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Standard addition flow method for potentiometric measurements at low concentration levels: Application to the determination of fluoride in food samples

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ABSTRACT

A standard addition method was implemented by using a flow manifold able to perform automatically multiple standard additions and in-line sample treatment. This analytical strategy was based on the in-line mixing of sample and standard addition solutions, using a merging zone approach. The flow system aimed to exploit the standard addition method to quantify the target analyte particularly in cases where the analyte concentration in the matrix is below the lower limit of linear response of the detector. The feasibility of the proposed flow configuration was assessed through the potentiometric determination of fluoride in sea salts of different origins and different types of coffee infusions. The limit of quantification of the proposed manifold was 5×10^{-6} mol L^{-1}, 10-fold lower than the lower limit of linear response of the potentiometric detector used. A determination rate of 8 samples h^{-1} was achieved considering an experimental procedure based on three standard additions *per* sample. The main advantage of the proposed strategy is the simple approach to perform multiple standard additions, which can be implemented with other ion selective electrodes, especially in cases when the primary ion is below the lower limit of linear response of the detector.

1. Introduction

The standard addition calibration method (SAM) is a well accepted analytical approach that consists in the addition of different amounts of the analyte to the sample, so that measurement is performed in the original matrix, permitting in this way to overcome potential sample matrix interferences that might occur during an analytical protocol [1].

In spite of the recognized advantages of SAM, this calibration method is an analytical procedure sporadically applied for routine laboratory work. Its implementation involves the selection of certain experimental parameters (e.g., initial concentration of the standard solutions and sample dilution factors), which sometimes requires extra work in order to optimize all of these variables before sample analysis. Apart from this, it is also necessary to prepare a set of standard addition solutions which make use of large amounts of reagents; SAM is therefore a quite labor intensive procedure.

With the development of flow analysis strategies, the automation of the SAM calibration procedure turned out to be a more easily performed analytical approach. By taking advantage of flow techniques features, namely the reproducible dispersion control of all solutions involved, and exploiting the versatility of the flow systems, several dedicated studies have been proposed for SAM implementation, based on different flow configurations [2–4] and data treatment [5–6]. Classification of some of these strategies was critically reviewed by Kościelniak et al. [1] and Assali et al. [7]. Another less exploited potentiality of the SAM procedure is to permit determinations when the analyte concentration is somewhat below the LOQ of the detector [8]. In this case, selected standard additions to the sample provide a final concentration of the analyte within the linear calibration range of the detector. This difficulty is sometimes found in potentiomentric determinations using ion selective electrodes. While, in most cases, potentiometric methods are generally based on a simple calibration curve approach, in some particular cases, when the selectivity [9,10] and/or response range of the ISE is not enough for certain complex matrices, SAM might be required.

In this context, the main objective of this work is to illustrate the potentiality of a flow system to execute automatically multiple standard additions to overcome matrix interferences and also to perform the determination at levels near or below the lower limit of linear response of the ISE detector. Furthermore, the approach herein proposed can be a good alternative to quantify lower levels

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of the analyte but with higher reproducibility and sampling rate, comparing to the slower and possibly drifting response usually observed in batch conditions for low analyte concentrations [11,12].

Fluoride determination was selected as a case study due to its recognized human health impact and to the current concern related to quantify its daily intake. The normal contribution of common foods to the daily ingestion of fluoride is very small as this ion is normally present at low concentration levels in food products. Tea and vinegar have been reported as good sources of fluoride in human diets [10,13]. Drinking water is normally the largest single contributor to the daily fluoride intake [14]. In some cases, fluoride can be intentionally added to water or table salt as a way to prevent dental decay, but an elevated exposure to fluoride can cause toxicity giving origin to dental and skeletal fluorosis [14]. The proposed approach aims to surpass the difficulty related to quantify low concentrations of fluoride, a problem often found in the determination of this analyte in sea salt and coffee beverages by potentiometric detection and in which, in some cases, the reported reference methodologies for fluoride determination does not fulfill the low ion concentrations levels. Therefore, the development of an automatic methodology, easily reproducible and able to quantify fluoride in different food matrices is of great utility.

2. Material and methods

2.1. Reagents and solutions

The reagents used were of analytical-reagent grade quality. All solutions were prepared with deionized water with a specific conductance of less than 0.1 $\mu S\ cm^{-1}$.

A standard stock solution of fluoride with a concentration of 0.2 $mol\ L^{-1}$ was prepared by rigorous weighing of previously dried NaF (Merck, 1.06449.1000) at 110 °C for 24 h. Standard solutions of fluoride of lower concentrations were obtained through rigorous dilution of the standard stock solution. All fluoride solutions were stored in polyethylene containers.

For preliminary studies, standard addition solutions were prepared in KNO_3 (Sigma-Aldrich, P8394) 0.2 $mol\ L^{-1}$ for ionic strength adjustment.

For the interferences studies and sample analyses, a TISAB solution was prepared with 1,2-diaminocyclohexane-tetraacetic acid monohydrate (CDTA, Fluka Analytical, 34588-25 G), 4,5-dihydroxy-1,3-benzenedisulfonic acid disodium salt monohydrate (Tiron, Fluka Analytical, 89460-100G) and sodium acetate (Merck, 1.06268.1000). The pH of the TISAB solution was set to 5.5 using a conc. HCl solution, (Merck, 109060.1000), measured with a pH combined glass electrode. The TISAB solution was stored in an amber container, avoiding exposure to the light.

Magnesium chloride hexahydrate (Merck, 5833.1000), aluminum nitrate solution (BDH, 14031), sodium metasilicate (Sigma-Aldrich, 338443), iron standard solution (Merck, 1.19781.0500) and calcium chloride dihydrate (Sigma-Aldrich, 223506) were also used for interference studies.

Samples of grounded roasted coffee and sea salt of different quality types and brands were purchased from local stores. One certified drinking water (National Water Research Institute, Calgary-93) and one sample of tap water used for an internal proficiency testing program were used to assess the accuracy of the proposed methodology.

2.2. Apparatus and flow diagram

The flow manifold assembled (Fig. 1) was based on commercially available equipment and aimed to be simple, capable of performing multiple standard additions and in-line sample treatment.

The flow manifold comprised three solenoids valves (NResearch Inc., P/N 161T031), an electrically actuated injection valve (VICI Valco, Cheminert TM NCX0076, Houston, USA) and a 4-channel peristaltic pump (Gilson, Minipuls 3, Villiers-le-Bel, France). The flow manifold was controlled via PC through a laboratory-made power drive and laboratory-made software designed in Visual Basic 6.0. The detector system consisted in a decimillivoltmeter (Crison, model 2002, Allela, Spain) and a combined fluoride selective electrode (Orion, 9609BNWP, Rockwood, USA) coupled to the flow system through a wall-jet flow cell configuration [15]. Two laboratory-made confluences as well as the electrode support were of polymethylmetacrylate (PMMA). All tubing used to connect between the different apparatus plus the reaction mixing coil, was made of polytetrafluoroethylene (PTFE) with 0.8 mm i.d. (Omnifit, Cambridge, U.K.). Tygon tubings (Gilson, Wisconsin, USA) with 1.00 and 1.52 mm i.d. were used to propel the solutions by the peristaltic pump.

Potentiometric signals were recorded using a chart recorder Kipp & Zonen (BD 111, Delft, The Netherlands).

2.3. Operation of the flow system

The operation of the flow system to perform sample analysis following the standard addition methodology comprised three steps (Table 1).

First, the injection valve (IV) is set to position "load" in order to fill the sample loop; simultaneously the peristaltic pump (PP) and the solenoid valve 1 (SV1) are activated. In this way the standard solution 1 (St1), which contains the lowest standard concentration of fluoride, is propelled towards the detector cleaning both mixing coils, MC1 and MC2, and establishing the baseline.

In the second step, the IV is electrically actuated to the position "inject" and SV2 is activated; in these conditions, the sample is dispensed towards the flow network through propulsion of St1 and, at the same time, the St2 is propelled into the MC1. At the end of this step, MC1 contains both plugs of standard addition solutions St1 and St2, and MC2 contains a mixture of the sample and St1.

Finally, at step 3, SV3 is actuated in order that standard solution 3 (St3), which contains the highest concentration of fluoride, is introduced into the flow system. During this step, the sample is merged sequentially in C2 with St1, then with St2 and finally with St3, being all mixtures performed at constant dispersion of both sample and standard addition solutions. Along the MC2, sample and each standard addition solution are mixed on the way to the detector. At this point, three analytical signals are obtained, corresponding to the sequential addition of each standard (St1, St2, and St3) to the sample. For the optimization of the flow system operation, a steady response of the fluoride selective electrode was targeted for each mixture of standard solution and model solution; with this objective, it was expected that the signal obtained should have three defined and consecutive plateaus, each one corresponding to each standard addition solution mixed with the model solution as mentioned (Fig. 2a). The difference in height, expressed in millivolts, between each plateau should be a consequence of the final concentration of each referred mixture. Additionally, this strategy of not performing a cleaning step between each addition, favoured an increase in the residence time of higher fluoride concentrations in the electrode surface, in order to achieve more easily near steady-state responses and a higher determinations rate.

At the beginning of each working day, the flow rate of the channels St1 and St3 were determined by weighing the amount of water aspirated during a fixed time interval (60 s) during step 3. The data retrieved allowed to calculate the dispersion of both sample and standard addition solutions after merging.

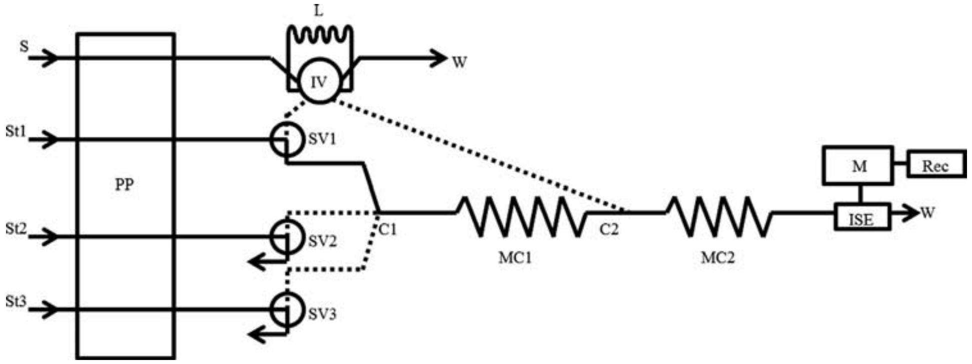

Fig. 1. Flow system developed for standard addition method for fluoride determination during Step 1: (—) filled channels; (–) empty channels. PP – peristaltic pump; S – sample; St – standard addition solution; L – sample loop; IV – injection valve; SV – solenoid valve; C – confluence; MC – mixing coil; ISE – ion selective electrode; M – millivoltmeter; Rec – recorder; and W – waste.

Table 1
Protocol sequence for the determination of fluoride using a standard addition methodology in a multicommuted flow system.

Step	Description	Time (s)	IV	SV1	SV2	SV3
1	Propelling of St1 towards the ISE to clean MC1 and MC2, and establishing the baseline. Sample loop is filled.	210	L	1	0	0
2	Propelling of St2 into the flow network.	50	I	0	1	0
3	Propelling of St3 into the flow network. Mixing of St1, St2 and St3 with the sample. Acquisition of signal.	210	I	0	0	1

L = load, I = inject; 0 – SV off; 1 – SV on; time selected using 3 ml sample loop and equal flow rates of sample and standard addition solutions, 1.0 ml min^{-1}.

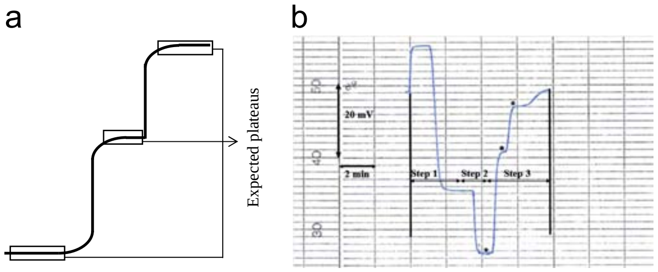

Fig. 2. Expected analytical signal with three plateaus corresponding to the three standard additions (a) and typical fiagram (b) obtained during the procedure of the flow system protocol with signal acquisition points (*).

Thereafter and before sample analysis, the calibration of the detector was performed following the protocol described in Table 1 but using deionized water instead of the sample. This procedure enabled to calculate the terms slope and intercept further used in the calculation of samples fluoride concentration. A typical signal obtained during the flow system operation is depicted in Fig. 2b. On a cycle basis operation, the signal registered during step 1 and step 2 (Fig. 2b), corresponds to the St3 solution of the former analysis being wasted, further establishment of the baseline with St1, and initial mixture of St1 with the sample. Thereafter, as already mentioned, during the step 3 each standard is mixed with the sample, generating the analytical signal with three plateaus.

The calculation of the fluoride concentration (C_A) was followed according to the approach discussed in the literature [9]. Considering the modified Nernst equation and a standard addition procedure based on merging zones approach, the fluoride concentration C_A was calculated using Eq. (1).

$$C_A = \frac{\text{intercept} \times D_A}{\text{slope} \times D_{St}} \tag{1}$$

where D_A and D_{St} are respectively the dilution factors of sample and standard addition solution. As it was referred before, the numeric values of these terms are calculated from the flow rate of the peristaltic pump channels respective to St1 and St3, while the numeric values of terms "intercept" and "slope" were obtained from the linear regression equation of the calibration of the potentiometric detector.

Precision and accuracy of the flow system were evaluated from the coefficients of variation (CV) and relative deviations (RD). For each dilution factor of the sample an analysis of variance was performed. The objective was to identify significant effects of the (i) sets of fluoride standard addition solutions and (ii) model solutions of fluoride on CV and RD values (two-way ANOVA and Tukey′s post-hoc test for multiple comparisons of the means).

2.4. *Samples preparation and analysis*

Four samples of sea salts, four samples of grounded roasted coffees and two waters were evaluated in terms of fluoride content. These matrices were selected as they usually present low content in fluoride. Furthermore, as these matrices are an important part of the human diet, they are good vehicles to increment the ingestion of this analyte in communities.

Moisture determination of sea salts and coffee samples were evaluated according to the AOAC protocols [16,17].

Sea salt sample solutions were prepared by simple dissolution of an exactly weighed (10 g) amount of salt in deionized water. Coffee infusions were prepared by pouring 50 mL of pre-heated deionized water into an exactly weighted amount (10 g) of coffee, with further boiling during 10 min. Infusions were allowed to cool down at room temperature and a total volume of 50 mL was made using deionized water. Prior to fluoride determinations, coffee infusions and sea salt solutions were filtered through a cellulose filter paper N°4 (Whatman, Kent, UK).

Water samples were used as received, with no further treatment.

3. Results and discussion

3.1. *Optimization of experimental variables*

Preliminary studies were performed comprehending signal stability and analytical response of the ISE after in-line mixing model solutions of fluoride with standard addition solutions. This approach aimed to assure the experimental conditions that best suited establishing the plateaus previously referred (Fig. 2a), as this feature is necessary in order to obtain fair precision and accuracy. Within this process of optimization, the experimental variables studied were: the dilution factor of the sample through the flow manifold system and the concentration range of sets of fluoride standard addition solutions. For these studies, model solutions of fluoride ranging from 1.00×10^{-5} to 2.00×10^{-4} mol L^{-1}, were prepared. The interval range of concentrations of model solutions selected aimed to evaluate the performance of the flow system for low concentrations of this ion. Sample dilution factors of ca. 3.0 (sample flow rate=0.5 mL min^{-1} and standard addition solution flow rate=1.0 mL min^{-1}) and 2.0 (sample flow rate=1.0 mL min^{-1} and standard addition solution flow rate=1.0 mL min^{-1}) were studied. A large dilution of the sample would not be feasible since the concentration change of the mixture between the sample solution and the standard addition solution would tend to be independent of the concentration of the model solution especially for the standard solution with a higher concentration; on the other hand, excessive dilution of the standard addition solution would require a higher sample loop volume. Additionally, the standard addition solutions sets were selected bearing that the highest standard addition solution would not be excessive comparing to the sample, while the lowest concentration of the standard addition should guarantee a minimum concentration of fluoride after mixing with the sample, in the linear interval of response of the electrode. Therefore, three sets of fluoride standard addition solutions were tested, each comprising three different concentrations of fluoride, Sets 1–2.00×10^{-4} mol L^{-1}, 3.00×10^{-4} mol L^{-1}, 6.00×10^{-4} mol L^{-1}, Set 2–2.00×10^{-4} mol L^{-1}, 6.00×10^{-4} mol L^{-1}, 1.00×10^{-3} mol L^{-1} and Set 3–2.00×10^{-4} mol L^{-1}, 6.00×10^{-4} mol L^{-1}, and 2.00×10^{-3} mol L^{-1}. For these preliminary studies, standard addition solutions were prepared in KNO_3, 0.2 mol L^{-1}. Loop sample volumes of 2.0 and 3.0 mL were tested aiming to assure that enough volume of sample was available to mix with each standard addition and provide a steady response of the ISE for the three additions. The results of these studies are presented in Tables 2 and 3.

According to the results obtained, it was clear that a fast stabilization of the ISE response was crucial to attain low relative deviations irrespective of the experimental conditions used. Higher concentrations of fluoride enable a faster response of the ISE; however, a compromise exists between higher concentrations of fluoride of the standard addition, and the dilution of the model solution within the standard addition solution (as a higher sample dilution will minimize the impact of the fluoride concentration of the sample over the mixture between sample solution and standard addition solution).

Results from ANOVA indicated that no significant differences were observed for CV and RD absolute values when all conditions (dilution factors of 2 and 3) were tested together. However, if sample dilution factor of 2 is analyzed separately, significant differences ($p < 0.004$) were detected for RD values. Under these conditions, Sets 1 and 2 were equivalent and RD absolute values of Set 3 were significantly higher. Furthermore, if one considers all the range of fluoride concentrations of the model solutions tested, Set 2 allows lower RD values. Therefore, the experimental conditions selected for further studies were sample dilution factor of 2 (total flow rate was 2 mL min^{-1}), sample loop of 3.0 mL and selected concentration of the standard addition solutions as Set 2.

3.2. *Effects of foreign ions*

Aluminum, iron, magnesium, calcium and silicon are among the metallic cations which tend to form complexes with fluoride [18]. For the potentiometric determination of fluoride, different TISAB solutions have been studied as decomplexing agents for the reliable determination of this analyte [19–21]. This issue is of great importance particularly in flow-injection determinations with potentiometric detection using a fluoride ISE, as efficient

Table 2
Results obtained for model solutions using a sample dilution factor of the sample of 3.0 and different sets of fluoride standard solutions[a].

Model solution $[F^-]$ (mol L^{-1})	Set 1			Set 2			Set 3		
	$[F^-]$ ($\pm$SD) (mol L^{-1})	CV (%)	MARD[b] (%)	$[F^-]$ ($\pm$SD) (mol L^{-1})	CV (%)	MARD[b] (%)	$[F^-]$ ($\pm$SD) (mol L^{-1})	CV (%)	MARD[b] (%)
1.00×10^{-5}	$9.84\ (\pm 0.69) \times 10^{-6}$	7.0	5.6	$8.91\ (\pm 2.91) \times 10^{-6}$	33	19	$1.03\ (\pm 0.12) \times 10^{-5}$	11	8.3
2.00×10^{-5}	$2.12\ (\pm 0.13) \times 10^{-5}$	6.4	7.1	$2.06\ (\pm 0.04) \times 10^{-5}$	1.2	2.3	$1.96\ (\pm 0.13) \times 10^{-5}$	6.5	5.3
5.00×10^{-5}	$5.01\ (\pm 0.15) \times 10^{-5}$	3.1	2.3	$4.82\ (\pm 0.21) \times 10^{-5}$	4.7	3.0	$4.87\ (\pm 0.14) \times 10^{-5}$	2.8	3.2
1.00×10^{-4}	$1.00\ (\pm 0.05) \times 10^{-4}$	4.7	3.5	$9.83\ (\pm 0.31) \times 10^{-5}$	1.4	4.2	$9.98\ (\pm 0.24) \times 10^{-5}$	2.4	1.6
2.00×10^{-4}	$1.99\ (\pm 0.06) \times 10^{-4}$	3.3	2.6	$2.09\ (\pm 0.02) \times 10^{-4}$	8.5	6.0	$1.94\ (\pm 0.04) \times 10^{-4}$	2.0	3.2

Set 1, Set 2 and Set 3 (see details in the text).

[a] Values are mean of three replicates.

[b] MARD are the mean of absolute values of the relative deviations of three replicates.

Table 3
Results obtained for model solutions using a sample dilution factor of the sample of 2.0 and different sets of fluoride standard solutions[a].

Model solution $[F^-]$ (mol L^{-1})	Set 1			Set 2			Set 3		
	$[F^-]$ ($\pm$SD) (mol L^{-1})	CV (%)	MARD[b](%)	$[F^-]$ ($\pm$SD) (mol L^{-1})	CV (%)	MARD[b](%)	$[F^-]$ ($\pm$SD) (mol L^{-1})	CV (%)	MARD[b] (%)
1.00×10^{-5}	$9.95\ (\pm 0.25) \times 10^{-6}$	2.5	1.8	$9.88\ (\pm 0.17) \times 10^{-6}$	1.7	1.6	$1.09\ (\pm 0.03) \times 10^{-5}$	2.4	9.1
2.00×10^{-5}	$1.79\ (\pm 0.23) \times 10^{-5}$	13	11	$2.00\ (\pm 0.06) \times 10^{-5}$	3.1	2.4	$2.32\ (\pm 0.15) \times 10^{-5}$	6.7	16
5.00×10^{-5}	$5.15\ (\pm 0.09) \times 10^{-5}$	1.7	3.1	$4.95\ (\pm 0.18) \times 10^{-5}$	3.6	2.5	$5.32\ (\pm 0.32) \times 10^{-5}$	5.9	6.4
1.00×10^{-4}	$1.01\ (\pm 0.03) \times 10^{-4}$	3.0	2.1	$1.02\ (\pm 0.01) \times 10^{-4}$	1.3	2.1	$1.02\ (\pm 0.02) \times 10^{-4}$	1.6	1.8
2.00×10^{-4}	$1.99\ (\pm 0.02) \times 10^{-4}$	1.0	0.9	$1.99\ (\pm 0.02) \times 10^{-4}$	0.8	0.7	$2.04\ (\pm 0.04) \times 10^{-4}$	2.1	2.0

Set 1, Set 2 and Set 3 (see details in the text).

[a] Values are mean of three replicates.

[b] MARD are the mean of absolute values of the relative deviations of three replicates.

and fast interferences suppression is required. For the application herein presented, standard addition solutions were prepared using the following TISAB solution composition, Tiron, 0.025 mol L^{-1}, sodium acetate, 1.0 mol L^{-1} and CDTA, 0.01 mol L^{-1}, pH 5.5. Main advantage of this TISAB solution composition is the ability to suppress the usual ion interferences for the potentiometric determination of fluoride, especially Al (III) [12].

Model solutions of fluoride with concentrations of 2.00×10^{-5} mol L^{-1}, 5.00×10^{-5} mol L^{-1} and 1.00×10^{-4} mol L^{-1} mixed with interfering cations at two levels with the following concentrations: interfering solution A (B): $[Al(III)] = 1.00\ (2.00) \times 10^{-5}$ mol L^{-1}; $[Si(IV)] = 3.00\ (6.00) \times 10^{-4}$ mol L^{-1}; $[Ca(II)] = 5.00\ (10.0) \times 10^{-2}$ mol L^{-1}; $[Mg(II)] = 5.00\ (10.0) \times 10^{-2}$ mol $\times L^{-1}$; and $[Fe(III)] = 1.00\ (2.00) \times 10^{-5}$ mol L^{-1}. The higher concentration of metallic cations selected for this study are equivalent to a 2-fold excess of the maximum concentration usually found in the different foods under evaluation [22–25].

For this study, two different (40 and 100 cm) mixing coil reactors MC2 were tested. Relative errors were lower than 6% in all assays, indicating a fast release of fluoride by using the referred TISAB solution. Therefore, the shorter mixing coil (40 cm) was selected for further experiments favouring higher determination rate and minimizing solutions consumption.

Under the selected experimental conditions, the flow system was evaluated with fluoride model solutions in order to evaluate the repeatability and accuracy [9]. Analyte solutions with concentrations ranging from 1.00×10^{-6} to 2.00×10^{-5} mol L^{-1} were analyzed. The results obtained showed a CV < 5% and an RD < 5% to concentrations down to 5.00×10^{-6} mol L^{-1} (data not shown). This concentration is 10 fold less the lower limit of linear response obtained in a low dispersion flow system using a tubular fluoride electrode [12]. Moreover, sample throughput (comprising three standard additions) was 8 samples h^{-1}. All potentiometric signals obtained were similar to the one depicted in Fig. 2b.

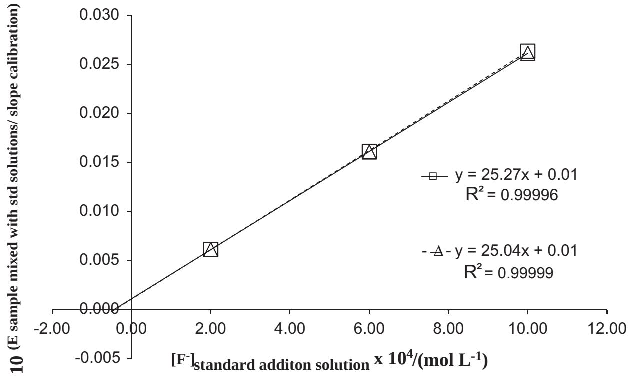

Fig. 3. Calibration curve obtained for a salt sample (two replicates). Linear regression equation for both analysis are presented indicating the terms slope and intercept used for calculation of fluoride concentration according to Eq. 1.

3.3. *Application to natural samples*

Sea salts, coffee infusions and two water samples, were analyzed using the proposed flow system with ISE detection. The protocol followed and the flow system used for the fluoride determination was as previously described respectively in Table 1 and Fig. 1. A typical calibration plot obtained for a salt sample analysis is presented in Fig. 3.

Table 4 shows the fluoride concentration determined in sea salts with different geographical origins and quality type characteristics. The fluoride concentrations found were within 7.70×10^{-6} to 5.15×10^{-5} mol L^{-1} and the precision of the determinations were within 7%. These results present a high variability between them probably due to the different types and origins of the sea salts analyzed. There is scarce information in the literature focusing the determination of fluoride in sea salts [26,27]. Therefore, the results obtained in this work were not critically evaluated with the ones reported in the literature. Furthermore, the values obtained of fluoride concentration in the analyzed samples were too low to be quantified following the reference procedures [28–30]. For this reason, recovery assays were performed, by previously doping sea salt sample solutions with a known amount of fluoride; the recovery test values were between 93.7% and 104%.

In relation to the fluoride content in coffee infusions, the concentrations obtained by the proposed methodology (Table 4) were in agreement with those reported by the World Health Organization for this matrix [14]. Among the different quality types of coffee analyzed, it was possible to see that the instant coffee presented higher fluoride content when compared with the other coffee infusions (Table 4). This result was in agreement with those previously reported by Warren et al. [31]. CV values were below 2%. To our best knowledge, no reference procedure was found for coffee infusions and therefore recovery assays were performed following the same approach used in salt samples; recovery test values were between 97.3% and 104%.

In order to test the accuracy of the fluoride determination by the flow system with the fluoride ISE detection, two waters were evaluated. In this case, with the experimental flow system conditions established, relative deviations lower than 3% were obtained (Table 5).

The signal profiles observed for all the samples analyzed were similar to the one presented previously (Fig. 2b).

4. Conclusion

A novel approach for the multiple standard addition method was implemented in a flow system. The main advantages of the

Table 4
Fluoride content (mol L^{-1})[a] and recovery test of sea salts and coffee infusions.

Sample (origin)	Mean (mol L^{-1}) ± SD	CV (%)	Recovery assays of spiked samples		
			Added amount (mol L^{-1})	Concentration found (mol L^{-1})	Recovery (%)[b]
Sea salt(Portugal)	$7.70\ (\pm 0.53) \times 10^{-6}$	7.0	2.00×10^{-5}	2.72×10^{-5}	97.9
Sea salt(France)	$2.74\ (\pm 0.02) \times 10^{-5}$	0.7		4.60×10^{-5}	93.7
Flower of salt(Portugal)	$5.15\ (\pm 0.12) \times 10^{-5}$	2.3		7.12×10^{-5}	99.4
Flower of salt(France)	$2.74\ (\pm 0.17) \times 10^{-5}$	6.1		4.80×10^{-5}	104
Regular coffee 1	$4.67\ (\pm 0.08) \times 10^{-6}$	1.7		2.46×10^{-5}	100
Regular coffee 2	$5.86\ (\pm 0.01) \times 10^{-6}$	0.2		2.65×10^{-5}	104
Beverage with 20% coffee	$5.26\ (\pm 0.05) \times 10^{-6}$	1.0		2.47×10^{-5}	97.6
Instant coffee	$1.52\ (\pm 0.01) \times 10^{-5}$	0.4		3.46×10^{-5}	97.3

[a] Mean value of three replicates.
[b] Mean value of two replicates.

Table 5
Fluoride content (mol L^{-1})[a] in waters.

Sample	Mean (mol L^{-1}) ± SD	CV (%)	Reference value	RD (%)
Drinking water	$1.33\ (\pm 0.03) \times 10^{-4}$	1.9	$1.30\ (\pm 0.05) \times 10^{-4}$	2.6
Tap water	$5.15\ (\pm 0.20) \times 10^{-5}$	3.4	$5.10\ (\pm 0.42) \times 10^{-5}$	0.9

[a] Mean value of three replicates.

proposed strategy are the simple approach to perform a widespread and valuable analytical procedure, the standard addition method, based on commercially available equipment that can be used with different ion selective electrodes in matrices where the primary ion is below the lower limit of linear response of the electrode.

The proposed automatic flow system coupled to a fluoride selective electrode was successfully applied for the quantification of fluoride in food samples, namely different types of sea salts, coffee infusions and waters.

The application of this flow strategy permitted to quantify levels of fluoride concentration down to 5×10^{-6} mol L^{-1}, 10-fold lower than the lower limit of linear response of the fluoride electrode.

The proposed methodology can be a good alternative for routine analysis of fluoride in food matrices with low fluoride concentrations.

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