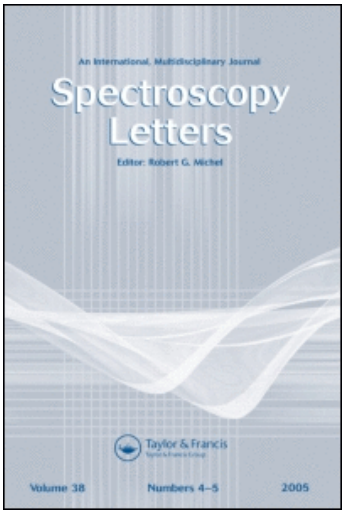

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Nonconventional Semiautomated Standard Addition Procedure Based on Membrane Micropumps for Flame Atomic Absorption Spectrometry

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Abstract: A manifold was studied that allowed the semiautomation of the standard addition method in flame atomic absorption spectrometry by use of two computer-controlled solenoid-based micropumps operating in a programmed way. One of the micropumps is used to inject several consecutive plugs of the sample solution while the other, which is operated out of phase, introduces plugs of a standard solution. In this way, a variant of the conventional standard addition method results. The theoretical background of the manifold is presented, and its ability to overcome classical interferences is experimentally verified. A modification of the procedure was proved to be suitable to deal with severe interferences, such as that produced by phosphate on calcium measurement,

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without the need of adding releasing agents. The reliability was assessed by use of certified reference materials. The manifold presented allows both sample and standard solutions as well as time to be saved. Thus, a four-point calibration graph can be obtained that uses 2 mL and 0.75 mL of sample and standard solutions, respectively.

Keywords: Automation, FAAS, micropumps, standard addition method

INTRODUCTION

The standard addition method (SAM) provides an excellent way to overcome some[1] of the interferences associated with atomic spectrometric methods, such as flame atomic absorption spectrometry (FAAS). However, the use of SAM in the conventional batch mode is time-consuming, as sample and standard solutions are first manually mixed in different proportions before the analytical signal can be obtained.

Present-day advances in both continuous flow methodology and automation offer interesting ways of facilitating FAAS in general[2] and SAM when required. Of particular interest are those approaches that, instead of introducing the solutions into the spectrometer by direct aspiration, use some type of remote-controlled propelling device for the purpose, which allows variable degrees of automation to be achieved. After the pioneering works of Tyson,[3,4] an excellent review was made by de la Guardia several years ago.[5] Recently, some papers have also addressed the subject from different points of view, the common denominator being the use of classical peristaltic pumps, coils, and valves.[6–10]

Although peristaltic pumps are the most frequently used propelling devices for analytical purposes,[11] interest has been aroused in recent years in the use of solenoid-based micropumps (MPs) which, similar to peristaltic pumps, are easily computer controlled. These low-cost, small propelling/injecting devices are versatile and have been shown to be particularly suitable for inclusion in flow manifolds.[12–16]

In this paper, a simple manifold for automating SAM based on the use of two computer-controlled MPs instead of peristaltic pumps is studied. The approach here presented is similar but not identical to SAM. The results demonstrate that this nonconventional semiautomated mode of operation presents advantages over the classical procedure, as even severe interferences can be solved.

MATERIALS AND METHODS

Reagents and Apparatus

All the measurements were obtained using a Perkin-Elmer Model 1100B (Norwalk, CT, USA) atomic absorption spectrometer. The transmittance analog signal was obtained directly from the motherboard of the instrument and

collected by a personal computer via a homemade data acquisition card, which also allowed the remote control of the pumps by means of voltage pulses. Calcium (II), copper (II), and iron (III) were used as analytes. Stock solutions of these elements (1000 μg mL^{-1}) were obtained from Panreac (Barcelona, Spain) and appropriate dilutions were made to obtain working solutions. The other chemicals used were obtained from Fluka (Buchs, Switzerland). Measurements were carried out at 422.7, 324.8, and 248.3 nm for Ca, Cu, and Fe, respectively, using ordinary hollow cathode lamps as the radiation sources. Air–acetylene flames were used exclusively, the nebulizer uptake rate being 15 mL min^{-1}.

Manifold

The manifold used is shown in Fig. 1. The pumps were two solenoid-operated membrane micropumps (Bio-Chem Valve Inc. Boston, MA, USA; reference 50SP12-125) that, theoretically, dispense 125 μL solution per stroke. The diaphragm of these micropumps (MPs) is maintained closed with an

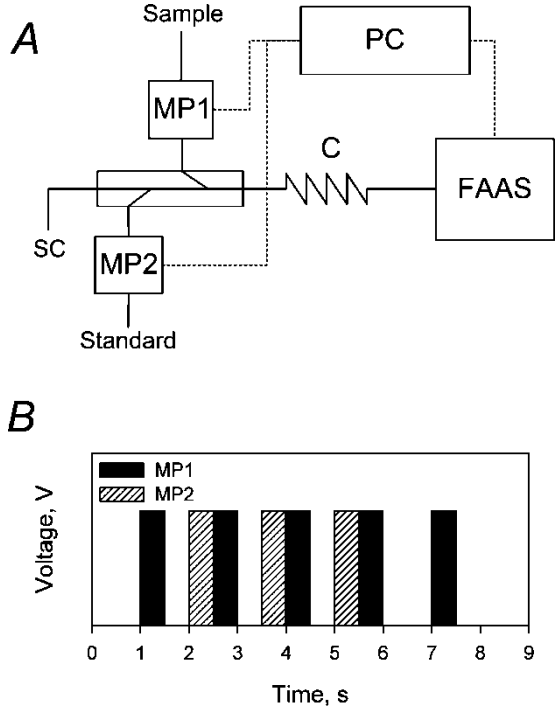

Figure 1. (A) Manifold used. MP1 and MP2, micropumps; SC, channel for compensating with solvent; C, connecting coil. (B) Timing diagram for MPs operation.

internal spring mechanism so that, when a voltage is applied, the solenoid pulls the diaphragm open. The fluid is dispensed from the pump when the voltage falls, the internal spring forcing the diaphragm to the closed position. The entire process, refilling and pushing liquid, requires 0.5 s. The MPs were operated by homemade software specifically developed for such a purpose. This software, written in C language, allowed the remote control of the pumps by means of square wave signals and included a number of parameters that can be selected by the operator, such as the time elapsing between the beginning of one stroke and the next, t_i, and the number of injections (strokes), n. In addition, it also permitted the direct plotting, in real time, of the absorbance–time profile on the computer screen and the absorbance–time data pairs to be saved in ASCII format for later handling. Other details are given elsewhere.[16]

Reaction coils and flow lines of 0.8-mm-i.d. PTFE tubing were obtained from Omnifit (Cambridge, UK). To connecting MPs, a Perspex piece was machined and suitably drilled at the Central Services of the University of Murcia. The micropump flow system was coupled to the flame atomic absorption detector equipment with the fluid outlet connected to the inlet of the nebulizer. It should be noted that, irrespective of the switching status of the pumps, the nebulizer is always fed with the same flow rate due to aspiration of solvent through the compensation channel included.

RESULTS AND DISCUSSION

Foundations of the Approach

Consider that, with MP2 stopped, several plugs of a sample solution of unknown concentration are introduced into the proposed manifold by means of a set of consecutive strokes (injections) carried out by pump MP1. The absorbance time profile thus obtained will depend on both the number of injections and the time elapsed between one injection and the following so that, as Fig. 2a shows, it would not be a set of narrow, well-differentiated peaks, but that resulting from the partial mixing of the analyte plugs propelled at each stroke. The pseudo-plateau thus obtained presents a number of oscillations whose amplitude depends on the t_i value used. It is clear that, provided the absorbance readings are within the linear response range of the spectrometer, the area under the absorbance–time profile, which is related to the amount of analyte reaching the detector, can be used as an analytical signal. Of course, very similar profiles could be obtained by using similar experiments involving repetitive injections made with conventional valves and peristaltic pumps, but the way here studied is more versatile and a single, easily computer-controlled, low-size, low-cost device is used.

Consider now that the experiment is modified in such a way that MP1 carries out a set of consecutive injections of a sample solution, as before,

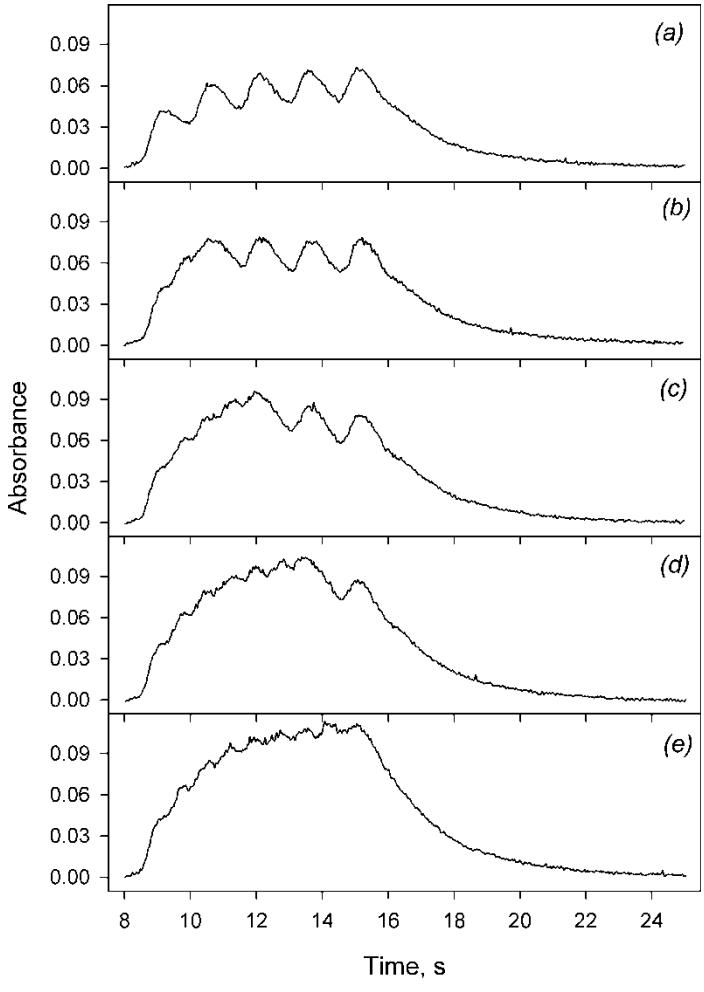

Figure 2. Absorbance–time profiles obtained by injecting plugs of analyte and standard solutions by means of MP1 and MP2, respectively, operated out of phase. Solutions containing 5 μg mL^{-1} calcium were used. For all graphs, five injections were carried out by MP1, while MP2 injected 0, 1, 2, 3, and 4 plugs leading to graphs *a*–*e*, respectively.

while MP2 carries out a set of injections of a standard solution. Both pumps operate at the same frequency, but they do not start at the same time, MP2 being out of phase as regards MP1. In this way, the plugs of standard solution are inserted between the plugs of the unknown solution (Figs. 2b–2e). According to this experiment, a plot of the number of injections, *n*, carried out by MP2 against the analytical signal should be a straight line, provided that the responses are within the linear response range and *n* is

below the number of plugs injected by MP1. The straight line thus obtained should allow the concentration of the unknown solution to be calculated. Such a calibration is, in fact, similar to the conventional standard addition method (CSAM) and, for simplicity and practical purposes, it could be named as a modified standard addition method (MSAM). It should be noted that an essential difference between both methods is that in the case of CSAM, the concentration of the solution reaching the detector increases with the number of additions. However, when using MSAM, the parameter that increases with the number of injections (strokes) carried out by MP2 is the amount of analyte passing through the detector, which is responsible for the subsequent increase in the analytical signal.

The extrapolation to the x-axis of the above-quoted straight calibration line should permit the concentration of the "unknown" solution to be calculated by means of the equation:

$$C_s = C_{st} \cdot \frac{n^*_{st}}{n_s} \cdot \frac{V_{st}}{V_s} = C_{st} \cdot \frac{n^*_{st}}{n_s} \cdot f_v$$

where n_s is the number of injections carried out by MP2, n^*_{st} is the value obtained for the intersection of the straight line with the x-axis, and f_v is the ratio between the volume delivered by both pumps at each stroke.

It is important to note that the procedure here described could at first glance be erroneously considered as complicated and difficult to carry out. On the contrary, the procedure is easy to use because the system is computer-controlled and the tasks of carrying out the injections and obtaining the analytical signal (area) are assumed by the software with no waste of time on the operator's part. The advantages of saving solutions and time (no manual mixing of solutions is required) are also clear.

Experimental Verification

The application of the above equation requires that the volumes (or the ratio of volumes) injected at each stroke by each pump be known exactly. Although both pumps used should theoretically deliver the same volume per stroke (125 μL), this was carefully measured. It was experimentally found that the volumes delivered by both pumps were not identical, the ratio between them, f_v, being 0.917, and this factor was used instead of unity for the rest of the experiments. It should be noted that in spite of the large number of strokes that were carried out, no alteration was observed in this ratio after intensive use of the micropumps.

The first step to verify the above reasoning was to obtain MSAM lines with solutions in the absence of a matrix effect. Figure 3 shows (line a) one of the graphs obtained to check the reliability of the approach, and Table 1 summarizes some experimental results made to confirm it.

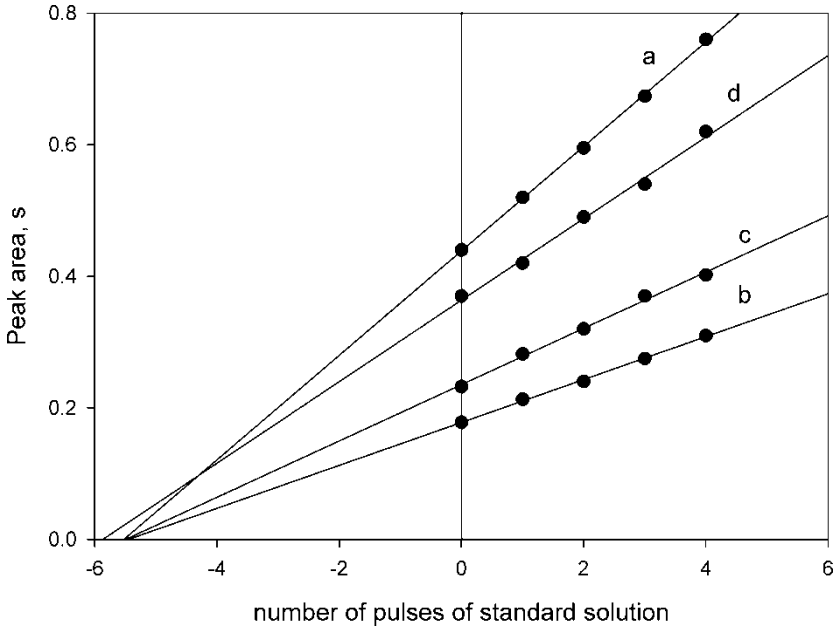

Figure 3. Several additions graphs obtained using MSAM. Line a was obtained using 5 μg mL^{-1} calcium solutions both as standard and analyte solutions; line *b*, *a* 5 μg mL^{-1} copper solution in the presence of 60% v/v ethanol; line *c*, *a* 5 μg mL^{-1} iron solution in the presence of 100 μg mL^{-1} cobalt; line *d*, similar to line *a*, but in the presence of 0.01 M phosphate. In this case, the MSAM variant based in the slope ratio is required to obtain correct results.

The suitability of MSAM as a semiautomatic alternative to the tedious CSAM when dealing with practical situations for which CSAM is normally used was then studied. It is well-known that CSAM is particularly suitable for correcting some nonspectral interferences, especially the transport interferences that often appear in FAAS, which include factors affecting the rate of liquid consumption, the efficiency of nebulization, and the fraction desolvated.[1] Thus, it is known that the presence of ethanol affects the FAAS determination of Cu(II), the analytical signal increasing as the proportion of ethanol in the sample increases. This is a typical interference that is corrected by CSAM and so it was thought that it could also be solved by MSAM, but more easily. This was indeed the case, and our results (see line b of Fig. 3 and Table 2) confirm the suitability of MSAM for dealing with this interference.

On the other hand, there are interferences that cannot be solved by CSAM, because they are dependent on the interferent/analyte ratio. Typical examples are the interferences caused by nickel or cobalt in the measurement of iron. In such cases MSAM proved effective because, due to the way in

Table 1. Results obtained for different calcium solutions using MSAM[a]

Sample ($\mu g\ mL^{-1}$)	Found[b]	
	n_{st}^{*c}	[Ca] calculated ($\mu g\ mL^{-1}$)
2.0	2.2 ± 0.1	2.0 ± 0.1
4.0	4.3 ± 0.1	3.9 ± 0.1
6.0	6.6 ± 0.1	6.1 ± 0.1
8.0	8.7 ± 0.1	8.0 ± 0.1

[a]A 5 $\mu g\ mL^{-1}$calcium solution was used as the standard solution.
[b]Mean value $\pm$ standard deviation (n = 5).
[c]Number equivalent of injections of the standard solution.

which the approach is carried out, the interferent/analyte ratio remains nearly constant in each experiment along the MSAM line. This was experimentally verified; the results shown in Table 2 demonstrating that MSAM effectively overcomes the interference.

In addition, it is well-known that CSAM cannot overcome certain severe interferences, such as that of phosphate or titanium in the determination of calcium, because at low analyte concentrations there is no linear relationship between signal and analytical concentration, and, consequently, the extrapolation in the absence of releasing agents leads to incorrect results. It was thought that the same would occur with MSAM, which is also an extrapolation-based method, and, indeed, its unsuitability to deal with these severe interferences was verified experimentally. However the manifold here presented allows this difficulty to be solved by means of a related semiautomatic approach based on two sets of experiments. For the first set of experiments, to obtain a pseudo-plateau for the analytical signal, MP1 is programmed to

Table 2. Results obtained for copper and iron in the presence of interferences

Analyte + interferent	[analyte] found[a] ($\mu g\ mL^{-1}$)	
	Procedure	
	Direct calibration	MSAM
5.0 $\mu g\ mL^{-1}$ copper + 20% ethanol	7.5	5.1
5.0 $\mu g\ mL^{-1}$ copper + 40% ethanol	9.2	5.0
5.0 $\mu g\ mL^{-1}$ copper + 60% ethanol	10.1	4.9
5.0 $\mu g\ mL^{-1}$ iron + 100 $\mu g\ mL^{-1}$ cobalt	3.3	5.0
5.0 $\mu g\ mL^{-1}$ iron + 200 $\mu g\ mL^{-1}$cobalt	2.5	5.1
5.0 $\mu g\ mL^{-1}$ iron + 500 $\mu g\ mL^{-1}$ cobalt	1.6	4.9

[a]Mean value for three measurements (standard deviation was in the ± 0.1 to ± 0.2 range).

carry out five injections of the sample solution, while MP2 remains stopped. In this way, an analytical signal, A_0, is obtained. Next, the measurement is repeated, MP1 injecting five plugs of the sample, as before, while MP2 introduces a single plug of a standard solution thus obtaining an analytical signal, A_1, which is somewhat higher than A_0. The difference between them is a net analytical signal related to the response of the spectrometer to the standard in the presence of interferent. If the measurements are repeated, using MP2 to inject an increasing number of standard solution plugs, a plot of the net analytical signals in this way obtained against the number of injections carried out by MP2 is a straight line whose slope, β_{st}, is easily calculated.

For the second set of experiments, the same procedure is followed, the only difference being that both pumps are used to inject sample solution. In this way, a plot of the net analytical signals against the number of injections carried out by MP2 allows a straight line with a slope, β_s, to be obtained. It should be noted that in each set of experiments, the interferent/analyte ratio does not significantly vary, so that the response factor of the spectrometer should not vary either. If such a hypothesis is valid, the following equations, where k is the response factor of the spectrometer, should be obeyed:

$$\beta_s = k \cdot V_s \cdot C_s$$
$$\beta_{st} = k \cdot V_{st} \cdot C_{st}.$$

Taking into account that both standard and sample solutions are introduced by the same pump (MP2), the volumes of sample and standard solutions are the same, and the sample concentration could be obtained by using the simple equation:

$$C_s = C_{st} \cdot \frac{\beta_s}{\beta_{st}}.$$

Table 3 shows the results of some experiments that were carried out to verify the above thoughts. All the experiments were made in the absence of the common releasing agents used for calcium determination. As can be seen and as expected, CSAM could not overcome the interference. However, the use of MSAM permitted correct results to be obtained, thus validating the above reasoning. It is important to emphasize again that most of the tasks involved in the described procedure are computer-controlled so that, by using suitable software, the intervention of the operator is kept to a minimum and a semiautomatic procedure results.

An additional verification of the suitability of this procedure to cope with the severe interference of phosphate in calcium determination was made by determining the calcium content of three certified reference materials, namely, CRM 063R (skim milk powder), SRM 1549 (nonfat milk powder), and SRM 8435 (whole-milk powder). Appropriate amounts of these samples were suspended (1% w/v) in a diluted nitric acid solution (1% v/v

Table 3. Results obtained for calcium in the presence of phosphate or titanium

	[analyte] found[a] ($\mu g\ mL^{-1}$)	
	Procedure	
Analyte + interferent	CSAM	MSAM[b]
5.0 $\mu g\ mL^{-1}$ + 0.01 M phosphate	4.1	5.1
5.0 $\mu g\ mL^{-1}$ + 0.05 M phosphate	3.7	5.0
5.0 $\mu g\ mL^{-1}$ + 5 $\mu g\ mL^{-1}$ titanium	3.6	5.0
5.0 $\mu g\ mL^{-1}$ + 10 $\mu g\ mL^{-1}$ titanium	3.1	4.9

[a]Mean value for three measurements (standard deviation was in the $\pm$ 0.1 to $\pm$ 0.2 range).
[b]The variant based in the slope ratio was used.

concentrated acid) and the calcium content was measured using three procedures, namely, direct calibration against aqueous standards, CSAM, and MSAM based on the slope ratios here discussed. No common releasing agents were used in any case. The comparison (Wilcoxon *t* test) of the results shown in Table 4 with those certified proved the absence of significant differences at the 95% confidence level, showing the reliability of the approach here studied.

CONCLUSIONS

The simple manifold here presented allows SAM to be carry out in a semiautomatic nonconventional way with a saving of time and reduced consumption of both sample and standard solutions. The replacement of classical peristaltic

Table 4. Results obtained for calcium determination in Standard Reference Materials (SRMs)

	Content[a] ($mg\ g^{-1}$)			
	Procedure			
Sample	Certified value	Direct calibration	CSAM	MSAM[b]
SRM 063R (skim milk)	13.49 $\pm$ 0.10	8.89 $\pm$ 0.15	12.35 $\pm$ 0.12	13.52 $\pm$ 0.12
SRM 1549 (nonfat milk)	13.0 $\pm$ 0.5	9.18 $\pm$ 0.19	12.43 $\pm$ 0.21	12.85 $\pm$ 0.19
SRM 8435 (whole milk)	9.22 $\pm$ 0.49	6.82 $\pm$ 0.21	8.82 $\pm$ 0.18	9.30 $\pm$ 0.21

[a]Mean value $\pm$ standard deviation for four experiments.
[b]The variant based in the slope ratio was used.

pumps, loops, and valves by MPs is confirmed to be an advantageous way for injecting, transporting, and mixing solutions for analytical purposes. In spite of the very large number of injections carried out by MPs, no changes in the volume delivered at each stroke was observed, which, in addition to the low-cost, small-size, and easy computer control of MPs, represents an additional advantage over peristaltic pumps that have the unavoidable drawback of the wear that occurs in the pump tubes.

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