

Espectrometría de infrarrojo y análisis en flujo para la determinación de compuestos químicos en alimentos

Infrared spectroscopy and flow analysis for the determination of chemical constituents in food products

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Abstract:

Two analytical methods were proposed for the determination of sodium chloride. A flow injection turbidimetric determination, based on the precipitation of silver chloride, was found to be a simple methodology to be implemented for routine analysis with a high sampling frequency, low reagent consumption and waste production in comparison with the reference methodology. On the other hand, the infrared methodology allowed NaCl determination with no reagent consumption. Both methodologies can be implemented for routine chemical analysis determinations in the food industry.

Keywords: FIA, IR-spectroscopy, food chemistry.

Resumen:

Son propuestos dos sistemas para la determinación de cloruro de sódio. Fue encontrado que un sistema de inyección de flujo por turbidimetría, basado en la precipitación de cloruro de plata, es una metodología simple para análisis de rutina con claras ventajas como una gran frecuencia de muestreo, un bajo consumo de reactivos químicos y una baja producción de residuos comparado con el método de referencia. Por otro lado, la espectroscopia de infrarrojo permite la determinación de NaCl sin la necesidad del uso de reactivos químicos. Ambas metodologías pueden ser implementadas para los análisis de rutina en una industria alimentaria.

1. Introduction

Salt, chemically identified as sodium chloride (NaCl) is a water-soluble compound, which dissociates into the sodium and chloride ions. The importance of both ionic species is well known. Sodium ions are required to maintain the pressure and the volume of the blood and are needed for the transmission of nerve impulses and for the metabolism of carbohydrates and proteins. Chloride ions are essential because they maintain the acid-base balance and are necessary for the formation hydrochloric acid in the stomach, needed in the digestive process. Salt is an ingredient extensively used in the food industry with important properties as bacteriostatic and flavouring agent. The influence of salt content on the characteristics of food products was well documented in several studies [1, 2, 3].

There are several methods used to detect sodium chloride, the classical volumetric determination [4] based on Mohr and Volhard methods is a precise method but is time consuming and difficult to perform for routine analysis. The potentiometric method is simple, economical and produces high sampling rate [5] but has the inconvenient of a lack of accuracy. Other methods - like flame-emission (FES) and atomic-absorption (AAS) spectrophotometry - are very precise but require high investment on equipment. Automated systems have already been developed for chloride determination using flow injection analysis (FIA), a methodology which presents significant advantages when compared to conventional methods, related to simplicity, automation, reduction in sample and

reagents consumption. Some of these systems involve the reaction between chloride and mercury thiocyanate with subsequent colorimetric measurement of iron thiocyanate [6]. In the recent years, a pronounced tendency towards “clean methods” was observed and efforts to replace toxic reagents as mercury induced the development of a simple and versatile turbidimetric procedure for chloride determination. The flow-injection turbidimetric determination of chloride is a method based on the precipitation of silver chloride and the measurement of the turbidity by a spectrophotometer under acidic conditions. This precipitation reaction was applied successfully for the determination of chloride in waters by Sartini et al. 1997 [7].

Infrared (IR) spectroscopy is a universal, versatile analytical technique for the structure elucidation of a large variety of organic, inorganic and biological samples. The principle of the IR spectroscopy is the measurement of the amount of IR radiation, which is absorbed (or emitted) by a sample as a function of the wavelength. The wavelengths of IR ranges it is divided into NIR infrared ($12.500 - 4000 \text{ cm}^{-1}$), MID-IR ($4000 - 100 \text{ cm}^{-1}$) and FAR-IR ($100 - 10 \text{ cm}^{-1}$); however for food analysis the most utilised are the NIR and MID-IR [8, 9].

The synergistic combination between FIA and IR, joins the features already presented for both systems and has been proposed as an analytical methodology that allows in-situ monitoring and real-time analytical determinations during the food manufacturing process [10].

The main objective of the present work was to compare the performance of a flow-injection (FIA) system and a Fourier transformed infrared (FTIR) spectroscopy procedure for the determination of sodium chloride in table and marine salt samples.

2. Materials and Methods

Samples, standards and reagents:

Samples of table and marine salt were collected from different sources: market places and from natural sea salt producers from Portugal. Solutions of salt samples were prepared by dissolving the appropriate mass of salt in deionised water, in some cases, samples were filtered prior analysis. All reagent solutions were prepared from analytical reagent grade chemicals and deionised water. A standardized stock NaCl solution with concentration of 0.1 mol L^{-1}

was prepared by dissolving 5.8448 g of NaCl in a 1000 mL of water, this solution was used to prepare eight chloride working standards ranging from 50 to 600 mg L^{-1} . A stock solution of 0.1 mol L^{-1} silver nitrate was prepared by dissolution of 8.5 g AgNO_3 in 500 mL of water. Subsequently, it was titrated against a standard NaCl solution.

The precipitation reagent was a $1 \times 10^{-2} \text{ mol L}^{-1}$ AgNO_3 solution in 2 mol L^{-1} HNO_3 and 1 g L^{-1} polyvinyl alcohol (PVA). To prepare this reagent, 0.25 g of PVA was dissolved in boiling water and allowed to cool. After cooling, 35 mL of concentrated HNO_3 ($d = 1.40$; 65%) and 0.42 g of AgNO_3 were added and the volume was made up to 250 mL with water.

A 2 mol L^{-1} HNO_3 solution was prepared from the appropriated dissolution of concentrated HNO_3 .

FIA system: The flow system set up comprised a manually operated injector commutator, a peristaltic pump (Gilson, Minipuls 3) with Tygon pumping tubes, an Ocean Optics cuvette holder with a flow cell (inner volume of $80 \mu\text{L}$ and 10 mm light path) connected to reaction coils built up with 0.8 mm i.d. PTFE tubing. The turbidity spectra were recorded using a fiber optic spectrophotometric system, composed of a CCD-array detector (Ocean Optics model HR4000) spectrophotometer with spectral range 200 – 1100 nm, a couple of optical fibers (id: $300 \mu\text{m}$) and a Mikropack DH-200 combined deuterium and tungsten/halogen light source. The spectrometer interfaced with a computer via USB port allowing the signal acquisition by the SpectraSuite Spectroscopy Operating Software.

The manifold for the turbidimetric determination of Cl^- in salt samples is depicted in Figure 1. The flow rate of the carrier solutions was set at 2.40 mL min^{-1} . The precipitating reagent in nitric acid solution and the working standard solutions (in the range of 50 to 600 mg L^{-1}) or samples are injected simultaneously into two carrier channels and mixed at the confluent point x. In this way, the sample zone is overlapped with the acidic silver chloride solution then the processed sample passes through the flow cell and the absorbance changes caused by the formed AgCl crystals are detected. The transient signal is monitored at 425 nm and maximum peak height is recorded. Quantification of the analyte is based on the interpolation of the recorded signal of the sample solutions on the calibration curve

established using standards of known concentrations.

Fourier infrared spectroscopy analysis:

All spectra were acquired using a spectrometer Perkin-Elmer (Spectrum BX) set up for mid-infrared measurement equipped with a horizontal one single reflection ATR Golden Gate (Specac, Germany). A 50 μL of standard or salt sample was deposited on the surface of an ATR Golden Gate. The spectra was registered between 4000 and 600 cm^{-1} and collected at a resolution of 4 cm^{-1} ; and 32 scans were co-added before Fourier transformation. A total of five replicates for each sample were registered.

Reference method: In order to evaluate the accuracy of the alternative methods (FIA and FTIR), the results obtained with the proposed methods were compared with those obtained by a reference volumetric procedure [4].

Data processing: Principal component analysis (PCA) and Partial Least Squares (PLSLV) regression were used for spectrum analysis [11]. Standard errors of calibration and cross-validation were calculated to assess the quality of the regression.

3. Results and Discussion

Optimization of the FIA system: A FIA system based on a turbidimetric reaction was designed and dimensioned to allow chloride determination over a wide concentration range (50 to 600 mg L^{-1}). Certain physical parameters, such as flow rate and coil dimensions (length and coil configuration), were studied. A knitted coil reactor of 200 cm was selected. The volume of the precipitating reagent (AgNO_3 prepared in PVA and HNO_3) was set to 60 μL . The sample volume was varied between 60 and 15 μL in order to study its influence on the sensitivity and repeatability of the system, the volume selected was set to 15 μL (see Figure 1). Other variables of the system like the wavelength for signal recording was optimized by maximizing the sensitivity and minimizing the coefficient of variation. The wavelength was varied from 390 to 450 nm and it was set at 425 nm. The potential interfering capacity from certain foreign ions, usually found in salt samples, was also evaluated in the turbidimetric chloride determination. The ions selected were: sulphates (SO_4^{2-}),

Iodide (I_2^-), and Bromide (Br^-) which might lead to precipitation reactions [12]. At several standard solutions containing chloride at 73 and 185 mg L^{-1} were added the interfering species at concentration level normally found in a sample of salt. The results are presented in Table 1.

Figure 1 Flow injection analysis (FIA) manifold for the turbidimetric determination of chloride: S, sample or standard; C1, water; C2, nitric acid (2 mol L^{-1}); R, silver nitrate (1 $\times 10^{-2}$ mol L^{-1}); L', loop sample (15 μL); L'', loop silver nitrate (60 μL); C, commutator; KR, 200 cm knitted reactor; λ , UV/VIS spectrophotometer; W, waste.

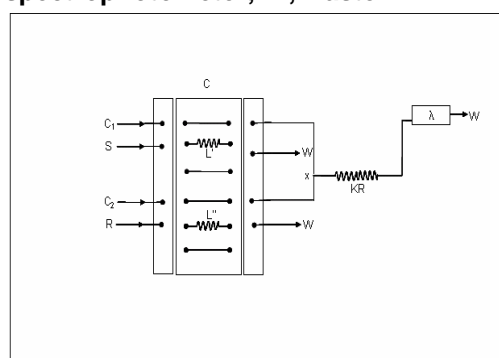


Table 1 Potential interfering species and their maximum tolerated concentration (%)

Chloride concentration mg L^{-1}	Molar Ratio: Interfering Species / Chloride		
	$1.2 \times 10^{-3} \text{SO}_4^{2-} / \text{Cl}^-$	$4.8 \times 10^{-4} \text{I}_2^- / \text{Cl}^-$	$8.5 \times 10^{-6} \text{Br}^- / \text{Cl}^-$
73	+3.1	-4.4	-9.1
185	-2.2	-2.8	-1.8

The result show that only the bromide interferes with the determination at the concentration studied causing signal alteration of the pure standard outside $\pm 5\%$.

The detection limit for chloride determination using the developed flow system was 30.8 mg L^{-1} ; it was calculated according the IUPAC recommendations [13].

A complete analytical cycle took about 90 s for any of the samples analysed. An analytical cycle is the sum of the time needed for each step until the signal is acquired. Thus, based on the time spent per cycle, the sample frequency was 40 determinations per hour. The overall consumption of reagents per hour of work was: 0.24 g of silver nitrate, 0.14 g of PVA and 90 ml of nitric acid (2 mol L^{-1}).

Application FIA and FTIR-ATR systems to salt samples: Two samples of table salt and eight marine salt samples were

analysed by the FIA, FTIR-ATR and the reference methodology. The results of the analyses are presented in Table 2.

Table 2 Determination of chloride in table and marine salt samples by flow injection analysis (FIA), FTIR-ATR and reference methods and corresponding relative deviation (RD)

Sample origin	Sample number	FIA \pm sd (% NaCl)	FTIR-ATR \pm sd (% NaCl)	Ref. Method \pm sd (% NaCl)	RD (%) ^a	RD (%) ^b
Spain	1	97.5 \pm 0.2	95.9 \pm 0.05	97.4 \pm 0.4	0.03	-1.5
Aveiro	2	93.3 \pm 0.9	85.8 \pm 0.04	94.7 \pm 0.8	-1.5	-9.4
Algarve	3	96.1 \pm 0.9	91.3 \pm 0.05	96.3 \pm 0.2	-0.2	-5.2
Aveiro	4	94.7 \pm 0.05	86.9 \pm 0.05	94.7 \pm 1.9	0.1	-8.2
França	5	95.2 \pm 1.1	92.8 \pm 0.04	96.9 \pm 0.5	-1.7	-4.2
Algarve	6	99.6 \pm 1.4	101.0 \pm 0.01	95.2 \pm 0.2	4.6	6.2
Aveiro-Artesanal	7	89.10 \pm 0.2	70.6 \pm 0.07	87.7 \pm 0.10	1.6	-19.5
Algarve-Cercicada	8	98.1 \pm 0.1	88.5 \pm 0.03	96.3 \pm 0.1	1.9	-8.0
Algarve	9	96.5 \pm 0.9	87.9 \pm 0.02	96.2 \pm 0.06	0.4	-8.5
Algarve	10	93.8 \pm 0.3	86.4 \pm 0.05	96.3 \pm 0.02	-2.5	-10.2

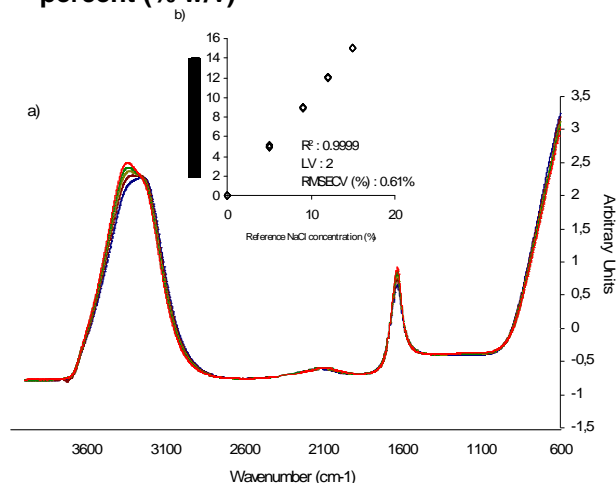
^a Relative deviations of the FIA method compared with the reference method

^b Relative deviations of the FTIR-ATR method compared with the reference method

The repeatability of the FIA system was assessed from 10 consecutive injections of one sample with three different dilution factors. The results in percentage were as follow: 90.5 \pm 0.8; 86.3 \pm 1.1 and 78.8 \pm 1.0. The relative standard deviations of the mentioned results were lower than 1.3%.

In relation to the FTIR-ATR system, the analyses of the spectrum of chloride working standard solutions ranging from 5 to 15 percent (% w/v) lead to the observed changes on the shape and position of the water bands in the frequencies of 3400 to 3140 cm⁻¹ and 1632 cm⁻¹ (see Figure 2). These changes allow the determination of the percentage of NaCl in ten samples of salt (Table 2). The repeatability of the determinations was assed by determination of NaCl using ten salt samples with two factors of dilution. The ANOVA analysis leads to the conclusion that there is no difference in the results obtained for samples accessed at different levels of dilution. The sample frequency using the infrared spectroscopy methodology was 20 determinations per hour.

Figure 2 a) Mid-infrared spectrum (a) and calibration curve (b) of chloride working standard solutions ranging from 5 to 15 percent (% w/v)



4. Conclusions

The developed FIA and FTIR methodologies allow determination of sodium chloride over a wide concentration range. The first aforementioned methodology offer several advantages: high sampling rate, low cost of implementation and simply interpretation of the results. On the other hand, the IR methodology does not make use of reagents and allows the detection, if necessary, of other constituents in the matrix by the analysis of one spectrum. The selection of any of these methodologies for food analysis will depend on the necessities, person's skill capacities and price that the food industry is willing to pay for the automatization of its analytical determinations.

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