CURRENT STUDIES OF BIOLOGICAL MATERIALS USING INSTRUMENTAL AND RADIOCHEMICAL NEUTRON ACTIVATION ANALYSIS

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ABSTRACT

Instrumental neutron activation analysis still remains the preferred option when analysing the trace element distribution in a wide range of materials by neutron activation analysis. However, when lower limits of detection are required or major interferences reduce the effectiveness of this technique, radiochemical neutron activation analysis is applied. This paper examines the current use of both methods and the development of rapid radiochemical techniques for analysis of the biological materials, hair, cow's mil, human's milk, milk powder, blood and blood serum.

INTRODUCTION

The use of neutron activation analysis (NAA) for trace element analysis in a wide range of materials is still increasing despite strong competition from other instrumental techniques. Within this technique, instrumental NAA (INAA) still remains the preferred option. However, when lower limits of detection are required or major interferences reduce the effectiveness of the technique, such as the quantities of interfering radionuclides produced by the matrix (^{24}Na, ^{32}P, ^{38}Cl, ^{42}K and ^{82}Br) in many biological materials, radiochemical NAA (RNAA) is applied. These two methods have been used recently in the radiochemical laboratories of CSIRO Division of Energy Chemistry to analyse samples of hair, human and cow's milk, blood and blood serum, both independently and in collaborative research projects with universities and government departments. In addition, rapid radiochemical separation techniques have been developed to hasten and improve RNAA techniques. This paper briefly examines these applications and developments.

EXPERIMENTAL

The INAA method used for trace element determination in biological materials is similar to that used for geological materials as described elsewhere in these proceedings (Fardy, this conference). However, when biological samples were irradiated for longer than 24 h (hairs, 1 to 5 d) they were encapsulated in prewashed, high purity, quartz vials. Where possible, liquid samples were lyophilised before irradiation.

Iodine determinations involved irradiation with resonance neutrons, epithermal neutron activation analysis (ENAA). Powdered samples (300-400 mg) were weighed into quartz vials and heat sealed. There were individually loaded into a cadmium box and placed in an aluminium rabbit for transport into the pneumatic transfer facility of Moata.$_1$ There were irradiated for 15 min at an epithermal flux of 2 x 10^{12} n cm^{-2} s^{-1} and, after a decay period of 20 min, counted for 1000 s on a 20% Ge(Li) detector. Iodine concentration measurements were obtained via the 443 keV peak for ^{128}I.

Initial radiochemical separation procedures entailed irradiating 150 mg of material in the X-6 of HIFAR and, after 2 h cooling, wet ashing in conc. H_2SO_4/HNO_3 and carrier solution. Elements were then separated using the APDC/$CHCL_3$ solvent extraction method of Nakahara and co-workers[1]. This procedure was modified later by replacing the solvent extraction step with a more rapid and selective technique based on the removal of metal ions by columns of C_{18}-bonded silica gel after selective complexation by a series of complexing agents[2,3].

RESULTS AND DISCUSSIONS

Hair analysis

Controversy still surrounds whether the trace element content of hair can be used to characterise hair from an individual in a forensic context, or to indicate specific nutrient requirements, certain diseases or contamination by the environment. Part of the problem involves sampling techniques and whether hair should be treated before analysis. Hair sampling and washing procedures used in this laboratory closely follow the recommendations of the IAEA Advisory Group[4].

Most collaborative studies have involved health monitoring of hospital patients suffering from heavy metal poisoning or under medical treatment for some disease. In a recent study of a young patient suffering from the extremely rare disorder of intestinal enterokinase deficiency, hair and blood serum samples were analysed by INAA to measure the selenium uptake as a result of treatment with Baker's yeast. Samples before, during and after treatment showed little variation with blood serum levels of 0.09 ± 0.02 $\mu g\ mL^{-1}$ and hair concentrations of 0.66 ± 0.03 $\mu g\ g^{-1}$. These values fall in the normal range for the Australian population of 0.04 - 0.13 $\mu g\ mL^{-1}$ and 0.3 - 6.4 $\mu g\ g^{-1}$, respectively, measured by this laboratory.

Current joint studies with the University of Sydney involve using INAA to measure the trace element profile of hair from a small population of Aborigines which suffer both physical and medical problems. It is hoped that both elemental levels and their ratios will locate the possible causes of these problems.

Iodine in milk products

While iodine is regarded as an essential trace element, its concentrations are usually so low (< 5 $\mu g\ g^{-1}$) that recorded values are scarse or dubious. This was verified recently when a number of local laboratories participated in a round-robin analysis of commercially available milk powders using colorimetric, gravimetric and XRF techniques. This produced values that varied by a factor up to four for each sample. However, iodine can be rapidly and accurately determined in milk products by ENAA[5]. This technique relies on the enhanced activation of iodine compared to the induced activity from the major intefering elements because its value for the resonance integral is so much larger. No loss of iodine occurs during the activation process since samples are sealed and counted in quartz tubes.

Samples of market cow's milk were selected randomly for testing and were lyophilised within 24 h of collection. Values for the iodine levels ranged from 0.058 to 0.12 $\mu g\ g^{-1}$. These compared favourably with the range of iodine concentrations reported for normal cow's milk[8] (0.014 - 0.270 $\mu g\ g^{-1}$). This

technique was also used on lyophilised human milk samples. The concentration of iodine for these samples fell within the reported range of 0.04 - 0.08 $\mu g\ g^{-1}$ recorded for human milk[8].

Trace elements in human milk and female blood plasma

A study of the effect of progestogen-only oral contraceptives on the vitamin content of the breast milk and blood plasma of Australian women by the Division of Biological and Health Sciences at Deakin University was extended to investigating its effect on trace elements in a collaborative study with the CSIRO Division of Energy Chemistry[6,7]. The design of the study also permitted the effects of the stage of lactation and maternal diet on milk composition to be observed. Trace element measurements involved use of both RNAA and INAA to yield data for Fe, Cu, Zn, Mn, Se, Co, Rb and Cs.

Results of this study have shown no effect of progestogen-only contraceptives on the milk or plasma concentrations of any of the trace element measured. However, the mean milk and plasma Fe and Zn concentrations changed significantly with the progression of lactation, 7 - 22 weeks post-partum, but the concentration of other trace elements did not.

The nutrient intake of some of the women influenced their milk composition more directly than others. While these effects were obvious in some women at normal dietary intake levels, only megadoses produced measurable response in others. The reason for these differences is still under investigation.

RNAA for biological materials

The determination of Mn, Cu and Zn in biological materials by RNAA, based on the solvent extraction procedure of Nakahara et al.[1], has been used successfully in these laboratories. However, the procedure is time-consuming and involves manipulations that result in unnecessary radiation exposure to the operator. A new procedure was developed based on the rapid removal of these metals ions by columns of C_{18}-bonded silica gel after selective complexation by 8-quinolinol (oxine), ammonium pyrrolidinedithiocarbamate (APDC) or cupferron (CUP)[2]. Recent investigations have extended this system to include other chelates, 1-(2-pyridylazo)-2-naphthol (PAN), 1-(2'-thiazolylazo)-2-naphthol (TAN), 4-(2-pyridylazo) resorcinol (PAR), diethylammonium diethyldithiocarbamate (DDDC), potassium ethyl xanthate (PEX), acetylacetone (AcAc) or thenoyltrifluoroacetone (TTA) solutions and to other metal ions of interest, As, Se and Mo. Each system was evaluated on the extent of metal ion removal by columns of C_{18}-bonded silica gel as a function of sulphate concentration and pH.

Results for the removal of As(III), As(V) and Mo(VI) in the presence of oxine, APDC or CUP showed that only Mo was quantitatively removed over a limited pH range in all chelate systems (2-6.5/oxine; 1-5/APDC; 1-3/CUP). Changing the sulphate concentration had little influence. As(III) was quantitatively retained in sulphate solutions of oxine and CUP but was fully removed by C_{18} columns from APDC solutions below pH of 7. Partial removal of As(V) occurred only in the APDC system.

The use of the azo complexing agents PAN and TAN was marred generally by the low solubility of these reagents and their metal chelates in sulphate solutions (0-18 - 1.44 M). Surprisingly, PAR, the resorcinol derivitive of PAN, was more effective as a separation medium despite its increased water solubility and that of the metal complexes.

Two additional sulphur donors, DDDC and PEX, were tested as chelating agents for Mn, Zn and Cu removal and showed similar results in low sulphate media. Cu was removed over the complete pH range 1 - 9, and Zn and Mn at pH values greater than 7 and 9.5, respectively. At higher sulphate concentrations, poor removal of all ions was observed from the PEX system. For the DDDC system, Mn behaved poorly, Zn was separated quantitatively over a reduced pH range but the behaviour of Cu was unchanged.

Both Cu and Zn were quantitatively separated on C_{18} columns over a limited pH range from either of the diketones, AcAc or TTA. However, only the TTA system was suitable for the complete recovery of Mn.

Recent observations of the role of Se in human and animal systems have renewed efforts in understanding the bioscience of this element and the need for accurate and precise method of analysis. Fast instrumental measurements of this element can be done via ^{77m}Se (t = 17.5 s). While its detection is sometimes subject to interferences, its short half-life precludes the use of radiochemical separations. Pre-irradiation separations via the C18-column have been examined. An investigation of the removal of Se as chelates of APDC, DDDC or PEX showed total removal was achieved in all systems above pH of 3.0. Although the APDC effectively removed Se in more acid solutions, these experiments were marred by the heavy precipitation of the reagent. Further investigations in the PEX system showed that Se was quantitatively removed from 1.44 M H_2SO_4 if solutions were recycled through C_{18}-columns with further addition of PEX between each cycle.

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