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Direct determination of iron and selenium in bovine milk by graphite furnace atomic absorption spectrometry

Poliana C. Aleixo, Joaquim A. Nóbrega*

Grupo de Análise Instrumental Aplicada, Departamento de Química, Universidade Federal de São Carlos, Caixa Postal 676, São Carlos, SP, 13560-970, Brazil

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Abstract

Milk is a complex sample containing high contents of organic compounds and its analysis generally involves digestion procedures that can be affected by losses and contamination. In the work here described it was developed a procedure for determination of Fe and Se in bovine milk. Samples were diluted using a mixture of water-soluble tertiary amines (10% v/v CFA-C). The tertiary amines had a favorable effect on the action of the autosampler and consequently on repeatability. Using this strategy the direct analysis of milk without any digestion procedure by graphite furnace atomic absorption spectrometry is feasible. Pyrolytic graphite tubes were used and all measurements were based on peak area of transient signals with background signal correction based on Zeeman effect. Palladium was used as chemical modifier for Se. The pyrolysis and atomization temperatures were 1300 and 2300 °C for Fe and 1500 and 2400 °C for Se, respectively. The procedure was applied for 13 bovine milk samples from different sources. The quantification was based on the standard additions method. The lifetime of the graphite tube was 300 and 250 heating cycles for Fe and Se, respectively.

Keywords: Bovine milk; Iron; Selenium; Tertiary amines; GF-AAS

1. Introduction

Milk is a close to ideal food essential for newborns due to its composition and availability. In addition to its macronutrients, i.e. protides, glucides, and lipids, milk also contains micronutrients, i.e. vitamins and elements, that are absolutely essential during the first months of a baby's life since it is the only source of nutrients. According to Coni, Alimonti, Bocca, La Torre, Pizzuti, and Caroli (1996), this is particularly true for micronutrients that are not stored by the fetus during its growth inside the uterus. These authors pointed out that essential elements could be divided into two groups: those with reserves usually sufficient to protect the baby from potential deficiencies during the first 4–6 months, and those which require immediate reintegration right from birth so as to reach the best rate of growth. Iron is a typical element of the first group and Se is representative of the second one. This clearly demonstrated the importance of the determination of these trace elements in milk. Human and cow's milk are relatively low in Fe ranging from 0.2 to 0.8 mg l^{-1}, but it is highly bioavailable mainly in human milk (Casey, Smith, & Zhang, 1995). On the other hand, Se concentration in milk is directly affected by levels in the food chain and, hence, reflects the food habits and the geochemical environment. Selenium concentration in cow's milk can vary as many as 2–1270 μg l^{-1} depending on the availability of this element in the food and geographical area. Nowadays there is an increasing knowledge about the role of Se in physiological processes, however despite its clear beneficial effects the Se intake is declining over the years in Europe (Rayman, 2002).

The determination of trace inorganic constituents in milk is not a trivial task because of the complexity of the emulsion. According to Jensen (1995), bovine milk contains around 3.4% of proteins, 2.8% of casein, 3.7% of fat and 4.6% of lactose; additionally, the elements are present as different compounds that can affect both the sample preparation and the measurement strategies. For example, about one-third of the Fe in human milk is

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* Corresponding author. Fax: +55-16-260-8350.
E-mail address: djan@terra.com.br (J.A. Nóbrega).

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associated with the low molecular weight aqueous fraction, one-third with the milk fat, and of the remainder, about 10% is found with the casein fraction (Jensen, 1995).

Most proposed procedures involve a step of digestion to eliminate the organic matrix. Owing to the complexity of this matrix, the procedure can involve several steps and contamination can become a serious obstacle for obtaining accurate data. A critical study of different procedures, such as dry ashing, hot-plate procedure, high-pressure ashing, open and closed vessels microwave-assisted, that could be applied for the determination of Zn in milk was presented by Krushevska, Barnes, Amarasiriwaradena, Foner, and Martines (1992). Despite the usefulness of these procedures, some of them are time consuming and it is attractive to investigate dilute-and-shoot procedures that could be properly applied for trace analysis of milk.

Direct procedures for milk analysis were proposed using different spectroanalytical techniques. Each one presents some difficulties. Quináia and Nóbrega (2000) demonstrated that the presence of fat compounds affects the performance of the autosampler in graphite furnace atomic absorption spectrometry (GF-AAS) and the pneumatic nebulization in the inductively coupled plasmas coupled to optical emission spectrometry (ICP-OES—Coni, Stacchini, Caroli, & Falconieri, 1990) or to mass spectrometry (ICP-MS—Stürup & Büchert, 1996). The formation of carbon residues causes a gradual deterioration of the graphite tube in GF-AAS, affects excitation conditions in ICP-OES and isobaric interferences in ICP-MS. In GF-AAS milk samples were diluted with a solution containing ethanol, nitric acid and hydrogen peroxide to remove the organic matter during the heating cycle (Viñas, Campillo, López-García, & Hernández-Córdoba, 1997). This procedure was successfully applied for the determination of Al, Cr, Mn, and Mo.

The addition of surfactant agents could also be employed to attenuate at least partially the undesirable effects. Wagley, Schmiedel, Mainka, and Ache (1989) have studied the direct determination of Se in milk introducing a suspension prepared in 3% v/v Triton X-100 and containing Rh + Mg as chemical modifier. More recently, this same surfactant was also used for determining nine elements, including Se, in different milk slurries by GF-AAS (Garcia, Lorenzo, Cabrera, Lopez, & Sanchez, 1999). Foster and Sumar (1995), in a review about Se determination in milk and infant formulae, emphasized that most AAS works involved hydride generation, that is critically dependent on a harsh sample decomposition treatment to convert all organoselenium forms to Se(IV).

The use of a chemical modifier for Se determination by GF-AAS is imperative due to its volatility, but the choice was not trivial in earlier works. In spite of the use of Ni as chemical modifier (Wagley et al., 1989), the main critical aspect that should be pointed out is that this modifier did not stabilize at the same degree different oxidation states and compounds of Se (Welz & Sperling, 1999). Despite of the mentioned 1800 °C pyrolysis temperature applied when the mixture Rh + Mg was used (Wagley et al., 1989), the use of Pd should be recommended owing to its general application in GF-AAS as an universal modifier and the reliability to prevent losses of all Se compounds up to a pyrolysis temperature of at least 1000 °C. The relatively high levels of total phosphate, i.e. around 150 mg l^{-1}, caused spectral interferences at the 196.0 nm resonance line and the use of a Zeeman background corrector is strongly recommended.

There is also a lack of information in the literature related to the direct determination of Fe in milk by GF-AAS. Bermejo, Dominguez, and Bermejo (1997), demonstrated the feasibility of a high-performance nebulizer for introduction of milk suspension in a flame atomic absorption spectrometer (FAAS) and determination of Fe. We had observed in preliminary experiments that the use of a conventional concentric nebulizer for non-diluted milk samples was not possible due to gradual clogging of the nebulizer and when the milk sample was diluted to overcome this effect the sensitivity of FAAS was not enough.

In a previous work it was proposed a dilute-and-shoot procedure for direct analysis of milk by ICP-MS (Nóbrega, Gélinas, Krushevska, & Barnes, 1997). The milk samples were diluted in a mixture of water-soluble tertiary amines and the resulting suspensions were introduced by pneumatic nebulization using a concentric nebulizer. Despite the good results obtained, the accuracy attained for Al, Cr, Fe, and Se was not suitable probably due to the occurrence of isobaric interferences.

In the work here described a procedure was developed for dilution of milk samples with a mixture of water-soluble tertiary amines and the suspension was introduced into the graphite tube by autosampler action. Iron and Se were determined in bovine milk using conventional heating programmes without any modifier or with Pd modifier, respectively.

2. Experimental

2.1. Apparatus

A Varian AA-800 atomic absorption spectrometer (Mulgrave, Victoria, Australia) was connected to a GTA-100 graphite furnace atomizer equipped with an automatic sampler, also from Varian. Pyrolytic graphite tubes (Part number 63-100011-00) were used. Background correction was based on the Zeeman effect with a transversal electromagnetic field. An ultra hollow cathode lamp and a hollow cathode lamp were employed for Se and Fe, respectively. Argon was used as purge gas at a flow-rate of 3.0 l min^{-1} during all stages, except during atomization when the gas flow was stopped. The operating conditions are summarized in Table 1. All measurements made used integrated absorbance with an integration time of 2 s. The graphite furnace temperature programmes used for Se and Fe are showed in Tables 2 and 3, respectively.

2.2. Reagents and samples

All reagents used were of analytical-reagent grade. Ultrapure water (specific resistivity 18 MΩ cm was produced using a Milli-Q water purification system (Millipore, Bedford, MA, USA). Selenium and Fe stock solutions (1000 μg ml^{-1}) were obtained from Merck (Darmstadt, Germany) and Carlo Erba (Italy), respectively, and diluted as necessary to obtain reference solutions. A mixture of tertiary amines (CFA-C, Spectrasol, Warwick, NY, USA) was used for dilution of milk samples. A solution of 2% m/v Pd in 1% v/v nitric acid of 99.999% purity (Aldrich, Milwaukee, WI, USA) was used as chemical modifier for Se.

Liquid milk samples were purchased in Brazil, Chile, Mexico, Uruguay, and the USA.

The standard reference material whole milk powder SRM 8435 was obtained from National Institute of Standards and Technology (Gaithersburg, MD, USA).

Table 1
Instrument parameters used in the determination of Se and Fe by GFAAS

Parameter	Se	Fe
Wavelength (nm)	196.0	248.3
Lamp current (mA)	15	15
Slit width (nm)	0.2	1.0
Sample volume (μl)	20	10

2.3. Milk sample preparation

All glassware was kept in 10% v/v nitric acid for at least 48 h and subsequently washed three times with ultrapure water before use. The milk samples, held in Tetra Pack©, were kept refrigerated at 4 °C after opening.

For determination of Se, 1.0 ml of liquid milk sample was transferred to a 5.0 ml volumetric flask and the volume was made up with 10% v/v of tertiary amines mixture (CFA-C). For determination of Fe in non-fortified milk, 0.5 ml of liquid milk sample was diluted in 10% v/v CFA-C in a 10.0 ml volumetric flask. For milk samples with chelate Fe added during processing, the dilution employed was 1+49 v/v in 10% v/v CFA-C. This CFA-C solution was also used as blank. The diluted samples were transferred to the autosampler cups for the measurements of Se and Fe.

2.4. Determination of Se and Fe

The first experiments were carried out with skim and low-fat milk samples. Direct measurements of Se and Fe in milk samples without any dilution were problematic due to the accumulation of fat compounds in the autosampler cup, irregular drop formation, accumulation of carbonaceous residues inside the atomizer, and high background values. Thus, dilution, with CFA-C was adopted to overcome all these difficulties.

The standard additions method was implemented by sequential introduction of 20 μl of diluted milk spiked with Se reference solutions containing 5, 10, and 15 μg/l prepared in 10% v/v CFA-C medium plus 10 μl (200 μg) of $Pd(NO_3)_2$ as chemical modifier into the graphite tube. Blanks containing 20 μl of CFA-C solution and 10 μl of chemical modifier were used. The temperature programme used is shown in Table 2. All measurements were made on triplicate.

For the determination of Fe in milk samples, the same procedure was adopted. A volume of 10 μl of milk diluted 1+19 or 1+49 v/v in 10% v/v CFA-C was spiked with Fe reference solutions containing 10% v/v CFA-C. The temperature programme used is shown in Table 3. All measurements were made in triplicate.

Table 2
Graphite furnace programme for determination of Se

Step	Temperature (°C)	Ramp time (s)	Hold time (s)	Read
Drying	120	55	5	No
Pyrolysis	1400[a]	10	6	No
Atomization	2400	0	2	Yes
Cleaning	2700	1	2	No

[a] 1500 °C for low-fat milk.

Table 3
Graphite furnace programme for determination of Fe

Step	Temperature (°C)	Ramp time (s)	Hold time (s)	Read
Drying	120	55	5	No
Pyrolysis	1200[a]	10	6	No
Atomization	2200[b]	0	2	Yes
Cleaning	2400	1	2	No

[a] 1300 °C for low-fat milk.

[b] 2300 °C for low-fat milk.

3. Results and discussion

3.1. Effect of dilution factor and the use of water-soluble tertiary amines

As already mentioned, the preliminary experiments focused on the possibility of milk introduction in the graphite tube without any dilution. This strategy was unsuitable due to poor repeatability caused by both carbon residues in graphite tube and fat residues in the autosampler capillary tube. The dilution of milk samples in water using different dilution factors was not effective for overcoming these effects. Taking into account these results, the effect of milk samples dilution with a 10% v/v water-soluble tertiary amines was evaluated. In a previous work it was demonstrated the benefits of using this solution in milk analysis by ICP-OES and ICP-MS (Nóbrega et al., 1997). The resulting suspension presented a pH around 10 that is convenient for milk samples and it was showed that there are no losses caused by hydrolysis. Using this solution, milk samples were diluted up to 1+49 and 1+4 v/v for Fe and Se, respectively. Dilutions equal or higher than 1+4 v/v were suitable to correct undesirable matrix effects, however when compared to Fe and Se absorbance signals obtained for acid-digested diluted samples it can be observed a loss of sensitivity of 30%. Best performances for Fe and Se were attained with 1+19 and 1+4 v/v dilutions, respectively. The performance was evaluated considering atomic (AA) and background (BG) signals, repeatabilities and graphite tube lifetimes. As it will be discussed later on, there is no deleterious effect of the tertiary amines solution on the tube lifetime. For milk samples enriched with chelate Fe the adopted dilution was 1+49 v/v when determining this analyte. It should be mentioned that even with this higher dilution factor the use of amines was mandatory and the dilution with water did not generate suitable working conditions for routine analysis of a large numbers of samples.

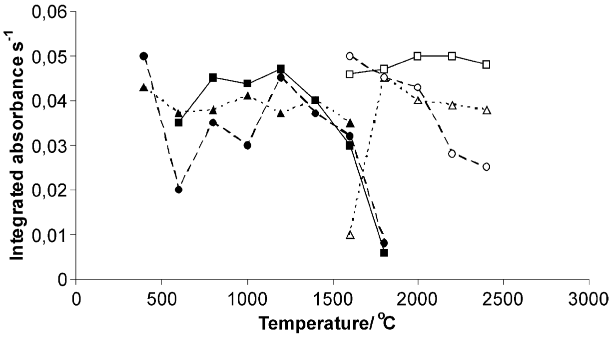

Fig. 1. Pyrolysis and atomization temperature curves for 30 $\mu g\ l^{-1}$ Se in low fat milk diluted 1+4 v/v in 10% v/v CFA-C. Pyrolysis (●, $T_{atomization} = 2200$ °C) and atomization (○, $T_{pyrolysis} = 500$ °C) curves without modifier; Pyrolysis (■, $T_{atomization} = 2400$ °C) and atomization (□, $T_{pyrolysis} = 1500$ °C) curves with 200 μg $Pd(NO_3)_2$; Pyrolysis (▲, $T_{atomization} = 2400$ °C) and atomization (△, $T_{pyrolysis} = 1400$ °C) curves with 32.5 μg $Pd(NO_3)_2$ + 10 μg $Mg(NO_3)_2$.

3.2. Chemical modifiers and heating programmes

The choice of chemical modifiers is also an important experimental parameter in GF-AAS. This is not so critical for Fe due to the thermal behavior of this element in the graphite atomizer. We have tested the effect of nitrate salts of Pd and Mg as chemical modifiers added to milk diluted samples. The best results considering thermal stability, AA and BG signals were reached without any modifier (Fig. 1). Even the use of Mg as recommended by Welz and Sperling (1999) was not advantageous.

The use of a chemical modifier for Se is essential due to its volatility, and according to the literature Pd seems to be the best choice. Fig. 2 shows pyrolysis and atomization temperature curves obtained for 1+4 v/v diluted milk samples with Pd, Pd+Mg, CFA-C, and without modifiers. It can be observed that the best sensitivity

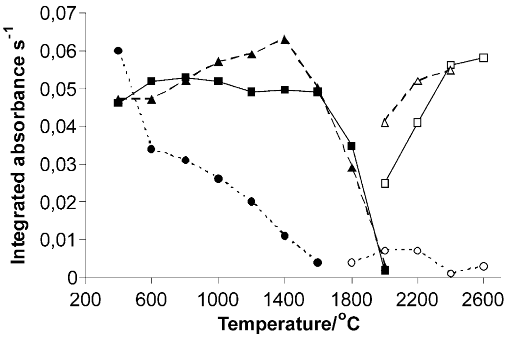

Fig. 2. Pyrolysis and atomization temperature curves for 10 $\mu g\ l^{-1}$ Fe in low fat milk diluted 1+9 v/v CFA-C. Pyrolysis (■, $T_{atomization} = 2000$ °C) and atomization (□, $T_{pyrolysis} = 1200$ °C) curves without modifier; Pyrolysis (▲, $T_{atomization} = 1800$ °C) and atomization (△, $T_{pyrolysis} = 1400$ °C) curves with 200 μg $Pd(NO_3)_2$; Pyrolysis (●, $T_{atomization} = 1800$ °C) and atomization (○, $T_{pyrolysis} = 1200$ °C) curves with 32.5 μg $Mg(NO_3)_2$.

Table 4
Results for the determination of Se and Fe in cow's milk samples (mean±standard deviation, $n = 3$)

Milk type and source	Fe (mg l^{-1})	Se (μg l^{-1})
Skim, New Jersey, USA	0.68±0.03	51±5
Skim, VillaHermosa, Mexico	0.61±0.01	66±11
Skim, Frutal, Brazil	1.14±0.03	46±7
Skim, Treze Tílias, Brazil	0.66±0.02	66±4
Low-fat, Carazinho, Brazil	0.95±0.02	56±6
Skim, Teutonia, Brazil	1.17±0.07	47±7
Skim, Feliz, Brazil	0.64±0.02	39±4
Skim, Montevideo, Uruguay	0.86±0.03	59±3
Skim, Carmelo, Uruguay	0.65±0.04	17±1
Whole, Brasilia, Brazil	Not measured	101±16
Whole, Colonia, Uruguay	Not measured	122±15
Whole, São Carlos, Brazil	Not measured	94±13
Whole, X Region, Chile	0.68±0.04	56±4

Table 5
Determination of Se and Fe (mg kg^{-1}) in fortified milks and in a standard reference material (mean ± standard deviation, $n = 3$)

Sample or SRM	Fe (mg l^{-1}) Label or certified value	Fe (mg l^{-1}) Determined value	Se (μg l^{-1}) Label or certified value	Se (μg l^{-1}) Determined value
Whole (chelate Fe)	6	8.1 ± 0.1	Not measured	Not measured
Whole (chelate Fe)	13	13 ± 1	Not measured	Not measured
Whole milk powder[a] NIST 8435	1.8 ± 1.1	1.8 ± 0.1	0.140 ± 0.02	0.131 ± 0.014

[a] Fe in mg g^{-1}.

Table 6
Detection limits for Se and Fe in different types of milk by GF-AAS ($n = 10$)

Sample	Fe (μg l^{-1})	Se (μg l^{-1})
Skim milk	0.39	1.2
Low-fat milk	0.49	2.4
Whole milk	0.60	2.5

and thermal stabilization were attained in Pd medium, however it should be pointed out that the solution of tertiary amines solution itself also exerted a thermal stabilization effect up to 1200 °C on Se. The pyrolysis temperature without losses of Se can be kept at 1400 °C in Pd medium. The maximum permissible temperature without losses was varied in less than 100 °C for whole, partially skimmed, and skimmed milk. The temperature recommended can be applied for all milk samples despite of its fat content. This was also observed for Fe determination (Fig. 1).

3.3. Determination of Fe and Se in cows milk

Iron and Se were determined in 13 milk samples after proper dilution with tertiary amines solution. Results are shown in Table 4. All data were obtained using the standard additions method since it led to more accurate results than those obtained using reference solutions prepared only in tertiary amines medium. The concentration of Fe varied from 0.61 to 1.17 mg/l and the Se concentrations are in the 17–122 μg/l range. The higher variability observed for Se in samples from different sources corroborates the effects of animal nutrition and soil chemical composition on the amount of this micronutrient in milk.

It can also be observed that the relative standard deviations are generally lower than 5%, which is suitable for routine analysis. The higher relative standard deviations observed for some samples for Se can be attributed to deviations originated by extrapolation of calibration curves using the standard additions method.

The results for two samples of whole milk enriched with chelate Fe and the standard reference material are showed in Table 5. The values determined for Fe in the two enriched milk samples are also in agreement with the indicative label values. Selenium was also determined in whole milk powder standard reference material and it was obtained a value of 0.140 ± 0.020 mg kg^{-1} that is in good agreement with certified value, 0.131 ± 0.014 mg kg^{-1}.

Detection limits ($3\sigma_{\text{blank}}$/slope, $n = 10$) experimentally determined for Se and Fe varied slightly depending on the sample matrix (Table 6). There is a degradation of the limit of detection from skimmed to whole milk owing to the complexity of the sample matrix mainly considering the amount of fats.

Graphite tube lifetimes were 250 and 300 for Se and Fe, respectively. These are at least 5-fold better than those obtained without using the tertiary amines reagent.

The main advantage of the proposed procedure is its applicability for determination of Fe and Se in milk without any tedious procedure for sample preparation. Depending on the dilution factor, the dilution could be performed directly into the autosampler cup and this single vessel strategy could still decrease the possibility of contamination.

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