Study of the effect of different iron salts used to fortify infant formulas on the bioavailability of trace elements using ICP-OES

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Abstract

Five different iron salts—sulphate, lactate, diphosphate, encapsulated sulphate, and EDTA-Fe(III)—were used to fortify an infant formula to study possible differences in iron bioavailability. The effect of iron fortification at two levels (0.5 mg Fe 100 kcal\textsuperscript{-1} and 1.5 mg Fe 100 kcal\textsuperscript{-1}) on the bioavailability of other important trace elements such as copper and zinc were also evaluated. An in vitro method based on element dialysability (i.e., the fraction available by absorption) to simulate newborn digestion was applied to study iron, copper and zinc bioavailability. Enzyme treatment was carried out in two stages involving pepsin at pH 5.0 followed by pancreatin at pH 7.0. The incubation times were short to mimic the transit of meal in an infant’s gastrointestinal tract. Iron, copper and zinc were determined using inductively coupled plasma atomic emission spectrometry using an axially configured device. The percentages of Fe, Cu and Zn dialysable at both iron fortification levels are discussed. From these results, EDTA-Fe(III) appears to be the most adequate salt for iron fortification of infant formulas.

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1. Introduction

Human milk has long been recognised as the optimal source of essential nutrients for the young infant. This is true if the mother’s diet is nutritionally adequate and the baby is fed with a sufficient amount of breast milk. Infant formulas are designed to cover all the nutritional needs of the human neonate where breast-feeding is not possible. The mineral composition of infant formulas is primarily based on the mineral content of human milk, but when an infant formula is designed it is important to take into account the bioavailability of the trace elements.

In general, iron in human milk is more bioavailable than iron in infant formula. For this reason, the iron content in fortified term infant formula (5–12 mg L\textsuperscript{-1}) is 12–22 times higher than that in human milk (0.3–0.5 mg L\textsuperscript{-1}). Although many studies have shown that iron fortification levels of 12–15 mg L\textsuperscript{-1} are beneficial to the infant, low levels of iron fortification may be adequate. Kaup (1998) found little difference in the amount of iron absorbed from formulas fortified with 6 mg L\textsuperscript{-1} iron and 12 mg L\textsuperscript{-1} iron.

Some trace element deficiencies in infants may be due to an inadequate trace element content in infant formulas. Daily feeding of iron fortified formulas may have negative effects on other mineral status, specially zinc and copper. The negative interaction of metal ions is one of the major dietary factors that causes low bioavailability of these nutrients (O’Dell, 1989). Iron supplementation is associated with a decrease in copper and zinc absorption. Zinc and iron may share common pathways for absorption, but they can compete for uptake into mucosal cells. Solomons (1986) has
proposed that chemically similar ions could compete in a common absorptive pathway. On the other hand, high dietary iron together with unsaturated fatty acids may stimulate the formation of highly reactive free radicals and hydroperoxides that can cause oxidative damage. Moreover, high dietary iron, acting as pro-oxidant, may be related to heart disease.

Iron salts differ in bioavailability as well as their pro-oxidant properties (Clemens & Mercurio, 1981; Theuer, Martin, Wallander, & Saret, 1973). Both in vivo and in vitro methods have been proposed for estimation of the bioavailability of trace elements. In vivo methods, using stable isotopes in humans (Ziegler et al., 1989; Davidsson et al., 1994; Fairweather-Tait, Fox, Wharf, & Eagles, 1995; Abrams, Wen, & Stuff, 1996; Fomon, Serfass, Nelson, Rogers, & Frantz, 2000a,b), give the best estimation of bioavailability. However, the use of radioisotopes is considered unethical in infant studies, and so in vitro methods have been preferred to investigate trace metal bioavailability. These methods consist basically of the simulation of gastrointestinal food digestion under fixed conditions. The methods measure the amount of element available for absorption (based on mineral solubility) and also simulate passive diffusion through the mucosa (based on element dialysability).

Miller, Schricker, Rasmussen, and Van Campen (1981) developed a method based on element dialysability to determine iron bioavailability. These methods have been used to predict the availability from different foods including milk or infant formulas. In vitro methods are simple, rapid, inexpensive, and easy-to-control alternatives to in vivo studies. Although in vitro digestion cannot accurately reflect the complexity of natural systems, information from these experiments regarding the effects of enzymes and pH may be applicable to the in vivo situation. Lööndral, Yuen, Glazier, and Litov (1993) have developed an in vitro method simulating digestion in the newborn infant in which several of the most important phenomena observed in vivo are mimicked. These aspects include the immaturity of the digestive mechanisms of the neonate in terms of enzymes and bile salts secretion, high postprandial pH of the stomach (5.0–6.0), and fast transit in the infant’s gastrointestinal tract.

To study the effects of different iron salts at different concentrations on iron, zinc, and copper bioavailability, the in vitro method proposed by Lööndral et al. (1993) was applied in this study (with some modifications). Milk samples were subjected to a two-stage digestion procedure reflecting the gastric and intestinal digestive processes. The intestinal digestion was carried out using dialysis membranes to determine the amount of dialyzed metal at the end of the digestion procedure. Inductively coupled plasma emission spectroscopy was used for the determination of trace elements in milk and in the dialysed fractions. Milk and dialysed fractions were subjected to a microwave-assisted digestion procedure prior to inductively coupled plasma atomic emission spectrometry (ICP-OES) analysis.

2. Materials and methods

2.1. Equipment, materials and reagents

An incubation chamber (Boxcult, SELECTA S.A., Barcelona, Spain), equipped with a Rotabit orbital-rocking platform shaker, coupled with an agitator Rotabit was used for the in vitro digestions. A pH-meter (Crisom 500, Crison Instruments, S.A., Barcelona, Spain) equipped with a LIQ-PLAST electrode (Hamilton, Barcelona, Spain) was used to determine pH during in vitro digestion. A Lyph-lock® 6 L freeze dry system (Labconco, Kansas, MO, USA) was used to pre-concentrate the dialysed samples. A domestic microwave was used for the wet acid digestion procedure prior to metal content determination using ICP-OES.

For ICP-OES, an Optima 3300, axial configuration, equipped with a spray chamber (Ryton, double pass, Scout-type) and a Gem Cone nebulizer (all from Perkin-Elmer, Norwalk, USA) was used for iron, copper and zinc measurements. The ICP-OES operating conditions were as follows: power 1300 W; nebulizer gas flow 0.95 L min⁻¹; auxiliary gas flow 15 L min⁻¹; peristaltic pump rate 1.5 mL min⁻¹; read time 5 s; integration time 60 s. The lines (nm) used were: Fe, 238.204; Cu, 224.700; Zn, 213.857.

All reagents were of analytical grade, and ultrapure water of 18 MΩ cm specific resistivity obtained from a Milli-Q purification system (Millipore Corp., MA, USA) was used throughout. Glass and polyethylene materials were soaked in 10% nitric acid for at least 48 h and then rinsed three times with ultrapure water and kept dry ready to use.

The digestive enzymes pepsin (porcine stomach mucosa; p-7000) and pancreatin (porcine pancreas; p-1750) were from Sigma (Sigma Chemical Co. Ltd., St. Louis, MO, USA) and working solutions were prepared daily. Pepsin solutions (gastric digestion) were prepared by dissolving 20 mg of pepsin in 100 mL of 0.1 M HCl, and pancreatin solutions (intestinal digestion) were prepared by dissolving 15 mg of pancreatin in 100 mL 0.1 M NaHCO₃.

Dialysis membranes (cut off 1 kDa) 45 mm wide, vol cm⁻¹ 6.42 mL (cellu-Sep®H1, Membrane Filtration Products, Texas, USA) were used in the intestinal digestion procedure. The membranes were soaked in 1 mM ethylene diaminetetraacetic acid (EDTA; BDH Ltd, Poole, UK) for several hours and rinsed with Milli-Q water before use.
Hydrochloric acid (36.5–38%; J.T. Baker, Philadelphia, USA), sodium bicarbonate (Sigma), sodium hydroxide (BDH) were used in the in vitro digestion procedure, while nitric acid (69–70%; J.T. Baker) and hydrogen peroxide (33%, Panreac, Barcelona, Spain) were used in the microwave-assisted acid digestion. Scandium (Perkin–Elmer), as internal standard, and a multielement stock standard solution (QC Std 21 at 100 µg mL⁻¹, Perkin–Elmer) were used for ICP-OES. A whole milk powder standard (Reference Material 1846 NIST; National Institute of Standards and Technology, USA) was used to assess the accuracy of the method.

2.2. Samples

A cows’ milk-based, non-mineral supplemented, powdered infant formula (protein content 12.0 g 100 g⁻¹ powder milk; whey/casein = 60/40) was provided by a Spanish infant formula manufacturer. The commercial formula is recommended for the full-term newborn infant during the first four to five months of life. Powders were reconstituted in Milli-Q water at 13.7 g 100 mL⁻¹ according to the manufacturer’s instructions.

2.3. Iron salts

Iron salts used for this study were selected in concordance with the directive 91/321/CEE. Some salts allowed by European legislation, i.e., ferrous lactate (C₈H₁₀FeO₆(δaq)); Fluka, Buchs, Switzerland), ferrous sulfate (FeSO₄·7H₂O; Panreac, Barcelona, Spain), ferric diphosphate (Fe₄(P₂O₇)₃; Riedel-de Haën, Steinheim, Germany) together with EDTA-iron (III) sodium salt hydrate and encapsulated ferrous sulfate (Aldrich, Milwaukee, USA) were studied. These two last iron salts were added due to the fact that they help prevent interactions between milk components and changes in the sensory characteristics of milk. All these compounds were added to the infant formula (non-mineral supplemented) at levels of 0.5–1.5 mg Fe 100 kcal⁻¹. These levels were chosen so that the final concentrations in the supplemented formulas were within the range established by the European Community.

2.4. In vitro gastrointestinal digestion method

The digestion procedure used in this work was based on the method of Miller et al. (1981), with modifications made to take into account the newborn gastrointestinal conditions (Lönnertdal et al., 1993). The in vitro gastrointestinal digestion procedure was performed in two stages, the first (pepsin) simulating gastric digestion, which occurs in the newborn stomach, and the second (pancreatin) simulating intestinal digestion.

In the fortification process, iron salts were added to a non-mineral supplemented powdered infant formula as a solid. Five replicates of 35 mL of milk were transferred to Erlenmeyer flasks and a sample retained for determination of the total element content. Two additional samples, one for each level of fortification, were retained to determine titratable acidity. Blanks for gastric and intestinal digestions were also performed in triplicate in each experiment to control possible contamination problems. The pH was adjusted to 5.0 (corresponding to the pH of newborn gastric digestion; Lönnertdal et al., 1993) using 5 M HCl, and 1.17 M HNO₃ solution (0.2 mg mL⁻¹ dissolved 0.1 M HCl) were added in the gastric stage. Samples were incubated at 37°C for 50 min in an orbital shaker at 100 rev min⁻¹. The gastric digestes were placed in an ice-water bath to cool down and stop the digestion procedure.

Prepared dialysis membranes were filled with 25 mL Milli-Q water and sufficient 1 M NaHCO₃ to obtain pH 7.0. This was the equivalent to the titratable acidity as defined by the number of equivalents of NaOH required to fix the gastric digest at pH 7. The membranes filled with NaHCO₃ were weighed and placed inside the Erlenmeyer flasks containing the gastric digestes and 2.34 mL of pancreatin solution (0.15 mg mL⁻¹) in 0.1 M NaHCO₃ were added. Finally the intestinal digestion was carried out in the incubation chamber at 37°C for 150 min.

2.5. Determination of Fe, Cu, and Zn by ICP-OES

Dialysates were pre-concentrated by freeze drying, and the organic matter was destroyed by microwave-assisted acid digestion prior to determination of metal concentrations. Fifteen milliliters of the dialysate sample were weighed and freeze-dried in open polytetrafluoroethylene vessels. One millilitre of the dialysate sample was added in the gastric stage. Samples were incubated at 37°C for 12 min (in two 6 min steps, with cooling in between). After cooling the vessels in an ice bath, 0.5 mL of H₂O₂ were added and the second stage in the microwave oven was carried out for 12 min at 310 W.

Iron, copper and zinc determinations were performed with ICP-OES using calibration curves in 2% HNO₃ over a concentration range of 0.25–0.75 mg L⁻¹.

The concentration of the metal in the solution within the dialysis membrane provides the dialysability data, and the dialysability percentage is calculated using the following equations in which [M]dialysed is the metal concentration within the dialysis membrane, Vdialysed is the dialysed volume, [M]milk is the metal concentration in the milk sample and Vmilk is the volume of the milk sample

\[ \text{mg dialysed} = \text{[M]dialysed} \times V_{\text{dialysed}}, \]
3. Results

Background equivalent concentration (BEC) was used to evaluate the instrumental limit of detection and is defined as the concentration of a solution that results in an analyte emission signal equivalent in intensity to that of the background emission signal at the measured wavelength. The BECs for each emission line were determined by calibration without background correction, followed by a sample analysis with the shutter to the optics closed. The BECs obtained were 0.001, 0.034, and 0.002 mg L\(^{-1}\) for Fe, Cu, and Zn, respectively.

3.1. Study of the acidity effect on the calibration curves

It is usually recommended to use acidified solutions when working with a ICP-OES, as well as an internal standard. Therefore, because the samples had been digested with nitric acid, a study on the effect of acidity on the calibration slopes was carried out. Two calibrations for Fe, Cu, and Zn were prepared in 2% and 20% H\(_2\)NO\(_3\), using Sc as internal standard at 0.1 mg L\(^{-1}\). The calibration curve slope was greater with the higher nitric acid concentration for all three studied elements. Lower limits of quantitation (LOQs) were found with 2% H\(_2\)NO\(_3\) than with 20% H\(_2\)NO\(_3\) (data not shown). Therefore, and due to the low content of these metals in dialyzates, the calibration curves were constructed routinely with 2% H\(_2\)NO\(_3\).

3.2. Accuracy

Because of unavailability of a reference material with certified metal content in gastric and intestinal digests, the accuracy of the method was studied only for total Fe, Cu, and Zn content.

3.3. Mass balance study

To check the accuracy of the Fe, Cu, and Zn determination in the in vitro digestion, a study of the mass balance was performed. The Fe, Cu, and Zn levels in the total milk, in the dialysed milk, and in the residue obtained after dialysis procedure, were determined (Table 2). Given the low concentrations of metals, and the complexity of the procedure, the mass balances obtained were considered adequate and minimal contamination or metal loss was assumed.

3.4. Precision of the method

The within-batch precision (\(n = 5\)) of the method was determined with aqueous standard solutions at different Fe, Cu and Zn levels (0.25, 0.50 and 0.75 mg L\(^{-1}\)), while the repeatability (\(n = 5\)) of the procedure was determined with a dialysate obtained after the in vitro digestion procedure of an infant formula sample. The results, expressed as relative standard deviation (RSD) of the concentration values of Fe, Cu and Zn obtained in the different solutions, are shown in Table 3. It was found that the precision of measurements was good (lower than 1%) for all concentration levels and for the three elements studied in both matrices.

3.5. Study of iron fortification

The proposed in vitro procedure was applied to a cows’ milk non-mineral supplemented powdered infant formula provided by a Spanish infant formula manufacturer. This infant formula was supplemented with...
Table 2
Mass balance study for in vitro digestion procedure for Fe, Cu and Zn

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>(A + B)</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe (mgL$^{-1}$)</td>
<td>0.02 ± 0.001</td>
<td>3.89 ± 0.052</td>
<td>3.91</td>
<td>3.32 ± 0.25</td>
</tr>
<tr>
<td>Cu (µg L$^{-1}$)</td>
<td>0.01 ± 0.002</td>
<td>0.33 ± 0.010</td>
<td>0.34</td>
<td>0.38 ± 0.07</td>
</tr>
<tr>
<td>Zn (mg L$^{-1}$)</td>
<td>0.03 ± 0.002</td>
<td>2.05 ± 0.133</td>
<td>2.08</td>
<td>2.85 ± 0.06</td>
</tr>
</tbody>
</table>

A: metal concentration in dialysed fraction; B: metal concentration in non-dialysed fraction; C: metal concentration in whole milk. The metal content is expressed as mean concentration ± standard deviation (n = 3).

Table 3
Precision of the method studied as the repeatability (n = 5) for aqueous standard solutions (0.25, 0.50, and 0.75 µg L$^{-1}$) of Fe, Cu, and Zn and precision of the overall procedure

<table>
<thead>
<tr>
<th>Element</th>
<th>Repeatability (%RSD) at standard solution concentrations (µg L$^{-1}$)</th>
<th>Precision (%RSD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.25</td>
<td>0.50</td>
</tr>
<tr>
<td>Fe</td>
<td>0.6</td>
<td>1.0</td>
</tr>
<tr>
<td>Cu</td>
<td>0.8</td>
<td>0.6</td>
</tr>
<tr>
<td>Zn</td>
<td>0.8</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Precision is expressed as relative standard deviation (RSD) (n = 5).

different iron salts to study the bioavailability of the iron as a function of the iron salts used in the fortification process. All salts were added to the infant formula powder at two supplementation levels, i.e., 0.5 mg Fe 100 kcal$^{-1}$ (level A) and 1.5 mg Fe 100 kcal$^{-1}$ (level B). The powder samples were reconstituted in Milli-Q water at a concentration of 13.7 g 100 mL$^{-1}$.

Bioavailability was expressed as the dialysable fraction of metal under the experimental conditions. The results of iron dialysability for the different iron salts at both fortification levels are summarised in Table 4, these values are expressed as dialysability percentage mean plus minus standard deviation (n = 5).

To study the possible interactions between the different iron salts added to the infant formula and the copper and zinc dialysability, copper and zinc concentrations were measured in all the dialysate samples. The results are also shown in Table 4.

4. Discussion

4.1. Influence of level of iron fortification in Fe, Cu and Zn dialysability

The iron concentration in the dialysates of infant formulas fortified with ferric diphosphate, at the two levels, and ferrous lactate at the lowest level of iron fortification were lower than the quantification limit of the method. There are no statistically significant differences (95% confidence level) between the iron dialysability percentage at the two fortification levels when using the other salts, the standard deviation with ferrous sulphate at 0.5 mg Fe 100 kcal$^{-1}$ being particularly large.

Due to its low levels in the sample used in supplementation, copper could not be detected at the lower level of iron fortification with encapsulated ferrous sulphate, Fe (III)-EDTA, and ferrous sulphate. The low copper values and the high variability of the results do not enable conclusions to be made on whether or not there is any significant effect on copper dialysability at the different fortification levels of these iron salts. Differences in dialysability between fortification levels were also not significant (95% confidence level) for ferrous lactate but were for ferric diphosphate.

With respect to zinc, the dialysability at the two levels of Fe(III)-EDTA and ferrous lactate were statistically different (95% confidence level). However, the effect of the different iron salts in the dialysability was different, with the higher Fe (III)-EDTA concentration giving lower zinc dialysability, but the higher concentration of ferrous lactate giving the higher zinc dialysability.

4.2. Influence of iron salts in fortification on Fe, Cu and Zn dialysability

To compare the dialysability obtained after fortification with the different salts tested, a statistical comparison of the standard deviation of measurements was...
Table 5
P-values after Cochran’s C and Barlett’s tests at 95.0% confidence level (α = 0.05) for variance check when comparing Fe, Cu and Zn dialysability using different iron salts

<table>
<thead>
<tr>
<th>Iron salt</th>
<th>Cochran’s C test</th>
<th>Barlett’s test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe</td>
<td>0.0040</td>
<td>0.0002</td>
</tr>
<tr>
<td>Cu</td>
<td>0.0590</td>
<td>0.0001</td>
</tr>
<tr>
<td>Zn</td>
<td>0.0009</td>
<td>0.0003</td>
</tr>
</tbody>
</table>

Table 6
Results from Multiple Range tests (95% confidence level) for Fe dialysability in an infant formula fortified with different iron salts

<table>
<thead>
<tr>
<th>Iron salt</th>
<th>Mean</th>
<th>Homogenous groups</th>
<th>Differences</th>
</tr>
</thead>
<tbody>
<tr>
<td>D</td>
<td>0.110</td>
<td>A–B *5.591</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>0.964</td>
<td>A–C *5.525</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>1.030</td>
<td>A–D *6.445</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>6.555</td>
<td>B–C –0.066</td>
<td>B–D 0.854</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C–D 0.920</td>
<td></td>
</tr>
</tbody>
</table>

A: Fe(III)-EDTA; B: ferrous lactate; C: encapsulated ferrous sulphate; D: ferrous sulphate.
* Denotes a statistically significant difference.

Table 7
Results from Multiple Range tests (95% confidence level) for Cu dialysability in an infant formula fortified with different iron salts

<table>
<thead>
<tr>
<th>Iron salt</th>
<th>Mean</th>
<th>Homogenous groups</th>
<th>Differences</th>
</tr>
</thead>
<tbody>
<tr>
<td>E</td>
<td>0.328</td>
<td>A–B *0.837</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>0.536</td>
<td>A–C 0.649</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>0.724</td>
<td>A–D –0.497</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>1.373</td>
<td>A–E *1.045</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>1.870</td>
<td>B–C –0.188</td>
<td>B–D *–1.334</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B–E 0.208</td>
<td>C–D *–1.146</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C–E 0.396</td>
<td>D–E *1.542</td>
</tr>
</tbody>
</table>

A: Fe(III)-EDTA; B: ferrous lactate; C: encapsulated ferrous sulphate; D: ferrous sulphate; E: ferric diphosphate.
* Denotes a statistically significant difference.

firstly made with the results obtained at the highest iron fortification level. The Cochran’s C and Barlett’s tests at 95% confidence level were applied. Results (Table 5) show that there are statistically significant differences in the standard deviations of dialysability percentages for copper, iron and zinc when using the different salts. Because there are statistically significant differences in the standard deviations, the Multiple Range test was applied to compare the means of the dialysability percentages. From Tables 6–8 it can be seen that the statistical procedure gives information about the statistically significant differences between each pair of iron salts used in the infant formula fortification. Thus, there are statistically significant differences between iron dialysability with Fe (III)-EDTA and the other salts (Table 6).

For copper dialysability there are three homogeneous groups with statistically significant differences among them (Table 7). One group comprises ferric diphosphate, ferrous lactate and encapsulated ferrous sulphate, the second comprises ferric diphosphate and encapsulated ferrous sulphate and the third comprises ferrous sulphate and Fe (III)-EDTA.

Two different homogeneous groups were founded for zinc dialysability (Table 8), one comprising ferrous sulphate, encapsulated ferrous sulphate and ferrous lactate and the other comprising ferrous lactate and Fe (III)-EDTA. The zinc dialysability of the infant formula supplemented with ferric diphosphate denotes a statistically significant difference among the other iron salts.

Fe(III)-EDTA is the most useful salt for iron fortification because it gives the highest iron bioavailability after in vitro digestions. For copper, both Fe(III)-EDTA and ferrous sulphate offer useful results, while zinc is more bioavailable when milk is fortified with Fe(III)-EDTA, ferric diphosphate and ferric lactate.

Thus, using an in vitro digestion procedure to mimic newborn gastrointestinal conditions, we conclude that Fe(III)-EDTA is the best iron salt for fortified infant formulas.

This can be explained by the high stability of the complex formed by EDTA with these metals. Iron-EDTA, like other EDTA-metal complexes, dissociates in the gastrointestinal tract to form iron, which is bioavailable, then the absorption of the metal ion and EDTA are independent. Because of this dissociation, information on other EDTA compounds would be relevant. Some studies related to the use of EDTA salts have been recently published (Heimbach et al., 2000).
However, no comparison has been reported in the literature on the metal dialysability of different iron salts to select those providing the highest dialysability values. Therefore, comparison of results of this study with those obtained by other authors is not possible.

The benefits of iron fortification to the infant have been studied extensively and continue to be identified. Iron sources that are more bioavailable to the infant could lead to the development of formulae containing less iron overall. Moreover, infant formula with new iron sources and lower iron levels may have less pro-oxidative potential and result in maximal trace element absorption. Although iron fortification of infant formulae leads to a reduction in the prevalence of anaemia, the level of fortification and type of iron salt are continually being evaluated.

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