

Flow systems exploiting in-line prior assays

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Abstract

An expert sequential injection system involving a prior assay is proposed for spectrophotometric determination of phosphate and eventually zinc in soil extracts. The result of phosphate determination is the basis for a concentration-oriented decision regarding to the need or not for zinc determination. Zinc was only determined if a threshold value (peak height corresponding to $5.0 \text{ mg l}^{-1} \text{ P}$) was surpassed. The methods involved formation of molybdenum blue and the Rhodamine 6G/ammonium thiocyanate/ Zn^{2+} ternary complex. Variations in the threshold value were $< 2\%$ during 4 h operating periods, false responses were not verified, and the analytical time was reduced in about 30%. Precise results (R.S.D. $< 3\% \text{ P}$ and $< 1\% \text{ Zn}$) in agreement with spectrophotometry and flame atomic absorption spectrometry were obtained. The innovation permits faster information processing, as well as a reduction in the number of measurements, number of analytical steps, laboratorial time, and consumption of sample and reagents, thus waste generation.

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1. Introduction

Fast processing of information associated to reliable and simple analytical procedures are among the objectives of the modern analytical chemistry. In this context, prior assays play a relevant role. A prior assay precedes the main determination and yields a binary answer, such as yes/no, absent/present, lower/higher than a pre-set threshold value, etc. which is taken into consideration for implementing eventual modifications in the course of further analytical steps.

In the traditional laboratory for large-scale chemical analysis, prior assays have been exploited, for example previous evaluation of unknown sample characteristics, and/or for screening purposes. A common example of this strategy is the rough analysis performed on an unknown sample. This preliminary information about its main characteristics permits the establishment of better analytical conditions, thus improving the reliability of the analytical results. Another potentiality of prior assays in the traditional laboratory refers to sample screening based on preliminary analytical data; only the samples to be submitted to the main analytical pro-

cess are selected, thus reducing the number of determinations to be carried out, and expanding the analytical capacity of the laboratory [1,2]. Exploitation of prior assays in the traditional laboratory is, however, limited, as they are usually carried out well before the main analysis being then considered as somewhat old-fashioned. Consequently, they are less useful when an immediate answer is required.

This aspect becomes no longer restrictive if prior assays are carried out in an automated analytical system. In this context flow analysis should be highlighted, as the flow analyzer is recognized as an excellent solution handling system [3], presenting favorable characteristics of sample throughput, consumption of samples and reagents, versatility, ruggedness, operating cost, etc. [4]. Moreover, it may provide an immediate answer [5], which is worthwhile in relation to real-time concentration-oriented decisions inherent to the expert systems. Decisions on the next steps of the analytical course are then fast and efficiently accomplished, as the software recognizes the preliminary measurement (usually a binary answer) and performs the required modifications in the manifold [6–18].

Prior assays are particularly compatible with unsegmented flow systems [19–22] where different processes such as stream redirecting, flow reversal, sample stopping, component additions to the manifold, etc. are reproducibly

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accomplished. The sample is then efficiently handled, system design is simplified, versatility is enhanced and timing is precisely controlled.

In view of the need for expanding the analytical capacity of the laboratory, the main purpose of the present work was to demonstrate the feasibility of expedite prior assays in an expert flow system in order to reduce the number of determinations, the consumption of samples and reagents, the analytical time and the generation of waste.

As an application, the spectrophotometric determination of phosphate and zinc in soil extracts using an expert sequential injection system was selected. This determination is important, e.g. in relation to studies on phosphate deficiency, usually a limiting factor on crop production in tropical regions [23]. High-levels of available phosphate may reduce the availability of zinc to the plants, leading to the appearance of the deficiency symptoms. In this way, zinc determination is only required when phosphate contents surpasses a threshold value.

2. In-line prior assays in flow analysis

Different flow systems with abilities to perform a pre-analysis, interpret it and set the conditions for further analysis through concentration-oriented feedback mechanisms have been proposed. On one hand, a qualitative pre-analysis is enough for gathering information on the presence of a given chemical species (or a set of similar species) in the sample [2], the approximate analyte content, the need for sample dilution, etc. On the other hand, reliable results are needed when feedback mechanisms based on strictly quantitative data are involved. This is important mainly in relation to, e.g. matrix matching and sample conditioning [7]. Regardless of the refinement of the prior assay, further analysis is always performed under optimized conditions.

Amongst the earlier applications involving prior assays in flow analysis, the simultaneous determination of metals in estuarine waters by ICP–OES should be highlighted [6]. In order to perform sample/standard matrix matching, the sodium content in the sample—thus, sample salinity—was roughly evaluated, allowing both the salt amount to be added to the sample and the salinity of the working standard solutions to be selected. Matrix interferences due to the salinity differences between sample and standard solutions were then minimized.

In-line individual sample conditioning based on prior measurements was proposed for the determination of total nitrogen in plants [7]. The Kjeldahl digests from a sample batch presented a pronounced variability in acidity, and an adjustment should be performed on every sample. For this task, a sequential injection system in the flow-batch configuration was designed with ability to add a variable yet known amount of an alkaline solution to the different samples. A prior sample monitoring was required to permit proper adjustment of the digest acidity. Further analysis

was then carried out under a constant acidity. The approach was also exploited for the determination of aluminum in plant digests [8]. Furthermore, an analogous strategy was developed for correction of the sample matrix in the spectrophotometric determination of Fe(III) in estuarine waters [9]. In order to get a constant ionic strength, different amounts of sodium chloride were added to the different samples, these amounts being defined according to a prior monitoring involving conductimetric detection.

Prior assays were also exploited for end point search in flow titrations [10]. Several measurements were done under different conditions attained by varying the titrant-to-titrant volumetric ratio. Regarding flow titrations, prior assays were also exploited to provide a rough estimation of the analyte content, then lessening the initial uncertainty interval and speeding up the entire titration—the approach was applied to spectrophotometric determination of iron in alloys [11].

Trial measurements were exploited for the determination of manganese in rocks by atomic absorption spectrometry [12]. The sample was extensively dispersed and a trial measurement was done. If this measurement was within the detector dynamical range, the process was stopped and the analyte concentration was determined. If the analytical signal was too low, the sample was handled again, but under lesser dispersion conditions, and so on. In this way, a suitable dispersion degree was selected for every sample. In-line prior assays referred then to successive sample dilutions.

An improved determination of calcium and potassium in plant digests by flow-injection atomic absorption and atomic emission spectrometry [13] should also be highlighted. A prior measurement was accomplished by injecting the sample into a straight manifold. If the analytical signal surpassed the detector dynamical range, the sample was in-line diluted by an optional confluent stream added immediately before the detector.

Another possibility of exploiting prior assays was recently demonstrated in the multisyringe flow determination of two analytes [14]. A robust and rapid continuous flow titrator, including a pre-dilution step, was developed. A quantitative dilution was achieved in two steps, first by splitting the concentrated sample stream and further, by dilution inside a mixing chamber. As the system was previously dimensioned, the need for diluting the standard solutions was avoided. A wide range of dilution degrees was easily implemented by software without mechanical reconfigurations of the manifold.

An expert multi-commuted flow analyzer for concentration and/or speciation of metal ions in natural waters was proposed [15]. For every assayed sample, the program decided if in-line concentration or speciation analysis was required and, if pertinent, to place an ion-exchange mini-column into the analytical path. After a prior measurement, a series of operating parameters were adjusted in order to provide suitable peak height measurements.

Regarding multiparametric determination, an expert flow-injection system for quality control of olive oil was

recently proposed [16]. The system compared the results obtained in the determination of total acidity, peroxide values and UV spectrophotometric measurements with the expected values, in accordance with the analyst skill and a previously defined mathematical model. If any of these results did not follow a logical pattern, the analysis was repeated after suitable modifications in the status of some of the involved devices. These repeated measurements can be considered as successive prior assays.

An expert sequential injection system exploiting a diode array spectrophotometer and multivariate calibration was proposed for the determination of calcium and magnesium in natural waters [17]. It was designed to permit real-time modifications in some operational parameters, according to results of a prior assay. Variations in sampled volumes, reagent addition order, and instant for getting the analytical signal were then implemented for every assayed sample.

An expert flow-injection system was also employed for reducing the uncertainty of the analytical results [18]. As the uncertainty may be dependent on the number of replicated measurements, exploitation of several prior assays proved to be valuable also for obtaining more precise mean results. The strategy involved real-time decisions—after three-fold sample manipulation, the uncertainty of the analytical results was estimated and compared with a preset value, the maximum allowed value. If the experimental uncertainty was lower than that value, next sample was run. Alternatively, the sample was run again and the uncertainty was re-evaluated. If the unfavorable situation persisted, an error message was sent.

3. Experimental

3.1. Methods

Phosphate determination involved reaction of *o*-phosphate with molybdate, yielding a heteropolyacid further reduced to molybdenum blue by ascorbic acid [24,25]. This chemical species was spectrophotometrically monitored at 700 nm. Zinc determination was based on formation of a ternary complex involving Rhodamine 6G, ammonium thiocyanate and Zn^{2+} (2:4:1 molar ratio) quantified at 575 nm [26]. Both methods were recently implemented in sequential injection systems for soil analysis [27].

3.2. Samples, standards, reagents

High purity de-ionized water (resistivity $>18.2 \text{ M}\Omega \text{ cm}$) obtained by a Milli-Q water purification system (Millipore) was used throughout.

The soil extracts were prepared by weighing ca. 5 g of sieved ($d < 1 \text{ mm}$) and air-dried soils into 125 ml Erlenmeyer flasks, adding 50 ml of a $0.05 \text{ mol l}^{-1} \text{ HCl}$ plus $0.012 \text{ mol l}^{-1} \text{ H}_2\text{SO}_4$ solution (Mehlich I extracting solution) and allowing the mixture to stand for about 30 min. Thereafter, the

flasks were shaken during 5 min, and the samples were allowed to decant for about 2 h (or overnight). Samples were then filtered (Whatman #1 paper filter) and stored in closed vessels [28].

Working standard solutions based on metallic zinc and potassium dihydrogen phosphate were prepared in the Mehlich I medium, covering the $0.0\text{--}20.0 \text{ mg l}^{-1} \text{ P}$ and $0.00\text{--}2.00 \text{ mg l}^{-1} \text{ Zn}$ ranges. The Mehlich I solution was also used as carrier and R_5 streams (Fig. 1).

For phosphate determination, reagent R_1 was a 1.0% (m/v) tetrahydrate ammonium heptamolybdate plus 0.5% (m/v) de monohydrate potassium oxalate solution; R_2 was a daily prepared 2.0% (m/v) ascorbic acid solution. For zinc determination, reagent R_3 (Fig. 1) was prepared by dissolving 0.01 g of Rhodamine 6G in 5 ml 96% (v/v) ethanol and filling the volume up to 100 ml with a 0.02% (v/v) Tween-80 solution; reagent R_4 was a 5.0% (m/v) ammonium thiocyanate solution, also 0.05 mol l^{-1} sodium picolinate plus 0.1 mol l^{-1} sodium pyrophosphate.

3.3. The expert sequential injection system

The flow system exploiting prior assay designed for phosphate and zinc determination in soil extracts is outlined in the Fig. 1. It comprised a model MP3 Ismatec peristaltic pump with a Tygon pumping tube, a 8-port Valco selecting valve, a model USB2000 UV–Vis ocean optics spectrophotometer, and accessories. The reaction conditions were optimized in order to permit the determination of both analytes in a single manifold (Fig. 1) (see also Table 1).

In the sampling position, a 0.21 ml sample aliquot was aspirated towards the holding coil. The selecting valve was then successively anti-clockwise operated allowing the sequential aspiration of 0.13 and 0.25 ml aliquots of reagents R_1 and R_2 , establishing a sample zone inside the holding coil. Thereafter, the flow was reversed and the selecting valve was switched in order to direct the sample zone towards detection. During transportation through the analytical path, mixing was improved, allowing the development of the involved chemical reactions. Sample passage through the flow cell resulted in a transient analytical signal with height proportional to the phosphate content in the sample. If the recorded peak height was lower than a threshold value (here

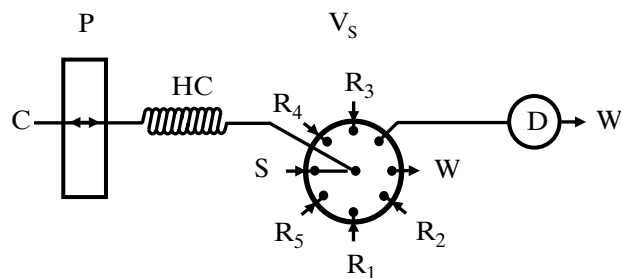


Fig. 1. Flow diagram: S sample; R_i reagent solutions; C carrier stream; P : pump; HC holding coil; V_S eight-port selecting valve; D detector; W waste; arrows indicate flow direction.

Table 1
Operating conditions

Step	Operation	Position of the selecting valve	Time (s)	Flow rate (ml min ⁻¹)
1	Propulsion of C towards HC and D	Waste outlet	80	9.0
2	Aspiration of R ₁ towards HC	R ₁ inlet	4	3.4
3	Aspiration of R ₂ towards HC	R ₂ inlet	4	3.4
4	Aspiration of S towards HC	S inlet	4	3.4
5	Aspiration of R ₃ towards HC	R ₃ inlet	4	3.4
6	Aspiration of R ₄ towards HC	R ₄ inlet	4	3.4
7	Aspiration of R ₅ towards HC	R ₅ inlet	4	3.4
8	Propulsion of solutions inside HC towards W	Waste outlet	80	10.2
9	Aspiration of S towards HC	S inlet	5	2.5
10	Aspiration of R ₁ towards HC	R ₁ inlet	3	2.5
11	Aspiration of R ₂ towards HC	R ₂ inlet	6	2.5
12	Propulsion of sample zone towards D (phosphate)	Outlet towards detection	40	7.4
13	Analysis of the generated signal: either go to step 9 or to step 14	–	–	–
14	Aspiration of S towards HC	S inlet	6	3.4
15	Propulsion of S towards W	Waste outlet	15	7.4
16	Aspiration of R ₃ towards HC	R ₃ inlet	2	2.5
17	Aspiration of R ₄ towards HC	R ₄ inlet	3	2.5
18	Aspiration of S towards HC	S inlet	5	2.5
19	Aspiration of R ₅ towards HC	R ₅ inlet	5	2.5
20	Propulsion of sample zone towards D (zinc)	Outlet towards detection	30	7.4

Table refers to the sequential injection system of Fig. 1. S: sample; C: carrier stream; HC: holding coil; D: detector. Steps: 1–8 system conditioning; 9–12 phosphate determination; 13 decision; 14–15 sample replacement; 16–20 zinc determination.

the analytical signal corresponding to 5.0 mg l⁻¹ P) the zinc determination was not needed, and next sample was run. Alternatively, if the prior assay yielded a signal higher than the threshold value, the steps related to zinc determination were carried out. For this task, R₃, R₄, S and R₅ aliquots (0.08, 0.13, 0.21, 0.21 ml) were sequentially aspirated towards the holding coil and, after flow reversal, analogously handled. After spectrophotometric monitoring of the formed ternary complex, next sample was placed for analysis. The entire process is summarized in Table 1.

4. Results and discussion

The versatility of the sequential injection system was essential in implementing prior assays with the purpose of reducing the number of measurements involved in the determination of phosphate and zinc in soil extracts (Table 2). Modifications in the manifold architecture were not required, and this is a positive aspect.

The proposed expert flow system was very stable, and baseline drift was not observed during extended operation periods. Consequently, the interval for successive runs of the 5.0 mg l⁻¹ P standard solution could be as large as 1 hour. Variations in the measurement related to this solution were always lower than 2%. As the proposed system operated during 4 h periods, signal corrections were not needed. Another positive aspect arising from the system ruggedness is that precise results were always obtained. For typical samples, relative standard deviation of results were estimated as <3 and <1% for phosphate and zinc determinations, respectively.

The entire analytical cycle (steps 9–20, Table 1) demanded 120 s to be completed meaning 30 samples per hour. Sampling rate could be as high as 67 samples per hour, depending on the need for zinc determination. Regarding lessening of the analytical time due to exploitation of the proposed strategy, a quantitative figure cannot be straightforward estimated, as the number of samples requiring the zinc determination is variable within the different sample lots, depending also on the focused agronomical problem. For most of the sample lots, a reduction in analytical time of about 30% has been verified by exploiting the proposed strategy.

Sampling rate could not be improved by lessening the time interval between successive sample injections. In fact,

Table 2
Typical results data in mg l⁻¹; uncertainties based on triplicate measurements

Sample	Phosphate	Zinc
1	1.84 ± 0.06	–
2	11.33 ± 0.17	0.06 ± 0.00
3	1.29 ± 0.06	–
4	0.76 ± 0.09	–
5	4.33 ± 0.29	–
6	1.41 ± 0.05	–
7	0.85 ± 0.02	–
8	8.96 ± 0.24	0.21 ± 0.01
9	6.37 ± 0.17	0.12 ± 0.01
10	3.97 ± 0.03	–
11	3.32 ± 0.05	–
12	2.15 ± 0.06	–
13	7.57 ± 0.21	1.18 ± 0.02
14	1.72 ± 0.02	–
15	1.42 ± 0.00	–
16	0.85 ± 0.00	–

carryover effects manifested themselves when the time intervals related to steps 12 or 20 were decreased to 20 s. It is important to note that the molybdate anion is a potential interfering species in the zinc determination [27]; therefore, the time interval related to step 12 is a critical parameter in the system design. In fact, when it was <38 s, a >2% between-method carryover coefficient [29] was observed.

Implementation of both methods in a single manifold did not impair accuracy, as their main analytical characteristics were maintained. After running seven already analyzed [25,30] soil extracts, statistical differences between methods were not found at the 95% confidence level.

False responses can be a drawback in screening analysis [31,32]. In order to verify the possibility of their occurrence in relation to the in-line prior assay, an additional experiment involving 16 already analyzed samples was carried out and revealed absence of false responses. This feature is another favorable consequence of the system ruggedness.

5. Conclusions

The proposed system can be considered as intelligent, as some features inherent to expert systems are exploited. It covers the need for the determination of phosphates and simultaneously is able to decide if the determination of zinc in the same digest has to be carried out or not. After applying this expert system to large sample batches it was verified that, as a whole, the proposed strategy includes an 'in-line screening' step. In fact, although the entire sample lot is not preliminarily run, nor the samples are previously selected for zinc determination, application of the strategy results in an expressive reduction in the number of measurements. This is a very positive aspect regarding laboratory management.

The logical evolution of this concept refers to the exploitation of prior assays in relation to multiparametric analysis. In this context, chemometrics algorithms would be important for efficient evaluation of the analytical signals. Experiments on this matter are presently in progress.

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