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Nutritional element analysis in infant formulas by direct dispersion and inductively coupled plasma-optical emission spectrometry

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Abstract

A method for direct determination of Ca, Cu, Fe, K, Mg, Mn, Na, P and Zn in infant formulas and milkpowders was developed using a water-soluble, tertiary amine:EDTA dispersant and analysis by inductively-coupled plasma–optical emission spectrometry (ICP–OES). Samples were prepared as slurries in aqueous dispersant (10%, v/v) incorporating internal standard (Lu) compensation for potential matrix effects and determined against external calibration standards. The instrument was radially configured and incorporated a charge-transfer device detector, facilitating simultaneous acquisition of multi-element and multi-line measurements. Method performance parameters were estimated and the overall technique found to be applicable to routine quality control monitoring of infant formulas and milkpowders. Advantages of this direct protocol are discussed with respect to improvements in sample throughput and analytical confidence.

Keywords: Trace elements; Infant formulas; Inductively-coupled plasma–optical emission spectrometry (ICP–OES)

1. Introduction

It is well established that the method of sample delivery to the plasma is a critical step in any analytical procedure incorporating the inductively-coupled plasma (ICP). The conventional delivery strategy is nebulization of dissolved samples following mineralisation. Traditional methods of sample preparation for infant formulas, milkpowders and liquid milks are well documented and include wet digestion or dry ashing (AOAC, 1995, method 984.27; Arnaud, Bouillet, Alary & Favier, 1992; Miller-Ihli, 1996; Vuchkova, Margitova & Arpadjan, 1996), microwave digestion (Borkowska-Burnecka, Szmigiel & Zyrnicki, 1996; de la Fuente, Guerrero & Juarez, 1995) and lyophilisation followed by ashing (Coni, Bocca, Coppolelli, Caroli, Cavallucci & Trabalza Marinucci, 1996).

An alternative and relatively new sample preparation and delivery technique is slurry nebulization, which involves the direct aspiration of diluted milk, reconstituted milk powder or infant formula into an AAS, AES, or ICP–optical emission spectrometry (OES) system. Compared to conventional mineralisation based procedures, slurry nebulization offers inherent benefits of simplicity and reduces sample manipulation, thereby minimising the potential for losses or contamination. This approach has been reported as successful for Ca, Mg, Na, K and Fe in infant formulas by FAAS and FES (de la Guardia, Salvador, Bayarri & Farre, 1986; Ruiz, Alegria, Barbera, Farre & Lagarda, 1995), Ca, Mg, K, Na, Cu, Fe, Zn, Mn and P in milk by DCP–OES (Sparkes & Ebdon, 1986), and for Ca, Mg, K, Na and P by ICP–OES (Nobrega, Gelinas, Krushevska & Barnes, 1997). In contrast, an earlier attempt to determine a range of trace minerals in human milk by slurry nebulization into an ICP–OES system failed (Coni, Stacchini, Caroli & Falconieri, 1990), although a more recent study of trace element speciation in milk has utilised direct injection of defatted milk onto a coupled HPLC-ICP–OES system (Bratter, Blasco, Negretti de Bratter & Raab, 1998).

We have recently reported the determination of Ca, Mg, Na, K, Fe, Zn, Cu, Mn, and P in milk and infant formulas by direct aqueous slurry nebulization and ICP–OES (McKinstry, Indyk & Kim, 1999). Although this study proved widely applicable, occasional failures were encountered in certain product types, in particular

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for Ca, Mg, P and Zn. The aim of the present study has been to extend this approach through utilisation of a water-soluble tertiary amine:EDTA dispersant, which provides enhanced casein dissociative properties.

2. Materials and methods

Instrumentation (Optima 3000, Perkin–Elmer), reagents, standards and sample preparation were as previously described (McKinstry et al., 1999), with the modification that working standards and samples were prepared in an aqueous solution (10% v/v) of mixed tertiary amines, containing EDTA (CFA-C Reagent, Spectrasol, Warwick, NY) adjusted to pH 8 (with conc. nitric acid).

2.1. Analysis

Instrumental operating parameters utilised for measurements are listed in Table 1.

Single level calibrations were routinely established for each element based on multi-element standards, followed by automated sequential analysis of samples. Standard and sample emission counts were acquired in triplicate, with software reduction to report means and standard deviations. An ammonia solution (3% v/v) was aspirated at an elevated flow rate (4 ml/min) for 20 s between samples and for 15 minutes at the conclusion of each sample set, prior to shut-down of the instrument.

2.2. Comparative methodology

Comparative data for an intralaboratory quality control sample have been obtained by FAAS following dry ashing-acid digestion for Ca, Cu, Fe, K, Mg, Na, and Zn (AOAC, 1995, method 985.35), by GFAAS following slurry sample preparation for Mn (Wagley, Schmiedel, Mainka & Ache, 1989), and by molybdovanadate colorimetry for P (AOAC, 1995, method 986.24). Certified reference materials (NIST 1846 and NIST 1549) were also analysed using the test protocol to assess analytical bias.

Further, a range of infant formula powders were subjected to ICP–OES analysis following dry-ashing mineralisation (AOAC, 1995, method 985.35), aqueous and CFA-C slurry sample preparation protocols. These samples were also analysed by an independent regulatory laboratory, using both FAAS and ICP–OES following conventional acid digestion.

In addition, a range of milk protein fractions (calcium caseinate, sodium caseinate, calcium and sodium ''proteinate' total milk proteins, rennet casein, lactic casein and acid casein) were submitted to ICP analysis, (i) following both slurry dispersion techniques and (ii) independently after conventional and microwave wet-digestion.

Table 1
Instrument operating parameters for ICP-OES[a]

Parameter	
RF power (W)	1350
Nebuliser flow (l/min)	0.80
Auxiliary flow (l/min)	15
Plasma flow (l/min)	0.50
Sample flow (l/min)	1.8
Source equilibration time (s)	15
Viewing height (mm)	15
Background correction	Manual point selection
Measurement processing mode	Area
Auto integration (s, min–max)	5–10
Read delay (s)	40
Rinse delay (s)	20
Number of replicates	3

[a] Lines used were: Ca 422.673, Cu 324.754, Fe 259.940, K 766.491, Mg 279.553, Mn 257.610, Na 588.995, P 213.618, Zn 213.856.

3. Results and discussion

Instrumental performance characteristics were as previously evaluated and demonstrated to be acceptable (McKinstry et al., 1999). A spectral study of authentic standards, as described previously, was performed in this study incorporating the CFA-C dispersant and confirmed the absence of significant inter-element interferences. Further, both intensity and precision of analyte signal measured in tertiary amine dispersant were equivalent to that in aqueous solution, consistent with the observations of Nobrega et al. (1997).

Method detection limits (MDL) for each element were estimated and are summarised in Table 2. The MDL for all elements, measured in a typical infant formula powder, were 1–3 orders below analyte levels expected in QC samples. Method precision data are summarised in Table 3.

Method precision [RSD (%)] ranged from 0.5 to 1.5 for within-run repeatability and 0.9 to 2.5 for between-run reproducibility. An additional indication of method

Table 2
Detection limits[a]

Element	MDL (mg/100 g)
Ca	10
Cu	0.015
Fe	0.014
K	14
Mg	1.2
Mn	0.002
Na	3.8
P	9.3
Zn	0.04

[a] Measured MDLs, $n = 10$, calculated as standard deviation$\times t$, where $t = 1.833$ from one-sided t-distribution at 95% confidence level (instrumental conditions as per Table 1).

precision, the Horwitz ratio, while primarily intended for estimates of inter-laboratory variability, is also useful in method validation (Albert & Horwitz, 1997; Boyer, Horwitz & Albert, 1985) and ranged 0.1–0.3 for all elements, compliant with established guidelines.

Table 3
Precision data

Element	RSD_r (%)[a]	RSD_R (%)[b]
Ca	0.5	0.7
Cu	1.5	2.5
Fe	0.8	1.3
K	0.6	0.8
Mg	0.5	0.7
Mn	0.6	1.4
Na	0.6	0.9
P	0.7	1.2
Zn	0.8	2.0

[a] Within-run method precision, $n=4$ (estimated with reference to in-house infant formula control powder).
[b] Between-run method precision, $n=13$ (estimated with reference to in-house infant formula control powder).

Table 4
Analyte recovery (%)[a]

	[Level (%)]		
Element	50	100	150
Ca	99.5	99.3	99.5
Cu	93.8	94.8	97.9
Fe	95.7	96.3	96.1
K	99.8	98.9	99.5
Mg	99.4	98.3	98.0
Mn	94.6	94.7	96.2
Na	99.5	99.2	99.1
P	101.0	100.5	100.9
Zn	98.0	97.5	97.7

[a] Values are means of between-run duplicate analyses.

RSD_r:RSD_R values for all elements ranged 0.4–0.6 and conform with accepted guidelines for the relationship between repeatability and reproducibility precision (Albert & Horwitz, 1997).

An estimation of analyte recovery was obtained by spiking the in-house control sample at the 50, 100, and 150% levels with authentic elemental standards, and the data, collated in Table 4, demonstrates quantitative recovery.

Three well-characterised reference samples were used to determine the accuracy of the overall method and the results are shown in Table 5.

The data show comparability of the test method with independent methods and compliance with SRM certified confidence intervals, indicating that an unbiased estimate of analyte level is achievable by the proposed method.

Additional independent comparative data against direct aqueous dispersion, conventional dry-ashing and acid-digestion protocols were obtained for a range of infant formulas, and these results are summarised in Table 6.

For the six infant formulas studied, the overall method means for each analyte were subjected to statistical analysis for variance, as illustrated in Fig. 1. The graphs represent a test for method comparability for each element, with overlap of mean standard error least significant difference (LSD) limits indicating an absence of significant difference ($p = 0.05$).

In general, there was acceptable comparability between all five independent methods for each of the nine elements, with no apparent systematic bias for the CFA-C direct dispersion method.

Freshly secreted milk is a complex and interactive structural system of water, lipids, proteins, carbohydrates, salts and minerals. During commercial processing of milk and infant formula, mineral-matrix interactions may be significantly affected, with particular significance

Table 5
Comparative data (mg/100 g)

	[QC sample ($n=10$)]		[NIST 1846 SRM ($n=11$)]		[NIST 1549 SRM ($n=8$)]	
Element	Test method[a]	Reference method[b]	Test method[a]	Certified[c]	Test method[a]	Certified[c]
Ca	655 (3)	642 (13)	363 (4)	367 (20)	1271 (3)	1300 (50)
Cu	0.452 (0.015)	0.431 (0.011)	0.514 (0.026)	0.504 (0.027)	0.061 (0.014)	0.07 (0.01)
Fe	7.45 (0.06)	7.86 (0.20)	6.09 (0.06)	6.31 (0.40)	0.166 (0.015)	0.178 (0.010)
K	793 (4)	794 (17)	712 (3)	716 (38)	1661 (8)	1690 (30)
Mg	60.6 (0.3)	57.9 (1.0)	54.7 (0.5)	53.8 (2.9)	121 (1)	120 (3)
Mn	0.160 (0.002)	0.168 (0.006)	0.031 (0.001)	0.040[d]	0.026 (0.002)	0.026 (0.006)
Na	232 (1)	223 (6)	220 (2)	231 (13)	483 (2)	497 (10)
P	430 (2)	429 (8)	255 (2)	261 (15)	1059 (11)	1060 (20)
Zn	4.01 (0.05)	3.80 (0.07)	6.21 (0.04)	6.00 (0.32)	4.81 (0.05)	4.61 (0.22)

[a] Test method: slurry nebulisation in CFA-C, ICP–OES. Shown as mean (SD).
[b] Reference method: dry ash, FAAS (Mn: GFAAS; P: spectrophotometry). Shown as mean (SD).
[c] Certified values are shown as mean (95% confidence interval).
[d] Indicative value only.

Table 6
Comparison of techniques (mg/100 g)[a]

Element		[Sample[b]]					
		A	B	C	D	E	F
Ca	1[c]	374	449	844	510	381	605
	2	375	463	847	485	427	608
	3	391	468	882	507	378	584
	4	379	468	838	522	391	604
	5	367	435	846	492	370	593
Cu	1	0.203	0.384	0.446	0.358	0.399	0.490
	2	0.199	0.394	0.450	0.354	0.404	0.480
	3	0.249	0.437	0.480	0.361	0.411	0.487
	4	0.178	0.373	0.410	0.332	0.379	0.451
	5	0.194	0.385	0.409	0.337	0.376	0.453
Fe	1	2.40	6.06	7.73	6.42	6.76	7.93
	2	2.45	6.18	7.82	6.53	6.86	8.00
	3	2.79	6.27	7.89	6.29	6.77	7.57
	4	2.45	5.96	7.33	6.27	6.62	7.57
	5	2.55	6.14	7.57	6.53	7.02	8.09
K	1	512	720	1035	466	510	711
	2	521	743	1040	478	596	718
	3	520	740	1060	472	499	674
	4	532	760	1050	500	544	742
	5	564	769	1068	512	570	764
Mg	1	40.0	53.7	77.0	47.8	38.4	56.5
	2	39.5	54.4	76.4	47.6	42.9	55.7
	3	40.2	54.3	77.2	47.0	36.6	52.6
	4	37.5	51.7	71.8	45.6	36.9	52.9
	5	40.1	54.1	75.5	45.1	38.1	54.3
Mn	1	0.404	0.085	0.120	0.078	0.083	0.112
	2	0.404	0.086	0.114	0.074	0.087	0.109
	3	0.411	0.094	0.121	0.076	0.080	0.108
	4	0.380	0.081	0.107	0.072	0.080	0.104
	5	0.405	0.092	0.106	0.073	0.079	0.108
Na	1	158	221	268	212	192	218
	2	197	230	270	215	215	219
	3	165	232	280	218	187	212
	4	166	235	277	228	209	231
	5	174	235	277	223	211	219
P	1	216	342	620	372	276	407
	2	214	337	601	342	295	392
	3	218	324	587	352	249	357
	4	220	331	591	371	274	387
	5	231	335	581	366	279	392
Zn	1	1.38	3.47	4.00	3.70	3.93	4.39
	2	1.37	3.48	3.90	3.65	4.15	4.23
	3	1.45	3.52	3.92	3.62	3.82	4.14
	4	1.56	3.59	4.20	3.74	3.95	4.49
	5	1.55	3.37	3.89	3.76	3.76	4.25

[a] Results are means of independent, duplicate, between-day analyses.
[b] A: soy-based infant formula (IF), oil-filled; B: goatmilk IF; C: milk-based follow-on, partially oil-filled; D: milk-based IF, oil-filled; E: whey-based IF, oil-filled; F: whey-based IF, partially oil-filled.
[c] 1: CFA-C slurry dispersion/ICP–OES test method; 2: slurry dispersion/ICP–OES method; 3: dry-ash sample preparation/ICP–OES method; 4: wet digestion sample preparation/ICP–OES method (independent laboratory); 5: wet digestion sample preparation/FAAS (independent laboratory).

for the casein-associated elements (de la Fuente, 1998; Guo, Hendricks & Kindstedt, 1998; May & Smith, 1998; Renner et al. 1989). Further, structural distribution among milk fractions and bioavailability of several divalent cations have been shown to vary significantly between human and bovine milk (Neville, Zhang & Allenn, 1995; Renner, 1983; Roig, Alegria, Barbera, Farre & Lagarda, 1999). Mineral compartmentalisation and interactions with proximates within milk and its derivatives may conceivably influence plasma spectroscopic analysis, through moderation of both atomisation and transport efficiencies.

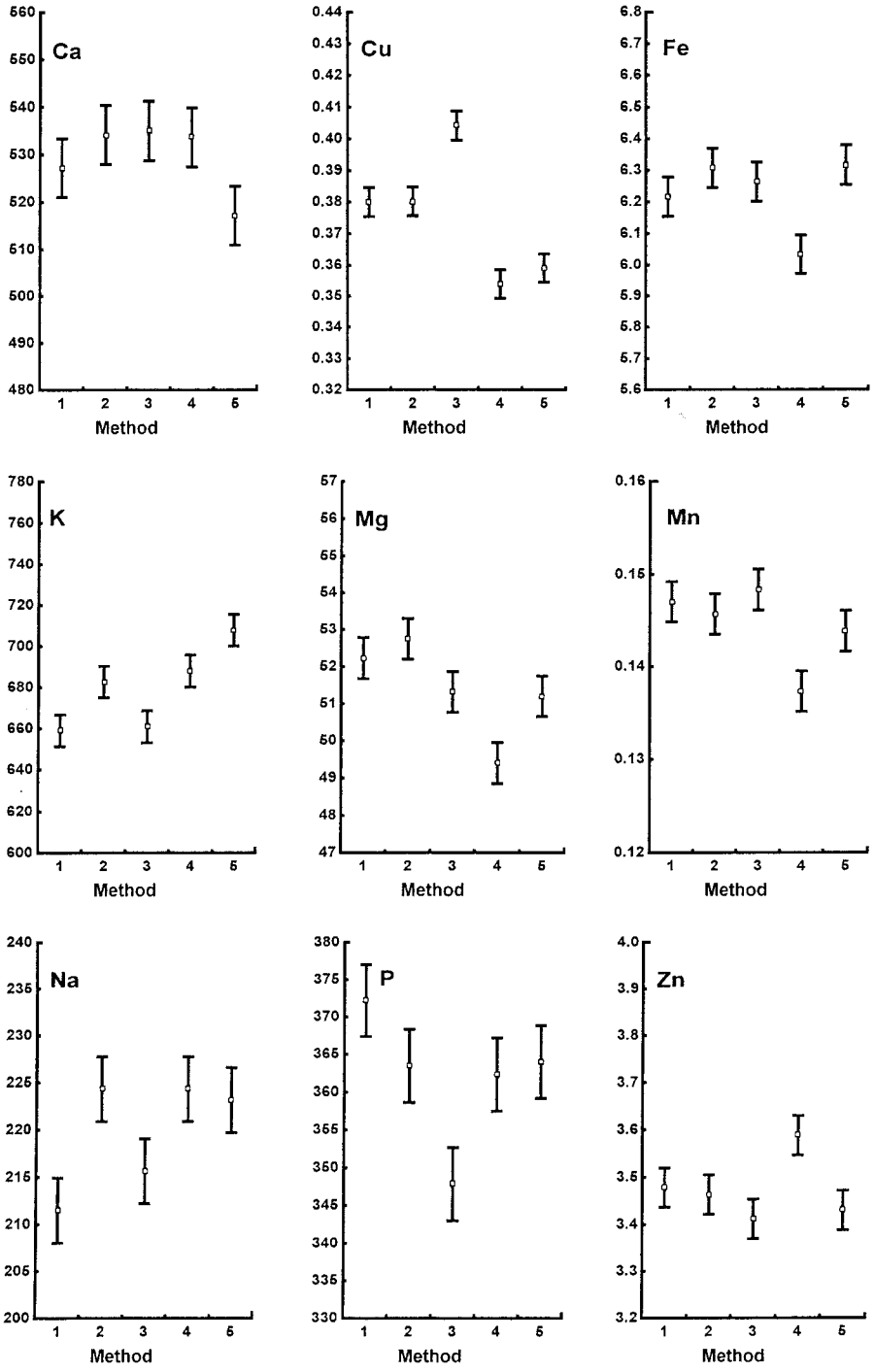

Fig 1. Comparison of methods. Error bar represents the least significant difference (LSD) limits derived from the standard error of the means ($p = 0.05$) (methods as described in Table 6; all units in mg/100 g).

The opaque, white appearance of milk results from spherical, hydrated aggregates of casein subunits, colloidal calcium phosphate and other ionic constituents, with micelle formation (30–300 nm) a function of Ca concentration (Creamer & MacGibbon, 1996; Lonnerdal & Atkinson, 1995). Since a clear, colourless solution is obtained by dilution of non-fat milk in the tertiary amine dispersant, it has been suggested that the reagent may function through dissociation of casein micelle macrostructure, thereby facilitating enhanced mobilisation of the casein associated elements (Ca, Mg, P and Zn) (Nobrega et al., 1997). In an attempt to test this hypothesis, a range of fractionated milk proteins were studied. Aqueous and tertiary amine slurry dispersion

techniques were compared against two independent laboratories employing conventional wet-digestion procedures. The proteins exhibited varying dispersant solubility, with caseinates entirely soluble in both solvents, yielding clear solutions. The total milk proteins produced relatively stable cloudy suspensions, while the caseins remained substantially insoluble in water, yet soluble in amine dispersant (after incubation at 60°C for 3 h). Table 7 summarises the results for three representative protein fractions.

There was substantial between-method comparability for all elements in the case of the fully soluble caseinate. However, the aqueous slurry protocol demonstrated unreliability, in particular for the caseins. This could be principally attributed to sample insolubility, compounded by reduction in Lu signal, suggesting its surface adsorption on insoluble casein. The equivalent data obtained for all proteins between direct dispersion in CFA-C and independent methods based on complete sample digestion, suggests that the tertiary amine indeed functions through a casein dissociative mechanism, which operates even in intact milk.

Table 7
Comparison of techniques for milk proteins (mg/100 g)

	[1[a]]	[2]	[3]	[4]
Calcium caseinate[b]				
Ca	1430	1350	1300	1470
Cu	0.06	0.07	0.12	0.11
Fe	0.9	0.8	1.0	nd[e]
K	9	2	3	5
Mg	9	8	9	10
Mn	0.099	0.093	0.101	0.091
Na	5	4	5	8
P	770	740	740	740
Zn	4.0	3.8	4.2	4.2
Total milk protein[c]				
Ca	1520	1450	1420	1560
Cu	0.06	0.05	0.10	0.08
Fe	1.4	1.3	1.5	nd
K	1	1	2	5
Mg	11	10	11	12
Mn	0.113	0.100	0.108	0.092
Na	5	4	4	10
P	710	680	680	630
Zn	2.0	3.0	2.3	1.8
Rennet casein[d]				
Ca	2850	2790	2710	2570
Cu	0.08	0.08	0.12	0.29
Fe	0.3	0.4	0.6	nd
K	14	18	16	78
Mg	94	92	98	176
Mn	0.118	0.113	0.118	nd
Na	6	10	9	75
P	1720	1700	1670	1340
Zn	11.4	10.8	11.3	0.9

[a] 1: microwave acid digestion, ICP–OES, ICP-MS for Cu, Zn, Mn (independent laboratory 1); 2: wet digestion, ICP–OES (independent laboratory 2); 3: direct slurry aspiration in CFA-C, ICP–OES; 4: direct slurry aspiration in H_2O, ICP–OES.

[b] Soluble, clear solution in both water and CFA-C dispersants.

[c] Partially soluble, cloudy suspension in both dispersants.

[d] Soluble in CFA-C dispersant, insoluble in water (60°C, 3 h).

[e] nd, < limit of detection.

For wholemilk and high-fat infant formula samples, the slurries have a cloudy appearance, indicating that lipid remains, at least partially, as an emulsion. However, since such slurries are stable over time, with no evidence of stratification, analytical performance remains uncompromised. It is particularly noteworthy that recoveries of the elements known to associate with the fat fraction (Zn, Cu and Fe) are quantitative, supporting the view that the amine dispersant may further function to dissociate interactions between protein, lipid and mineral.

As summarised previously, a direct dispersion sample pretreatment in conjunction with a simultaneous charge-transfer detector based ICP-OES, facilitates significant operational advantages for milk and infant formula analysis (McKinstry et al., 1999). The direct introduction of intact milk dilutions into a plasma source relies on the integration of sample destruction, atomisation and excitation, essentially within a single operation. There are several physical attributes of a slurry which influence its stability, homogeneity, transport, and nebulization efficiency and these properties have been reviewed recently (Ebdon, Foulkes & Sutton, 1997).

The analysis of milk by direct nebulization of aqueous solutions into the ICP has previously been reported to be compromised by poor accuracy and sensitivity, attributed both to fat content and aerosol droplet size (Coni et al., 1990; Emmett, 1988). In contrast, other studies have emphasised the importance of ensuring milk protein dissolution under alkaline slurry conditions (Dean, Ebdon & Massey, 1987; Sparkes & Ebdon, 1986; Sturup & Buchert, 1996). The restricted applicability of these strategies has been variously attributed to memory effects, polyatomic interferences and partial analyte insolubility. The water-soluble tertiary amine:EDTA dispersant utilised in the present study overcomes most matrix associated deficiencies of earlier direct dispersion schemes and confirms the efficacy of this reagent as reported recently (Nobrega et al., 1997). The present study has demonstrated the technique to be applicable to a wide range of milks, milk proteins and infant formulas, although certain dry-blended formulas containing supplemental calcium carbonate remain intractable to this protocol.

Despite the complexity of the milk colloidal system, this study has demonstrated successful slurry aspiration during pneumatic nebulization. Milk is considered an ideal candidate for such a sampling protocol, since its particle size generally falls below 5μm (Guo et al., 1998; Sparkes & Ebdon, 1986). The cone-spray, high solids nebuliser has been demonstrated in this study to tolerate

milk slurries containing 1% w/v solids, while others have sucessfully applied the technique with up to 10% w/v solids content (Sparkes & Ebdon, 1986). A recent study of slurry nebulization, as applied to environmental soil and sediment samples, found that extensive pretreatment and ultrasonication of the sample probe were mandatory to ensure slurry stability and homogeneity (Ploegaerts, Baeten & Hoenig, 1997). In contrast, the present study confirmed the stability of milk dispersions without ultrasonication, with no significant change in measured element concentrations over 48 h.

Provided a dilute sample slurry displays transport and atomisation efficiencies equivalent to those of working standards, then a direct external calibration may be appropriate (Ebdon et al., 1997; Sparkes & Ebdon, 1986). However, to account for potential sample matrix effects, an internal standard calibration technique using Lu was incorporated in the protocol, as described previously (McKinstry et al., 1999).

The potential of slurry nebulisation protocols, concurrent with recent developments in charge-transfer detectors, optics and nebulisers, has been identified in a recent review of plasma spectroscopy (Pennebaker et al., 1998). Although conventional mineralisation techniques overcome matrix associated effects through manipulative decomposition procedures, a direct slurry technique offers expedient advantages, provided its analytical performance may, as indicated in this study, be demonstrated to be independent of matrix effects.

4. Conclusion

The described method, based on a tertiary amine: EDTA sample dispersion protocol, demonstrates enhanced analytical performance attributable to its protein solvation properties, as compared to aqueous slurry nebulisation. Whether this enhancement is mediated through a true chaotropic or dissociative mechanism is not clear, but the technique is evidently applicable to routine compliance monitoring of most infant formulas. The recommended scheme is as direct as previous slurry methods, enhances overall slurry stability for a wide range of samples, results in higher recovery of specific elements (particularly Ca, Mg, Cu, Zn and P) and improves overall method precision.

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References

Albert, R., & Horwitz, W. (1997). A heuristic derivation of the Horwitz curve. *Analytical Chemistry, 69*(4), 789–790.

AOAC (1995). *Official methods of analysis* (16th ed.). Washington, DC: AOAC.

Arnaud, J., Bouillet, M. C., Alary, J., & Favier, A. (1992). Zinc determination in human milk by flameless atomic absorption spectrometry after dry-ashing. *Food Chemistry, 44*, 213–219.

Borkowska-Burnecka, J., Szmigiel, J., & Zyrnicki, W. (1996). Determination of major and trace elements in powdered milk by inductively coupled plasma atomic emission spectrometry. *Chemica Analityczna, 41*(4), 625–632.

Boyer, K., Horwitz, W., & Albert, R. (1985). Interlaboratory variability in trace element analysis. *Analytical Chemistry, 57*, 454–459.

Bratter, P., Blasco, I. N., & Negretti de Bratter, V. A. (1998). Speciation as an analytical aid in trace element research in infant nutrition. *Analyst, 123*, 821–826.

Coni, E., Bocca, A., Coppolelli, P., Caroli, S., Cavallucci, C., & Trabalza Marinucci, M. (1996). Minor and trace element content in sheep and goat milk and dairy products. *Food Chemistry, 57*, 253–260.

Coni, E., Stacchini, A., Caroli, S., & Falconieri, P. (1990). Analytical approach to obtaining reference values for minor and trace elements in human milk. *Journal of Analytical Atomic Spectrometry, 5*, 581–586.

Creamer, L. K., & MacGibbon, A. K. H. (1996). Some recent advances in the basic chemistry of milk proteins and lipids. *International Dairy Journal, 6*, 539–568.

Dean, J. R., Ebdon, L., & Massey, R. (1987). Selection of mode for the measurement of lead isotope ratios by inductively coupled plasma mass spectrometry and its application to milk powder analysis. *Journal of Analytical Atomic Spectrometry, 2*, 369–374.

de la Fuente, M. A., Guerrero, G., & Juarez, M. (1995). Manganese and zinc analysis in milk by microwave oven digestion and platform graphite furnace atomic absorption spectrometry. *Journal of Agricultural and Food Chemistry, 43*, 2406–2410.

de la Fuente, M. A. (1998). Changes in the mineral balance of milk submitted to technological treatments. *Trends in Food Science and Technology, 9*, 281–288.

de la Guardia, M., Salvador, A., Bayarri, P, & Farre, R. (1986). Rapid atomic spectrometric determination of sodium, potassium, calcium and magnesium in powdered milk by direct dispersion. *Analyst, 111*, 1375–1377.

Ebdon, L., Foulkes, M., & Sutton, K. (1997). Slurry nebulisation in plasmas. *Journal of Analytical Atomic Spectrometry, 12*, 213–229.

Emmett, S. E. (1988). Analysis of liquid milk by inductively coupled plasma mass spectrometry. *Journal of Analytical Atomic Spectrometry, 3*, 1145–1146.

Guo, M. R., Hendricks, G. M., & Kindstedt, P.S (1998). Component distribution and Interactions in powdered infant formula. *International Dairy Journal, 8*, 333–339.

Lonnerdal, B., & Atkinson, S. (1995). Nitrogenous Components of Milk. In R. D. Jensen, *Handbook of Milk Composition* (pp. 351–368). London: Academic Press.

May, R. J., & Smith, D. E. (1998). Effect of storage and various processing conditions on the amount of ionic calcium in milk. *Milchwissenschaft, 53*(11), 605–608.

McKinstry, P. J., Indyk, H. E., & Kim, N. D. (1999). The determination of major and minor elements in milk and infant formula by slurry nebulisation and inductively coupled plasma–optical emission spectrometry (ICP–OES). *Food Chemistry, 65*(2), 245–252.

Miller-Ihli, N. J. (1996). Trace element determinations in foods and biological samples using inductively coupled plasma atomic emission spectrometry and flame atomic absorption spectrometry. *Journal of Agricultural and Food Chemistry, 44*, 2675–2679.

Neville, M. C., Zhang, P., & Allenn, J. C. (1995). Minerals, ions and

trace elements in milk. In R. G. Jensen, *Handbook of milk composition* (pp. 577–592). London: Academic Press.

Nobrega, J. A., Gelinas, Y., Krushevska, A., & Barnes, R. M. (1997). Direct determination of major and trace elements in milk by inductively coupled plasma atomic emission and mass spectrometry. *J. Anal. At. Spect., 12*, 1243–1246.

Pennebaker, F. M., Jones, D. A., Gresham, C. A., Williams, R. H., Simon, R. E., Schappert, M. F., & Denton, M. B. (1998). Spectroscopic instrumentation in the 21st century: excitement at the horizon. *J. Anal. At. Spect., 13*, 821–827.

Ploegaerts, G., Baeten, H., & Hoenig, M. (1997). Introduction of solid environmental samples as a slurry into inductively coupled plasma-emission spectrometry: an alternative for routine analyses? *Analusis, 25*, 336–340.

Renner, E. (1983). Minerals and trace elements in milk. In E. Renner, *Milk and dairy products in human nutrition* (pp. 190–197). Germany: W-GmbH.

Renner, E., Schaafsma, G., Scott, K.J., Hurrell, R.F., Berrocal, R., Neeser, J.R., Schweizer, T.F., Hilpert, H., Traitler, H., Colarow, L., & Lindstrand, K. (1989). Micronutrients in milk and infant formula. In *Micronutrients in milk and milk-based food products*, E. Renner. Elsevier Applied Science, pp 23–32, 246–256.

Roig, M. J., Alegria, A., Barbera, R., Farre, R., & Lagarda, M. J. (1999). Calcium dialysability as an estimation of bioavailability in human milk, cow milk and infant formulas. *Food Chemistry, 64*, 403–409.

Ruiz, C., Alegria, A., Barbera, R., Farre, R., & Lagarda, M. J. (1995). Direct determination of calcium, magnesium, sodium, potassium and iron in infant formulas by atomic spectroscopy. Comparison with dry and wet digestions methods. *Die Nahrung, 39*, 497–504.

Sparkes, S., & Ebdon, L. (1986). Slurry atomisation for agricultural samples by plasma emission spectrometry. *Analytical Proceedings, 23*, 410–412.

Sturup, S., & Buchert, A. (1996). Direct determination of copper and iodine in milk and milk powder in alkaline solution by flow injection inductively coupled plasma mass spectrometry. *Fresenius Journal and Analytical Chemistry, 354*, 323–326.

Vuchkova, L., Margitova, L., & Arpadjan, S. (1996). Comparative study on the determination of trace elements in milk powder and cheese products using modifier-free ETAAS and ICP-AES. *Analytical Laboratory, 5*(1), 41–45.

Wagley, W., Schmiedel, G., Mainka, E., & Ache, H. (1989). Direct determination of some essential and toxic elements in milk and milkpowder by graphite furnace atomic absorption spectrometry. *Atomic Spectroscopy, 10*(4), 106–111.