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Analytical Methods Section

Laser ablation–inductively coupled plasma–mass spectrometry (LA–ICP–MS) for the multielemental analysis of biological materials: a feasibility study*

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Multielemental analyses of biological reference materials (hair, mixed diet and milk powder) by inductively coupled plasma–mass spectrometry (ICP–MS) were performed. Sample mobilisation directly from the solid by pulses from a free-running ruby laser was compared to conventional pneumatic nebulisation of the sample solution following acidic digestion. Although inferior in both accuracy and precision, laser ablation ICP–MS offered rapid semiquantitative analysis with little or no sample preparation.

INTRODUCTION

Inductively coupled plasma mass spectrometry (ICP–MS) is well established as a method for multielemental analysis and the determination of isotope ratios (Date & Gray, 1988; Durrant, 1992*a*). The advantages of the technique, such as sub-ng ml^{-1} detection limits, rapid samples throughput, wide linear dynamic range (at least six orders of magnitude) and direct calibration against aqueous standards, are widely acknowledged (Date & Gray, 1988; Hieftje & Vickers, 1989). However, samples are usually digested prior to analysis and these digestions present a number of difficulties. Any sample handling involves the risk of contamination or the loss of volatile elements or both. Sample matrices are typically dissolved in nitric acid. Use of this solvent for sample digestion is advantageous for ICP–MS analyses because it gives relatively few spectral interferences (Gray, 1988). However, some biological matrices resist digestion in nitric acid alone. For example, milk powder requires a multistage digestion using nitric acid, perchloric acid and hydrogen peroxide (Emmett, 1988).

To by-pass such difficulties, numerous sample introduction methods other than pneumatic nebulisation have been used. Electrothermal vaporisation (Gregoire *et al.*, 1992), direct sample insertion (Karanassios & Horlick, 1989), flow injection (Dean *et al.*, 1988), spark ablation (Ivanovic *et al.*, 1992), hydride generation (Wang *et al.*, 1988) and laser ablation (LA) (Gray, 1985) have all been used with ICP–MS. The last of these, which involves the removal of sample material directly from the solid by ablation with laser pulses, has received considerable recent attention (Van Heuzen, 1991; Durrant, 1992*b*), and geological applications have been the principal interest (Imai, 1990; Durrant & Ward, 1993). However, the only extant works on biological analysis by LA–ICP–MS are by Ward *et al.* (1990); Ward *et al.*, (1992) and Durrant (1992*b*). Of these, a multielemental analysis of leaf material, National Institute of Standards and Technology (NIST) 1571 Orchard Leaves and 1573 Tomato Leaves, was accomplished using an element-for-element calibration against a matrix-matched standard (Bowen's Kale). Similarly, 14 elements (Al, P, K, Ca, Cr, Mn, Fe, Ni, Cu, Zn, Rb, Sr, Ba and Pb) were determined in Japanese National Institute of Environmental Studies (NIES) Tea Leaves and Pepperbush, and nine elements (Na, Mg, Al, P, K, Cr, Mn, Fe and Zn) were determined in International Atomic Energy Agency (IAEA) All Milk Powder (Durrant, 1992*b*).

In this work, multielemental analyses of three biological reference materials by LA–ICP–MS are outlined. Comparison is made with conventional pneumatic nebulisation (PN–ICP–MS). Established digestion procedures were used for the latter. The reference materials analysed were: Chinese Reference Hair SINR 0920, NIST Mixed Diet 8431a and NIST Non-fat Milk

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Powder 1549. Although, as mentioned above, analysis of milk powder by LA–ICP–MS has been reported (Durrant, 1992*b*), new data are presented here. The aim is to illustrate the diversity of biological matrices that may be analysed by the technique, and to compare LA–ICP–MS with the more established PN–ICP–MS.

MATERIALS AND METHODS

Apparatus

PN–ICP–MS

A VG PlasmaQuad™ was used with a Jarrell Ash cross-flow nebuliser discharging into a Scott double-pass spray chamber. Rapid scan mode, using 2048 channels of the multichannel analyser, a dwell time of 250 μs per channel and 120 sweeps per analysis, was used. Other system parameters are given in Table 1.

LA–ICP–MS

A JK 2000™ ruby laser was used with the Surrey prototype ICP mass spectrometer. The ablation system has been described by Gray (1985), with modifications as reported elsewhere (Durrant, 1989; Ward *et al.*, 1992). Literature describing the mass spectrometer is also available (Date & Gray, 1983). System parameters are shown in Table 2.

The ion optics may be successfully optimised on the $^{12}C^+$ response, which results from CO_2 that is desorbed from the gas transfer lines and entrained from the atmosphere (Durrant, 1989; Ward *et al.*, 1992).

In principle, optimal plasma conditions—cell flow and forward r.f. plasma power—must be found for each matrix. Compromise conditions, based on previous signal-to-background response studies, were used in this work (Durrant, 1989; Ward *et al.*, 1992).

Sample preparation

PN–ICP–MS

Hair. Between 250 and 500 mg of the dried material was wet digested using concentrated nitric acid in Teflon digestion vessels at 450°C for 24 h.

Diet. A microwave-heating assisted nitric acid digestion described previously was used (Ward *et al.*, 1990).

Milk. The nitric acid–hydrogen peroxide–perchloric acid procedure reported by Emmett (1988) was used.

LA–ICP–MS

Materials were dried at 85°C for 2 h, then ball-milled in a tungsten mill with a carbonaceous binder (20%, m/m) for 5 min. The binder used was methyl methacrylate (Elvesite 2013). As mixed human diet and milk powder are rather pale, ablation by the laser is poor even at high (1 J) laser energies. To increase laser–sample coupling for these samples, 1% (m/m) of a high purity graphite was added to each, together with the binder, before ball-milling. The resulting powders were each pressed in aluminium X-ray fluorescence cups to 10 tonnes, producing a disc of about 3 cm diameter.

Table 1. System parameters for multielemental analysis with PN–ICP–MS

Spectrometer	VG PlasmaQuad™
Plasma conditions	
Coolant flow	14 litres min^{-1}
Nebuliser flow	0·75 litres min^{-1}
Forward r.f. plasma power	1·3 kW
Reflected power	< 20 W
Interface	
Loadcoil-extraction aperture separation	10 mm
Extraction aperture diameter	1·0 mm
Skimmer aperture diameter	0·7 mm
Ion optics	optimised on a multielement solution at *m/z* 59 (Co) and *m/z* 209 (Bi).
Sample introduction	
Solution uptake rate	1 ml min^{-1}

Procedure

PN–ICP–MS

Analyses at dilutions of 1 : 9 and 1 : 99 were made for minor and major elements, respectively. Multielement solutions at 100 ng ml^{-1} in 1% (v/v) nitric acid were used as standards. A blank of 1% (v/v) nitric acid was also used. Reagent blank corrections were made after the concentration calculations had been performed.

LA–ICP–MS

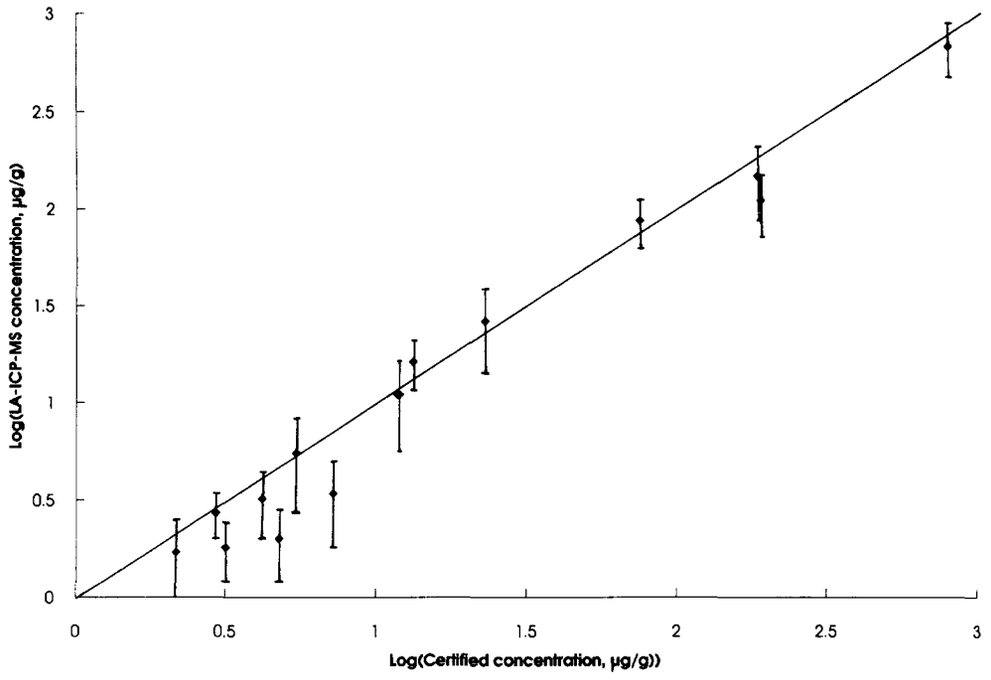
To obtain quantitative data, element-for-element calibration against individual sensitivities from the certified values of elements in matrix-matched standards was

Table 2. System parameters for multielemental analysis with LA–ICP–MS

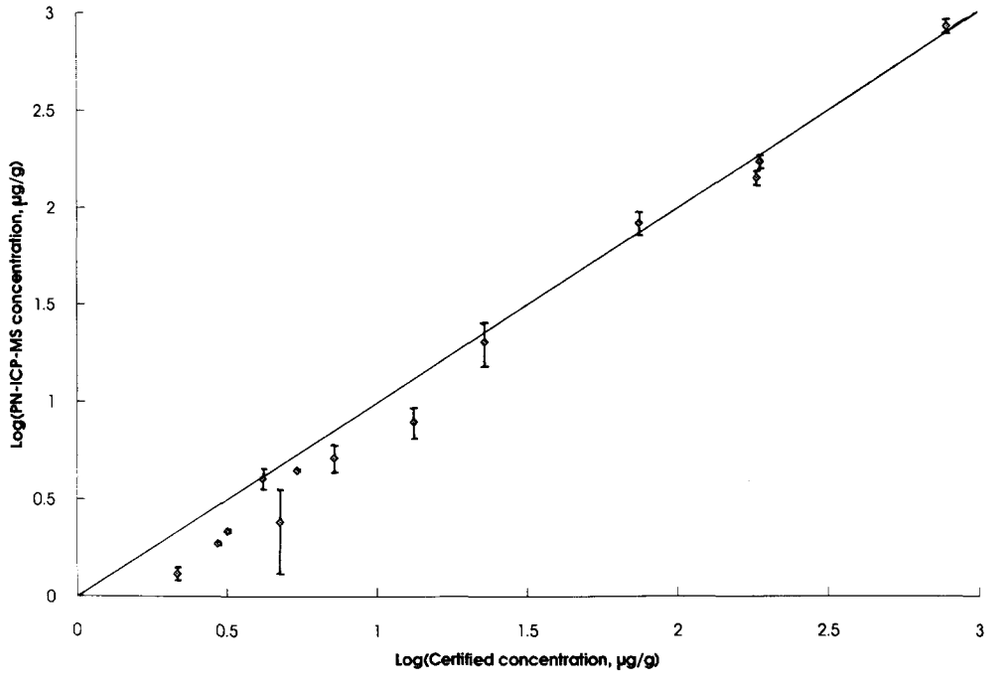
Spectrometer	Surrey prototype
Plasma conditions	
Coolant flow	14 litres min^{-1}
Cell flow	0·8 litres min^{-1}
Forward r.f. plasma power	1·5 kW
Reflected power	< 20 W
Interface	
Loadcoil-extraction aperture separation	10 mm
Extraction aperture diameter	1·0 mm
Skimmer aperture diameter	0·7 mm
Ion optics	Optimised on $^{12}C^+$
Scan details	
m/z range	4–240
Dwell time per channel	50 μs
Sweeps per integration	300 or 600
Laser	JK 2000™ ruby using free-running pulses

Ablation regime

Sample	Laser pulses (J)	Standard
SINR 0920 (hair)	6 × 0·3	NIES 5
NIST 8431a (diet)	20 × 0·1	IAEA H-9
NIST 1549 (milk)	10 × 1	IAEA A11



(a)



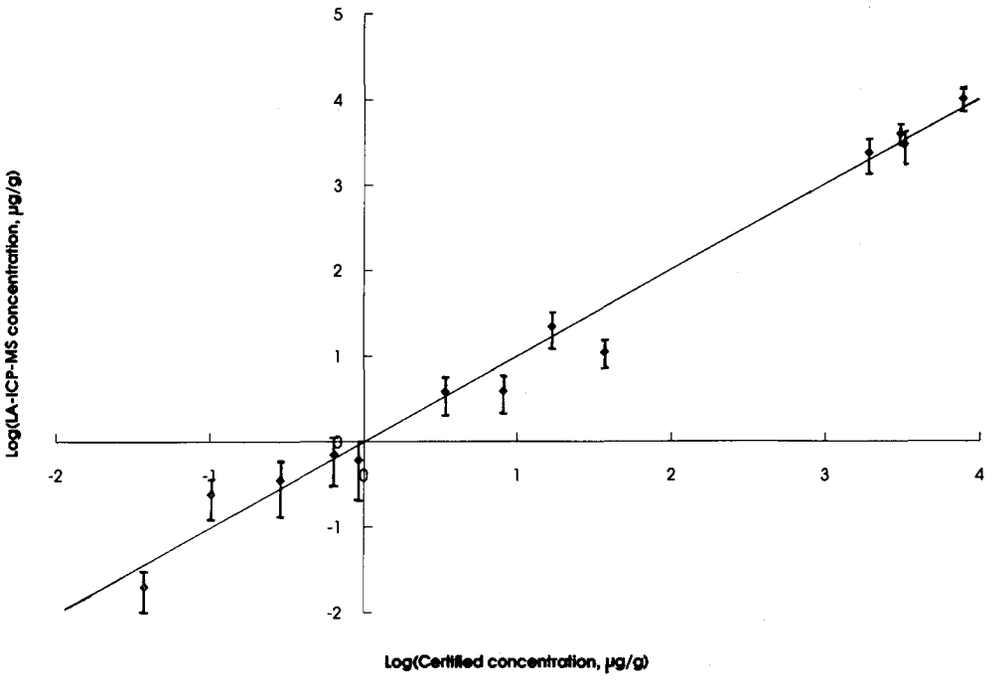
(b)

Fig. 1. (a) log (LA–ICP–MS concentration) and (b) log (PN–ICP–MS concentration) as a function of log (certified concentration) for the elements Mg, Al, P, K, Ca, Cr, Mn, Ni, Cu, Zn, Sr, Ba, Hg and Pb in SINR 0920 Chinese reference hair. Element-for-element calibration based on responses from NIES 5 human hair reference material, with full internal standardisation based on ^{57}Fe (r^2 = 0·971 and 0·992 for (a) and (b), respectively).

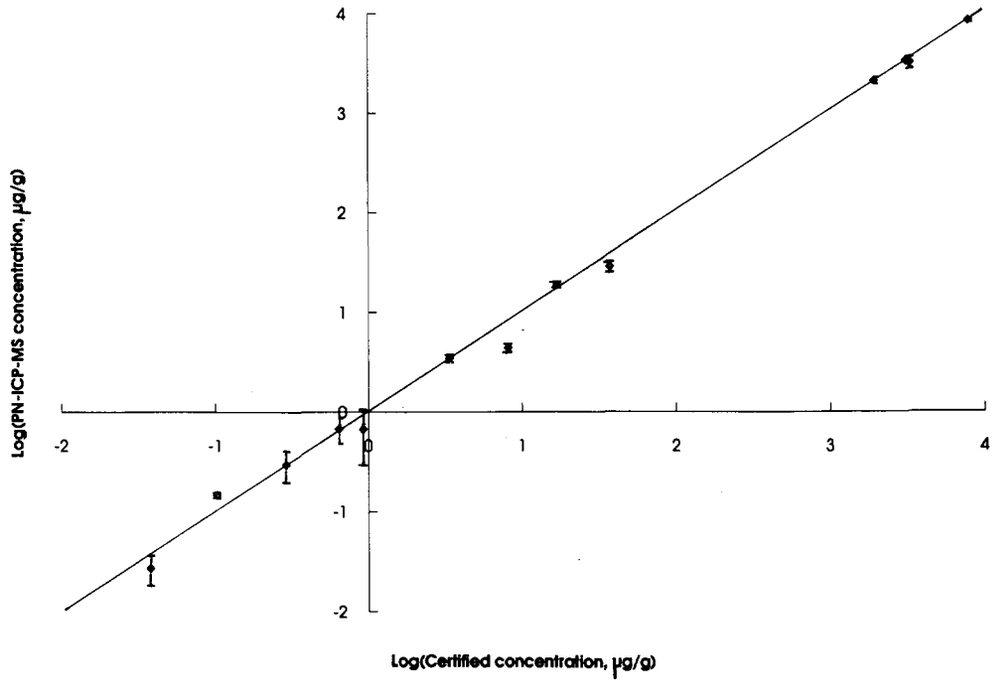
used. For the hair, diet and milk these were Japanese NIES SRM no. 5, IAEA H-9 and IAEA RM A11, respectively.

The laser energy is set empirically to give good signal-to-background ratios while avoiding saturation effects. Higher laser energies lead to material condensing on the cone or skimmer aperture or both, and may also overload the plasma, thus overwhelming its ionising capacity. At a lower mass transfer rate a less serious effect, the saturation of individual responses of isotopes of the matrix, may occur. This is tolerable if these isotopes are not of analytical interest, or if an isotope of lower abundance is available. The ablation regime used with each sample is given in Table 2.

Full internal standardisation was employed. This involved normalisation of the integrals obtained by replicate analyses (of a standard or sample) to a selected integral, and normalisation of sensitivities from



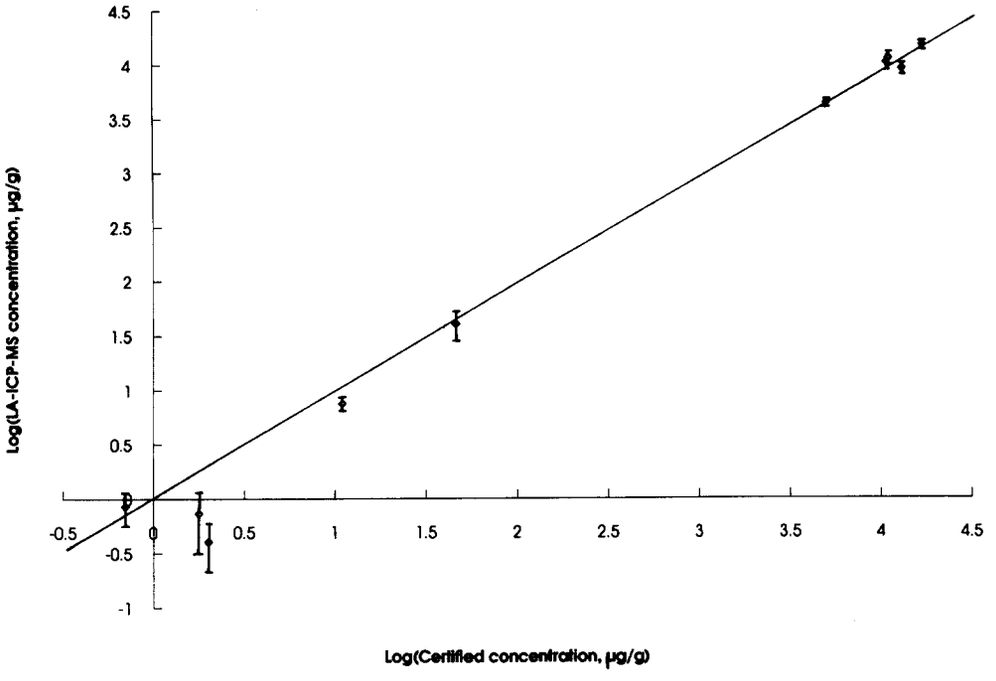
(a)



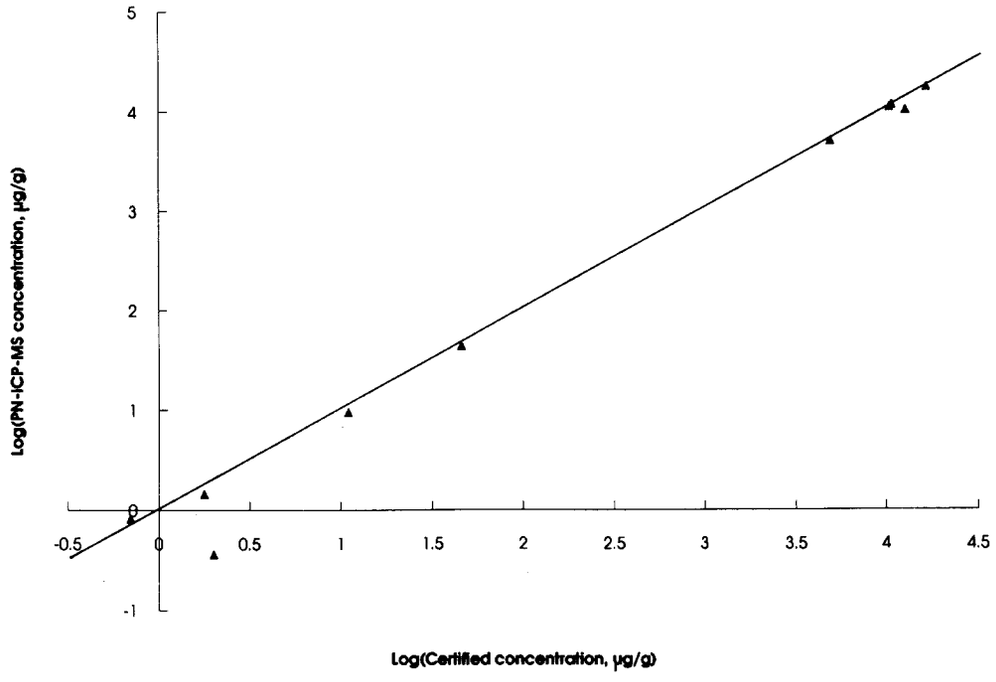
(b)

Fig. 2. (a) log (LA–ICP–MS concentration) and (b) log (PN–ICP–MS concentration) as a function of log (certified concentration) for the elements Na, P, K, Ca, Cr, Mn, Fe, Co, Ni, Cu, Zn, As and Mo in NIST 8431a mixed diet. Element-for-element calibration based on IAEA H-9 mixed human diet, with full internal standardisation based on ^{26}Mg (r^2 = 0·984 and 0·997 for (a) and (b) respectively).

a known elemental concentration in the sample. The former *between-replicate* internal standardisation should improve (reduce) precisions, percentage relative standard deviations, by accounting for local changes in sample density or laser energy between replicates. This type of internal standardisation is simple to apply because the concentration of the element used for internal standardisation need not be known. A minor isotope of a major element may often be used, for example, ^{26}Mg or ^{57}Fe. Such elements are also easy to determine by other methods, such as atomic absorption spectrometry, allowing *sample-to-standard* internal standardisation, which is based on a known concentration in both standard and sample, and should improve accuracy. An alternative to measuring the concentration of the element chosen as an internal standard is to add an exact, known mass of a suitable element at the milling stage. A preliminary LA–ICP–MS analysis of



(a)



(b)

Fig. 3. (a) log (LA–ICP–MS concentration) and (b) log (PN–ICP–MS) as a function of log (certified concentration) for the elements Na, Al, P, Cl, K, Ca, Fe, Cu, Zn and Rb in NIST 1549 non-fat milk powder. Element-for-element calibration based on IAEA A11 milk powder, with full internal standardisation based on ^{26}Mg (r^2 = 0·989 and 0·988 for (a) and (b), respectively).

the original material would show whether the internal standard element was present. This might prove a more convenient approach to the analysis of practical samples.

Only a limited number of concentrations are reported; the criteria used were that each element be certified at a sufficiently high concentration in the calibration standard to obtain reasonable sensitivity and that its concentration be known in the sample material. Thus, poor agreement between the LA–ICP–MS and the certified concentrations of the test materials would likely be due to errors in the former.

RESULTS AND DISCUSSION

Concentration data obtained for the hair, diet and milk matrices are shown in Figs 1(a,b), 2(a,b) and 3(a,b), respectively. Plots of the logarithm (base 10) of the determined concentrations are given as a function of

the logarithm of the certified concentrations. LA–ICP–MS data and PN–ICP–MS data are displayed in graphs designated (a) and (b), respectively.

From the figures we see that the LA–ICP–MS data are, in general inferior in both accuracy and precision to those obtained by PN–ICP–MS. Thus, the PN–ICP–MS data points are usually closer to the ideal line (gradient 1, passes through origin). This is reflected in the least squares fit correlation coefficients (figure captions). Only for milk powder are the values of r^2 for the LA–ICP–MS and PN–ICP–MS data similar. The hair and diet samples are complex matrices. The latter contains fats, sugars and proteins, which are perhaps not well mixed by the ball-milling procedure. Moreover, laser–sample coupling is not strong, even though graphite is included in the mix. High repetition rate Nd : YAG lasers operating in the Q-switched mode may be more effective here. However, use of Q-switched pulses may also result in greater memory effects, probably due to the resuspension of ablated material deposited on the cell walls and gas lines to the ICP. This difficulty, therefore, will also have to be addressed.

The error bars (one standard deviation, $n = 4$) of the LA–ICP–MS data points are typically larger than those obtained with PN–ICP–MS. No error bars are shown in Fig. 3b because they typically fall within each data symbol. For the hair analysis, precisions of 20 to 40% relative standard deviation (RSD) were obtained by LA–ICP-MS, which compares unfavourably with the typical 2 to 10% RSD obtained by PN–ICP–MS. For mixed diet the RSDs were typically 20 to 40% and 5 to 10% for LA–ICP–MS and PN–ICP–MS, respectively. For milk powder the respective RSDs were typically *c.* 10% and *c.* 5%.

As would be expected the concentration data tend to show poorer precisions at lower concentrations. The inferiority of the LA–ICP–MS precisions results from the variation of the ablation process, which depends upon many laser and sample characteristics. Obtaining identical ablated masses requires identical laser energies, polarisations and angles of incidence. The sample must have a uniform surface, density, thermal conductivity, etc. Even though sample and standard are matrix-matched, and full internal standardisation is used, this ideal is not closely realised. It should be remembered too, that trace elements may not be homogeneously distributed within the test material or standard matrices and that the ablated mass is very small (sub-mg per analysis). This mass may be compared to the minimum 250 mg or so typically recommended as a sample mass when analysing biological reference materials.

CONCLUSIONS

Semiquantitative analyses of diverse biological matrices are possible by LA–ICP–MS based on element-for-element calibration, using matrix-matched standards. Precisions, however, are generally inferior to those obtained by PN–ICP–MS.

The difficulties of finding well characterised standards are well known and have been discussed previously (Ward *et al.*, 1992). However, the speed and ease of sample preparation and the high throughput may offset these disadvantages in particular applications. More specifically, pale matrices show poor laser–sample coupling, which is only partly improved by the incorporation of a dark material (Durrant, 1992*b*). Complex matrices containing sugars, fats and proteins yield less accurate concentration data than simpler materials. The next challenge is to improve the quality of the analysis of such complex materials by LA–ICP–MS.

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REFERENCES

Date, A. R. & Gray, A. L. (1983). Development progress in plasma source mass spectrometry. *Analyst*, **108**(1283), 159–65.

Date, A. R. & Gray, A. L. (eds) (1988). *Applications of Inductively Coupled Plasma Source Mass Spectrometry*. Blackie, Glasgow, pp. 254.

Dean, J. R., Ebdon, L., Crews, H. M. & Massey, R. C. (1988). Characteristics of flow injection inductively coupled plasma mass spectrometry for trace analysis. *J. Anal. At. Spectrum*, **3**(2), 349–54.

Durrant, S. F. (1989). The application of laser ablation for solid sample introduction to inductively coupled plasma-source mass spectrometry. PhD Thesis, University of Surrey, Guildford, UK.

Durrant, S. F. (1992*a*). Inductively coupled plasma–mass spectrometry for biological analysis. *Trends in Anal. Chem.*, **11**(2), 68–73.

Durrant, S. F. (1992*b*). Multi-elemental analysis of environmental matrices by laser ablation inductively coupled plasma mass spectrometry. *Analyst*, **117**(10), 1585–92.

Durrant, S. F. & Ward, N. I. (1993). Rapid multielemental analysis of Chinese reference soils by laser ablation inductively coupled plasma-source mass spectrometry. *Fresenius' J. Anal. Chem.*, **345**(7), 512–17.

Emmett, S. E. (1988). Analysis of liquid milk by inductively coupled plasma mass spectrometry. *J. Anal. At. Spectrom.*, **3**(8), 1145–6.

Gray, A. L. (1985). Solid sample introduction by laser ablation for inductively coupled plasma source mass spectrometry. *Analyst*, **110**(5), 551–6.

Gray, A. L. (1988). Inductively coupled plasma source mass spectrometry. In *Inorganic Mass Spectrometry*, ed. R. Gijbels & R. Van Grieken. John Wiley, New York, Ch. 6, pp. 257–300.

Gregoire, D. C., Lamoureux, M., Chakrabarti, C. L., Al-Maawali, J. & Byrne, J. P. (1992). Electrothermal vaporization for inductively coupled plasma mass spectrometry and atomic absorption spectrometry: Symbiotic analytical techniques. *J. Anal. At. Spectrom.*, **7**(4), 579–85.

Hieftje, G. M. & Vickers, G. H. (1989). Developments in

plasma source/mass spectrometry. *Anal. Chim. Acta*, **216**(1/2), 1–24.

Imai, N. (1990). Quantitative analysis of original and powdered rocks and mineral inclusions by LA–ICP–MS. *Anal. Chim. Acta*, **235**(2), 381–91.

Ivanovic, K. A., Coleman, D. M., Kunz, F. W. & Schuetzle, D. (1992). Spark ablation of solid samples for analysis by inductively coupled plasma–mass spectrometry. *Appl. Spectrosc.*, **46**(6), 894–9.

Karanassios, V. & Horlick, G. (1989). A computer-controlled direct sample insertion device for inductively coupled plasma–mass spectrometry. *Spectrochim. Acta*, **44B**(12), 1345–60.

Van Heuzen, A. A. (1991). Analysis of solids by laser ablation inductively coupled plasma mass spectrometry (LA–ICP–MS)–I. Matching with a glass matrix. *Spectrochim. Acta*, **46B**(14), 1803–17.

Wang, X., Viczian, M., Lasztity, A. & Barnes, R. M. (1988). Lead hydride generation for isotope analysis by inductively coupled plasma mass spectrometry. *J. Anal. At. Spectrom.*, **3**(6), 821–7.

Ward, N. I., Abou-Shakra, F. R. & Durrant, S. F. (1990). Trace elemental content of biological materials: A comparison of NAA and ICP–MS analysis. *Biol. Trace Elem. Res.*, **26–27**, 177–87.

Ward, N. I., Durrant, S. F. & Gray, A. L. (1992). Analysis of biological standard reference materials by laser ablation inductively coupled plasma mass spectrometry. *J. Anal. At. Spectrom.*, **7**(7), 1139–46.