The study of the composition of human milk has attracted a lot of interest, since it represents the most suitable pattern of nutrients for the younger infant. In the present study, the concentration of iron, copper and zinc was measured in the colostrum of 50 Brazilian mothers. The samples were collected from the day of birth to the fourth day postpartum. The levels of trace elements were determined by total reflection X-ray fluorescence analysis (TXRF) with synchrotron radiation. The accuracy of the measurements was calculated by determining the concentration of the elements in a standard solution (ICP-multiplelement standard solution) resulting in values ranging from 3.7 to 6.1% for the elements to be considered. We obtained the following values (mean ± S.D.): 1.72 ± 1.01 mg/L Fe; 0.54 ± 0.29 mg/L Cu; 6.97 ± 2.82 mg/L Zn. The concentrations of the determined elements were comparable with previously reported colostrum values. We concluded that TXRF is an adequate technique for evaluating trace elements in colostrum, providing multielement analysis in a single measurement, and requiring no pre-concentration of the sample. The technique can be optimized in further studies in order to evaluate a wide range of trace elements in the human milk, in a greater number of colostrum samples.

Key Words: iron; copper; zinc; colostrum; TXRF.

INTRODUCTION

Several deficiencies in term and pre-term newborns have been associated with deficiencies in iron, copper, and zinc (Bhatia and Rassin, 1988; Gurr, 1981; Haschke et al., 1995). These deficiencies may have adverse effects on the growth and the intellectual development of the newborn, can cause skin-related diseases, and increase the newborn mortality (Flynn, 1992; Olivares and Uauy, 1996; WHO/IAEA, 1989). For this reason, there is interest in determining the concentration of iron, copper, and zinc in human milk, particularly because...
expressed human milk is frequently used in hospitals to feed low birth-weight infants.

Although considerable knowledge exists about the physiological significance of trace elements in human nutrition, and significant contribution about trace elements content of human colostrum has been made during the past several years (Bates and Prentice, 1994; Allen et al., 1991; Escrivão, 1993; Arnaud and Favier, 1995; Alaejos and Romero, 1992; Lönnertal, 1997; Gouveia, 1983; Goes, 1999; Turan et al., 2001; Silvestre et al., 2000, 2001) there is an increasing need for sensitive and reliable methods to identify and quantify the trace elements in human milk.

The total reflection X-ray fluorescence (TXRF) technique is an extremely useful tool in trace elements determination. Its main advantages over the other methods are the low detection limits and the simultaneous determination of different elements employing small amounts of sample (Klockenkämper, 1992; Wobrauschek, 1994). There is no report in the literature of studies performed with the TXRF technique in colostrum samples. The purpose of this study was to apply TXRF for determination of trace elements in samples of colostrum from mothers of term infants.

**MATERIALS AND METHODS**

*Subjects*

The study was descriptive and transversal (Hennekens and Buring, 1987), undertaken after approval by the Ethics Commission of the Fernandes Figueira Institute/Oswaldo Cruz Foundation—IFF/FIOCRUZ—Rio de Janeiro, Brazil. Fifty lactating mothers, who delivered full-term infants in the maternity of Instituto Fernandez Figueira/FIOCRUZ, consented to participate in this study. All mothers were between 20 and 38 years of age; 1.8±0.4 pregnancies (parity) and 21.2±3.6 wt/ht^2 (body mass index). Following the definition of colostrum adopted by the Institute of Medicine/NAS (1991), the colostrum samples were collected in the period from the first to the seventh day postpartum, although the actual collection was made until the fourth day postpartum, which is the internation period for lactating mothers.

The colostrum samples were collected by manual expression during a regular feeding of the child, using disposable gloves. About 2mL of milk from both breasts was collected between 10am and 2pm during the day. Immediately after collection, the milk was transferred to sterile polypropylene vials, previously cleaned with distilled water, nitric acid at 5% and de-ionized water, and then immediately frozen at a temperature of −20°C, for later analysis.

*Sample Preparation and Elemental Analysis*

The samples were thawed and homogenized using a vortex shaker prior to subsampling. 0.5mL samples of milk were transferred to polyethylene vials free of trace elements, and treated with 0.5mL of HNO₃ and 0.5mL of H₂O₂ at 60°C for 5 days with the vials sealed. After this, the solution was left to dry and the volume recovered to 0.5mL with a solution of HNO₃ 0.1M as diluting agent. Added to the sample was 50μL of solution (40μg/g Ga) of gallium (Ga)—which was used as an internal standard of reference for the TXRF measurements—in order to obtain a concentration in the order of 10ppm of Ga in the solution.
An aliquot of 5 μL of the solution was placed using a pipette onto a per spex support (used as a reflector) and subsequently submitted to drying in a vacuum drier for 3 h, obtaining spots of approximately 5 mm in diameter.

Measurement of the samples by TXRF was carried out at the National Laboratory of Synchrotron Light (LNLS), Campinas, São Paulo State, Brazil. All samples were processed in duplicate, and the final value was taken as the mean of the results obtained running each sample three times. A white beam of irradiation with a maximum energy of 20 keV filtered by 0.5 mm of aluminum, with an angle of incidence of 1.0 mrad was utilized to excite the sample. The characteristic X-rays were detected by a silicon–lithium (Si–Li) detector with a resolution of 165 eV for 5.9 keV energy linked to an electronic system with a multichannel analyzer (Canberra). The distance between the detector and sample was fixed at 6.0 mm, using a tantalum (Ta) collimator with an aperture of 1.0 mm in order to limit the dead time of the measurements to a minimum value of 15%.

The spectrometer sensitivity was determined using five multielement standards with different concentrations containing Al, Si, Ca, Ti, Cr, Fe, Ni, Zn, Ga and Se. The standards were prepared from mono-elemental solutions (induced coupled plasma (ICP) standard), supplied by Sigma. The accuracy of the measurements was calculated by determining the concentration of the elements of a standard solution (ICP-multielement standard solution—MERCK). The measurement time was 300 s for the samples and 150 s for the standards. The spectrums were analyzed by a quantitative analysis program (quantitative X-ray analysis system—QXAS) distributed by the International Atomic Energy Agency (IAEA), which gives the fluorescent count intensities for each element and the associated uncertainty.

For the analysis of the results, central tendency and variation measures were used; as well as averages and standard deviations.

In order to detect outlying values (discrepancies), the values for Fe, Cu, and Zn were represented in a box plot, where the outlying observations were visualized (Hair et al., 1995).

RESULTS AND DISCUSSION

Analysis of minerals and trace elements in colostrum based on data from conventional techniques, such as atomic absorption spectrometry (AAS), neutron activation analysis (NAA), and inductively coupled plasma atomic emission spectrometry (ICP-AES) require the use of several samples, but often the volume available for determination is small. Therefore, analytical techniques that allow simultaneous determination of more than one mineral are of great importance. TXRF is a highly sensitive technique, allowing the processing of a multielement analysis from a single measurement. In addition, it uses direct irradiation from the sample in such a way that it does not undergo chemical treatment. Consequently, the risk of manipulation-related contamination is reduced (Liendo et al., 1999).

In the present study, 50 samples were analyzed in an approximate time period of 4 h 30 min, that is, each sample in about 5 min. This result shows the importance of the utilization of TXRF in comparison with other techniques, especially in population studies in which a large sample universe is demanded and—depending on the study’s object—only small sample quantities can be collected. The detection limits for the analysis of trace elements in colostrum by TXRF are below 10 ng/mL. These results therefore suggest that TXRF provides detection of elements at ppb level, and can be used for the analysis of trace elements in...
The precision of the method, which was demonstrated by the low coefficient of variation (3.7–6.1%) in the energy range of interest, allows the results to be compared with other analytical methods.

As seen in Table 1, the concentrations of iron, zinc, and copper show wide variations, which are more accentuated for the elements iron and zinc. These individual variations can be related to age, parity, nutritional state, and socio-economic level, and to the rapid and intense changes that occur in the concentrations of these elements at the start of lactation (Worthington-Roberts and Williams, 1997; Calil et al., 1992; Picciano, 1998).

We can also see in Table 1 that the number of samples was lower for the elements iron and zinc than for copper, the reason being the exclusion of outlying observations (vide statistical methods).

Different research studies that employ other analytical methods and evaluate the levels of iron, copper and zinc in colostrum of mothers of term infants (37 weeks) also found quite variable values for these elements (Carias et al., 1997; Triago et al., 1997; Fransson et al., 1984; Caramia et al., 1984; Greco et al., 1984; França et al., 1985; Torrealba et al., 1987; Trugo et al., 1988).

Data from the literature referring to the usual concentration of iron, copper and zinc in colostrum in which different analytical methods were used are presented in Table 2.

The comparison of the average concentration of the trace elements iron, copper and zinc in colostrum from the population studied with the average values of the same elements reported in the literature (Table 2), indicated a good accuracy of the method and a good reproducibility of measurements in colostrum. The precision of the method was demonstrated by the low (3–6%) coefficient of variation obtained when reproducing the analysis in a given day, on different days, and by different operators. Iron was the only element whose observed average concentration in colostrum was above (1.72 mg/L) that encountered in other studies using different analytical methods.

As all possible care was taken during the collection and analysis of the samples (two measurements in each case), it is probable that the variability encountered in the results, in terms of iron concentration, reflects a biological characteristic of the studied population rather than interferences related to contamination during the analysis. In fact, studies performed on colostrum of Brazilian women report similar high values (Escrivão, 1993; França et al., 1985). The probable associated factors that could explain such a finding deserve investigation.

As for copper and zinc, we can observe from Table 2 that the values of these elements proved to be similar to those values from colostrum in lactating women residing in different countries and obtained using different analytical techniques.

### TABLE 1
Concentration of minerals (mg/L) in the colostrum of mothers of term infants

<table>
<thead>
<tr>
<th>Mineral (mg/L)</th>
<th>Iron</th>
<th>Copper</th>
<th>Zinc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of samples</td>
<td>37</td>
<td>50</td>
<td>48</td>
</tr>
<tr>
<td>Mean ± S.D.</td>
<td>1.72 ± 1.01</td>
<td>0.54 ± 0.29</td>
<td>6.97 ± 2.82</td>
</tr>
<tr>
<td>Minimum</td>
<td>0.25</td>
<td>0.20</td>
<td>2.41</td>
</tr>
<tr>
<td>Maximum</td>
<td>3.76</td>
<td>1.63</td>
<td>14.47</td>
</tr>
</tbody>
</table>
On the basis of the presented results, it is possible to verify that the use of the TXRF technique enabled the determination of zinc, copper and iron in colostrum samples and proved to be a quick, relatively simple and accurate method when compared with atomic absorption spectrometry. This analytical technique can be

<table>
<thead>
<tr>
<th>Reference</th>
<th>Method</th>
<th>Iron (mg/L)</th>
<th>Copper (mg/L)</th>
<th>Zinc (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present study</td>
<td>TXRF</td>
<td>1.72 ± 1.01</td>
<td>0.54 ± 0.29</td>
<td>6.97 ± 2.82</td>
</tr>
<tr>
<td>Lamounier et al. (1989). Brazil</td>
<td>AAS</td>
<td>—</td>
<td>—</td>
<td>4.7 ± 1.0</td>
</tr>
<tr>
<td>Vega-Franco et al. (1987). Mexico</td>
<td>AAS</td>
<td>0.85</td>
<td>0.70</td>
<td>—</td>
</tr>
<tr>
<td>Sharda et al. (1983). India</td>
<td>AAS</td>
<td>0.72 ± 0.23</td>
<td>9.32 ± 1.63</td>
<td></td>
</tr>
<tr>
<td>Triago et al. (1997). Venezuela</td>
<td>ICP-AES</td>
<td>0.49 ± 0.14</td>
<td>0.52 ± 0.15</td>
<td>7.1 ± 2.5</td>
</tr>
<tr>
<td>Carias et al. (1997). Venezuela</td>
<td>AAS</td>
<td>0.82 ± 0.23</td>
<td>2.56 ± 0.52</td>
<td></td>
</tr>
<tr>
<td>Rajalakshmi and Srikantia (1980). India</td>
<td>AAS</td>
<td>—</td>
<td>5.32 ± 0.31</td>
<td></td>
</tr>
<tr>
<td>Trugo et al. (1988). Brazil</td>
<td>AAS</td>
<td>1.04 ± 0.62</td>
<td>7.26 ± 3.03</td>
<td></td>
</tr>
<tr>
<td>Greco et al. (1984). Italy</td>
<td>AAS</td>
<td>0.5–0.8</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Bhandari et al. (1985). India</td>
<td>AAS</td>
<td>—</td>
<td>2.2 ± 0.85</td>
<td></td>
</tr>
<tr>
<td>Mandic et al. (1997). Croatia</td>
<td>AAS</td>
<td>—</td>
<td>8.86 ± 2.46</td>
<td></td>
</tr>
<tr>
<td>Escrivão (1993). Brazil</td>
<td>AAS</td>
<td>1.25 (&gt;SE level)</td>
<td>0.63 (&gt;SE level)</td>
<td>11.19 (&gt;SE level)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.17 (&lt;SE level)</td>
<td>0.51 (&lt;SE level)</td>
<td>10.42 (&lt;SE level)</td>
</tr>
<tr>
<td>Fransson et al. (1984) Sweden/Ethiopia</td>
<td>AAS</td>
<td>0.47 ± 0.19 (Eth.)</td>
<td>0.37 ± 0.20</td>
<td>6.59 ± 2.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.50 ± 0.12 (Swe.)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>França et al. (1985). Brazil</td>
<td>AAS</td>
<td>1.65–50.0</td>
<td>6.2–18.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>—</td>
<td>0.6 ± 0.1</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Torrealba et al. (1987). Venezuela</td>
<td>AAS</td>
<td>0.97</td>
<td>8.0 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>Feeley et al. (1983). USA</td>
<td>AAS</td>
<td>0.50–0.80</td>
<td>8.0–12.0</td>
<td></td>
</tr>
</tbody>
</table>

1AAS—Atomic absorption spectrophotometry. ICP-AES—plasma-induced atomic emission spectrophotometry.

On the basis of the presented results, it is possible to verify that the use of the TXRF technique enabled the determination of zinc, copper and iron in colostrum samples and proved to be a quick, relatively simple and accurate method when compared with atomic absorption spectrometry. This analytical technique can be
optimized for the evaluation of a wide range of trace elements in samples of human milk, with very low detection limits, high precision and speed. TXRF is a highly sensitive technique for multielement analysis that can easily be applied to samples of biological and medical interest, especially in population studies aiming to identify women with inadequate levels of trace elements in the maternal milk and, therefore, the infants at risk of developing specific nutritional deficiencies.

ACKNOWLEDGEMENTS

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REFERENCES


