Between-method carryover in flow analysis

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Abstract

Between-method carryover due to reagent replacement in flow systems devoted to multiparametric determinations is critically discussed. For this task, a model system involving the spectrophotometric procedure for iron speciation in natural waters based on the known reaction of Fe(II) with 1,10-phenanthroline was initially designed. Furthermore, other systems for different bi-parametric assays were also highlighted. Potentialities and limitations of the different strategies for compensating and/or reducing between-method carryover are discussed, and guidelines for laboratory management are withdrawn.

Keywords: carryover, flow analysis, spectrophotometry, iron speciation, random access reagent

1. Introduction

Carryover reflects the contribution of one assay to the next in terms of analytical signal. It should be minimized and/or compensated in order to keep analytical accuracy. In flow analysis, it is usually the main limiting factor in sampling rate, harming the reagent consumption, especially in procedures involving a continuously flowing reagent stream. Mentioning the degree of carryover is then recommended when specifying sample throughput [1]. Classical sources of carryover are the overlap between successive sample zones, the sample residues inside the analytical path, and detector memory effects.

Sample overlap is a consequence of zone broadening related to the continuous dispersion process occurring during sample transport towards detection. The effect is minimized by improving the design of the flow set up and/or by lessening the sampling rate [2]. Sample residues remaining in the analytical path are generally associated with the sampling step, especially when a sampler is available. Portions of the sample are then retained - usually inside the sampling probe - influencing next assay. Carryover may also manifest itself in poorly rinsed systems where sample portions remain inside specific sites of the manifold [3]. Memory effects are noted when restrictions inherent to the detection unit impair the baseline restoration before sample monitoring. A fraction of the analytical signal related to the previous sample is then added to the net analytical signal. The drawback occurs mainly in relation to detectors characterized by high damping factors [4] and/or low temporal performance [5].

Two kinds of carryover have been reported, the within-method and between-method carryover [6]. The former is inherent to flow-systems devoted to single analyte determinations and is efficiently compensated by applying Eq. 1 [7 -10]:

$$R'_n = R_n - k\,R_{n-1} \qquad (1)$$

where R = reading related to measurement of the n^{th} sample; R' = reading corrected for carryover compensation; k = carryover coefficient, experimentally evaluated by processing two sample solutions with known analyte concentrations or a sample plus the blank [7]. Alternatively, only one sample solution can be used, and the difference between the first measurement and that obtained after several successive sample processing is considered [11].

The potentiality of Eq. 1 for compensating within-method carryover in flow-injection analysis was recently demonstrated in a turbidimetric procedure for determination of potassium in fertilizers [10]. The sample throughput underwent a 4-fold increase simply by setting a constant carryover degree of 7 %.

Between-method carryover is inherent to multiparametric determinations and has been discussed mainly in relation to clinical chemistry [6,12]. In flow analysis, this kind of carryover may be a limiting factor in e.g. sequential determinations [13,14] (including speciation [15,16]), particularly in connection with random reagent access [6,13]. This approach has usually been accomplished by exchanging specific reagents at every analytical cycle; to this end, merging zones [17] and sandwich techniques [18] have been exploited. Between-method carryover as a potential source of inaccuracy in flow analysis has not been however studied deeply.

The main purpose of the present work was then to investigate the between-method carryover in unsegmented flow systems in order to understand how it may affect the measurement of the next sample and to provide guidelines for management of laboratories devoted to large scale analyses. A multi-commuted set up was selected as a model system, as it can efficiently mimic a flow-injection system [19]; in this way, more general conclusions are gathered.

As a potential source of between-method carryover, the intermittent addition of reagents was investigated. The study was carried out with a model system involving the known reaction of Fe(II) with 1,10-phenanthroline. As applications the spectrophotometric speciation of iron in natural waters [20], as well as the sequential determinations of nitrogen and phosphate in plants [21], and fructose and glucose in syrups [22] were highlighted.

2. Experimental

2.1. Samples, standards, reagents

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All solutions were prepared with analytical-grade chemicals and distilled/deionised water. Natural water samples were collected into polyethylene bottles, filtered through 0.45 μm cellulose membrane filters and analysed in the same day [23]. Plant digests and syrups were prepared as described [21,22].

The 1000 mg l^{-1} Fe(III) stock standard solution was based on iron fillings, and the Fe(II) solution - freshly prepared - was based on $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$. Working standards within 0.00 and 10.00 mg l^{-1} Fe were prepared by water dilutions of the above-mentioned solutions. The sample carrier stream (C - Fig. 1) was water and the alternating streams R_1' and R_1" were water and a daily prepared 1.0 % (w/v) ascorbic acid solution, respectively. Reagent R_2 was a 0.12 % (w/v) 1,10-phenanthroline solution prepared in 0.05 mol l^{-1} acetic acid/acetate buffer solution, pH 4.7 [20].

2.2. Apparatus

The flow set up comprised a model IPC-8 Ismatec peristaltic pump, an electronically operated double-loop injector [21], a model 482 Femto spectrophotometer with a 10-mm optical path, 80-μl inner volume flow cell, a model 111 Kipp & Zonen recorder, 161TO31 NResearch three-way solenoid valves, and accessories. Sampling loops, coiled reactors (2-cm winding diameter) and transmission lines were built up with 0.5 mm i.d. Teflon tubing.

The sample was always aspirated to fill a sampling loop (Fig. 1) and any switching of the double-loop injector intercalated the selected sample volume into the carrier stream. Reagent exchange was done in synchronism with the injector operation by switching valves V_1 and V_2. This permitted successive selected sample aliquots to be handled in the presence and absence of the reducing agent. As the sample was injected twice, the established sample zones were processed under two different conditions, depending on the presence of R_1' or R_1" (added at point x) downstream. After mixing with R_1' (or R_1"), the sample zone reached next confluence point y, where reagent R_2 was added allowing involved chemical reactions [20] to proceed inside the main reaction coil B_2. Passage of the sample through the flow-cell resulted in a transient variation in the monitored absorbance, quantified at 512 nm and recorded as a peak. When the sample was handled in the presence of R_1', the recorded peak height was proportional only to the Fe(II) concentration and when ascorbic acid was directed towards the analytical path, the recorded peak height reflected both the Fe(II) and Fe(III contributions [20].

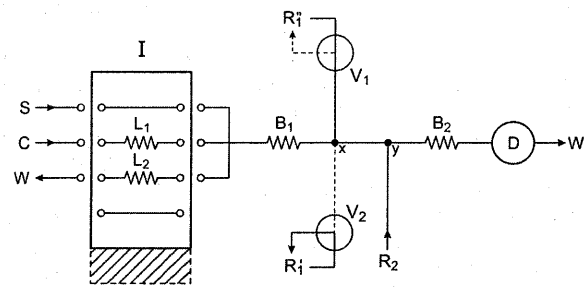

Fig. 1. Flow diagram. S = sample (> 2 ml min^{-1} through 100-cm L_1 or L_2 loop); I = injector (alternative position: dashed area); C = carrier stream (3.4 ml min^{-1}); R_1' and R_1" = intermittent reagents (1.0 ml min^{-1}); R_2 = colour forming reagent (1.0 ml min^{-1}); V_i = three-way solenoid valves; B_1 and B_2 = delay (50 cm) and reaction (100 cm) coils; D = detector (512 nm); W = waste; x, y = confluence points.

The system was dimensioned to provide limited sample dispersion [2]; therefore the carrier-to-confluent flow rate ratios were selected as high as possible yet suitable to provide good mixing conditions. Sampling loops were selected as 100 cm (ca. 300 μl) as a compromise between sensitivity and sampling rate. Coil length and total flow rate were set to permit quantitative reaction development, suitable sampling throughput and low hydrodynamic pressure.

2.3. Procedure

In the initial experiments, the model system in Fig. 1 was used with a 50-cm B_1 delay coil. Both injector and V_i valves were simultaneously switched. This situation is herein referred to as the first strategy. The model system was further modified: the B_1 coil was set as short as possible (10 cm), as no chemical reaction took place inside it, and reagent exchange was done before sample injection. This approach is herein referred to as the second strategy. Influence of the time elapsed between valve and injector switching was investigated. After system dimensioning, the main figures of merit were evaluated. As carryover manifested always as very pronounced, the system was not directly applied to iron speciation in natural waters. Another strategy involved the design of very simple systems derived from that in Fig. 1 but without alternating streams. The R_1 channel was removed for Fe(II) determination and restored for Fe(II) plus Fe(III) determination. In this latter situation, herein referred to as the third strategy, the ascorbic acid reagent flew continuously through it.

The analytical characteristics inherent of the different strategies were evaluated and the systems were applied to the analysis of natural waters, plant digests and syrups. The latter analyses were carried out by using flow set-ups similar to those already described [21,22].

3. Results and Discussion

The model system was suitable to investigate between-method carryover effects, as a stable baseline was always noted and precise measurements (r.s.d. < 1 %) were attained regardless if the quantified fluid element was at the central, front or trailing portions of a dispersed solution. As R_1" was added in an alternating fashion to allow Fe(III) reduction, reagent residues affected the following Fe(II) determination, ideally carried out in absence of any reducing agent.

A linear relationship between absorbance and iron concentration was observed for single standard solutions of both Fe(II) or Fe(III). Reduction of Fe(III) inside the main reactor B_2 was quantitative, as no statistical differences between angular coefficients of Eqs 2 and 3 were found at the 95 % probability level. The related analytical equations (n = 6) were:

$$h = (0.006 \pm 0.004) + (0.063 \pm 0.001)\, C_{Fe(II)},\ r = 0.9997 \quad (2)$$

$$h = (-0.004 \pm 0.005) + (0.065 \pm 0.001)\, C_{Fe(III)},\ r = 0.9996 \quad (3)$$

where, h = peak height, in absorbance; $C_{Fe(II)}$ and $C_{Fe(III)}$ = Fe(II) and Fe(III) concentrations, in mg l^{-1}.

Figure 2 was obtained by using Fe(II) standard solutions to mimetic all the involved solutions [24]; therefore the recorder tracings reflect the relative volumetric contributions of the different solutions. This figure refers to the first strategy and shows that about 4 % of R_1' (or R_1") are still present at the central portion of the next sample zone during its passage through the flow-cell. As a consequence, about 96 % of R_1" (or R_1') concentration are reached. These data are usually acceptable for sequential determinations. In the present procedure, the 4 % lessening in ascorbic acid concentration did not affect the Fe(III)

determination, but the remaining ascorbic acid manifested itself as a pronounced between-method carryover source in the Fe(II) determination. This confirmed that the 50-cm delay coil (B_1 - Fig. 1) was not enough for proper reagent exchange before arrival of the following sample zone to the confluence point x.

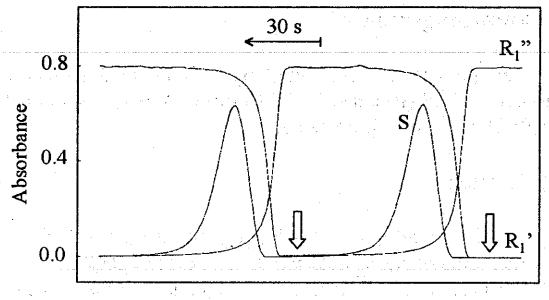

Fig. 2. Recorder tracing – first strategy. Figure refers to the system in Fig. 1 with a 50-cm delay coil and simultaneous operation of the injector and V_i valves. Arrow = instant of sample injection/valve switching; S, R_1' and R_1" = sample and intermittent reagents. For each tracing, an Fe(II) solution was used instead of the specific solution and water was placed in the other channels, except for the R_2 channel that directed the colour forming reagent. Fe(II) concentrations = 10.00 or 30.00 mg l^{-1} for mimics the sample or R_1 reagents, respectively.

In order to circumvent this drawback, the delay coil was increased: system simplicity was maintained, two different situations for sample processing were efficiently attained and manual operation of the injector was feasible. It should be stressed that most of the previously proposed strategies for sequential determinations and/or speciation are amenable with this strategy. In the model system with a 100-cm delay coil, the reagent replacement started ca 10 s before sample arrival at confluence point x, and was almost complete (about 98 %) at the centre of the sample zone during its passage through the detector. Schlieren noise due to the establishment of concentration gradients along the R_1'/R_1" boundaries was not observed. Under these conditions, however, application of the system to iron speciation in natural water samples could not be recommended because, in spite of the low contributions of R_1" to the sample zone related to Fe(II) determination, a pronounced carryover effect was observed. This is explained by recalling that a very low amount of ascorbic acid is enough for partial although erratic reduction of Fe(III). Moreover, doubling the delay coil length led to a 40 % reduction in sampling rate meaning a pronounced increase in the reagent consumption.

Another possibility was to promote the reagent exchange (V_1 or V_2 switching) before sample injection. Implementation of this second strategy required however a higher degree of system automation, as precise timing control for sample and reagent insertion was required. As the delay coil was not needed, sampling rate - thus reagent consumption - was improved. A noteworthy feature of this second strategy is the enhanced versatility. As an example, one could stress that the delay time could be selected at will without any system reconfiguration.

Fig. 3 refers to the system without a delay coil and with the sample injected 25 s after reagent exchange. Almost quantitative reagent replacement at the central portion of the sample zone was attained. Analysis of the peak shaped signals does not reveal any overlap between successive sample zones. This feature was guaranteed by lessening the sampling frequency. In this situation, carryover effect due solely to between-method carryover was not observed.

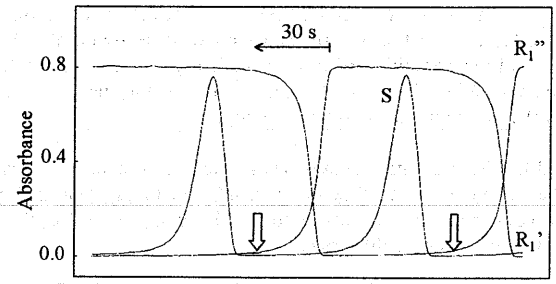

Fig. 3. Recorder tracing – second strategy. Figure refers to the system in Fig. 1 without the delay coil; sample injected 25 s after reagent exchange. Symbols and conditions as in Fig. 2.

Exploitation of the second strategy cannot be recommended for iron speciation indeed. Even with an ascorbic acid concentration less then 1 % of its initial concentration a 25 % carryover degree is still observed (Fig. 4). Parallel experiments, confirmed that selecting an injection delay larger than 100 s could eliminate this carryover, but this led again to a pronounced drop in sampling frequency. The low amount of ascorbic acid remaining in the flow line during next sample handling was still enough to reduce a significant portion of Fe(III) leading to inaccurate results for Fe(II), as emphasized in Tab. 1. It should be noted that the "envelope" in this figure does not tend to reach baseline. This is probably due to adherence effects of the coloured products on the tubing inner walls, connectors and flow-cell; the phenomenon has not been noted in real analysis because the standard solutions are not added continuously.

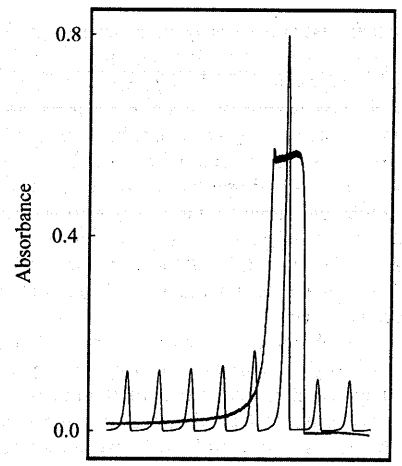

Fig. 4. Between-method carryover. Figure refers to the system in Fig. 1 operated according to the conditions specified in Fig. 3, but using the solutions required for iron speciation. Recorded peaks correspond to successive injections of a 2.00 mg l^{-1} Fe(II) plus 10.00 mg l^{-1}(Fe(III) solution. "Envelope" tracing obtained by placing a 10.00 mg l^{-1} Fe(III) solution as sample carrier stream (C – Fig. 1), and adding the ascorbic acid only once.

Regarding accuracy, Tab. 1 permits a comparison of the results for Fe(II), total Fe and Fe(III) determination obtained with the system in Fig. 1 (second strategy), and with the two-separate system approach (third strategy). Differences between strategies are evident, especially with regard to Fe(III), more affected by between-method carryover effects. Depending on the Fe(II)/Fe(III) concentration ratio in the sample, a relative deviation sometimes as high as 1300 % was observed in the Fe(II) quantification. Results obtained with the third strategy are in agreement with those obtained by a reference procedure [20].

At this point, it should be recalled that the concentration of ascorbic acid in the R_1" reagent cannot be lessened at will, in view of the possibility of having oxidising species in the natural water samples [23]. Therefore, it was decided to run the samples by the two-separated systems strategy.

Table 1. Comparative results. Data (mg l^{-1} Fe) obtained by using the two-independent system approach and the system in Fig. 1 without the delay coil and with a 25-s delay time. Results for Fe(III) obtained by difference.

Sample	Third strategy			Second strategy		
	Fe(II)	Total Fe	Fe(III)	Fe(II)	Total Fe	Fe(III)
1	2.08	4.19	2.11	4.15	4.34	0.19
2	4.21	6.68	2.46	6.81	6.94	0.13
3	2.13	3.66	1.53	3.62	3.87	0.24
4	0.20	1.03	0.82	0.84	0.95	0.11
5	0.38	8.16	7.78	5.30	8.26	2.96
6	1.00	4.83	3.83	4.75	4.98	0.23
7	0.43	7.29	6.86	5.56	6.75	1.19

Regarding other applications, between-method carryover effects were not observed in relation to syrup analysis. It manifested itself however in the sequential determination of nitrogen and phosphate in plant digests. In fact, reagent residues impaired the determination of nitrogen (as ammonium), and the drawback was circumvented either by exploiting the second strategy (a double delay time before nitrogen determination) or by using the less usual sodium molybdate reagent [21].

4. Conclusions

Intermittent addition of reagent is an important parameter affecting sampling rate in the flow system of Fig. 1. Remaining ascorbic acid influencing the Fe(II) determination impaired its application to iron speciation in natural waters; therefore, the first two investigated strategies cannot be recommended for this analysis and two systems should be used. Under the practical point of view, this is not a pronounced limiting aspect, especially for large sample lots. In fact, a number of samples can be run for Fe(II) and, after modifying the system design, run again for Fe(II) plus Fe(III). The total analytical time is about the same in relation to sequential determinations, but laboratory management becomes cumbersome.

The spectrophotometric determination of fructose and glucose in syrups [22] was not limited by this aspect, because it involves sample handling under two different pH values. As two buffer reagents with high buffering capacity were exchanged, the strategy became less susceptible to between-method carryover. In fact, a 4 % residue is not enough to impair the procedure, and a flow system similar to that in Fig. 1 can be recommended. As a general rule, the effect of a reagent residue is less relevant when the next reagent overcomes it.

An intermediate situation is that related to sequential determination of nitrogen and phosphate in plant digests, for which different possibilities to circumvent between-method carryover are available.

In short, when between-method carryover is concerned, there are different strategies for management of the laboratory dedicated to large-scale analysis. The first strategy (R_1'/R_1" exchange simultaneously with sample injection - need for a delay coil) is the most simple and can be recommended for large sample batches. Manual operation of the system is feasible. The second strategy (R_1'/R_1" exchange before sample injection - no delay coil) is more efficient to circumvent between-method carryover, but the resulting system is more complex, requiring external computer control and several discrete devices in the manifold. Enhanced versatility is attained: in fact, the delay time can be re-set at will without the need for modifying the system architecture. For extreme situations - like the investigated one in relation to iron speciation - the possibility of using separate flow manifolds should be taken in consideration.

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