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Isotopic and Nuclear Analytical Techniques in Biological Systems: A Critical Study

PART IX. NEUTRON ACTIVATION ANALYSIS

(Technical Report)

Prepared for publication by

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Isotopic and nuclear analytical techniques in biological systems: A critical study—IX. Neutron activation analysis (Technical Report)

Synopsis

Neutron activation (NAA) methods applied to the analysis of biological samples are reviewed. Methods covered include instrumental neutron activation (INAA), prompt gamma NAA(PGNAA), radiochemical NAA(RNAA) and pre-irradiation separation methods (CNAA). The use of epithermal neutrons, fast neutrons, and applications to in vivo studies are also discussed.

Introduction

Neutron activation analysis (NAA) is primarily a method of trace element determination (concentrations < 0.01%) although minor and major elements may be determined in some applications. The method originated with Hevesy and Levi in 1936 (ref. 1) but did not become a practical method of analysis until the development of the nuclear reactor (1940s) as a high intensity neutron source. The introduction of the NaI(Tl) scintillation γ-ray detector (1950s) and the high resolution Ge(Li) γ-ray detector (1960s) established NAA as a major method of trace element determination in biological materials. Several recent texts and monographs cover activation analysis in general and NAA in particular (refs. 2-9). This review covers only neutron methods and their applications to biological materials.

Principle of Neutron Activation Analysis

The principle of activation analysis is that a particle (neutron, proton, α-particle, etc.) or photon (γ-rays, bremsstrahlung) induces a nuclear reaction in an atom of a target element. The product of the reaction is detected and quantified by prompt photon or particle emission or, more commonly, by its decay properties, if radioactive. For NAA, in which neutrons are used, several nuclear reactions are possible depending on the target nucleus and the neutron energy. In the case of Al, as an illustration, the major nuclear reaction channels are:

$$^{27}Al + {}^{1}n \rightarrow [^{28}Al^{*}] \begin{cases} \rightarrow \gamma + {}^{28}Al & (n,\gamma) \text{ reaction} \\ \rightarrow {}^{1}H + {}^{27}Mg & (n,p) \text{ reaction} \\ \rightarrow {}^{4}He + {}^{24}Na & (n,\alpha) \text{ reaction} \\ \rightarrow 2\,{}^{1}n + {}^{26}Al & (n,2n) \text{ reaction} \\ \rightarrow {}^{1}n + {}^{27}Al & (n,n) \text{ elastic scattering} \end{cases}$$

Where $[^{28}Al^{*}]$ represents the compound nucleus (excited nuclear state of ^{28}Al) resulting from neutron capture which has a very short lifetime (less than 10^{-13} s). Except for the elastic scattering (n,n) reaction, the reaction products are radioactive. When the target element has more than one stable isotope similar reactions may be possible with all isotopes. Other more limited neutron reactions used in NAA are induced fission, (n,f), for fissionable elements (U, Pu, Th) and inelastic neutron scattering, (n,n'γ), in which a radioactive isomeric state of the target nuclide is measured (e.g., ^{77m}Se, ^{111m}Cd, ^{204m}Pb). Most applications of NAA to determine trace elements in biological materials utilize the (n,γ) reaction because of the generally higher sensitivity achieved compared to other reactions. All (n,γ) reactions with stable isotopes, with the exception of $^{4}He(n,\gamma)^{5}He$, are exoergic and thus have zero neutron threshold energies. Also, (n,γ) reaction cross sections (σ) for thermal (<0.1 eV) and epithermal (0.1 - 100 eV) neutrons are generally much higher than for endoergic (n,p), (n,α) or (n,2n) reactions which have threshold energies and which normally occur only with fast neutrons. These properties plus the fact that low energy neutrons (<100 eV) are much more abundant than fast neutrons in typical reactor neutron irradiation facilities generally result in higher analytical sensitivity for (n,γ) reactions for a given target nuclide.

In most NAA methods the product nuclide is radioactive and the induced activity, A_t (in Bq, or disintegrations per second) at the end of irradiation of time t_i in seconds is related to the number of target nuclide atoms, N (hence the mass of element), the reaction probability or cross section, σ (in cm^2), the neutron flux, ϕ (in $cm^{-2}s^{-1}$), and the decay constant, λ (in s^{-1}), of the induced nuclide by:

$$A_t = N\,\sigma\phi\,(1 - e^{-\lambda t_i})$$

The maximum induced activity, A_{max}, obtained for an irradiation time much longer than the half life of the product nuclide (in practice about seven half-lives), is given by:

$$A_{max} = N\sigma\phi$$

In non-destructive or instrumental NAA (INAA), the most commonly used technique, the product radionuclide is normally quantified, depending on its decay mode and half life, by high resolution γ-ray spectroscopy using intrinsic Ge, Ge(Li) or Si(Li) detectors or in some cases by NaI(Tl) scintillation spectrometry. Other techniques used include β-γ and γ-γ coincidence spectrometry, Compton suppression γ-ray spectrometry, delayed neutron counting and nuclear track analysis (NTA). For some elements (e.g., B, Cd) greatly enhanced sensitivities can be achieved by direct measurement of the prompt capture γ-rays emitted by the transient compound nucleus rather than the radioactive (or stable) reaction product (prompt gamma NAA-PGNAA).

In principle, INAA (and PGNAA) is an absolute method of analysis and the concentration of an element (i.e. N) can be calculated if the absolute disintegration rate, A_t, is measured and if ϕ and σ are known. Absolute analysis is rarely used because ϕ and σ vary with neutron energy. Reactor neutron flux distributions as a function of neutron energy are generally not precisely known (and may vary with time or reactor-core configuration) and reported σ values, or excitation functions, often show large variations (e.g, ± 20%) depending on the method of determination. Many routine methods employ either comparator standards of known mass of the elemental compound or use Standard Reference Materials (SRMs) of known elemental concentrations. The sample and the standard are irradiated and counted under identical conditions, thus:

$$\frac{(A_t)_{sa}}{(A_t)_{st}} = \frac{P_{sa}}{P_{st}} = \frac{W_{sa}}{W_{st}}$$

where P_{sa}, P_{st} refer to measured γ-intensities (i.e., count rates) for the sample and standard and W_{sa}, W_{st} are the weights of the element in the sample and standard, respectively. An alternative approach (k_0 method) is to use a single comparator (i.e., ^{197}Au standard) to compute a k_0 normalizing factor for all elements measured. The k_0 factor is experimentally determined for each element and incorporates cross section and other information on the pertinent nuclear reaction (see refs. 10-12). Use of this factor with appropriate neutron flux (thermal/epithermal) values and γ-ray detector efficiencies allow the concentrations of each element to be determined using the single comparator. Applications of the k_0 method to biological samples have been recently reviewed (refs. 13,14).

Some trace or minor elements (e.g., P, S, B, Pb, Be, Cd) may not be measurable by thermal neutron INAA, or cannot be determined with sufficient sensitivity for analysis of biological samples because their (n,γ) products do not emit γ-rays (e.g., ^{35}S, ^{32}P, ^{45}Ca), the reaction-product half lives are too short (<1 s) or excessively long (<100 a) for accurate and sensitive measurement, or the σ for the reaction is too small. The detection limit using a specific (n,γ) reaction is also strongly influenced by the composition of the sample because the γ-ray peak to be measured is superimposed on a Compton continuum resulting from inelastic scattering in the detector of γ-rays from other induced radionuclides (e.g., ^{24}Na, ^{42}K, ^{38}Cl) in the sample. For some elements (e.g., B, Cd) measurement of the prompt γ-ray emitted during de-excitation of the compound nucleus during on-line irradiation (PGNAA) can be used. For other elements chemical separation of the element can be performed before irradiation (chemical separation NAA; CNAA), or the induced radionuclide can be separated from the sample after irradiation (radiochemical NAA; RNAA).

A major advantage in the INAA of biological matrices is that the matrix elements C, H, N, O produce negligible γ-ray activity to interfere with the determination of trace elements. The $^{13}C(n,\gamma)^{14}C$ and $^{2}H(n,\gamma)^{3}H$ reactions produce very small activities of the non-γ emitters ^{3}H and ^{14}C and the $^{18}O(n,\gamma)^{19}O$ and $^{15}N(n,\gamma)^{16}N$ reactions give very short lived ^{19}O (27 sec) and ^{16}N (7.1 s). All of these reactions have extremely low thermal neutron cross sections. However, the high concentration of P in many biological tissues (e.g., bone) reduces the detectability of some γ-emitters because ^{32}P from the $^{31}P(n,\gamma)^{32}P$ reaction, although not a γ-emitter, gives a bremsstrahlung continuum in the 0-1.7 MeV region of the γ-ray spectrum. Similarly, traces of Na, K and Cl in biological samples may produce high activities of ^{24}Na (15 h), ^{42}K (12 h) and ^{38}Cl (37 min).

Methodology and Instrumentation

Nuclear reactors are the most important neutron sources because of the high stable neutron fluxes and ample irradiation volumes available. More than 300 research reactors with NAA capability are operational world-wide (approx. 100 in Europe and the U.S., respectively). Large research reactors at centers such as ILL-Grenoble (57 MW), Oak Ridge National Laboratory (HIFAR - 100 MW), National Institute of Standards and Technology (20 MW) and the University of Missouri (10 MW) provide intense neutron fluxes (up to 10^{16} $cm^{-2}s^{-1}$). However, smaller (0.1-2 MW) and less complex reactors such as the SLOWPOKE (Canada) and TRIGA (U.S.A.) reactors located at many universities and research institutes world wide provide suitable neutron fluxes (10^{10}-10^{13} $cm^{-2}s^{-1}$) for most biological applications of NAA. The high thermal-neutron and epithermal-neutron fluxes make the reactor the neutron source of choice for NAA methods using the (n,γ) reaction as well as for a few fast neutron methods. Fast neutron sources are generally preferred for threshold (n,p), (n,α) or (n,2n) reactions for elements that cannot be determined conveniently by the (n,γ) reaction (e.g., O, N, C) and the most important sources are Cockroft Walton (or Van de Graaff) accelerators which generate high energy neutrons via a charged particle reaction, e.g. $^{3}H(^{2}H,n)^{4}He$ which produces 14.7 MeV neutrons. Other fast neutron sources, particularly for *in-situ* or *in-vivo* NAA include ^{252}Cf (spontaneous fission nuclide; 2.64 a half life) and isotopic neutron sources utilizing (α,n) or (γ,n) reactions with ^{9}Be, e.g., ^{241}Am-Be or ^{238}Pu-Be sources.

Typical INAA methodology is to irradiate samples (which can be solid or liquid) and elemental standards (or a monitor in the k_0 method) for a time determined by the half-life of the radionuclide or radiological considerations and the composition of the sample. Unwanted short-lived nuclides are allowed to decay for a predetermined period and the γ-ray spectra are recorded on a Ge or Ge(Li) detector coupled to a computer (or a multichannel analyzer). Gamma-ray spectra of irradiated biological samples are typically complex (up to several hundred γ-ray peaks); hence highly stable electronics and corrections for losses at high count rates (ref. 15) are required to achieve the required high energy resolution (typically 1.5-2.0 keV at 1333 keV). A variety of computer routines exist for conversion of γ-ray spectra to radionuclide activities and elemental concentrations. In RNAA, the sample is dissolved, following irradiation of either wet or dry biological tissues, by oxidative decomposition depending on sample matrix (e.g., H_2O_2-HNO_3; H_2SO_4-HNO_3; HNO_3-$HClO_4$ for wet ashing; solid Na_2O_2 oxidation; or high temperature ashing). Following dissolution, non-radioactive elemental "carriers" are added and the analyte element plus radionuclide is separated with the radiochemical purity necessary for quantification of the nuclide. The yield of the separation is determined from the "carrier" recovery which can be determined chemically or by re-irradiation and counting. Separation of the radionuclide of interest in a radiochemically pure state allows the use of very high efficiency (but low resolution) counting, e.g., large volume or well-type NaI(Tl) solid scintillation detectors with essentially 4π counting geometry. The highest sensitivity for a given element and neutron flux/irradiation time/sample size can thus be achieved by RNAA.

Epithermal NAA (ENAA) has also been used for the determination of trace elements in biological materials (refs. 16,17). In ENAA the sample is irradiated in an irradiation facility and/or capsule that filters out thermal neutrons (either Cd metal or B as boron carbide and boron nitride) thus enhancing the resonance, or epithermal, neutron (n,γ) reaction contribution relative to thermal neutron (n,γ) activation. Thus a radionuclide produced by (n,γ) reaction with a large resonance integral, I, (in barns) relative to thermal

neutron cross section, σ, can be measured with a lower detection limit in biological materials because major contributions to the Compton background are typically ^{24}Na or ^{46}Se which have lower I/σ ratios. Iodine and cadmium can be thus determined instrumentally in some biological samples by ENAA when they cannot be determined by INAA (refs. 16,17).

Several authors have used very short half-life (n,γ) products (e.g., 0.8 s-^{8}Li; 11.1 s-^{20}F) in the analysis of biological materials (refs. 18). Cyclic INAA systems in which repeated cycles of irradiation/counting allow improved counting statistics for the short-lived radionuclides have been described (refs. 19-21). Use of short-lived nuclides is particularly valuable in *in vivo* studies, particularly for the determination of Ca via 8.7 min- ^{49}Ca and P via the $^{31}P(n,\alpha)^{28}Al$ (2.2 min) reaction (ref. 23). Use of very short half-life radionuclides often requires γ-ray spectroscopy at very high count rates and very stable "loss free" counting systems have been developed for this application (ref. 15).

Fast neutron activation analysis (FNAA) has some distinct advantages over thermal or epithermal INAA for some biomedical applications. Although the major elements of biological materials, C, H, N, O, etc., cannot be determined via thermal (n,γ) reactions, (an advantage in trace element determination by INAA), N and O may be determined by (n,p) or (n,α) reactions using 14 MeV neutrons from a neutron generator. More important applications of fast neutron generators to biomedical research, however, are the in vivo determination of body burdens of Ca, P, N, Na, and K by either (n,γ), (n,p), (n,2n) or prompt-gamma (n,γ) reactions (refs. 24,25).

Prompt gamma neutron activation analysis (PGNAA) differs from other activation methods in that the prompt capture γ-rays are measured rather than the radioactive product. Experimentally a γ-ray detector is mounted at 90° angle to a sample irradiated by a collimated neutron beam (thermal or fast) extracted from the reactor core. Although a large number of elements can be determined in principle by PGNAA (refs. 26,27), the most important *in vitro* applications to biological samples have been the determination B and Cd because the reaction products of the high cross-section $^{10}B(n,\alpha)^{7}Li$ and $^{113}Cd(n,\gamma)^{114}Cd$ reactions are stable, thus precluding their determination by INAA. Limitations of the PGNAA technique with reactor neutrons are related to the high background (hence shielding) and relatively low neutron fluxes at beam stations close to the reactor. Recently, however, facilities have been constructed at KFA-Jülich in Germany, at the NIST reactor in the U.S.A and at other high-flux reactor facilities which produce "cold" neutrons (<0.005 eV) in liquid O_2 or cooled (30°K) D_2O and which alleviate this problem significantly. Because (n,γ) cross sections show an inverse relationship to neutron velocity, cross sections for cold neutrons (<0.005 eV) are larger than for thermal neutrons (0.025 eV). Also, cold neutrons can be extracted in guide tubes much more efficiently than thermal neutrons which allows sample irradiation facilities to be placed in more remote, lower background, locations. Applications of PGNAA using cold neutrons have been described by Rossbach (ref. 28) and this technique shows considerable potential.

Non-reactor neutron sources are extensively used in *in-vivo* NAA or PGNAA, techniques (ref. 23) which measure total body N, skeletal Ca or body burdens of other elements (e.g., Cl, K, Na, P, Cd). In this technique whole body or specific organs are irradiated with fast neutrons from multiple ^{238}Pu-Be, or ^{241}Am-Be (α,n) sources, ^{252}Cf sources or 14.7 MeV neutrons from a Cockroft-Walton generator. Hospital facilities using ^{238}Pu-Be or ^{241}Am-Be have been described by Ellis (ref. 23), Krishnan, et al. (ref. 29), ^{252}Cf sources by Ryde et al. (ref. 30) and pulsed 14 MeV neutron generators by Mitra et al., (ref. 25) and Weinlein et al., (ref. 31). Multiple large volume NaI(Tl) scintillation detectors in whole body or organ counters measure either prompt γ-rays for N from $^{14}N(n,\gamma)^{15}N$, for H from $^{1}H(n,\gamma)^{2}H$ and for Cd from $^{113}Cd(n,\gamma)^{114}Cd$ or delayed γ-emission (INAA) for Ca from $^{48}Ca(n,\gamma)^{49}Ca$, for P from $^{31}P(n,\alpha)^{28}Al$ and γ rays of other short half-life radionuclides.

Detection Limits:

Detection limits for NAA vary widely from element to element because the sensitivity of the method (i.e., induced radionuclide activity (Bq)/g element) for a given element is partly a function of the reaction cross section, σ, and the half life of the induced radionuclide, both of which are related to the fine structure of the

nucleus. Hence, unlike X-ray fluorescence (XRF) and inductively-coupled plasma-mass spectrometry (ICP-MS) in which the sensitivity of the method is a smooth function of atomic number (XRF) or atomic mass (ICP-MS), there is no direct relationship of NAA sensitivity to atomic properties (i.e., atomic number, Z; or mass number, A, for a given element). Also, detection limits for trace elements in biological materials are significantly different for RNAA and INAA with the former representing ultimate practical detection limits. In RNAA, separation of the radionuclide of interest after irradiation allows the activity of the radionuclide to be determined with high efficiency (i.e., 4π geometry) with low resolution detectors under low spectral background conditions. Theoretical detection limits have been calculated (ref. 32) in which saturation activity for a given neutron flux, ϕ, zero decay time, and 100% efficient measurement of the induced activity is assumed. More practical limits (in g element/sample) have been calculated by Guinn and Hoste (ref. 33) using practical conditions of $\phi = 10^{13}$ $cm^{-2}s^{-1}$; t_i = 5 h followed by counting for 100 min on a Ge(Li) detector to give 30 counts in the full-energy γ-ray peak. These are given in Table 1.

Table 1: Detection limits (in g) by RNAA (adapted from ref. 33).

10^{-9}-10^{-8}	10^{-10}-10^{-9}	10^{-11}-10^{-10}	10^{-12}-10^{-11}	$<10^{-12}$
Al Sn	Ba Nd Te	Sb I W	Au Re	Dy
Ca Ti	Cl Sc Tb	As Ga V	Ir Rh	Eu
Ce Y	Gd Se Th	Br La Yb	Lu Sm	Ho
Cd Zr	Ge Sr Tm	Cs Hg	Mn	In
Cr W	Hf Ta	Co Pd		
Mg		Cu Ru		
Mo		Er Na		

The detection limits in Table 1 are generally lower (assuming a minimum sample size of 1 g) than those obtainable by atomic emission spectroscopy (ICP-AES) (10^{-7}-10^{-11} g/g) and are comparable to detection limits for many elements determined by ICP-MS (10^{-9}-10^{-11}g/g). In contrast to RNAA, INAA detection limits are matrix dependent, even though the major elements of biological materials (C, H, N, O) have low neutron absorption cross sections and have negligible (n,γ) radionuclide contributions to sample activity. The presence of minor elements (e.g, P, Cl, S, Ca, Na, K) and large cross-section trace elements in a sample result in a composition and concentration-dependent Compton continuum under the measured γ-ray peak in the irradiated sample. Hence, detection limits for INAA in biological materials are generally several orders of magnitude poorer than for RNAA (refs. 7,34). If the approximate compositions of samples are known, detection limits for INAA can be calculated for typical biological materials for given irradiation and measurement conditions (refs. 35,36).

Advantages and Limitation of NAA

The major advantages of NAA, particularly INAA are *a*) the relative freedom from matrix effects and interferences, *b*) high accuracy and *c*) very low or zero blank contributions. Because nuclear reactions and decay processes are virtually unaffected by the chemical and physical structure of the material during and after irradiation, the composition of the matrix has little influence on the induced activity. Biological materials have low neutron absorption cross sections (unless high concentrations of elements such as Cd and B are present). Thus neutron attenuation in the sample during irradiation is generally very small and can be corrected for, if present. Also, γ-ray attenuation during counting of small volume irradiated biological samples in *in vitro* analysis is small, except at energies below 100 keV and can be corrected for in the case of low energy γ-rays or large sample volumes (e.g. *in vivo* NAA). The absence of matrix effects thus allows the use of standards that have different composition (and even physical state) from that of the sample. Interferences may arise through production of the radionuclide via fast neutron reactions on other elements (e.g., (n,p), (n,α) and (n,2n) reactions) or from coincident γ-rays during counting. Thus, the determination of Al in biological matrices via the $^{27}Al(n,\gamma)^{28}Al$ reaction is interfered with by the $^{28}Si(n,p)^{28}Al$, and more importantly, by the $^{31}P(n,\alpha)^{28}Al$ reaction. In most biological tissues the P content is generally much higher than the Al content (e.g., brain and bone tissue) and the $^{31}P(n,\alpha)^{28}Al$ may make the INAA determination of

Al impossible. In some cases corrections can be made if the concentration of interfering element (e.g, P) is low and is known, or is measured by another nuclear reaction, e.g, $^{31}P(n,\gamma)^{32}P$, but pre-irradiation separation of Al is preferred (ref. 37). Similar problems arise in the determination of Mn in blood i.e. ($^{55}Mn(n,\gamma)^{56}Mn$; $^{56}Fe(n,p)^{56}Mn$). In general, however, these interferences are exceptions and only are significant where the concentration of the interfering element is several orders of magnitude greater than that of the analyte because (n,p) and (n,α) fast neutron reaction-cross sections are usually lower than (n,γ) cross sections and fast neutron fluxes are much lower than thermal neutron fluxes in a well moderated reactor irradiation position.

Sample pretreatment is minimal in RNAA and INAA and often involves only packaging the sample in a irradiation container (quartz, polyethylene or Al foil). For ultratrace analysis this is an important advantage over such competing techniques AAS or ICP for which the sample must normally be in solution, thus introducing the potential for contamination from reagents and equipment (blank problem) and the loss of analyte through adsorption, volatilization, coprecipitation, etc. Post-irradiation chemistry can be done in RNAA after addition of non-radioactive carriers to the radionuclide "tagged" sample thus eliminating blank contributions and providing a mechanism for chemical yield determination of the separation process (i.e. to evaluate losses during decomposition or in separations).

The independence of the nuclear reaction of chemical parameters in NAA of biological materials and the absence of blank contributions are the major reasons for the high accuracy of the technique, particularly in RNAA when the signal to noise (i.e. peak/background) ratio is maximized. Becker et. al., (ref. 38) have considered all sources of random and systematic error in the determination of trace elements in NIST botanical SRMs. They showed that 95% confidence intervals ranged from less than ±1% to less than ±10% of the mean for many elements at the sub ppm level in both RNAA and INAA. The high accuracy and sensitivity of NAA is a major reason for its extensive use in the certification of biological samples for trace elements. For example up to 1986, 2262 elemental determinations had been reported in the literature for NIST SRM 1577, Bovine Liver, and 3113 determinations for NIST SRM 1571, Orchard Leaves, (ref. 39) of which 28% and 38%, respectively, were obtained by NAA.

Another advantage of NAA is the determination of isotopic ratios of certain elements (e.g., S, Cu, Zn, Pb) via different nuclear reactions. Applications of isotopically enriched stable tracers of Zn (e.g., ^{64}Zn, ^{68}Zn and ^{70}Zn) in nutritional studies thus allow calculations of different metabolic pathways (refs. 40,41).

The disadvantages of NAA include the need for access to a nuclear reactor for trace element studies, the need to work with radioactive materials and the length of analysis time for many elements in INAA. Compared to XRF, or ICP-AES and ICP-MS, INAA is less labor intensive but analytical throughput is lower (refs. 34,42,43). A major disadvantage of NAA for trace element research in biomedical studies is the lack of speciation or oxidation state information. The excited state (compound) nucleus formed during irradiation de-excites following neutron capture by prompt ($<10^{-13}$s) γ-ray emission (of energy E_γ) which imparts a recoil energy to the nucleus of mass M ($= E_\gamma^2/2Mc^2$) of 10eV to greater than 1000 eV. This recoil energy is sufficient to rupture covalent bonds and will leave the recoiled atom in a different location and oxidation state. Hence, speciation/oxidation state information must be obtained by pre-irradiation separation of species as in chemical separation NAA (CNAA). Examples are the speciation of As and Se in biological fluids (refs. 44-46). Other problems encountered in RNAA include sample decomposition during irradiation via radiolysis and change of oxidation state of elements during irradiation either by recoil of the radionuclide or radiolytic reactions in solution, as for

$$Hg^{2+} + 2e = Hg^0$$

or the reduction of Se^{IV} or Se^{VI} compounds to Se^0 during irradiation. These effects must be addressed during post-irradiation chemistry by cycling the sample and carrier through oxidation-reduction cycles to bring the induced radionuclide and the carrier to a common oxidation state.

Applications of NAA

The major applications of INAA and RNAA to biological materials have been to the determination of trace elements for metabolic, nutritional, toxicological, bioavailability and disease related studies. The most exhaustive reviews of the NAA literature are those published biennially by Ehmann, et al., (refs. 47-49) and the reader should consult these sources together with reviews on NAA in biology and medicine (refs. 2,9,50-52). Much of the earlier literature (1960s-1970s) can be characterized as demonstrating the capabilities of the NAA multielement method and in establishing baseline elemental abundance data for many biological matrices. As Iyengar (ref. 53) has pointed out, however, much of the early data collected in medical studies is of doubtful value because of the lack of coordination between the analyst and the sample collector. Thus contamination during sample collection and the non-representative nature of many samples (e.g. organ biopsies) invalidates many of the data. This situation, however, is not unique to the development of NAA and the involvement of the analyst in the overall project from planning, initiation, sampling and analysis is relatively recent. Table 2 lists some recent representative applications of INAA and RNAA to the determination of trace elements in biological materials and application to biomedical problems.

Table 2: Application of INAA and RNAA to In Vitro Studies.

Matrix Analyzed	Descripton of Project	Method	Elements Analyzed	References
Metabolic and Nutritional Applications				
Blood, milk	Uptake of Se in infants in infant-mother pairs	INAA	Se	54
Urine	speciation of Se and data of total Se, TMSe, selenite	CNAA	Se	45,46, 55
Various tissues	Combined INAA (^{77m}Se) and tracer ^{75}Se) to study Se metabolism in the rat	INAA/ tracer	Se	56, 6l
Human milk	Survey of minor and trace elements in human milk from different countries using INAA, RNAA, ICP-AES and AAS	INAA RNAA	Cl, Co, Fe, Hg Sb, Se, Zn Cd, Mo, Cu, Mn V, Sn, I, As	57
Hair, other tissues	Metabolic behavior of Se and Cd and deposition in hair of guinea pig	INAA	Se, Cd l7 other elmenets	58
Embryos	Early incorporation of V in embryogenesis in ascidians	CNAA	V	59
Blood Serum	Use of pre- and post-irradiation separations of V	CNAA/ RNAA	V	60
Bile	Effect of antirheumatic Au preparations on ll trace elements in bile and plasma	INAA	Au other	62
Various	Use of activatable tracers in metabolic studies	INAA/ RNAA	^{70}Zn, ^{197}Au ^{65}Cu	40, 4l, 62 63
Rumen, feces	Use of rare earth elments and Cr as inert markers in bio-digestibility studies in animals	INAA	Cr, La, Sm Yb	64, 65

Table 2 (continued)

Matrix Analyzed	Elements Descripton of Project	Method	Analyzed	References
Toxicological Applications				
Hair	Determination of trace elements in human hair as indicators of pollution or toxicity	INAA INAA RNAA	10 elements Cu, other Cd	66, 67, 68 76 69
Liver, blood others	Use of INAA to monitor pollutant effects through environmental specimen banking	INAA	10-23 elements	70,71
	Comparison of RNAA and INAA in tissue analysis RNAA	INAA/	Cr, As, Sn Se, Mo, Sb, Ag	72
Bone	Cd incorporation in bone in toxicity studies	INAA/ RNAA	Cd	69, 73
Plant tissues	Application of epithermal NAA to Cd and As determination	ENAA ENAA	Cd As	74 75
Hair	Use of RNAA to determine correlation between toxic elements in hair and lung, kidney and liver	RNAA	Hg, Cd, As	77
Disease States and Trace Elements				
Brain	Trace elements in sub-cellular fraction of brain from Alzheimers and control patients	INAA	13 elements	78
Brain	Comparison of INAA and ICP-AES for determination of elements in brain tissues	INAA	8 elements	79
Hair	Trace elements in hair of normal and sickle-cell anemia patients	INAA	12 elements	80
Brain, nails	Correlation between elements in nails and brain of Alzheimers patients	INAA	15 elements	81
Blood	Determination of trace elements in blood platelets	INAA	Se, Zn Fe	82
Breast tissue	Determination of trace elements in normal and cancerous tissues	INAA	21 elements	83
Lung	Determination of trace elements in lung, trachea, bronchus and subpleura by combination of INAA and RNAA	INAA RNAA	50 elements	84
Various	Review of trace element correlation with amylotrophic lateral sclerosis and other neurological disease	NAA	Al, Se, Co, Mn, Fe, Cr, Cu, Zn, Mg, Rb, Cs, Sb	85

Table 3: Applications of NAA to *In-Vivo* Studies.

Application of Technique	Elements Determined	Reference
Use of INAA with fast neutron sources for determination of bone mass using ^{49}Ca	Ca	23, 30, 86, 87
Use of PGNAA to measure total N and protein content of whole body and organs	N	88, 89, 90, 96
Determination of Cd in kidney and liver by PGNAA	Cd	91, 92
Determination of Cd and Pb by PGNAA in occupationally exposed workers	Cd, Pb	93
Multielement determinations *in vivo* using combined PGNAA and INAA	Ca, N, P, Cl Na, K	22, 23
Design of facilities for *in vivo* INAA and PGNAA	various	25, 29, 31, 75
Combined INAA/PGNAA with tomographic techniques	several	97-99

Applications of *in vivo* methods have increased considerably in the past 10 years, such that determination of whole (or partial) body Ca in skeletal osteoporosis and demineralization studies and total body nitrogen have become routine procedures (refs. 23,,86-90). Table 3 lists recent in vivo applications of NAA and PGNAA, including Ca and N determinations. A unique application of NAA to in vivo studies is neutron activation tomography which combines photon or neutron absorption tomography with neutron activation and has been recently reviewed by Spyrou and co-workers (refs. 97-100).

Future Directions

Neutron activation analysis is a mature and established trace element technique and advances are likely to be improvements or modification of existing methods. In INAA and PGNAA the development of "cold" neutron sources at KFA in Germany, NIST in the USA and at other high flux reactors is likely to lead to greater sensitivity for biological trace element research (ref. 28). *In vivo* methods which are already extensively used for Ca and N skeletal and whole body determinations of Ca and N show promise of providing spatial distributions of elements when combined with tomographic techniques (refs. 97-100). Another very promising application of NAA is the combination of biochemical and immunoassay techniques and NAA for the determination of specific proteins. Thus polyacrylamide gel electrophoresis (PAGE) has been combined with NAA to identify specific protein hosts for trace elements (refs. 101-103) and enhanced immunoassay with colloidal Au has been used to isolate a given protein followed by detection of the Au-tagged immunoreagent by INAA (ref. 101).

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