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Analytical Methods Section

A DPCSV method for the determination of nickel in infant formulas

R. Barbera, M. J. Esteve, R. Farre & J. C. Lopez

Nutrition and Food Chemistry, Faculty of Pharmacy, University of Valencia, Avda. Vincente Andres Estellés s/n, 46100 Burjassot, Valencia, Spain

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A useful method for nickel determination in infant formulas is described. After dry digestion of the sample, a differential pulse cathodic stripping voltammetry (DPCSV) method is applied. After checking the absence of interferences, the analytical parameters of the method (linearity, detection and quantitation limits, precision and accuracy) were determined. The values obtained show that the method studied is reproducible, accurate and sensitive enough to be useful for the analysis of infant formulas.

INTRODUCTION

There is substantial evidence that nickel is essential to animals, and the same is probably true in the case of humans (Nielsen, 1988). However, the specific biochemical functions of nickel in higher animals (including man) have not yet been defined. For this reason this element has still not been accepted as essential (Nielsen *et al.*, 1992) and there are no reliable data on which to base estimates of human requirements (NRC 1989).

On the other hand, up to now the cases of nickel toxicity described have been related to work activities rather than food intake. The most frequent symptoms are skin and respiratory allergies and there is also an increased risk of cancer of the nasal cavities, lung or larynx (Mastromatteo, 1967; Sunderman, 1968).

The purpose of this work is to establish a method for nickel determination in infant formulas. This work is a part of a larger study on trace elements in infant formulas carried out for the purpose of estimating trace element intake, including both essential and toxic elements, of artificially fed infants in Spain.

The interest in ascertaining the nickel content of foods in general and of infant formulas in particular, is due to nickel's possible essentiality and to the positive skin reactions that oral intake has been observed to produce in some nickel-sensitive individuals.

Since the nickel content in infant formulas is very low, between 20 and 50 ng g^{-1}, sensitive techniques need to be used for its measurement. Electrothermal atomic absorption spectroscopy (ETAAS) is being proposed by the IUPAC Subcommittee on Environmental and Occupational Toxicology of Nickel as the standard technique for trace nickel determinations in biological samples. But the determination of nickel in food matrices by atomic absorption techniques has the drawback of non-specific background absorption. Alegria *et al.* (1988) pointed out that if the non-specific background absorption is not corrected, the values obtained are inaccurate. Background absorption is also found to be very high for nickel, equal to atomic absorption, which cannot be removed by pretreating the milk by ashing (see Casey and Neville (1987) who applied the graphite furnace atomic absorption method to the determination of nickel content in human milk; they report that good background correction is essential for this analysis).

On the other hand, voltammetric techniques can satisfy the requirements for the determination of nickel content in infant formulas. These techniques are adequately sensitive and free of interferences (Flora & Nieboer, 1980; Pihlar *et al.*. 1981)

The aim of this work was to study a Differential Pulse Cathodic Stripping Voltammetry (DPCSV) method for measuring the nickel content of infant formulas and to present it as an alternative to AAS techniques. Voltammetric methods are simple, rapid and inexpensive, and free from the interferences detected by AAS procedures.

The DP-procedure was applied, after prior interfacial accumulation by an adsorption layer of dimethylglyoximate at the hanging mercury drop electrode, organic matter was destroyed by incineration. The different pulse mode (DP) improves the base line, making the evaluation of the peaks more precise than with a non-DP procedure.

EXPERIMENTAL

Materials

All reagents were reagent grade. Distilled—deionized Millipore-Milli Q water was used. All glassware was

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soaked in 10% (v/v) HNO_3 for 24 h and rinsed with deionized water before use.

Apparatus

This consisted of Methrom 663VA stand fitted with a polarecord 626, with a Work-Hang Mercury Drop Electrode (HMDE), a reference electrode Ag/AgCl–KCl 3M and a Pt auxiliary/counter electrode. The temperature-programmed furnace (Hereaus M 1100/3) was fitted with a Eurotherm M821 programmer.

Method

The voltammetric method for nickel content determination of infant formulas involves the following steps:

- Selection of instrumental polarographic conditions;
- Influence of the amount of dimethylglyoxime (DMG) in the cell on peak intensity;
- Determination of analytical parameters: linearity, accuracy, sensitivity and precision.

Instrumental conditions

The selection of instrumental operating conditions was carried out using aqueous solutions of nickel.

To 4% HNO_3 (10 ml), Nickel (200 ng). 0·043 M DMG (50 μl) and ammonium chloride (pH = 9·2) (5 ml) were added and the volume was completed to 25 ml.

Instrumental conditions, except for the one being studied, were fixed. Peak intensities were measured as a function of the variable studied.

The results obtained allowed us to propose the following instrumental conditions, in order to obtain reliable and sensitive Ni contents:

Mode: Differential Pulse Cathodic Stripping Voltammetry. Pre-electrolsis time: 1 min with stirring (3000 rpm) followed by 2 min rest.
Initial potential: −0·72 V; final potential: −1·32 V.
Modulation amplitude: 50 mV.
Scan rate: 10 mV s^{-1}.
Current sensitivity (commensurate with concentration): 2–10nA.

Applying the reported conditions, polarograms similar to the one shown in Fig. 1 were obtained.

Dimethyl glyoxime concentration

As can be seen in Fig. 2, an increase in DMG content in the cell increases the peak intensity. However, the value of 250 μg in the cell was chosen because at this concentration the intensity of the peak was high enough to measure the nickel content of infant formulas at the levels usually present.

After selection of the instrumental conditions and the most appropriate dimethylglyoxime concentration

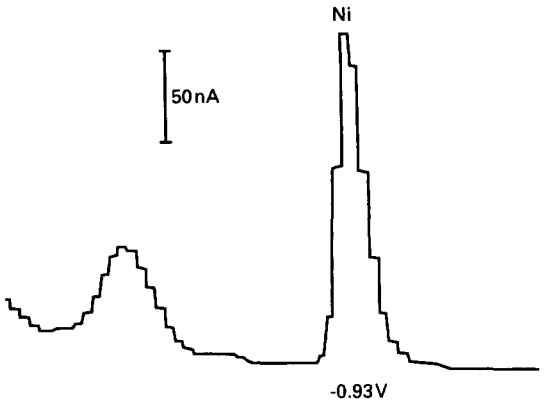

Fig. 1. Voltammogram of an infant milk formula: Ni.

for the use of nickel in aqueous solutions, the study of the interferences and the determination of analytical parameters were carried out using infant formulas. Samples of infant formulas produced from milk and also on vegetable bases, available in Spain, were supplied to us by the manufacturers.

Prior to the application of the DPCSV methods, it was necessary to destroy the organic matter, because as has been reported by two other authors (Hasse & Schramel, 1983; UTO *et al.*, 1985) this step is critical in voltammetric methods.

Dry organic matter destruction

The organic matter of 10 g of sample was destroyed by ashing in a temperature-programmed furnace. The temperature was increased slowly, at a rate of 50°C h^{-1}, to obtain a final temperature of 450°C. This temperature was maintained for 24–48 h. In order to complete the digestion, the residue was soaked with conc. nitric

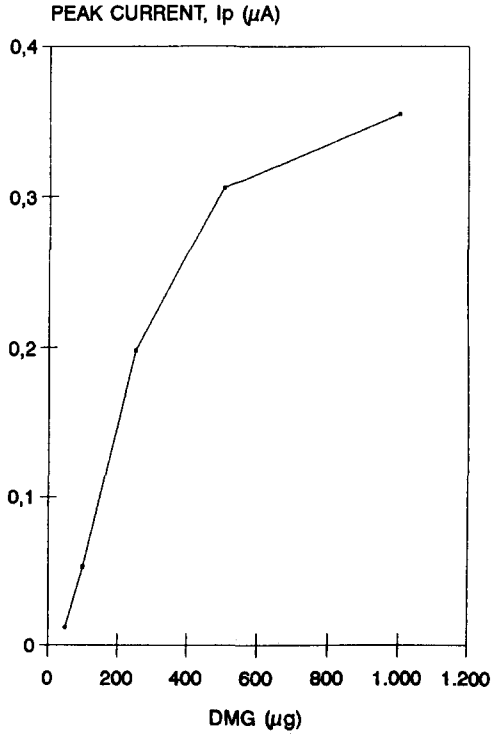

Fig. 2. Dependence of signal height on DMG.

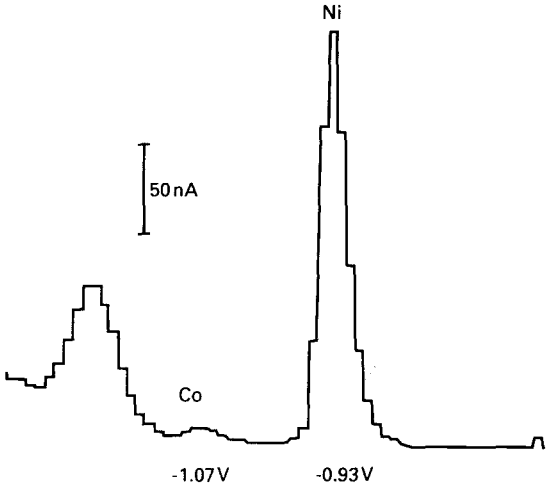

Fig. 3. Voltammogram of an infant milk formula: Ni and Co.

acid (sp. gr. 1·4) (1 ml). Between 48 and 72 h were needed to destroy the organic matter of the sample completely.

White ash was dissolved by adding (1 ml) concentrated nitric acid and the volume was completed to 25 ml with water. To an aliquot (10 ml) of this solution DMG 0·043M (50 μl) and ammonium chloride (pH = 9·2) (5ml) were added; the volume was completed to 25 ml with water and the solution was transferred to the polarographic cell. Instrumental conditions described above were applied. The standard addition method was applied. Each measurement was repeated three times.

Interference

The presence of Cd, Cu, Fe, Pb and Zn in the range of concentrations found in infant formulas does not interfere with the determination of Ni, since the reduction of Ni(II) from adsorbed $Ni(DMG)_2$ was performed by alteration of the electrode potential in the catholic direction (Pihlar *et al.*, 1981). The non-interference of these elements has been checked in this study by applying the procedure to solutions of digested infant formulas.

Only the presence of large amounts of cobalt (II), which reacts similarly to Ni, with DMG, leads to a partial overlapping of the peaks, since the peak for $Co(DMG)_2$ is only about 140 mV more negative than the peak for $Ni(DMG)_2$, (see Fig. 3).

It has been verified that cobalt does not interfere in nickel determination, because no overlapping of the peaks was observed when 300 ng of Ni and 300 ng of Co were present simultaneously in the cell and usually the concentrations of cobalt in infant formulas are ten times lower than the concentrations of nickel.

Analytical parameters

Linearity

A linear range was obtained for the amount of nickel in the cell between 0 and 400 ng ($y = 0{\cdot}76x - 5{\cdot}88$, $r = 0{\cdot}99$). Applying the reported conditions, 50 ng of Ni in the cell gave a peak intensity of 30 nA, clearly differentiated from the base line.

Precision (relative standard deviation)

Instrumental precision was checked from ten consecutive measures of an infant formula sample, mean concentration 39·5 ng g^{-1}. The value found was 5·7%.

The precision of the method was calculated from the analysis of ten homogeneous samples of an infant formula, mean concentration 31·25 ng g^{-1} of Ni. The value found was 8·7%.

The precision depends basically upon the reproducibility of the digestion procedure, dilutions of the sample and the addition of standard solutions to the cell. The values obtained were acceptable, taking into account the low levels of nickel in the analysed samples of infant formulas.

Detection and quantitation limits

Detection (LOD) and quantitation (LOQ) limits were calculated according to the definitions $x_b + 3\tilde{O}_b$ and $x_b + 10\tilde{O}_b$, respectively (ACS, 1980), where x_b was the field blank and $\tilde{O}_b$ the variability in the field blank. For a total of eight blanks, LOD= 14·5 and LOQ= 23·7 ng g^{-1}.

These limits depend upon the reproducibility of the procedural blank rather than instrumental noise. The major source of nickel in the blanks comes from the reagents. Although the purification of analytical grade reagents would lower the blanks and therefore the detection limits, the values obtained with the conditions described here were adequate for Ni determination in infant formulas.

Accuracy

Accuracy was estimated by applying the proposed method to a certified reference sample (Citrus leaves SRM 1572). It was impossible to analyse a powdered milk reference sample because NBS was not certified for Ni and in the case of BCR, the Ni content was too low.

For these reasons and in order to take into account the possible matrix interference, recovery assays were also carried out. Results obtained are shown in Table 1.

Recovery values of 98·5 ± 9·3% show the adequacy of the studied method for the determination of nickel content in infant milk formulas.

Table 1. Accuracy assays in nickel determination

Sample	Number of samples	Nickel (ng g^{-1}) Present	Added	Found	Recovery %
Infant[a] formula	9	39·5 ± 8·7	100	135·5 ± 9·5	98·5 ± 9·3
Citrus leaves SRM1572	3	600 ± 300		590 ± 40	

[a] Infant formula was analysed 9 times at the unspiked level and an additional 9 analyses were carried out on the spiked samples. Results are the confidence intervals at a 95% confidence level.

CONCLUSIONS

The values obtained in the determination of analytical parameters show that the proposed DPCSV method is useful for determining the nickel content of infant formulas. It is sensitive enough to measure nickel levels usually present in infant formulas, the precision is adequate and the accuracy acceptable.

The voltammetric method described and applied to infant formulas was free of interferences and when compared with atomic absorption procedures this method offers a substantial advantage. The DPCSV method is an alternative to atomic absorption spectroscopy when the nickel concentration is greater than 1 $\mu g\,kg^{-1}$, in the case that good background correction is available. However, when the level is below 1 $\mu g\,kg^{-1}$, the voltammetric method is the best choice for Ni determination.

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