Available online at www.sciencedirect.com
SCIENCE DIRECT®
Talanta 67 (2005) 651–657
Talanta
www.elsevier.com/locate/talanta

FIA-potentiometry in the sub-Nernstian response region for rapid and direct chloride assays in milk and in coconut water

Iranaldo Santos da Silva [a], Eduardo Mathias Richter [b], Claudimir Lúcio do Lago [b], Ivano G.R. Gutz [b], Auro Atsushi Tanaka [a], Lúcio Angnes [b,*]

[a] *Departamento de Química, CCET, Universidade Federal do Maranhão, São Luís, MA, Brazil*
[b] *Departamento de Química Fundamental, IQ, Universidade de São Paulo, Av. Prof. Lineu Prestes, 748, 05508900 Sao Paulo, SP, Brazil*

Received 4 January 2005; received in revised form 15 March 2005; accepted 17 March 2005
Available online 18 April 2005

Abstract

A simple and reliable FIA-potentiometric system for rapid assays of chloride in certain food samples is described and evaluated. The system is constituted by an aquarium air pump to propel the carrier solution, a manually operated injector, a homemade dialysis flow cell, a solid-state chloride detector (Ag/AgCl), a reference electrode and a multimeter connected to a microcomputer for data acquisition. The dialysis unit enables direct analysis of liquid food samples without any other previous treatment. The principal novelties are the precision (R.S.D. of 1.2% for whole milk) and rapidity (90 determinations/h) of FIA measurements near and below the lower end of the linear (Nernstian) response region of the chloride ion-selective electrode (ISE), with an estimated detection limit (3 s) of 0.4 mg L^{-1} Cl^- in the sample injected in donor stream. Data of peak potential versus sample chloride concentration (donor stream) was accurately fitted with a quadratic polynomial over the range between 4 and 1000 mg L^{-1} ($r^2 = 0.9999$) and used as a calibration curve. The method was applied to the determination of chloride in milk and in coconut water samples. The validation of the results was done by comparison with a NIST reference material (milk) or by capillary electrophoresis (coconut water). For all analysis, no significant difference at a 95% confidence level was observed.

Keywords: Chloride ISE; FIA; In-line dialysis; Sub-Nernstian response; Milk analysis; Coconut water analysis

1. Introduction

Chloride determination finds widespread application in clinical, environmental, industrial, food and beverage analysis [1–15]. This ion is a vital element for humans, animals and plants, but the narrow limits between deficiency and excess require the precise control in many situations. Sodium chloride is a major inorganic constituent of blood, but relatively small excess is pointed as the main cause of hypertension [1]. In soils, the accurate determination is important due to the potential toxicity of this ion on some plants and also because it increases the osmotic pressure around the plant roots, which drastically inhibits water uptake [5]. In the industry, there are many applications where the determination of chloride is crucial [8,9]. The analysis of chloride in foods [10–12] and in beverages [13–15] is also of major importance.

In the milk industry, chloride analysis plays an important role because it permits to detect diseases such as mastitis [16] and to avoid fraudulent addition of salt to increase the specific gravity [12]. Sub clinical mastitis can be indicated by the increase of chloride content in milk, even in the absence of the characteristics symptoms of this disease [17,18]. In this case an increase of the chloride content is observed, caused by the marked reduction in the amount of milk produced. This is also valid in humans, and some authors state that breastfeeding increases the risk of postnatal mother-to-child HIV transmission when the mastitis disease is not detected and quickly controlled [19,20].

Another important food sample where chloride is the predominant anion is green coconut water. This refreshing beverage, extracted from the nut of *Cocus nucifera*, is well known

* Corresponding author. Tel.: +55 11 3091 3828; fax: +55 11 3091 2150.
E-mail address: luangnes@iq.usp.br (L. Angnes).

0039-9140/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2005.03.010

as an important alternative for oral rehydration [21] or even for patient intravenous hydration in remote regions [22]. A survey in the literature shows that there is a lack of studies of chloride quantification in this sample. Besides the presence of vitamins, sugars, organic acids, fatty acids, amino acids and different minerals [23], a serious interference for chloride quantification by potentiometry with an ion selective electrode (AgCl or Ag_2S/AgCl) in this sample is sulfite [24], which is added as a preservative to bottled Brazilian coconut water.

Various techniques are available for the determination of chloride in foods and beverages including direct potentiometry [3,11,14], potentiometric titration [6,8,13], photometry [15], spectrometry (ICP-OES) [16], conductometry [18] and ion chromatography [25]. The association of potentiometry and flow injection analysis is an attractive alternative [3,4,12] because it incorporates the combination of these two techniques. Unfortunately, for the majority of the samples, the complexity of the matrix hampers the direct determination. The simplest way found to eliminate (or minimize) interferences is to separate the analyte through dialysis [2,5,9] thus avoiding more expensive and time consuming separation techniques like ion chromatography [25] or capillary electrophoresis [26].

For solid-state ion selective electrodes, the chloride response (potential variation as a function of the logarithm of the chloride activity) presents Nernstian behavior at concentration levels above 5 mg L^{-1} under equilibrium conditions (batch measurements) and a gradual deviation from linear behavior with a decrease in the slope below this limit (apparently sub-Nernstian response) [27–29]. Under FIA operation, useful measurements can be made down to about 10 μg L^{-1} [27]; however, the Nernstian behavior is displaced to a higher chloride activity (or concentration, at constant ionic strength) region in comparison with batch operation. At the lowest end, a linear relationship of potential versus concentration sets in, as explained by Trojanowicz and Matuszewski [29]. However, in the FIA methods for chloride proposed by these authors [27,29], they preferred to work in the Nernstian region.

The present contribution is the first to explore, with real food samples, the advantages of a potentiometric FIA method operating in the sub-Nernstian region. In-line dialysis is the only pre-treatment applied to whole milk and coconut water samples directly injected in the FIA system.

2. Experimental

2.1. Reagents, solutions and samples

All chemicals were of analytical grade and employed without further purification. The reagents 2-[*N*-morpholino] ethanosulfonic acid (MES), L-histidine (HIS) and, *N*-cetyl-*N*-*N*-*N*-trimethylammonium bromide (CTAB), utilized in the capillary electrophoresis were purchased from Merck (Germany). The bromide from CTAB was exchanged by hydroxide with an anion-exchanger-III column (Merck) [30,31], resulting in *N*-cetyl-*N*-*N*-*N*-trimethylammonium hydroxide (CTAH). The running buffer was composed by 30 mmol L^{-1} MES/HIS and 0.2 mmol L^{-1} CTAH. The CTAH was added to promote the inversion of the direction of the electroosmotic flow. The solutions were prepared with ultra pure water ($R \geq 18$ MΩ cm^{-1}, NANOpure, Barnstead, USA). For potentiometric analysis a 20.00 g L^{-1} chloride stock solution was prepared from 6.5915 g of previously dried NaCl, which was diluted with water up to 200 mL. Working solutions were prepared by dilution of this stock solution in the range of 4.0–1000 mg L^{-1}. The acceptor solution utilized in the detector line was constituted by 0.1 mol L^{-1} sodium nitrate and 0.002 mol L^{-1} nitric acid, providing a slightly acid electrolyte with constant ionic strength. In the sample (donor) line, water with 0.5% Triton-X 100 was used as carrier. The surfactant increases the lifetime of the dialysis membrane in the presence of the studied samples. Milk and coconut water samples were purchased in a local supermarket and injected after simple dilution with water. The reference milk sample (NIST 1549) was purchased from National Institute of Standards and Technology (NIST, USA).

2.2. Instruments and apparatus

The potentiometric measurements were performed with a 16-bit multimeter (model HC 608, Hung Chang, Seoul, Korea) interfaced to a microcomputer through a RS-232 serial port [32,33]. This instrument comes with proprietary software (Protec 608) for data acquisition, display, and saving as text files that can be imported by spreadsheet programs or a graphical package like Origin 5.0 (Microcal, Northampton, MA, USA), used here for data analysis and plotting. The input impedance of the multimeter in the 0.5 and 2.5 V ranges is 10^9 Ω, satisfactory for potentiometric measurements with ISEs, with exception of glass electrodes. For multimeters with lower input impedance, the use of a voltage follower (constructed with a FET operational amplifier) is advisable. Anyway, no commercial potentiometer for ISEs is required.

Capillary electrophoresis studies were performed utilizing a homemade instrument equipped with a contactless conductivity detector (CCD) operating at a frequency of 600 kHz [34]. The CCD was positioned 10 cm from the end of the 50 cm long fused-silica capillary with 75 μm bore (J&W, Agilent Technologies, São Paulo, Brazil). The samples were injected by gravity at 100 mm during 15 s. The separation voltage used in all studies was +25 kV.

2.3. Flow system

The two-channel flow system with in-line dialysis for potentiometric chloride analysis is depicted in Fig. 1. Liquid displacement was promoted by pressurization of the bottles of the carrier and the acceptor electrolyte with help of a double channel aquarium air pump [35]. Flow rates were controlled at the outlet of each bottle with adjustable pinch valves of the

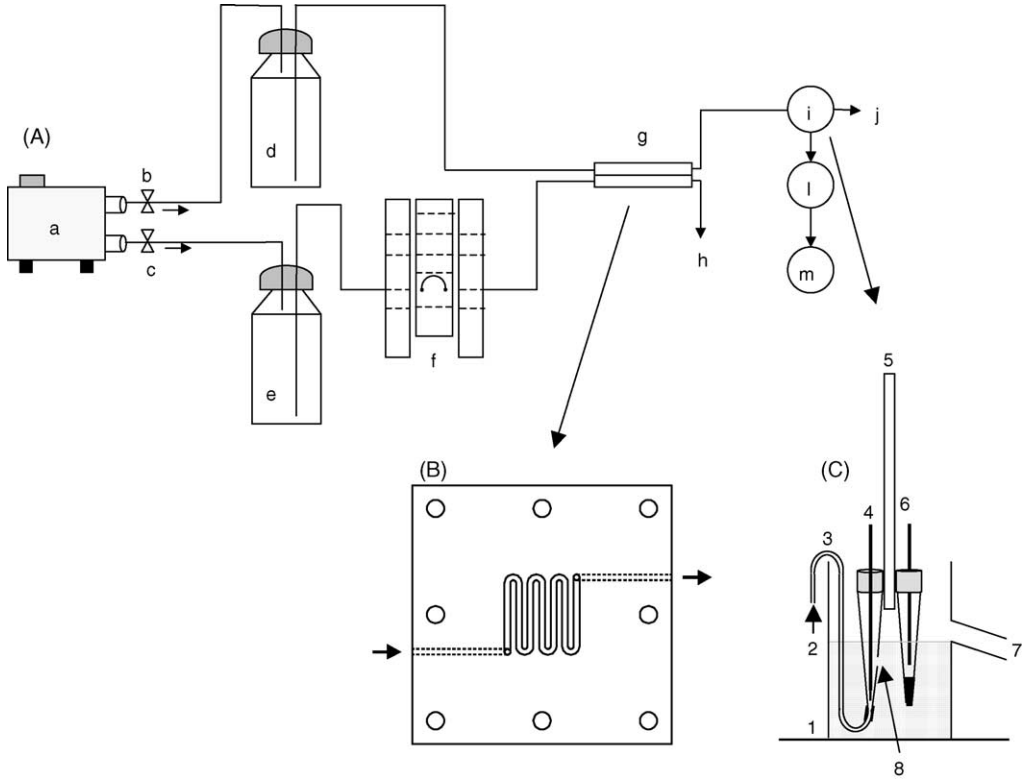

Fig. 1. (A) Flow injection manifold used for chloride determinations: (a) aquarium air pump; (b and c) aquarium flow regulator valves; (d) solution carrier flask of the detector; (e) solution carrier flask of the sample; (f) injector; (g) dialysis unit; (h and j) waste; (i) home made flow cell; (l) multimeter; (m) computer. (B) Superior view of the dialysis unit (4.5 cm × 4.5 cm). (C) Schematic flow cell: (1) cut polypropylene measuring cylinder; (2) inlet; (3) polyethylene tube (Ø = 1.2 mm); (4) working ISE electrode; (5) acrylic rod; (6) reference electrode (Ag/AgCl); (7) waste; (8) outlet hole (and contact between working and reference electrode).

type used to control the airflow of aquarium pumps. A home-made rotary injection valve was utilized to insert the samples in the flow stream. The dialysis cell was made from Plexiglas. The membrane was sandwiched between two blocks with a sinusoidal channel engraved on its surface, one block being the reflected image of the other. The channels are 0.5 mm deep, 1 mm wide and 10 cm long. At the outlet of the dialysis cell, the acceptor flow of electrolyte enters a conical tube (a 100 μL Eppendorf pipette tip) housing the indicator electrode. A perforation in the region of wider section of the tip serves as outflow and electrolytic contact with an external low volume batch containing the reference electrode, assembled in a second Eppendorf pipette tip, as shown in Fig. 1C. The fluid interconnections of the parts of this system were done with 0.8 mm inner diameter PTFE tubing.

2.4. Electrodes

The construction of the miniaturized reference electrode ($Ag/AgCl_{sat.\,KCl}$) was described in detail elsewhere [36]. The manufacture of the indicator electrode starts with a careful cleanup of the silver wire (5 cm length × 1 mm diameter). The central 3 cm of the wire were painted with varnish. A flexible electric wire was soldered to one end of the silver wire and this region was covered with Teflon tape. Sufficient tape was wrapped to form a stopper that fits tightly into the widest end of the pipette tip, aligning the wire inside it. A film of silver chloride was electrolytically formed on the free end of the silver wire by dipping it into a 0.1 mol L^{-1} hydrochloric acid and applying +300 mV versus $Ag/AgCl_{KCl(sat.)}$, having a Pt wire as auxiliary electrode of the potentiostat. After 15 min, a bulky AgCl film was observed on the silver wire.

3. Results and discussion

The potentiometric response of silver-based ISE to chloride ions is well known and it is a consensus that the response of these electrodes follows the Nernst equation. The solubility product of silver chloride dictates the lower end of linear region of the potential change with the logarithm of the chloride activity. Measurements require some equilibration time, that becomes longer at lower chloride concentrations [4]. It is also well known that for flow measurements, factors like sample dispersion and insufficient equilibration time are responsible for a shift of the linear region to higher concentrations. The situation becomes worse with in-line dialysis, as used in this work, because a dilution of the chloride sample plug from the donor to the acceptor stream is inevitable. The dilution

factor depends on cell and membrane geometries and always increases with the flow rates [5,9].

In order to evaluate the performance of the silver-based ISE constructed in our lab, preliminary experiments were done in a batch cell. Fig. 2 depicts a calibration curve, where a Nernstian slope of 57 ± 3 mV was found in the range from 10 to 860 mg L^{-1}. In this and all other potentiometric measurements reported here, the initial potential in the absence of added chloride (but with traces of chloride from the electrolyte and the dissolution of the AgCl of the ISE) was arbitrarily set to zero.

Selection of working conditions for this electrode under FIA conditions with in line dialysis revealed that the volume injected and the flow rate of both streams are responsible for the greatest effects.

Fig. 3 shows the potentiometric responses over a wide concentration range (4–1000 mg L^{-1} of Cl^- injected in the donor stream) obtained with four different injected volumes (50, 100, 200, and 300 μL) and at two different sets of flow rates (1.0 mL min^{-1} on both sides of the membrane and 3.0 mL min^{-1} of acceptor stream and 4.0 mL min^{-1} of donor stream).

Fig. 3A and C show that, the lower flow rate and the higher the injected sample volume, the wider is the nearly linear response to the logarithm of chloride ions concentration (I = constant) at low log[Cl^-] values. There is, thus, a compromise between sample throughput and linearity of response. By plotting the same data in non-logarithmic

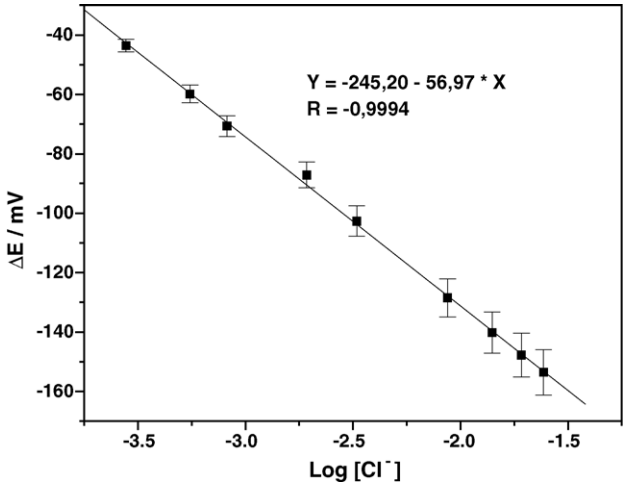

Fig. 2. Analytical curve obtained for experiments in batch. Graph obtained by injecting chloride standard solution into a 0.1 mol L^{-1} of $NaNO_3$ + 0.002 mol L^{-1} of HNO_3 solution. Concentration range: 10–860 mg L^{-1} (n = 3).

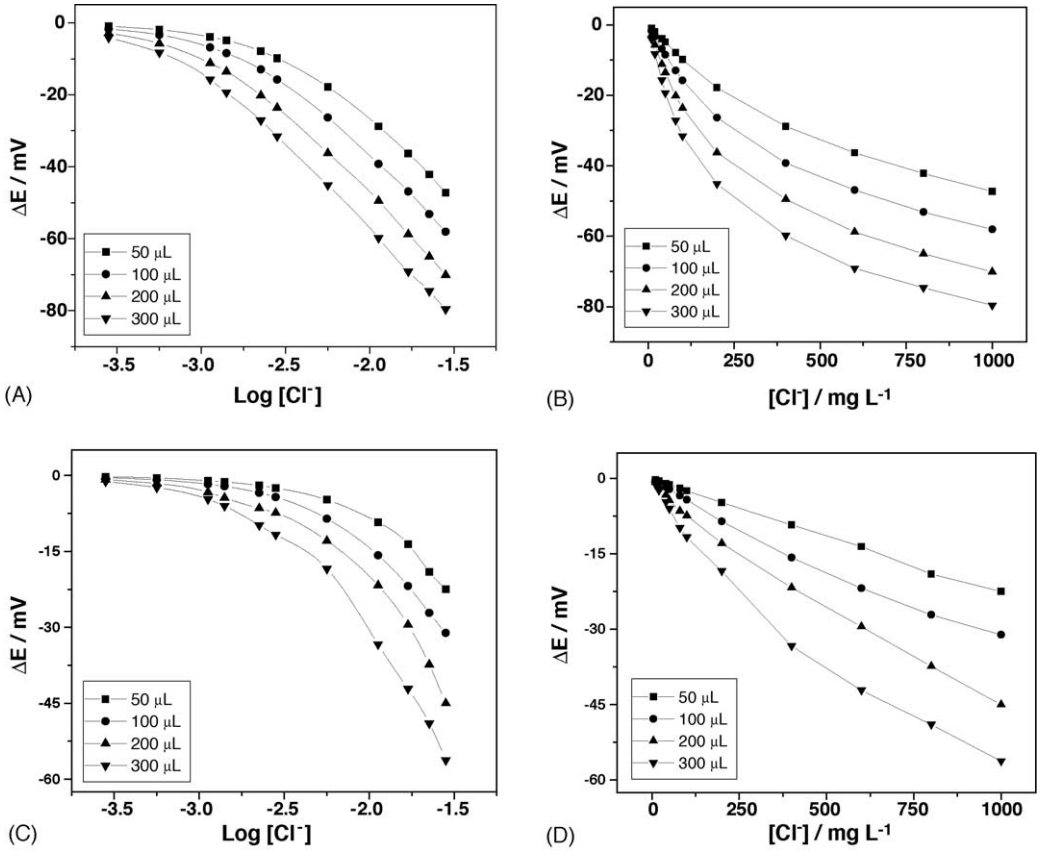

Fig. 3. Influence of the sampling loop (50, 100, 200 and 300 μL) and flow rates combination in FIA-potentiometric system with in line dialysis. Plots obtained with two flow rates: 1.0/1.0 ml min^{-1} (A and B) and 3.0/4.0 ml min^{-1} (C and D) presented in logarithmic (A and C) and linear (B and D) abscissas. Concentration range injected in the donor stream (4–1000 mg L^{-1}). Carrier solution: 0.1 mol L^{-1} of $NaNO_3$ and 0.002 mol L^{-1} of HNO_3 in the acceptor line and 0.5% of Triton-X 100 in the carrier solution, in the sample line.

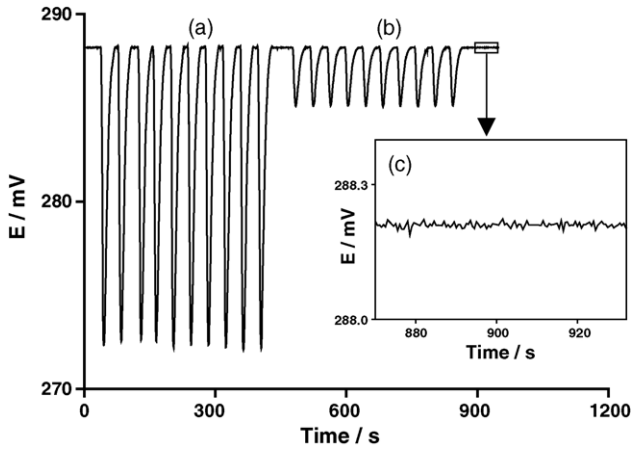

Fig. 4. Signals for repetitive injections of milk sample diluted 2.5 times (a) and 80 mg L^{-1} of chloride standard solution (b) in FIA-potentiometric system with in line dialysis. The inset depicts the very low noise present in the baseline (c). Flow rate: 3.0 and 4.0 ml min^{-1} for acceptor and donor lines, respectively; sampling loop: 50 μL; R.S.D. calculated: (a) for the milk sample = 1.2% and (b) for chloride standard solution = 0.56%. Other conditions: see Fig. 3.

concentration scale, a nearly linear behavior is observed at the higher flow rate (Fig. 3D), especially for smaller injected sample volumes. This is a definite advantage to be explored when a higher analytical frequency of the method is a goal, as usual in FIA. For the chloride concentration range from 4 to 1000 mg L^{-1}, excellent fit was obtained with a quadratic relationship ($r^2 = 0.9999$) at the experimental condition of 50 μL of sample injection, 3.0 mL min^{-1} donor flow and 4.0 mL min^{-1} acceptor flow. At this condition, an analytical frequency of 90 injections h^{-1} was achieved (peaks not shown in Fig. 3D are similar to those in Figs. 4 and 5),

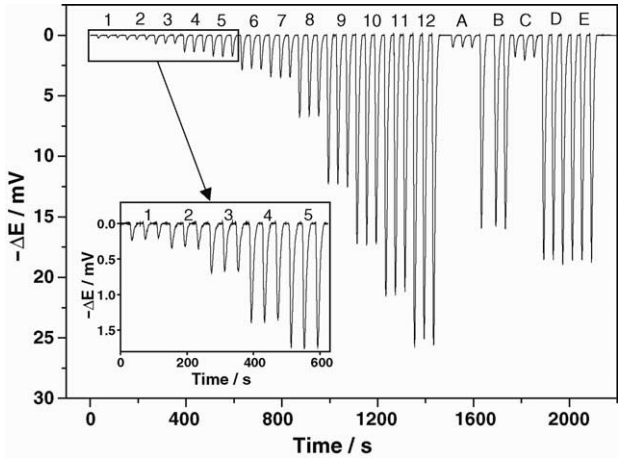

Fig. 5. Potentiogram for consecutive injections in FIA-potentiometric system with in line dialysis of 12 chloride standards solutions: (1) 4, (2) 10, (3) 20, (4) 40, (5) 50, (6) 80, (7) 100, (8) 200, (9) 400, (10) 600, (11) 800 and (12) 1000 mg L^{-1}; certified milk sample (A); two milk samples, one diluted 2.5 times (B) and other diluted 25 times (C); coconut water diluted 2.5 times, directly after opening of a canned sample (D) and after long time of contact with air (E). Other conditions: see Fig. 3.

far superior than those reported previously for chloride quantification systems comprising a dialysis unit [5,9]. A second advantage of the work with smaller samples at higher flow rates is the marked decrease of interference of ions like bromide, iodide or sulfide, as demonstrated by Trojanowicz and Matuszewski [29].

The good performance of the FIA method at sub-Nernstian region with on-line dialysis and high sample throughput motivated its application to complex real samples. In the first studies involving milk samples, a progressive decrease in sensitivity was observed, probably due to some clogging of the dialysis membrane by organic substances (e.g., lipids) present in the milk or coconut water. The addition of Triton X-100 to the donor stream prevents this contamination of the membrane, allowing the injection of hundreds of samples without decrease in the dialysis rate. Fig. 4 presents the potentiometric response of repetitive injections of milk sample diluted 2.5 times (a) and 80 mg L^{-1} of chloride standard solution (b). The relative standard deviations were calculated as 1.2% for the milk sample and 0.56% for the chloride standard solution ($n = 10$).

The very low noise level in the measurements (the baseline was enlarged in the inset of Fig. 4) is noteworthy and the pulsation-free flow achieved by propelling the carrier streams with aquarium pumps [34] contributes for this result, which would be less favorable with a peristaltic pump. The in-line dialysis was very effective to avoid electrode poisoning and no other pretreatment was required for the whole milk and the coconut water samples. Variation in the ionic strength on any side of the dialysis membrane or changes of the counterion from one sample to another, are parameters that influence the efficiency of the dialysis, as detailed in the literature [5]. In our study, the acceptor solution of 0.1 mol L^{-1} sodium nitrate plus 0.002 mol L^{-1} nitric acid keeps the ionic strength almost constant. Experiments were made with and without added electrolyte to the donor stream. In both cases, good results were obtained for standards and real samples of coconut water and milk. In these samples, where the principal counterion is potassium and chloride is the predominant anion, there was no need for ionic strength adjustment, because any effect is taken into account by the quadratic model fitted to the calibration points. The accuracy of the analysis of a reference material and the intercomparison of the results with capillary electrophoresis confirms this statement, as shown in Table 1. It is worthwhile to add that only about 1.4% of the chloride in the donor stream (4 mL min^{-1}) is transferred to the acceptor stream (3 mL min^{-1}) by the dialysis unit under continuous flow operation, as determined by capillary electrophoresis of the emerging flows. This is still a good compromise between sensitivity (still adequate for the samples under consideration), selectivity (no sample pretreatment required) and analytical frequency. For samples with much lower chloride levels, at the price of lower analytical frequency, the efficiency of dialysis can be increased by injecting larger sample plugs, lowering the flow rates or using a dialysis unit with larger contact area or more permeable membranes

Table 1
Concentration of chloride in four milk and five coconut water samples determined by the proposed FIA-potentiometric procedure with in line dialysis and it comparison with certified sample (milk) and with capillary electrophoresis technique for coconut water ($n = 3$)

Milk	Certified value ($mg\,L^{-1}$)	FIA-potentiometry ($mg\,L^{-1}$)
NIST Milk 1549	10900 ± 205	11333 ± 185
Sample A	–	1377 ± 7
Sample B	–	1242 ± 10
Sample C	–	1114 ± 21
Coconut water	**CE* ($mg\,L^{-1}$)**	**FIA-potentiometry ($mg\,L^{-1}$)**
Sample A	1401 ± 15	1394 ± 9
Sample B	1061 ± 52	1084 ± 8
Sample C	1379 ± 61	1325 ± 27
Sample D	1964 ± 63	1905 ± 34
Sample E	1704 ± 81	1708 ± 34

(e.g., the substitution of the membrane used in this work by Cuprophane has doubled the transference of chloride).

Fig. 5 presents a series of potentiometric signals obtained for a sequential injection (in triplicate) of 12 standards solutions (1–12), a certified milk sample (A), a commercial milk sample diluted 2.5 times (B), the same sample diluted 25 times (C) and a commercial coconut sample diluted 2.5 times, before (D) and after (E) long exposure to the air for oxidation of sulfite (used as a preservative) to sulfate.

The excellent repeatability of the measurements even in the region of lowest concentration of chloride (4–30 $mg\,L^{-1}$ of Cl^- in the standards injected in the donor stream) becomes evident in the inset of Fig. 5. This figure confirms that about 40 s are required for recording a peak, what results in a frequency of 90 injections h^{-1}. The results for the chloride quantification in four milk and five coconut water samples are listed in Table 1. Validation of the FIA-potentiometric method for the milk samples was achieved with a certified milk sample (NIST 1549); no significant difference was observed at 95% confidence level. For the coconut water samples, no certified material is available and an intercomparison with capillary electrophoresis was elected for validation. At the 95% confidence level, no significant difference was observed in the results obtained by both techniques.

It is worthwhile to say that, although the more expensive and complex technique of capillary electrophoresis has presented a lower precision, as indicated by the standard deviations of the peak areas, it provides, in one run, the concentration of other anions, like sulfate, nitrate and acetate. Sulfite ion, used as a preservative in bottled coconut water, was not observed in the electropherograms, possibly because it was completely oxidized to sulfate during sample manipulation with exposure to atmospheric oxygen. An evidence of the oxidation is the presence of a higher sulfate peak in bottled coconut water (~100 $mg\,L^{-1}$) than in samples extracted directly from coconuts (<20 $mg\,L^{-1}$). The stability constants of the silver complexes with sulfite are sufficiently high to render the bisulfite ion an interfering ion for the chloride ISE. In this study, no interference was observed (Fig. 5D and E), possibly by the same reason no peak is found in the electropherogram (the donor and the acceptor streams are not degassed). Anyway, for samples with higher levels of sulfite, its interference can be overcome by addition of an excess of hydrogen peroxide.

4. Conclusions

The FIA-potentiometry method here proposed for chloride combines many advantages in comparison with the previously described ones. The measurements in the sub-Nernstian region by fitting a second degree polynomial to analytical curve allows high sample throughput (90 analyses/h) and reduces the effect of interfering ions, inherent to flow potentiometry with Cl^- ISE [29]; in-line dialysis suffices as sample pretreatment to protect the detector from poisoning and impairs the sensitivity to a level that still is entirely compatible with the aimed applications (4–1000 $mg\,L^{-1}$ of Cl^-). The instrumentation is very simple and inexpensive, with a digital multimeter acting as potentiometer and data acquisition device. Reduced size of the equipment, fast set-up, simple calibration, and low reagent and power consumption are other favorable attributes, especially for field application.

Acknowledgements

This work was supported by the Brazilian Foundations FAPESP, CNPq and CAPES. Authors thank Prof. Marcia A.M.S. da Veiga for kind donation of the certified milk sample, for Prof. Dennys Corbo for lending us the dialysis unit, and Dr. José G. Brito for the English revision.

References

[1] K. Hermansen, Br. J. Nutr. 83 (2000) 113.
[2] J.F. van Staden, Talanta 38 (9) (1991) 1033.
[3] A. Andrade-Eiroa, J.A. Erustes, R. Forteza, V. Cerda, J.L.F.C. Lima, Anal. Chim. Acta 467 (2002) 25.
[4] W. Frenzel, Fresenius Z. Anal. Chem. 335 (1989) 931.
[5] M. Miró, W. Frenzel, Analyst 128 (2003) 1291.
[6] H. Hara, Y. Wakizaka, S. Okazaki, Anal. Chem. 58 (1986) 1502.
[7] J.L.F.C. Lima, L.S.M. Rocha, Int. J. Environ. Anal. Chem. 38 (1990) 127.
[8] M.A. Climent, E. Viqueira, G. de Vera, M.M. Lopez-Atalaya, Cement Concr. Res. 29 (6) (1999) 893.
[9] A.N. Araújo, M.B. Etxebarria, J.L.F.C. Lima, M.C.B.S.M. Montenegro, R.P. Olmos, Fresenius J. Anal. Chem. 351 (1995) 614.
[10] E. Sucman, J. Bednar, Can. J. Anal. Sci. Spectrosc. 47 (3) (2002) 66.
[11] R. Pérez-Olmos, R. Herrero, J.L.F.C. Lima, M.C.B.S.M. Montenegro, Food Chem. 59 (1997) 305.
[12] J.L.F.C. Lima, C. Derlue-Matos, M.C.V.F. Vaz, Cienc. Technol. Aliment. 2 (2000) 234.

[13] J.A. Vieira, I.M. Raimundo Jr., B.F. Reis, M. Conceição, B.S.M. Montenegro, A.N. Araújo, J. Braz. Chem. Soc. 14 (2003) 259.
[14] J.C.B. Fernandes, G.D. Neto, J.J.R. Rohwedder, L.T. Kubota, J. Braz. Chem. Soc. 11 (4) (2000) 349.
[15] S. Morais, M.I. Alcaina-Miranda, F. Lázaro, M. Plantá, A. Maquieira, R. Puchades, Anal. Chim. Acta 353 (1997) 245.
[16] J. Naozuka, M.A.M.S. Veiga, P.V. Oliveira, E. Oliveira, J. Anal. At. Spectrom. 18 (2003) 917.
[17] H.L. De Clercq, J. Mertens, D.L. Massart, J. Agric. Food. Chem. 22 (1974) 153.
[18] F.V. Silva, G.B. Souza, L.F.M. Ferraz, A.R.A. Nogueira, Food Chem. 67 (1999) 317.
[19] J.F. Willumsen, S.M. Filteau, A. Coutsoudis, M. Newell, N.C. Rollins, H.M. Coovadia, A.M. Tomkins, AIDS 17 (2003) 407.
[20] S. Filteau, Vaccine 21 (2003) 3377.
[21] J.G. Woodroof, Coconuts: Production, Processing, Products, Avi, CT, 1970.
[22] D. Campbell-Falck, T. Thomas, T.M. Falck, N. Tutuo, K. Clem, Am. J. Emerg. Med. 18 (1) (2000) 108.
[23] U. Santoso, K. Kubo, T. Ota, T. Tadokoro, A. Mackawa, Food Chem. 2 (1996) 299.
[24] C.F. Campos, P.E.A. Souza, J.V. Coelho, M.B.A. Gloria, J. Food Proc. Preserv. 20 (1996) 487.
[25] A. Alcazar, P.L. Fernandez-Caceres, M.J. Martin, F. Pablos, A.G. Gonzalez, Talanta 61 (2) (2003) 95.
[26] Y.S. Fung, K.M. Lau, Talanta 45 (4) (1998) 641.
[27] T. Altunbulduk, H.M.Z. Kocker, W. Frenzel, Fresenius J. Anal. Chem. 351 (1995) 593.
[28] M. Trojanowicz, W. Matuszewski, Anal. Chim. Acta 138 (1982) 71.
[29] M. Trojanowicz, W. Matuszewski, Anal. Chim. Acta 151 (1983) 77.
[30] E.M. Richter, J.A.F. Silva, I.G.R. Gutz, C.L. Lago, L. Angnes, Electrophoresis 25 (2004) 2965.
[31] R.A.A. Munoz, E.M. Richter, D.P. de Jesus, C.L. Lago, L. Angnes, J. Braz. Chem. Soc. 15 (4) (2004) 523.
[32] E.M. Richter, F.R.P. Rocha, L. Angnes, Quim. Nova 27 (2) (2004) 313.
[33] E.M. Richter, D.P. de Jesus, C.A. Neves, C.L. do Lago, L. Angnes, Quim. Nova 26 (6) (2003) 839.
[34] J.A.F. da Silva, C.L. do Lago, Anal. Chem. 70 (1998) 4339.
[35] R.C. Matos, I.G.R. Gutz, L. Angnes, R.S. Fontenele, J.J. Pedrotti, Quim. Nova 24 (6) (2001) 795.
[36] J.J. Pedrotti, L. Angnes, I.G.R. Gutz, Electroanalysis 8 (1996) 673.