

Simultaneous determination of potassium and total fluoride in toothpastes using a SIA system with two potentiometric detectors

R. Pérez-Olmos*, J.C. Soto, N. Zárate, I. Díez

Lab. de Química Analítica, EUITI, Universidad del País Vasco, Plaza de la Casilla 3, 48012 Bilbao, Spain

Received 17 May 2007; received in revised form 19 November 2007; accepted 20 November 2007

Available online 26 November 2007

Abstract

A sequential injection analysis (SIA) system has been developed for the first time to quantify potassium and total fluoride in toothpastes and gels used to prevent both dentinal hypersensitivity and dental caries. To enable this simultaneous determination, potentiometric detection, using a conventional fluoride electrode and a tubular potassium selective electrode, formed by a PVC membrane containing valinomycin as ionophore, was carried out. A manifold that uses a three-way solenoid valve was designed. The former under binary sampling conditions, provides reproducible mixing ratios of two solutions. This fact facilitates that the system automatically generates, on-line, the calibration curves required by the analytical procedure. The calibration ranged from 1.0×10^{-4} to 1.0×10^{-3} mol L⁻¹ for both potassium and total fluoride determinations. The R.S.D. (11 readings) resulted to be less than 1.5% for both determinations. Off-line studies related to the dissolution of the solid samples, the transformation of monofluorophosphate in fluoride, the elimination of organic matrix interference onto the plastic membrane of the potassium electrode, and the selection of the most adequate TISAB solution for fluoride determination, were also considered. A sampling rate of 18 samples h⁻¹ for both determinations was attained, their precisions and accuracies being statistically indistinguishable from those achieved by atomic emission spectroscopy (for potassium determination) and by a conventional batch potentiometry (for total fluoride determination) adopted as reference techniques.

© 2007 Elsevier B.V. All rights reserved.

Keywords: Toothpaste; Potassium and fluoride simultaneous determination; SIA; Potentiometric detection

1. Introduction

It is well known that the fluoride ion has a critical role in preventing tooth decay. Toothbrushing with fluoride ion-containing toothpaste is by far the most common way of caries control. Nowadays, the main sources of fluoride in toothpaste are sodium fluoride and monofluorophosphate (MFP). Different methods of analysis for the determination of fluoride plus MFP (after transformation in fluoride by hydrolysis in presence of hydrochloric acid) in toothpaste have been developed. Some of these methods were potentiometric procedures in batch analysis [1–17]. On the other hand, the fluoride selective electrode was used as potentiometric detector in flow injection analysis (FIA) [18–20] and SIA [21] flow techniques. To a lesser extent, gas chromatography [22,23] and atomic absorption spectrometry [24] were used. Some works, comparing these methods, have been

published [25–27]. To determine simultaneously fluoride and MFP in toothpaste, ion-chromatography [28–32] and capillary electrophoresis [25,33–35] have been proposed. Since FIA is a very effective technique for the automation of chemical analysis and it makes possible multiparametric detection, two FIA manifolds were developed for the simultaneous determination of fluoride and MFP using spectrophotometric detection [36], and fluoride, MFP and orthophosphate using both spectrophotometric and potentiometric detection [37].

Dentinal hypersensitivity is a common unpleasant sensation and many people complain about having teeth that are sensitive to hot or cold liquids or to tactile stimuli. Most cases can be easily treated at home, by brushing with a toothpaste containing a potential desensitizing agent. At present, potassium nitrate is the most utilized desensitizer contained in the toothpastes marketed in many countries. In spite of this, other potassium salts such as chloride [38], oxalate [39,40] or citrate [38] have been recently used in clinical trials. A method based on the use of a nitrate selective electrode for the determination of potassium nitrate in products, used for the treatment of dentinal hypersensitivity, has

* Corresponding author. Tel.: +34 946 014 428; fax: +34 946 014 300.

E-mail address: qappeolr@lg.ehu.es (R. Pérez-Olmos).

been published by some of us [41]. Simultaneous determination of nitrate and MFP in toothpaste, by ion-chromatography, has been developed [42,43].

Nowadays, it is possible to find in the market toothpastes containing compounds used to prevent both caries and dental hypersensitivity. For that reason, in this work and for the first time, an economic, rapid and simple method for the on-line simultaneous determination of total fluoride and potassium contained in toothpastes based on a SIA manifold is proposed. It uses a commercial fluoride selective electrode and a tubular potassium selective electrode, constructed in the laboratory, as potentiometric detectors.

2. Experimental

2.1. Reagents and solutions

For the preparation of the membrane of the potassium tubular potentiometric detector, valinomycin ref. 94675, bis(2-ethylhexyl) sebacate ref. 84818, potassium tetrakis(4-chlorophenyl)borate ref. 60591, all of them from Fluka (Steinheim, Switzerland), and tetrahydrofuran ref. 1.09731 from Merck (Darmstadt, Germany) were used. For the conductive support, a mixture (1:1.2 w/w) of graphite powder ref. 4206 from Merck and a non-conductive epoxy resin (1 g of Araldite M with 0.4 g of hardener REN HY 5162) from Vantico España S.L. (Barcelona, Spain) were utilized.

In the flow system proposed, a TISAB solution was used as ionic strength adjuster/carrier solution, and also as inner electrolyte solution in the reference electrode for both determinations. The ionic strength adjuster for the fluoride and the potassium determinations was a TISAB solution of pH 8.2. This solution was prepared by dilution of 8.4 mL of hydrochloric acid, ref 1.00317, in 500 mL of deionized water and by dissolving 24.2 g of tris(hydroxymethyl)aminomethane, ref. 1.08382, and 23.0 g of sodium tartrate 2-hydrate, ref. 1.06663, all of them from Merck. This mixture was under stirring and heating, and transferred to a volumetric flask of 1000 mL. The volume was adjusted to 1000 mL with deionized water. The ionic strength adjuster solution contained a 1.0×10^{-8} mol L⁻¹ potassium fluoride (Merck ref. 1.04994). The stock solution

used to prepare, on-line, the calibration curves of both ions was a 1.0×10^{-3} mol L⁻¹ potassium fluoride solution prepared by rigorous weighing out of the solid in deionized water.

A 0.1 mol L⁻¹ potassium chloride stock solution (Merck ref 1.04933) was prepared for the potassium determination by using atomic emission spectroscopy. Less concentrated solutions, for the preparation of the calibration curves, were obtained after dilution of the previous one. A 1.0 mol L⁻¹ hydrochloric acid solution was also prepared. For the fluoride batch determination a 0.1 mol L⁻¹ sodium fluoride stock solution (Merck ref. 1.06449) was prepared and a TISAB solution, ten times more concentrated than the solution utilized in the SIA system, was used.

All solutions were prepared with deionized water (conductivity <0.1 μ S cm⁻¹) and analytical grade chemicals were used without any additional purification.

2.2. Apparatus and electrodes

Two Orion 720 potentiometers, sensitivity ± 0.1 mV, from Orion Research (Cambridge, USA), were used for measuring the difference of potential. The reference electrode was a double junction AgCl/Ag (Orion 90-02-00) containing in the outer compartment a solution formed by 1 mL of the TISAB/carrier solution and 10 mL of a 1 mol L⁻¹ sodium chloride solution diluted in 100 mL of deionized water. The potassium selective electrode was a tubular potentiometric detector with PVC membrane and without internal reference solution. The membrane preparation was carried out following a previously described [44] methodology, by mixing 1% (w/w) of valinomycin, as ionophore, 65.8% (w/w) bis(2-ethylhexyl)sebacate, as plasticizer, 0.2% (w/w) potassium-tetrakis(4-chlorophenyl)borate, as membrane additive, and 33.0% (w/w) of PVC previously dissolved in tetrahydrofuran. The membrane solution was directly applied and deposited in the conductor surface, made up of a mixture of epoxy resin with graphite powder, of the central electrode hole. The fluoride selective electrode was an Orion 9409. Both selective electrodes were connected to home-made tubular stainless steel ground electrodes in order to eliminate static electricity pulses. The fluoride selective and the reference elec-

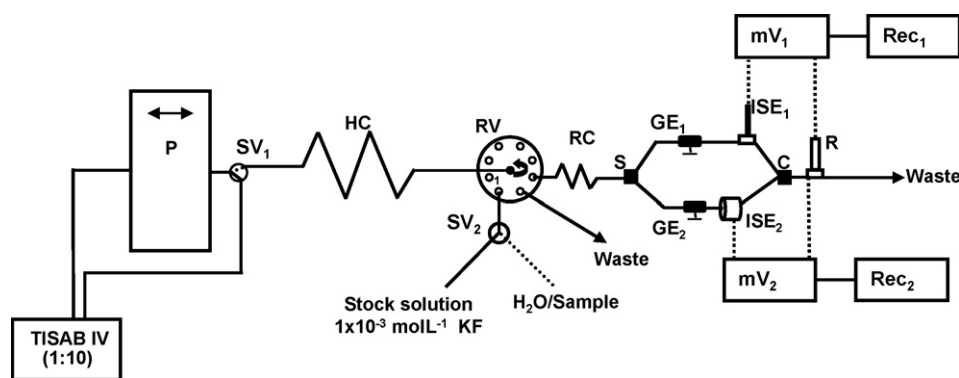


Fig. 1. Schematic representation of the potentiometric SIA system developed. P: peristaltic pump; RV: multiposition rotatory valve; SV₁, SV₂: three-way solenoid valves; HC: holding coil; RC: reaction coil; S: splitting; C: confluence; GE₁, GE₂: ground electrodes; ISE₁: fluoride selective electrode; ISE₂: potassium tubular electrode; R: reference electrode; mV₁ and mV₂: decimilivoltmeters; Rec₁ and Rec₂: recorders.

trodes were fitted in home-made plastic supports constructed, with slight modifications, according to Ref. [45].

The flow system depicted in Fig. 1 is formed by a peristaltic pump Gilson Minipuls 3 (Villiers-le-Bel, France) with a PVC pumping tube (i.d. 1.65 mm) of the same brand, and an electrically actuated multiposition fluid selection 8-port valve, from Valco Instruments (Houston, USA), model Cheminert™ C25-3188 D. In order to achieve maximum reproducibility of fluid drive in the manifold, a three-way solenoid valve (SV₁) NResearch 161 T031 (Stow, USA) was placed between the peristaltic pump and the holding coil. This valve was activated by establishing electric contact on an electronic switch placed in a defined position of the rotating head of the peristaltic pump [46]. Another three-way solenoid valve (SV₂), of the same brand, connected to the lateral port 1 of the rotatory valve, was used for the on-line preparation of the calibration curves. The manifold was connected with PTFE tubes (i.d. 0.8 mm). The holding coil was 200 cm long and the reaction coil 50 cm. Some auxiliary laboratory-made devices, i.e. joints and grounding electrodes, were made from Perspex and stainless steel tubing [45]. Two single channel stripchart recorders Kipp & Zonen BD11 (The Netherlands) were used for the simultaneous data acquisition. All the SIA system operations were controlled by a computer through an Advantech PCL-711S interface card and a program written in Quick Basic 4.5.

The determination of potassium by atomic emission spectroscopy, adopted as reference method, was performed with a PFP 7 flame photometer from Jenway Ltd. (Dunmow, UK). A Meditronic centrifuge, an ultrasonic bath 3001208 Ultrasons and a magnetic stirrer 243 Agimatic, all of them from Selecta (Barcelona, Spain), were used for the sample treatment. Glass or plastic volumetric material was also used when necessary.

2.3. Procedures

2.3.1. Sample treatment

In a first step, toothpaste or gel samples (2 g) were exactly weighed into a 50 mL centrifuge tube, made of polypropylene, and 20 mL of a hydrochloric acid solution (2 mol L⁻¹) were

added. The tube was introduced into an ultrasonic bath where it was heated (40 °C) and sonicated for 10 min. Afterwards, it was centrifuged (2500 rpm) for 4 min, and an aliquot of 10.0 mL of the supernatant was exactly pipetted into a 50 mL volumetric flask and made up to volume with deionized water. In a second step, the solution was transferred to a 250 mL separating funnel where 25 mL of ethyl ether and 25 mL of ethylacetate were added. After shaking and resting for 5 min, the aqueous phase was collected and an exact volume (5.0 mL) was pipetted into a 50 mL volumetric flask and, finally, the volume was completed with deionized water.

2.3.2. Developed SIA procedure

Initially, placing the peristaltic pump in propulsion mode, the transmission channels between the multiposition valve and each detection system were filled with the carrier solution. This operation mode was maintained until a stable baseline was obtained for both electrodes. The steps required for getting the two analytical signals are shown in Table 1. The first three steps of the calibration procedure and the first two steps of the measurement procedure correspond to the substitution of the solutions that fill the SIA manifold.

Taking into account the potassium and total fluoride concentrations labelled on the toothpaste and gel samples, calibration solutions of 1.0×10^{-4} , 3.0×10^{-4} , 7.0×10^{-4} and 1.0×10^{-3} mol L⁻¹ for potassium and total fluoride determinations were prepared automatically in the interior of the manifold for each selection of port 1 by means of the SV₂ on/off cycles [47]. Small standard potassium fluoride plugs were aspirated into the HC by activation of the SV₂ (on) in an alternating mode with plugs of the deionized water when the SV₂ was in the off position. The mutual dispersion between both solutions during the aspiration and the sending stages to the potentiometric detector, through port 3, enables the simulation of the different standard solutions with intercalation the above-mentioned concentrations. To obtain these standard solutions, the SV₂ was activated during 10, 30, 70 and 100% of each on/off cycle time applied to the SV₂. For the potassium and fluoride calibrations 250 μL of stock solution (corresponding to 4 cycles of the SV₂)

Table 1
System operation

RV	SV ₂ state	Volume (μL)	Flow rate (mL min ⁻¹)	Flow direction	Event
Potassium and fluoride selective electrode calibration					
1	ON	150	1.3	R	Stock potassium and fluoride
1	OFF	150	1.3	R	Water
2	–	500	4.0	F	Waste
1	ON	250	1.3	R	On-line potassium and fluoride standard preparation
	10%				
	30%				
	70%				
	100%				
3		2350	1.5	F	Potassium and fluoride measurement
Potassium and fluoride determination					
1	OFF	150	1.3	R	New sample
2	–	300	4.0	F	Waste
1	OFF	250	1.3	R	Potassium and fluoride sample volume
3		2350	1.5	F	Potassium and fluoride measurement

were aspirated and sent to both detectors through port 3 at a flow rate of 1.5 mL min^{-1} . Once the standard solutions were obtained, on-line, and taking into account that the propulsion mode was already selected, the carrier solution was directly mixed with the corresponding standard solution. For the analytical determination of potassium and total fluoride in toothpaste samples, port 1 was selected and filled up with the off-line treated sample to be measured. After that, $250 \mu\text{L}$ of this sample solution were aspirated into the HC and sent to the potassium and fluoride detectors through port 3 at a flow rate of 1.5 mL min^{-1} .

2.3.3. Reference procedures

2.3.3.1. Potassium determination. The toothpaste sample was dissolved according to the first step of the sample treatment and 1.0 mL of that solution was diluted 100 times with deionized water and directly aspirated in a Jenway PFP7 Flame Photometer. Potassium concentration was obtained by means of calibration curves with concentration ranging from 5.0×10^{-5} to $2.5 \times 10^{-4} \text{ mol L}^{-1}$ of potassium, prepared from a $5.0 \times 10^{-3} \text{ mol L}^{-1}$ potassium chloride standard solution, also prepared by rigorous dilution of the $1.0 \times 10^{-1} \text{ mol L}^{-1}$ potassium chloride stock solution. All the solutions were treated with 0.5 mL of a $1.0 \times 10^{-1} \text{ mol L}^{-1}$ hydrochloric acid solution. A blank assay was also carried out.

2.3.3.2. Total fluoride determination. An aliquot of 25.0 mL of the solution obtained according to the first step of the sample treatment was pipetted in a plastic beaker and other 25.0 mL of TISAB IV solution were added, the fluoride selective and reference electrodes were immersed and the fluoride concentration was determined by direct potentiometry.

3. Results and discussion

3.1. Optimization of the flow system

Optimization of the operational conditions was performed by analyzing the influence caused by varying concentration, volume and flow rates of the solutions. The intensity and repeatability of the potentiometric signals obtained were studied either by varying the injection volume for the two determinations in the interval $100\text{--}550 \mu\text{L}$ and the propulsion rate in the interval $1.2\text{--}3.5 \text{ mL min}^{-1}$ in a way, whereby the sampling rate was

not diminished. With the increase of the injection volume, an increase in the intensity of analytical signals was verified for volumes up to $250 \mu\text{L}$ for potassium and fluoride. For greater injection volumes no significant improvement in the intensity of signals was evidenced and it was also observed that volumes smaller than $250 \mu\text{L}$ provided dispersion of the sample zone and consequently less linearity. This injection volume was obtained for a maximum flow rate of 1.5 mL min^{-1} for both ions.

To evaluate the analytical usefulness of the developed SIA system it was decided to devise a simple and quick procedure for the simultaneous determination of potassium and total fluoride in gels and toothpastes used against caries and dentinal hypersensitivity. Some assays were carried out to study the possibility of using a unique carrier solution to adjust the ionic strength in both determinations. The linear concentration ranges, slopes and reproducibility of the measurement were observed when phosphate buffer solution (pH 5.5) and TISAB II and III (pH 5.5) and TISAB IV (pH 8.2) buffer solutions from Orion Research were used. For the fluoride determination the best results were obtained with TISAB IV, which confirm the results previously obtained by some of us [11] in batch determination of fluoride in toothpastes containing silica or alumina. In those occasions, the CDTA contained in TISAB II and III was unable to break completely complexes formed by fluoride and Si (IV) or Al (III) ions, which could be achieved by sodium tartrate contained in TISAB IV. Unfortunately, TISAB IV buffer solution resulted to be not adequate as carrier in the potassium determination, since the potassium electrode showed lower slope and narrower linear concentration range, due to the interference action of the high sodium concentration contained in the TISAB IV solution. For that reason, and taking into account the value of the potentiometric coefficient of the potassium selective electrode, a new TISAB solution, 10 times less concentrated, was prepared. This solution was used as TISAB and carrier solution for both determinations, these showing adequate results. It is essential for the carrier solution to contain a small concentration of KF, since it stabilizes the baseline, guaranteeing constant conditioning of the electrode surface between consecutive injections and minimizing the dissolution of the electrode membrane surface. In this way, the electrode operational characteristics were improved and its lifetime extended [48]. Since a unique TISAB/carrier solution and a unique reference solution in the outer compartment of the double junction reference electrode could be used for

Table 2
Figures of merit of the developed potentiometric (SIA) system

	Potassium determination	Fluoride determination
Linear concentration range (mol L^{-1})	5.2×10^{-5} to 5.0×10^{-2}	8.3×10^{-5} to 5.0×10^{-2}
Regression equation ^a	$E = 58.1 \log [C] + 115.3$	$E = -57.4 \log [C] - 121.7$
Quadratic correlation coefficient (R^2)	0.9996	0.9995
Electrode slope and standard deviation (mV decade^{-1})	58.1 ± 0.6	57.4 ± 0.4
Practical limit of detection (mol L^{-1}) ^b	9.4×10^{-6}	4.3×10^{-5}
Relative standard deviation (%) ^c	1.3	1.2
Sampling rate (sample h^{-1})	18	18

^a E : potentiometric signal (mV); $\log [C]$: logarithm of analyte concentration (mol L^{-1}).

^b Determined according to IUPAC recommendations [50].

^c Obtained for 11 replicates at $10^{-3} \text{ mol L}^{-1}$ analyte standard solutions.

both potentiometric determinations, the SIA system adopted the configuration shown in Fig. 1, which enables the simultaneous determination of potassium and total fluoride.

The general working characteristics of both electrodes were evaluated after coupling them to the SIA system, and using the TISAB/carrier solution mentioned above. After selecting the optimum injection volume and the propulsion flow rate, the characteristics of both electrodes were determined by constructing repeatedly calibration curves for both ions with concentrations ranging from 5.0×10^{-6} to 5.0×10^{-2} mol L⁻¹ and the results obtained are shown in Table 2. In the case of potassium determination, it was not observed any significant baseline drift. On the contrary, during fluoride determination, a baseline drift of 8 mV h⁻¹ was obtained at the beginning of the work, when the system was not continuously in use. A similar effect was pointed out by other authors [49].

Having optimized the SIA system and once the characteristics of both electrodes in flow conditions were known, the automation of on-line calibration and sample determination procedures were carried out as previously described in Section 2.3. The use of a solenoid valve under binary sampling conditions provides reproducible mixing ratios of two solutions [47]. The system enables the on-line preparation of the calibration solutions from the stock solution using the SV₂ placed in port 1. The analytical signals were obtained with an intensity of 95% comparatively to those obtained with the conventional analysis of the same calibration solutions, prepared manually.

3.2. Study of sample treatment

Despite the complexity of the toothpastes, fluoride selective electrode gives adequate results during the potentiometric

determination of sodium, tin or zinc fluoride. However, at the beginning of this work some considerations, previously reported [12], related to the quantitative extraction of Na₂FPO₃ contained in some toothpaste samples and also to its own hydrolysis, were taken into account. On the one hand, during the ageing of these products part of the Na₂FPO₃ can be hydrolyzed to fluoride which may react with calcium or magnesium, liberated from the abrasives contained in the dentifrices to form sparingly soluble salts. Consequently, the quantitative transfer of total fluoride into the water solutions is difficult to achieve. Some authors have suggested that the extraction step could be carried out properly and in a short space of time by addition of acid. On the other hand, the hydrolysis of Na₂FPO₃ follows the ionisation of the salt in aqueous solution and it is also well known that the excess of acid catalyzes this reaction. Heating the solution can also help to this process. For all these reasons, some preliminary studies to dissolve the samples were carried out in this work. Initially, 20 mL of different concentration (1–4 mol L⁻¹) hydrochloric acid solutions were added onto three samples of toothpaste and the corresponding slurries were sonicated during different times (5–30 min) in an ultrasonic bath at different temperatures (30–70 °C). After that, the samples were centrifuged at different speeds (1500–3500 rpm) and times (1–10 min). The potassium and total fluoride concentrations were determined by applying the reference methods and from the data obtained it is possible to affirm that the best result was obtained sonicating at 40 °C for 10 min, using a 2.0 mol L⁻¹ hydrochloric acid solution and centrifuging at 2500 rpm for 4 min. These three samples of known concentration were specially prepared by a manufacturer and kindly donated to us. Since the presence of surfactant agents in some samples of toothpastes causes a negative effect on the plastic membrane of the potassium electrode, a drift in the poten-

Table 3
Potassium determination in dentifrices and gels by simultaneous application of the developed SIA potentiometric and reference AES methods

S ^a	LLC ^b	Developed SIA method			Reference AES method			RE ^f	F ^g
		X ^c	R.S.D. ^d	R ^e	X ^c	R.S.D. ^d	R ^e		
D1	19.6	18.0 ± 0.7	3.9	100.9	17.5 ± 0.4	2.3	97.8	2.8	3.06
D2	20.5	18.9 ± 0.2	1.1	101.3	20.1 ± 0.4	2.0	101.4	-1.0	4.00
D3	19.3	18.8 ± 0.4	2.1	104.0	19.4 ± 0.5	2.6	101.9	-3.1	1.56
D4	19.3	19.3 ± 0.6	3.1	101.8	19.6 ± 0.7	3.6	99.9	-1.5	1.36
D5	19.3	17.5 ± 0.4	2.3	100.6	17.8 ± 0.3	1.7	100.4	-1.7	1.78
D6	19.3	18.2 ± 0.5	2.7	99.5	18.3 ± 0.4	2.2	101.3	-0.5	1.56
D7	20.3	18.5 ± 0.3	1.6	101.7	18.4 ± 0.2	1.1	103.1	0.5	2.25
D8	19.3	17.0 ± 0.5	2.9	102.6	17.3 ± 0.7	4.0	98.3	-1.7	1.96
D9	19.3	19.0 ± 0.6	3.2	100.5	18.8 ± 0.4	2.1	101.3	1.1	2.25
D10	19.3	19.6 ± 0.6	3.1	101.2	19.1 ± 0.7	3.7	99.7	2.6	1.36
D11	9.4	9.2 ± 0.3	3.3	100.7	9.0 ± 0.2	2.2	101.5	2.2	2.25
G1	38.7	37.5 ± 0.8	2.1	100.5	37.8 ± 0.5	1.3	100.7	-0.8	2.56
G2	19.3	18.5 ± 0.5	2.7	99.5	18.9 ± 0.2	1.0	100.3	-2.1	6.29
G3	38.7	37.9 ± 0.7	1.8	99.2	37.1 ± 0.3	0.8	98.0	2.1	5.44
G4	38.7	37.0 ± 0.3	0.8	102.7	37.5 ± 0.6	1.6	102.0	-1.3	4.00

^a Type of sample: D1–D11 for dentifrices; G1–G4 for gels.

^b Labelled potassium concentration (g kg⁻¹).

^c Mean potassium concentration and standard deviation (g kg⁻¹).

^d Relative standard deviation (%).

^e Mean spike recovery corresponding to 3 additions of KF standard (%).

^f Relative error of the developed SIA method vs. the reference method (%).

^g The critical *F*, considering a 95% confidence level and 5 degrees of freedom, for a two-tailed test is 7.15.

tial values was observed. Consequently, they were eliminated by liquid–liquid extraction using a mixture of 50% ethyl ether and 50% ethyl acetate in a sample/solvent ratio of 1:1.

3.3. Validation of the proposed procedure

Hence, 15 samples of gels and toothpastes commercialized in Europe were treated as described above and the resulting solutions were analyzed by the developed SIA procedure. As the authors could not find any reference or recommended method for the determination of potassium in this type of products, the resulting solutions were simultaneously analyzed by atomic emission spectroscopy, adopted as reference technique. In the case of total fluoride determination, the results attained were compared with those obtained by application of a manual version of the potentiometric procedure. The results furnished by simultaneous application of the proposed and reference methods are summarized in Tables 3 and 4. In each case, the precisions were evaluated by application of both the treatment sample step and the analytical procedure, to 6 samples of the same product and were expressed in terms of their relative standard deviations. From the data obtained, it is possible to affirm that the precisions of both SIA potentiometric determinations were good, since the mean relative standard deviations were 2.5% for the potassium and 2.7% for the total fluoride determinations. To test whether the potentiometric and reference methods differ in their precision, a significance (two-tailed) *F* test was carried out. The calculated *F* values were less than the critical *F* value for all the samples analyzed. Hence, there is no statistically significant difference between the two standard deviations at the 95% confidence level [51]. Standard potassium and fluoride additions were used to evaluate the accuracy of the methods. Four

identical samples of all the toothpastes analyzed were prepared, and their potassium and total fluoride determined, by application of the SIA developed procedure. One sample received no added potassium and fluoride ions, and additions of 1, 2 and 3 mL of a standard solution $5.0 \times 10^{-2} \text{ mol L}^{-1}$ in sodium fluoride and 1.0 mol L^{-1} in potassium chloride were made to the remaining samples before applying the sample treatment. This assay was carried out in duplicate and the average percentages of spike recovery for the three different additions were calculated. These were 101.3% and 100.1% for potassium and total fluoride, respectively. As it can be observed in Tables 3 and 4, these data were very similar to those supplied when the reference methods were carried out. On the other hand, no discrepancies in potassium and total fluoride contained in the commercial samples were observed, since the mean percentage of differences between the developed and reference methods ranged from -3.1% to 2.8% for potassium and from -2.5% to 3.8% for total fluoride determination.

A statistical comparison was carried out in order to establish whether the developed SIA potentiometric method could be accepted as giving reliable results. The *t*-test with multiple samples (paired by differences) was performed at a $P = 0.05$ confidence level. The *t*-test adopted the null hypothesis and it was two-tailed since we were interested in both $\bar{x}_D < 0$ and $\bar{x}_D > 0$, being \bar{x}_D the mean difference between the methods. The *t*-data paired test was applied using the formula $t_{\text{calc.}} = \bar{x}_D \sqrt{n} / S_X$, where S_X is the standard deviation of the differences. When substituting for $t_{\text{calc.}}$ they were found to be -0.91 and 0.89 for potassium and total fluoride, respectively. Since the calculated values were less than the tabulated value ($t = 2.14$), there is no statistically significant difference between the proposed potentiometric SIA method and the reference methods.

Table 4

Total fluoride determination in dentifrices and gels by simultaneous application of the developed SIA and batch potentiometric methods

S ^a	LLC ^b	Developed SIA method			Reference ISE method			RE ^f	F ^g
		X ^c	R.S.D. ^d	R ^e	X ^c	R.S.D. ^d	R ^e		
D1	1.45	1.15 ± 0.06	5.2	99.6	1.13 ± 0.04	3.5	100.5	1.8	2.25
D2	1.00	0.93 ± 0.05	5.4	101.0	0.91 ± 0.07	7.7	101.6	2.2	1.96
D3	1.00	0.96 ± 0.04	4.2	97.2	0.94 ± 0.02	2.1	100.3	2.1	4.00
D4	1.00	0.99 ± 0.02	2.0	99.8	0.98 ± 0.05	5.1	99.0	1.0	6.25
D5	2.50	2.35 ± 0.05	2.1	103.3	2.37 ± 0.07	2.9	100.4	-0.8	1.96
D6	2.50	2.16 ± 0.03	1.4	100.7	2.13 ± 0.05	2.3	100.3	1.4	2.78
D7	1.50	1.40 ± 0.04	2.8	100.6	1.38 ± 0.07	5.1	99.4	1.4	3.06
D8	2.50	2.27 ± 0.07	3.1	99.0	2.33 ± 0.05	2.1	101.9	-2.5	1.96
D9	2.50	2.65 ± 0.05	1.9	101.5	2.68 ± 0.08	3.0	99.6	-0.7	2.56
D10	1.50	1.41 ± 0.02	1.4	99.6	1.45 ± 0.05	3.4	100.5	-2.7	6.25
D11	1.50	1.46 ± 0.03	2.0	98.9	1.48 ± 0.06	4.0	99.8	-1.3	4.00
G1	1.00	1.11 ± 0.03	2.7	101.1	1.07 ± 0.05	4.7	100.8	3.7	2.78
G2	2.50	2.44 ± 0.03	1.2	100.4	2.35 ± 0.07	3.0	100.5	3.8	5.44
G3	2.60	2.65 ± 0.07	2.6	98.5	2.61 ± 0.05	1.9	99.7	1.5	1.96
G4	1.50	1.52 ± 0.03	2.0	100.2	1.49 ± 0.05	3.3	102.3	2.0	2.78

^a Type of sample: D1–D11 for dentifrices; G1–G4 for gels.

^b Labelled total fluoride concentration (g kg^{-1}).

^c Mean total fluoride concentration and standard deviation (g kg^{-1}).

^d Relative standard deviation (%).

^e Mean spike recovery corresponding to 3 additions of KF standard (%).

^f Relative error of the developed SIA method vs. the reference method (%).

^g The critical *F*, considering a 95% confidence level and 5 degrees of freedom, for a two-tailed test is 7.15.

The limits of detection of the proposed SIA determinations including the sample treatment were established by its application (11 times) to a toothpaste specially prepared by a local manufacturer, without potassium and fluoride ions in its formulation, and calculating their standard deviations. Considering ($\mu + 3\sigma$ blank) as the definition of the Analytical Methods Committee [52], they resulted to be 0.16 g kg^{-1} for potassium determination and 0.15 g kg^{-1} for fluoride determination. The limits of quantification adopting a ($\mu + 10\sigma$ blank) criterion were 0.19 and 0.17 g kg^{-1} for the potassium and fluoride determinations, respectively.

4. Conclusions

The study undertaken showed that the possibility of coupling two potentiometric detectors, selective to potassium and fluoride, to a SIA system have permitted, for the first time, the multiparametric determination of both ions in toothpastes and gels used to simultaneously prevent dentinal hypersensitivity and caries. By implementing a sequential injection set-up with an additional binary sampling approach, the versatility of the system allowed to on-line generate solutions for automatic calibration resulting in a remarkable decrease on the reagent consumption. The system was easy to operate, it provided adequate sampling rates, and the developed potentiometric method resulted to be precise and accurate with results statistically in accordance with those obtained by applying the reference methods.

Acknowledgements

This work was supported by the University of the Basque Country (Spain) through Project UPV 171.363-E-14795/2002. One of the authors (N.Z.) wishes to thank to this university for the Ph.D. Grant 98/13828/01. We also thank to the Department of Mechanical Engineering for constructing the plastic cell for housing the electrode of conventional configuration and to Lacer S.A., Spain (Barcelona), for the toothpaste samples kindly provided.

References

- [1] N. Shane, D. Miele, *J. Pharm. Sci.* 57 (1968) 1260–1262.
- [2] E.J. Bushee, D.K. Grissom, D.R. Smith, *J. Dent. Child* 38 (1971) 279–281.
- [3] C. Martin, B. Mandrou, *Trav. Soc. Pharm. Montpellier* 33 (1973) 229–232.
- [4] T.S. Light, C.C. Capuccino, *J. Chem. Edit.* 52 (1975) 247–250.
- [5] M. Friedman, *Pharm. Acta Helv.* 56 (1981) 9–13.
- [6] D.W. Cook, *Aust. Dent. J.* 26 (1981) 299–303.
- [7] F.N. Hattab, *J. Dent.* 17 (1989) 77–83.
- [8] M.T. Orzáez, P. García, *Alimentaria* 217 (1990) 43–45.
- [9] P.W. Heidbüchel, *Pharm. Acta Helv.* 66 (1991) 290–296.
- [10] M. Ivanova, R. Christova, *Anal. Lab.* 4 (1995) 47–50.
- [11] R. Pérez-Olmos, E. Resines, E. Arza, F. Rico, A. Rios, *Ind. Farm.* (1997) 59–63.
- [12] M. Pavic, D. Carevic, Z. Cimerman, *J. Pharm. Biomed. Anal.* 20 (1999) 565–571.
- [13] R.J. Casella, H.C. Araújo, A.J. da Silva, J. Ademario, M.B. Cruz, M.D. Campos, *Anal. Lett.* 33 (2000) 819–829.
- [14] M.R. Ganjali, V. Akbar, M. Ghorbani, P. Norouzi, A. Ahmadi, *Anal. Chim. Acta* 531 (2005) 185–191.
- [15] M.R. Ganjali, P. Norouzi, M. Adib, A. Ahmadi, *Anal. Lett.* 39 (2006) 1075–1086.
- [16] M.R. Ganjali, A. Ghesmi, M. Hosseini, M.R. Pourjavid, M. Rezapour, M. Shamsipur, M. Salavati-Niasari, *Sens. Actuators B: Chem.* B105 (2005) 334–339.
- [17] M.R. Ganjali, S. Rasoolipour, M. Rezapour, P. Norouzi, A. Tajarodi, Y. Hanifehpour, *Electroanalysis* 17 (2005) 1534–1539.
- [18] R.-J. Stefan, J.F. van Staden, H.Y. Aboul-Enein, *Pharm. Acta Helv.* 73 (1999) 307–310.
- [19] L. Gámiz-Gracia, M.D. Luque de Castro, *J. Pharm. Biomed. Anal.* 22 (2000) 909–913.
- [20] Y. Zhou, A. Yan, H. Xu, K. Wang, X. Chen, Z. Hu, *Analyst* 125 (2000) 2376–2380.
- [21] J.F. van Staden, R.-J. Stefan, S. Birghila, *Talanta* 52 (2000) 3–11.
- [22] *Off. J. Eur. Communities* L291 (1983) 37–40.
- [23] C. Pham Huy, F. Nadji, M. Postaire, M. Hamon, *Ann. Pharm. Fr.* 49 (1991) 139–150.
- [24] M. Gómez, M.A. Palacios, C. Cámara, *Microchem. J.* 47 (1993) 399–403.
- [25] E. Skocir, A. Pecavar, A. Krausja, M. Prosek, *J. High Resol. Chromatogr.* 16 (1993) 243–246.
- [26] S.A. Black, G.P. Matthews, *Anal. Proceed.* 26 (1989) 67–69.
- [27] P.A. Compagnon, *Sci. Tech. Pharm.* 12 (1983) 495–499.
- [28] J.J. Potter, A.E. Hilliker, G.J. Breen, *J. Chromatogr.* 367 (1986) 423–427.
- [29] D. Murawski, *J. Chromatogr.* 546 (1991) 351–367.
- [30] N. Yazo, S. Nakashima, T. Nakazato, N. Ueda, H. Kodama, A. Tateda, *Anal. Chem.* 64 (1992) 1499–1501.
- [31] D.J. Pinkman, M.P. Knapp, *J. Liq. Chrom. Rel. Technol.* 23 (2000) 1099–1108.
- [32] V.F. Samanidou, C.K. Zacharis, I.N. Papadoyannis, *J. Liq. Chrom. Rel. Technol.* 25 (2002) 803–818.
- [33] D. Jara, S. Borrós, *Afinidad* 460 (1995) 393–398.
- [34] P. Wang, S.F.Y. Li, H.K. Lee, *J. Chromatogr. A* 765 (1997) 353–359.
- [35] P. Wang, S.F.Y. Li, *J. Chromatogr. A* 781 (1997) 457–466.
- [36] D.G. Themelis, P.D. Tzanavaras, *Anal. Chim. Acta* 429 (2001) 111–116.
- [37] P.D. Tzanavaras, D.G. Themelis, *Anal. Chim. Acta* 467 (2002) 83–89.
- [38] D. Hu, Y.P. Zhang, P. Chaknis, M.E. Petrone, A.R. Volpe, W. de Vizio, *J. Clin. Dent.* 15 (2004) 6–10.
- [39] J.C. Pereira, A.D. Segala, D.G. Gillam, *Dent. Mater.* 21 (2005) 129–138.
- [40] F.L. Pillon, I.G. Romani, E.R. Schmidt, *J. Periodontol.* 75 (2004) 1461–1464.
- [41] R. Pérez-Olmos, P. Bezares, J. Pérez II, *Farmaco* 55 (2000) 99–103.
- [42] J.M. Talamage, T.A. Biemer, *J. Chromatogr.* 410 (1987) 494–499.
- [43] S.S. Chen, H. Lulla, F.J. Sena, V. Reynoso, *J. Chromatogr. Sci.* 23 (1985) 355–359.
- [44] S. Alegret, J. Alonso, J. Bartroli, J.L.F.C. Lima, A.A.S.C. Machado, *Anal. Chim. Acta* 164 (1984) 147–152.
- [45] S. Alegret, J. Alonso, J. Bartroli, A.A.S.C. Machado, J.L.F.C. Lima, J.M. Paulis, *Quim. Anal.* 6 (1987) 278–294.
- [46] A.N. Araújo, R.C.C. Costa, J.L.F.C. Lima, *Anal. Sci.* 15 (1999) 991–994.
- [47] B.F. Reis, B.F. Giné, E.A.G. Zagatto, J.L.F.C. Lima, R.A. Lapa, *Anal. Chim. Acta* 293 (1994) 129–138.
- [48] A. Andrade-Eiroa, J.A. Elustes, R. Forteza, V. Cerdá, J.L.F.C. Lima, *Anal. Chim. Acta* 467 (2002) 25–33.
- [49] J. Alpizar, A. Crespi, A. Cladera, R. Forteza, V. Cerdá, *Electroanalysis* 11 (1996) 1051–1054.
- [50] IUPAC, *Pure Appl. Chem.* 53 (1981) 1907–1912.
- [51] J.C. Miller, J.N. Miller, *Statistics for Analytical Chemistry*, Second edition, Ellis Horwood Ltd., Chichester, UK, 1988.
- [52] Analytical Methods Committee, *Analyst* 112 (1987) 199–204.