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Determination of chloride in milk using sequential injection automated conductimetry

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Abstract

A new conductimetric sequential injection procedure is proposed for the automatic chloride determination in milk. The preparations of milk samples are time consuming procedures. The use of a sequential injection system coupled to a dialysis camera permitted easy automation and improved process control over the parameters, giving high throughput analyses. In this work, a fast and reliable automated sample addition method is described. An untreated sample of milk (142 μl) was injected with a standard reference solution (750 μl) in a carrier stream and dialysed for the conductimetric chloride determination. Potential interferences and ideal work conditions were appraised. Data acquisition and device control were achieved by computer. Results were precise (r.s.d. < 1.0%) in agreement with both the official titration procedure and with the milk standard reference materials. The system was designed for conductimetric samples addition analysis, and no major problems were observed. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Sequential injection analysis; Chloride; Milk; Sample addition; Animal health

1. Introduction

Improvements in nutrition and animal health studies require more detailed determinations. This paper uses a chloride determination in milk as an example of possible laboratory automation with the use of a sequential injection system for such purposes.

Analyses using sequential injection systems (SI) have shown a continuous growth with applications in several areas since their proposal in 1990 (Ruzicka & Marshal, 1990). This technique presents a great potential for on-line measurements in many routine laboratories due to the simplicity and convenience with which the sample manipulation can be automated. The system uses an on-line configuration and a selector valve to introduce the wash solution, the sample and the reagents inside a reaction coil, where a well defined sample zone is formed (Gübeli, Christen & Ruzicka, 1991; Rvticko, 1992; 1991; Ruzicka, 1992). Reversing the flow, sample and reagents are pushed towards the detector. The selector valve can be used for different purposes during the analyses: to introduce additional reagents and standard solutions, or to be coupled to mixing or dilution cameras. The sequential injection theory and properties were extensively studied (Gübeli et al., 1991; Ruzicka, 1992; Ruzicka & Marshal, 1990) and have been used broadly in several areas, owing to the advantages offered in automation and the control of the process (Alpízar, Crespi, Cladera, Forkeza & Cordia, 1996; van Staden, du Plesis & Taljaard, 1997).

The chloride determination in milk is a useful parameter to establish the degree of subclinical mastite that can occur in the cattle. Usually it can be made by qualitative or comparative methods (Olmos & Lima 1990), manually (AOAC, 1995) or using flow injection potentiometric titrations (Ferreira, Lima & Rangel, 1994). Typical levels of chloride in cases of mastite are normally high. Because milk is a complex matrix, and usually requires extensive sample treatment for the analytical determination of chloride, the use of a dialysis unit as a part of the manifold system not only removes interferences (such as suspended solids and milk proteins), but also facilitates automated dilution (van Staden, 1993,

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1995) allowing an on-line sample treatment. When coupled to a conductimetric detector, sequential injection facilitates the chloride determination in milk samples. Procedures using sequential injection system have been employed for several purposes (such as Oms, Cerdá, Cladera, Cerdá & Forteza, 1996). However, caution should be taken when a conductimetric detector is used. Due to the low selectivity of this detector, the presence of other concomitant ions in solution could contribute to the total conductivity, interfering in the results. A sample addition method (Rizov & Ilcheva, 1995) in association with a sequential injection system was developed and evaluated in this work in order to minimise the matrix interference.

2. Materials and methods

2.1. Reagents and solutions

All solutions were prepared from analytical grade reagents and distilled–deionized water, with a specific conductivity less then 0.1 $\mu S\ cm^{-1}$, was used throughout in this work.

A 1.0 mol l^{-1} Cl^- stock solution was prepared from solid sodium chloride, previously dried at 100°C for 120 min. This stock solution was used to prepare reference solutions containing 0.0; 0.5×10^{-2}; 1.0×10^{-2} and 5.0×10^{-2} mol l^{-1} Cl^- in H_2O medium. To study the effect caused by other ions in the conductimetric measure without standard addition approach, solutions containing the main concomitants, in concentrations normally found in milk, were prepared: 0.50×10^{-2} mol l^{-1} Cl^- plus 1.8×10^{-2} of Na^+; 0.50×10^{-2} mol l^{-1} Cl^- plus 1.0×10^{-2} of PO_4^{2-}; 0.50×10^{-2} mol l^{-1} Cl^- plus 4.0×10^{-2} of Mg^{2+}; 0.50×10^{-2} mol l^{-1} Cl^- plus 3.6×10^{-2} of K^+ and 0.50×10^{-2} mol l^{-1} Cl^- plus 2.7×10^{-2} mol l^{-1} of Ca^{2+} (based on, respectively: CH_3COONa; $NaH_2PO_4.H_2O$; $MgSO_4$; K_2SO_4 and $CaSO_4.2H_2O$, Merck, diluted in H_2O medium).

2.2. Instrumentation

The sequential injection system experimental set-up, schematically depicted in Fig. 1, was constructed using two peristaltic pumps with variable rotation (Ismatec, Switzerland), and a six-way electrically actuated solenoid valve (NResearch, USA). The manifold was built with 0.8 mm i.d. Perspex tubing. Tygon tubing (0.5 mm i.d.) was used for pumping the solutions. The dialysis unit consisted of a single unit of 150×49×28 mm. The path length of both donor and acceptor streams was 70 mm and the path was made with a semi-tubular groove with an i.d. of 0.3 mm (i.e. 0.3 mm wide and 0.3 mm deep). Between the entrance and exit sides of the grooves was inserted a Technicon premount dialysis membrane type C, which allowed the milk introduction without previous treatment. The detection was made by a conductimeter (Fisher Scientific, USA) with a home made flow cell with 50 mm diameter and 37×5 mm of conduction path (Fig. 1). Data acquisition and device control were achieved using a PCL 711-S interface board (American Advantech, USA) connected to an Ashford PC computer (USA). The time, injection volume and data acquisition and storage were developed in Quick Basic® software package (Microsoft, USA).

2.3. Sequential injection system

Fig. 2 is a flow chart diagram of the program developed to control the conductimetric determination of $C1^-$ using the system outilined in Fig. 1. Pump 1 was connected to the selector valve, allowing that the sample of "in-natura" milk (142 µl) and standard solutions (750

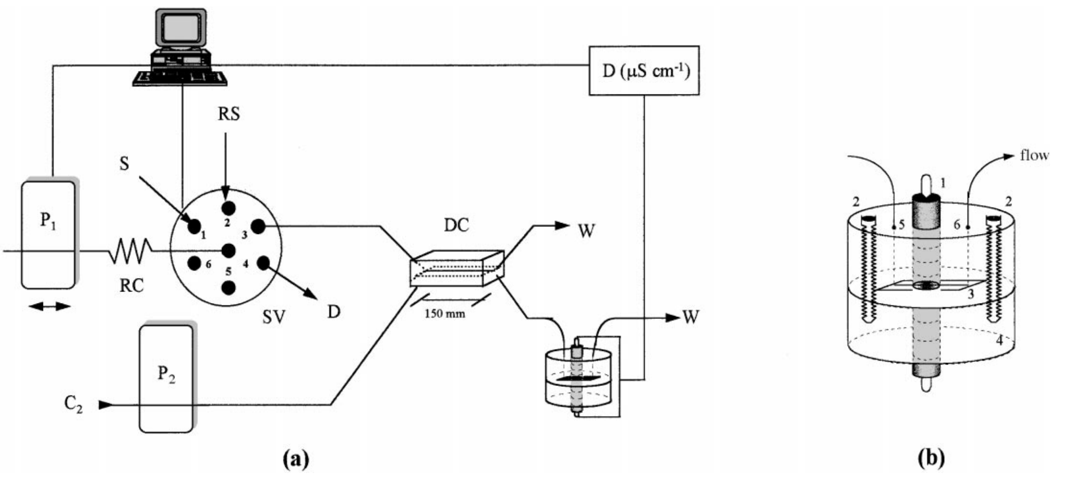

Fig. 1. (a) Sequential injection system. S, sample (142 µl); RS, reference solution (750 µl); C_1 and C_2, carrier streams (H_2O–4.3 and 1.4 ml min^{-1}, respectively); RC, reaction coil (3.0 ml); SV, selector valve; P_1 and P_2, peristaltic pumps, DC, dialyser unit; D, detector and W, waste. (b) Flow cell for conductimetric determination. 1, electric contact; 2, screws; 3, rubber; 4, perspex body; 5 and 6, in/out flow.

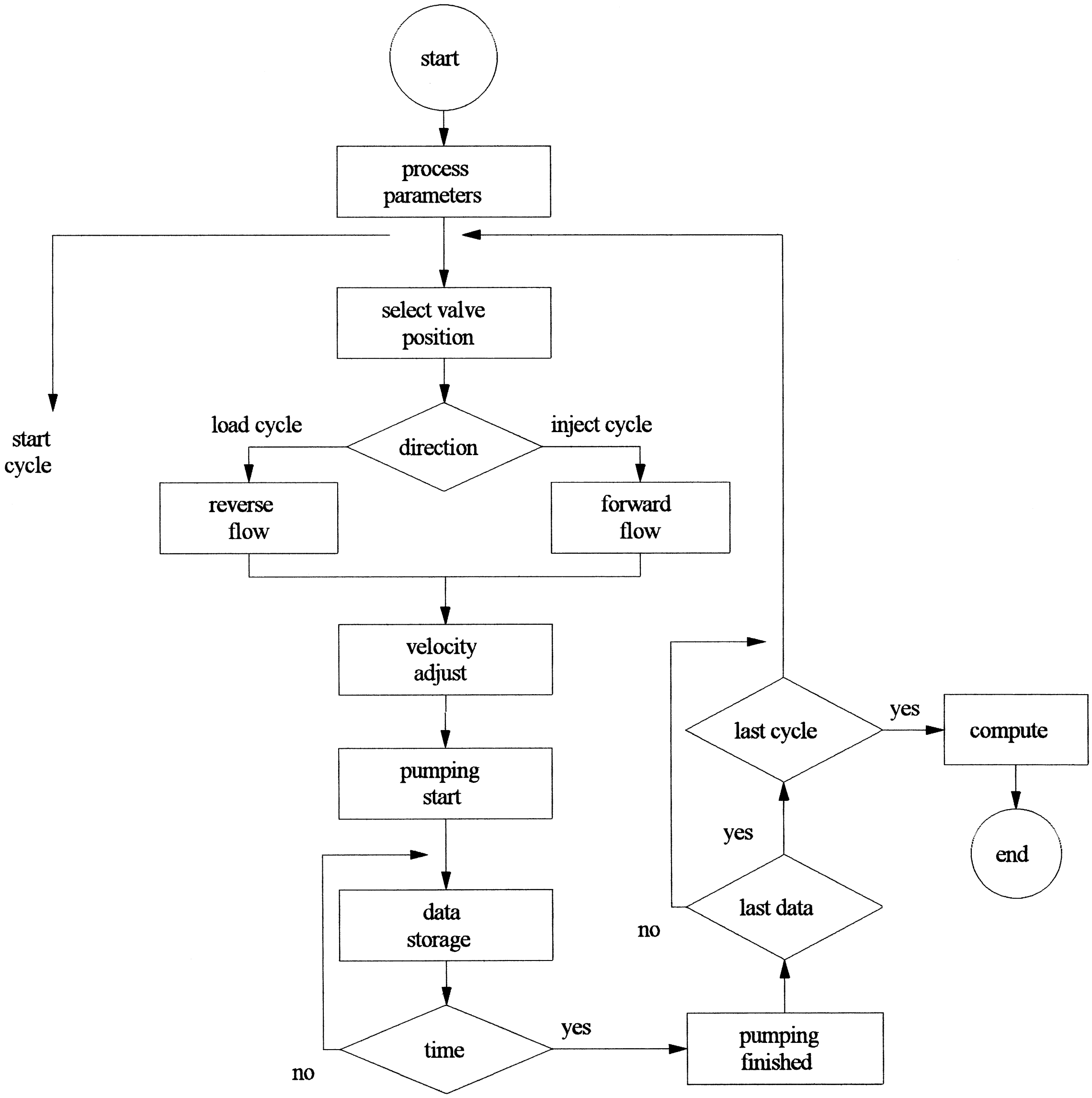

Fig. 2. Operating program for conductimetric determination.

μl) to be sequentially aspirated, mixed, and then transported to the holding coil (RC, 3.0 ml). After, sample and reference solutions were addressed, by the C_1 carrier solution (water), to the dialysis unit where the chloride ions present in the donor solution were spread across the membrane and the solution carrier to the waste. The diffused chloride ions were collected by the C_2 carrier stream (water) and carried toward the conductimetric cell, and then to the waste. The conductance values were collected every 1 s and stored for latter data treatment. The device operational sequence for the conductimetric determination by sequential injection is summarised in Table 1.

2.4. Reference procedure

For the chloride determination in milk, the volumetric method using silver (I) solution as titrant is recommended (AOAC, 1995) and was used for comparison purposes in this work. Certified reference materials (NIST milk powder 8435 and AIEA A-11 milk powder) were used for analytical accuracy.

2.5. Standard addition procedure

The standard addition approach was adopted (Rizov & Ilcheva, 1995) in order to avoid matrix interference. Firstly, different types of chloride saline matrices were studied to determine the interference due to the counter-ion passage through the dialysis membrane in the slope of the sample addition curve. Solutions of 0.5×10^{-2}, 1.0×10^{-2} and 1.5×10^{-2} mol Cl^- l^{-1} (based on KCl, NH_4Cl and NaCl) were added to a sample of milk that had previously been set by an official titration procedure (AOAC, 1995). The chloride concentrations were then compared.

Table 1
Analytical sequence employed by system proposed

Analytical sequence	Open port	Time (s)	Flow rate (ml min^{-1})	Pumping direction[a]
Cleaning step[b]				
Reference solution aspiration	2	15	1.7	a
Discarding reference solution	4	20	5.1	b
Sample addition step				
Sample aspiration	1	5	1.7	a
Reference solution aspiration	2	20	2.5	a
Reading step				
Pumping the sample zone towards the dialysis unit	3	60	4.3	b

[a] a: reverse; b: forward.
[b] Start data acquisition.

3. Results and discussion

The incorporation of a dialysis unit to a flow system allowed an on-line sample treatment eliminating undesirable effects caused by suspended solids and macromolecules in milk, such as proteins and colloids. The use of dialysis is simple and reproducible, and its use coupled to a sequential injection system eliminates manual pretreatment and other separations, which are time consuming, boring and expensive when used in routine laboratories. Using the proposed system, costs were reduced because only water was used as reagent.

Dialysis is a separation process that depends on the differential transport of solutes of different ionic or molecular sizes across a semi-permeable membrane. The selective transport of the analytes in the dialysis unit is ruled by the membrane that acts as a molecular sieve. This mass transport, known as deficient (van Staden, 1993, 1995), can be used to produce a wide linear range in systems where manual dilution would be necessary due to the high analyte concentration. As the amounts of chloride in milk are normally high (Olmos & Lima, 1990), the deficiency related to the mass transport was used as a diluter agent, and any manual dilution steps were needed.

The dialysis of chloride was studied with water as the carrier in both the donor and acceptor streams, and without sample addition procedure. Concentrations frequently achieved in milk were separately added to a constant amount of chloride and the mass transfer through the semi-permeable dialysis membrane was settled by the conductimeter. The experimental results, presented in Table 2, revealed that the higher concentrations of K^+ and Ca^{2+} in milk caused serious conductimetric interference, obviated by the low selectivity of the membrane. In order to avoid this effect, the standard addition approach was then adopted (Rizov & Ilcheva, 1995).

Defined concentrations of chloride salts were automatically added to the sample, and each sample's zone showed the same final composition, except for the chloride concentration. In spite of its relevance, it is evident that this procedure is difficult and relatively slow. The SI conductimetric system proposed in this paper was employed to reduce these difficulties. A series of additions were accomplished and the results plotted in order to find the unknown sample analyte concentration. The data acquisition was done automatically by the same interface board that was used to control the system (Fig. 1). The operating program determined the chloride concentration automatically after each sample addition procedure. The transient conductimetric signal and the standard sample addition curve are presented in Fig. 3 [(a) and (b), respectively].

In view of the probability that the chloride saline matrix presented differences in the sample addition curve, so altering the results, solutions of KCl, NH_4Cl and NaCl salts were evaluated as the reference solution to be added and the milk sample was also determined by the official titration procedure (AOAC, 1995). It was observed that, when the potassium or ammonium salts were used, the final milk chloride presents different concentration of the titration value. It could be caused by the molar conductivity of the ions K^+ and NH_4^+ (73.50 Scm^2 mol^{-1}) higher than the Na^+ conductivity (50.10 Scm^2 mol^{-1}), resulting in curves with different slopes (NaCl: $y = 4.88 + 2186x$; $R = 0.999$; KCl: $y = 5.34 + 2980x$; $R = 0.998$; NH_4Cl: $y = 5.82 + 3040x$; $R = 0.998$) (Table 3). The results, when KCl or NH_4Cl solutions were used, were 16% lower than the titration value. When NaCl was used as matrix salt, the results presented better agreement with the titration values (Table 3).

After the optimisation of work conditions, the SI system was evaluated regarding accuracy, precision and detection limit. The precision of the SI system was determined by 10 times analysis of a milk sample in a standard addition curve. In all cases, the r.s.d. was $<1.5\%$. The turn around analytical cycle was 120 s,

Table 2
Interfering study of milk concomitants on Cl^- conductivity using dialysis membrane and the SI system, without sample addition[a]

Concomitant added	Concentration (mol l^{-1})	Detected Cl^- (mol l^{-1})	Deviation from Cl^- concentration (%)
Na^+	0.018	0.052	+4.0
PO_4^{3-}	0.010	0.049	−2.0
Mg^{2+}	0.040	0.051	+2.0
K^+	0.036	0.082	+64.0
Ca^{2+}	0.027	0.062	+24.0

[a] Chloride standard solution = 0.050 mol l^{-1}.

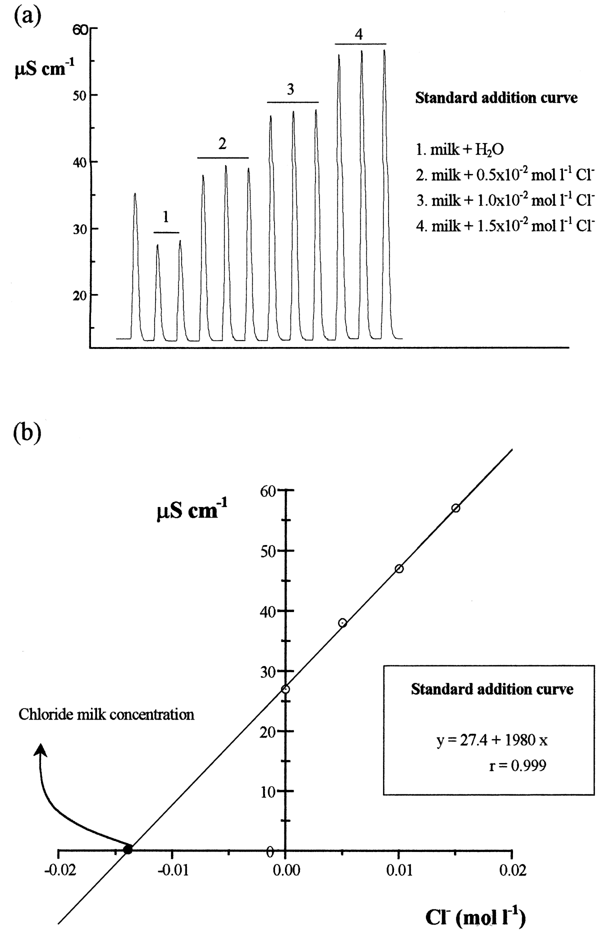

Fig. 3. Sample addition method for chloride milk determination. (a) Conductimetric recorder. From the left, 0.0; 0.5×10^{-2}; 1.0×10^{-2} and 1.5×10^{-2} mol^{-1} Cl$^-$; (b) Data treatments.

Table 3
Influence of chloride saline matrix on the results

Chloride saline matrix	mol l^{-1} Cl$^-$	Titration value	Deviation from titration value (%)
NH_4Cl	0.035	0.042	−16.0
KCl	0.035		−16.0
NaCl	0.040		−4.76

including the cleaning step, required to avoid the base line increase among the standards and samples (Table 1, Fig. 3). The response of the proposed SI system for the conductimetric chloride determination in certified and fresh milk samples was evaluated and compared with the reference (titration) method. The medium results presented smaller amounts of Cl$^-$ when the determination was made by SI may be due to the presence of the sodium counter ion. When the *t*-test was applied, no statistical difference was observed at 95% of probability (Tables 3 and 4). The detection limit, 0.71×10^{-3} mol l^{-1} Cl$^-$, was determined after 10 consecutive chloride standard additions in water (Fernandes & Rangel, 1998). The results confirmed the viability of the proposed methodology for determination of chloride in milk, without previous and laborious sample treatment.

Table 4
Chloride milk concentration[a]

Sample	mol l^{-1} Cl$^-$	
	SIA	Titration
1	0.032(0.002)[b]	0.036(0.001)
2	0.032(0.006)	0.036(0.001)
3	0.040(0.001)	0.042(0.001)
Reference material		Certified value
NIST 8435	0.22(0.02)	0.24(0.01)
AIEA A-11	0.24(0.05)	0.26(0.05)

[a] Determined by the proposed procedure (SI), by reference titration procedure (AOAC, 1995), and certified materials.

[b] Values in parentheses are the r.s.d. based on three replicates.

4. Conclusions

Using the chloride milk determination as an example, this work demonstrated the possibility of SI on-line implementation on animal nutrition and animal health studies. It presented the ability to perform the automation of the pre-treatment of the samples for complex analyses, which usually involves several mechanical stages and/or several reagents, thus simplifying and increasing its versatility. The conductivity detection has been proved to be suitable to use in combination with sequential injection technique. Separation and pre-dilution of the analyte take place by using an ion permeable membrane. With the on-line automated sample addition, data acquisition was performed with great ease. The system is fully computerised and can be applied in other kinds of nutrition and animal production studies.

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