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Analytical Methods Section

Sample preparation and handling

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Ideally sample preparation converts the sample matrix to a form which during analysis minimises any dependency of analyte response upon variations in sample composition without losses of endogenous analyte or adventitious contamination. Examples of the practical limitations to achieving this ideal are discussed with reference to trace element analyses of body tissues, tissue-fluids, excreta and of diets by using atomic spectrometry.

Simple procedures with minimum handling are recommended for those analyses for which contamination is the major problem. For example, aluminium may be measured in serum and milk, by electrothermal atomisation and atomic absorption following dilution with water and chemical modification with oxygen in the graphite tube furnace. More complex procedures such as acid digestion in open vessels or in sealed vessels at high pressures are used for measurements of selenium or mercury in tissues, diets etc., which are seriously affected by matrix interferences and volatilisation losses.

1 INTRODUCTION

Ideally, sample preparation will convert the sample matrix to a form which, during analyses, minimises any dependency of analyte response upon variations in sample composition. Furthermore this will be accomplished without either losses of endogenous analyte or adventitious contamination. In practice this ideal is approached only with careful attention to detail, a high degree of practical skill and a good understanding of the physical and chemical variables which affect the preparation and measurement components of the analytical method. The procedures used to prepare samples for analysis are determined by the physical and chemical properties of both the sample and the analyte, the concentration of the analyte and the sensitivity and specificity of the analytical technique. These procedures range from a very simple aqueous dilution to acid digestion followed by some means of separating the analyte from the bulk matrix. Examples of these procedures will be discussed with reference to elemental analysis by plasma source mass spectrometry and by atomic absorption spectrometry, which together encompass the most commonly used analytical methods for studying micronutrients in foods, beverages and biological tissues.

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2 SIMPLE DILUTION METHODS

Accurate analyses by atomic spectrometric methods which use pneumatic nebulisers for introduction of aerosols of samples require the distribution of diameters of aerosol droplets of samples and standards to be closely matched. Droplet-size distribution is affected by the surface tension and viscosity of the liquid nebulized. For many liquid samples, e.g. milk, whole blood, blood serum, a simple dilution will suffice to match surface tension and viscosity to those of the calibrating standards. The classic example of this is the measurement of copper and zinc in blood serum by flame atomic absorption spectrometry (FAAS) following dilution with 6% v/v butan-1-ol reported some twenty years ago (Meret & Henkin, 1971). This method has proved extremely robust and reliable in this laboratory over that time using a 1 + 9 dilution for blood-serum and a 1 + 24 dilution for whole-blood.

The sensitivity of FAAS limits the range of applications of high dilutions. The much more sensitive technique of inductively coupled plasma source mass spectrometry (ICP-MS) enables many elements to be determined in solution at nanomolar concentrations (low micrograms per litre). The data in Fig. 1 were obtained with a 1 + 24 dilution of whole blood using a diluent containing 1% v/v Triton X100 plus $NH_4OH/(NH_4)_2EDTA/(NH_4)_2HPO_4$ (Delves, 1988). These show elemental concentrations ranging from nanomolar (I, Ba, Sn) via micromolar (Pb, Rb, Zn, Cu, Br) to millimolar (Na,

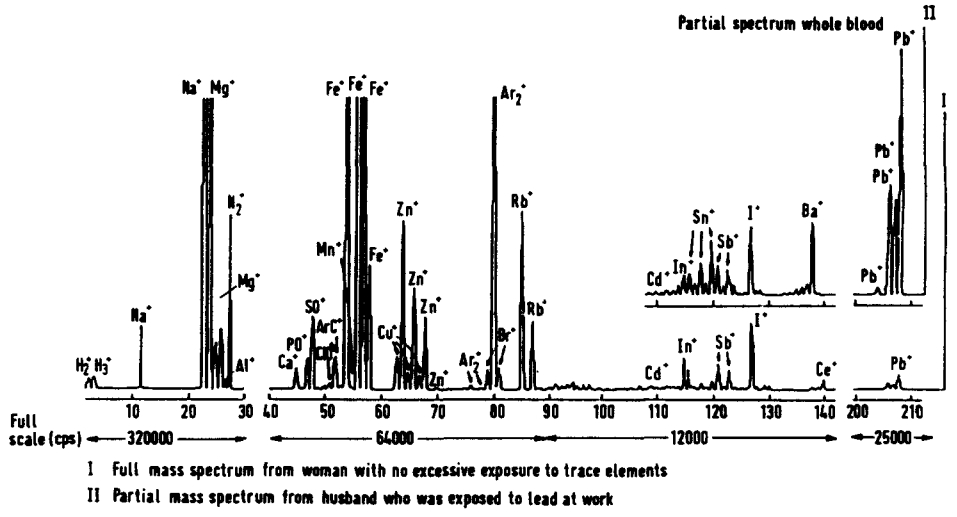

Fig. 1. Mass spectrum of whole blood (reproduced from *Chemistry in Britain*, 1988, **24**, 1009–1012 with permission of RSC).

Mg, Ca, Fe). These data were generated over a 10-min period at an uptake of 0·8 ml min^{-1} of diluted sample which represents only 320 μl whole-blood. This simple preparation, with suitable internal standardisation, affords accurate analyses of total concentrations of most elements >80 amu. At lower masses there are many matrix-derived mass spectral overlaps, discussion of which is beyond the remit of this paper. This preparation procedure enables diluted blood specimens to be nebulised using a Meinhard nebuliser for at least 5 h without blockage. This procedure has been used successfully for accurate measurements of lead isotope ratios in blood, diets and environmental samples for source apportionment studies (Campbell & Delves, 1989).

3 DILUTION AND CHEMICAL MODIFICATION OF SAMPLE MATRIX FOR ELECTROTHERMAL ATOMISATION AND ATOMIC ABSORPTION (ETA-AAS)

Few clinical/nutritional laboratories have ICP-MS instrumentation so that there are relatively few published applications compared with those reporting data obtained by the equally sensitive and most commonly used technique of atomic absorption spectrometry using electrothermal atomisation (ETA-AAS). Most of the sample preparation procedures for ETA-AAS are concerned with overcoming physical and chemical interferences in either condensed phase—liquid/solid—or in the vapour phase. Chemical modification of the matrix to control these interferences is done using either liquid or gaseous reagents.

3.1 Gaseous phase modification of matrix

Elemental analyses of organic-rich matrices by ETA-AAS require some degree of sample oxidation. A simple but often neglected way to achieve this without contamination is to oxidise the matrix using an O_2 atmosphere in the graphite tube furnace (Beaty & Cooksey, 1978). At temperatures of 500–550°C, and with a low volume flow (50 ml min^{-1}) of O_2 within the graphite tube furnace the organic constituents of 2 μl whole blood (10 μl of 1 + 4 dilution) will be completely oxidised in about 30 s. The significant reduction in the subsequent background interferences from light scattering by carbonaceous residues is only one advantage. The major advantage of ashing with oxygen is the complete elimination of any accumulation of carbonaceous residues which are always present following pyrolysis in an argon atmosphere. Depending upon the number and type of specimens analysed, these residues affect to varying degrees the rates of carbothermal reduction of condensed and vapour phase metal oxides to gaseous free atoms. Elimination of the residues with oxygen ashing leads to more reproducible rates of atom formation. It is of course essential to remove all chemisorbed oxygen from the graphite as CO by heating at 950°C with an argon internal gas flow (300 ml min^{-1}) for 25 s. Failure to do this will result in rapid degradation of the graphite surface. With correct desorption of CO, tube life-times in excess of 200 firings are easily achieved for determinations such as lead in blood (Shuttler & Delves, 1986).

3.2 Liquid phase chemical modification for ETA-AAS

At temperatures used for ashing and desorption of O_2 (500–950°C) many elements, e.g. Pb, Cd, Se, have partial vapour pressures which are sufficient to cause severe analyte losses. The addition of reagents, in the liquid phase, to convert volatile elements to thermally stable compounds was initially proposed by Ediger (1975) who described this as matrix modification. One

of the earlier used modifiers—$NH_4H_2PO_4$—serves a dual purpose in analysis of foods and biological samples: the removal at low temperatures of chlorine as volatile NH_4Cl and the formation of phosphates of elements such as Pb, Cd which are thermally stable at 850–950°C. The use of Pd as a 'universal modifier' has received much attention since its original application by Shan and Ni (1987). The reduction by the graphite surface of Pd salts to metallic Pd enables many elements, e.g. Se, Te, to form intermetallic compounds which are thermally decomposed only at temperatures in excess of 1000°C, thus allowing electrothermal/chemical decomposition of the sample matrix without their loss.

The influence of the residual matrix after ashing on analyte sensitivity by ETA-AAS, called rotational interferences, may also be overcome by the addition of a suitable chemical modifier. The use of $Mg(NO_3)_2$ in the analysis of Al in serum achieves two goals. Firstly it prevents volatilisation losses of $Al(OH)_2^+$ species and secondly it forms a spinel type compound ($MgOMgAl_2O_4$) which stabilises aluminium to ~1300°C (Kantor *et al.*, 1983). The net effect is a compensation of rotational interferences so that calibration curves obtained with a serum matrix are parallel to that from an aqueous standard solution (Fig. 2) (Fellows, 1990).

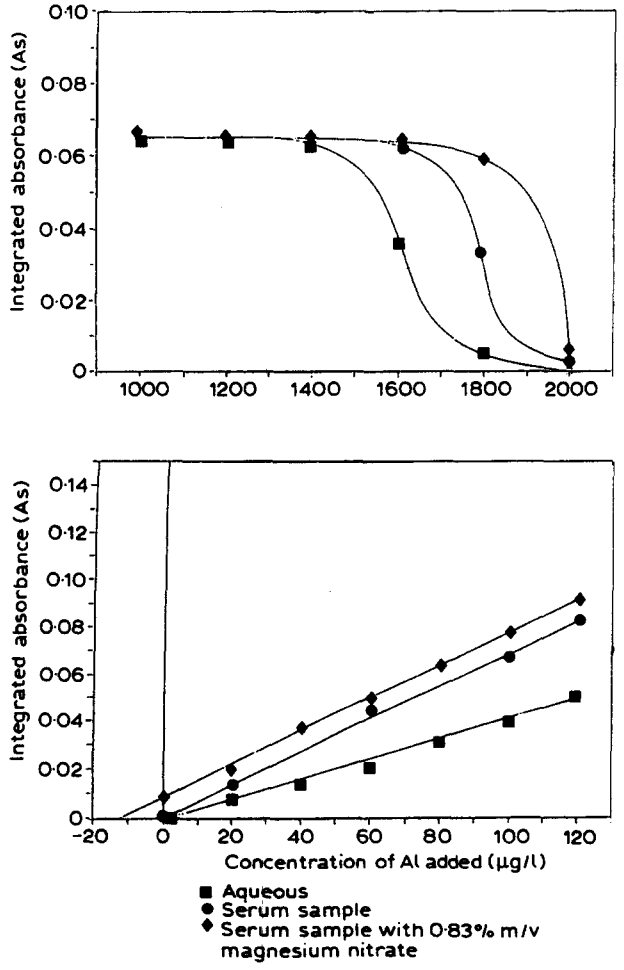

Fig. 2. $Mg(NO_3)_2$ as chemical modifier for aluminium.

It must be remembered that modification of the chemical properties of the matrix is only part of the overall strategy of sample preparation for ETA-AAS. Differences in surface tension and viscosity between sample solutions and calibrating standards demand different conditions for accurate and precise deposition of microlitre volumes into the furnace. It is therefore often more convenient to use matrix-matched standards for calibration. For example, measurements of very low levels of Al in serum are easily accomplished by using a simple 1 + 2 aqueous dilution and by calibrating against a similarity diluted pool of bovine serum with serial additions of Al equivalent to 5 to 30 µg/litre. Chemical modification is done using gaseous O_2 followed by desorption of CO as described earlier and a L'vov platform is used with the ETA-AAS system. The method is accurate (Fig. 3) and has a within-run precision (RSD) of 7% at 4 µg/litre. This level of analytical performance has enabled the observed small increases in serum Al following the consumption of soya milk, citrated fruit juices and other foods without any added aluminium to be measured accurately for bioavailability studies (Sieniawska, 1990).

3.3 L'vov platform, ETA-AAS

A L'vov platform (1978) is a small 15 mm × 4 mm × 0·8 mm rectangular disc of pyrolytic graphite with a central trough for sample containment. The platform is placed within a graphite tube furnace and ideally is heated only by radiation from the tube wall which is electrically heated. The role of the L'vov platform in ETA-AAS is to delay atom formation until the spatial and temporal variations in the temperature within the graphite furnace are minimal. In this way the temperature-dependent rates of chemical reactions and of diffusion which affect free atom populations are controlled as closely as possible. It has been shown that

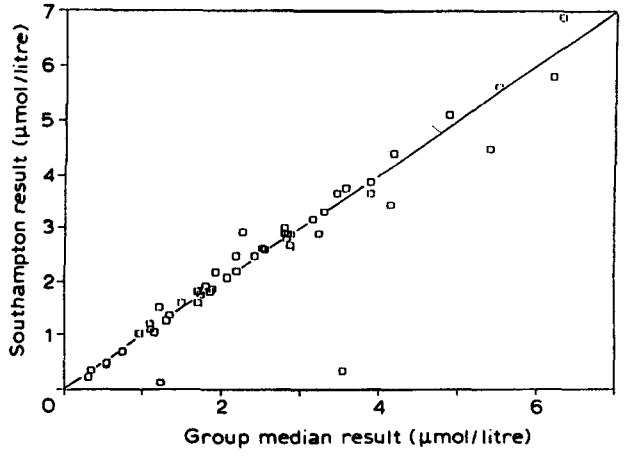

Fig. 3. Serum aluminium external quality assurance, April 1989–February 1991.

the optimum 'time window' for measurement of free atoms in a graphite tube furnace is between 1·1 and 3·0 s after initiation of the atomisation stage (Falk, 1988). The atom profiles of most analyte elements may be arranged to occur within this time window, by appropriate combinations of electrothermal conditions and chemical modifications.

4 ACID DIGESTION

Most solid samples of foods and biological specimens may be converted to relatively simple solutions amenable to trace element analysis by digestion with a mixture of nitric and sulphuric acids (19 + 1) in open vessels at temperatures of 150°C. Typical quantities would be 1 g sample, 5 ml conc. HNO_3, 0·25 ml conc. H_2SO_4. This has proved successful for measurement of aluminium in a wide range of diets and food products (Delves *et al.*, 1989). For many elements it is essential to use high purity reaction vessels, e.g. quartz conical flasks, to avoid contamination. Other suitable materials which include glassy carbon and perfluoralkenes (at lower temperatures) have been discussed in excellent reviews by Angerer and Schaller (1988) and Knapp (1988). It has long been claimed that in order to measure selenium accurately in biological materials or foods by hydride generation and atomic absorption it is necessary to incorporate perchloric acid in the digestion process. However, detailed comparisons of HNO_3/H_2SO_4 and $HNO_3/HClO_4/H_2SO_4$ digestion mixtures have shown that $HClO_4$ is not needed for accurate measurements of selenium in liver, skin, diets, faeces, kidney, urine and blood (Fairhurst *et al.*, 1987). In view of the potential hazard of using $HClO_4$ this is somewhat reassuring.

The biggest disadvantage of open-vessel digestion is the long digestion times and the need to use relatively large volumes of acid. Both of these are overcome by using microwave energy heating for acid digestion in closed vessels at elevated pressures. Kingston and Jassie (1988) have reviewed the principles and many applications of this technique. In this laboratory it has been shown that mixtures of 0·5 ml blood and HNO_3 (>1 ml) give completely clear solutions after only 5 min heating at 300 W, <160 psi. Furthermore there is no loss of added mercury. One problem with this technique which needs attention is the appearance of a rapid pressure peak (>200 psi) from the initial evolution of gases during digestion. This is overcome by using two stages for microwave heating: a low power stage to minimise pressure peaks followed by a higher power stage to complete the digestion process. It is also worth noting that with repeated use some vessels may exhibit slight leakage of acid vapour with accompanying losses of volatile elements such as mercury.

5 SEPARATION OF ANALYTE AND MATRIX

5.1 Solvent extraction

The removal of trace elements from the bulk matrix by solvent extraction of their lipophilic complexes with organic ligands is rarely used with modern instrumental analyses. However, a related and equally old procedure (Egan *et al.*, 1981) has recently proved useful for measuring aluminium in foods with a high fat content. Extraction of butter, margarine and cheese samples with petroleum ether yields an organic phase and an inorganic residue. The latter is easily and safely oxidised with concentrated nitric acid for elemental analysis and the former may be stripped of any aluminium (or other metals) by extraction with 1M HNO_3 (Delves *et al.*, 1989). This simple procedure avoids the hazards of attempting to use oxidative acid digestion of high fat content materials.

5.2 Ion exchange

Pre-concentration by ion exchange separation on microcolumns using continuous flow techniques is a very attractive means of extending analyses down to lower analyte concentrations (Bysouth *et al.*, 1990). A useful clinical example is the measurement of low concentrations of aluminium in dialysis concentrates (Suchak, 1990). These sample solutions have dissolved solids of 35–40% m/v so that any dilution to allow direct analysis would be too great to be of practical value. However, ion exchange on 'Chelex 100' at pH 5·5 removes all of the Al^{3+} in solution and subsequent elution into 1M HNO_3 yields a solution amenable to direct accurate analysis by ETA-AAS.

6 CONTAMINATION CONTROL

If the analytical result is to have any value at all then it is essential to minimise adventitious contamination with trace elements at all of the stages of collection, preparation and analysis. Elements such as zinc and aluminium are ubiquitous and most analysts are aware of problems of contamination from these elements. However, beryllium is present in laboratory tissue-paper at very low levels but nonetheless sufficient to compromise analysis of tissue biopsy specimens and not many people know this. Every material with which the specimen to be analysed comes into contact must be regarded as a potentially serious source of contamination. Analytical control must therefore start with the equipment used for specimen collection and continue throughout all stages of sub-sampling, preparation and analytical measurement. All collection materials must be tested before use and be shown to be suitable for the assay(s) required.

Venesection using plastic disposable syringes fitted with stainless steel needles, after cleaning the skin with alcohol-impregnated swabs, is generally acceptable for most trace-element assays. The notable exceptions are cobalt, chromium, manganese and nickel which are released from the stainless steel into the blood at levels which can exceed natural physiological concentrations. Thus detection of deficiency states of these elements from blood analyses is not possible unless plastic cannulae are used (Versieck & Cornelis, 1989) or some alternative collection procedure is devised. Subsequent storage in glass containers (including commercially available evacuated collection tubes) can produce elevated aluminium concentrations. Other elements can be imported to the blood specimen from anti-coagulants (Cu, Zn, Co); rubber stoppers (Zn); orange plastic stoppers (Cd). Clear polyethylene, polypropylene and polycarbonate containers are the most suitable for storing specimens for trace element analyses.

Collection of diets, faeces and urine also needs careful control to avoid contamination. Acid-washed polyethylene or polypropylene containers are the most suitable vessels for collection and storage. Homogenisation of diets, faeces etc, requires the use of titanium- rather than steel-bladed blenders to avoid contamination with chromium and other elements in the steel. It is also necessary to replace other components, e.g. copper bushes and some plastic components, with those made from PTFE.

The most important aspect of contamination control is good laboratory hygiene. Aluminium is present in dusts at concentrations of 2–4 mg kg^{-1} and great care is needed at every stage of the analysis to avoid contamination with this element. The problems of trace-element contamination and procedures for dealing with them are excellently discussed by Zieff and Mitchell (1976) and by Behne (1980).

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