pumped and re-circulated to their respective reservoirs (Fig. 1a). Then the valves V_S, V_R, and V_B are simultaneously switched ON for a time interval of 1.50 s and the working solutions (S, R, and B) are pumped towards the MC to fill the channels between the valves and the chamber. Then immediately, the discard valve V_W is opened for 5.0 s and then any solution inside the MC is emptied using the peristaltic pump (PP) for aspiration. This channels filling procedure is very important and must be carried out whenever there is a change of the reservoir liquids.

The additions of the sample or standard working solution (S), reagent (R) and buffer (B) into the MC were performed switching ON valves V_S, V_R and V_B, respectively. The homogenization of the

solutions in the MC is constantly performed by a stirring bar (SB) under the action of the magnetic stirrer (MS).

For in-line blank preparation in the MC, valves V_R and V_B are simultaneously switched ON for 1.65 s and 3.30 s, respectively. Reagent and buffer are brought into the MC. The in-line preparation of calibration solutions (0.10–1.00 mg L^{-1} of boric acid) in the MC were performed using a standard working solution of 3.00 mg L^{-1} of boric acid, which itself was prepared by adequate dilution of a stock solution of 10.00 mg L^{-1}. In these preparations, valves V_S, V_R and V_B are simultaneously switched ON and standard working solution, reagent and buffer are sent into the MC. The valve V_R is switched ON for 1.65 s and the valves V_S and V_B are switched ON for times, which vary in proportion to the standard solution concentration being prepared.

The procedure for in-line preparation of the sample is similar to the preparation of calibration solutions. The difference is that the samples are used instead of the standard working solution. The timing diagram for sample analysis is shown in Fig. 1b.

For monosegment formation of blank, calibration solution, or sample (air–sample–air), valve V_M is switched ON for 1.65 s, time for aspiration of about 50 μL air (present in L) plus about 200 μL of fluid (present in the MC) to the DC. Afterwards, the excess of fluid in the MC is discarded and the "loop" (L) is filled with air by switching ON valve V_W for 3.80 s.

In Fig. 2 the formation stages of monosegmented sample are represented. Fig. 2a illustrates the step of addition and homogenization of the fluids (sample, reagent and buffer) in the MC. In Fig. 2b is shown early formation of the sample monosegment, which occurs after the valve V_M to be switched ON. The formation of the sample monosegment is finished in Fig. 2c when 50 μL of air plus 200 μL of sample is aspired to the delay coil (DC). Soon afterwards, the valve V_W is switched ON and the excess of fluid in the MC is discarded.

The cleaning step of the MC and DC is carried by switching ON valve V_B for 5.45 s, to add buffer to the MC. Soon afterwards, V_M is switched ON for 1.65 s time for aspiration of about 50 μL air (present in L) and about 200 μL of buffer (present in the MC) to the DC, formatting the air–buffer–air monosegment in the DC. The buffer excess in the MC is discarded and the "loop" (L) is filled with air by switching ON valve V_W for 3.80 s. This step is carried out twice in order to ensure the cleaning of both the MC and the DC.

Analytical signal measurement is performed when sample, calibration solution or blank monosegment reaches the flow cell, fulfilling its optical path with 200 μL of processed fluid. At this moment, the valve V_M is switched OFF, stopping the flow inside the inline DC to keep air bubbles completely out of the optical path. It is not necessary to remove the bubbles because they will not interfere on absorbance measurements. Finally, the absorbance at 420 nm is recorded in steady state (and during the pre-determined sampling time) for further data treatment. During any analytical signal measurement a new monosegment is prepared concomitantly inside the MC and thus, neither sampling throughput nor sensitivity is impaired.

The valve V_M is controlled by time. This value (1.65 s) represents the time necessary for one monosegment (200 μL fluid, and 50 μL air) to be drawn into the DC. Since the volume between the MC and D, and the rate of flow are constant, the reaction time provided for by the DC is also held constant. The synchronised time settings for valve V_M and valve Vw then permit that absorbance from the sample, blank or calibration solution is always stop flow mode measured at 5 min from mix, without interference of the air portion of the monosegment. The normal use of opto-switches for detection of the monosegment [29] at the flow cell in this arrangement is not necessary, which simplifies the MSFB manifold.

It is worth noting that when the number of samples to be analysed are finished, several cleaning monosegments (air-buffer-air) are continuously prepared and added to delay coil until

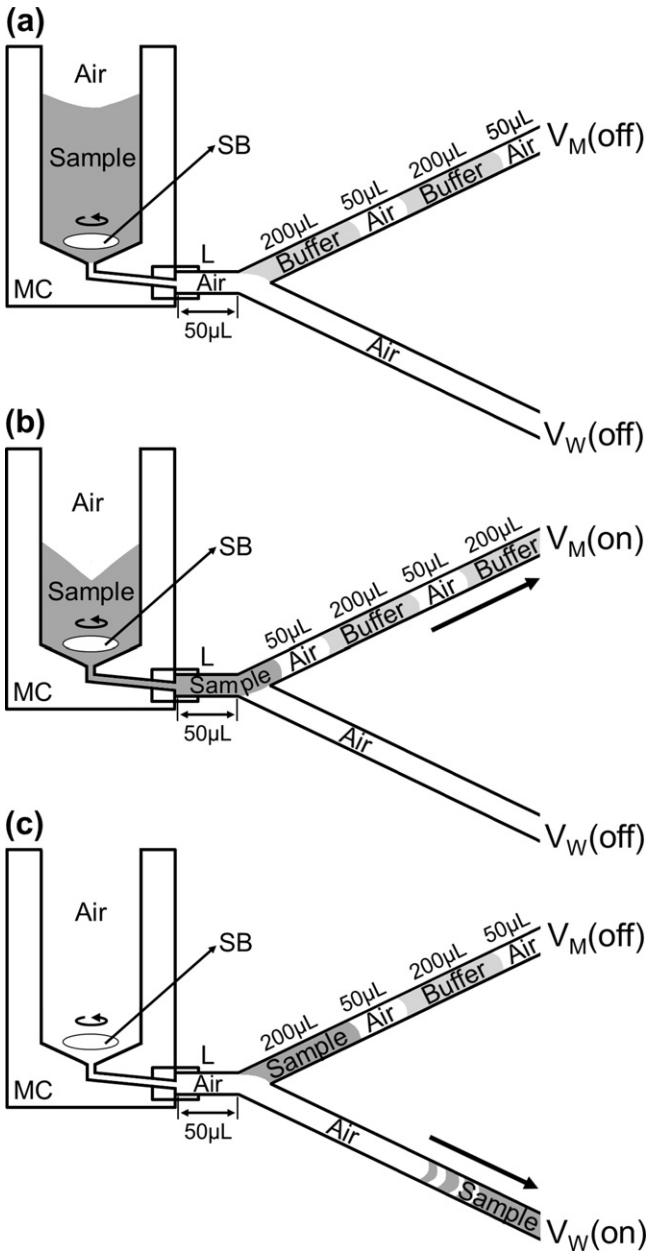

Fig. 2. Stages of formation of monosegment. (a)–(c) V_M and V_W, solenoid valves monosegmented and waste, respectively; MC, mixing chamber; SB, stirring bar; L, "loop" for introduction of air.

measurement of the absorbance yielded by the last sample. This procedure is important to keep residence coil always feed in a systematic way, avoiding possible changes in the flow aspiration rate through detector and consequent losses in the timing synchronism among absorbance measurements. Notwithstanding, distilled water may be also used instead buffer solution for the preparation of cleaning monosegments.

2.6. Analytical procedure of the reference method

For comparison, the proposed MSFB performance was evaluated against a manual reference UV–vis spectrophotometric azomethine-H method analyzing the plant extract samples. Calibration solutions were prepared from 0.1 to 1.0 mg L^{-1} [27]. The analytical signals were measured at a maximum absorbance of around 420 nm. The analysis of each sample was performed in

Table 1
Results for boron determination in plant extract (mg kg^{-1}) using the proposed MSFB and the spectrophotometric reference method. The values of uncertainty have been estimated by using the expression $\pm t_{N-1}s/\sqrt{N}$, where N is the number of replicate measurements, t_{N-1} is the statistic parameter often called Student's t (with $N = 5$, at 95% level of confidence) and s is the standard deviation.

Samples	MSFB system	Reference method
1	14.3 ± 0.2	14.7 ± 0.2
2	18.8 ± 0.1	18.1 ± 0.3
3	41.2 ± 0.3	40.7 ± 0.2
4	31.7 ± 0.2	32.1 ± 0.1
5	33.6 ± 0.1	32.8 ± 0.1
6	13.5 ± 0.1	13.9 ± 0.3
7	16.0 ± 0.2	16.2 ± 0.1
8	8.7 ± 0.1	9.1 ± 0.1
9	7.8 ± 0.3	7.3 ± 0.2
10	4.3 ± 0.2	4.1 ± 0.1

quintuplicate and the concentrations were calculated from the analytical curve.

3. Results and discussion

3.1. Monosegmented flow-batch features

The length of the delay coil was set at 240 cm of tube (2.0 mm internal diameter), enough to permit the reaction of boron with azomethine-H to reach equilibrium when the monosegment (air-sample-air) is being transported towards to detection. This corresponds to a residence time of 300 s.

The use of two consecutive steps of cleaning with the buffer monosegment (between bubbles) was perfectly adequate, because no carry over was observed.

3.2. Analytical application

A good analytical curve was obtained with the MSFB system for the determination of boron in plant extract samples by the azomethine-H method. The regression equation was $A = 0.0061 + 0.7741\,C$; where A is the analytical response and C is the analyte concentration in mg L^{-1} of boron. The squared linear correlation coefficient, r^2 was 0.998 in the range between 0.10 and 1.00 mg L^{-1}. The calibration curve was statistically validated by variance analysis (ANOVA), showing no lack of fit at a 95% confidence level. The limit of detection (LOD) was 0.008 mg L^{-1}. The LOD for the method was calculated based on the criteria established by IUPAC [28], the LOD was evaluated as three times the standard deviation of the blank measurement.

Table 1 presents the results obtained for the proposed MSFB and those obtained for the reference spectrophotometric method. They show a good agreement between the obtained values by using both methods, attesting the accuracy of MSFB. Actually, at a confidence level of 95%, no statistical difference was observed between them when the paired t-test was applied. In terms of precision, the obtained values by the proposed and reference methods were similar. The overall standard deviation ($n = 5$) obtained in the determinations for both methods was 0.6 mg kg^{-1}. The MSFB presented an analytical frequency of 120 samples per hour for the azomethine-H method.

Table 2 presents some analytical features of the proposed MSFB and others automated systems, selected by authors, described in the literature for determination of boron in plant extract samples by the azomethine-H method. The proposed MSFB has a lower limit of detection (LOD = 0.008 mg L^{-1}). It was observed that the its LOD was 2.5 times lower than that reported for MCFA [24] and 58 times lower than that for MFA [23]. The sampling rate of the MSFB was similar to MCFA, and 3.4 times higher than MFA. The elimination of a carrier fluid, in-line preparation of calibration solutions, and a lower volume of monosegment than previous automated systems are further advantages.

Table 2
Parameters from different automatic systems.

	MCFA [24]	MFA [23]	MSFB
Detection limit (mg L^{-1})	0.02	0.47	0.008
Sampling rate (h^{-1})	120	35	120
Volume of monosegment (μL)	350	600	200
Carrier fluid	Present	Present	Absent
Preparation of calibration solutions	Off-line	Off-line	In-line

4. Conclusion

A novel automated system, the MSFB, which combines the favourable characteristics of flow-batch and monosegmented analysers was successfully developed. The system enabled the use of a flow-batch system for slow kinetic reactions without impairing either sensitivity or sampling throughput. The system was applied for spectrophotometric determination of boron in plant extracts, which involve a slow reaction between boron and azomethine-H.

In general, the MSFB presents better analytical features than either MCFA or MFA [23,24] automated systems, i.e. detection limit, sampling rate, elimination of the carrier fluid, preparation in-line of calibration solutions, and a lower volume of monosegment.

In the case of the MSFB system is used in applications that requires other analytical procedures, after the additions in the mixing chamber, (sequential treatments required before the sample is detected) other devices or components can be simply added to the system (such as mixing chambers, valves, tubes and delay coils). Thus, the MSFB system presents the favourable characteristics as flexibility, versatility and robustness as a traditional flow-batch system, and has potential for other use in determinations.

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