Multisyringe flow injection analysis system for automation of standard addition calibration method

João Rodrigo Santos a, Marcela A. Segundo a,*, Josè L.F.C. Lima a, Mauro Korn b

a REQUIMTE, Serviço de Química-Física, Faculdade de Farmácia, Universidade do Porto, R. Aníbal Cunha, 164, 4099-030 Porto, Portugal
b Dept. de Ciências Exactas e da Terra, Universidade do Estado da Bahia, R. Silveira Martins, 2555, 4195-001, Salvador, BA, Brasil

A R T I C L E   I N F O

Article history:
Received 16 March 2009
Accepted 25 March 2009
Available online 1 April 2009

Keywords:
Standard addition method
Multisyringe flow injection analysis
Potentiometry
Chloride determination
Electroplating bath
Milk

A B S T R A C T

In the present work, the multi-channel features of multisyringe flow injection analysis (MSFIA) were exploited for the first time to implement calibration based on standard addition method (SAM). For this, standard solutions containing different concentrations of target analyte were placed in each syringe of the multisyringe and connected to a flow network where in-line mixing of sample and standard through a merging zone approach was established prior to detection of analyte. Using this strategy, artifacts reported before in SAM using flow injection analysis were avoided as the concentration of the analyte in the resulting mixture was related to the dilution of sample and added standard within the system, and the concentration of all matrix components was kept constant during all measurements. The feasibility of the proposed MSFIA system was assessed through application to potentiometric determination of chloride ion in electroplating bath and milk samples. Results obtained for samples \( n=15 \) were not statistically different from those provided by titrimetric procedures, with an excellent throughput (20–31 samples h \(^{-1} \)), comprising four-level addition of chloride ion.

© 2009 Elsevier B.V. All rights reserved.

1. Introduction

The chemical composition of most samples is complex, presenting a multitude of components that may interfere when determining a specific target analyte. Consequently, fastidious sample treatment is usually employed intending to overcome potential matrix interferences and increase the reliability of the analytical result, namely when dealing with rather selective detectors such as ion selective electrodes. In this case, unreliable results are frequently caused by the differences that may exist between the composition of standards and samples (such as variable and/or high ionic strength, for instance). In order to overpass this problem, analytical procedures based on standard addition method (SAM) are used [1]. This calibration method presents as main advantage the accounting of sample matrix interferences by measurement of analytical signals provided by solutions containing a constant amount of sample and a variable amount of analyte species. However, this procedure is labor intensive, requiring large amounts of sample and reagents and providing very low determination rates.

The developments of automatic techniques based on flow injection analysis concept [2] have attenuated the aforementioned disadvantages. Nowadays, many examples exploiting automatic methodologies for performance of simple tasks, such as sample handling, reagent addition, dilution, and mass-transfer based operations, can be found with applications to different areas [3,4]. The implementation of standard addition methods through flow injection techniques is not an exception, and it has been accomplished using flow injection analysis (FIA) [5,6], sequential injection analysis (SIA) [7], and multicommutation [8] flow schemes.

In this context, multisyringe has been introduced as a novel tool for liquid management that was successfully applied to the implementation of multisyringe flow injection analysis (MSFIA) [9,10]. The utilization of multisyringe apparatus allowed the delivery of low (µl magnitude), precise volumes with reproducible flow rate and high versatility concerning liquid management. The versatility is conferred by the multi-channel capability of this liquid driver, where up to 4 channels can be operated simultaneously, allowing selective introduction of solutions into the flow network. Despite the number of publication (about 80) describing applications of MSFIA to analysis of real samples, the utilization of potentiometric detectors is scarce [11–13] and the implementation of SAM in this type of flow systems has not been reported so far.

Therefore, in the present work, the main objective is to take advantage of the multi-channel features provided by MSFIA to implement SAM by placing standards with different concentration in each syringe and by assembling a flow network that allows the in-line mixing of sample and standard through a merging zone approach. In this way, the concentration of the analyte in the resulting mixture depends on the dilution of sample and added standard within the system, enabling also that the concentration of all matrix components are kept constant during all measurements. The concentration of the analyte is obtained through graphic extrapolation of the resulting

© 2009 Elsevier B.V. All rights reserved.
data. To illustrate the applicability of the proposed strategy, the potentiometric determination of chloride ion in electroplating baths and in milk samples was chosen. In the first case, the standard addition method is necessary due to the variable ionic strength of each sample. For milk samples, the diversity of products commercially available, including components to enhance their flavor or nutritional value, justifies the application of the standard addition method.

2. Material and methods

2.1. Reagents and solutions

Reagents used were of analytical-reagent grade quality or similar. Solutions were prepared with deionized water with a specific conductance of less than 0.1 \( \mu \text{s} \cdot \text{cm}^{-1} \) obtained through a purification system Milli-Q RG (Millipore, Bedford, MA).

Chloride stock solutions were prepared by rigorous weighing of previously dried NaCl at 110 °C for 24 h. Diluted standard solutions were obtained through rigorous dilution of the stock solution. For evaluation of the chloride tubular electrode and determination in electroplating baths, standard solutions also included KNO\(_3\) 0.1 M for ionic strength adjustment. For milk samples, standard solutions containing KNO\(_3\) 0.2 M and H\(_2\)SO\(_4\) 0.05 M were applied.

For the construction of the tubular selective electrode, non-conductive epoxy resin was prepared through mixing Araldit M (ref. 10951, Fluka, Buchs, Switzerland) and hardener Ren HY5162, 100:40 (w/w). Conductive epoxy silver based resin was prepared through mixing Epo-Tek 410E (part A) and hardener Epo-Tek 410E (part B) 15:1.6 (w/w). Conduction epoxy resin was prepared through mixing Epo-Tek 410E (part A) and hardener Ren HY5162, 100:40 (w/w). Conductive epoxy silver based resin was prepared through mixing Epo-Tek 410E (part A) and hardener Epo-Tek 410E (part B) 15:1.6 (w/w).

Electroplating baths were prepared according to Lowenheim [14] and analyzed without previous treatment. Milk samples were purchased at local market and they were also analyzed without previous treatment.

2.2. Apparatus and electrodes

Standard solutions were propelled through the flow network by means of a multisyringe piston pump (Crison Instruments, Altea, Spain) equipped with syringes of 10 mL (Hamilton, Switzerland). Each syringe is connected to a three-way commutation valve (N-Research, Caldwell, NJ) that allows the access to two different channels (solution flasks or flow network), corresponding to position off or position on, respectively. One extra commutation valve was included for sample exchange (V5). For propulsion of samples, a peristaltic pump (Minipuls 3, Gilson, Villiers-le-Bel, France) equipped with PVC tubing (0.10 cc/m, allowing a maximum flow rate of 0.70 mL min\(^{-1}\)) was used. The flow assembly also included a lab-made confluence (four side channels of 0.3 mm i. d. and one main channel with 0.8 mm i. d.), connected to a mixing coil (1 = 35 cm). Polytetrafluoroethylene (PTFE) tubing (0.8 mm i. d., Omnifit, Cambridge, U. K.) was used for connecting the different devices as depicted in Fig. 1.

For the potentiometric measurements, a decimillivoltmeter (model 2002, Crison, Altea, Spain) and a double-junction reference electrode (Russell, model 90-0029) were used. The reference electrode was coupled to the flow system using an adaptor of polymethylmetacrilate (PMMA) specifically machined for this purpose [15]. As indicator electrode, a tubular shaped chloride ion-selective electrode without inner reference solution was used. The tubular electrode consisted of a sensing element made up by AgCl/Ag\(_2\)S sensor pellet [16]. For incorporation of the ion sensor pellet in the flow system, a disposable PMMA cell from Kartell (Noviglio, Italy), ref. PN 1961 (1.0 cm width \( \times \) 1.0 cm depth \( \times \) 4.5 cm height) was filled up to 3/4 of its capacity with non-conductive epoxy resin and the sensor was completely immersed in the resin. The resin was then cured for one day at 40 °C. Subsequently, a flow channel with an internal diameter of 0.8 mm was drilled throughout the cell. Finally, the extremities of the previous hole were enlarged to 3.0 mm i. d. and 3.0 mm depth in order to fit a small piece of flexible PVC tubing for direct coupling of the electrode to the flow system tubing. A schematic representation of a tubular shaped chloride selective electrode is depicted in Fig. 1a.

A personal computer, running lab-made software written in QuickBasic 4.5 (Microsoft, Redmond, WA), controlled the multisyringe operation (number of steps and direction of piston displacement, position of all commutation valves) by a serial port. The peristaltic

Fig. 1. Schematic representation for MSFIA determination of chloride ion based on standard addition method. MS, multisyringe; PP, peristaltic pump; Vi, three-way commutation valves; S, sample; Sti, chloride standard solution; C, 6-way commutation point; MC, mixing coil; mV, decimillivoltmeter; ISE, chloride ion selective electrode; E\(_{\text{ref}}\), reference electrode; W, waste. For V1–V4, position on corresponded to the system and position off corresponded to solutions flask. For V5, position on was assigned to waste and position off was assigned to flow network. a) detailed representation of flow through, tubular shaped ISE.
pump control (flow direction and rotation speed) and data acquisition (at 4 Hz) were performed by the same software using a PCL-711 interface card (Advantech, Taipei, Taiwan).

Besides the decimillivoltmeter and the reference electrode, a conventional, lab-made AgCl/Ag2S electrode was also used for the reference batch methodologies.

2.3. Operation of MSFIA system

The flow system designed to perform the standard addition method and evaluated for chloride determination without any sample pre-treatment is depicted in Fig. 1. The analytical cycle is described in Table 1. First, the four syringes are filled with the standard solution (St1–St4) while the peristaltic pump is activated to allow introduction of new sample through the pumping tubing, towards waste (V5 in position on). Next, sample and all standard solutions are dispensed towards the flow network in order to fill their respective channels (V5 is now in position off). Afterwards, the peristaltic pump is stopped and standard solution St1 (containing the lowest concentration of added chloride) is dispensed, filling the mixing coil and reaching the detector to establish the baseline. In the following steps, the standard addition procedure takes place. A defined volume of sample and standard solution St1 are propelled by the peristaltic pump and the respective syringe, simultaneously. These two solutions merge at the confluence point and they are further mixed towards the detector, where the analytical signal is acquired. Next, the peristaltic pump is stopped and standard solution St1, containing the lowest concentration of added chloride, is propelled for establishing the baseline again. These two last steps are repeated three more times, with replacement of St1 in the first step by each of the other standards (St2, St3, St4). At the end of this cycle, four analytical signals are obtained, corresponding to the addition of each standard.

Before sample analysis, the MSFIA system was calibrated using also the protocol described in Table 1. Water was used as sample and the slope S was calculated from the data obtained, considering the dilution factor of standards. The flow rate in the peristaltic pump was determined by weighing the amount of water aspirated during a fixed time interval, with concomitant operation of the multisyringe.

2.4. Calculation of chloride concentration in samples

Considering a standard addition procedure based on merging streams where the dilution factor of sample (Di) and standard (Di) are defined by the relative contribution (fi) of each stream to the total flow rate (f) (Eq. (1)), the mathematical expression for the potentiometric determination of the target analyte can be obtained from the modified Nernst equation [17] for ion selective electrodes (Eq. (2)).

\[ D_i = \frac{f_i}{f} \]  
\[ E = k + S \times \log C \]  

The analyte concentration Ca attained when a stable potential value is reached is given by the total amount of analyte (nA) per unit of volume (Eq. (3)). Considering that the total volume (Vt) in contact with the ISE is composed by an aliquot of sample (Vs) and other from standard (VSt) and considering also that nA can be expressed in terms of volume and concentration of both sample (Ca) and standard (CSt), (Eq. (3)) can be rearranged to (Eq. (4)):

\[ E = k + S \times \log \left( \frac{n_A}{V_i} \right) \]  
\[ E = k + S \times \log \left( \frac{C_{St} \times V_{St} + C_A \times V_A}{V_{St} + V_A} \right) \]  

By further mathematical rearrangement, Eq. (5) is obtained. Then, considering that for any given time interval, Eq. (1) can also be written as Eq. (6),

\[ 10^{\frac{E}{S} - \frac{E_k}{S}} = \frac{C_{St} \times V_{St}}{V_t} + \frac{C_A \times V_A}{V_t} \]  
\[ D_i = \frac{V_i}{V_t} \]
Table 2
Results obtained for model solutions by addition of 4 or 2 standards using experimental conditions selected for analysis of electroplating baths.

<table>
<thead>
<tr>
<th>Model solution</th>
<th>4 standard additions (S1–S4)</th>
<th>2 standard additions (S1 and S4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Cl] / M</td>
<td>[Cl] /calculated / M</td>
<td>RD /%</td>
</tr>
<tr>
<td>0.010</td>
<td>0.011 ± 0.005</td>
<td>10</td>
</tr>
<tr>
<td>0.020</td>
<td>0.019 ± 0.003</td>
<td>−5.0</td>
</tr>
<tr>
<td>0.050</td>
<td>0.050 ± 0.003</td>
<td>0.0</td>
</tr>
<tr>
<td>0.100</td>
<td>0.101 ± 0.005</td>
<td>1.0</td>
</tr>
<tr>
<td>0.500</td>
<td>0.486 ± 0.008</td>
<td>−2.8</td>
</tr>
<tr>
<td>1.00</td>
<td>0.95 ± 0.02</td>
<td>−5.0</td>
</tr>
<tr>
<td>1.50</td>
<td>1.49 ± 0.04</td>
<td>−0.7</td>
</tr>
<tr>
<td>2.00</td>
<td>2.01 ± 0.05</td>
<td>0.5</td>
</tr>
<tr>
<td>2.50</td>
<td>2.49 ± 0.07</td>
<td>−0.4</td>
</tr>
</tbody>
</table>

RD = relative deviation; [S1] = 2.00 × 10−3 M; [S2] = 1.00 × 10−2 M; [S3] = 2.00 × 10−2 M; [S4] = 3.00 × 10−2 M.

By combining these two equations, the following expressions are obtained:

\[ 10^{1-1} = \frac{C_A}{C_0} + \frac{C_A}{D_A} \]  

\[ 10^0 = \frac{1}{10^{1-1} \times D_{St}} \times C_{St} + \frac{C_A}{\frac{10^{1-2}}{D_A}} \]  

In this case, the concentration of analyte in the sample \( (C_A) \) can be calculated from the slope and intercept obtained from Eq. (8):

\[ C_A = \text{intercept} \times 10^{1-1} \times D_A = \frac{\text{intercept} \times D_A}{\text{slope} \times D_{St}} \]  

2.5. Reference method

The determination of chloride ion in electroplating baths was performed according to the reference procedure based on Mohr reaction [18] and the titration end point was detected potentiometrically.

The AOAC reference method was adopted for the determination of chloride ion in milk samples [19]. Milk samples were previously diluted with nitric acid (0.3 M) and further titrated with silver nitrate, using also potentiometry for detection of the end-point.

3. Results and discussion

In the present work, the main objective was to implement the automatic addition of standards containing the target analyte (chloride, in this case) to the sample, without previous manipulation of sample (e.g. dilution, ionic strength adjustment, etc.). Considering that the multi-channel configuration of the MSFIA apparatus enables the independent introduction of solutions into the flow manifold, standards containing different concentrations of chloride ion and KNO₃ 0.1 M as ionic strength adjustor were placed in each syringe, providing the addition of chloride at four concentration levels. To introduce sample into the flow system, a peristaltic pump was chosen for two reasons. First, the change of sample is simpler, as the pumping tubing was connected to a three-way commutation valve that diverted the flow towards waste, allowing exchange of sample without contamination of the flow network and detection system. This feature also enables a decrease of the time taken for sample exchange as unnecessary washing of flow network is avoided. Secondly, it would take a long time for performing washing steps to prevent carry-over between samples, if they were introduced directly through one of the syringes.

Taking into account the recommendations made by Koscielniak and Koza[6] concerning the maintenance of the quantity of interferent components, the mixture of sample and standard must be performed in such a way that the respective dilution factors should be the same throughout all additions. Therefore, a confluence point was introduced into the flow network to allow the mixing of sample and standard through a merging zones approach. In this case, the dilution factor was controlled by the ratio between the total flow rate and the flow rate of propulsion of sample (section 2.4, Eq. (1)). Therefore, the analytical cycle consisted of two main steps: 1) addition of standard + detection of potentiometric signal; 2) return to signal baseline. In this case the carrier chosen was the most diluted chloride solution applied as a stable signal is obtained whenever the measured species is present on the background flowing stream. Moreover, to avoid sudden changes of baseline, the propulsion flow rate towards the detector was kept constant (Table 1).

Preliminary studies for signal stability evaluation after mixing sample and standard solution were performed using mixing coils (Fig. 1, MC) with different length (25, 35 and 50 cm). The concentration of chloride ion in the standard addition solutions were 0.002 M, 0.01 M, 0.02 M and 0.03 M, while model solutions containing 0.05 M, 0.5 M and 1.5 M of chloride ion were used as sample. The total flow rate was of 6.0 mL min⁻¹, with a sample flow rate of 0.12 mL min⁻¹ and added standard flow rate of 5.88 mL min⁻¹, providing dilution factors of 50 and 1.02, respectively. The volume of standard added was fixed at 600 µL. Using these experimental conditions, repeatable analytical signals were obtained, with signal variation lower than 0.4 mV, showing good mixing conditions and low electrical noise. Therefore, a reactor length of 35 cm was selected for further studies.

To evaluate the repeatability and accuracy of these operation conditions, model solutions containing between 0.010 and 2.50 M of chloride ion were tested as sample (Table 2). Relative deviations <5% from the true value were obtained for samples containing more than 0.02 M. However, precision was not good for low concentration values (RSD >15% for 0.02 M model solution). Acceptable results (RSD <5%) were obtained for model solutions containing more than 0.1 M, which is therefore the minimum recommended amount to be present in samples.

These operation conditions were successfully applied to electroplating baths (Table 3), as exemplified in Fig. 2. In this case, SAM calibration is recommended due to the high (and variable) ionic
strength of samples, whose interference cannot be overcome by sample dilution. To evaluate the accuracy of the proposed methodology, samples were also analyzed by a reference method, and relative deviations < 3.5% were found. A paired t-test was performed on the data obtained for these samples and a t-value of 0.478 was calculated. The comparison between this value and the tabulated t (2.570, P = 0.05, df = 5) indicates no significant difference for the mean concentration obtained by the two methods [20].

Good repeatability was also attained, with RSD < 3.0% (n = 12, meaning the repetition of 3 analytical cycles) for all samples tested. Sample throughput was excellent, considering that it took 114 s to obtain the analytical signals corresponding to four standard additions (the repetition of 3 analytical cycles) for all samples tested. The results obtained for the four chloride ion standards and calibration curve obtained from this data are shown in Fig. 2.

As expected, the results obtained for Solution 1 revealed the strong influence of milk constituents over the electrode response as a continuous potential drift was observed. Solution 2 yielded similar results and it was not possible to obtain repeatable analytical signals when a milk sample was processed. Therefore, the composition of the standard solutions was changed in order to minimize matrix effects and to allow a suitable electrode conditioning. Standard solutions composition studied were 1) KNO₃, 0.2 M (control); 2) NaNO₃, 1.0 M + acetic acid/acetate buffer, 1 M, pH 5; 3) KNO₃, 0.5 M + H₂SO₄, 0.05 M; 4) KNO₃, 0.5 M + H₂SO₄, 0.005 M; 5) KNO₃, 0.5 M + H₂SO₄, 0.0005 M; 6) KNO₃, 0.5 M, HNO₃, 0.1 M; 7) KNO₃, 0.5 M, HNO₃, 0.01 M; 8) KNO₃, 0.5 M, HNO₃, 0.001 M. These solutions were previously applied for potentiometric chloride determination in milk samples through standard addition method (Solution 2) [21] and on the reference procedure, where samples were previously diluted in nitric acid [19].

As mentioned before, the established conditions are suitable for determination in samples containing more than 0.1 M of chloride ion, which is higher than the concentration usually found in milks. Furthermore, as occurs for batchwise application of SAM, the concentration of target analyte added must be adapted to the level expected to be found in samples. Hence, for application to milks, some adjustments were performed, namely the decrease of dilution factor of sample (to 20 times), the utilization of lower concentration of chlorides in the standards added, the utilization of a higher concentration of KNO₃ (0.2 M) for ionic strength adjustment and decrease of total flow rate to 5.0 mL min⁻¹. As depicted in Fig. 3, it was not possible to obtain repeatable analytical signals when a milk sample was processed.

Table 4
Results obtained for model solutions by addition of 4 or 2 standards using experimental conditions selected for analysis of milk samples.

<table>
<thead>
<tr>
<th>Model solution [Cl⁻] [M]</th>
<th>4 standard additions (St1-St4)</th>
<th>2 standard additions (St1 and St4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl⁻ [experimental] [M]</td>
<td>RD [%]</td>
<td>Cl⁻ [calculated] [M]</td>
</tr>
<tr>
<td>1.00×10⁻³ M</td>
<td>1.17 (±0.36)×10⁻³ M</td>
<td>17</td>
</tr>
<tr>
<td>3.00×10⁻³ M</td>
<td>3.12 (±0.42)×10⁻³ M</td>
<td>40</td>
</tr>
<tr>
<td>5.00×10⁻³ M</td>
<td>4.75 (±0.21)×10⁻³ M</td>
<td>50</td>
</tr>
<tr>
<td>1.00×10⁻² M</td>
<td>1.00 (±0.04)×10⁻² M</td>
<td>0.0</td>
</tr>
<tr>
<td>3.00×10⁻² M</td>
<td>2.89 (±0.04)×10⁻² M</td>
<td>3.7</td>
</tr>
<tr>
<td>5.00×10⁻² M</td>
<td>4.75 (±0.07)×10⁻² M</td>
<td>50</td>
</tr>
</tbody>
</table>

RD = relative deviation; [St1] = 3.00×10⁻³ M; [St2] = 6.00×10⁻³ M; [St3] = 9.00×10⁻³ M; [St4] = 12.0×10⁻³ M.

Table 5
Results obtained for chloride determination in milk samples using MSFIA standard addition method or reference methodology.

<table>
<thead>
<tr>
<th>Sample</th>
<th>[Cl⁻] [experimental] [M]</th>
<th>[Cl⁻] [reference] [M]</th>
<th>RD [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk 1 (with strawberry flavor)</td>
<td>2.80 (±0.19)×10⁻² M</td>
<td>2.85 (±0.05)×10⁻² M</td>
<td>-1.8</td>
</tr>
<tr>
<td>Milk 2 (low fat content)</td>
<td>3.03 (±0.20)×10⁻² M</td>
<td>3.04 (±0.04)×10⁻² M</td>
<td>-0.3</td>
</tr>
<tr>
<td>Milk 3 (high fat content)</td>
<td>2.85 (±0.12)×10⁻² M</td>
<td>2.85 (±0.03)×10⁻² M</td>
<td>0.0</td>
</tr>
<tr>
<td>Milk 4 (medium fat content)</td>
<td>2.95 (±0.13)×10⁻² M</td>
<td>2.89 (±0.03)×10⁻² M</td>
<td>2.1</td>
</tr>
<tr>
<td>Milk 5 (biologic)</td>
<td>3.37 (±0.13)×10⁻² M</td>
<td>3.31 (±0.01)×10⁻² M</td>
<td>1.8</td>
</tr>
<tr>
<td>Milk 6 (enriched with calcium)</td>
<td>2.97 (±0.14)×10⁻² M</td>
<td>2.95 (±0.02)×10⁻² M</td>
<td>0.7</td>
</tr>
<tr>
<td>Milk 7 (with chocolate and vitamins)</td>
<td>3.50 (±0.18)×10⁻² M</td>
<td>3.43 (±0.02)×10⁻² M</td>
<td>2.0</td>
</tr>
<tr>
<td>Milk 8 (with chocolate)</td>
<td>2.81 (±0.09)×10⁻² M</td>
<td>2.89 (±0.04)×10⁻² M</td>
<td>-2.8</td>
</tr>
<tr>
<td>Milk 9 (with chocolate)</td>
<td>3.40 (±0.20)×10⁻² M</td>
<td>3.51 (±0.04)×10⁻² M</td>
<td>-3.1</td>
</tr>
</tbody>
</table>

RD = relative deviation.

a n = 12.
b n = 3.
application of HNO₃ or H₂SO₄. For further studies standard solutions St1-St4 were prepared using Solution 3 (KNO₃, 0.5 M + H₂SO₄, 0.05 M) as at this pH value (pH = 1) a higher ionic strength is attained compared to the utilization of nitric acid.

In the assays performed using acidic media, the formation of a precipitate was noticed, due to the protein content of milk. This aspect did not allow the application of sample dilution factors lower than 20, as a larger amount of protein precipitate was formed, causing clogging of flow tubing when a sample dilution factor of 10 was applied. Therefore, a sample dispersion factor of 20 was selected for further assays.

Considering the concentration range to be found in milk samples, model solutions containing between 0.001 and 0.05 M of chloride ion were tested as sample (Table 4) in order to evaluate the repeatability and accuracy of operation with lower dilution factor of sample. Relative deviations <5% were obtained for model solutions containing more than 0.003 M, but precision was not acceptable for the lowest concentrations tested (RSD>13% for 0.003 M model solution). Good results (RSD <5%) were obtained for model solutions containing at least 0.005 M, which is therefore the minimum recommended amount to be present in samples when applying the modified protocol.

The modified protocol was successfully applied to different types of milk samples (Table 5), as exemplified in Fig. 4. To evaluate the accuracy of the proposed methodology, samples were also analyzed by a reference method, and relative deviations <3.1% were found. A paired t-test was performed on the data obtained for these samples and a t value of 0.206 was calculated, which was compared to the tabulated t (2.306, P=0.05, df=8), indicating also no significant difference for the mean concentrations obtained by the two methods [20].

Repeatability was acceptable, though not as good as that obtained for the previous strategy, (RSD<7%, n = 12). Sample throughput was also good as it took 180 s, providing a determination throughput of 20 samples h⁻¹. This value can be also enhanced to 27 sample h⁻¹ if only two standards are added (Table 4).

4. Conclusions

For the first time the standard addition method was successfully implemented using multisyringe flow injection analysis technique.

The strategy proposed here avoided the errors presented in previous flow based automatic systems [6] as the amount of sample processed is the same for all standard additions performed, making the quantity of interferent species constant for all measurements performed. Moreover, the proposed flow system can be considered more flexible compared to previous automatic flow based systems as the dilution factor of sample can be easily adjusted through software control, by changing the flow rate ratio for sample and added standards.

As reported here, conditioning of sample can be tailored according to its composition by adding any other component to the standard solutions, such as salts for ionic strength adjustment or acids/bases for pH control and masking of interfering species.

Finally, the major advantage of the present flow system is the excellent throughput (20–31 sample h⁻¹) that includes all operations necessary for SAM: sample dilution, addition of standard, mixing of solutions and measurement of analytical signal at four different concentration levels of added analyte.

Acknowledgements

The authors wish to thank GRICES (Portugal) and CAPES (Brazil) for financial support to exchange research work.

References