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Multielement Determination in Small Samples of Human Milk by Inductively Coupled Plasma Atomic Emission Spectrometry

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ABSTRACT

Inductively coupled plasma atomic emission spectroscopy (ICP-AES) was used for routine analysis of small samples of human milk. The concentrations of calcium (Ca), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), phosphorus (P), and zinc (Zn) were determined in 203 milk samples from postpartum women at different stages of lactation after stepwise digestion in HNO_3, $HClO_4$, and H_2O_2 under heat. Validation of the procedure was achieved using certified reference material of bovine liver (NBS 1577) with mean recoveries of 103.5%. The concentrations of the above elements in milk matrix were comparable with previously reported values. The analytical results from breast milk will provide reference information for mineral studies of Brazilian mothers and breast-fed infants.

Index Entries: ICP; human milk; Ca; Cu; P; Mg; Mn; Fe; Zn.

INTRODUCTION

Inductively coupled plasma, either as atomic emission spectrometry (ICP-AES) or mass spectrometry (ICP-MS), is an extremely useful tool in mineral determination. Its advantages over other methods are principally

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owing to low detection limits and simultaneous determination of several elements. This last characteristic is fundamental for many areas of research, notably nutrition and toxicology *(1)*.

In nutritional sciences and toxicology, the study of one element by itself is not enough to understand interactions among minerals. Using the more common method of atomic absorption spectroscopy, the study of more than one element requires individual samples for each reading, which can be limited by the availability of the analyte. In studies of mineral metabolism, sample size for multielement determination in human breast milk becomes a serious limitation *(2)*.

Although ICP has been used before for determinations of several elements in milk of different species *(2–7)*, its application to studies of human milk, so far, has been limited. In multielement determination of human milk, researchers used large or unspecified volumes *(2,6,8)*, which are not suitable for mineral metabolism research. The sampling method poses limitations regarding quantity of the milk collected, and the results are not sufficiently specific to be used in scientific research. Although large volumes are sometimes unfeasible to collect when dealing with exclusively breast-fed infants of poor mothers, pooled or unspecified volumes are not suitable for most nutritional and metabolic studies.

The objective of this study was to apply ICP-AES for multielement determination of calcium (Ca), copper (Cu), phosphorus (P), magnesium (Mg), manganese (Mn), iron (Fe), and zinc (Zn) in human milk.

MATERIALS AND METHODS

The human milk samples were from our ongoing research projects dealing with nutrient changes in human milk as affected by constitutional and environmental variables. The lactating mothers were all from the city of Brasilia and were recruited as volunteers who donated milk samples after informed consent. The milk samples (2–10 mL) were collected by manual expression. Preventive measures to avoid contamination were secured by cleaning the nipples with distilled and deionized water, and by using properly cleaned glassware. All glassware was rendered clean by washing in acid and rinsing with EDTA solution followed by distilled and deionized water, as routinely done in our laboratory. After collection, samples were divided in aliquots and stored at –20°C until analysis.

Before analysis, samples were thawed and thoroughly homogenized in a vortex. An aliquot of 2 mL of milk was accurately weighed to the nearest decimal point with an analytical scale, and mixed with 7 mL of HNO_3 (Suprapur, Merck, Darmstadt, Germany). The mixture was heated at 110°C until it reached a volume of approx 1 mL. After cooling, 2 mL of concentrated $HClO_4$ (Suprapur, Merck) were added to the digests and again heated until the liquid turned to a dark-brown color. The digests

were cooled, and 2 mL of H_2O_2 (30%) (Suprapur, Merck) were added. The mixture was heated to 70°C until the digests turned clear and had a volume of approx 1 mL. After cooling, the digests were quantitatively transferred to a calibrated test tube and brought to a volume of 10 mL with double-distilled and deionized water.

For the determination of the chosen elements (Ca, Cu, Fe, Mg, Mn, P, and Zn), an ICP Spectroflame model FVM03 with a focal distance of 75 cm (Kleve, Germany) was used. A concentric Meihard (Kleve, Germany) nebulizer was operating under the following conditions: pressure, 38 psi; flux of cooling gas, 13 L/min; flux of auxiliary gas, 0.5 L/min; sample injector flow, 1 mL/min, and forward power of 1.1 kW. The emission lines of the studied elements were as follows (in nm): Ca (317.93), Cu (324.75), Fe (259.94), Mg (269.87), Mn (257.61), P (214.91), and Zn (213.85).

Multiple element standards were prepared from standard solutions for atomic absorption spectroscopy (Merck) after appropriate dilution of the chosen elements. Standard Reference Material 1577 Bovine Liver (National Institute of Standards and Technology, Washington, DC) was used for quality assurance using an identical procedure on 0.25 g of sample. All samples were processed in duplicate, and the final value was taken as the mean of running each sample three times.

RESULTS AND DISCUSSION

The accuracy of the technique here assessed is shown in Table 1. Overall mean recovery was 103.5 % (SD 11). The analytical results for human milk are shown in Table 2, and the distribution of the concentrations of chosen minerals is presented in Fig. 1.

The routine analyses of human milk in nutritional and/or toxicological studies sometimes require several samples, and under certain circumstances, only a small volume is available for determination of a number of nutrients, including minerals. Therefore, analytical techniques that allow simultaneous determination of more than one mineral are of great importance. On the other hand, ICP-AES also shows high sensitivity, accuracy, low matrix effect, and simple operation *(1)*. With regard to the chosen minerals, the study of multielemental concentrations in milk matrices has been done only experimentally, i.e., in few or in pooled milk samples *(2,4–8)*. Data on multielement determinations with individual samples of human milk have only been obtained with instrumental neutron activation analysis *(9)*.

The mineral concentrations in human milk are not uniform, and have been found to vary with postpartum age and environmental variables (socioeconomic status, season of the year, country, and so on). Some of the elements studied here were found to be influenced by environmental variables, such as socioeconomic status (Ca, Fe, Zn) or postpar-

Table 1
Quality-Control Determination of a Number of Elements in Certified Material (NBS 1577) (μg/g)

Element	P	Ca	Mg	Fe	Cu	Zn	Mn
Limit of detection	1.2	0.12	0.094	0.151	0.012	0.057	0.004
Mean	12063	44	545	262	185	152	10.7
SD	753	19	29	17	13	11	0.6
CV %	6.2	13	5.2	6.7	7.3	7.4	5.9
Certified value							
mean	-	123*	605*	270	193	130	10.3
SD	-	-	-	20	10	10	1.0
Recovery, (%)		117	90	97	96	117	104
Number of samples	12	15	15	15	15	15	15

*Not certified.

Table 2
Summary of Determination of Mineral Concentration in Human Milk (μg/g)

Element	P	Ca	Mg	Fe	Cu	Zn	Mn
Number of samples	203	203	203	203	203	203	171*
Mean	123.8	238.4	26.5	0.56	0.26	1.75	0.0126
SD	25.0	48.1	5.3	0.50	0.12	0.93	0.0089
CV, %	20.2	20.2	20.1	89.3	44.7	53.3	70.5
Median	122.4	237.3	26.2	0.42	0.25	1.60	0.0104
Minimum	50.7	127.6	15.5	0.10	0.05	0.27	0.0043
Maximum	204.9	405.0	43.4	3.52	0.94	6.04	0.0708

*Number of samples above detection limit.

tum age (Zn, Cu), whereas others (Ca, Mg) are more stable and would be affected only in late lactation *(2,9,10)*.

The comparison of the current results with previous studies that focused on multielemental determination of minerals and used standard reference material for quality control of the technique shows that only two studies met such requirements: the WHO *(9)* study done with milk from six countries (Guatemala, Hungary, Nigeria, Philippines, Sweden, Zaire), and the work of Durrand and Ward *(2)* from England.

Although the WHO *(9)* study used several techniques, including instrumental neutron activation analysis, the work of Durrand and Ward *(2)* used ICP-MS to determine 18 elements in pooled samples (0.5 L) of human milk.

Although the results of those studies, and including the present one (Table 3) showed no discrepancies, there were small differences, which were mostly related to the different environmental and constitutional origins of the samples. The comparison of the current results with both studies shows that minimum values were in very good agreement. In almost all cases, they fell between the reported values. For maximum concentrations, only Fe was shown to be 75% than both Durrand and Ward *(2)* and WHO *(9)*.

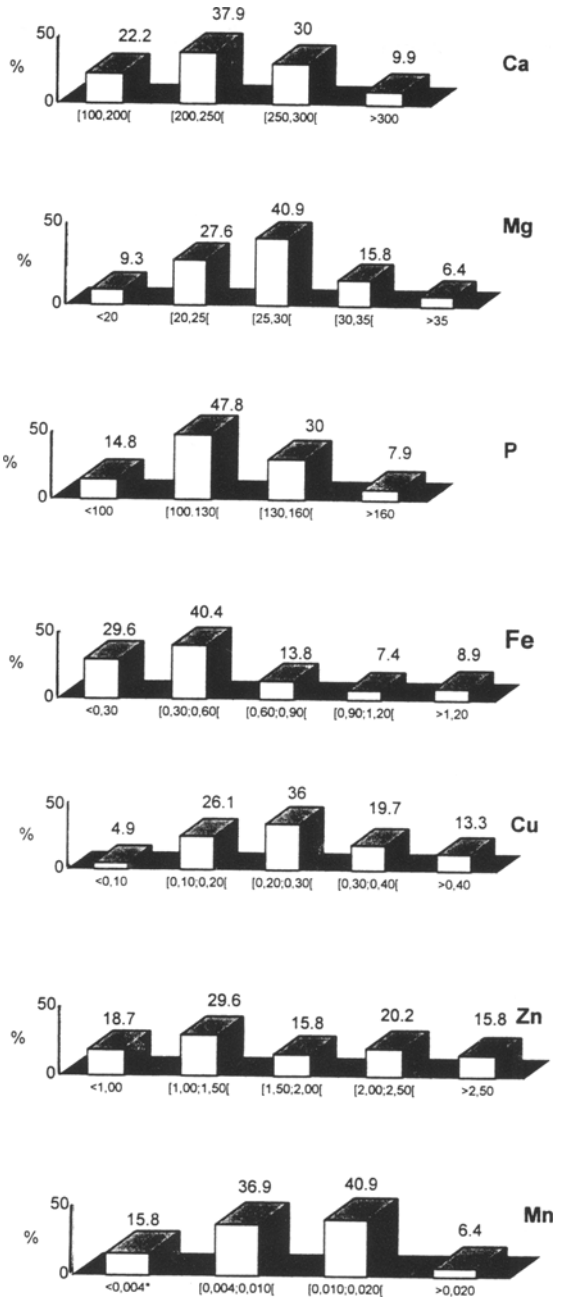

Fig. 1. Relative frequency distribution of concentrations (μg/g) of Ca, Mg, P, Fe, Cu, Zn, and Mn in human milk of Brazilian women.

ICP-AES for mineral determination in human milk can be used for elements in major and minor concentrations. The use of this analytical technique on a large number of samples of breast milk from Brazilian women was not only feasible, but also provided reference data for Ca,

Table 3
Comparison of Range Values Between the Current and Two Other Studies That Reported Multielement Determinations in Human Milk (Minimum–Maximum in μg/g)

Reference	P	Ca	Mg	Fe	Cu	Zn	Mn*
This study	51 - 205	128-405	15 - 43	0.1 - 3.5	0.05- 0.94	0.27- 3.04	4.3 -70.8
Durrand and Ward *(2)*	43.4-202	97.5-257	20.1-49.1	0.286-1.92	0.082-1.138	0.39-7.184	5.0 - 20.0
WHO *(9)* **	86 - 385	147-496	9.5-62.5	0.101-1.99	0.057-0.715	0.27-10.66	1.06-153.4

*μg/L.
**Data from reference analytical laboratory.

Cu, P, Mg, Mn, Fe, and Zn with good accuracy. Such results should serve to indicate the mineral status of women and nutritional parameters for breast-fed infants.

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