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Direct determination of Cu and Zn in fruit juices and bovine milk by thermospray flame furnace atomic absorption spectrometry

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Abstract

In the present work, thermospray flame furnace atomic absorption spectrometry (TS-FF-AAS) was employed for Cu and Zn determination in bovine milk and fruit juice samples without any pretreatment. TS-FF-AAS system was optimized and a sample volume of 300 μl was injected into the carrier stream ($0.014\,mol\,l^{-1}$ HNO_3 at a flow rate of $0.4\,ml\,min^{-1}$), and it was introduced into a hot Ni tube. The detection limits obtained for Cu and Zn in aqueous solution were 2.2 and $0.91\,\mu g\,l^{-1}$, respectively, and $3.2\,\mu g\,l^{-1}$ for Cu in a medium containing water-soluble tertiary amines. The relative standard deviations varied from 2.7 to 4.2% ($n = 12$). Sample preparation was carried out by simple dilution in water or in water-soluble tertiary amines medium. Accuracy was checked by performing addition-recovery experiments as well as by using reference materials (whole milk powder, non-fat milk powder, and infant formula). Recoveries varied from 97.7 to 105.3% for Cu and Zn. All results obtained for reference materials were in agreement with certified values at a 95% confidence level.

Keywords: FAAS; Flame furnace; Thermospray; Fruit juices; Milk; Cu and Zn

1. Introduction

Flame atomic absorption spectrometry (FAAS) is the most widely used technique for elemental determination [1], owing to its simple set up, low running costs, robustness, and good selectivity. For some applications FAAS is not suitable due to its relatively low sensitivity. This is mainly caused by the low efficiency of conventional pneumatic nebulizer and the short residence time of the sample in the flame [2]. The sensitivity of FAAS can be improved by increasing the efficiency of the aerosol generation/transport and by increasing the residence time of free analyte atoms in the absorption volume [3].

Gáspár and Berndt [2] proposed a technique namely thermospray flame furnace atomic absorption spectrometry (TS-FF-AAS) that achieves complete sample introduction and also increases the residence time ofthe sample in the flame. In this system, a Ni tube is placed on a standard burner head of a FAAS equipment. The sample to be nebulized is introduced into the tube via a ceramic capillary that is heated only by the flame of the spectrometer [1]. The end of the capillary reaches approximately 900 °C, which results in a very high temperature gradient along the capillary tip. The sensitivity for different elements is increased in up to one order of magnitude. This technique was only recently proposed and there are only few applications in the literature employing TS-FF-AAS.

Pereira-Filho et al. [4] investigated the use of TS-FF-AAS technique for determining Cd, Cu, and Pb in plant and animal tissues by slurry sample introduction. Simultaneous in situ sample digestion and analytes determination were performed in the thermospray flame furnace. The authors proposed this technique as a simple low-cost alternative to electrothermal atomization AAS in a graphite tube, which is commonly used for slurry analysis.

The application of TS-FF-AAS technique for direct analysis of slurry samples can help to improve two frequent difficulties in analytical procedures: quantification of metals

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at trace levels and time spent in sample preparation. These problems are frequently achieved in quality control of foods.

The determination of metals in foods and beverages is important due to their essential or toxic action. Copper and zinc are known to be essential nutrients, but both can be toxic depending on the concentration [5]. Zinc constitutes about 33 $\mu g\ g^{-1}$ of an adult body mass and it is essential as a constituent of many enzymes involved in several physiological functions, such as protein synthesis and energy metabolism [6]. An adult human body contains about 1.5–2.0 $\mu g\ g^{-1}$ of Cu [7], which is essential as a constituent of some metalloenzymes and is required in hemoglobin synthesis and in the catalysis of metabolic oxidation [8]. In this sense, considerable interest has been focused on Cu and Zn determination in foods and beverages [6].

Bovine milk and fruit juices are widely consumed due to flavor and nutritive value. Milk is a major source of nutrients for newborns and infants [9] and juices are largely consumed in all ages. Both products are good sources of vitamins and minerals. Copper and Zn concentrations in fruit juice and bovine milk can vary widely depending on source, manufacturing process, and raw material. Licata et al. [10] obtained Cu and Zn concentrations around 0.1–735 $\mu g\ kg^{-1}$ and 25–4960 $\mu g\ kg^{-1}$, respectively, when working with several whole raw bovine milk samples. In fruit juices, the concentration of Cu and Zn can vary from $\mu g\ kg^{-1}$ to $mg\ kg^{-1}$ range. This aspect makes difficult the employment of conventional FAAS for direct determination of these elements. Moreover, direct aspiration of the fruit juices and milk can cause flame fluctuations and accumulation of solid deposits on the burner head [11].

Traditional methods of sample preparation for infant formulas, milk powders, liquid milks, and juices are well documented and include wet digestion or dry ashing [12–14], microwave digestion [15–17], and lyophilisation followed by ashing [18].

Compared to conventional mineralisation procedures, slurry introduction offers inherent benefits such as simplicity and speedness, thus reducing sample manipulation, thereby minimizing losses or contamination [19–21].

It is important to develop fast and accurate procedures for determination of essential nutrients in foods. This paper reports the employ of TS-FF-AAS technique for direct determination of Cu and Zn in bovine milk and juice samples without any preliminary sample preparation except dilution.

2. Experimental

2.1. Instrumental

A Varian SpectrAA-640 flame atomic absorption spectrometer (Varian, Mulgrave, Australia) equipped with deuterium lamp background corrector was used. The instrumental spectrometer parameters for Cu and Zn determination were set according to the manufacturer recommendations. In all experiments an air–acetylene flame, flowing at 13.5 and 2.0 $l\ min^{-1}$, respectively, was used. Hollow cathode lamps of Cu and Zn (Varian) were used. Copper and Zn were measured at the wavelengths 324.7 and 213.9 nm, respectively.

2.2. Arrangement of the TS-FF-AAS system

The system consisted of a peristaltic pump with eight channels (Ismatec, Labortechnik Analytik, Glattbrugg-Zürich, Switzerland) furnished with Tygon tubes, a lab-made commutator injector, and a thermospray flame furnace unit, which included the ceramic capillary (0.5 mm i.d., 2.0 mm o.d., and 100 mm of length) and the Ni tube (10 cm) which is located on the burner by the lab-made stainless steel support with four ceramic pins fixed. The tube was laid on these pins and could be moved into and out of the flame. For properly reaching the atomization temperature for Cu and Zn inside the tube, six holes of 2 mm diameter were drilled in the bottom part of the tube. Another orifice was drilled at 90° to the bottom holes for inserting the thermospray capillary. The capillary tip was about 1 mm inside the tube furnace. The manifold was assembled with 0.5 mm i.d. PTFE tubing.

2.3. Reagents

All solutions were prepared using analytical-reagent grade and deionized water. All glassware and polypropylene flasks was washed with soap, soaked in 10% (v/v) nitric acid and rinsed with deionized water prior to use.

Reference solutions of Cu and Zn containing 100 $\mu g\ l^{-1}$ were prepared by diluting the standard stock solutions containing 1000 $mg\ l^{-1}$ (Tec-Lab, Hexis, São Paulo, Brazil) with deionized water.

A 0.014 $mol\ l^{-1}$ HNO_3 solution was prepared by diluting concentrated HNO_3 (Merck, Darmstadt, Germany).

Solutions containing 10 and 20% (v/v) water-soluble tertiary amines were prepared by diluting the stock solution (CFA-C, Spectrasol, Warwick, NY, USA). The pH was adjusted to 8.0 by adding HNO_3.

2.4. Samples

Different commercial juices (apple and peach mixed with soy-beans, cashew, citric fruits, orange/acerola, and peach) and whole milks were analyzed. These samples were bought in a local market.

Standard reference materials produced by National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA), whole milk powder (SRM 8435), non-fat milk powder (SRM 1549), and infant formula (SRM 1846) were used to check the accuracy of the method.

2.5. *Optimization of flow rates, sample volume, and measurement time*

A peristaltic pump was employed for propelling the solutions and slurries through the thermospray capillary. The effects caused by flow rate (0.25–0.65 ml min^{-1}) and sample volume (50–300 μl) on sensitivity were evaluated as well as the effect of measurement time (30–210 s) on sensitivity. Copper and Zn solutions containing 100 μg l^{-1} were used in these studies.

2.6. *General procedure*

The established conditions were utilized for determining Cu and Zn in reference materials and samples. Masses of SRM milk powder from 20 to 400 mg were weighed in 15 ml polypropylene flasks and they were diluted to 10 ml with CFA-C 10% (v/v) at pH 8 for Cu determination and with water for Zn determination.

For commercial milk samples, an 1:1 (v/v) dilution was performed for Cu determination using CFA-C 20% (v/v) pH 8. For Zn, a volume of 200 μl of sample was diluted by adding 9.8 ml of deionized water. Analysis of juices was performed after diluting 1:10 or 1:5 (v/v) with deionized water for Cu and Zn determination, depending on the type of juice.

The slurry was pumped at a flow rate of 1.2 ml min^{-1} through the 300 μl sample loop. After the commutation, the sample was injected into the carrier stream (0.014 mol l^{-1} HNO_3 at a flow rate of 0.4 ml min^{-1}) being transported towards the thermospray capillary.

Calibrations were performed using reference solutions. Delay and measurement times were 30 and 60 s, respectively.

Samples were spiked with different concentrations of Cu and Zn for evaluating recoveries at the set experimental conditions, and all transient signals were measured in peak area mode.

3. Results and discussion

3.1. *Optimization of flow rate, sample volume, and measurement time*

The influence of the flow rate used for introducing the solution into the hot Ni tube on sensitivity was studied in the 0.25–0.65 ml min^{-1} range using 100 μg l^{-1} of Cu and Zn solutions. The use of higher flow rates were not feasible because when flow rates higher than 0.65 ml min^{-1} were employed the residence time of the flowing liquid in the heated tip of the capillary was too short for evaporation [2]. Results obtained are shown in Fig. 1. Similar behavior was observed for both elements, however transient signals for Zn were greater than those observed for Cu due to its higher volatilization. This system is more effective for elements with low volatilization temperature, because the temperature inside the tube is approximately 900 °C [2]. A hotter flame (N_2O/C_2H_2) was not used due to the melting point of the Ni tube. By increasing the flow rate from 0.40 to 0.65 ml min^{-1}, a decrease of 19 and 65% for Cu and Zn signals, respectively, were observed. In conventional thermospray systems higher sample flow rates provide higher signals because the larger sample amount introduced into the atomizer [22]. According to Pereira-Filho et al. [4] in TS-FF-AAS system a smaller flow rate provides higher sensitivity, because higher flow rates decreased the temperatures inside the tube furnace. On the other hand, at too low flow rates, the signals can be reduced due to the erratic and uncontrolled sample vaporization. Additionally, if higher flow rate is used the measurement time would considerably increase, thus causing a reduction of the analytical throughput [2].

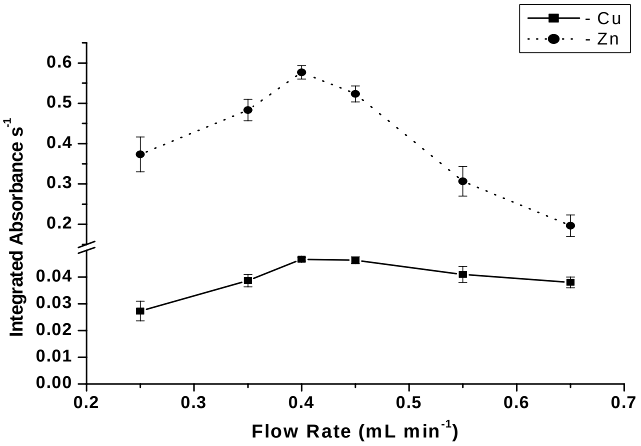

Fig. 1. Effect of the flow rate on Cu and Zn transient signals. Sample volume: 200 μl; 100 μg l^{-1} Cu and Zn.

The sample volume was varied from 50 to 300 μl range and the results obtained are shown in Fig. 2. Peak area is proportional to the sample volume because if a larger sample volume is introduced into the tube flame furnace, the atom population in the absorption volume is greater.

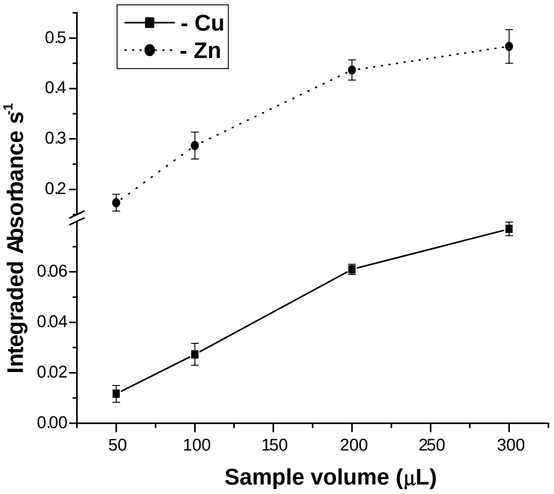

Fig. 2. Effect of sample volume on Cu and Zn transient signals. Flow rate: 0.4 ml min^{-1}; 100 μg l^{-1} Cu and Zn.

Table 1
Analytical characteristics of the Cu and Zn measurements by TS-FF-AAS

Parameter	Cu aqueous	Cu in CFA-C	Zn aqueous
Regression equation	$y = 0.0064x - 0.0352$	$y = 0.0041x - 0.0079$	$y = 0.1685x + 1.0606$
R^2	0.9997	0.9996	0.9963
Linear range ($\mu g\,l^{-1}$)	Up to 400	Up to 400	Up to 200
LOD ($\mu g\,l^{-1}$)	2.2	3.2	0.91
LOQ ($\mu g\,l^{-1}$)	7.3	10.7	3.0
Precision (%)[a]	3.5	4.2	2.7

[a] $n = 12$.

The measurement time was determined for a transient signal obtained using 300 μl of a solution containing 100 $\mu g\,l^{-1}$ Zn and it was around 210 s. The greatest transient signal is reached between 30 and 90 s. The time necessary to the solution reaches the flame furnace is 30 s. Higher measurement times affect negatively the analytical throughput. Delay and measurement times were fixed at 30 and 60 s, respectively. For avoiding memory effects, the system was cleaned by introducing 1% (v/v) H_2O_2 solution after each measurement. The use of this solution avoids the gradual accumulation of carbon and sugar residues in the ceramic capillary tip. This accumulation was only observed when measuring milk and juice samples. The sampling frequency was 15 h^{-1}; however, it should be emphasized that milk and juice samples were introduced by simple dilution of the samples without any digestion step. Consequently, the analytical frequency can be seen as the actual analytical throughput.

3.2. Figures of merit

The figures of merit for Cu and Zn can be seen in Table 1. Calibration curves were obtained with aqueous reference solutions for both analytes. A water-soluble tertiary amines medium (CFA-C, pH 8) was also used for Cu. The calibration graphs were linear up to 400 and 200 $\mu g\,l^{-1}$ for Cu and Zn, respectively. The limit of detection (LOD) was defined as the concentration corresponding to three times the standard deviation of 10 measurements of the blank divided by the slope of the analytical curve [23].

The LOD's attained using TS-FF-AAS were lower than those attained by conventional FAAS (75.7 and 11.1 $\mu g\,l^{-1}$ for Cu and Zn, respectively). Taking into account the LOD's established, the ratios Cu/Zn are 2.4 and 6.8 in TS-FF-AAS and FAAS, respectively. This is in disagreement with the expected behavior since TS-FF-AAS should lead to better improvement for more volatile elements. However, the measurements of LOD's are critically affected by blank fluctuations and experimentally, it was observed that these were more critical for Zn due to the lower wavelength (213.9 nm) when compared to Cu (324.7 nm). The repeatability for 12 consecutive signals for aqueous solution containing 50 $\mu g\,l^{-1}$ of Cu and Zn were 3.5 and 2.7%, respectively, and 4.2% for Cu in CFA-C medium.

3.3. Determination of Cu and Zn in milk and juice samples

External calibration with aqueous reference solutions was employed. This strategy can be adopted when solutions and slurries present as similar as possible transport and atomization efficiencies. For Cu determination in milk samples a CFA-C solution was used as diluent. This strategy was important for avoiding fast accumulation of carbon residues in the capillary tip without requiring excessive dilution of the samples. Milk samples must be diluted in alkaline or neutral medium to prevent protein precipitation. The chemical form of the analyte in the matrix may be critical for FAAS analysis. Depending on the chemical form, a variation in the atomization degree is expected [19]. The reagent CFA-C seems to promote total or partial dissociation of casein micelles, causing an increase in the metal concentration in the aqueous phase and minimizing matrix effects [19,21]. Copper and Zn are mainly bound to casein micelles in bovine

Table 2
Copper and Zn in juice and milk samples: addition-recovery experiments

Sample	Cu ($\mu g\,l^{-1}$)			Zn ($\mu g\,l^{-1}$)		
	Added	Found	Recovery (%)	Added	Found	Recovery (%)
Whole milk A	50	49.1	98.2	50	49.8	99.6
Whole milk B	80	80.8	101.0	30	30.5	101.6
Citric fruits juice	30	29.3	97.7	70	72.5	103.6
Orange/acerola juice	80	81.0	101.3	20	20.8	104.0
Peach juice	50	50.6	101.2	40	40.3	100.8
Cashew juice	70	71.4	102.0	60	63.2	105.3
Apple juice with soy-bean	100	99.1	99.1	80	83.5	104.4
Peach juice with soy-bean	40	40.9	102.3	50	51.4	102.8

Table 3

Copper and Zn determination in standard reference materials, juice and milk samples by TS-FF-AAS

Sample	Cu (μg l^{-1})	Zn (μg l^{-1})
Whole milk A	161.7 ± 6.5	3.92 ± 0.22[a]
Whole milk B	153.1 ± 2.4	3.69 ± 0.03[a]
Citric fruits juice	90.0 ± 7.3	101.6 ± 4.0
Acerola/orange juice	117.1 ± 6.5	84.2 ± 2.5
Peach juice	245.0 ± 11.8	904.4 ± 37.1
Cashew juice	85.3 ± 4.9	68.5 ± 2.1
Apple juice with soy-bean	335.7 ± 16.7	691.4 ± 17.9
Peach juice with soy-bean	384.3 ± 17.8	1097.3 ± 25.2
Whole milk powder (SRM 8345)[b]	0.48 ± 0.02[c]	29.9 ± 0.6c[c]
Non-fat milk powder (SRM 1549)[d]	0.71 ± 0.08[c]	44.5 ± 0.7[c]
Infant formulae (SRM 1846)[e]	4.97 ± 0.23[c]	61.3 ± 1.6[c]

[a] Concentrations in μg ml^{-1}.

[b] Certified values: Cu, 0.46 ± 0.08 μg g^{-1} and Zn, 28.0 ± 3.1 μg g^{-1}.

[c] Concentrations in μg g^{-1}.

[d] Certified values: Cu, 0.7 ± 0.1 μg g^{-1} and Zn, 46.1 ± 2.2 μg g^{-1}.

[e] Certified values: Cu, 5.04 ± 0.27 μg g^{-1} and Zn, 60.0 ± 3.2 μg g^{-1}.

milk [24]. The dilution of milk with CFA-C for determining several analytes by ICP OES was previously recommended [19].

Since Zn is found in greater concentrations, further sample dilution can be performed when this analyte is determined. In this case, CFA-C was not necessary for Zn determination due to extensive dilution.

3.3.1. Recovery studies

Recoveries for Cu and Zn in different samples were evaluated by adding different concentrations of both elements to commercial samples of milk and fruit juices. Results are given in Table 2 and recoveries varied from 97.7 to 105.3% for both analytes. Sample dilutions were varied from 1:1 to 1:50 (v/v) and they were dependent on the analytes concentration. The 1:50 (v/v) dilution was only adopted for Zn determination in milk. In general, no matrix effects were observed for Cu and Zn even for 1:1 (v/v) dilution. However, interference effects were observed when the samples were introduced without any dilution. However, in order to avoid losses in sensitivity when Cu was determined in milk samples, which were diluted 1:1 (v/v), the use of CFA-C was required to guarantee quantitative recoveries. It could be supposed that these elements could be directly determined in these samples by FAAS since it seems that this technique presents enough sensitivity. However, despite proper sensitivity for some samples, it is not normally possible to perform these measurements without any sample pre-treatment and thus, even a simple dilution deteriorates the detection power.

3.3.2. Sample analysis

The results obtained for three standard reference materials (whole milk, non-fat milk, and infant formula) and eight commercial samples (two samples of whole milk and six juices) are given in Table 3. According to a *t*-test, all determined and certified values are in agreement at a 95% confidence level.

Thermospray flame furnace AAS technique presented satisfactory results for Cu and Zn determination in bovine milk and fruit juices. This technique presents better sensitivity than flame-AAS. This improvement in sensitivity is probably caused by two main features: the increase of the residence time of the atomic cloud in the absorption volume and the total sample introduction of the thermal aerosol [1].

No reagent was added for determining Zn in all samples and Cu in juice samples. Water-soluble tertiary amines solution was used for preparation of milk samples for Cu determination.

In the proposed system, the flame penetrates into the Ni tube and when the slurry sample reaches the furnace tube, the solid particles of the sample are destroyed by a hot and oxidizing atmosphere and the analytes are atomized.

Copper and Zn concentrations were similar in two commercial milk samples. For commercial juices, the analytes concentration varied from 85.3 to 384.4 μg l^{-1} for Cu and from 68.5 to 1097.3 μg l^{-1} for Zn. It can be seen in Table 3 that Cu and Zn concentrations are greater to those juices containing soybeans in their composition. As it can be seen there is a pronounced variation of Cu and Zn concentrations for different samples and determination by FAAS is not feasible for some of them because the sensitivity could not be enough and normally, it is not possible to introduce fruit juice and milk samples without any dilution.

4. Conclusions

Copper and Zn were determined in bovine milk and fruit juice samples by TS-FF-AAS technique after a simple dilution with deionized water or CFA-C 10% (v/v). No pretreatment was required, thus minimizing the time and reagents consumed in the analysis. Copper and Zn determination in these samples was feasible due to the low detection limits obtained and the reduced dilution of samples. The detection power is better than that reached by FAAS and lower than that obtained by ETAAS, however, TS-FF-AAS presents lower cost than this latter technique and can be as easily implemented as FAAS. Additionally, TS-FF-AAS can expand the applicability of the widely available FAAS instruments.

External calibration with aqueous reference solutions was successfully used indicating the absence of matrix effects. The proposed procedure is simple and results can be rapidly obtained for quality control in food industries.

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