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Automation of iron and copper determination in milks using FIA systems and colourimetric detection

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This paper describes a flow injection manifold with a colourimetric detection system that enables the determination of iron and copper in different types of milks, namely cow milk and infant formula powdered milks. The methodology used is based on the formation of coloured complexes produced by the reaction of iron (II) with 1,10-phenanthroline and copper (II) with 1,5-diphenylcarbazide. The samples were digested with nitric and sulphuric concentrated acids and inserted in the FIA system without additional treatment. The pH adjustment was carried out inside the manifold including the addition of the reagents needed to form the absorbing species. The sampling rate of both species was never lower than 120 determinations h^{-1}. The results obtained were compared with those given by the reference methods, and the relative deviation was less than 5 and 4% for the determinations of iron and copper respectively. The precision of the results evaluated by the relative standard deviation (RSD%) was less than 0.5% for iron and 2% for copper determinations. © 1998 Elsevier Science Ltd. All rights reserved.

INTRODUCTION

The importance of iron and copper in food as contributing to healthy growth and development of the human body is widespread. Iron is essential for synthesis of the haemoglobin molecule and copper acts as an important metallic activator of several enzymes (Jeness, 1984). As cow milk presents a rather low concentration of iron and copper, whereas infant formula powdered milks are fortified with these elements, the determination of these species in milks is common in routine analyses.

Several works on the determination of these ions in milks using different analytical methods have already been published. The most common methods are atomic absorption spectrometry with electrothermic atomization (Byrd and Butcher, 1993; Jorhem, 1993; Mingorance *et al.*, 1993; Fuente *et al.*, 1995), hydride-generation (Jochum *et al.*, 1995), or performing a pre-concentration over a column of ion exchange separation using flame atomization (Aziz-Alrahman, 1994). Other methods are also referred to, such as inductively coupled plasma atomic-emission spectrometry (Carrion *et al.*, 1994; Negrett-de-Braetter *et al.*, 1995), UV/Vis spectrophotometry with a catalytic effect on the potassium bromate–indigo carmine (Shraydeh *et al.*, 1994) and constant potential amperometry (Rao *et al.*, 1991).

Burguera *et al.* (1987), have developed a flow injection analysis manifold (FIA) as an automatic method for the determination of Fe and Cu in infant formula powdered milks, using as detector an atomic absorption spectrophotometer with standard additions, to avoid the low sensitivity obtained with flame atomization.

The reference methods proposed for the determination of both species in milks used colourimetric measurements based on the formation of coloured complexes after a digestion procedure. The reagent used in Fe determination is 1,10-phenanthroline (Manuel Suisse, 1973) or batophenathroline (FIL 103 A, 1986), and sodium diethyldithiocarbamate (FIL 76 A, 1980; Manuel Suisse, 1993) in the determination of Cu. In all reference methods the adjustment of the sample (after digestion) to the colourimetric measurement is complex and time-consuming, what grounds the development of automatic methods based on FIA systems.

This work refers to the development of a FIA system which enabled determinations of Fe and Cu in several types of milk, using a spectrophotometer as detector. The colour reagent used for Fe was 1,10-phenanthroline

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and 1,5-diphenylcarbazide for Cu. After digestion, samples were directly injected into the FIA system and its preparation before measurement performed inside the flow system.

MATERIALS AND METHODS

Reagents and solutions

Solutions were prepared with deionized water (specific conductivity less than 0.1 μS cm^{-1}) and all reagents were analytical grade.

The Fe (II) stock solution (200 mg litre^{-1}) was prepared from ammonium iron (II) sulphate hexahydrate and the solution of 10 mg litre^{-1} Cu (II) was obtained from a stock solution of 1000 mg litre^{-1} (Spectrosol, BDH ref. 1413 D). All solutions used for the calibration curves were prepared by dilution of stock solutions.

The 1,10-phenanthroline 0.01% (w/v) and 1,5-diphenylcarbazide 0.01% (w/v) solutions were prepared from the corresponding solids and dissolved in 1.5 M acetate solution obtained from sodium acetate-3-hydrate, and in a buffer solution with pH = 9, obtained by the pair boric acid/potassium tetrahydroxoborate, respectively.

Sample preparation

The powdered milk samples were prepared according to manufacturers instructions and submitted to digestion according to the reference methods, resulting in solutions with acidity between 0.4 and 0.9 M.

In the determination of Fe, the sample was directly injected into the FIA system, whereas in the determination of Cu, owing to the reaction with 1,5-diphenylcarbazide at a high pH level (pH = 9), a small amount of a NaOH saturated solution was added in the digest before the sample was injected into the FIA system. More concentrated NaOH solutions were not used in the automatic system so that the lifetime of the injection valve and of the propulsion PVC tubes could be increased.

Instrumentation

A Jenway 6100 spectrophotometer equipped with a Hellma 178.713 flow cell (8 μl of optical volume) and connected to a Kipp & Zonnen BD 111 chart recorder was used as detector in the FIA manifold. The solutions were propelled using Gilson Minipuls 3 (Gilson France S.A., Vallas Le Bel, France) peristaltic pumps and pumping tubes of the same brand. The absorbance measurements of the coloured complexes were performed at 512 and 495 nm for the determination of Fe and Cu, respectively.

Omnifit teflon tubings (0.8 mm i.d.) connected by Gilson end-fittings and joints were used in the construction of the manifold. The confluences were constructed of perspex as previously reported (Alegret *et al.*, 1987).

The samples and standards were inserted into the carrier stream by means of a Rheodyne, model 5020, six-port injection valve.

For the reference procedure, a Hitachi, U-2000 spectrophotometer was used.

Reference procedure

The Fe and Cu ions present in milk samples were determined by colourimetry, as recommended by the Manuel Suisse des Denrées Alimentaries, after prior samples digestion. These determinations were carried out with controlled pH and *o*-phenanthroline and sodium diethyldithiocarbamate were used as colour developing reagents in the determination of Fe and Cu, respectively. The absorbance measurements of the formed complexes were performed at 512 and 436 nm, respectively.

RESULTS AND DISCUSSION

In order to determine Fe and Cu in milk samples, a FIA manifold (Fig. 1) was developed optimizing each parameter separately and setting the remainder. This procedure aimed for adjustment to the best conditions in terms of sensitivity, detection limit, reproducibility, sampling rate and reagent consumption.

In the Fe determination, an injection volume of 250 μl, determined by titration of a sample obtained from 10 consecutive injections of a solution with a known concentration (Lima and Rangel, 1990), was selected amongst three injection volumes (500, 250 and 175 μl) tested, since it presented the best compromise between the reproducibility and sampling rate.

After injection in water used as carrier stream ($Q_1 = 1.2$ ml min^{-1}), a solution of ascorbic acid 0.1% (w/v) was added ($Q_2 = 1.2$ ml min^{-1}) at confluence X and its concentration was determined in order to provide a slight excess of the reducing agent to assure that all Fe

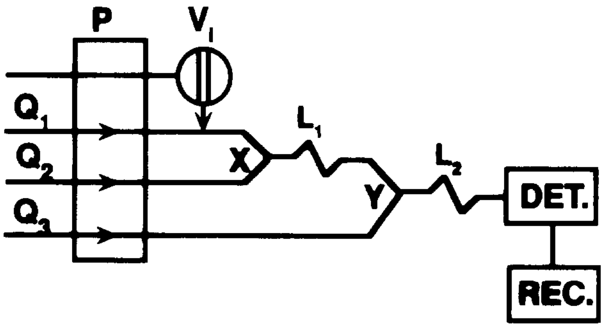

Fig. 1. Flow injection manifold for Fe (A) and Cu (B) determinations in milks. Q: flow rates (Q_1 and $Q_2 = 1.2$ ml min^{-1} for A and 1.7 ml min^{-1} for B; $Q_3 = 2.4$ ml min^{-1} for A and 3.4 ml min^{-1} for B); P: peristaltic pump; V_i: injection volume (250 μl for A and 50 μl for B); X and Y: confluence points; L: tube lengths ($L_1 = 10$ cm for A and 30 cm for B; $L_2 = 400$ cm for A ad 235 cm for B); DET: detector; REC: recorder.

(III) was reduced to Fe (II). Ascorbic acid was used instead of hydroxylammonium chloride, as indicated in the reference procedure, because it was a more powerful reducer in very acid solutions ($\text{pH} < 1$) (Mortatti *et al.*, 1982).

The reduction process occurred inside reactor L_1 which was only 10 cm long this being sufficient to accomplish full reduction and provide a lower dispersion. The reduction of Fe (III) to Fe (II) was considered complete when solutions of Fe (II) and Fe (III) with the same concentrations ($4\,\text{mg litre}^{-1}$) presented equal absorbance values.

As Fe (II) reaction with 1,10-phenanthroline occurred at a pH between 2 and 9, the pH adjustment was performed by the addition of 1.5 M sodium acetate solution ($Q_3 = 2.4\,\text{ml min}^{-1}$) at confluence Y since the acidity of the samples after being digested was between 0.4 and 0.9 M. Concentrations of sodium acetate solutions from 0.75 to 1.50 M (with fixed background of phenanthroline) were tested, and 1.5 M solution was selected because it was the lowest concentration allowing pH adjustment of any sample inserted in the system regarding the different acidity levels of the samples after digestion. Several solutions with different concentrations of 1,10-phenanthroline were added in channel Q_3 and 0.01% (w/v) was selected because it was the lowest concentration enabling a maximum sensitivity.

The coloured complex was formed in reactor L_2, length (400 cm) being selected in consideration of reproducibility and sampling rate. Both reactors L_1 and L_2 were coiled to improve radial mixing and minimize the dispersion of the sample plug (Tijssen, 1980).

In Cu determination, the same basic FIA configuration was used and optimized following the same criteria.

An injection volume of 50 μl was selected from different volumes tested (35, 50, 75 and 125 μl) according to the optimization of iron considering its reproducibility and sampling rate.

The sample was injected into a 1.2 M NaOH solution used as carrier stream ($Q_1 = 1.7\,\text{ml min}^{-1}$) and it flowed to confluence X in which a solution having the same concentration was added ($Q_2 = 1.7\,\text{ml min}^{-1}$). At confluence Y, a solution of 1,5-diphenylcarbazide was added. The concentration of this solution was changed within the range 0.005–0.015% (w/v), increasing sensitivity up to 0.01% (w/v) which was the selected concentration.

Different lengths of reactors L_1 and L_2 were tested being selected $L_1 = 30\,\text{cm}$ and $L_2 = 235\,\text{cm}$ regarding reproducibility, sensitivity and sampling rate.

The developed flow injection methodologies led to rather reproducible results of Fe and Cu. Successive calibration curves carried out throughout each day, and also from day to day, presented similar values of slope and intercept. Therefore, no periodic calibrations between batches of sample determinations were necessary. Additionally, no baseline drift was observed, meaning that eventual problems of coloured complex retention on the flow cell walls did not occur.

The determination limits were also estimated according to IUPAC (Analytical Methods Committee, 1987) recommendations and the values found were $0.035\,\text{mg litre}^{-1}$ for iron and $1.41 \times 10^{-3}\,\text{mg litre}^{-1}$ for copper. In both determinations, these manifolds enabled a sampling rate of 120 samples h^{-1}.

Determination of iron and copper in milks

The determination of Fe and Cu was obtained from 12 samples of different types of milk.

The mean values obtained with the FIA system (C_f) when compared with the standard values given by the reference method (C_r) showed a relative deviation (RD) less than 5% for Fe and 4% for Cu (Table 1). A $C_f = C_0 + SC_r$ relation was established for each of the two species (Table 2). Considering that the slope is close

Table 1. Determinations of Fe and Cu in different kinds of milk, using the FIA manifold (C_f), the reference method (C_r) and the corresponding relative deviation (RD)

Sample	Fe			Cu		
	C_f (mg litre^{-1})	C_r (mg litre^{-1})	RD (%)	C_f (mg litre^{-1})	C_r (mg litre^{-1})	RD (%)
1[a]	7.9 ± 0.5	8.3 ± 0.7	−4.8	0.404 ± 0.005	0.41 ± 0.01	−1.5
2[a]	10.32 ± 0.09	9.9 ± 0.9	+4.2	0.408 ± 0.004	0.404 ± 0.005	+1.0
3[a]	13.5 ± 0.3	14.2 ± 0.7	−4.9	0.406 ± 0.005	0.416 ± 0.005	−2.4
4[a]	10.4 ± 0.2	10.5 ± 0.7	−1.0	0.403 ± 0.008	0.415 ± 0.006	−2.9
5[a]	8.8 ± 0.5	8.9 ± 0.7	−1.1	0.449 ± 0.003	0.435 ± 0.005	+3.2
6[a]	8.30 ± 0.06	8.2 ± 0.3	+1.2	0.29 ± 0.01	0.28 ± 0.01	+3.6
7[a]	12.6 ± 0.1	12.1 ± 0.4	+4.1	0.650 ± 0.009	0.66 ± 0.02	−1.5
8[a]	8.23 ± 0.04	8.2 ± 0.4	+0.4	0.448 ± 0.005	0.456 ± 0.005	−1.8
9[a]	10.93 ± 0.04	10.53 ± 0.04	+3.7	0.438 ± 0.004	0.426 ± 0.005	+1.2
10[b]	2.90 ± 0.02	2.99 ± 0.02	−3.0	0.118 ± 0.004	0.116 ± 0.005	+1.7
11[b]	1.58 ± 0.01	1.6 ± 0.3	−1.3	0.139 ± 0.002	0.136 ± 0.005	+2.2
12[b]	3.27 ± 0.05	3.15 ± 0.01	+3.8	0.129 ± 0.002	0.126 ± 0.005	+2.4

Mean and standard deviation of six determinations.
[a] Samples of different kinds of powdered milks.
[b] Cow milk samples.

Table 2. Results obtained in the determination of Fe and Cu in milk samples (n = 12)

Element	C_0[a]	S[a]	r[b]	$t_{0.025}$[c]	$t_{0.025}$[d]	RSD%[e]
Fe	1.001	0.025	0.996	−0.335	2.201	0.5 (8.23)
Cu	0.982	4.842×10^{-3}	0.998	+0.561	2.201	1.3 (0.45)

[a] Parameters of the equation $C_f = C_0 + SC_r$.
[b] Correlation coefficient.
[c] Calculated values for a two-tail t-test.
[d] Tabulated values (95% confidence level).
[e] Relative standard deviation obtained from 12 consecutive injections of a milk sample.
The mean concentration value obtained in the experiment is indicated in parentheses.

to the unit and the intercept is close to zero, it can be found that both methodologies are in good agreement. The precision of the present methodology was tested by estimating the relative standard deviation (RSD%) (Table 2) of 12 replicate determinations of a milk sample with a concentration close to the average value of the concentration range found in the samples analysed.

CONCLUSIONS

The flow injection system developed for the determination of Fe and Cu in milk samples is a good alternative to the reference methods as it provides comparable results with a sampling rate of 120 determinations h^{-1}. After digestion the samples are inserted in the FIA system without additional treatment and adjustment of the sample to the colourimetric measurement is carried out inside the system thus reducing duration.

The developed FIA systems may be easily introduced, present high sampling rates and require instrumentation already available in analytical laboratories for milk control.

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REFERENCES

Alegret, S., Alonso, J., Bartroli, J., Machado, A. A. S. C., Lima, J. L. F. C. and Paulis, J. M. (1987) Construction of equipment for potentiometric determinations in flow injection analysis. *Quimica Analytica* **6**, 278–292.

Analytical Methods Committee (1987) Recommendations for the definition, estimation and use of the detection limit. *Analyst* **112**, 199–204.

Aziz-Alrahman, A. M. (1994) Ion exchange separation-atomic-absorption-spectrophotometric method for the determination of microgram quantities of copper, iron and zinc in infant milk formula: powder form. *Analytical Letters* **27**(2), 411–428.

Burguera, M., Burguera, J. L., Garaboto, A. M. F. and Alarcón, O. M. (1987) Determination of iron and copper in infant formula powdered milks by flow injection atomic absorption spectrometry. *Quimica Analytica* **6**, 427–435.

Byrd, E. D. and Butcher, D. J. (1993) Determination of trace elements in biological standard reference materials by graphite-furnace atomic-absorption spectrometry with solid and slurry sampling. *Spectroscopy Letters* **26**(9), 1614–1624.

Carrion, N., Itriago, A., Murillo, M., Eljuri, E. and Fernandez, A. (1994) Determination of calcium, potassium, magnesium, iron, copper and zinc in maternal milk by inductively coupled plasma atomic-emission. *Journal of Analytical Atomic Spectrometry* **9**(3), 205–207.

Fuente, M. A., Guerrero, G. and Juárez, M. (1995) Determination of copper in skim milk by GFAAS using palladium-magnesium modifier and L'vov Platform. *Atomic Spectroscopy* **16**(5), 219–223.

Jeness, W. (1984) *Dairy Chemistry and Physics*. John Wiley and Sons, New York.

Jochum, F., Fuchs, A., Cser, A., Menzel, H. and Lombeck, I. (1995) Trace mineral status of full-term infants fed human milk, milk-based formula or partially hydrolysed whey protein formula. *Analyst* **120**(3), 905–909.

Jorhem, L. (1993) Determination of metals in foodstuffs by atomic-absorption spectrophotometry after dry ashing: NMKL inter-laboratory study of lead, cadmium, zinc, copper, iron, chromium and nickel. *Journal of AOAC International* **76**(4), 798–813.

Lima, J. L. F. C. and Rangel, A. O. S. S. (1990) Determination spectrophotométrique du phosphore total et inorganique dans les vins par la technique d'injection dans un flux. *Journal of International Science Vigne Vin* **24**, 49–61.

Mingorance, M. D., Perez-Vazquez, M. L. V. and Lachica, M. (1993) Microwave digestion methods for the atomic-spectrometric determination of some elements in biological samples. *Journal of Analytical Atomic Spectrometry* **8**(6), 853–858.

Mortatti, J., Krug, F. J., Pessenda, L. C. R. and Zagatto, E. A. G. (1982) Determination of iron in natural waters and plant material with 1,10-phenanthroline by flow injection analysis. *Analyst* **107**, 659–663.

Manuel Suisse des Denrées Alimentaires (1973) 5th edn. Vol. 2.

Negrett-de-Braetter, V. E., Recknagel, S. and Gawlik, D. (1995) Speciation of selenium, iron and zinc in human milk whey: the use of instrumental neutron-activation analysis (INAA) to corroborate element profiles measured with inductively coupled plasma atomic-emission spectrometry (ICP AES). *Fresenius Journal Analytique Chemie* **353**(2), 137–142.

Norme FIL internationale 76 A (1980) Lait et produits laitiers. Determination de la teneur en cuivre.

Norme FIL internationale 103 A:1986. Lait et produits laitiers. Determination de la teneur en fer.

Rao, D. S., Pantulu, P. C., Sudheendranath, C. S., Rao, M. B. and Krishnan, C. P. A. (1991) Successive complexometric estimation of iron and copper in milk by constant potential amperometry. *Journal of Food Science and Technology* **28**(3), 164–166.

Shraydeh, B. F., Abu-Eid, M. A., Zatar, N. A., Abu-Obeid, A., Khamis, M. and Kanan, K. (1994) A sensitive catalytic method for the determination of copper by its catalytic effect on the potassium bromate-indigo carmine reaction. *Instrument Science and Technology* **22**(4), 355–363.

Tijssen, R. (1980) Axial dispersion and flow phenomena in helically coiled tubular reactors for flow analysis and chromatography. *Analytica Chimica Acta* **114**, 71.