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Detecting and circumventing sources of inaccuracy in flow analysis*

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Abstract: Mechanized procedures for chemical analyses are susceptible to systematic errors usually difficult to trace. This holds also for flow analysis that although accepted worldwide may sometimes yield inaccurate results. The present work focused on the development of a simple system able to detect and circumvent sources of inaccuracy. The versatility inherent in the multicommuted flow analyzers was exploited for real-time characterization of the source of inaccuracy and overcoming the problem for every assayed sample. Specific situations where the analytical results are more susceptible to inaccuracy are emphasized.

INTRODUCTION

The high sampling rate inherent to flow analysis has been seldom fully exploited, and the expression "my system processes hundreds of samples per hour but I have just a few of them" [1] is often valid. Moreover, the analytical results are sometimes hindered by systematic errors often difficult to trace. In this context, systems can be designed in order to take advantage of the high sampling rate for detecting and circumventing potential sources of inaccuracy.

A typical situation refers to the partial overlap of sample and reagent zones. This is more likely to occur in sequential injection [2] or in flow-injection systems with sandwich techniques [3]. In these systems, samples and reagents are sequentially inserted, and the analytical signal corresponds to a region of the sample zone with self-optimized sample and reagent volumetric fractions [4]. However, this region is not necessarily associated with the highest accuracy, especially when large reagent excess is required. The drawback can be circumvented by exploiting merging zones [5] or by intercalating sample plugs in tandem with reagent plugs [6].

Inaccuracy may also be associated with the incompleteness of the involved chemical reactions. Although a flowing sample can be monitored without attaining chemical equilibria [7], the completion degree of the chemical processes should be constant for the assayed samples and reference solutions. Without a suitable matrix matching, inaccurate results can arise as a consequence of differences in kinetics of the processes to which sample and reference solutions are submitted. In addition, full reaction development is required for masking purposes. Thus, the flow system should be designed to permit the sources of inaccuracy to be real-time detected and compensated. For this task, successive measurements at different time intervals, additions of different amounts of the masking agents and/or analyte spiking can be exploited.

Another situation refers to use of mini-columns of solid-phase reagents such as enzyme cartridges, ion-exchangers, slightly soluble or immobilized reagents. If the reagent is not renewable, its efficiency may undergo a continuous lessening [8] leading to systematic errors. A simple strategy to cir-

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cumvent this drawback is to perform periodic additions of reference samples during the analyses of large sample batches.

The aim of the present work was therefore to present a flow system able to overcome the above-mentioned hindrances. The versatility inherent to the multicommuted systems [6] was exploited for real-time characterization of the source affecting the analytical results as well as for circumventing the problem for every sample being analyzed. As examples, some spectrophotometric determinations currently carried out at the authors' laboratories were selected.

EXPERIMENTAL

The flow system

The flow system (Fig. 1) comprised a peristaltic pump, a spectrophotometer furnished with a 10-mm optical path, 80-µl inner volume flow cell, four computer-controlled three-way solenoid valves, Perspex connectors and accessories [5,6]. Transmission lines and coiled reactors (15-mm winding diameter) were built up with 0.7-mm i.d. polyethylene tubing. System control, as well as data acquisition and processing were performed by means of software written in Microsoft Visual Basic 3.0. Details of interfacing were already described [6].

The system was operated by switching the three-way valves. Every solution was either introduced into the analytical path or allowed to recycle, depending on the status of the corresponding valve. The design synthesizes earlier proposed strategies exploiting multicomutation such as merging zones [5], binary sampling [6], zone sampling [9], zone trapping [10] and random-access reagent selection [11,12]. In all experiments, signals were compared statistically considering the confidence level of 95%.

Partial overlap of sample and reagent zones

The spectrophotometric determination of iron with 1,10-phenanthroline (phen) exploiting the sandwich technique [13] was studied by applying the valve timing scheduled in Table 1 (steps 1a–4a). System washing by the carrier stream was accomplished in the first step. The reducing reagent was introduced into the analytical path by switching the valves V_1 and V_3 (step 2a). The sample was further introduced by switching the valves V_1 and V_2 (step 3a). Thereafter, valves V_1 and V_4 were simultaneously operated for the introduction of the phen plug neighboring the sample plug. Next, the situation specified in

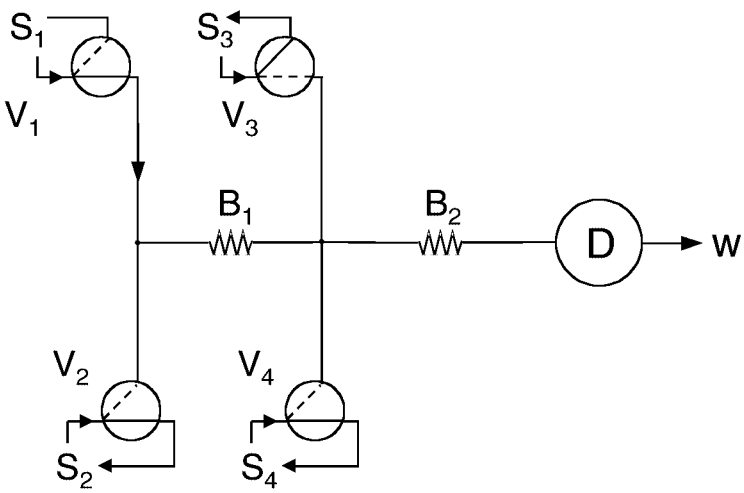

Fig. 1 Flow diagram of the proposed system. Si: solutions; Vi: three-way solenoid valves; B_1, B_2: reactors; D: spectrophotometric detector; W: waste. Flow rates: 3.5 ml min^{-1} (S_1) and 1.0 ml min^{-1} (S_2–S_4).

Fig. 1 was restored, and the carrier stream pushed the sandwiched zones toward detection. Mixing, Fe(III) reduction and colored complex formation occurred as the sample zone was transported toward detection. For establishment of the tandem stream, steps 2b–4b were 10-fold repeated, and the time for introducing the solutions (plug volumes) was accordingly reduced in order to maintain the total volume constant. The merging zones approach was implemented by modifying the valve timing course in order to permit the simultaneous introduction of S_2, S_3, and S_4 into the analytical path.

Matrix matching

The molybdenum-blue reaction [14] was exploited in the experiments to demonstrate the effect of the reaction rate on the accuracy in the presence of pronounced matrix differences. The flow system in Fig. 1 was operated according to the valve timing in Table 2 that provided two conditions for reaction development.

Table 1 Valve timing related to Fe(II)-phen reaction. Valves V_i direct the S_1, S_2, S_3, and S_4 solutions (carrier, sample, reducing reagent, and phen); 0 or 1 corresponds to the valve status in Fig. 1 and to the alternative one.

Step	V_1	V_2	V_3	V_4	t (s)
a. Sandwich					
1	0	0	0	0	—
2	1	0	1	0	6
3	1	1	0	0	6
4	1	0	0	1	6
b. Tandem flow					
1	0	0	0	0	—
2	1	0	1	0	0.6
3	1	1	0	0	0.6
4	1	0	0	1	0.6
c. Merging zones					
1	0	0	0	0	—
2	1	1	1	1	6

Table 2 Valve timing related to molybdenum-blue formation. S_1, S_2, S_3, and S_4 correspond to carrier, sample, ammonium molybdate, and ascorbic acid solutions. Other symbols as in Table 1.

Step	V_1	V_2	V_3	V_4	t (s)
a. Monitoring of reaction development					
1	0	0	1	1	—
2	1	1	1	1	10
3	0	0	1	1	10
4	1	0	0	0	35
5	0	0	0	0	20
b. Strategy for detecting matrix effects					
1	0	0	1	1	—
2	1	1	1	1	10
3	0	0	1	1	23
4	1	1	1	1	10
5	0	0	1	1	3
6	1	0	0	0	10
7	0	0	1	1	20

For monitoring the chemical reaction, all solutions were let to recycle (Table 2a) when the central portion of the processed sample was passing through the flow cell (stopped-flow approach). Furthermore, the system was operated to permit sample processing with (Table 2, steps 4b–7b) or without (Table 2, steps 2b and 3b) including a trapping period, thus providing analytical signals referred to two mean sample residence times.

Solid-phase reagents

Nitrate determination based on reduction to nitrite by copperized cadmium fillings [15] was selected to demonstrate the effect of the continuous lessening of the reduction efficiency when unrenewable solid-phase reagents are employed. The system was operated according to Table 3a, permitting the periodical insertion of the reference solution S_3 within the samples. For recalibration, S_3 aliquots were introduced in tandem with plugs of the carrier solution S_1 (Table 3b). The variations in S_3 volumetric fraction (0.00, 0.20, 0.40, 0.60, 0.80, 1.00) were attained by modifying the V_1–V_3 timing, and maintaining the total length of the binary string [6].

Reagents and solutions

All solutions were prepared with deionized water and analytical grade chemicals. Solutions (S_1–S_4) employed in each system are described below.

In the experiments related to Fe(II)-phen formation, the carrier solution (S_1) was a 0.10 mol l^{-1} acetate buffer (pH 5.0) solution. Iron(III) solutions (5.00 mg l^{-1}) prepared with 0, 50, 100, or 160 mg l^{-1} Cu(II) in 0.10 mol l^{-1} HCl were used to simulate the samples (S_2). The reducing reagent (S_3) was a 1.0% (m/v) ascorbic acid solution. The color forming reagent (S_4), 0.12 or 0.25% (m/v) phen, was prepared in water.

Studies related to molybdenum-blue formation were carried out by using 1.0% (m/v) ammonium molybdate (S_3) and 1.0% (m/v) ascorbic acid (S_4) solutions. Reference solutions (S_2) within 2.50 and 10.0 mg l^{-1} P (as KH_2PO_4) were prepared in 0.25 mol l^{-1} $HClO_4$. A perchloric acid solution with the same concentration was used as carrier stream (S_1). In order to investigate the influence of sample acidity, 5.00 mg l^{-1} P solutions were prepared in 0.10, 0.25 and 0.40 mol l^{-1} $HClO_4$.

Nitrate determination in waters involved reduction to nitrite inside a copperized cadmium mini-column, followed by a diazo-coupling reaction [15]. The mini-column and the chromogenic reagent (S_4) were prepared as previously described [12,15]. The carrier stream (S_1) was a 1.0% (m/v) ammonium chloride plus 0.2% (m/v) sodium tetraborate solution (pH 7.8) also 0.01% (m/v) Na_2EDTA. The ref-

Table 3 Valve timing related to nitrate determination. S_1, S_2, S_3, and S_4 correspond to carrier, sample, reference, and reagent solutions. Other symbols as in Table 1. For recalibration, the volumetric fraction of the reference solution was varied from 0.00 to 1.00, by modifying the sampling times t_1 and t_2 ($t_1 + t_2 = 10$ s).

Step	V_1	V_2	V_3	V_4	t (s)
a. Monitoring of the mini-column efficiency					
1	0	0	0	1	—
2	1	1	0	1	10
3	0	0	0	1	23
4	1	0	1	1	10
5	0	0	0	1	23
b. Recalibration					
1	1	0	1	1	t_1
2	0	0	0	1	t_2
3	0	0	0	1	23

erence solution (S_3) was 0.500 mg l^{-1} N–NO_3^-. The river water samples (S_2) were filtered through 0.45-μm cellulose acetate membrane and analyzed in the same day without any further treatment [14].

RESULTS AND DISCUSSION

Partial overlap of sample and reagent zones

Inaccurate results are sometimes obtained in systems based on sequential introduction of sample and reagent aliquots especially when reagent excess is required. In these systems, the overlap of the dispersed zones comprises different fluid elements with distinct sample and reagent amounts, and the signal maximum corresponds to the optimized volumetric fractions. Interfering species may consume the reagent, modifying its concentration in the considered fluid element. Therefore, a larger amount of reagent is "selected" by the system to compensate the reagent consumed by the side reaction. A higher reagent amount means a lower sample contribution at the fluid element yielding the analytical signal. The recorded peak maximum corresponds then to another portion of the overlapped zones with different sample and reagent volumetric fractions.

The aspect was exemplified by the interference of copper in the iron reaction with phen [13]. When the proposed system was operated in the sandwich configuration (Table 1, steps 1a–4a), modifications in analytical signals due to addition of the interferent manifested themselves (Fig. 2). Copper reacts with phen without yielding a pronounced absorbance increase. In the presence of the interferent, the peak maximum is therefore delayed and reduced (Fig. 2), affecting accuracy.

The hindrance is minimized by designing the flow system in such a way that a given reagent volume always interacts with the sample. This is inherent to systems exploiting tandem flow [6] where a preselected number of sample plugs interacts with a given reagent volume under improved mixing conditions. This holds also in flow systems with the confluent streams, including those with merging zones. Overlapping of the established zones guarantees the required reagent excess. This was confirmed by operating the system in Fig. 1 according to the valve switching scheduled in Table 1 (steps 1b–4b or 1c–2c) and using 0.12% (m/v) phen as S_4. Inaccuracy due to addition of copper underwent a pronounced reduction when tandem flow or merging zones were exploited (Table 4), reflecting better reagent utilization.

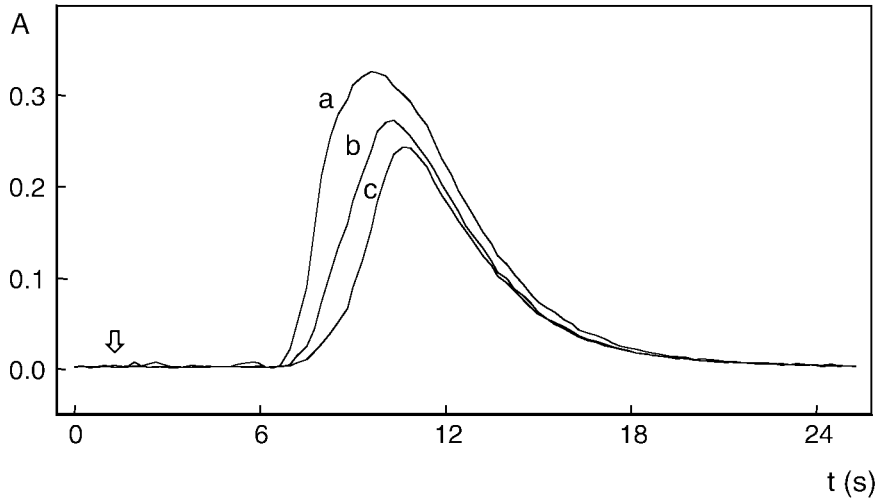

Fig. 2 Influence of interferent addition in the iron determination. Recorded tracings a, b, and c correspond to additions of 0.00, 100, and 160 mg l^{-1} Cu. Arrow specifies the instant of sample insertion into the system. Figure refers to the system in Fig. 1 with S_1 = 5.00 mg l^{-1} Fe, S_4 = 0.12 m/v phen, B_1 = 5 cm, B_2 = 100 cm, and λ = 512 nm, operated according to Table 1.

Table 4 Iron determination in 5.00 mg l^{-1} Fe solutions with different copper concentrations. Data correspond to means and uncertainties (n = 3)

Copper concentration (mg l^{-1})	Sandwich technique	Tandem flow	Merging zones
0	5.01 ± 0.05	4.98 ± 0.03	4.96 ± 0.05
50	4.64 ± 0.01	4.97 ± 0.02	4.96 ± 0.09
100	4.09 ± 0.09	4.92 ± 0.07	4.95 ± 0.05
160	3.59 ± 0.03	4.35 ± 0.03	4.03 ± 0.01

An alternative way to circumvent the above-mentioned drawback is to use a higher reagent amount. In the present procedure, interference effects were reduced in about 90% by increasing the phen concentration to 0.25% m/v. The strategy is, however, not always recommended because the expected copper content in the sample batches seldom reaches the interference threshold value. Moreover, phen solubility—0.30% m/v, 25 °C—is another limiting factor.

The proposed system is able to deal with this aspect. For sample batches where copper contents are within a specified range, the system can be operated according to any of the strategies in Table 1. For batches with high variability in interferent content, operation of the system with tandem flow or merging zones should be preferred. The strategy exploiting merging zones (Table 1, steps 1c–2c) is the fastest, as a single sampling step is concerned. It should be emphasized that tandem flow can be implemented by operating the system with a single pumping tube [6].

Matrix matching

Without a suitable matrix matching, inaccurate results can be a consequence of differences in kinetics of the processes to which sample and reference solutions are submitted. The aspect was exemplified in the determination of phosphate in plant digests based on the molybdenum-blue formation [14]. As the rate of heteropolyacid reduction by ascorbic acid diminishes by increasing the sample acidity (Fig. 3), inaccurate results can be observed for some samples in batches with pronounced variability in acidity. In routine analyses, it has been noted that acidity of the digests are usually within 0.1 and 0.4 mol l^{-1} $HClO_4$, and thus the reference solutions are prepared in 0.25 mol l^{-1} $HClO_4$ that corresponds to the sample mean acidity. Therefore, if the system in Fig. 1 provides a too short mean sample residence time, the analytical signal will be strongly affected by variations in acidity, resulting in positive and negative systematic errors. The effect was surpassed by increasing the mean sample residence time to about 40 s. For this task, the most concentrated portion of the sample zone was stopped inside the flow cell by switching of valve V_1 (Table 2).

It is always possible that a longer time is required for some specific samples, and a general procedure should be available to deal with unsuitable sample/reference matrix matching. With the proposed system, the sample was submitted to two processing times (Fig. 4). For every injected solution, the higher recorded peaks correspond to a situation where the sample zone was trapped during 10 s inside the main reactor [10], and the lower one reflects the situation without trapping. When matrix differences are not significant, concentrations estimated by considering the first and second analytical signals tend to be in agreement. An statistical test can be real time performed to evaluate this aspect, and the final concentration is the mean of both results, that is inherently more accurate because it is based on results obtained under different conditions [16]. The strategy succeeded in detecting the inaccuracy source, as can be concluded after statistic analysis of data related to Fig. 4: only the b set is characterized by results in agreement with each other (95% confidence level) regardless of the processing time. In the routine praxis, it has been verified that only a few samples have been run with longer processing times. For these samples, the proposed system is able to permit different strategies to be implemented, such as the increase in trapping period to 35 s (Fig. 3), or the sample processing with higher dispersion. After some

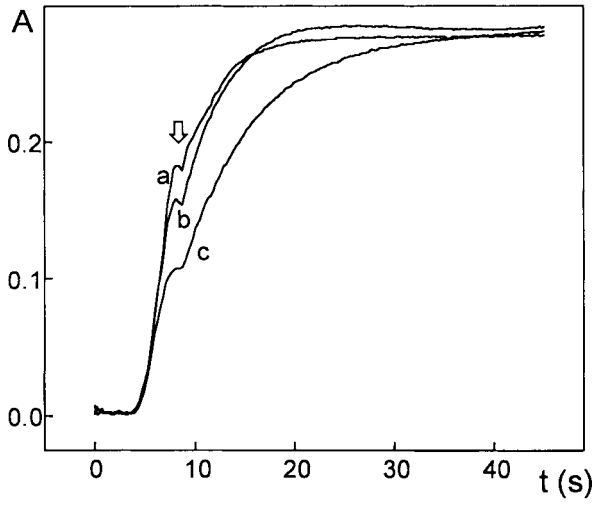

Fig. 3 Influence of sample acidity on the rate of molybdenum blue formation. Figure refers to 5.00 mg l^{-1} P solutions in 0.10 (a), 0.25 (b), and 0.40 (c) mol l^{-1} $HClO_4$ introduced into the system of Fig. 1 with B_1 = 5 cm, B_2 = 100 cm and λ = 690 nm, operated according to Table 2a. Arrow specifies the instant of sample trapping inside the flow cell.

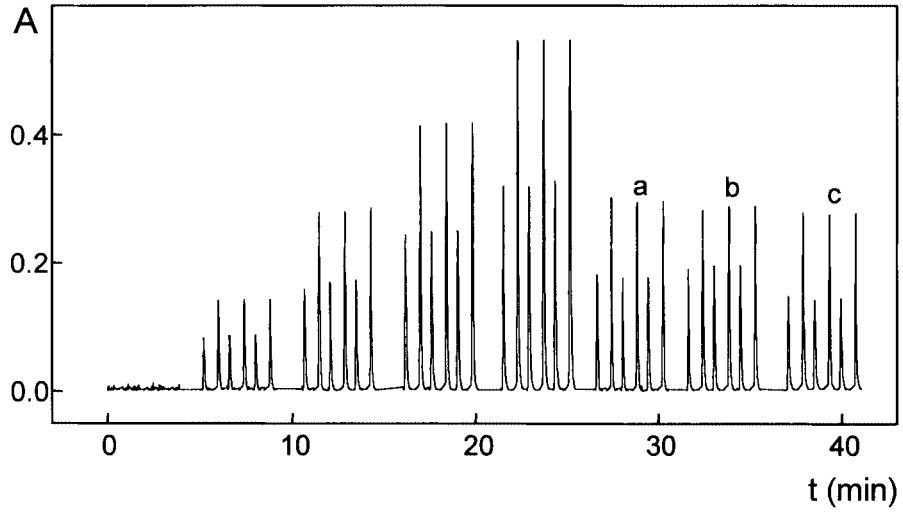

Fig. 4 Detecting matrix effects in the phosphate determination. Figure refers to the system in Fig. 1 with B_1 = 5 cm, B_2 = 100 cm, and λ = 690 nm, operated according to Table 2b. Signals in triplicate correspond to 10 and 20-s residence times. From left, signal recorded sets for 0.00, 2.50, 5.00, 7.50, and 10.0 mg l^{-1} P in 0.25 mol l^{-1} $HClO_4$, followed by 5.00 mg l^{-1} P solutions in 0.10 (a), 0.25 (b), and 0.40 (c) l^{-1} $HClO_4$.

modifications, the system could also implement the standard addition method for circumventing the influence of the sample matrix.

Solid-phase reagents

Analytical procedures involving solid-phase reagents may be susceptible to inaccuracy due to the slow consumption or modification of the immobilized reagent that may lead to a continuous drift in the analytical signal. The proposed set-up is able to deal with this limitation by processing a reference solution after every assayed sample and correcting for eventual deviations. The simplest way is to apply a correction coefficient estimated as the ratio of recorded peak heights (initial measurement/actual measurement) corresponding to the reference solution. When the variation in reference signal surpasses a preset value, the analytical curve should be renewed. In extreme situations, reconditioning or even replacement of the solid-phase reagent is needed.

The above-mentioned aspect has been verified in the large-scale analysis of natural waters for nitrate (Fig. 5). The slight drift in the reference signal (Fig. 5a) is due to the continuous lessening in reduction efficiency of the mini-column caused by the organic matter in the samples [15]. After proper correction, the results were in agreement with those obtained by a procedure involving the standard addition method. Regarding Fig. 5a, the correction procedure was suitable for the first nine samples; for the tenth one, an unacceptable deviation in results was observed even after applying the correction coefficient. In this situation, the system permitted another analytical curve to be attained by using a single reference solution. This was done by stepwise varying the sample volumetric fraction (Fig. 5b). In the routine praxis of this laboratory, the correction coefficient is usually applied when the decrease in peak

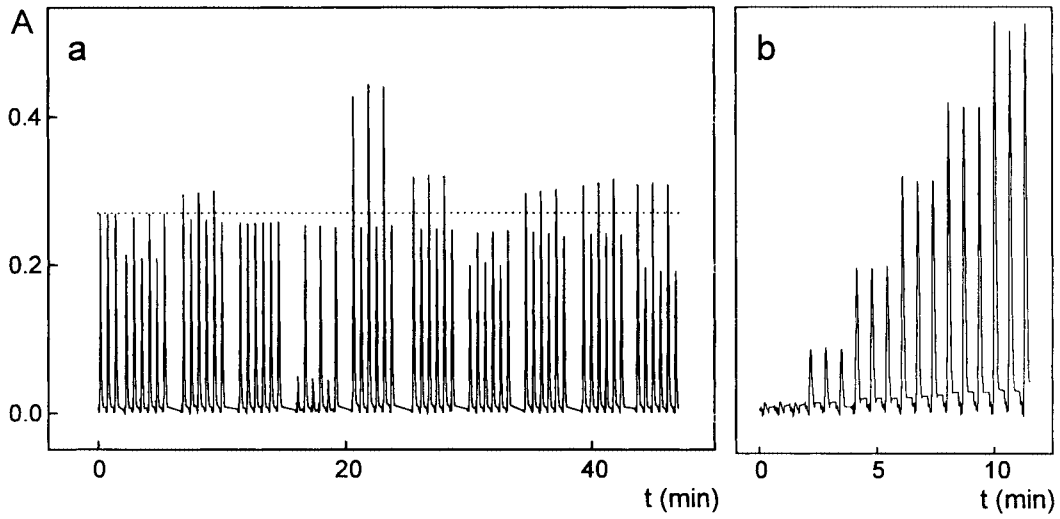

Fig. 5 Detecting the continuous lessening in efficiency of the reducing mini-column. Figure refers to the system in Fig. 1 with B_1 = 50 cm, B_2 = 100 cm, and λ = 535 nm, operated according to Table 3. (a) From left, signals recorded in triplicate for a 0.500 mg l^{-1} N (as nitrate) reference solution followed by signals for 10 natural water samples intercalated with signals for the same reference solution. Dashed line indicates the initial peak height related to the reference solution. (b) Calibration run based on different S_3 volumetric fractions (0.00, 0.20, 0.40, 0.60, 0.80 and 1.00).

height related to the reference solution is lower than 10% relatively to the initial measurement (dashed line: Fig. 5a). Renewal of the analytical curve is done for deviations within 10 and 25%; and column reconditioning is performed for deviations higher than 25%.

Incomplete masking

Measurements in flow analysis are often performed without attaining chemical equilibrium, but quantitative reaction development is required for masking purposes. When kinetics of the masking reaction is a potential source of inaccuracy, the system should be able to perform measurements under two timing conditions, e.g., measuring the processed sample directly or after a trapping period. If results are in agreement with each other, masking was already quantitative and the final result should be estimated as the mean of the previous ones. If the results are different, the trapping period should be increased, results evaluated again, and so forth.

Moreover, there are methods where sensitivity is hindered by the addition of a masking agent due to the involved chemical equilibria. The masking concentration should be defined as a compromise between the acceptable sensitivity drop and the maximum allowed concentration of the interfering species. A typical example is the spectrophotometric determination of calcium and magnesium in plant digests with CPC as the color-forming reagent [17]. Under alkaline conditions, CPC reacts with calcium and magnesium yielding colored complexes with similar wavelengths for maximum absorption. EGTA and 8-hydroxyquinoline are often used as masking reagents, and their concentrations are chosen as a compromise between sensitivity and selectivity.

With the proposed system using the lowest volumes of masking solutions, trial measurements are done for roughly estimating the analyte contents. Thereafter, interferent additions are performed and their influence on the analytical signal is taken into consideration, allowing different masking concentrations to be selected. With the approach, different analytical curves are used and the sensitivity is usually enhanced. In addition, the consumption of masking agents is reduced thus minimizing the difficulties related with waste disposal.

CONCLUSIONS

The proposed system is robust, easily operated, and attractive for routine analysis, and has proven to be a powerful tool for minimizing systematic errors. It yields precise measurements characterized by relative standard deviations usually lower than 2.0% under all the investigated situations. During 8-h continuous operation periods, no baseline drift was observed regardless of the involved analytical procedures. Implementation of the strategies for detecting and circumventing potential sources of inaccuracy leads to an improvement of the result reliability and does not impair the analytical capacity of the laboratory.

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