
UNIT 13 RADIOANALYTICAL METHODS

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13.1 INTRODUCTION

Earlier, in Unit 12, you have studied fundamentals of radioactivity, decay laws, measurement methods and methods of production of radioisotopes and radiotracers including its availability within India. In this unit you will study about applications of radioactivity which has found wide applications in basic researches in physics, chemistry, biology and medicine including agriculture, industry, nutrition research, environment related research, forensic science etc. However, in this unit we will limit ourselves mostly with analytical applications of radioisotopes. In general, nuclear analytical methods based on the measurement of radioactivity may be classified into two broad groups: first based on the direct measurement of radioactivity in the system and second by making it radioactive by the bombardment of the sample in a nuclear reactor or accelerator facility or by adding radioactivity in the system. A broad classification of various nuclear analytical methods is presented in Fig. 13.1. Ever since the discovery of radioactivity, several techniques such as neutron activation analysis (NAA), isotope dilution analysis (IDA), radiometric titration (RT), radiochromatography (RC), radioimmunoassay (RIA), etc have been developed during last few decades. Out of all these methods, NAA is the most common. It is primarily based on the principle of irradiation of the sample with a neutron beam in a nuclear reactor and thus making it radioactive. However, in case of radiotracer techniques such

as IDA, RT, RC and RIA sample is made radioactive by the addition of radiotracers of suitable radiation type and half life. A comparative account of advantages and limitations of each of the techniques will be presented.

Objectives

After studying this unit, you should be able to:

- state a choice whether it is essential to adopt a radioanalytical method,
- describe uniqueness of radioanalytical methods,
- identify the suitable radioisotope or radiotracer for the analysis,
- list the suitable radioanalytical method for the analysis,
- predict the concentration in an analyte by isotope dilution analysis,
- explain principle, methodology and applications of IDA,
- describe the basic principle of neutron activation analysis,
- demonstrate NAA technique for the determination of concentration,
- describe sensitivity and detection limits of NAA,
- list typical applications of NAA,
- distinguish between the of radiometric titrations and other titrimetric methods,
- identify metal ions/organic constituents by radiochromatography,
- explain methodology of radioimmunoassay and list its advantages, and
- compare detection limits and sensitivity of NAA and IDA with other trace analysis methods.

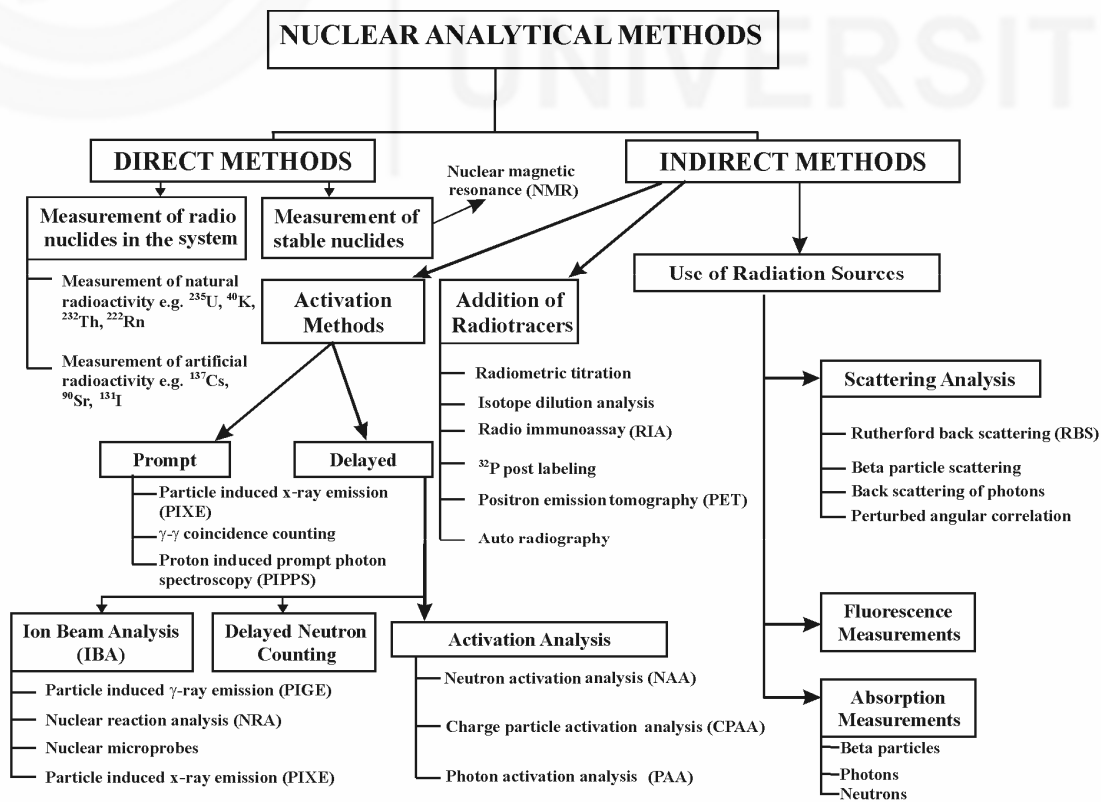


Fig.13. 1: Classification of nuclear analytical methods based on the measurement of radioactivity

13.2 RADIOTRACER TECHNIQUES

Application of radioactivity is best illustrated by the use of radiotracers in various disciplines such as chemistry, physics, biology, agriculture, medicine, industry, etc. Looking to the tremendous scope of radiotracers, it is important to understand basic principle involved in radiotracer methodology. George de von Hevesy, a Hungarian chemist and co discoverer of neutron activation analysis, is credited with being first to use radiotracers for determining solubility product of lead substances in water. Hevesy was awarded 1943 Nobel Prize in chemistry for his pioneering work. He was also the first person to use radiotracers to follow biological processes, tracing the movement of radionuclides from soil into plants and movement of food through animal systems. Production of radionuclides in nuclear reactors gave a great impetus to tracer concept. This concept has made further crossroads with the development of multitracers in 1990s.



George de von Hevesy

A radiotracer is defined as a chemical specie having one or more radionuclides as cation, anion or one of its components so that it may be used as a marker to follow the course of a chemical reaction or physical process or to show the location of a substance in an area. The activity of radionuclide is monitored to follow the process under investigation. Basic principle, on the basis of which a radiotracer works, is that it mixes well with its own element or compound and behaves chemically in a similar manner except for its radiation emitting property. Radiotracers can be used qualitatively for identification only, as simple markers or to quantitatively determine the amount of a component in a mixture.

13.2.1 Choice of Radiotracers

It is not possible to use all the radionuclides especially with too long (>10 y) or too short ($<$ few min) half lives as radiotracers. Similarly, type of emitted radiation is also an important criterion. In order to make use of a radionuclide as tracer certain assumptions and criteria have to be made.

The most important assumption is that the radiotracer should mix perfectly well with the system under investigation. It implies that the radiotracer should preferably be in the same chemical form (ionic or covalent) as the analyte with which it is being mixed. It means that the tracer will behave as an indistinguishable from the nonradioactive material except for the emitted radiation. Further, it should not adversely affect any component by way of radiation damage which means that the energy of radiation should not be too large to cause bond damage. Of course tracer is present in small amounts so that its radioactivity does not significantly affect the system in physical appearance or otherwise. Different isotopes of a radiotracer differ in mass between the active and non active species. Though isotopes of different masses show different reaction rates kinetically because of isotope effect and suitable corrections should be made but these are not really significant for heavy elements. NOTE: It may be noted that by substituting 'H with ^2D , isotopic mass effect is quite significant whereas for other elements of higher mass, it will be insignificant. Thus, main criteria for the choice of a radiotracer are type of radiation emitted, and half-life of radioisotope including its availability.

13.2.2 Factors Affecting Choice of Radiotracers

Several factors as depicted in Fig. 13.2 should be considered while choosing a suitable radiotracer. Most important of these is the half-life of the radioisotope. It should not be too short or too long. However, it could be in the range of a few minutes to a maximum of couple of years so that there is sufficient activity present throughout the experiment for measurement of activity and good counting statistics. A general

criterion could be that the half-life should be at least as long as the duration of the experiment.

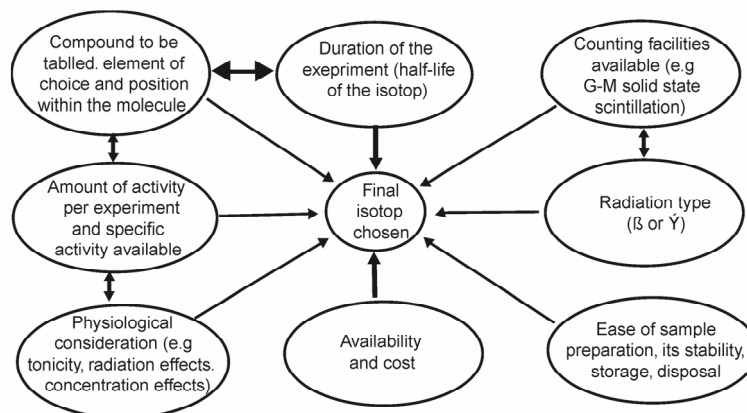


Fig. 13.2: Illustration of factors affecting the choice of radiotracer

Too long half-life means low specific activity and it may result in problems with disposal, storage, long term environmental contamination and excessive radiation dosage to the worker. It may be noted that the half-life of a tracer is also decided by the usage. For example, if an iodine isotope is needed for imaging thyroid gland then ^{128}I ($t_{1/2} = 25$ min) may be used whereas for any chemical studies long lived isotopes such as ^{131}I ($t_{1/2} = 8$ d) or ^{125}I ($t_{1/2} = 59.7$ d) should be recommended depending on their ease of availability. Similarly, using sodium isotope as biological marker in human studies, ^{24}Na ($t_{1/2} = 15$ h) should be used whereas for chemical studies long lived ^{22}Na ($t_{1/2} = 2.6$ y) may be used.

Another important consideration is the type of radiation emitted by the radionuclide especially with respect to penetrating power, ease of measurement and potential radiation damage. Generally an α emitting isotope is least preferred because of low penetration power associated with high ionization and radiation damaging effects. For thick and dense samples, γ emitting radiotracer should be a necessary choice because of its high penetrating power and ease of detection. In principle β emitters may be used as tracer for thin samples such as thin films and surfaces but these are not preferred. In general, β or γ emitters are considered as best choice for chemical studies because of ease of detection by using Geiger-Muller or scintillation counters.

Potential chemical and physical interactions of the tracer with the system under study should also be a matter of consideration. Attention must also be paid to the possible interactions of the other components of the tracer solution with the system because not all the isotopes can be obtained in radiochemically pure form with high specific activity. Generally radiotracers are available as chloride or nitrate salt solution though sometimes sulphate or phosphate salt solutions are also available. Chemical form of radiotracer depends on the separation procedure adopted.

Some other practical considerations are easy availability within the country and the cost of the desired tracer, the accessibility of the appropriate detector system, and the availability of laboratory certification and counting equipments. GM counter is the most easily available in a laboratory though NaI (TI) based scintillation counter is also available quite easily. Similarly, fume hood requirements may also restrict the use of an isotope as tracer. As already mentioned in section 12.4.3 of Unit 12 of this course, radiotracers in India are available from the Board of Radiation and Isotope Technology (BRIT), Mumbai though these are prepared in the Isotope Division of the Bhabha Atomic Research Centre (BARC), Trombay, Mumbai.

Besides analytical applications, radiotracers have been used for a variety of investigations such as leak detection, location of blockages in industrial systems, wear studies of machine tools and engine components, oil well tracer logging, ground water studies, silt movement in the sea bed, thickness gauge in steel rolling mills etc. Thus now days radiotracers are finding increasing applications in medical diagnosis and agriculture.

SAQ 1

Which one of the radioisotopes of manganese (^{54}Mn , $t_{1/2} = 312 \text{ d}$; ^{56}Mn , $t_{1/2} = 2.56 \text{ h}$) should be used as radiotracer for the determination of manganese in a steel sample in a chemical laboratory? Explain the reason.

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SAQ 2

Which one of the following radioisotopes should be used as radiotracer for the type of studies mentioned below?

^{128}I ($t_{1/2} = 25 \text{ min}$), ^{131}I ($t_{1/2} = 8 \text{ d}$), and ^{125}I ($t_{1/2} = 59.7 \text{ d}$)

- a) For the determination of solubility product of PbI_2 using radiotracer method.

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- b) For the treatment of thyroid cancer using a radiopharmaceutical.

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- c) For assaying of thyroid hormones by RIA.

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SAQ 3

- a) Which one of the following is the most important characteristic property of a radiotracer technique?

- i) Multielement analysis
- ii) Trace analysis
- iii) Sensitivity
- iv) Nondestructive character

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- b) Which of the following is TRUE or FALSE for a radioanalytical method?
- i) Radiation hazards
 - ii) Less specific
 - iii) Limited applicability
 - iv) Serious chemical interferences

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13.3 ISOTOPE DILUTION ANALYSIS (IDA)

It is a widely used quantitative analytical technique of considerable value in complex mixtures of compounds in organic syntheses, biochemical systems and for the determination of inorganic elements in geological and biological samples. It was first developed by George de von Hevesy and R. Hobbie in 1932 though many innovative developments were made by J. Tolgyessy, J. Ruzicka and J. Stary. It has the advantage of not requiring the separation of a component to be determined quantitatively from an analyte mixture. The method of IDA has two alternatives, direct isotope dilution analysis (DIDA) and inverse/reverse isotope dilution analysis (I/RIDA).

13.3.1 Principle and Equation

It is primarily based on the principle that the specific activity of a mixture of stable and radioisotope remains unchanged during chemical processing. Accurately known amount of specific activity of a radiotracer is added to an aliquot of analyte mixture containing the sought component. After addition, the resultant specific activity decreases. Chemical separation procedure is carried out where the component need not be recovered quantitatively but it must be in chemically pure form with well defined composition and in sufficient amount so that it can be weighed. Separation may be carried out by any of the following separation procedures:

- Solvent extraction of chelate complexes formed with specific reagent
- Precipitation using specific reagent
- Ion-exchange separation
- Electrodeposition or sorption process

After equilibration the desired constituent is separated, weighed and its activity is measured.

Sp. activity of tracer (SA) + Analyte Mixture = Sp. Activity of isolated part

Here, specific activity is defined as $SA = \text{Activity} / \text{Mass in mg}$
 $= A \text{ counts per min (R)} / m.$

In general, the separation is performed by chemical separation methods as mentioned already. Of all, solvent extraction is the most widely used separation method because of its simplicity and ease in handling.

Let us consider a complex sample of m g containing m_1 g of the component of interest. A radiotracer with total activity of A counts per min having a mass of m^* is added to the analyte so that its specific activity becomes $SA_1 = A / m^*$. After chemical separation, if m_1 g component containing both the active and inactive components is isolated and its activity is measured as A_1 counts per min. Its specific activity will be

$SA_2 = A_1/m_1$ and will remain constant regardless of how much of the pure component is isolated. Following balance sheet can be formulated.

	Before mixing	After mixing (before purification)
Amounts	m_1 (inactive) + m^* (active)	$m_1 + m_2$ (mixture)
Sp. Activity	SA_1	SA_2

After purification, only a fraction (not quantitative) is isolated. Since specific activity of the component of interest will remain same before and after purification, it follows that

$$m^* SA_1 = (m_1 + m^*) SA_2 \quad \dots (13.1)$$

On solving this equation for the analyte of interest m_1 , we get

$$m_1 = m^* (SA_1/SA_2 - 1) \quad \dots (13.2)$$

Thus, percentage amount of the component of interest in m g sample may be calculated.

$$\text{Percentage of unknown} = \frac{100 \times m^*}{m} \left(\frac{SA_1}{SA_2} - 1 \right) \quad \dots (13.3)$$

Since all the quantities on RHS can be determined experimentally, the amount of unknown in a given sample can be determined in appropriate units of % or ppm in case of trace abundance. This is the equation to be followed for DIDA. Following conditions must be fulfilled to carry out IDA procedure;

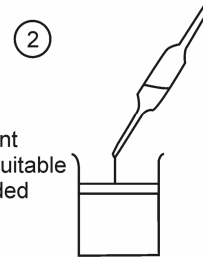
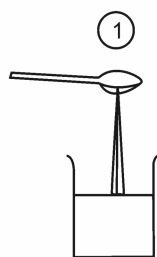
- The radioactive tracer must be radiochemically pure. It means that the tracer must not be contaminated with any other radioisotope.
- The radiotracer must be chemically identical. It means that it must be in ionic or covalent form as the analyte.
- The radiotracer must be uniformly distributed in the sample. It means that the mixture must be stirred thoroughly.
- Suitable separation procedure must be developed before hand to isolate the component in pure form.
- Half-life of the radiotracer must be sufficiently long so that it should be feasible for chemical separation.

A schematic illustration of various steps in IDA determination is shown in Fig. 13.3. This procedure is also called direct isotope dilution analysis (DIDA). In an alternate method called **inverse isotope dilution analysis** (IIDA) amount of radioactive substance (m^*) in the sample solution with activity A_1 can be determined by adding inactive component (m_1) and some fraction of the pure component having activity A_2 is isolated. In this case mass of radioactive substance (m^*) is determined using the equation:

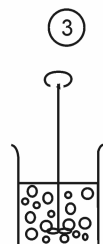
$$m^* = (A_1/A_2)m - m_1 \quad \dots (13.4)$$

where, m and A_2 are the weight of recovered sample and its activity respectively. Thus quantity of the radioactive substance initially present in the sample or its specific activity is calculated. It has the advantage that quantity of specific radioactive component of a sample without comparing it with a known radioactive standard can be determined. This procedure is especially useful for determining the amount of radionuclide present in a complex mixture of radionuclides, say spent nuclear fuel or reactor irradiated sample.

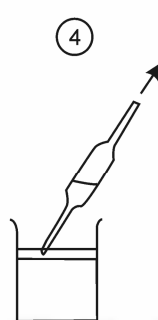
A small amount (m^* g) of radiotracer of known specific activity (SA_1) is added to the solution of complex mixture containing an unknown quantity (m_1 g) of same compound. Solution is warmed to make sure of isotopic exchange



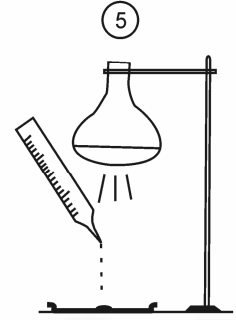
Organic solvent containing a suitable reagent is added



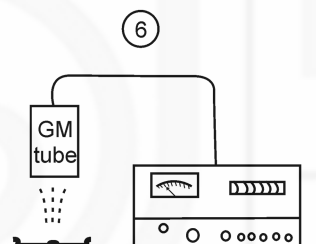
Two-phase aq.-organic solvent mixture is stirred, and radiotracer is quantitatively extracted



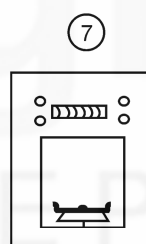
Aliquot of organic phase is withdrawn on a planchet



Evaporated to dryness.



Sample is counted (R_2) using a GM counter



Planchet is weighed (m).



From specific activity ($SA_2 = R_2/m$) unknown weight (m_1 g) is calculated by

$$m_1 = \left(\frac{SA_1}{SA_2} - 1 \right) m^*$$

Fig. 13.3: Schematic illustration of sequence of steps for the determination of an element or a compound in a complex mixture by IDA

Solved Example 1: A 0.6 cm^3 solution containing $7.5 \mu\text{Ci}/\text{cm}^3$ of ^{59}Fe was injected into the blood stream of a horse. After equilibration, 1.0 cm^3 of blood was taken out and it gave 13239 counts in 3 min. Calculate the blood volume in the body of horse.

Solution $7.5 \mu\text{Ci}/\text{cm}^3 = 7.5 \times 10^{-6} \times 3.7 \times 10^{10} \text{ counts/s} = 27.75 \times 10^4 \text{ cps}$
 Total activity added to the blood = $0.6 \times 27.75 \times 10^4 = 166.5 \times 10^4 \text{ dps}$
 Activity of blood taken out = $13239/3 \times 60 = 73.55 \text{ cps}$
 Let the volume of blood in the body of horse be $X \text{ cm}^3$

As the amount of solution added to the horse body is insignificant compared to the large blood volume, it is not necessary to use the Eq. (11.2). Instead, we can assume the constancy of total activity because of long half life of radiotracer i.e. 44.5d

$$73.55 X = 166.5 \times 10^4 \quad \text{or} \quad X = 2.26 \times 10^4 \text{ cm}^3 = 22.6 \text{ dm}^3$$

Ans. Horse body contains 22.6 dm^3 of blood

Solved Example 2: Isotope dilution analysis was employed for the determination of glycine in 5.83 g protein hydrolysate mixture by using ^{14}C labelled compound. 5.3 mg labeled compound with an activity of 39856 cpm was added to the mixture. The counter had background counts of 11790 counts in 90 min. After thorough mixing, the solution was passed through an ion-exchange column when 3.7 mg pure glycine was

Radioanalytical Methods

Solution

The counter had a background of 11790 counts in 90 min

or Background count rate = 131 cpm

Amount of radiolabeled tracer added, m^* = 5.3 mg

Supervising Assistant Secretary, 1111 G.A. (39856-131)

$$\begin{aligned}\text{Specific Activity of the radiotracer added, SA}_1 &= \frac{39725}{25.7} \\ &= 39725/25.7 \\ &= 1545.7 \text{ cpm/mg}\end{aligned}$$

Amount of pure fraction of glycine = 23.7 mg

$$\begin{aligned}\text{Net Activity of glycine} &= 10900 \text{ counts in } 50 \text{ min} = 218 \text{ cpm} - 131 \text{ cpm}(\text{background}) \\ &= 87 \text{ cpm}\end{aligned}$$

Specific Activity of separated fraction of glycine = $87 / 3.7 = 23.5$ cpm/mg

$$\text{Amount of glycine in mixture} = 5.3 (1545.7/23.5 - 1) = 343.1 \text{ mg}$$
$$\begin{aligned}\text{Percent amount of glycine in the mixture} &= (0.343/5.83) \times 100 \\ &= 5.89\%\end{aligned}$$

13.3.2 Instrumentation

An advantage of IDA method is that it does not require any specific or costly instrumentation except simple radioactivity measurement equipments such as G.M. counter or scintillation gamma ray spectrometer which are good for gross activity measurements. Of course one requires a good electronic balance for accurate determination of the mass of the recovered sample. For chemical separation procedure a radiochemistry laboratory with glove box and fume hood facilities are also required. Also the laboratory must have license to acquire radioisotopes from BRIT, Mumbai. When stable isotopes are used, the diluted natural isotopic ratio is related to the concentration of the amount and isotopic composition of the separated isotope that was added. In that case measuring instrument is mass spectrometer and the technique is called isotope dilution mass spectrometry (IDMS). This technique of IDMS is, however, less common and requires a more sophisticated laboratory.

13.3.3 Applications

IDA is one of the most widely used analytical technique for trace element analysis in a wide variety of complex matrices of geological, biological, environmental, archaeological, and others. IDA procedure has been used for the determination of compounds that are of interest in organic and biochemistry and inorganic industrial samples. Methods have been developed for the determination of diverse substances such as vitamins, insulin, sucrose, penicillin, amino acids, thyroxin, etc. Also the IDA has been widely used for the determination of inorganic elements in biological systems such as for the study of interaction of inorganic elements in organisms, role of elements in disease and regulation of elements by the body. It has been employed for several quantitative analytical problems that are otherwise difficult to handle by other analytical methods. Because of its advantage that quantitative recovery is not required, IDA is especially useful. Some typical situations are considered below;

- a) *A quantitative isolation of the element or compound is difficult* – This could be exemplified by the determination of iodide in a mixture of halides as its quantitative separation is almost impossible. ^{128}I ($t_{1/2} = 25$ min) or ^{131}I ($t_{1/2} = 8$ d) may be used as a radiotracer which is formed by radioactive neutron capture of ^{127}I . Radiotracer with known specific activity could be added to the halides mixture or any other sample containing halides. All the halides may be precipitated by the excess addition of AgNO_3 solution. To the precipitate sulfuric acid and MnO_2 may be added which would oxidize I^- to I_2 which may be sublimed by heating and condensing vapours on a cold surface. The mass and

activity of the condensed sample may be determined and thus the amount of I^- or iodine may be determined.

- b) *More rapid analysis is required* – Rapid determination of Co in steels can be carried out by IDA using carrier free ^{60}Co of known activity. A small sample of steel is extracted from the furnace melt, dissolved in acid and to this tracer solution is added. From the resultant solution arbitrary amount of cobalt metal is electro deposited, and its mass and activity are determined. Thus, amount of cobalt in original steel sample is routinely determined.
- c) *It is impossible to obtain the entire sample for analysis* – Total blood volume in human body may be determined by IDA using ^{51}Cr ($t_{1/2} = 27.7$ d), ^{59}Fe ($t_{1/2} = 44.5$ d) or even ^{32}P ($t_{1/2} = 14$ d). The procedure involves removal of a few cm^3 of blood from the body, addition of tracer of known activity and re injection into the body. Tracer binds itself with the hemoglobin in the blood cells. After equilibration for a few hours (approximately 2.5 h), a small aliquot of blood is removed for the counting of activity. From these measurements, total blood volume is determined. Similarly, total amount of water in a pond or lake and of oil in a huge container may be determined.

13.3.4 Advantages and Limitations

As already mentioned radiotracers have special advantages compared to routine chemical or instrumental methods of analysis. Some special advantages of IDA are;

- a) Isotope dilution analysis is more sensitive than many other analytical methods depending on the availability of radiotracer of desired specific activity. For certain elements such as Zn, Ag, Hg, IDA has better detection limits than even neutron activation analysis. Of course sensitivity of IDA technique depends on the availability of carrier free or high specific activity radiotracer.
- b) Where other instrumental methods fail to achieve these limits for complex matrices, IDA using appropriate radiation detectors can measure much smaller amounts.
- c) Radiotracers also have potential specificity due to their unique decay properties and hence chemical interferences are of little consequence.
- d) Quantitative separation is not necessary. In many wet chemical analyses involving trace amounts, it is almost impossible to achieve quantitative yield. However, yield should be more than 50% without too many losses.
- e) Nuclear detection equipments used for IDA are less expensive than many modern instruments. Instrumentation is quite simple because only gross activity measurement is required.
- f) IDA can be used for speciation studies in environmental and biological samples.
- g) Decay process of radiotracers is not affected by ambient conditions of temperature, concentration of reagents, pH, etc and these could be used for determining dyes in natural water systems.

Besides advantages, IDA has following limitations;

- i) IDA is essentially a destructive method. Since the sample is not preserved, it has a limited applicability.
- ii) The method can be used for the determination of a single element at a time whereas for several samples multielemental methods are preferred.
- iii) IDA has limited applicability for the elements for which suitable radiotracers are available.

iv) Blank levels may vary considerably from μg (10^{-6} g) to pg (10^{-12} g).

13.3.5 Substoichiometric Isotope Dilution Analysis (SIDA)

A variation in isotope dilution analysis method was suggested by J. Ruzicka and J. Stary in 1963. The necessity to determine specific activity makes IDA a difficult technique for trace determination because it is necessary to isolate sufficient weighable amount of substance. This difficulty is overcome in substoichiometric IDA where a standard with known amount of analyte and the unknown solution are subjected to an identical separation process. In this process same amount of reagent is added which is a little less than that required stoichiometrically (substoichiometric) in both standard and the unknown solutions so that same amount of analyte is extracted in both cases. Ideally the amount of radiotracer added to the unknown should be approximately of the same order. An illustration of the principle of SIDA is shown in Fig. 13.4

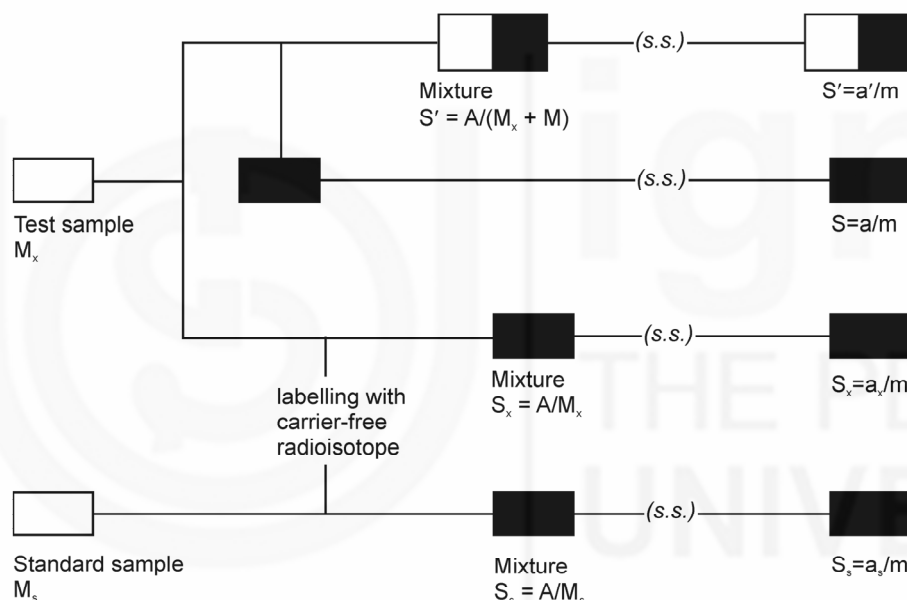


Fig. 13.4: Illustration of principle of substoichiometric isotope dilution analysis

Since masses of portions counted are identical, the specific activities SA_1 and SA_2 in Eq. (13.3) can be replaced with the measured activities A_1 and A_2 of the two portions. This eliminates the quantitative determination step for the samples. Having known the amount of reagent exactly (as in the composition of reaction product) the amount of substance (m_{cal}) isolated by performing the isolation step can be determined. Unknown amount of component (m) in the analyte may be calculated by using the eqn.

$$m = m_{\text{cal}} \times (A_1/A_2) - m_o \quad \dots (13.5)$$

where, m_o is the amount of activity added. An essential aspect of the method is the availability of a suitable reagent which is added in equal amounts in the standard and sample solution.

An important advantage of substoichiometric method is the enhanced selectivity of the procedure caused by competing ability of the unreacted substance towards the impurities which are kept away from reacting with the reagent and passing into the same phase as the substance of interest. The technique has been widely used for the accurate and specific determination of a large number of elements in a variety of complex matrices. Several other variations of IDA have been developed e.g. sub and super equivalence IDA. These methods have lost popularity because of complex chemical procedures.

SAQ 4

Why long lived radioisotopes are preferred for isotope dilution experiment? If at all you have short lived isotope only in your laboratory, what precautions should be followed?

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13.4 ACTIVATION ANALYSIS (AA)

Techniques of activation analysis include the use of nuclear reactions (section 12.4.1 of Unit 12) induced by neutrons, photons or charged particles such as protons, deuterons, alpha particles and other heavy ions in a nuclear reactor or accelerator facility to produce radionuclides. When a target element is bombarded with a neutron beam, a compound nucleus with higher mass number in excited state is formed. It may undergo instantaneous decay to form a product nucleus finally emitting delayed β or γ rays. This technique is called neutron activation analysis (NAA). However, if the bombarding particle is photon, it is called photon activation analysis (PAA) or if the sample is bombarded with charged particles such as protons, deuterons or alpha particles then it is called charged particle activation analysis (CPAA). If bombarding particle induces x-ray emission via interaction with the electron shells of the target element, the analytical method is known as particle induced x-ray emission (PIXE). Of all the activation analysis methods, NAA using reactor neutrons remains the most widely used analytical method. The technique of NAA was discovered by George de Van Hevesy and Hilde Levy in 1936 and used for the determination of Dy in a rare earth mineral by using ^{222}Rn .Be neutron source. Later charged particle activation analysis (EPAA) was discovered by G T Seabory and Livingood in 1938 for the determination of Ga impurity in iron by deuteron induced reaction. Here we shall describe principle, methodology including instrumentation and applications of NAA in detail.

13.4.1 Principle of NAA

The technique of NAA involves two steps; irradiation of a sample with neutrons and subsequent measurement of the induced radioactivity. The radionuclides are characterized by their characteristic γ -ray energy and half-life as illustrated in Fig. 13.5.

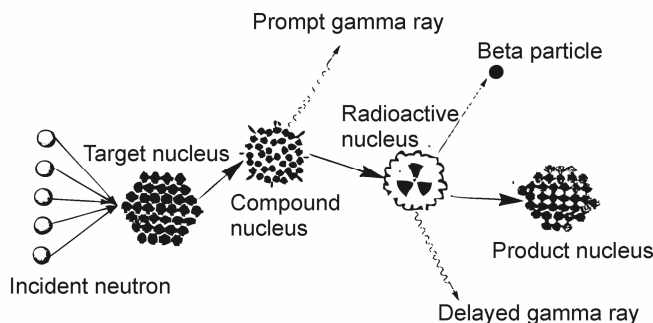


Fig. 13.5: Basic principle of Neutron Activation Analysis

In this process, the rate of formation of the product atoms (N_p) is represented as;

$$\frac{dN_p}{dt} = N \sigma \phi - \lambda N_p = N \sigma \phi - \lambda N_1 \quad \dots (13.6)$$

where N is the number of target atoms of an element in the sample $\left[\frac{iw N_A}{M} \right]$,

i is the isotopic abundance; w is the concentration of element; M is the mass of the target atom; N_A is the Avogadro number;
 σ is the thermal neutron absorption cross section in barns ($b = 10^{-24} \text{ cm}^2$);
 ϕ is the neutron flux ($\text{n cm}^{-2} \text{ s}^{-1}$), and

λ is the decay constant (s^{-1}) $= \left(\frac{0.693}{t_{1/2}} \right)$ and $t_{1/2}$ = half life of the product nucleus.

On integration, Eq. (13.6) yields

$$\lambda N_1 = A = N \sigma \phi (1 - e^{-\lambda t_i}) \quad \dots (13.7)$$

where t_i is the irradiation time. Since the sample is delayed for time t_d before counting using a counter with efficiency ϵ , and gamma-ray abundance γ , the complete equation can be written as;

$$A = \left(\frac{wiN_A}{M} \right) \sigma \phi (1 - e^{-\lambda t_i}) e^{-\lambda t_d} \epsilon \gamma = \left(\frac{wiN_A}{M} \right) \sigma \phi S D C \epsilon \gamma \quad \dots (13.8)$$

where $S = (1 - e^{-\lambda t_i})$ is saturation factor, $D = (e^{-\lambda t_d})$ is delay factor, and $C = (1 - e^{-\lambda t_c}) / \lambda$ is the correction factor for decay during counting time t_c .

Activity (A) is measured in terms of peak area after subtracting background. γ activity of radioisotopes is measured using an HPGe detector though it could also be measured by using a scintillation detector. Thus, concentration of an element may be determined by rearranging the left part of Eq. (13.8) as;

$$w = \frac{AM}{i N_A \sigma \phi (1 - e^{-\lambda t_i}) e^{-\lambda t_d} \epsilon} \quad \dots (13.9)$$

This way of calculating the concentration of an element is called the **Absolute Method**, where σ , ϕ , t_i , t_d and ϵ are the experimentally determined parameters. After calculating w , the concentration of an element in the sample, its mass may be determined. However, the absolute method has uncertainties in the values of ϕ and σ . It is well known that these may vary under experimental conditions depending on the location of the sample in the reactor or irradiation can. In order to eliminate these uncertainties, a **Comparator Method** has been suggested where a standard (primary and/or secondary) with known concentration of element is simultaneously irradiated along with the sample under similar experimental conditions. Both are counted using the same detection system in the same geometry. Thus, an equation similar to Eq.13.8 can be written for the activity of standard (A') as;

$$A' = \left(\frac{w' i N_A}{M} \right) \sigma \phi (1 - e^{-\lambda t_i}) e^{-\lambda t_d'} \epsilon \gamma = \left(\frac{w' i N_A}{M} \right) \sigma \phi S D' C \epsilon \gamma \quad \dots (13.10)$$

Dividing LHS of Eq. 13.8 by Eq. 13.10, we get

$$\frac{A}{A'} = \frac{w}{w'} \times \frac{e^{-\lambda t_d}}{e^{-\lambda t_d'}} \quad \dots (13.11)$$

This is applicable only if $t_{1/2}$ of the radionuclide is comparable with delay time (t_d) and counting time (t_c). However, if $t_{1/2}$ of the radionuclide is long enough compared to delay and counting times then, it can be further simplified to

$$\text{Conc. of element in sample}(S_a) = \text{Conc. of element in standard}(S_t) \times \frac{\text{Activity in sample}}{\text{Activity in standard}} \dots (13.12)$$

When many elements are to be determined in each sample, preparation of individual standards for each element becomes tedious and impractical. Some workers use synthetic multielemental standard which are difficult to prepare and have its own limitations. Thus, either multielemental standards or the use of a single comparator for all the elements becomes necessary. In Eq. 13.12, activity is replaced by specific activity to have the uniformity while considering masses of the sample and the comparator standard. The standard/certified reference materials (SRM/CRM) are available from various agencies such as the National Institute of Standards and Technology (NIST, USA), International Atomic Energy Agency (IAEA, Vienna), National Institute of Environmental Studies (NIES, Japan), Institute of Nuclear Chemistry and Technology (INCT, Poland), wherefrom RMs of a variety of matrices such as plant leaves, tissue or dust particulate soils and rock powder are available. However, these are quite expensive. Therefore, many laboratories prepare their own standards by spiking with μg amounts of standard aq. solutions prepared from AR/GR/HP grade salts on to SiO_2 /cellulose or Whatman filter paper strip.

In order to achieve better accuracy and to eliminate errors due to secondary standard, a **Monostandard (k_o) Method** has been proposed. In this method a single element such as Au, Mn, Co with 100% isotopic abundance is used as a comparator for multielement NAA. Several schemes have been devised to accomplish this and the most important of these is k_o method. following equation is used to calculate the concentration (w) using this method.

$$w(\mu\text{g/g}) = \frac{A_{sp}}{A_{sp}^*} \frac{1}{k_o} \frac{(f + Q_o^*(\alpha))\epsilon^*}{(f + Q_o(\alpha))\epsilon} \dots (13.13)$$

where A_{sp} is the specific activity, f is the thermal to epithermal flux ratio
 Q_o is the ratio of the resonance integral to the thermal neutron cross section;
 ϵ is the detector efficiency; α is the deviation of the epithermal flux from ideality

$$k_o = \text{the } k_o \text{ factor} \left(\frac{M^* i \gamma \sigma_{th}}{M i^* \gamma^* \sigma_{th}^*} \right); * \text{corresponds to the comparator element}$$

The k_o factor is an experimentally determined value that contains the values for the nuclear constants needed in the activation analysis. In Eq. 13.13 a new term K_{anal} is introduced so that it may now be written as

$$\rho(\mu\text{g/g}) = \frac{A_p' p}{A_{sp}^*} \cdot \frac{I}{K_{anal}} \text{ where } K_{anal} = k_o \frac{(f + Q_o)}{(f + Q_o)^*} \cdot \frac{\epsilon}{\epsilon^*} \dots (13.14)$$

where A_p' is the peak area of the i^{th} element corrected for saturation, cooling and decay during counting and normalized for 1 g sample. The k_o factors for many nuclides have been determined and published using gold as a comparator element.

13.4.2 Neutron Sources

A wide range of devices is used to produce neutrons needed for activation analysis. Some are sophisticated and extremely expensive, while others are rather simple and modest in cost. In Table 13.1 are listed some commonly used neutron sources. These may be classified into three groups: isotopic and machine type sources and nuclear reactors. As evident from Table 13.1, isotopic neutron sources are characterized by low flux, mobile and have low cost. These are good for

Table 13.1: Common Sources of Neutrons for NAA

Reaction	Half Life	Average Neutron Energy (MeV)	Neutron Yield (ns ⁻¹ Ci ⁻¹ , unless otherwise stated)
I. Isotopic Sources			
(i) Photo neutron (γ, n) ⁸⁸ Y with ⁹ Be ¹²⁴ Sb with ⁹ Be	106.6 d	0.16	1x10 ⁵
(ii) Alpha emitter (α, n) ²³⁹ Pu with ⁹ Be ²²⁶ Ra with ⁹ Be ²⁴¹ Am with ⁹ Be	60.2d 2.4x10 ⁴ y 1600 y	0.02 3.5 3.6	1.9x10 ⁵ $\approx 10^7$ 1.1x10 ⁷
(iii) Spontaneous fission ²⁵² Cf	433 y 2.64 y	3.5 2.3	2.2x10 ⁶ 2.3x10 ¹² ns ⁻¹ g ⁻¹
II. Machine Type Source			
(i) Cockroft-Walton n-generator ³ H(d, n) ⁴ He	-	14.7	10 ⁸ – 10 ¹¹ n/s
(ii) Cyclotron 10 μ A of 30 MeV deuterons on Be [²³⁹ Pu(α, n) ²⁴² Cm]	-	Broad distribution	2x10 ¹¹ ns ⁻¹
III. Nuclear Reactors			
APSARA (1956) } CIRUS (1960) } BARC, Dhruva (1985) } Mumbai	-	Broad distribution	$\left. \begin{array}{l} \sim 10^{11} \\ \sim 10^{12} \\ \sim 10^{13} \\ \text{s}^{-1} \end{array} \right\} \text{ n cm}^{-2}$
Kamini (1999) – IGCAR, Kalpakkam			$\sim 10^{10}$

demonstration experiments and for the determination of major constituent elements such as Al, Mn and Cu in bauxite, pyrolusite and pyrite respectively. However, these have limited life and are not good for trace element analysis. On the other hand machine type sources have the advantage of being put off when not in use. On the other hand reactor neutrons are most widely used for NAA work. These have wide energy spectrum as illustrated in Fig. 13.6. Neutrons may be classified on the basis of energy viz. thermal (0.025 eV), epithermal (1 eV-1 keV) and fast (>1MeV). Various activation methodologies have been developed depending on the energy of neutrons. A brief description of these methodologies is described here.

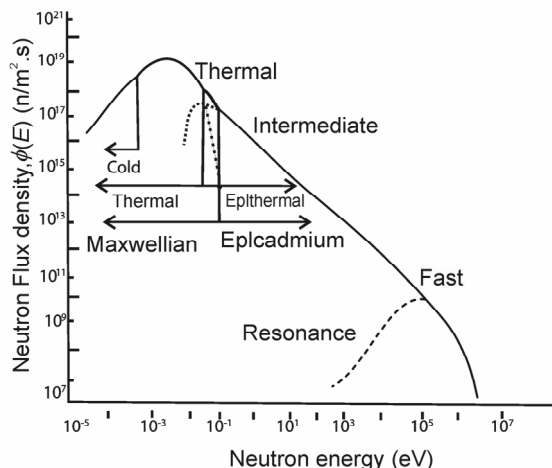


Fig. 13.6: Neutron energy spectrum in a nuclear reactor

Energy of neutrons plays an important role as different types of reactions viz. (n, γ) , (n, p) , (n, α) , (n, n') , $(n, 2n)$ are possible depending on the energy of neutrons as already mentioned earlier in section 12.4.1 of the preceding Unit 12. Schematic layout of the production of 14 MeV fast neutrons as produced in Cockroft-Walton neutron generator using the reaction $d(t, n)^4\text{He}$ is shown in Fig. 13.7.

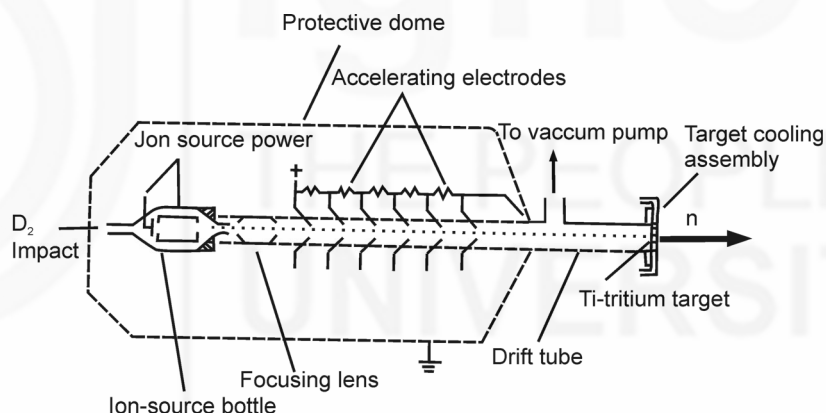


Fig. 13.7: Schematic diagram of a 14 MeV neutron generator developed by Cockroft and Walton

SAQ 5

Explain if ^{241}Am -Be neutron sources can be used for following studies. Mention how?

- a) For the determination of major constituents in an ore such as Mn in pyrolusite or Al in bauxite

.....
.....

- b) For the demonstration of half life determination experiment of ^{56}Mn ($t_{1/2} = 2.56$ h) or ^{128}I ($t_{1/2} = 25$ min)

.....
.....
.....

- c) For the determination of Cr or Co in steel sample.

- A. **Thermal NAA:** Thermal neutrons with energy 0.025 eV can induce radiative capture (n,γ) reactions in the target nuclei and delayed γ-rays from the sample are detected. An advantage of using thermal neutrons is their higher cross section compared to those for higher energy neutrons. At higher flux of thermal neutrons, TNAA offers potentially higher sensitivity for a large number of elements except lighter elements such as C, N, O etc. and potentially toxic elements such as Tl, Pb, Ni, etc. TNAA can be used for the determination of concentration of up to 60 elements at trace and ultra trace level can be determined in a wide variety of samples.
- B. **Epithermal NAA:** Epithermal neutrons are narrowly defined as those having energy in the range of 0.1 to 1.0 eV. In practice the lower end of the neutron energy range used in ENAA is closer to 0.5 eV, which is the Cd cutoff energy (~ 0.55 eV) and the higher end of the range extends through the resonance neutrons up to several keV. Epithermal neutrons, like thermal neutrons, also induce (n, γ) reactions and delayed gammas are detected for many elements. These neutrons have limited applicability for a number of elements such as Ag, As, Au, Ba, Br, Cs, Eu, Ga, Gd, Hf, Ir, La, Ni, Rb, Sm, Sn, Sr, Ta, Tb, U, W and Yb which can be determined with higher sensitivity.
- C. **Fast NAA:** The fast neutrons encompass the widest range of energy, 0.5 – 20 MeV. Neutrons of this energy range do not induce (n,γ) reactions but instead are responsible for (n, p), (n, α), (n, n') and (n, 2n) reactions. Mostly FNAA is performed using 14 MeV neutrons. There are specially useful for the determination of oxygen using the reaction $^{16}\text{O}(n, p)^{16}\text{N}$.

An illustration of various types of neutron induced reaction is already illustrated in section 12.4.1 of preceding Unit 12.

Solved Example 3: 100 mg piece of a gold ornament containing 10% copper as impurity was irradiated with thermal neutrons at $2.1 \times 10^{11} \text{ n/cm}^2/\text{s}$ for 2 days producing ^{198}Au ($t_{1/2} = 2.7 \text{ d}$) and ^{64}Cu ($t_{1/2} = 12.7 \text{ h}$). Calculate the activity of these nuclides in terms of counts/s at the end of irradiation and after a delay of 2 d and 4 d. How long will it take for the decay of activity due to ^{64}Cu to 0.1% of its original activity? ^{197}Au is monoisotopic and its thermal neutron absorption cross section (σ) is 99 b whereas ^{63}Cu is 30.9% abundant and its σ value is 4.4 b.

Solution 100 mg piece of gold ornament will contain 90 mg gold or ^{197}Au and 10 mg of copper or $0.309 \times 10 = 3.09 \text{ mg } ^{64}\text{Cu}$
Using the Eq. (13.8) Activity due to ^{198}Au at the end of irradiation

$$= 90 \times 10^{-3} \times 6.02 \times 10^{23} \times 2.1 \times 10^{11} (1 - 0.5985) / 197$$

$$= 22.96 \times 10^8 \text{ cps}$$
 Activity after a delay of 2 d $= 22.96 \times 10^8 \times e^{-(0.693 \times 2 / 2.7)}$

$$= 13.74 \times 10^8 \text{ cps}$$
 Activity after a delay of 4 d $= 22.96 \times 10^8 \times e^{-(0.693 \times 4 / 2.7)}$

$$= 8.22 \times 10^8 \text{ cps}$$

Activity due to ^{64}Cu at the end of irradiation $= 3.09 \times 10^{-3} \times 6.02 \times 10^{23} \times 4.4 \times 10^{-24} (1 - 0.073)$

$$= 2.53 \times 10^7 \text{ cps}$$
 Activity after a delay of 2 d $= 2.53 \times 10^7 \times e^{-(0.693 \times 48 / 12.7)}$

$$\begin{aligned} &= 18.4 \times 10^5 \text{ cps} \\ \text{Activity after a delay of 4 d} &= 2.53 \times 10^7 \times e^{(0.693 \times 96/12.7)} \\ &= 13.4 \times 10^4 \end{aligned}$$

$$\begin{aligned} \text{Using the formula } t &= 1/\lambda \ln N_0/N \\ \text{After substitution, time interval} &= 12.7/0.693 \ln 2.53 \times 10^7 \times 0.1/100 \\ &= 185.8 \text{ h} = 7.74 \text{ d} \end{aligned}$$

13.4.3 Interferences

NAA is based on nuclear phenomenon and hence no chemical interferences are possible. Possible nuclear interferences are primary, secondary and second order interference reactions, gamma ray spectral interference, neutron self-shielding, γ -ray self-attenuation, true and random coincidences during gamma ray measurement. Some of these are described in following lines.

- A. **Primary Interferences** It arises when the same radionuclide is formed from other than the analyte element. For example, in the case of ^{56}Mn produced by (n, γ) reaction, the same radionuclide is also produced by $^{56}\text{Fe}(n, p)$ and $^{59}\text{Co}(n, \alpha)$ reactions on iron and cobalt respectively. Thus, determination of Mn in steel containing iron and cobalt may be erroneous if higher energy neutrons are present. If the sample is irradiated with thermal neutrons only or Cd/B shielding is used then these interfering reactions may be eliminated. Other examples of such interferences are in the determination of Al in presence of Si and P in soil or Na in presence of Al and Mg in biological samples.
- B. **Spectral Interferences:** Gamma-ray spectral interferences occur when similar γ -ray energies are produced by other radionuclides produced by other elements. For example: 846.8 keV of ^{56}Mn is often interfered by 844 keV of ^{27}Mg . Similarly 279.2 keV of ^{203}Hg is interfered with 279.5 keV of ^{76}Se . In such cases other indicator gamma rays should be used or one should wait for the decay of the other radio nuclide. For example, ^{56}Mn has $t_{1/2} = 155 \text{ min}$. and it should be counted after an hour so that ^{27}Mg ($t_{1/2} = 9.45 \text{ min}$) may undergo decay during this period.

Other types of less common interferences are due to successive capture and secondary particles from primary reaction. For example, in the determination of manganese by (n, γ) reaction producing ^{56}Mn is also produced by a successive reaction, $^{54}\text{Cr}(n, \gamma) ^{55}\text{Cr} \beta^-$, $^{55}\text{Mn}(n, \gamma) ^{56}\text{Mn}$. Similarly while determining nitrogen in proteins via $^{14}\text{N}(n, 2n) ^{13}\text{N}$ and $^{13}\text{C}(p, n) ^{13}\text{N}$.

SAQ 6

A bauxite sample was analyzed for Al, Si, Fe and O by neutron activation analysis. Mention the type of neutron (energy) used and type of interferences, if any, in the determination of each of these elements.

- a) Aluminum (Al)

.....

- b) Silicon (Si)

.....

- c) Iron (Fe)

.....

13.4.4 Sensitivity and Detection Limits

The sensitivity of an element in NAA is defined as counts μg^{-1} for particular experimental conditions viz. t_i (irradiation), t_d (delay) and t_c (counting) durations besides neutron flux (ϕ) of the irradiation source, neutron absorption cross section (σ) and counting efficiency (ϵ) of the detector system. Depending on the nuclear properties such as isotopic abundance (i) and neutron absorption cross section (σ) of the isotope of interest, the above parameters (t_i , t_d and t_c) can be decided. From the activity Eq. (13.8), it is clear that the main parameters for sensitivity is σ , ϕ and ϵ . Since σ is fixed for a target nucleus that can not be changed, high flux neutrons and so also a detector of high efficiency are needed. Because a sample may contain isotopes that are not active, the fraction of the atoms that are radioactive must also be known. Therefore, isotopic abundance (i) of the nuclide of interest plays an important role in deciding the detection limits. Rate of production of activity of a radionuclide depends on neutron flux (ϕ) and the time of irradiation in a nuclear reactor (t_i). For a particular target being irradiated in a neutron device, however, activity will increase exponentially. A comparison of the production of activity in three different neutron devices is illustrated in Fig. 13.8 where X-axis is the irradiation time in terms of half-life of the product and Y-axis is the activity. It is observed that highest activity is obtained for irradiation in a high flux reactor. In NAA detection limit varies from pg to mg depending on the element of interest, gamma ray background, sensitivity and sample matrix.

Detection limit is a statistical concept, based on the ability of a measurement method to determine an analyte in a sample matrix, regardless of its source of origin. Without a precise statistical definition, it is impossible to determine a numerical value for the limit of detection (LOD). In general, all LODs are defined in terms of 3σ where σ is standard deviation for the specimens. Thus detection limit (L_D) is defined as 3σ of the standard deviation of the background counts (C_b) under the peak and is calculated using the expression

$$L_{D(\text{counts})} = 2.71 + 3.29 \times \sqrt{C_b} \quad \dots (13.15)$$

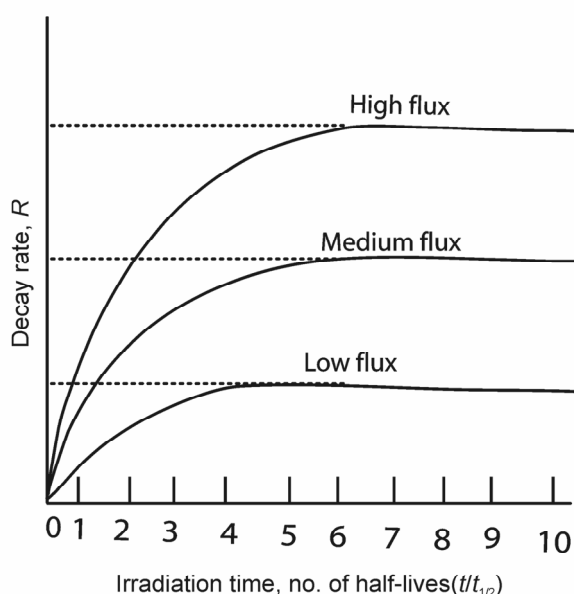


Fig. 13.8: Variation of activity with neutron flux

The counts are then converted to $\mu\text{g g}^{-1}$ by using S (sensitivity) as sample mass (g). Also activity may be taken as 10,000 counts with 0.1% statistical error and thus the amount or sensitivity may be calculated under experimental conditions. The typical detection limits using a flux of $10^{13} \text{ n cm}^{-2} \text{ s}^{-1}$ are available from the literature. For elements like Eu and Dy, the L_D is about 1 pg whereas for Mn, In and Lu, it is 10 pg under normal experimental conditions of INAA. However, sensitivity can be improved if RNAA procedure is followed whereby interfering radionuclides are removed and thus background is lowered.

13.4.5 Classification

The NAA technique can broadly be classified as instrumental and radiochemical NAA depending on whether the sample is counted directly after irradiation or after chemical separation and the resultant activity is used for calculating the concentration of an element. Many methodological developments have taken place primarily due to the advancement in instrumentation, methodology and data processing procedures as illustrated in Fig. 13.9. Some of these methodologies are described here.

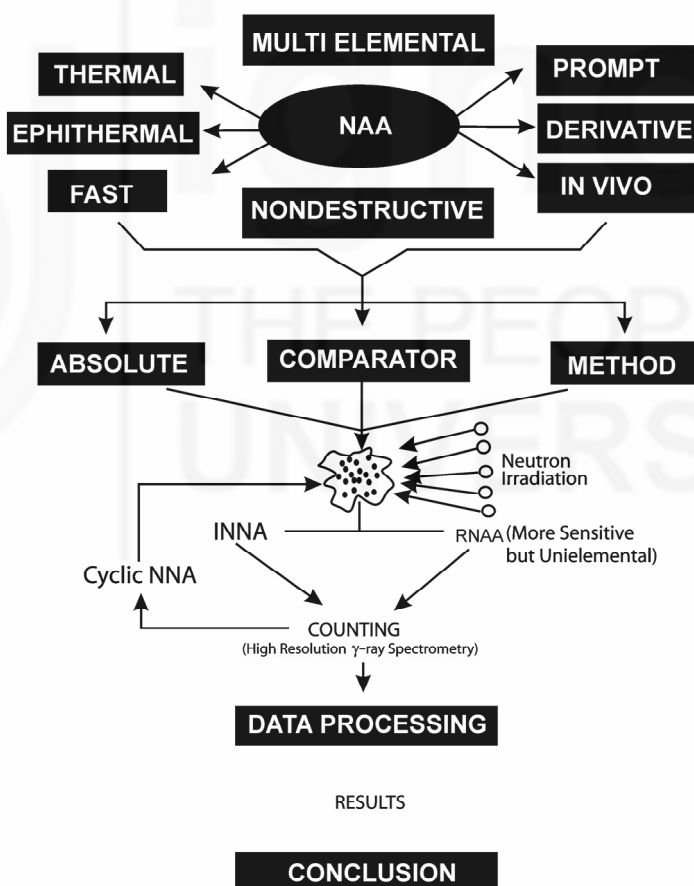
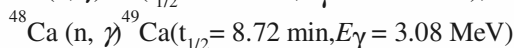
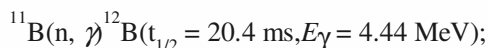


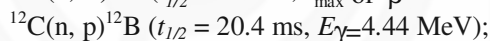
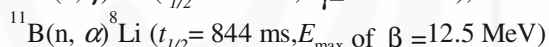
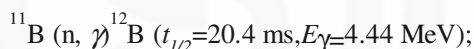
Fig. 13.9: Various methodologies of NAA

- A. **Prompt Gamma (PG) NAA:** In this methodology, first used in 1969, prompt gamma rays emitted during (n, γ) reaction as illustrated in Fig. 13.5 are counted. An essential feature of this technique is that detector system has to be placed in close vicinity of the irradiation facility so that γ rays are directly counted. It is frequently used for analysis and is especially suitable for the determination of lighter elements such as H, B, N, P, S, C and Si and elements having high neutron absorption cross section e.g. Hg, Cd, Sm and Gd. A majority of PGNAA work is carried out using isotopic sources though counting facility can be setup at the reactor site as well. It is being increasingly used as a rapid

instrumental, non-destructive and multielemental analysis technique. A further advancement of PGNA is *in-vivo* PGNA where the entire body or a segment of the patient/subject is irradiated with $^{241}\text{Am-Be}$, ^{252}Cf or an accelerator based neutron source followed by counting of prompt/delayed gamma rays. PGNA has been widely used due to improved analytical sensitivity and detection limits especially by using low energy guided neutron beams. It's most clinical use is the *in vivo* determination of ^{10}B concentration needed to measure the radiation dose received by patients undergoing neutron capture therapy for tumours.



- B. Short Irradiation Activation Analysis** This involves irradiation of the sample for a very short duration (1-600s) and counting after a delay of 20 to 100 ms. It is particularly useful for the determination of elements with very short half life of the order of few seconds. It has the advantage of low turn around time and high throughput. A fast irradiation measurement system (FIMS) using TRIGA Mark II reactor has been developed. Short time instrumental NAA, with high throughput, sensitivity and accuracy without matrix interferences can be achieved in spite of the initial high count rate from both short and long lived nuclide activation. Some typical examples are;



Short time NAA ($t_i = 30 \text{ s}$) for the analysis of trace elements in honey has been performed. The technique has the advantage of being free from radiation hazards.

- C. Cyclic Neutron Activation Analysis (CNAA):** In case of elements with short-lived activation products, sensitivity is often hampered by low activity and thus reliability of the measurement is at stake. This difficulty is overcome by repeating the whole cycle of irradiation and counting several times and subsequently summing up the γ -ray spectra as typically illustrated in Fig. 13.10.

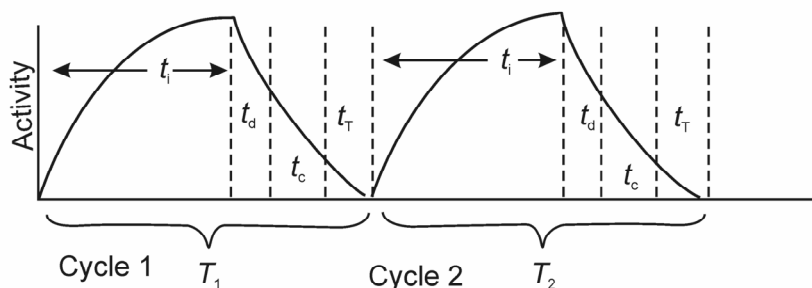


Fig. 13.10: Schematic illustration of cyclic neutron activation analysis

Its basic theory has been developed and an equation derived for the total activity;

$$A = \frac{N \phi \sigma k}{\lambda} \left(1 - e^{-\lambda t_i}\right) \left(1 - e^{-\lambda t_c}\right) \left(e^{-\lambda t_d}\right) \left[\frac{n}{1 - e^{-\lambda T}} - \frac{\left(e^{-\lambda T}\right) \left(1 - e^{-n\lambda}\right)}{\left(1 - e^{-\lambda T}\right)^2} \right] \quad \dots (13.16)$$

where A = total cumulative counts recorded in n cycles,

k = factor containing the detector efficiency and branching ratio for the decay path,

t_i = irradiation time for each cycle,

t_c = counting time for each irradiation,

t_d = delay time period to counting for each irradiation,

T = the delay period from the end of a previous delay period to the end of the next delay period (cycle time, s), and

n = number of cycles.

Thus, having statistically significant counts, sensitivity is enhanced. A typical example of this technique is the determination of aluminium using $^{27}\text{Al}(n, \gamma) ^{28}\text{Al}$ ($t_{1/2} = 2.3$ min) in bauxite using ^{241}Am -Be neutron source.

D. Derivative Neutron Activation Analysis (DNAA): This method also called as Molecular Activation Analysis is used to enhance the sensitivity of NAA for more elusive elements, which are otherwise difficult to be determined by NAA. It has the advantage of being useful for studying chemical speciation. In this technique, an element or chemical entity to be determined (U) is complexed with, or exchanged for another element, which has higher sensitivity for NAA. Also the product radionuclide should have an interference free photopeak in low background region. It is desirable that the compound formed should be stoichiometric and it should be easily separable quantitatively. A DNAA procedure has been developed for the determination of phosphorus. It is complexed as molybdovanadophosphoric acid which is then extracted in MIBK and the activity of ^{52}V ($t_{1/2} = 3.4$ min) is measured. Thus phosphorus which is otherwise difficult to be determined by NAA, can be determined indirectly.

E. *In Vivo* (IV) NAA: It has been suggested that NAA can be used to determine whole body elemental levels in living organisms. Ever since IVNAA has become an established technique in nuclear medicine, it is being widely used for biomedical analysis. IVNAA has found applications in basic physiological studies, clinical diagnosis of disease and monitoring of therapeutic interventions for diseases. In fact future developments in NAA depend on the diagnostic medical applications using *in vivo* activation.

$^1\text{H}(n, \gamma) ^2\text{H}$ ($E_\gamma = 2.22$ MeV)

$^{31}\text{P}(n, \gamma) ^{32}\text{P}$ ($E_\gamma = 0.08$ MeV)

F. Chemical Treatment before Irradiation: In some cases chemical separations are required before irradiation as a special measure because the concentration of analyte in the sample is too small or if speciation studies are to be carried out. This is often referred to as chemical neutron activation analysis (CNAA). These may be of following types;

- a) **Preconcentration NAA:** It is especially useful in case of water samples because of low levels of analyte elements present (at μg or ng or even pg/cm^3). For this purpose, water is passed through an ion exchange resins or some other adsorbent where the analyte elements are retained. In other cases, measured volume of water can be dry ashed whereby the sample size is reduced and analyte elements are concentrated.
- b) **Chemical Neutron Activation Analysis (CNAA):** It was developed for determining trace amounts of analyte elements such as As and Hg in a variety of matrices including water, sedimentary rock, plant, animal organs etc. In this case, a chemical procedure is followed whereby trace elements are separated before irradiation and thus sensitivity is enhanced.

- c) **Speciation Neutron Activation Analysis (SNAA):** Chief limitation of NAA is that it can not be used for speciation studies. However, its scope can be further extended by chemical separation of specie(s) prior to irradiation and then determining the elemental content. This is essential because the toxicity of an element depends significantly on its physicochemical form. There is an increasing interest in studying speciation by NAA because of its low detection limits. It involves two steps; first the separation of species from the sample followed by the detection of element by NAA. A variety of methods involving liquid chromatography, reversed phase chromatography, ion exchange, hydride generation, liquid-liquid extraction, solid phase extraction, coprecipitation etc. have been developed for the separation of various inorganic, organic and organometallic species. The main advantage of SNAA includes simultaneous speciation of elements such as Cl, Br and I, which are rather difficult to be determined by other techniques. A SNAA method has been developed in combination with HPLC for the determination of low levels of five arsenic species, As(III), As(V), monomethylarsonic acid (MMA), dimethylarsenic acid (DMA) and arsenobetaine (AsB) in natural water samples.

SAQ 7

Which of the following parameters are responsible for higher sensitivity in neutron activation analysis? Explain briefly.

- a) Neutron absorption cross section (σ)

.....
.....

- b) Isotopic abundance (i)

.....
.....

- c) Neutron flux (ϕ)

.....
.....

- d) Counter efficiency (ϵ)

.....
.....

SAQ 8

53.8 mg steel sample was analyzed for its manganese content by neutron activation analysis following comparator method. 68.5 mg standard steel sample containing 0.96% manganese was irradiated along with the sample in the ^{252}Cf source and counted under the same experimental conditions. Activity of the sample and the standard counted for 5 min were 16597 and 21983 counts respectively.

- i) Calculate the % amount of manganese in the steel sample.

.....
.....

- ii) What are the possible interferences in this analysis and how these could be minimized or eliminated?

.....

- iii) Is the irradiation source right for this analysis?

.....

- iv) What will be the detection limit for the determination of manganese by this method?

.....

13.4.6 Instrumentation

General procedure of NAA can be divided into several stages viz sampling, sample preparation, sample packing, irradiation and activity measurements, calculation of results, data processing and its interpretation. Of these irradiation and activity measurement are the two main steps of NAA which will be discussed here. Neutron irradiation can be carried out with any of the neutron sources described in Table 13.1. Isotopic neutron sources can be used for some specific studies involving analysis of major constituents in bauxite or pyrolusite or alloys. Most widely used sources are nuclear reactors. In India three nuclear reactors at the Bhabha Atomic Research Centre (BARC), Mumbai are APSARA, CIRUS and Dhruva which are used for NAA work. The γ -ray spectrometry forms the basis of NAA. However, a method based on β -activity measurement has been developed for the determination of P via ^{32}P . In principle, a scintillation gamma ray spectrometer can be used but it has limited applicability when radiochemical separation is carried out. Generally an HPGe detector is used because of better performance in terms of energy resolution, peak to Compton ratio, efficiency and linear response of pulse height and energy of gamma photons. It consists of HPGe detector with all its electronic accessories as schematically shown in Fig. 13.11. Radionuclides are identified by prior calibration of the spectrometer using standard sources. In some cases, however, half life of the radionuclide is also followed.

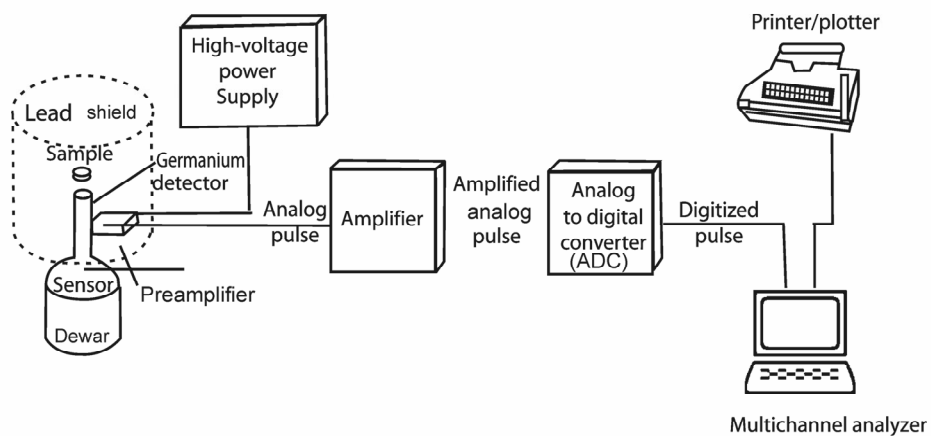


Fig. 13.11: Schematic of counting set up for high resolution gamma spectrometry

Characteristic E_γ , $t_{1/2}$, and other characteristics of common elements determined by NAA are listed in Table 13.2. A photographic illustration of the HPGe detector surrounded with lead shielding, NIM Bin with HV supply, ADC and MCA including computer are shown in Fig. 13.12.

Target Element (Nuclide)	Isotopic Abundance (%)	Product Nuclide	Half Life ($t_{1/2}$)	Cross Section σ (b)	Energy E_γ (keV)
Al (^{27}Al)	100	^{28}Al	2.241m	0.23	1779
As (^{75}As)	100	^{76}As	26.3h	4.3	559, 657
Au (^{197}Au)	100	^{198}Au	2.7d	98.8	412
Ba (^{138}Ba) and (^{130}Ba)	71.7 0.1	^{139}Ba ^{131}Ba	83.1m 12d	0.36 8.8	(166), 373, 496
Br (^{81}Br)	49.5	^{82}Br	35.4h	3.31	554, 776
Ca (^{48}Ca)	0.187	^{49}Ca	8.718m	1.1	3084
Ce (^{140}Ce)	88.5	^{141}Ce	33d	0.6	145
Cl (^{37}Cl)	24.4	^{38}Cl	37.2m	0.43	1643, 2167
Co (^{59}Co)	100	^{60}Co	5.27y	37.2	1173, 1332
Cr (^{50}Cr)	4.35	^{51}Cr	27.8d	15.9	320
Cs (^{133}Cs)	100	^{134}Cs	2.06y	30	605, 796
Eu (^{151}Eu)	47.8	^{152}Eu	13.5y	5900	244, 1408
Fe (^{58}Fe)	0.33	^{59}Fe	44.6d	1.2	1099, 1291
Hf (^{180}Hf)	35.1	^{181}Hf	42.4d	10	482
Hg (^{202}Hg)	29.7	^{203}Hg	46.6d	3.8	279
K (^{41}K)	6.88	^{42}K	12.5h	1.30	1524
La (^{139}La)	99.9	^{140}La	40h	8.93	487, 1596
Mg (^{26}Mg)	11.17	^{27}Mg	9.46m	0.038	1014
Na (^{23}Na)	100	^{24}Na	15h	0.5	1368
Mn (^{55}Mn)	100	^{56}Mn	2.58h	13.3	846
P (^{31}P)	100	^{32}P	14.3d	0.19	1708(β^-)
Rb (^{85}Rb)	72.1	^{86}Rb	18.7d	0.91	1077
Sb (^{123}Sb)	42.8	^{124}Sb	60d	3.3	603, 1691
Sc (^{45}Sc)	100	^{46}Sc	83.8d	27.2	889, 1120
Se (^{74}Se)	0.89	^{75}Se	120d	52	264
Sr (^{84}Sr)	0.56	^{84}Sr	65.2d	1.4	514
Th (^{232}Th)	100	^{233}Th (^{233}Pa)	27d	7.4	312
Ti (^{50}Ti)	5.18	^{51}Ti	5.76m	0.179	320
V (^{51}V)	100	^{52}V	3.74m	4.9	1434
Zn (^{64}Zn)	48.6	^{65}Zn	244d	0.76	1115



Fig. 13.12: Photographic representation of the HPGe counting set up with lead shielding and associated electronics

Typical gamma ray spectra of short and long lived radionuclides formed in Neem leaves along with its experimental conditions are shown in Figs. 13.13 and 13.14 respectively. Such spectra can be used for simultaneous determination of 25-30 elements in complex biological samples.

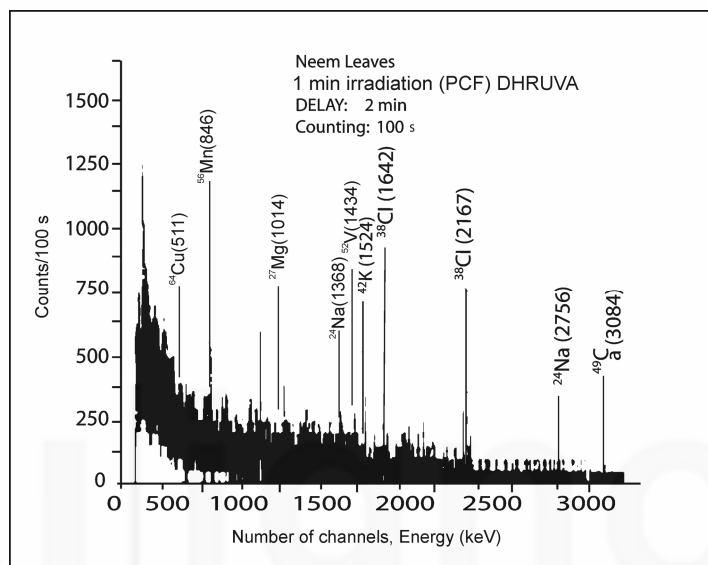


Fig. 13.13: Typical gamma ray spectrum of short lived nuclides in Neem leaves

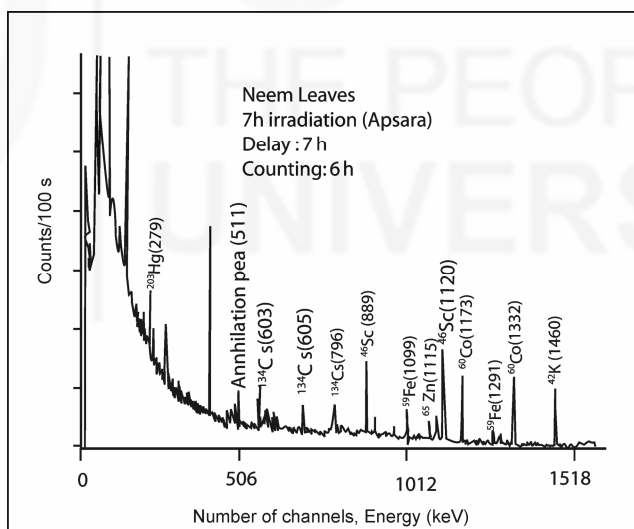


Fig. 13.14: Typical gamma ray spectrum of long lived radionuclides in Neem leaves after irradiation in APSARA reactor

Calculation and Data Processing: As can be seen from the gamma ray spectra of irradiated samples of natural matrix such as Neem leaves in Fig. 13.13 and 13.14, these are complex with closely lying multiple peaks. The continuum under the peak is due to Compton Effect from the γ -rays of higher energies and the background. The peaks show Gaussian distribution as the first contribution to the photo peak in a semiconductor detector is due to statistical fluctuations in sharing the absorbed energy between ionization and heating the crystal network. It is not practical to analyze these spectra manually. With the development of high resolution semiconductor detectors and 4, 8 and 16 K multichannel analyzers, γ -ray spectrometry along with computers with suitable software has become an important tool for the detection of natural and artificial radioactivity and activation analysis of complex samples. Important steps in the computerized analysis of photo peaks involve;

- Smoothening of experimental data
- Peak searching
- Selection of fitting intervals
- Peak energy calculation
- Peak area calculation

The superimposition of photo peaks on each other or the high Compton background makes it difficult to precisely determine the net counts in the region of interest (ROI). The base area is mostly taken as trapezoidal summation of the average counts in each channel giving the background over which the photo peak is situated as illustrated in Fig. 13.15.

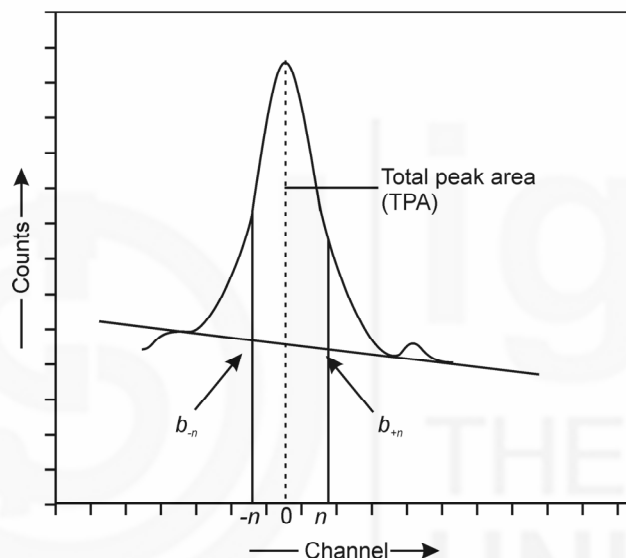


Fig. 13.15: Illustration of activity calculation from total peak area calculation

For NAA, the most important of all the various factors mentioned above is peak area calculation. Typically peak area may be calculated as in Eq. (12.13), derived for single channel analyzer. However, while using MCA several methods have been proposed to determine the total peak area (TPA) represented as:

$$A = \sum_{-n}^{+n} \alpha_i - \frac{n+1}{2} (b_{+n} + b_{-n})$$

where, n = number of channels on right (+) and left (-) of the peak channel (o),

α_i = total counts in i channels,

$b_{\pm n}$ = background counts in $2n$ channels as determined from a straight line drawn between the channels to left and right to peak channel.

Several softwares have been developed whereby closely lying multiplets can be resolved into individual photo peaks.

SAQ 9

Which type of nuclear counting instrument system will be suitable for following type of analysis?

- a) Determination of P by isotope dilution analysis.

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- b) Determination of Mn in steel by NAA.

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.....
Multielemental trace analysis of lunar samples.

.....
.....
Assay of steroids in the blood of an athlete.

13.4.7 Applications

Importance of trace element analysis (TEA) in today's context of technological globalization is vital. A physicist working in the field of high purity semiconductor materials, a geologist in mineral exploration, an analytical chemist dealing with the problems related to trace elements in biological systems or heavy metal pollutants in environment, a physician treating the patient suffering from a disease like Ca deficiency or Cd toxicity in bones, a curator in a museum interested in studying historical monuments and a criminologist dealing with the murder case, all require a suitable analytical technique to solve their problems at their respective work place. Though there are several alternative techniques such as x-rays fluorescence (XRF), inductively coupled plasma mass spectrometry (ICP-MS), energy dispersive x-ray fluorescence (EDXRF), but the only common answer to all these problems is NAA provided a nuclear reactor is available? It is possible to determine up to 60 elements with high sensitivity, specificity, accuracy and precision. NAA has found wide applications for macro, micro, trace and ultra trace element analysis in the samples corresponding to diverse fields such as archaeology, biomedicine, animal and human tissues, environmental science, forensics, geology and cosmo chemistry, industrial products, nutrition, quality assurance of analysis and certification of reference materials (RM). Some important applications are primarily related with biology, nutrition and medicine as illustrated in Fig. 13.16. NAA is now a well accepted routine analytical method for determining trace and ultratrace constituents in small size (just few mg) samples. We shall discuss some typical applications in following lines.

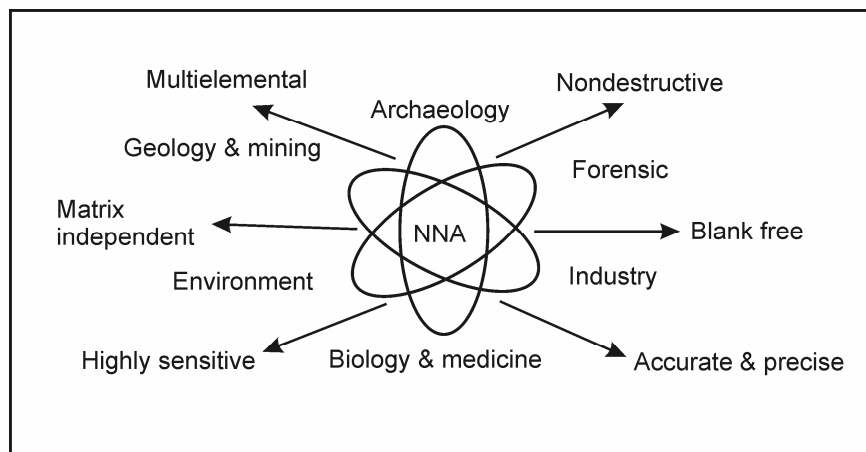


Fig. 13.16: Applications of NAA in various disciplines

- i) **Forensic science:** Non-destructive nature along with simultaneous multielemental analysis in small amount of sample by NAA has proved to be a boon in forensic studies. Some marker elements such as Ba, Sb, Cu, Ag, Fe, Br, Zn and K are analyzed depending on the type of sample. A typical case of multiple murders involving arsenic poisoning in USA has been solved by NAA. Positive detection of Ba and Sb in the firearm discharge residue swapped from the hands has proved to be of great importance in criminal cases. NAA has been used by forensic experts to find the muzzle distance of bullet fire and differentiate entry and exit hole in certain cases. Most important elements from forensic point of view are As, Hg and Pb whose analysis may help in deliberate poisoning or accidental ingestion cases. Another area of application of NAA in forensic science is the possibility of trace element characterization of specimens to establish probability of source correspondence. Looking to the potentials of NAA, Central Forensic Science Laboratory (CFSL), Kolkata has set up NAA unit at the Analytical Chemistry Division of BARC, Mumbai.
- ii) **Environmental Sciences:** NAA has made significant contributions in the determination of heavy metals and other inorganic pollutants in a variety of matrices such as hair, nails, suspended particulate matter, fish, etc. NAA has been accepted as a basic analytical tool by environmentalists against its earlier recognition as a nuclear technique. INAA has been used for analyzing hair and fish to monitor occupational exposure and aquatic environmental pollution. A large population exposed to subsoil water contaminated with arsenic showed clinical features of arsenic toxicity called arsenicosis. On the other hand airborne particulate matter (PM) from industrial and vehicular emissions has been associated with a wide variety of environmental problems including detrimental health effects. INAA has been employed for determining up to 30 elements in the total suspended particulates (TSP) in the ambient air of Indian metropolitan cities of Delhi, Mumbai, Chennai, Kolkata, Hyderabad and Nagpur where significant differences in elemental contents were found depending on geographical location. There could be different aspects of such studies where biosphere and urban/industrial monitoring could be carried out by analyzing hair, air/water/soil or fish. Analysis of fish from coastal areas of Indian ocean has shown significant differences in elemental contents which are attributable to industrial activity in that region.
- iii) **Biology, Nutrition and Medicine:** It is now well recognized that in addition to the principal body building elements such as Ca, Mg, P, Na, K, etc. a number of trace elements, including V, Cr, Mn, Fe, Co, Cu, Zn, Se, Mo, Rb and I are essential for growth and health in humans. Just as important, however, are some toxic elements for the same group of species e.g. As, Br, Ag, Cd, Sb, Ba, Pb though to widely differing extent. Activation analysis has proved to be a very effective technique for the determination of most of these trace elements. The collaboration between activation analysts and medical researchers has resulted in a much deeper understanding of the function of trace elements in the body. Variation in trace element levels has been observed in hair, blood and tissue samples of nasal-pharynx cancer patients and so also in tissue and blood of breast cancer patients at different histopathological stages. NAA has been used in the determination of trace element levels in human blood and excision biopsy samples of breast cancer to correlate with clinical histopathological stages and further correlated it with dietary intake. Another biologically important area is nutritional studies where NAA is being extensively used because of its multielemental character. Trace element evaluation of some medicinal herbs has been carried out whereby variation in trace element contents of same plant species from different origins may be attributed to ecological and geographical variations. This may be further used for the development of drug formulation.

13.4.8 Advantages and Limitations

NAA is now a routinely employed analytical method because of following advantages:

- Universal applicability for major, minor and trace element analysis in a wide range of matrices for up to 60 elements.
- Virtual absence of any blank or chemical interference.
- High specificity and low detection limits for many elements.
- Non-destructive and multielemental character.
- Radiochemical NAA, though destructive, provides interference free determination in low detection limits.
- Requirement of only a few mg amount of sample for analysis.

Despite these advantages, NAA has many limitations as follows:

- Requires nuclear reactor which makes it less accessible and cost effective.
- Long turn around time especially for long lived radionuclides.
- No information on chemical speciation as NAA provides total element concentration.
- Some environmentally important elements such as Pb, Tl, Ni and low Z elements (H, C, N, etc.) cannot be determined.
- It cannot be automated or only partial automation possible after irradiation.

13.5 COMPARISON OF NAA AND IDA WITH OTHER METHODS

The development of analytical instrumentation over the past few decades has undergone a sea change and allowed to detect trace elements at sub-ppb or even lower levels. As recently as early 1960s, trace element determinations were predominantly carried out by traditional wet chemical methods and instrumental methods. It wasn't until the development of atomic absorption spectrophotometry (AAS) in late 1960s that the clinical community realized about necessity of a highly sensitive trace analysis technique that could be automated. With the advent of ICP technology, ICP-AES and ICP-MS were developed after 1980s. As a result NAA, AAS, and ICP-AES or ICP-MS or even XRF are in tough competition. Today NAA has emerged as a front-runner between various analytical techniques for trace element determination. A comparison of NAA with other trace element analysis methods especially with regard to size and type of samples is given in Table 13.3.

Table 13.3: Comparison of NAA and IDA with Other Trace Analysis Methods

	Accuracy	Sensitivity	Sensitivity	Distribution	Multielement	Sample Size	Sample Type	Routine	Turn Around time	Accessibility	Costs (\$)	Costs (£)
INAA	+	0	0	–	+	s-k	S,L	+	–	–	0	0
RNAA	+	+	+	–	0	s,m	S,L	+	–	–	–	–
IDA	+	0	+	–	0	m	S,L	+	+	+	0	+

XRF	0	–		–	+	s	S	+	+	+	+	
TXRF	0	0	+	–	+	s,m	L	+	+	+	0	+
SXRF	0	+		+	+	s	S	–	–	–	–	
PIXE	–	0		+	+	s	S	+	–	–	–	
ICP-ES	0	0	+	–	+	s,m	L	+	+	+	0	+
AAS	–	0	+	–		s,m	L	+	+	+	0	+
ICP-MS	0	0	+	–	+	s,m	L	+	+	0	–	0
ICP-IDMS	+	0	0	–		s,m	L	0	+	0	–	–
SSMS	–	0		–	+	s	S	+	+	+	+	

Scores: + = positive, 0 = Average, – = Negative, Sample size: s = mg, m = g, k = kg
Sample type : S = solid, L = aqueous solution

It may be mentioned that IDA is another ideal technique for trace element analysis though it has limitations of being unelemental and destructive. On the contrary INAA is essentially nondestructive as integrity of the sample is not changed in any manner by prechemistry or the addition of any foreign material before irradiation. Thus the problem of contaminants due to reagents introduced unlike AAS or ICP is completely avoided. The analytical approach of NAA for most of the elements of interest is primarily an instrumental technique. In some cases though, post irradiation chemistry may be required. Thus the technician time per sample analysis is low, producing an efficient, low cost analytical approach. NAA is a multi-element technique in that many elements can be analyzed simultaneously in a given sample by recording γ spectrum without changing or altering the apparatus as is necessary in AAS. NAA is fast in that for many elements, several samples can be irradiated at a given time and counted later on following a given decay schedule. A significant advantage of NAA is its sensitivity to trace elements. The sensitivity obtained by NAA is a function of the neutron absorption cross section (σ in cm^2) of the element in question, available neutron flux (ϕ in $\text{n cm}^{-2} \text{s}^{-1}$), irradiation time, resolution and efficiency of the detector, matrix composition, and the total sample size. Hence, increasing neutron flux and irradiation time, and the major advances in detector technology especially with regard to increased efficiency and resolution have pushed the detection limits of most elements of interest to the very low levels (10^{-12} g). It is observed that NAA shows much lower detection limits for most of the elements compared to AAS and XRF. With the availability of research reactors, NAA is now a more acceptable technique to the scientific community and industry. This is evident from the fact that more research papers have been published on trace element analysis using NAA during last decade compared to any other instrumental technique mentioned above.

Isotope dilution analysis (IDA) is primarily a destructive analytical method where it is essential to dissolve the sample and hence its identity is lost. Sensitivity of IDA is often restricted by the specific activity of the radiotracer. Since availability of a suitable radiotracer is very essential, it is not universally applicable. Instrumentation required for IDA work is much simpler and less costly than that required for NAA work. Availability of nuclear reactor puts another limitation for NAA whereas radiotracer based IDA can be carried out at any place far away from the reactor location.

13.6 RADIOMETRIC TITRATIONS (RT)

Radiometric titrations are based on the detection of radioactivity in a separable species used to indicate the endpoint of a titration. It has the unique advantage of requiring no indicator. Radiotracer may be added to the titrant or the unknown analyte sample. Sole purpose of the use of radioactivity is for detection of end point and as such it does not participate in the titration process. At the end of reaction, there is sudden change in activity level wherefrom end point may be determined. It is of special importance where suitable indicators are not available in conventional titrimetry (neutralization, redox, precipitation and complexometric titrations). However, it is essential to have a separable phase and that puts great limitation on its use though in principle it can be used for all types of titrations. Therefore, most successful radiometric titration procedures have been developed for the precipitation reactions.

13.6.1 Procedure

Typical experimental arrangement of radiometric titration consists of a titration vessel containing filter disc for phase separation as shown in Fig. 13.17 along with scintillation detector and lead shielding. A syringe is used to move supernatant solution into and out of the counting chamber which may be a scintillation detector. A typical example of radiometric titration is for the determination of halides using ^{110}Ag ($t_{1/2} = 252$ d) as tracer where corresponding silver halide is precipitated. In order to understand this method you may consider the titration of 10 mL of 1mM NaCl solution containing Cl^- with 1 mM solution of $^{110}\text{AgNO}_3$ to follow the reaction;

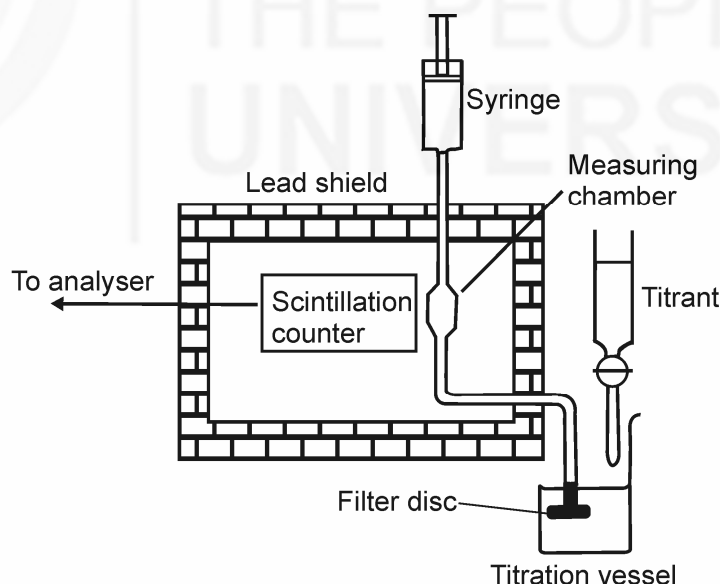


Fig. 13.17: Illustration of experimental set-up for radiometric titration

where $K_{sp} = 1.82 \times 10^{-10}$ for AgCl. Activity of the supernatant solution is monitored after equilibrium is attained following the addition of titrant. Initially solution containing NaCl has no activity. With the addition of increasing amount of $^{110}\text{AgNO}_3$, supernatant will have very little or insignificant activity because all the activity would have gone to the precipitate of $^{110}\text{AgCl}$. After the end point, however, activity of $^{110}\text{AgNO}_3$ will come into the solution and keep increasing with every additional drop. Titration data are plotted as shown in Fig. 13.18 from where equivalence point is the intercept of the two straight lines of the curve. Conversely, NaCl may be

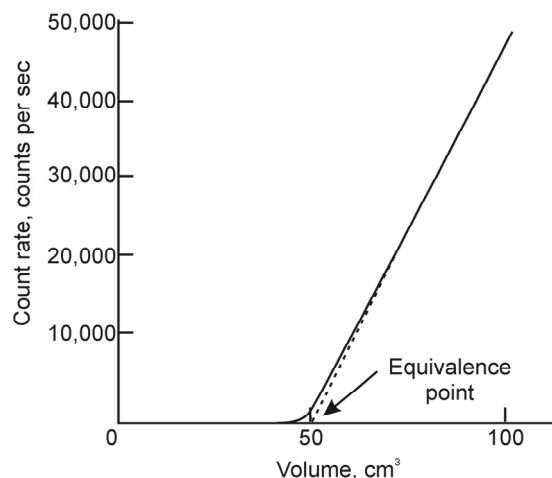


Fig. 13.18: Radiometric titration curve using radiometric titrant

labeled with ^{36}Cl ($t_{1/2} = 3 \times 10^5 \text{ y}$) and its activity in solution may be monitored. In that case, however, plot shape will be reversed. In other words, activity will show decreasing trend with the addition of each drop of AgNO_3 causing precipitation of Ag^{36}Cl . At the end point, however, activity will become constant. End point can be determined from the point of inflexion. It may be noted that ^{36}Cl is not a suitable radiotracer because of its long half-life.

As there is no method for titrating phosphate with a normal indicator, it may be conveniently determined by titrating with barium nitrate solution. For this purpose Na_3PO_4 labelled with ^{32}P ($t_{1/2} = 14.3\text{d}$) is used and a GM counter may be employed for counting the activity. Instead of cumbersome apparatus, a number of centrifuge tubes may be taken. A graph may be plotted for a graph of counting rate on y-axis against volume of barium nitrate solution added as the x-axis, from the graph, concentration of sodium phosphate is calculated.

13.6.2 Advantages and Limitations

This method has an advantage that the detection of end point does not depend on the chemical reaction employed unlike the normal titration where indicator such as phenolphthalein, methyl orange, ferroin, etc participate in the reaction. In principle, any titration system in which phase separation can be affected is good for radiometric detection provided a suitable radiotracer is available. A major limitation of radiometric titration is the requirement of phase separation method which of course is automatic in precipitation titrations. For other types of titrations, special phase separation methods will have to be employed.

13.7 RADIOCHROMATOGRAPHY

Radiochromatography is based on paper or thin layer chromatography though it could also be used in ion-exchange column or gas chromatography as well as in electrophoresis. It uses a radiotracer as cation or anion or radiolabelled organic compound where separated cation, anion or a compound is identified on the basis of characteristic radiation emitted by the radionuclide. Thus, any chromatographic separation can be carried out using radiotracers. However, no developer/chromogenic reagent or detector is used to study the movement of initial spot except nuclear counting equipment or autoradiography. In this way distribution of activity is measured which increases sensitivity of the measurement by several fold.

Radiochromatography has played an important role in the discovery of some trans-plutonium elements by Glen T. Seaborg. The identity of each element of 5f series was established beyond any doubt by the sequence of their appearance analogous to the

sequence of the corresponding 4f elements. There are many instances of the use of radiochromatography for analytical purposes.

Let us consider typical example of complete separation of the alkali metal ions Na^+ , K^+ , Rb^+ , and Cs^+ by using respective tracer solutions.

Requirements: Acid washed asbestos paper, dil. HCl as solvent, Radiotracer solution of ^{22}Na ($t_{1/2} = 2.6$ y), ^{42}K ($t_{1/2} = 12.36$ h), ^{86}Rb ($t_{1/2} = 18.6$ d) and ^{137}Cs ($t_{1/2} = 30.1$ y), Well type NaI(Tl) scintillation gamma ray spectrometer.

Procedure: Asbestos paper strip of 40 cm×3cm was taken and starting 3 cm from one end, lines were drawn using pencil at an interval of half cm up to 30 cm. each line was numbered. The glass chamber is filled with dil HCl up to a height of 5cm. Spotting was done on the strip using radiotracers at about 3 cm from one end in the centre. It was hung such that one half of its bottom remains dipped in solvent. The solvent starts rising till it stops rising any further in about an hour. The paper is removed from the chamber and dried in a drying chamber. Strips are cut along the lines, kept in a polythene bag and each strip is counted for 50 seconds in the well type scintillation counter. The activity of the strips (Y-axis) is plotted against the strip number (X-axis). Peaks observed for individual radioisotopes are used for calculating R_f values. Following values were obtained for individual alkali metal cations.

Na^+	K^+	Rb^+	Cs^+
0.86	0.77	0.72	0.45

In an unique application speciation studies of sulphur, S^{2-} , SO_3^{2-} , and SO_4^{2-} have been identified by using ^{35}S ($t_{1/2} = 87.2$ d) as tracer and GM counting system.

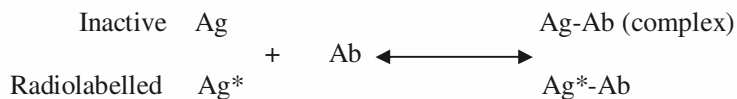
13.8 RADIOIMMUNOASSAY

Radioimmunoassay (RIA) developed by Rosalyn Yalow and Soloman Berson (1958) is an extensively used popular technique for assaying biologically important substances such as hormones, vitamins, drugs, virus, etc. in extremely small amounts of body fluids such as blood. For this unique discovery Dr Rosalyn Yalow was awarded Nobel Prize in Medicine in 1977 where insulin was determined using ^{131}I as tracer. RIA is an extensively used technique in medical diagnostic laboratories worldwide for the determination of hormones (B, TA, TSH, etc.), drugs, viruses, and other organic species.

13.8.1 Principle

It is based on a specific reaction between an antigen (Ag) and an antibody (Ab) by using radiolabelled antigen (Ag^*). An antigen is a simple bio molecule with molecular weight of a few hundred Daltons e.g. small steroids, drugs, thyroid hormones, or macromolecules such as virus, bacteria etc. It should be able to react with an antibody, a complex protein bio molecule with molecular weight of over a million Dalton and synthesized within a living system so as to nullify the effect of a foreign attacking substance. For example, if a living system encounters a new virus capable of causing a disease, it will try to resist the virus. One mechanism of resistance is by activation of the immune system to synthesize antibodies. The antibody binds with the antigen, in this case the virus, to form an antigen-antibody (Ag-Ab) complex.





Once antigen is bound to the antibody, the virus is no more active and it is metabolized and excreted from the body. The reaction of the antigen with the antibody is highly specific i.e. an antibody produced against a particular antigen will normally react with that antigen only. This may be compared with the lock and key system made for each other. Moreover, the equilibrium constants of such reactions are very high, of the order of 10^9 - 10^{12} dm³ per mole. These antigen-antibody reactions have been used to develop a series of highly specific and sensitive assays known as immunoassay. Some of these assays are capable of measuring analytes down to even femtomole (10^{-15} M) concentrations in the presence of related analogues.

13.8.2 Methodology

Let us consider an analyte A with a binder B which can bind with A to form a complex A-B. A conventional methodology to determine analyte concentration is to add excess amount of reagent to form product which may give a direct measure of the analyte. For example, to determine the amount of Ag in a solution, excess amount of HCl is added to form the precipitate of AgCl which is weighed and thus amount of silver present in unknown sample is calculated. An alternative method could be to use radiolabelled analyte and determine the activity of the product as in IDA. However, RIA differs in that instead of excess amount of reagent, only a limited or a little lesser amount of reagent is added as in substoichiometric isotope dilution analysis. Thus, only a fraction of analyte will get a chance to bind. In IDA, analyte is antigen (Ag) which is radiolabelled and the binder reagent is antibody (Ab). Determination of unknown concentration of antigen is based on the fact that the inactive and radiolabelled antigen compete physicochemically for the binding sites on the antibodies as illustrated in Fig. 13.19. With increasing amount of analyte, this fraction or its activity decreases. In case of RIA, both inactive antigen (Ag) and radiolabelled antigen (Ag*) are taken in limited amount and compete with a fixed (but lesser than required for stoichiometric reaction) amount of antibody (Ab). The concentration of inactive Ag is varied. In the absence of inactive Ag, all the Ab will be consumed to form Ag*-Ab and may be considered as initial activity. As inactive Ag is added, fraction of Ag*-Ab and hence its activity will decrease. Thus, the amount of radioactivity associated with the complex is inversely related to the concentration of Ag. By using known amounts of Ag as standard, a standard curve is set up as shown in Fig 13. 20. This may be used.

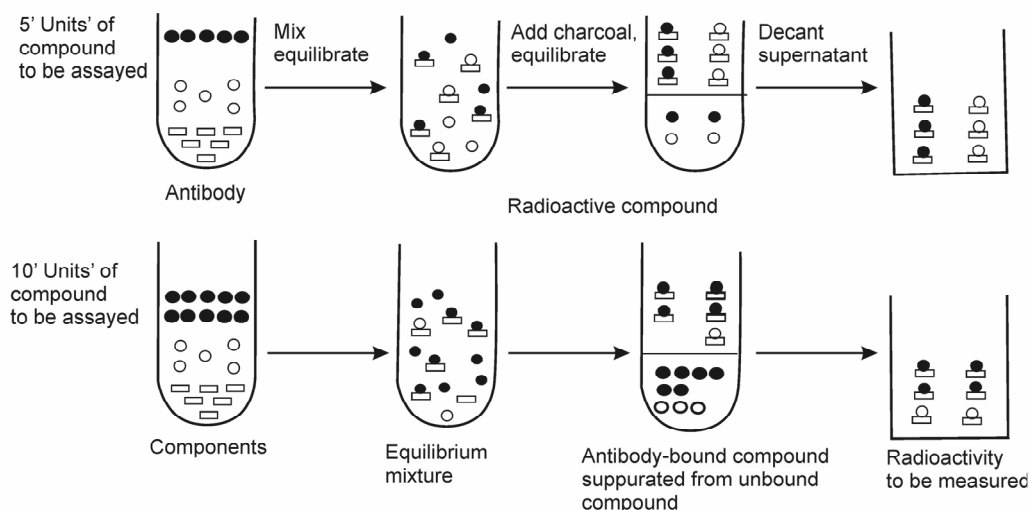


Fig. 13.19: Diagram showing principle of radioimmunoassay

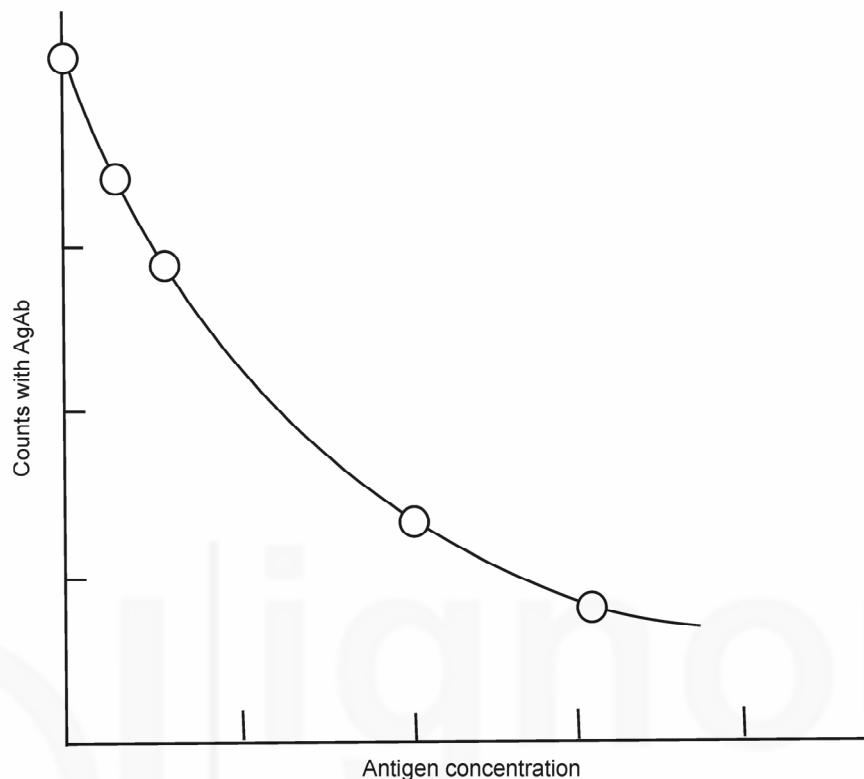


Fig. 13.20: Standard curve for radioimmunoassay used for the determination of an antigen

For the determination of unknown concentration of the antigen by counting the activity of Ag^*-Ab complex.

13.8.3 Procedure

It involves a radiolabelled antigen (Ag^*) along with inactive antigen (Ag) analyte of known concentrations (standards) and an antibody (Ab)-the reagent. An important point to note is the requirement of an efficient and specific separation procedure for the complex $\text{Ag}-\text{Ab}$ free from antigen. Various steps to be followed are:

Antibody (Ab): It is the key reagent in RIA and efficiency of assay depends on the amount of Ab used. These molecules come as immunoglobulins with high molecular weight of $\sim 150,000$ Daltons. These are prepared by injecting Ag into laboratory animals such as rabbit, guinea pig, goat, sheep, chicken, monkey, etc. The antigen against which antibodies are required, is emulsified with a mineral oil and injected into the animal. Secondary injections or booster doses are given after 3-4 weeks interval. Normally good quality antibodies are produced after 3 to 4 such injections. Afterwards blood of immunized animals is collected and screened for antibody response. The serum, a white yellow liquid left after removal of red blood cells, is separated and assayed for antibody content and their specificity checked. It is essential to have pure antibodies because any impurity will show cross reactivity and it will overestimate the antigen concentration.

Radiolabelled Antigen (Ag^*): A highly purified antigen is required for radiolabelling without any loss in immunoreactivity. It acts as a tracer and prepared by introducing the radioisotope to the antigen. Most commonly used radioisotope is ^{125}I ($t_{1/2} = 59.7$ d, $E_\gamma = 35$ keV) because of its low penetration and availability in high specific activity and radiochemical pure form. Besides, it can be easily introduced into molecules such as tyrosine residues. Because of low energy gammas, it can be counted using thin and small solid scintillation counter. Another radioisotope of iodine ^{131}I ($t_{1/2} = 8$ d) can also

be used. Other isotopes commonly used are ^{14}C ($t_{1/2} = 5730$ years) and ^3H ($t_{1/2} = 12.3$ years) both of which are β emitters and must be counted by liquid scintillation methods.

Standards: RIA being a comparator method, response of unknown is compared with that of a set of standards of known concentrations. Since analyte concentrations are in the range of nano or pico mole, very high dilution is necessary. These are prepared in serum matrix to make them identical to the unknown sample.

Separation System: It is the most important step in RIA procedure especially because of low concentrations involved. As already pointed out earlier, antigen is comparatively a smaller molecule whereas antibody is a macromolecule. Hence, Ag-Ab will differ in size considerably from the free antigen. A variety of physicochemical methods are employed for the separation of Ag-Ab complex from antigen. Most widely used method is precipitation of Ag-Ab by using polyethylene glycol. Another method is the solid phase separation technique. Some experimental factors such as pH, ionic strength, and high temperature, composition of the incubating medium buffer, heparin, urea, and high bilirubin concentrations are of special importance. The separated complex is assayed for its activity using a scintillation counter. Finally concentration of antigen is calculated.

13.8.4 Applications

The technique offers several advantages, most important of them being sensitivity and specificity. Very small quantity of the sample (less than 0.1 mL) is required for the measurement. Analysis by RIA does not require any sample preparation or prior extraction of the analyte except the separation of blood serum. Samples may be collected at remote places far away from laboratory and sent for analysis at a central place. Being an *in vitro* technique no radioactivity enters into patient's body. Substances routinely determined using radioiodine labeled antigens include thyroid related hormones (T_3 , T_4 , TSH), digestive hormones (gastrin), sex hormones (FSH, LH, HCG), insulin and other compounds as listed in Table 13.4.

Table 13.4: Selection of Compounds Analyzed by RIA

Drugs	Steroids	Polypeptide hormones
Amphetamines	Anabolics	Adrenocorticotrophic hormone (ACTH)
Barbiturates	Androgens	Follicle Stimulating Hormone (FSH)
Diazepam	Corticosteroids	Glucagon
Digoxin	Oestrogens	Growth hormone
Gentamicin	Progesterones	Insulin
Methadone	Steroid glucuronides	Luteinizing hormone (LH)
Morphine		Prolactin
Nicotine		T_3 , T_4 , TSH
Penicillins		Vasoactive substances (rennin, norpinephrine, angiotensin)
Prostaglandins		
Tetrahydrocannabinol		
Tubocurarine		

In India, reagent kits are available for the assay of hormones such as T_3 , T_4 , TSH, etc from the Radiopharmaceuticals Division of the Board of Radiation and Isotope Technology (BRIT), Navi, Mumbai an organ of the Department of Atomic Energy,

13.9 SUMMARY

Radioanalytical methods using radiotracers and activation analysis are more sensitive and specific because of ease of detection of nuclear radiation. Being dependent on nuclear property there is complete absence of chemical interferences. Details of isotope dilution analysis (IDA), radiometric titration, radiochromatography and radioimmunoassay including their principles of methodology, applications, advantages and limitations are discussed. The principle of neutron activation analysis, its methodology including instrumentation and typical applications are discussed. Instrumental neutron activation analysis (INAA) has the special advantage of being nondestructive and multielemental besides being blank free and hence very useful for forensic and archaeological studies. However, INAA is suited for the determination of total elemental content only but not suited for speciation studies. A comparative account of NAA and IDA with other trace analysis methods is presented. These methods are preferred in spite of the fact that specially licensed laboratory and radiation safety measures are required.

13.10 TERMINAL QUESTIONS

1. Indicate your choice of radioisotope for following type of studies;
 - a) Uptake of manganese by plants, ^{56}Mn or ^{54}Mn
 - b) Determination of sodium in plant leaves by NAA, ^{22}Na or ^{24}Na .
 - c) Determination of age of a rock by dating, ^{14}C or ^4He
 - d) Radiochromatography experiment, ^{64}Cu or ^{66}Cu
2. What type of radiotracer technique should be used for following analyses/processes.
 - a) Qualitative analysis of cation in a mixture,
 - b) Determination of TSH,
 - c) Determination of Ba,
 - d) Blood volume in a patient
3. What type of neutron source will be adequate for the determination of following?
 - i) Manganese in pyrolusite sample,
 - ii) Oxygen in a rock sample,
 - iii) Antimony in gun shot powder,
 - iv) Gold content in an ornament.
4. For which type of following determinations isotope dilution analysis can be used? Indicate the radiotracer used.
 - i) Cr content in electroplating bath,
 - ii) Ag content in photographic solution,
 - iii) Cd content in the emission of thermal power plant,
 - iv) Hg contamination in a pond.

5. Match the characteristic property in Table B with the radiotracer technique in Table A.

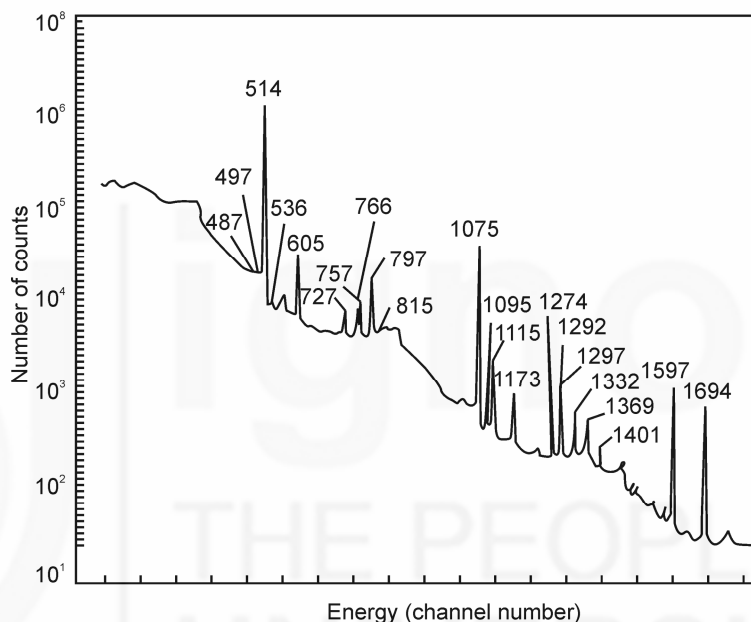
Technique (Table A)	Characteristic Property (Table B)
A. Substoichiometric IDA	1. No indicator required
B. Prompt gamma NAA	2. R_f value
C. Radiochromatography	3. Antigen-Antibody complex
D. Radiometric titration	4. Quantitative recovery not required
E. Radioimmunoassay	5. Determination of Ca

6. In an organic synthesis of p-aminobenzoic acid (PBA) crude product was analyzed for its purity by isotope dilution analysis using ^{14}C labeled PBA. Initially 25 mg of PBA with an activity of 23586 counts in 5 min was added. After equilibration, the mixture was acidified and extracted with an organic solvent. 15 mg purified product was separated and counted. It gave 12732 counts in 30 min. Calculate the % amount of PBA in the crude mixture.
7. Compare scintillation and HPGe detector systems for gamma spectrometry with respect to resolution, efficiency, cost and other factors. Explain why these systems cannot be used for the detection of beta particles?
8. A steel sample weighing 53 mg was analyzed for its manganese content by neutron activation analysis following nuclear reaction $^{55}\text{Mn}(n, \gamma)^{56}\text{Mn}$ ($t_{1/2} = 2.58$ h). The sample was irradiated for 6 h with thermal neutrons at 1.5×10^{12} n cm $^{-2}$ s $^{-1}$ in a nuclear reactor and counted for its gamma activity after a delay of 45 min whence it gave 36597 counts in 3 min. The counter had background count rate 6 cpm. Target nuclide has 100% isotopic abundance and neutron absorption cross section of 13.3 barns.
9. Iodine content of a branded iodized salt was determined by isotope dilution analysis method. To a 12.5 g iodized salt was added radiolabelled 7.3 mg sodium iodide containing ^{131}I ($t_{1/2} = 8$ d) with specific activity of 3.65×10^4 cps. After chemical separation 3.6 mg pure AgI was separated and it showed an activity of 10685 cps. Calculate the amount of iodide present in the salt.
10. Compare advantages and limitations of absolute, comparator and k_0 methods used for the calculation of elemental concentration by NAA.
11. A piece of aluminium foil was analyzed for indium impurity by NAA by using the reaction $^{115}\text{In}(n, \gamma)^{116}\text{In}$ ($t_{1/2} = 54.2$ min) whereby primary reaction was $^{27}\text{Al}(n, \gamma)^{28}\text{Al}$ ($t_{1/2} = 2.24$ min). 6.7 mg piece of foil was irradiated at 10^{12} n cm $^{-2}$ s $^{-1}$ for 20 min. The sample was counted after a delay of 10 min and gave 327 cpm. Target nuclide has isotopic abundance of 95.7% and $\sigma = 45$ b. Calculate concentration of In impurity in aluminium foil. Also calculate % content of Al if activity due to ^{28}Al was 26986 cpm.
12. Wherefrom following may be procured?
- Radiotracers
 - Standard Reference Materials
 - Neutron irradiation

13. Calculate the smallest amount of gold that can be determined by NAA using the reaction

$^{197}\text{Au}(n, \gamma) ^{198}\text{Au}$ in a 50 mg gold foil irradiated at $3.7 \times 10^{12} \text{ n cm}^{-2} \text{ s}^{-1}$ for 6 h. Given that target nuclide is 100 % abundant and $\sigma = 99 \text{ b}$. The sample was counted after 1 h delay and the lowest detectable activity for ^{198}Au is 100 dps.

14. In following figure is given a high resolution gamma spectrum of a sample with indicated energies of photo peaks expressed in keV. Identify the peaks with their respective radionuclides.



15. A sample containing 8.3 μg Zn was irradiated with thermal neutrons for a week at $2.3 \times 10^{12} \text{ n cm}^{-2} \text{ s}^{-1}$ inducing the reaction $^{64}\text{Zn}(n, \gamma) ^{65}\text{Zn}$ ($t_{1/2} = 245 \text{ d}$). Target nuclide had isotopic abundance of 48.9% and its thermal neutron absorption cross section is 0.50 b. Calculate induced activity in terms of Curies. How much activity will be left after a week?
16. 3.8 mg sodium chloride was irradiated in a nuclear reactor for 1 d at $3.7 \times 10^{11} \text{ n cm}^{-2} \text{ s}^{-1}$ inducing (n, γ) reactions to produce ^{24}Na ($t_{1/2} = 14.9 \text{ h}$) and ^{38}Cl ($t_{1/2} = 37.2 \text{ min}$). Target nuclide of ^{23}Na monoisotopic and ^{37}Cl had isotopic abundance of 24.23 % with their neutron absorption cross sections 0.53 b and 0.43 b, respectively. It took 1 h to transport the sample before the counting could be started. Calculate the activity of both the radionuclides in cps if the counter efficiency was 30%.
17. In an isotope dilution analysis experiment, 15 mg radiolabelled analyte with specific activity $0.57 \mu\text{Ci/mg}$ was added. After equilibration and chemical separation 2.76 mg pure analyte having specific activity $0.019 \mu\text{Ci/mg}$ was isolated. Calculate the amount of analyte in the sample.

13.11 ANSWERS

Self Assessment Questions

- ^{54}Mn . Any chemical procedure may require 1-2 h during which ^{56}Mn will decay.
- a) ^{131}I b) ^{128}I c) ^{126}I

3. a) [iii] sensitivity
b) [i]. True [ii]. False [iii]. True [iv]. False
4. A chemical experiment takes time during which radioisotope should not decay significantly. If at all, short lived radiotracer is used then decay correction should be employed.
5. a) Yes b) Yes c) No
6. a) and c) Al and Fe- Thermal neutrons, b) Si- Fast neutrons, d) O- 14 MeV neutrons

^{28}Al is also produced by $^{28}\text{Si}(n, p)^{28}\text{Al}$.

Further iron can also be determined by the reaction $^{56}\text{Fe}(n, p)^{56}\text{Mn}$ using fast neutrons.

7. (a) and (b)
8. a) 92.3%
b) Being steel sample, it will contain iron and cobalt which are likely to generate ^{56}Mn via $^{56}\text{Fe}(n, p)^{56}\text{Mn}$ and $^{59}\text{Co}(n, \alpha)^{56}\text{Mn}$ due to fast neutron component.
c) ^{252}Cf source is suitable for analysis.
d) 1.45 mg
9. 109 ng/g

Terminal Questions

1. a) ^{54}Mn b) ^{24}Na c) ^{14}C d) ^{64}Cu
2. a) Radiochromatography
b) Radioimmunoassay
c) Radiometric titration
d) Isotope dilution analysis
3. a) Isotopic neutron source such as $^{241}\text{Am-Be}$
b) Cockcroft-Walton neutron generator
c) Nuclear reactor
d) Reactor/ ^{252}Cf source
4. a) Yes, ^{51}Cr b) Yes, ^{110}Ag c) No d) Yes, ^{203}Hg
5. A- 4, B- 5, C-2, D-1, E-3
6. 5.67%
7. Scintillation detector has low resolution but higher efficiency with moderate cost. It does not require much of maintenance.

HPGe detector has much higher resolution but low efficiency. It is costlier and requires continuous supply of liquid nitrogen along with strong lead shielding.

Radioanalytical Methods

8. 109 ng/g
9. 400 ppm
10. *Absolute Method*: This method gives highly accurate results provided σ and ϕ values are known with certainty. Since that is not the case, it is not a practical method.

Comparator Method: It is a practical method with least experimental problems. However, comparator standards are quite costly and difficult to be procured.

 k_o Method: This method is most ideal, experimental part is difficult to be performed as it requires lot of calibration work.
11. In- 65.4 ppb, Al- 99.4%
12. a) BRIT, Mumbai
b) NIST (USA) and IAEA (Vienna)
c) BARC, Mumbai
13. 1.45 ng
14. Identify the radionuclides and elements with the help of data in Table 13.2.
15. 23.3 kCi
16. Activity of ^{24}Na = 1.48×10^6 cps and of ^{38}Cl = 1.45×10^6 cps.
17. 435 mg

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