

# Determination of Calcium, Potassium, Magnesium, Iron, Copper and Zinc in Maternal Milk by Inductively Coupled Plasma Atomic Emission Spectrometry\*

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A simple and rapid method was developed for the determination of Ca, P, Mg, Fe, Cu and Zn in maternal milk samples by inductively coupled plasma atomic emission spectrometry. Milk samples were emulsified with ethoxy nonylphenol to 0.03% m/v. The emulsified samples were diluted 10-fold with nitric acid and to 1% v/v. Aqueous solutions with the same amount of emulsifier and acid were used as calibration standards. The accuracy of the proposed method was assessed using the National Institute of Standards and Technology Standard Reference Material 1549 Non-Fat Milk Powder. No significant difference at the 95% confidence level was observed. The precision of the method when applied to real samples was in the range 0.3–2% expressed as %RSD, with no internal standard. Maternal milk samples from 50 women residing in Caracas, Venezuela, collected at three different lactation stages (on the third, seventh and twenty-first days postpartum) were analysed.

**Keywords:** Inductively coupled plasma atomic emission spectrometry; human milk; trace element determination

Human milk has long been regarded as the optimal source of essential nutrients for the young infant, if the maternal diet is nutritionally adequate and a sufficient amount is consumed. Accurate data on the concentration of trace elements in human milk throughout lactation are important both for formulating nutritional requirements and for obtaining baseline levels leading to an understanding of the physiology of milk secretion.<sup>1</sup> For this reason, sensitive, rapid and precise methods for the determination of trace elements in human milk are necessary.

Inductively coupled plasma mass spectrometry (ICP-MS) and ICP atomic emission spectrometry (AES) have been used in the past for the determination of trace elements in milk.<sup>2–9</sup> Both techniques have analytical characteristics that are applicable in the routine determination of elements in milk, *i.e.*, low detection limits, long linear working ranges and the capability of simultaneous multi-element determinations.

Different sample treatments, such as dry and wet digestion and decomposition with microwave heating in open and closed vessels have been used. Their efficiency is based on the time required and the completeness of the decomposition. Emmett<sup>2</sup> reported trace metal determination in liquid and powdered milk by ICP-MS. Samples were digested by a wet acid procedure and the results obtained compared well with the reference values. The decomposition method was lengthy. Dry ashing<sup>3–5</sup> and wet acid digestion<sup>6,7</sup> procedures were also used for the determination of trace metals in liquid and powdered milk by ICP-AES.

Human milk is a complex colloidal system that can be difficult to dissolve completely. Krushevska *et al.*<sup>8</sup> carried out a comparison of several dry ashing and wet digestion procedures for the determination of Zn in milk samples using ICP-AES. They reported that the wet dissolution procedure with a hot-plate is time-consuming and prone to contamination due to the large amounts of reagent required. The use of microwave systems increased the speed of sample dissolution compared with classical digestion, but did not completely eliminate the requirement for chemical reagents. High-pressure digestion with a high-temperature programme destroys the organic carbon almost completely with only HNO<sub>3</sub>. Zinc losses were observed at high temperatures when a dry ashing pro-

cedure, was used, without an ashing aid, especially in the presence of chlorides.

Direct dilution of sample is simple, it can be automated and is less time-consuming than alternative procedures, such those mentioned above. Durrant and Ward<sup>9</sup> determined 18 elements in milk samples by ICP-MS. The samples were simply diluted to 2% v/v in aqueous acidic solution, but the accuracy of the method is not very clear. Emmett<sup>2</sup> in an attempt to directly analyse milk samples diluted with water, reported a poor accuracy that was attributed to the fatty nature of the milk. Coni *et al.*<sup>5</sup> also tried direct aspiration of the liquid milk samples into an ICP torch. They obtained an analytical signal lower (as much as one third) than those obtained by aspiration of the digested milk sample. This was associated with the rather large average droplets size characteristic of the un-treated milk and the incomplete atomization of components during residence in the plasma. They suggested that this method of determination is impracticable, due to the difference in viscosity and surface tension values between real samples and aqueous calibration solutions.

It has been reported<sup>10–13</sup> when using flame atomic absorption spectrometry that the addition of surfactants to aqueous sample solutions improves the analyte sensitivity. It reduces the average droplet size in aerosols produced by pneumatic nebulizers due to a depression of the surface tension. Nevertheless, Bertagnolli *et al.*<sup>14</sup> have reported that the use of surfactants in ICP, does not improve sensitivity. They have mentioned that only the sample transport process is better, thereby allowing a more stable plasma when using high sample uptake rates. One of the useful uses of surfactants in atomic spectrometry has been the emulsification of oil samples in water, for the direct introduction of emulsions in both flame<sup>15–18</sup> and ICP<sup>19–22</sup> atomizers. The finely divided particles are uniformly dispersed in the water phase and the sample behaves like an aqueous solution. In this way, the use of emulsions without prior destruction of the organic matrix has become an alternative method for sample introduction in atomic spectrometry.

The aim of this investigation was to develop a direct, rapid and simple method for the determination of Ca, P, Mg, Fe, Cu and Zn in human milk by ICP-AES by direct aspiration of emulsified samples. The accuracy was assessed by analysing the National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 1549 Non-Fat Milk Powder.

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## Experimental

### Apparatus

A Jobin-Yvon Model JY24 inductively coupled plasma spectrometer was used. Experimental parameters are presented in Table 1.

### Reagents and Materials

All reagents were of the highest purity available. Milli-Q de-ionized water (Millipore) was used throughout the work. Nitric acid was Merck Suprapure grade. Ethoxy nonylphenol was obtained from Etoxil of Venezuela. Standard solutions were prepared from 1000  $\mu\text{g ml}^{-1}$  stock solutions (BDH) of each element. A 100  $\mu\text{g ml}^{-1}$  phosphorus standard stock solution was obtained by dissolving  $\text{K}_2\text{HPO}_4$  (BDH) in high-purity water.

### Cleaning of the plastic- and glass-ware

All laboratory glass-ware and polystyrene plastic-ware containers were acid-washed overnight in 3% v/v nitric acid and rinsed repeatedly with de-ionized water before drying at 60 °C. Once dried, all plastic ware was heat-sealed in clean plastic bags to prevent further contamination.

### Samples

A total of 50 lactating mothers provided milk samples for this investigation. All were residents of the city of Caracas (Venezuela). Maternal milk samples were collected at three

**Table 1** Instrumental parameters

ICP atomic emission spectrometer	Jobin Yvon Model JY-24
Nebulizer	Meinhard C-type
Spray chamber	Scott-type
Forward power/W	1000
Sample delivery	Peristaltic pump (Sorvall, type 49061)
Coolant flow rate/ $\text{l min}^{-1}$	12
Nebulizer flow rate/ $\text{l min}^{-1}$	0.7
Sample flow rate/ $\text{l min}^{-1}$	0.5
Observation height	15 mm above load coil
Analytical lines/nm	Ca II 393.66 Cu II 324.754 Fe II 239.562 Mg II 279.553 P I 213.618 Zn II 213.856

**Table 2** Analysis of SRM materials by direct ICP-AES methods

Element	Concentration	Certified	Found
Zn	$\mu\text{g g}^{-1}$	$46.1 \pm 2.2$	$45.0 \pm 0.7$
Cu	$\mu\text{g g}^{-1}$	$0.7 \pm 0.1$	ND*
Fe	$\mu\text{g g}^{-1}$	$1.78 \pm 0.02$	$1.72 \pm 0.12$
Ca	% m/m	$1.30 \pm 0.05$	$1.29 \pm 0.03$
Mg	% m/m	$0.120 \pm 0.003$	$0.118 \pm 0.005$
P	% m/m	$1.06 \pm 0.02$	$1.05 \pm 0.03$

\* ND = Not detected.

**Table 3** Concentration ranges of elements in maternal milk collected 3, 7 and 21 days postpartum

Day	Concentration/ $\mu\text{g ml}^{-1}$					
	Ca	Mg	Fe	Cu	Zn	P
3	107–388	20.8–47.6	0.22–0.74	0.30–0.78	2.6–11.6	35–169
7	192–393	20.2–39	0.22–0.57	0.32–0.72	2.0–5.75	136–278
21	144–330	19.8–32.5	0.22–0.55	0.32–0.65	1.01–4.3	105–234

different lactation stages (on the third, seventh and twenty-first days postpartum). Samples were collected in the early morning into previously prepared, acid-washed, 20 ml containers from INDEMEDICA. Ethoxy nonylphenol aqueous solution (25% m/v) was added as an emulsifying agent (final concentration 0.30% m/v). The samples were stored at  $-15^\circ\text{C}$  prior to analysis.

### Procedure

The maternal emulsified milk samples were diluted 10-fold with nitric acid to 1% v/v (final concentration 0.030% m/v ethoxy nonylphenol and 1% v/v nitric acid). Aqueous standard solutions for calibration purposes were prepared using identical acid and ethoxy nonylphenol concentrations as for the samples.

The milk reference material (SRM 1549 Non-Fat Milk Powder) was reconstituted by suspending 2 g of the dried material in 25 ml of 0.03% ethoxy nonylphenol solution and then homogenized by ultrasonic agitation. The iron concentration was determined in this solution. For the determination of Zn however, this solution was diluted 8-fold, and 40-fold for Ca, Mg and P. No agglomeration of solid particles was observed under these conditions.

## Results and Discussion

### Optimization of Analytical Conditions

Under ideal conditions, freezing is the best long-term preservation method. However, during freezing, some irreversible processes may take place because of denaturation of proteins and re-distribution of elements due to rupture of cell walls by ice crystals. An oily layer on the top of the liquid milk is produced which cannot be re-dispersed by mechanical procedures such as stirring, shaking or ultrasonic treatment.

Ethoxy nonylphenol is a non-ionic surfactant that is completely soluble in water. It is not affected by the presence of Ca, Mg and Fe salts.<sup>23</sup> This surfactant has been successfully used by Murillo *et al.*, to emulsify lubricating oils<sup>21</sup> and crude oil<sup>22</sup> samples for the determination of metals by ICP-AES.

The concentration of surfactant was evaluated to obtain the most stable milk emulsion. For this, the chosen criterion was the re-dispersion of the oil layer into the whole milk sample so as to produce a homogeneous and stable emulsion. The homogenization of the emulsion was visually evaluated. The ethoxy nonylphenol concentration was varied from 0 to 0.53% m/v. The optimum emulsifier concentration selected was 0.3%; concentration greater than 0.3% m/v did not improve the emulsion stability. At this optimum concentration, stable emulsions are formed which can be kept under refrigeration for a long time. Reconstruction of such samples is carried out by manual shaking. The emulsified sample was diluted 10-fold with nitric acid prior to nebulization (final concentration 0.030% m/v ethoxy nonylphenol and 1% v/v nitric acid). Reproducibility of the analyte signal was checked using samples that had not been frozen.

In order to obtain representative portions of the sample, it was better to add the emulsifier before sampling. By adding ethoxy nonylphenol to the samples, several limitations associated with milk fat separation during sample storage under frozen conditions could be avoided. Also, ethoxy nonylphenol enabled direct sample introduction without blockage of the

nebulizer. The introduction of emulsions has improved transport and atomization efficiency of milk sample in ICP-AES. This may be associated with the reduction of the average size of the droplets.<sup>24</sup> The technique also overcomes the problems associated with viscosity and surface tension differences between real samples and aqueous calibration solutions.<sup>5</sup>

#### Detection Limit

The detection limits were calculated based on the Kayser definition<sup>25</sup> with a signal-to-noise ratio of 3. The detection limits obtained were 0.05, 0.0089, 0.0079, 0.023, 0.023 and 0.055  $\mu\text{g ml}^{-1}$  for Zn, Cu, Fe, Ca, Mg and P, respectively.

#### Analysis of an SRM by the Proposed Method

To assess the accuracy of the proposed method, the NIST SRM 1549 Non-Fat Milk powder was analysed. The results obtained are presented in Table 2. The significance test<sup>26</sup> indicated no significant difference at the 95% confidence level between the observed and certified values for the elements determined.

#### Analysis of Maternal Milk Samples

The proposed method was applied to the determination of Ca, Cu, Fe, Mg, P and Zn in 50 maternal milk samples. The concentration ranges found are presented in Table 3. The precision was found to be satisfactory (between 0.3 and 2%). It can be seen that, element concentration is higher in the early period of lactation, namely colostrum (third day) and in transitory milk (seventh day postpartum). Similar results have been found by other workers.<sup>27,28</sup>

#### Conclusions

The method described, enables the rapid and direct determination of Ca, Mg, Fe, Cu, Zn and P in whole maternal milk and similar materials by ICP-AES.

The addition of ethoxy nonylphenol to milk samples bypasses several limitations associated with milk fat separation during storage of frozen samples. This surfactant also facilitates direct sample introduction without nebulizer blockage. Aqueous standard solutions, prepared under identical acid and surfactant concentrations as the samples, were adequate for calibration purposes and complete recovery was obtained. For real samples, reproducibility of the proposed method (as %RSD) varied from 0.3 to 2%. Good accuracy was obtained with no internal standard.

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#### References

- Barnes, L. A., Mauer, A. M., Anderson, A. S., Dallman, P. R., Forbes, E. B., Nichols, B. L., Roy, C., Smith, N. J., Walker, W. A., and Winick, M., *J. Pediatr. (St. Louis)*, 1978, **2**, 591.
- Emmett, S. E., *J. Anal. At. Spectrom.*, 1988, **3**, 1145.
- Feely, R. M., Eitenmiller, R. R., Jones, J. B., and Barnhart, H., *J. Pediatr. Gastroenterol. Nutr.*, 1983, **2**, 262.
- Feely, R. M., Eitenmiller, R. R., Jones, J. B., and Barnhart, H., *J. Clin. Nutr.*, 1983, **37**, 443.
- Coni, E., Stacchini, A., Caroli, S., and Falconieri, P., *J. Anal. At. Spectrom.*, 1990, **5**, 581.
- Suzuki, K. T., Tamagawa, H., Hirano, S., Kobayashi, E., Takahashi, K., and Shimojo, N., *Biol. Trace Elem. Res.*, 1991, **28**, 109.
- Li, J., Yoshinaga, J., Suzuki, T., Abe, M., and Morita, M., *J. Nutr. Sci. Vitaminol.*, 1990, **36**, 65.
- Krushevska, A., Barnes, R. M., Amarasiriwardena, C. J., Foner, H., and Martines, L., *J. Anal. At. Spectrom.*, 1992, **7**, 851.
- Durrant, S. F., and Ward, N. I., *J. Micronutrient Anal.*, 1989, **5**, 111.
- Kodama, M., and Miyagawa, S., *Anal. Chem.*, 1980, **52**, 2358.
- Yan, Z.-y., and Zhang, W., *J. Anal. At. Spectrom.*, 1989, **4**, 797.
- Mora, J., Canals, A., and Hernandis, V., *J. Anal. At. Spectrom.*, 1991, **6**, 139.
- Ruiz, A. I., Canals, A., and Hernandis, V., *J. Anal. At. Spectrom.*, 1993, **8**, 109.
- Bertagnolli, J. A., Neylan, D., and Hammargren, D. D., *At. Spectrosc.*, 1993, **14**, 1.
- Berenguer, V., and Hernández, J., *Quím. Anal.*, 1977, **31**, 81.
- Berenguer, V., Guinon, J. L., and De la Guardia, M., *Anal. Chem.*, 1979, **294**, 416.
- Polo-Diez, L., Hernandez-Mendez, J., and Pedraz-Penalva, F., *Analyst*, 1980, **105**, 37.
- De la Guardia, M., Salvador, A., and Berenguer, V., *Analisis*, 1980, **8**, 488.
- Lord, C., *Anal. Chem.*, 1991, **63**, 1594.
- Borszeki, J., Knapp, G., Halmos, P., and Bartha, L., *Mikrochim. Acta*, 1992, **108**, 157.
- Murillo, M., Gonzalez, A., Ramirez, A., and Guillén, N., *At. Spectrosc.*, in the press.
- Murillo, M., and Chirinos, J., paper presented at the XXVIII Colloquium Spectroscopicum Internationale (CSI), York, UK, June 29–July 4, 1993.
- Martinez, A. A., *Introducción a la Química de Superficies y Coloides*, Alhambra, S.A. Madrid, 1977.
- Becher, P., *Emulsions: Theory and Practice*, Reinhold, New York, 2nd edn., 1965, ch. 6 and 7.
- Liteanu, C., and Rica, I., *Statistical Theory and Methodology of Trace Analysis*, Wiley, New York, 1980, p. 255.
- Miller, J. C., and Miller, J. N., *Statistics for Analytical Chemistry*, Wiley, New York, 1985, pp. 52 and 82.
- Casey, C. E., Hambidge, K. M., and Neville, M., *Am. J. Clin. Nutr.*, 1985, **41**, 1193.
- Casey, C. E., Neville, M., and Hambidge, K. M., *Am. J. Clin. Nutr.*, 1989, **49**, 773.

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