CHAPTER 19

Radiochemical Techniques

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19.1 Introduction 689
19.2 Basic Nuclear Properties 690
A. Radioactive Decay 691
B. Radiation Units 694
C. Interaction of Radiation with Matter 694
D. Stability of Radioactive Compounds 698
19.3 Measurement of Radioactivity 700
A. Methods Based on Ion Collection 700
B. Methods Based on the Scintillation Technique 703
C. Statistics of Radioactive Measurements 713
19.4 Analytical Application of Radioactivity 715
A. Techniques Involving the Measurement of Added Radioactivity 716
B. Methods Based on Induction of Radioactivity 723
19.5 Laboratory Experiments 730
A. Experiment I: Determination of the Operating Characteristics of a Geiger-Müller Tube 730
B. Experiment II: Determination of Chemical Quench in Liquid Scintillation Counting 731
C. Experiment III: Radiochromatography 732
General References 733
References 733

19.1 INTRODUCTION

Scientists have always searched for a method of analysis which is accurate, specific, precise, economical, and easily adaptable to various laboratories.

Radiotracer techniques, as an analytical tool, come close to satisfying such criteria. These methods are particularly convenient for the determination of a variety of elements and compounds which cannot be estimated by standard analytical procedures. The principal limitation of these techniques is the health hazard involved in laboratories where great amounts of radioactive substances are used.

The basic principle of radioisotope methods is that a radionuclide, when mixed with the stable form of the isotope, can be characterized by radiation detection equipment. Thus, qualitative and quantitative inferences can be made regarding the elemental composition of the compound to be analyzed.

19.2 BASIC NUCLEAR PROPERTIES

Ionizing radiation, which includes γ rays, neutrons, β and α particles, is a characteristic phenomenon of unstable nuclides, distinguishing them from the others. Such various kinds of radiation result from a series of processes

TABLE 19.1: Nuclear Particles and Their Properties of Interest

Name	Symbol	Relative mass units[a]	Charge
Electron	e^-, β^-	5.4388×10^{-4}	-1
Positron	e^+, β^+	5.4388×10^{-4}	$+1$
Neutron	n	1	0
Proton	p	0.9986	$+1$
Deuteron	d	1.9980	$+1$
Triton	t	2.9969	$+1$
Alpha	α	3.9948	$+2$
Photon	γ	0	0

[a] 1 mass unit = 1.6747×10^{-27} kg.

which take place within the nucleus of the atom. The orbiting electrons are, in certain instances, also involved in these processes. Radioisotopes can thus be defined as elements in which the nuclei of the atoms contain either more or fewer neutrons than are present in the naturally occurring stable isotopes of such elements. Such unstable nuclei tend, in time, to change into stable configurations by the various processes collectively known as radioactive decay. The emission of a charged particle (β^-, β^+, α, etc.) produces a change in the electric charge of the nucleus. The product nucleus is chemically a different element which has a lower energy content than the parent species.

Experimental work has shown that the most common radiations have properties similar to those outlined in Table 19.1. The difference in energy is distributed between the radiations which are emitted. Invariably, the emission of β^- and β^+ particles is accompanied by uncharged nucleons of

very small mass called neutrinos which carry part of the energy. Neutrinos interact only to a negligible extent with matter and play no part in the useful application of isotopes. The transformations observed when these particles are emitted may be understood by considering specific examples. One of the isotopes of uranium contains in its nucleus 92 protons and 146 neutrons and hence is called uranium-238. To reach stability the above nucleus may spontaneously emit an α particle (i.e., a helium nucleus) and become an isotope of thorium according to the following reaction:

$$^{238}_{92}\mathrm{U} \rightarrow {}^{234}_{90}\mathrm{Th} + {}^{4}_{2}\mathrm{He}$$

The ^{234}Th which is formed in the above disintegration is itself radioactive and decays by the emission of a β^- particle. Because electrons do not exist in the nucleus as such, it must be assumed that they are created at the instant of emission. The net result is that a parent neutron in the nucleus is converted to a proton and an electron which is ejected.

In some instances of radioactive decay (α, β^-, or β^+ decay) the emergent particles may not have their full energy. Consequently, the resultant nucleus remains in an excited state. The energy of excitation is eventually released in a form of electromagnetic radiation commonly known as γ ray or X-ray. The emission of such radiation involves a simple rearrangement of the various nucleons and does not cause a change in the number of protons or neutrons.

A. RADIOACTIVE DECAY

Once a radioisotope is formed, it may decay at any time thereafter. The rate of decay has been found to be independent of the experimental conditions. Changes in temperature, pressure, humidity, and even the chemical state of the substance have been shown not to affect the decay rate. However, there is no way of knowing when any given radioactive nucleus will disintegrate. It was demonstrated experimentally and theoretically that radioactive decays are random in occurrence and can be described in terms of probabilities. This probability of decay per unit time is called the decay constant, λ. If we suppose that there were N atoms at time t, then the average number that decay in a time interval dt is

$$-dN = \lambda N\, dt \tag{19.1}$$

or

$$\frac{dN}{N} = -\lambda\, dt \tag{19.2}$$

Integration of (19.2) from $t = 0$ to t, yields

$$\ln \frac{N}{N_0} = -\lambda t \tag{19.3}$$

$$N = N_0 \exp(-\lambda t) \tag{19.4}$$

where N is the number of radioactive atoms not yet decayed at the time t and N_0 is the number of radioactive atoms in the sample at time $t = 0$. The time required for the number of atoms to diminish to one-half the original number is called the physical half-life, T, of the particular radioisotope. But since

$$\ln \frac{N}{N_0} = -\lambda t \tag{19.5}$$

and since where $N = (\frac{1}{2})\, N_0$, $t = T$,

$$\ln(\tfrac{1}{2}) = -\lambda T \tag{19.6}$$

$$T = \frac{0.693}{\lambda} \tag{19.7}$$

By substituting from Eq. (19.4) into Eq. (19.2), one obtains

$$\frac{dN}{dt} = -\lambda N_0 \exp(-\lambda t) \tag{19.8}$$

Using common logarithms, Eq. (19.4) may be written as

$$\log N = \log N_0 - \frac{\lambda t}{2.303} \tag{19.9}$$

and if one sets $y = \log N$, $t = x$, and $\log N_0 =$ constant b, Eq. (19.9) follows the standard slope intercept form of a straight line:

$$y = mx + b \tag{19.10}$$

where $m = \lambda =$ the slope of the line. The half-life of a particular isotope may thus be very useful for identification purposes.

If the radiations from two different isotopes are detected simultaneously, then the total activity observed at any time is equivalent to the sum of the individual activities. The plot of the observed activity on semilog paper will not result in a straight-line relationship. However, it is possible to decompose the curve into two straight lines after the almost complete decay of the short-lived component. In Fig. 19.1 the total activity from two radioisotopes is plotted as a function of time. The decay relationship of the shorter-lived isotope was deduced after subtracting the activity of the longer-lived component from the total activity after extrapolation to zero time. Another useful concept is that of average life, τ, of all the atoms of a particular radionuclide. The actual life of any particular atom can be any value between zero and infinity. The average life of a large number of atoms is, however, a definite and important parameter. From Eq. (19.1) the life span of dN atoms is evidently t. Therefore, τ can be expressed as follows:

$$\tau = \frac{\int_{N=0}^{N=N_0} t\, dN}{\int_{N=0}^{N=N_0} dN} \tag{19.11}$$

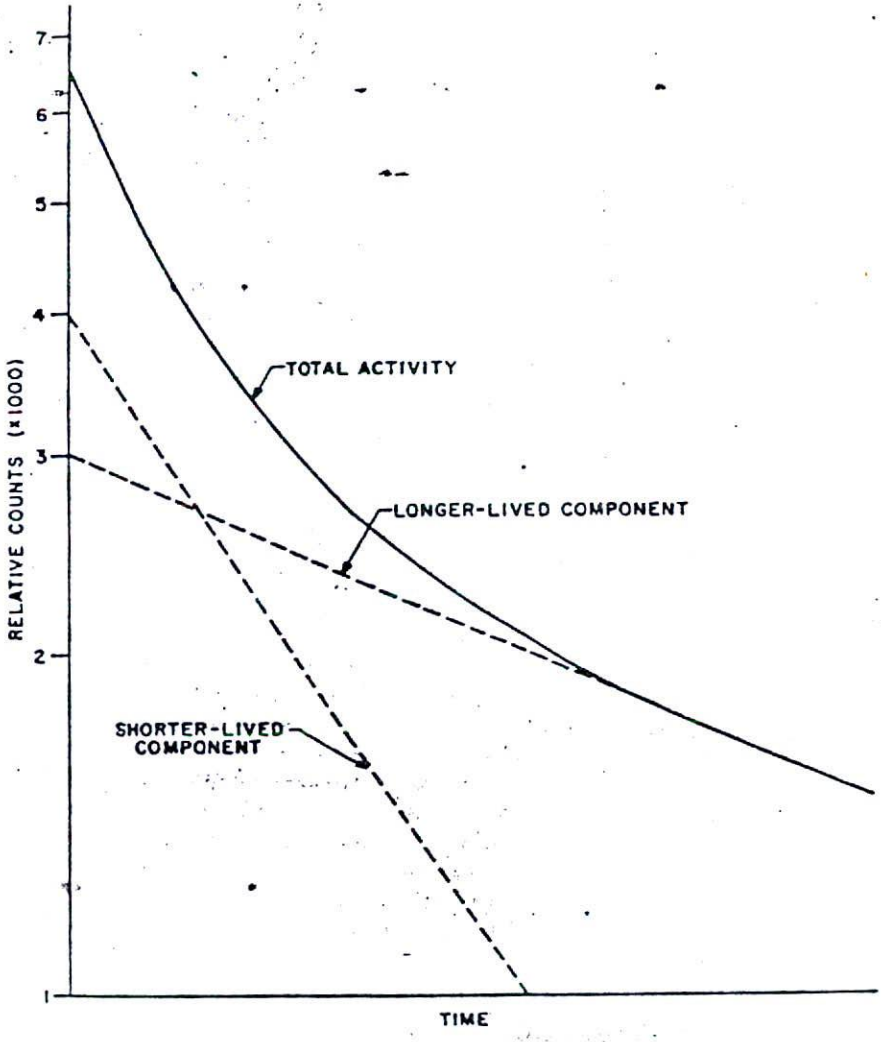

FIGURE 19.1: Decay curve of a mixture of two radioisotopes.

From Eq. (19.8), Eq. (19.11) can be rewritten

$$\tau = \frac{1}{N_0} \int_{t=0}^{t=\infty} \lambda N_0 \exp(-\lambda t) t \, dt . \tag{19.12}$$

Integration of the above equation gives

$$\tau = \frac{1}{\lambda} = \frac{T}{0.693} = 1.443T \tag{19.13}$$

The average life finds useful application in calculations involving the total number of particles emitted by a certain quantity of radioactive material. For example, the total disintegrations of 1 mCi of ^{24}Na ($T = 14.97$ hr) during complete decay would be 3.7×10^7 disintegrations per second (equal to 1 mCi) multiplied by the average life of a radiosodium atom, i.e., 7.77×10^4 sec. This would be 2.88×10^9 disintegrations. From this last value, the total energy released by the source and absorbed by the surrounding medium could be calculated.

While the physical half-life is of great interest for the pharmaceutical chemist in order to select the appropriate isotope for a particular experiment, the average life calculations will give an indication regarding the stability of the labeled compound used.

B. RADIATION UNITS

There are two concepts dealing with radiation units. The measurements that concern the physical condition of the isotope are expressed as disintegration rates, while those that pertain to biological radiation damage are expressed as radiation dose. The curie unit is defined as that amount of radiactive material decaying at the rate of 2.2×10^{12} disintegrations per minute (dpm). The roentgen unit, R, is defined as "that quantity of X or gamma radiation such that the associated corpuscular emission per 0.001293 gram of air produces, in air, ions carrying one electrostatic unit of quantity of electricity of either sign." Because the definition of the roentgen unit is limited to the effects of γ or X-radiation, it becomes of little importance for the analytical chemist since most of the measurements performed are based on the rate of degradation rather than the biological effects observed.

C. INTERACTION OF RADIATION WITH MATTER

All charged particulate radiation of any type loses its energy by interaction with the surrounding matter in essentially the same way. The energy of the particles is sufficiently high to attract or repulse an electron within the target atom. Thus, when particulate radiation passes through matter, ion pairs are produced as a result of the removal of an electron from a neutral atom, leaving behind a positively charged ion.

1. Interaction of α Particles

All α particles from a given isotope have the same energy (monoenergetic) and approximately identical ranges. This range is extremely short, thus resulting in a high specific ionization. The specific ionization of α radiation is at least 25 times higher than for β radiation having the same energy. This is due to the large mass and double charge of the α particle. The importance lies in the fact that to detect α particles, the energy of radiation must be transmitted directly into the sensitive volume of the detector. This presents a serious limitation to the use of α-emitting isotopes in analytical chemistry. The approximate range ($\pm 15\%$) of α particles in materials other than air is given by the Bragg-Kleeman rule:

$$R = (3.2 \times 10^{-4}) R_{\text{air}} \frac{\sqrt{A}}{\rho} \tag{19.14}$$

where R = range in experimental material
R_{air} = range of the particular α particle in air
A = atomic weight of material
ρ = density in grams per cubic centimeter

The range in mixtures and compounds may be determined by use of the equation:

$$A = n_1\sqrt{A_1} + n_2\sqrt{A_2} + n_3\sqrt{A_3} + \cdots n_n\sqrt{A_n} \tag{19.15}$$

where $n_1, n_2, n_3, \ldots, n_n$, are the fractional compositions.

2. Interaction of β Particles

The β spectrum, on the other hand, is continuous and the energies of the particles vary up to a maximum energy, E, characteristic of the isotope. The shapes of typical spectra are illustrated by Fig. 19.2. The spectral shapes of negative and positive electrons differ due to the Coulomb interaction of electron and nucleus in case of positron emission. The range R is almost a linear function of E_m for energies above 0.5 MeV[1]:

$$R(\text{mg/cm}^2 \text{ of Al}) = 520E_m \text{ (MeV)} - 90 \tag{19.16}$$

Experimentally, Katz and Penfold[2] found that the following relationship holds well for a range of energies from 0.01 to 3 MeV:

$$R(\text{mg/cm}^2 \text{ of Al}) = 412^n \tag{19.17}$$

where n equals $1.265 - 0.0954 \ln E_m$.

The absorption of negatrons in any medium was found to be approximately exponential when the absorber thicknesses are not too great:

$$I = I_0 \exp(-\mu x) \tag{19.18}$$

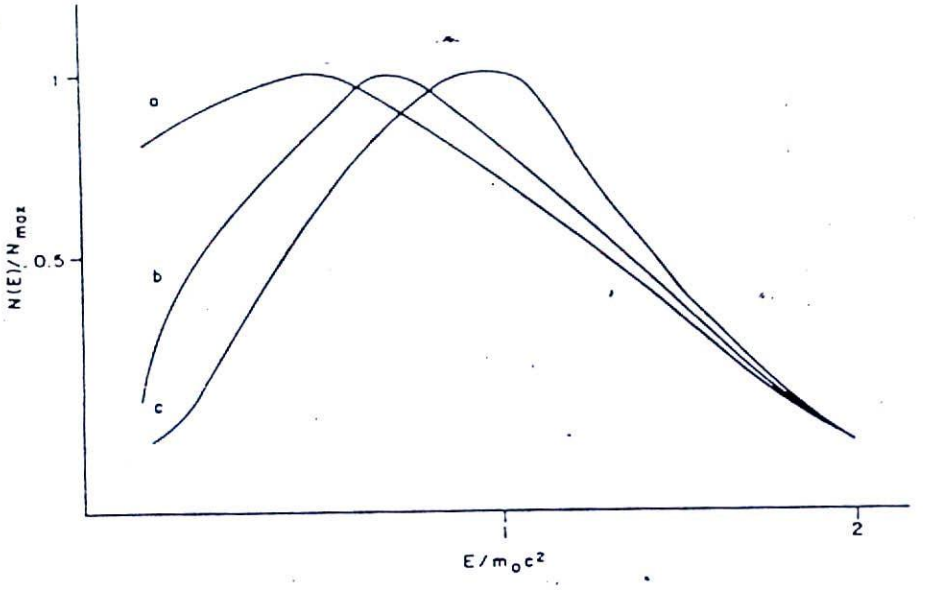

FIGURE 19.2: Dependence of energy distribution of particles on atomic number Z of residual nucleus. $N(E)$ is the number of particles per unit energy interval; E is energy. Normalized distributions for $E_- = 2m_0c^2 = 1.02$ MeV. a: β^-, $Z = 40$; b: $Z = 0$ hypothetical); c: β^-, $Z = 40$. Courtesy of Nuclear Chicago Corporation.

where I is the transmitted energy or counting rate, I_0 is the value before absorption, μ is the mass absorption coefficient (in square centimeters per gram), and x is the thickness (in grams per square centimeter). It was found[1] that the value of μ was related to E_m by

$$\mu = \frac{17}{E_m^{1.14}} \text{ cm}^2/\text{gm of Al} \qquad (19.19)$$

where E_m is in million electron volts. It should be borne in mind that the above relationship holds true only for a particular experimental setup. The effect of geometry, particularly of absorber position relative to source and detector, as well as the atomic number of the absorber appreciably affect the calculation of the mass absorption coefficient using Eq. (19.19).

The positive β particles, or positrons, consume their kinetic energy in forming ion pairs in a manner similar to that of negatrons. In this instance the attractive forces are responsible for the ionization observed. When the positrons lose their kinetic energy, they combine with any orbital electron to produce an annihilation radiation consisting of two γ-ray photons given

off at a 180° angle to one another. The energy of each photon is equal to 0.511 MeV, which is equivalent to the transformation of the rest mass of each electron to electromagnetic radiation.

3. Interaction of γ Rays

Gamma and X-rays have a much lower probability of interaction with matter than particulate radiation has, and the mechanism of such interaction is quite different from that of α or β particles. The latter interact directly to produce ion pairs, while the action of electromagnetic radiation is indirect. For a parallel or collimated beam of γ rays, the intensities at various points in the absorbing medium can be calculated using the relationship given in Eq. (19.18). In this instance μ represents the total absorption coefficient whose value depends on the kind of material and the energies of the photons in the beam. Linear absorption coefficients, which are sometimes used, are in units of reciprocal centimeters. Mass absorption coefficients may be calculated by dividing the latter by the density of the absorbing media. They are useful in comparing effects in materials of different density.

The total absorption coefficient is composed of three contributing components:

a. Photoelectric Interaction. Photoelectric interaction is important for low-energy photons and materials having a high atomic number. In this situation the photon interacts with an orbiting electron, transferring the entire energy of the quantum to the particle, thus ejecting it. The kinetic energy of the ejected electron is equivalent to the energy of the incident photon less the binding energy of the electron. The photoelectric absorption coefficient is related to the incident photon energy and the atomic number of the material by the following equation:

$$\tau = 0.0089 \frac{\rho}{A} 4.1 Z \left(\frac{12.4}{E}\right)^n \tag{19.20}$$

where τ is in reciprocal centimeters, ρ is the density of the material, E is the energy of the photon in thousands of electron volts, Z is the mean atomic number, A is the mean atomic weight, and n is a constant well defined for elements up to iron in the periodic table (value of 3.05 for N, C, and O and 2.85 for the elements from Na to Fe).

b. Compton Interaction. Compton interaction is of importance where the energy of the photon is of higher energy and the atomic number of the material is of a lower value. Hence, the photon imparts only a portion of its energy to an orbiting electron, thus resulting in the distribution of the energy between a free electron and a lower-energy photon. This results in a situation where the absorption has to be computed on the basis of the ejected electron and the scattered photon. The latter can also give rise to Compton or photoelectric interactions in the absorbing medium. The total Compton absorption

coefficient, σ, can be calculated using the Klein-Nishina[3] theory of the process:

$$\sigma = NZ(f_a + f_s) \qquad (19.21)$$

where N is the number of atoms per cubic centimeter, Z is the atomic number and f_a and f_s are functions of the energy.

c. Pair Production. Pair production is of importance where the incident photon has an energy of at least 1.02 MeV. In this instance the γ ray is converted, in the vicinity of a nucleus, to a positron and negatron. This conversion requires an energy equivalent to the rest mass of the two particles ($2m_0C^2 = 1.02$ MeV). Any additional electromagnetic energy is imparted to the formed electrons as kinetic energy. The pair-production absorption coefficient, κ, is calculated according to the following equation:

$$\kappa = \underline{\alpha} N Z^2 (E - 1.02) \qquad (19.22)$$

where $\underline{\alpha}$ = proportionality constant
N = number of atoms per cubic centimeters
Z = atomic number
E = energy of incident photon in million electron volts

It is beyond the scope of this book to delve into the various details of the absorption of radiation by matter. However, a good understanding of the subject should be emphasized before interpreting γ-ray spectra where activation analysis is used as a tool by the analytical chemist. The choice of the proper counting equipment is primarily based on the nature of the interaction of radiation with matter; for example, it would be foolhardy to count a very weak β particle such as that of tritium in a GM counter. The entire energy of the particle would be dissipated in the air before reaching the sensitive volume of the detector.

D. STABILITY OF RADIOACTIVE COMPOUNDS

Of prime importance to the analytical chemist using radiotracers as reagents is the determination of their stability before and during experimentation. The measure of degradation as a result of self-radiation is expressed in terms of G(-M) values. This refers to the number of molecules permanently transformed per 100 eV of energy absorbed. The primary degradation is brought about by interaction of a molecule with a nuclear particle. If such a molecule happens to be labeled, then a radioactive impurity results. It is reported that, in certain situations, one β particle from ^{14}C may destroy up to 5000 molecules. To overcome such a problem, the pharmaceutical chemist has a number of options available when storing radioactive materials:

1. Spreading the compound in a monomolecular layer form, thus allowing most of the emitted particles to escape. An example of such technique is

the storage of ^{60}Co-cyanocobalamin, and ^{14}C-chlorophyll having high specific activities.

2. Dilution of the labeled material with nonradioactive form or a different substance, chosen to make it easier to separate the labeled compound again when required. Benzene has been a solvent of choice for the storage of tritriated molecules.

TABLE 19.2: Relative Stability of Some Radioactive Compounds*

Compound	Specific Activity, mCi/mM	Age, Days	Storage	Decomposition, %	Ref.
Cholesterol-4-^{14}C	2.5	540	Solid in air	40	4
Choline chloride (methyl-^{14}C)	1.8	270	Solid in air	63	4
Dextran-^{14}C sulfate (20 glucose units per molecule)	3	21	Solid in air	100	5
L-Methionine-^{35}S	100	60	Solid in air (dry)	20	6
L-Phenylalanine-^{14}C(U)	304	105	In 0.01 N HCl	14	6
Succinic acid-2,3-T	58,000	30	Solid	100	7
9,10-Dimethylbenz-anthracene-9-^{14}C	9	30	Benzene solution	20	6
9,10-Dimethylbenz-anthracene-T(G)	3,250	390	Benzene solution	37	6

* Courtesy of Nuclear Chicago Corporation.

Table 19.2 demonstrates the relative stability of some radioactive compounds when stored for various periods of time. It is possible to predict the degree of self-decomposition from the following equation:

$$P_d = f\bar{E}S_a(5.3 \times 10^{-9})G(\text{-M}) \tag{19.23}$$

where P_d = initial percentage decomposition per day
f = fraction of the radiation energy absorbed by the system
$\bar{E}$ = mean energy of the emission in electron volts
S_a = initial specific activity of the compound in millicuries per millimole

The foregoing relationship remains linear, provided the magnitude of degradation is less than 10% and the storage time is short when compared to the half-life of the isotope. For greater details on the decomposition rates and G(-M) values, the reader is referred to the tables published by the Radiochemical Center, Amersham.[8] In view of the various problems introduced in experimentation when a radioactive impurity is present, it

becomes mandatory for each chemist to evaluate the degree of radiocompound purity before each experiment. This can be accomplished by means of chromatographic or electrophoretic methods. Usual tests such as melting point determination, boiling point, refractive index, etc., are inadequate since they are not sufficiently sensitive to measure radiation decomposition.

19.3 MEASUREMENT OF RADIOACTIVITY

The determination of radioactivity in organic and inorganic compounds is a rather complex problem in which the best method varies with the particular radioisotope, sample volume, and the specific experiment at hand.

TABLE 19.3: Properties of Some Isotopes Commonly Used in Radiochemistry

Isotope	Half-life	β Energy, MeV	γ Energy MeV
^{14}C	5740 years	0.155	—
^{3}H	12.26 years	0.0186	—
^{35}S	87 days	0.168	—
^{32}P	14.3 days	1.71	—
^{36}Cl	3×10^5 years	0.714	—
^{82}Br	36 hours	0.44	0.78, 0.55, 0.62, 0.70, 1.47
^{131}I	8.06 days	0.61, 0.25, 0.81	0.36, 0.72
^{125}I	57.4 days	—	0.035
^{45}Ca	164 days	0.25	—
^{22}Na	2.6 years	0.54 (β^+)	1.28
^{24}Na	15 hours	1.39	1.37, 2.75
^{75}Se	121 days	—	0.024, 0.136, 0.265, 0.280, 0.58
^{42}K	12.4 hours	3.53, 2.01	1.52

Since α-emitting compounds are rarely used as tracers, the pharmaceutical chemist will be mostly concerned with three other classes of isotopes: (a) the very weak β emitters such as tritium; (b) moderately weak and strong β emitters such as ^{14}C, ^{35}S, and ^{32}P; and (c) the γ emitters such as ^{131}I, ^{125}I, ^{60}Co, etc. No one method is generally superior for measuring all isotopes used in analytical chemistry. Table 19.3 summarizes a number of isotopes which are in fairly common use in the biological and analytical fields.

All three classes of isotopes can be counted either by some method of ion collection, scintillation technique, or solid-state measurement.

A. METHODS BASED ON ION COLLECTION

All instrumentation associated with the detection and measurement of radioactivity require two basic elements: a detector element and a recording

system. The detector element converts the radiative energy into electrical pulses which are passed either directly or indirectly to the recording system. The pulses are then displayed or registered in the form of counts.

The basis of ion-collection measurements is the fact that when ionizing radiation interacts with the molecules of a gaseous medium, ion pairs are formed. The behavior of a counting circuit such as the one shown in Fig. 19.3, when ionizing radiation strikes the sensitive area between the two

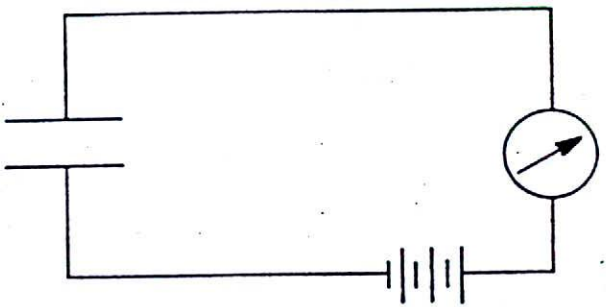

FIGURE 19.3: Simple circuit of ionization detector.

parallel plates or electrodes, depends upon two factors, i.e., the amount of ionization produced by radiation and the potential impressed across the plates by the battery. These relationships are illustrated in Fig. 19.4. Thus, gas detectors can be used in some form to detect any of the isotopes under consideration. If the change in current is the criterion of measurement, the detector is called an ion chamber. However, if the potential change across the electrodes is used as the desired criterion, then the instrument is called a gas counter. Geiger-Müller tubes and proportional detectors are examples of gas counters. A typical gas detector consists essentially of a chamber in which the walls form one electrode (cathode) and a central probe or wire forms the anode (Fig. 19.5). A suitable gas is introduced into the chamber, and a potential applied between wall and central electrode. A solid or liquid sample is usually separated from the sensitive volume of the counting chamber by means of a thin membrane. However, it is possible in certain instances to introduce the sample directly within the sensitive area of the detector. At a relatively low potential (50–100 V) a region of saturation current is established in which the ions produced in the gas, as a result of radiation interaction with the molecules, result in a current flow which is directly proportional to the number of events taking place within the chamber. Because the current flow will differ from one isotope to the other due to the difference in the average energy release, the apparatus must then be calibrated for every individual isotope. An ideal gas in the chamber must (a) be chemically inert, i.e., it must not attack the chamber components chemically; and

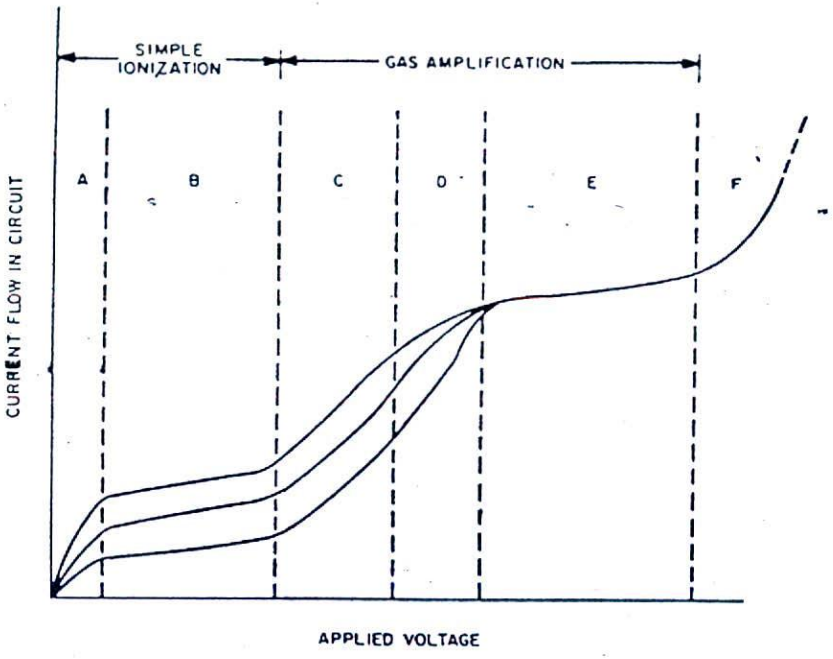

FIGURE 19.4: Effect of increased potential on current flow in an ionization detector. A, recombination region; B, current saturation region; C, proportional region; D, limited proportionality region; E, Geiger-Müller region; F, continuous discharge region.

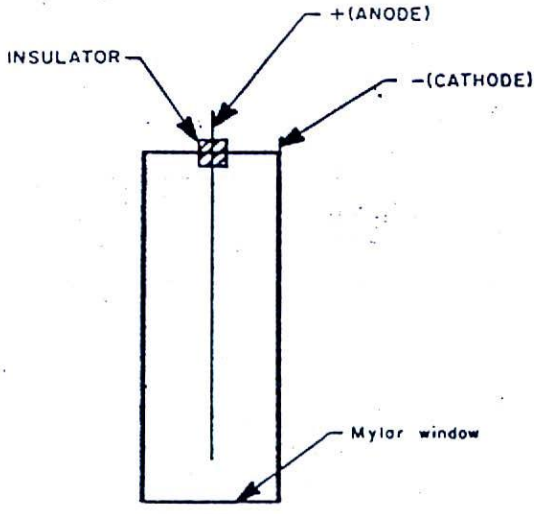

FIGURE 19.5: Longitudinal section of a typical GM tube.

(b) not be easily ionized such that the applied potential between the electrodes result in a current flow without the presence of radioactivity. Typical gases are usually low molecular weight hydrocarbons or mixtures of them. Helium and argon are also sometimes added. At such low voltages the current can be measured by dc amplifiers or vibrating-reed electrometers. An alternative way is the measurement of the rate at which the current charges a condenser to a particular preset potential.

If the applied voltage is raised above the ion-chamber plateau, a limited multiplication of energy results across the counter. There is a direct proportional relationship between the average energy of the events and the energy output of the counter. Thus, a small change in potential of the electrode results when some ions reach the central anode. The change is amplified and detected by suitable circuits and a voltage plateau is observed for ideal monoenergetic emitters. However, all β emitters have a wide range of energies up to E_{max}. In such instances the circuit amplifier is designed in such a way that all pulses above a certain energy level produce a constant output to be recorded. The amplifier, in turn, must have an input sensitivity of 1 mV or less. The above system is called a proportional counter and is very useful in counting and differentiating between α- and β-emitting isotopes.

If the voltage across the counter is increased further, a different plateau is reached and the counting is said to be done in the Geiger region. All radiation, regardless of the number of primary ion pairs produced, will produce the same current flow and the counter is no longer able to differentiate between α and β radiation. This is due to the production of an avalanche whereby gas amplification will have reached its maximum value. The only advantage of counting in the Geiger region over that in the proportional region is that much less sensitive amplifiers are needed because of the comparatively large pulses produced in the former situation. However, counting rates above 25,000 cpm cannot be measured in the GM counter since the counter is essentially in a state of continuous discharge above that rate. Statistical corrections will also have to be made to account for the lost pulses which happen to occur during the "dead" time of the detector. In this instance the effects of the previous event play a major role in the detection process and the counter is known to be "dead" since it will not respond to a newly formed pulse until complete recovery. The dead time of each Geiger tube will vary depending upon the pressure and quality of the gas, as well as the materials of the chamber. On the other hand, proportional counting is generally less sensitive to impurities and to variations in filling pressure.

B. METHODS BASED ON THE SCINTILLATION TECHNIQUE

These methods are probably considered to be the most important from the analytical standpoint. The basis of the scintillation technique rests on

the production of light photons resulting from radiation interaction with matter. This concept is not new in any sense and was used by many investigators in the first part of this century to measure the radioactivity of α-emitting radioisotopes. The scintillator used at that time was zinc sulfide and the detector was the human eye. The technique was soon abandoned after the introduction of the Geiger-Müller counter until the development of the photomultiplier tube, after which scintillation spectrometry progressed along two different lines.

I. Liquid Scintillation Spectrometry

A sample prepared for liquid scintillation spectrometry consists of several components: the radioactive materials to be counted, a solvent system, and a proper scintillator. The type of solvents used depends on the chemical or physical nature of the substances in question. The function of such solvent is the transfer of energy from the point of radioactive emission to a scintillator molecule. An efficient solvent system must exhibit the following characteristics: (a) be transparent to the light photons emitted, (b) not freeze at low temperature under counting conditions, and (c) be able to dissolve or suspend the sample to be counted. Aromatic hydrocarbons such as benzene, xylene, and toluene are frequently used as solvents. However, other hydrocarbons are also used. Potential solvents are usually rated by comparison to toluene.

On the other hand, a good scintillator must have the following characteristics: (a) efficiently produce light photons upon transfer of the energy from the solvent to the scintillator molecule (the wavelength of the light output should also be compatible with sensitivity of the photomultiplier tube) (b) sufficient solubility at working temperatures, and (c) chemical stability.

Scintillators are classified into two groups which are usually mixed together in the solvent system. Primary scintillators, such as *p*-terphenyl, 2,5-diphenyloxazole (PPO), and 2-phenyl-5-biphenyl-oxadiazole (PBD), are routinely used. Secondary scintillators, such as 1,4-bis-2-(5-phenyloxazolyl)-benzene (POPOP) and α-naphthylphenyloxazole (α-NPO), are usually added in trace quantity to a solution containing one of the common primary scintillators. Their function is to convert the photons which were emitted at shorter wavelengths into longer wavelengths which are closely matched to photomultiplier response. A new scintillator, 2,5-bis-2-(5-*t*-butylbenzoxazolyl)thiophene (BBOT), has been suggested recently as a replacement for both PPO and POPOP. Having a fluorescence maximum of 4350 Å, BBOT thus shows a better match to typical glass-faced photomultiplier response than either PPO or *p*-terphenyl.

To estimate the proper concentration of a scintillator in a particular liquid scintillation system, a known amount of a radioactive substance, such as

^{14}C-toluene, is added to the selected solvent. Increasing amounts of a certain scintillator are then gradually added, and the count rate is observed after each addition. The optimal amount to be used is that quantity which produces a maximal pulse height response. A comparison of the various possible combinations has been reported by Rapkin.[9] Table 19.4 summarizes some of the commonly used "cocktail" mixtures in liquid scintillation spectrometers.

Any process which interferes with the performance of a liquid scintillation counting solution is referred to as "quenching." Color quenching arises from the absorbance of the emitted light photons, thus resulting in a lower detected pulse. It can often be minimized by bleaching or decolorization. Biological samples are usually solubilized in a strong quaternary ammonium solution such as hyamine 10-X and then bleached with a trace of hydrogen peroxide. Sodium borohydride has also been suggested as a bleaching agent. Other methods of eliminating color include passage over activated charcoal, precipitating protein material with trichloroacetic acid, and combustion of the sample in question to some form of radioactive gas that could be quantitatively collected and counted. The extent of color quenching could be estimated by measurement of the absorptivity of the solution at a particular wavelength, and correlating the above value with that of the observed pulse height. However, this method is only approximate, since the relationship is not linear at high concentrations of colored material.

Chemical quenching, on the other hand, involves interference with the process of energy transfer between the solvent and scintillator molecules. Dissolved oxygen is probably the most common quenching agent. The radioactive solutes themselves also show some degree of quenching. Thus, concentration becomes an important factor. To determine if a particular radioactive compound produces a chemical quench, increasing amounts of the compound are dissolved in the particular liquid scintillation system, and the count rates are observed after each addition. A linear relationship between the concentration and the recorded count rates per unit weight is an indication that the chemical quench is not concentration dependent.

Because of the variation in the degree of quench between the different samples, it becomes mandatory to convert the relative counts into absolute values expressed as dpm. This is effected in a variety of ways, each having its own limitations. The use of an internal standard, such as ^{14}C-toluene, has been most common. External standards, such as ^{137}Cs and ^{133m}Ba have recently been introduced. The principle of the latter technique depends on the interaction of the γ-ray photon with the materials of the scintillator solution thus producing a light photon. A third technique is based on the fact that quench, whether color or chemical, results in a shift of the pulse height spectrum. The ratio of the counts observed between two pulse height analyzers is an indication of the degree of quench. When a set of quenched

TABLE 19.4: Composition of Various Scintillation Mixtures

Number	PPO, g	POPOP, g	BBOT, g	Naphthalene, g	Xylene, ml	Toluene, ml	Dioxane, ml	Cellosolve, ml	Anisole, ml	1,2-Dimethoxy-ethane, ml
1	5	0.5	—	—	—	1000	—	—	—	—
2	7	0.3	—	100	—	—	1000	—	—	—
3	15	0.6	—	—	—	—	750	—	125	125
4	10	0.5	—	50	—	—	833	166	—	—
5	3	0.1	—	—	1000	—	—	—	—	—
6	10	0.5	—	80	—	143	428	428	—	—
7	10	0.5	—	80	143	—	428	428	—	—
8	—	—	4	—	—	1000	—	—	—	—
9	—	—	4	—	1000	—	—	—	—	—
10	—	—	4	50	—	—	835	165	—	—

standards of the particular isotope is counted, the various counts occurring between the two selected channels will vary according to the degree of quench. Thus, a standard curve which could be used to correct the observed count of the counted sample is established. Instruments containing up to four counting channels are available today. Computer programs are also provided for the direct conversion of cpm to dpm. It is beyond the scope of this book to detail the various statistical approaches designed for such conversion. Insoluble radioactive compounds are sometimes counted by suspending them in a thixotropic gel powder. Cab-O-Sil and Thixin R, a castor oil derivative, have been successfully used for the counting of radioactive samples absorbed on silica gel G. Thus, liquid scintillation spectrometry could effectively be used in conjunction with thin-layer chromatography to solve complex analytical systems.

A novel approach to counting β particles by the scintillation technique is the use of plastic phosphors. Some models consist of a thin-layer plastic phosphor coupled to a photomultiplier tube. Self-absorption corrections and careful standardization are empirical in this case. Attempts have also been made to use scintillating plastic counting vials and scintillating plastic flow cells. Scintillating plastic beads, immersed in aqueous solutions, have been used to count ^{45}Ca. Most recently, ion-exchange resins which possess fluorescent or scintillating properties were reported.[10] In this case the radioactive ion can be removed from the solution by the resin; the latter is then washed and counted directly in a liquid scintillation counter.

The photomultiplier tube, which serves as the scintillation detector, is probably the most critical part of the system. The "Venetian blind" type of photomultiplier is essentially used in most liquid scintillation counters. It is characterized by having a quartz face, since the latter has less radioactivity than glass. It is also more transparent in the ultraviolet region where scintillators exhibit their principal emission. The quality of the photomultiplier is primarily responsible for the performance of the entire system, and an inherent amount of electronic noise is unavoidable if the tube is not sufficiently cooled. The efficiency^2/background ratio is thus drastically improved. A 50% counting efficiency for tritium with 500 cpm background will yield an E^2/B figure of 5, whereas a 25 cpm background results in an improved value of 100. A modern liquid scintillation counter is a coincidence-type machine based on the principle that the sample is interposed between two photomultipliers which examine it simultaneously. Any radioactive event resulting in a sufficient number of photons will trigger a pulse in both tubes. Thus, a record of the event is made only when both tubes pulse within a given coincidence resolving time. If a pulse originates in only one tube, but not the other, such a pulse is considered as noise, and it is not counted. The chance coincidence rate is calculated from the following equation:

$$a = 2n_1n_2\tau/60 \qquad (19.24)$$

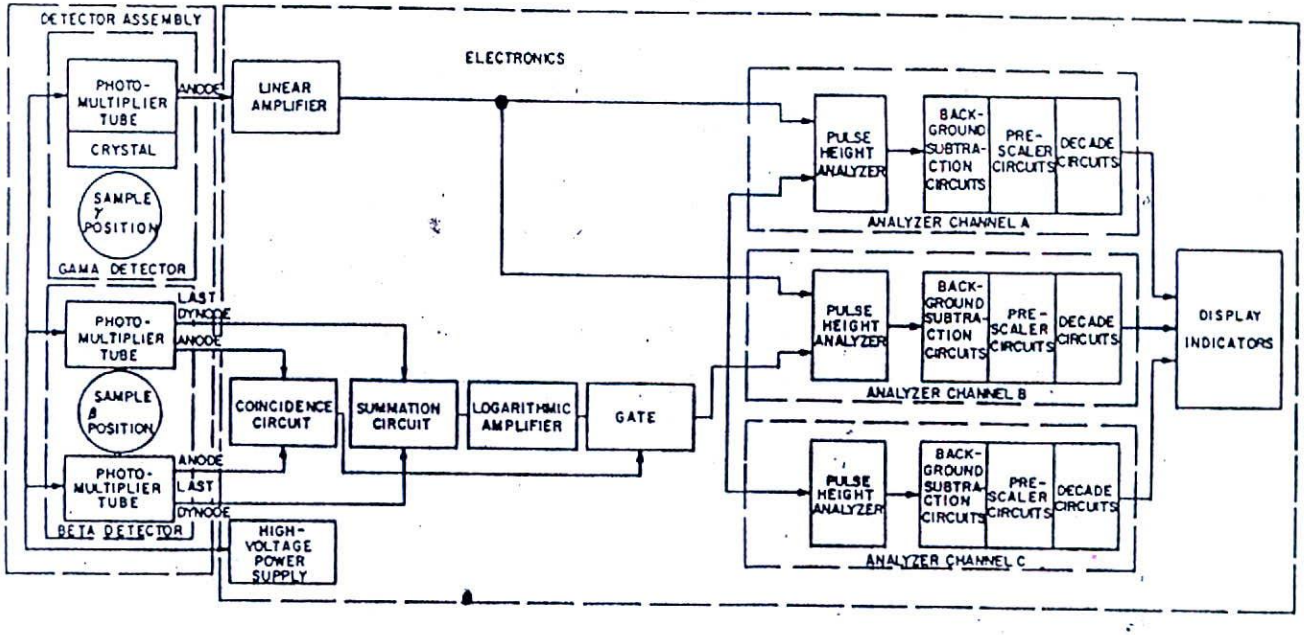

FIGURE 19.6: Block diagram of a liquid scintillation spectrometer. Courtesy of Picker Nuclear Corporation.

where a = chance coincidence rate in cpm
n_1 = noise rate of photomultiplier 1 in cpm
n_2 = noise rate of photomultiplier 2 in cpm
τ = coincidence resolving time in seconds

If we assume that each of the two photomultiplier tubes has a noise rate of 10,000 cpm and that the coincidence resolving time of the circuit is 6×10^{-7} sec, the chance coincidence rate would then be equivalent to

$$a = 2 \times 10^4 \times 10^4 \times (6 \times 10^{-7})/60 = 2 \text{ cpm}$$

However, other sources of background counts arise from cosmic rays, radioactive contamination in the shield, and inherent radioactivity in the glass of the containers. The obvious problem of coincidence circuitry is that the light output of the scintillator is divided between two photomultipliers, and, as such, the output signal is cut in half. Figure 19.6 shows the block diagram of a Liquimat liquid scintillation spectrometer. The logarithmic system demonstrated in the diagram has the advantages of simplicity over linear instrumentation. The major disadvantage is that it cannot efficiently be used for the quench correction of suspended samples.

2. γ-Scintillation Spectrometry

A γ-scintillation system consists of a scintillation detector, high-voltage power supply, preamplifier, amplifier, discriminator, and scaler. Figure 19.7 shows the arrangement of the various units mentioned above. The scintillation detector consists of a solid scintillator which emits photons of visible light upon interaction with the incident γ radiation. The photons

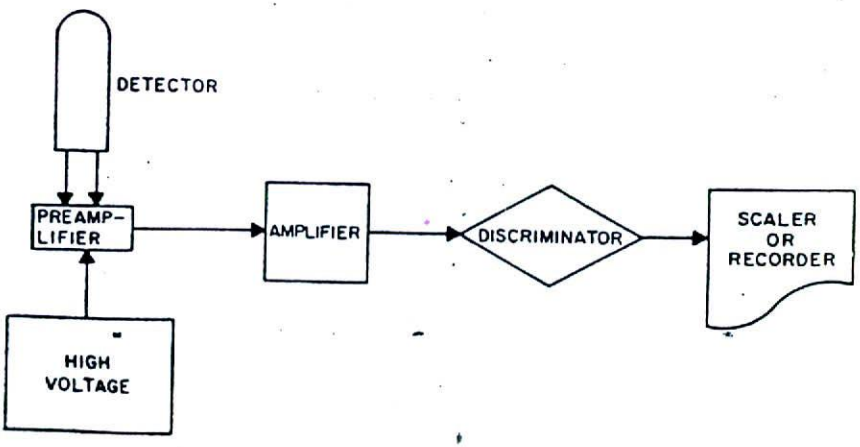

FIGURE 19.7: A typical scintillation system.

are then amplified by a photomultiplier. Because the photoelectric absorption coefficient varies approximately as the fifth power of the atomic number, the scintillation crystals were designed to consist of sodium iodide or other alkali halides. Table 19.5 demonstrates the different characteristics of various scintillators. The photons produced by the crystal are reflected by the crystal housing and strike the photocathode of the photomultiplier. The number of photoelectrons is directly proportional to the number of incident light photons. Thus the photomultiplier-scintillator system will deliver a

TABLE 19.5: Characteristics of Common Scintillators*

Scintillator	Density, g/cm³	Wavelength of Emission, Å	Light Yield	Decay Time, μsec	Remarks
Anthracene	1.25	4450	1.00	0.025	Large crystal, not clear
Stilbene	1.16	4100	0.73	0.007	Good crystals
Terphenyl	1.23	4150	0.55	0.012	Good crystals
Naphthalene	1.15	3450	0.15	0.075	Good crystals
ZnS(Ag)	4.1	4500	2.0	1	Small crystal, poor transparency
NaI(Tl)	3.67	4100	2.0	0.25	Excellent crystals, but hygroscopic
CsI(Tl)	4.51	white	1.5	1	Excellent crystals
p-Terphenyl in xylene	0.87	3700	0.48	0.007	Liquid solution
Terphenyl in polystyrene	1.06	4000	0.30	0.005	Plastic solution

* Courtesy of Baird Atomic Inc.

burst of charge proportional in size to the radiant energy dissipated in the scintillator. This is due to the fact that for every 100 eV of radiant energy, the crystal emits approximately two photons.

Because there are several ways in which γ rays may intersect with matter, the observed γ spectrum is complicated. The decay scheme of a commonly used isotope such as ^{137}Cs is given in Fig. 19.8. Since the γ energy is less than 1.02 MeV, no pair production is anticipated. Ideally, such spectrum as seen by a detector should show only three peaks: a low-energy X-ray peak due to the deexitation of ^{137}Ba to the ground state, a compton edge, and a photopeak. However, a slightly modified spectrum is observed under normal counting conditions Fig. 19.9. The differences observed are caused by the characteristic scintillation detector. Statistical broadening of the photoelectric peak arises primarily from the spread of the relatively small numbers of photoelectrons formed at the photocathode. The degree of broadening is a measure of the accuracy of the system. The smaller the broadening, the more accurate is the determination of the energy peak. This in turn is referred to as the resolution of the photopeak. Resolution is

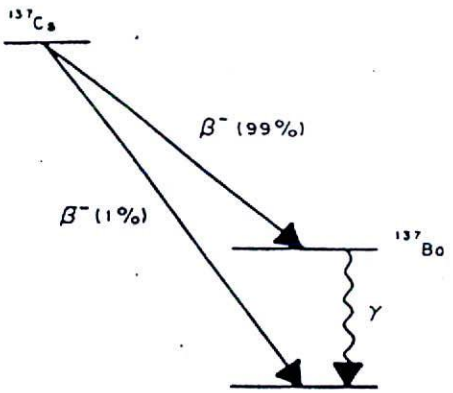

FIGURE 19.8: Decay scheme of ^{137}Cs.

defined as "the full width of the peak as measured at half the maximum counting rate divided by the pulse height at the peak." Thus, the lower the resolution, the greater the ability to differentiate between two photopeaks with energies close to each other; it also follows that the higher the energy, the better the resolution. For example, if the full width of the photopeak at half maximum counting rate for ^{137}Cs is equivalent to 66 keV, the resolution of the detector system would then be equivalent to 66/662 or 10%. However, in the usual γ-ray energy range (0.1–3 MeV) detection and photopeak efficiency decrease with increasing energy.

The Compton scatter is dependent on the size of the crystal. The larger the size, the greater is the probability for scattered Compton rays to be recaptured and thus interact photoelectrically. Thus, the size of the scintillator crystal

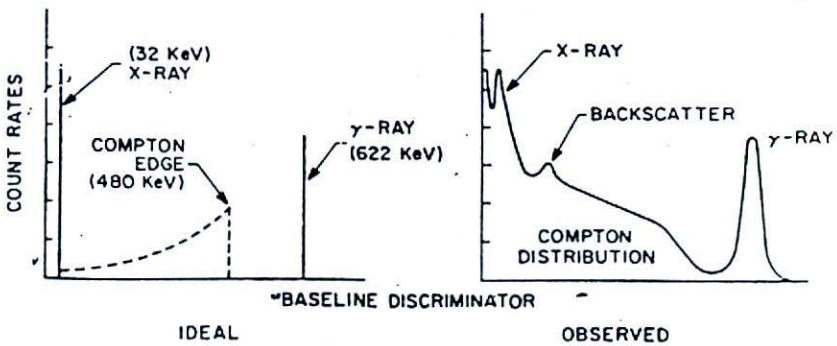

FIGURE 19.9: Ideal and observed spectra of ^{137}Cs.

determines the sensitivity of the detector. However, this does not improve the resolution which is associated with the performance of the photomultiplier.

The phenomenon of backscattering is due to the deflection and interaction of the emitted γ rays with the shielding material surrounding the scintillator crystal. The spectrum observed in Fig. 19.9 is called a differential spectrum. It is obtained by measuring the output pulses occurring between two discriminators which were separated by a small "window." When the lower

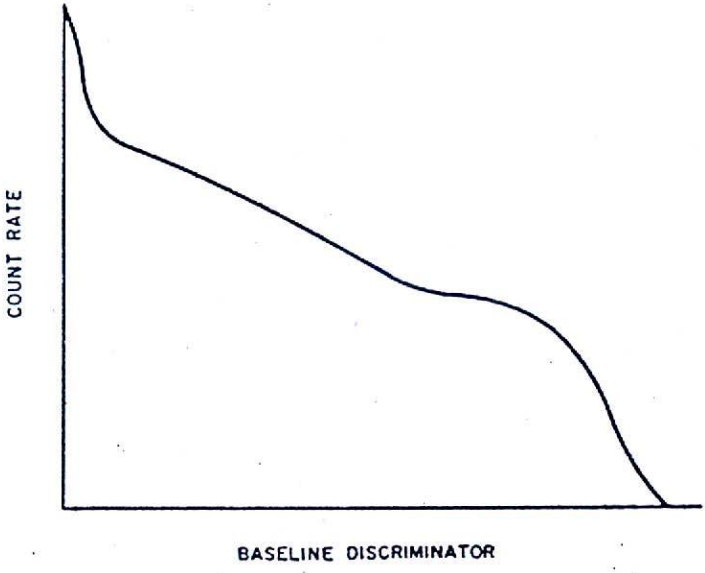

FIGURE 19.10: Integral spectrum of ^{137}Cs.

discriminator is advanced, either manually or automatically, in the differential mode, the above spectrum is observed. Newer pulse height analyzer systems incorporate up to 5024 of such windows, and the various pulses are simultaneously and automatically sorted into each channel. An analog to digital converter digitizes the information present in each channel, and the output tape relays the number of counts per channel.

If, however, only one discriminator is varied in selective steps, by recording the counts at each discriminator setting and plotting the observed count rate at each setting, an integral spectrum such as the one illustrated in Fig. 19.10 results. This is due to the fact that a threshold circuit will only accept all pulses exceeding a selected pulse height level and will reject all those which are smaller in amplitude. The plateau region in Fig. 19.10 corresponds to the "valley" observed in Fig. 19.9, immediately prior to the photopeak.

The choice of integral versus differential discrimination as a mode of counting depends largely on the experiment at hand. For identification and counting of multilabeled preparations, it becomes mandatory to use a differential spectrometer. An added advantage is the low background observed in such instances. The major disadvantage is that the counting rate might be small enough that extended periods of time will be necessary to count an individual sample in order to reach a particular level of statistical accuracy. Since a slight shift in high voltage will result in a variation of the base line of the discriminator or the gain of the amplifier, a large difference in the counting rate will be observed. Thus, the stability of the electronic components determines the accuracy of a certain counting system. The drift (in volts) of the photopeak is particularly dependent on the line voltage, temperature of the room, operating point, and the magnitude of the count rate. For integral analysis, stability is effected by counting the radioactive sample in the plateau region. On the other hand, the drift occurring in differential operation is difficult to determine since the counting rate is already differentially related to the base-line voltage of the discriminator. An estimation of such drift could be done by measuring the changes in the count rate in the slope region of the integral curve for a particular discriminator setting. The slope corresponds to the location of the photopeak.

Multidimensional γ-ray spectroscopy has been recently used for the identification and measurement of complex mixtures of radionuclides. It is based on the property of such nuclides to decay through emission of two or more γ rays in sequence. Thus, a multidimensional analyzer analyzes the γ rays received in coincidence and stores them in a memory according to the energy lost by interaction with the detector, which consists of two sodium iodide crystals. This new approach has helped the determination of many radionuclides present in very trace amounts in biological materials without the need of chemical separation.

C. STATISTICS OF RADIOACTIVE MEASUREMENTS

The fact that the decay of radioactive atoms is a random process introduces certain limits of error which vary according to the number of emitted photons or particles. The larger the number of events, the smaller the error, and vice versa. This is often referred to as indeterminate error. On the other hand, errors resulting from the instability or uneven performance of electronic equipment, sampling inaccuracy, and various other factors is referred to as determinate errors. Thus, it becomes necessary to analyze the measurement variations to draw conclusions with regard to the reproducibility of such measurements. The normal distribution of the latter approximately follows the Poisson statistics. The normal distribution function P_x can

be written as

$$P_x = \frac{1}{s\sqrt{2\pi}} \exp[-(x - m)^2/2v] \tag{19.25}$$

where P_x = the normal distribution function, defined in such a manner that the quantity $P_x\,dx$ gives the probability that the value x lies between x and $x + dx$
v = the variance of the measurement = (standard deviation)^2
s = standard deviation
m = true mean value of the distribution

When the relationship between P_x/dx and x is studied, the well-known bell-shaped normal curve results. However, the true mean m is never known exactly, and as such the observed mean $\bar{x}$ is always used in calculations. The standard deviation, or the measurement of spread of data, is estimated by

$$s = \sqrt{\frac{\sum_{i=1}^{n}(x_i - \bar{x})^2}{n - 1}} \tag{19.26}$$

where n is the number of observations. However, if the same set of measurements was repeated, a different value for $\bar{x}$ is observed, and the standard deviation from the mean is then called standard error, which is given by the relation

$$\text{S.E.} = s/\sqrt{n} \tag{19.27}$$

Another common type of measurement error which is usually referred to in statistics is called the probable error, r. This is defined as the error which is as likely to be exceeded as not. Thus, a 1% probable error for a certain value $\bar{x}$ means that any one measurement will have an equally good chance of falling between the values of $\bar{x} \pm 1\%$. The relationship between the probable error and the standard deviation is

$$r = 0.6745s \tag{19.28}$$

where r is the probable error and s is the standard deviation.

A different approach for calculating the standard deviation of a set of measurements is the application of Poisson distribution. This law describes most of the counting observations made in experimental nuclear physics. It is possible to correlate the Poisson distribution and the normal distribution, whereby

$$s = \sqrt{m} \tag{19.29}$$

where m is always an integer. Essentially, the two ways mentioned for calculating the standard deviation should yield closely identical results. If

they disagree appreciably, a Chi-square test of goodness of fit is recommended. This test determines the probability of deviation where a set of measurements is repeated and compared to the assumed distribution of those events observed in the first trial.

Because any particular radioactive measurement will combine several errors, such as those of background counts, instability of detector, randomness of disintegration, etc., the error of the sum or difference could not be simply added or subtracted. This is due to the fact that the errors may partially cancel each other. The law of combining independent errors of measurement is

$$s = \sqrt{s_{obs}^2 + s_{backg}^2} \tag{19.30}$$

where s = combined standard deviation
s_{obs}^2 = standard deviation of the observed count rate
s_{backg}^2 = standard deviation of background rate

A different kind of error is the one observed when highly radioactive compounds are counted by means of a GM detector. The response of such a detector will not follow a linear relationship since some of the events will occur during the dead time of such a detector. In this instance the true count rate is given by

$$N = \frac{n}{1 - nT} \tag{19.31}$$

where N = true count rate
n = observed count rate
T = resolving time of the detector

In summary, when designing a particular radioactive experiment, the investigator should bear in mind several important criteria:

1. The expected quantities of radioactivity to be measured
2. The efficiency of the instrument available or selected
3. The background contribution to each measurement
4. The amount of time allocated for the counting of each individual sample

19.4 ANALYTICAL APPLICATION OF RADIOACTIVITY

A great number of techniques are available today for the application of isotopes in analytical pharmaceutical chemistry. The scope and dimensions of such techniques are unlimited. Since the first requirement of a tracer is that it must contain a radionuclide, the application of radioactive techniques will essentially fall into two categories.

A. TECHNIQUES INVOLVING THE MEASUREMENT OF ADDED RADIOACTIVITY

A certain amount of radioactive tracer is mixed or incorporated with the unknown sample at some predetermined stage during the analysis. Several variations of this technique are available.

1. Yield Determination

The radioisotopic yield determination is applicable when the quantitative separation of a particular compound from a solution mixture is difficult or time consuming. The complexity of such a mixture, as well as the presence of interfering substances, may yield uneven amounts of the compound upon extraction. The principle of this method depends on the addition of a minute amount of a radioactive tracer having the same chemical composition as that of the unknown compound to the crude mixture. Amounts, usually less than 1 μCi, are thoroughly mixed with the original mixture, which is then subjected to the extraction process. The total amount of radioactivity observed after extraction represents the fraction of the unknown compound removed from the mixture. The sample is then purified and analyzed by a standard method of analysis. The true amount of the sought compound is then calculated by applying the correction factor from the radioactivity measurement. The technique is especially successful when determining biologically important compounds in human or animal tissues. Two essential limitations are applicable in these instances: (1) availability and cost of the labeled compound and (2) the specific activity of the isotope. Although a great number of labeled compounds are available commercially, it is quite possible that for a certain application, such as the determination of a particular drug metabolite, it may be uneconomical to purchase the labeled form of the metabolite. If the compound contains nonexchangeable hydrogen atoms, it could then probably be labeled by the Wilzbach technique.[11] The compound to be labeled is exposed for several days to several curies of tritium gas in a sealed container. Some of the hydrogen atoms will exchange with those of tritium. The resulting radioactive compound is then purified to remove any labile tritium atoms. It is also possible to derivatize the unknown compound by means of a radioactive reagent. In this situation the reaction must be quantitative, reproducible, and with known yields. On the other hand, the specific activity of the added tracer determines the accuracy of the technique. Since the amount originally added is relatively negligible in comparison to the amount of unknown present in the mixture, no correction is made for the amount of tracer in the final computations. However, if the labeled compound is available at lower specific activities only, its weight will have to be subtracted from the final answer. From a statistical standpoint the maximum amount of the tracer

added should not exceed the weight of the unknown. This restriction thereby limits the total amount of radioactivity which could be added to the mixture.

Example

During the analysis of sulfanilamide in a complex triple sulfa mixture, 10,000 dpm of ^{35}S-labeled sulfanilamide was thoroughly mixed with 100 mg of the preparation. Extraction was effected using 100 ml of chloroform. The corrected activity, as measured in a liquid scintillation counter, was found to be 80 dpm/ml. The amount of sulfanilamide in the extract was measured spectrophotometrically and found to be 100 μg/ml.

1. What is the percentage of sulfanilamide in the unknown mixture?
2. If the specific activity of the labeled reagent is known to be 1 μCi/mg and the number of desired counts are 500 dpm/ml after extraction, what is the minimum amount of sulfanilamide which can be analyzed by this method?

Answers:

1. Efficiency of extraction = (80 × 100)/10,000 = 0.80; total amount of sulfanilamide in 100 mg of mixture = 100 μg × 100 ml × 1/0.80 = 12.5 mg; and percentage of sulfanilamide in mixture = (12.5 × 100)/100 = 12.5%.
2. The maximum amount of sulfanilamide which can be analyzed is equivalent to the total weight of labeled sulfanilamide added. This is calculated in the following way:

$$\text{Weight of labeled reagent} = \frac{\text{total observed counts}}{\text{specific activity} \times \text{yield}}$$

$$= \frac{500 \times 100 \text{ dpm}}{2.2 \times 10^6 \text{ dpm/mg} \times 0.8} = 3.01 \times 10^{-2} \text{ mg}$$

2. Isotope Dilution Analysis

The basic principle of isotope dilution analysis depends on the fact that if a known amount of radiotracer is mixed with an unknown amount of the same unlabeled compound, the extent of dilution could be measured in terms of the reduction of the specific activity of the original radiotracer. Various modifications of this technique have been reported. However, all those variations must meet certain requirements, namely:

1. The absolute purity of the radiocompound must be ascertained before the mixing process.
2. Complete equilibrium of the tracer with unknown compound in the mixture.

3. The amount of material separated after the mixing must be chemically pure.

4. The smaller the mass of added radioactive tracer, the greater the statistical accuracy of the method. The major advantage of isotope dilution analysis is that no quantitative recovery is required during any step of the analysis.

a. Direct Isotope Dilution Analysis. This method involves the simple addition of a known amount of radiotracer to an unknown quantity of the same compound in a complex mixture. After thorough mixing, a sample of the compound in question is separated and the final specific activity is determined. Thus, any decomposition of the compound at any part of the procedure will not affect the outcome of the results. The following equation is used to estimate the mass of the unknown:

$$M_u = M_r\left(\frac{S_0}{S_f} - 1\right) \tag{19.32}$$

where M_u = mass of unknown
M_r = mass of radiotracer added
S_0 = original specific activity of radiotracer
S_f = final specific activity of the diluted compound

Example

Penicillin is to be determined in a pharmaceutical sample. One milligram of tritium-labeled penicillin having a specific activity of 250,000 cpm/mg was added to the preparation. The specific activity of a separated sample after mixing was found to be 1000 cpm/mg. If the counter efficiency for tritium is 40%, what is the mass of unknown penicillin?

Answer:

Substituting in formula (19.32),

$$M_u = 1\left[\frac{250{,}000}{1000 \times (1/0.4)} - 1\right]$$

$$= 99.0 \text{ mg of penicillin}$$

b. Inverse Isotope Dilution Analysis. It is not always possible, for practical and economical reasons, to use a radiotracer similar in chemical composition to that of the unknown. In such instances the compound being determined is reacted in a quantitative and reproducible way with a radioactive reagent of known specific activity. A known amount of carrier is then added. The carrier will consist of the nonradioactive derivative of the unknown which is previously prepared and purified. Statistically, the larger the amount of

carrier added, the more accurate is the determination. The final specific activity is determined after dilution, and as the stoichiometry of the reaction between the reagent and compound is known, then

$$M_u S_r = (M_u + M_c) S_f \tag{19.33}$$

where M_u = mass of unknown radioactive derivative
M_c = mass of added carrier
S_f = final specific activity of the diluted compound
S_r = initial specific activity of the reagent

Then the mass of compound present can be calculated from the above formula, where

$$M_u = M_c \left(\frac{S_f}{S_r - S_f} \right) \tag{19.34}$$

It is very important to have excess of the radioactive reagent when the reaction is performed. Equally important is the removal of all traces of the excess reagent before determining the final specific activity.

Example

An unknown compound A is reacted quantitatively with a radioactive reagent B having a specific activity of 10 mCi/mM. One hundred milligrams of carrier derivative is mixed with the preparation, and the final specific activity of a separated sample was found to be 1 μCi/mM. What is the mass of compound A if the molecular weight of the latter is 200 and that of the derivative is 300?

Answer:

From Formula (19.34),

$$M_u = 100 \left(\frac{1}{10{,}000 - 1} \right)$$

$$= 100 \times 10^{-4} \text{ mg}$$

Mass of unknown compound $= 100 \times 10^{-4} \times (200/300) = 66 \times 10^{-4}$ mg.

c. Double Inverse Isotope Dilution Analysis. In situations whereby it is either impractical or impossible to determine the initial specific activity of the reagent, an inverse isotope dilution could still be performed. This is achieved by carrying two reactions, each using a different amount of carrier. By solving the simultaneous equation for M_u in formula (19.34), the following formula should apply:

$$M_u = \frac{M_{c_2} S_{r_2} - M_{c_1} S_{r_1}}{S_{r_1} - S_{r_2}} \tag{19.35}$$

where M_u = mass of unknown derivative
M_{c_1} = mass of carrier in first reaction
M_{c_2} = mass of carrier in second reaction
S_{f_1} = final specific activity in the first reaction
S_{f_2} = final specific activity in the second reaction

Example

One hundred milligrams of ^{35}S-chlorpromazine were injected into a rat, and the metabolite chlorpromazine sulfoxide was separated from the urine. Two 1-ml urine samples were taken and 10 mg of carrier sulfoxide was added to the first, while 20 mg of the same carrier was added to the second sample. The respective final specific activities were found to be 3000 dpm/mg and 1600 dpm/mg. What is the amount of chlorpromazine sulfoxide in 1 ml of urine?

Answer:
Using Eq. (19.35),

$$M_u = \frac{(20 \times 1600) - (10 \times 3000)}{3000 - 1600} = 1.41 \text{ mg}$$

d. Double Label Isotope Dilution Analysis. This technique is of particular application in complex steroid analysis. Corticosterone, cortisol, aldosterone, and testosterone have been successfully determined by this method. A known amount of labeled steroid (usually ^{14}C- or ^{3}H-labeled) is added to the unknown sample. After sufficient equilibration time, the steroids are chemically separated from the sample. The separated mixture is then subjected to a reaction with a reagent carrying a different label (usually acetic anhydride or thiosemicarbazide). The acetates or thiosemicarbazides are then separated by chromatography and converted into more stable derivatives. The activity of the two labels is determined by liquid scintillation counting. The reasoning behind the use of two labels is that while the first label resolves the problem of yield, the second label determines the absolute amount of steroid in the particular sample.

In all reactions involving a derivative formation, careful thought should be given when selecting the proper reagent. Complexation and salt formation reactions are undesirable. Unknown amines and hydroxy compounds can be acetylated with ^{3}H-acetic anhydride; unknown acids can be esterified with ^{14}C-diazomethane or converted to an amide with a radioactive amine; unsaturated compounds and phenols can be brominated with radiobromine; ^{131}I-pipsyl chloride (*p*-iodo benzene sulfonylchloride) has been successfully used for the determination of many amines such as histamine and amphetamine.

3. Radiometric Methods of Analysis

Radiometric analysis is a technique involving the formation of a radioactive compound whose solubility is markedly different from the original radioactive material. Its main advantages over the conventional methods are (1) it can be adapted to unweighable tracer amounts of the unknown and (2) there is no interference from any suspended solids in the original unknown. Several variations of this technique have been used in which an insoluble radioactive solid is made to dissolve, or a phase transfer of radioactive gases and solids is effected.

a. Gravimetric Methods. The principal requirement in this type of analysis is the quantitative precipitation of the unknown with an excess amount of radioactive reagent. The radioactivity of the precipitate or filtrate is an indication of the amount reacted. The method is mostly applied for the study of the coprecipitation phenomenon. This is accomplished by adding a radioactive form of the compound suspected to coprecipitate in a mixture and then performing the analysis and determining the amount of radioactivity in the precipitate. Electrodeposition methods have also been used for the gravimetric study of interfering substances.

b. Titrimetric Methods. These are essentially based on the incremental addition of a titrant to form a radioactive precipitate. The radioactivity of the supernatant solution is continuously monitored after each addition until an end point indicating the end of the reaction is reached. Figure 19.11 shows the relationship between the volume of $^{110}AgNO_3$ titrant added to a solution of sodium chloride and the level of radioactivity in the supernatant. The normal problems encountered in this type of determination are as follows:

1. The accuracy will vary according to the time of counting. A longer time will yield accurate results but will also slow down the titration.
2. According to the law of mass action, the solubility of the precipitate is directly affected by the product-ion concentration and accordingly the end point will not be constant.

It is possible, in certain instances, to count the precipitate directly by either centrifuging the particles or by filtering them. Care should be taken, especially near the end point, to thoroughly wash the precipitate to remove any cross contamination from the radioactive titrant.

Alternative approaches include the mixing of the unknown substance with a trace of its radioactive form. The titration is carried as usual and the end point is observed when nearly all radioactivity disappears from the supernatant.

If the precipitate is soluble to an extensive amount, an immiscible solvent is used to extract it. Certain cations have been analyzed using this technique. The sensitivity is in the range of 10^{-6} g.

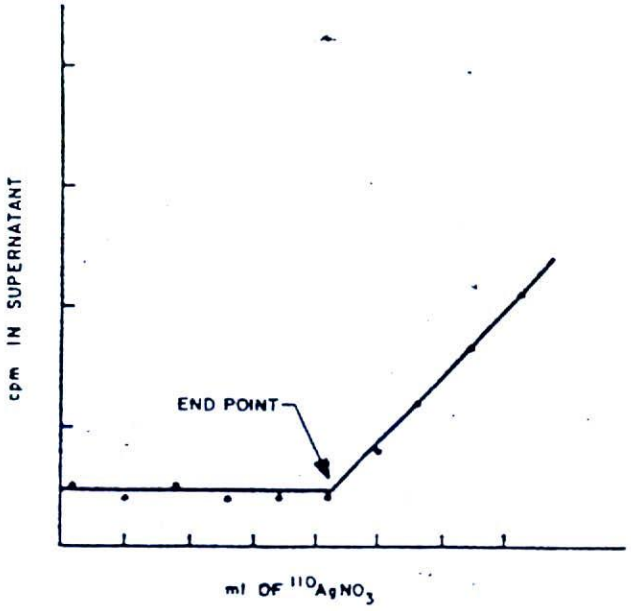

FIGURE 19.11: Radiotitration of chloride with silver nitrate.

c. Chromatographic Methods. The introduction, and extensive use, of thin-layer chromatography in the past few years has opened up new frontiers to radiochemical analysis. Chromatographic methods are usually associated with autoradiographic techniques. Autoradiography is a procedure describing the detection of radiation or radioactive substances which interact with a photographic emulsion and produce a darkening in the respective area. It is now possible to detect the presence of compounds having an activity of few dpm. The thin-layer plate is usually superimposed with an X-ray film for a certain exposure period, after which the emulsion is developed and fixed. The length of exposure is dependent on the concentration of the radioactive compound and the temperature of the system. The lower the temperature, the shorter the exposure time.

The use of radiotracers in chromatographic separations has helped in the determination of the extent of spot trailing and the losses by irreversible adsorption. In the latter situation the detected spot is quantitatively transferred to a liquid scintillation counting vial containing a thixotropic gel. The suspension is counted and the total activity is compared to a standard. The per cent loss is then estimated accordingly. Radiochromatography has also been extensively used for the qualitative determination of unknown

compounds. Thus, compounds such as steroids can be labeled with ^{14}C-acetic anhydride, spotted on a chromatogram, developed in a suitable solvent system, and easily detected by autoradiography or a 4π chromatographic scanner. Similarly, amines, hydroxy compounds, organic acids, unsaturated compounds, and phenols can be reacted with the proper labeled reagent prior to application on the chromatogram. It is more suitable, sometimes, to form the derivatives on the chromatogram itself. Thus, amino acids can be detected by exposure to ^{131}I-methyl iodide. Quantitative estimation of the unknown compound is feasible if the initial specific activity of the labeling reagent is known. The following example will illustrate the above point.

Example

Cation A is known to react quantitatively and in equimolecular amounts with hydrogen sulfide. The chromatogram containing a spot of cation A is exposed for a length of time to a saturated atmosphere of ^{35}S-hydrogen sulfide having a specific activity of 10 million dpm/mM. The radioactivity in the spot is then counted and found to be 600 dpm. What is the quantity of cation A on the chromatogram?

Answer:

Since cation A reacts in equimolecular amounts with H_2S, the specific activity of the unknown is equivalent to that of the reagent, whereby

$$\frac{\text{Total activity of unknown}}{\text{Weight of unknown}} = \text{specific activity of reagent}$$

$$\text{Weight of unknown} = \frac{600}{10{,}000{,}000} = 6 \times 10^{-5}\ \text{mM}$$

The activity on the chromatogram is usually determined by either direct counting of the paper via liquid scintillation counter or by elution of the spot. The sources of error in each technique lie in the fact that the geometry of the paper can hardly be duplicated or that the elution is incomplete. A more accurate but drastic approach, is the digestion of the paper with a suitable reagent, and the eventual counting of the solution.

An excellent review on the application of isotopes in various chemical analysis has been presented by Reynolds and Ledicotte.[12] The published tables exemplify the use of radiotracers in inorganic and organic analysis.

B. METHODS BASED ON INDUCTION OF RADIOACTIVITY

Radioactivity can be induced in an atom when the nucleus of such an atom is bombarded with other nuclear particles. This process is called activation analysis. In principle it is quite similar to some other methods of instrumental analysis in that energy in some form is introduced into an unknown

nucleus and the characteristic radiation is detected. The simplest form of activation analysis involves the irradiation of a certain material with a source of neutrons. This material is then placed in front of a detector to characterize and count the emitted radiation. The above technique has several advantages:

1. Sensitivity: Several elements, in the range of ppb, can successfully be analyzed by this method. Table 19.6 shows the various detection limits obtained when different elements are bombarded with 14 MeV neutrons from a positive-ion accelerator. Greater sensitivities are observed when the samples are irradiated in a nuclear reactor.
2. Speed and Accuracy: Samples can be analyzed in a few minutes without much preparation. The accuracy of this technique is comparable to, if not better than, other instrumental methods.
3. Nondestructiveness: This is probably the major advantage in view of the fact that repeat analyses can be easily made to confirm the first analysis or improve the precision. Thus, activation analysis offers a powerful experimental and analytical tool for biological, medical, and chemical research workers.

1. Production of Neutrons

Since most of the applications of activation analysis deal primarily with neutron bombardment, we are mostly concerned with the sources of such neutrons. The three basic types of neutron sources are nuclear reactors, various types of accelerators, and isotopic sources.

The nuclear reactor is the greatest producer of neutrons due to the radioactive fission of uranium. It is the most versatile and as such the most sensitive system for analyzing various elements. The obvious problems are the high cost and large size. Most recently, certain arrangements have been established in which a particular sample is irradiated in a nuclear reactor thousands of miles away and the resulting data are directly transmitted by teletype to the concerned individual.

Accelerator sources include cyclotrons, Van de Graaffs, and Cockroft-Waltons. The Cockroft-Walton is a positive-ion, low-energy accelerator having an accelerating voltage of 100–200 kV. It produces 14-MeV neutrons as a result of bombarding tritium targets with deuterons. This type of accelerator is probably the most used in activation analysis laboratories due to its relative simplicity and cost. Figure 19.12 shows the Cockroft-Walton accelerator at the irradiation facility of the Faculty of Pharmacy, University of Alberta. Several radiation hazards are associated with this type of installation, namely, protection from the produced high-energy neutrons and the tritium contamination. Concentrations of up to 100 Ci of tritium are easily accumulated in the instrument after a relatively short operating period.

TABLE 19.6 Detection Limits of Various Elements by Neutron Activation Analysis*

Element	Product half-life	γ-ray energy, MeV	Irradiation time, min	Counting time, min	Detection limit, μg
Aluminum	9.45 min	0.834	5	10	6
Antimony	16.4 min	0.51	5	10	7
Arsenic	48 sec	0.139	1	2	4
Barium	2.60 min	0.662	5	10	1
Beryllium	0.82 sec	3.2 (β^-)	0.05	0.1	110
Boron	0.84 sec	13 (β^-)	0.05	0.1	100
Bromine	4.8 sec	0.20	0.1	0.2	60
Cerium	55 sec	0.740	1	2	9
Chromium	3.74 min	1.433	5	10	10
Cobalt	2.58 hr	0.845	5	10	50
Copper	9.73 min	0.51	5	10	9
Fluorine	29.4 sec	0.200	1	2	24
Gallium	1.1 hr	1.07	5	10	20
Germanium	48 sec	0.139	1	2	5
Hafnium	19 sec	0.215	1	2	80
Indium	20.7 min	0.155	5	10	30
Iron	2.58 hr	0.845	5	10	30
Iodine	13.3 days	0.368, 0.650	5	10	80
Lead	0.80 sec	0.57	0.05	0.1	110
Magnesium	14.97 hr	1.368, 2.754	5	10	80
Manganese	3.74 min	1.433	5	10	40
Mercury	42 min	0.159	5	10	20
Molybdenum	15.5 min	0.51 (β^+)	5	10	30
Nickel	10.47 min	0.059	5	10	260
Nitrogen	10.47 min	0.51 (β^+)	5	10	90
Oxygen	7.35 sec	6.1	0.2	0.4	30
Palladium	4.75 min	0.188	5	10	4
Phosphorus	2.27 min	1.78	5	10	8
Platinum	1.4 hr	0.337	5	10	240
Potassium	7.7 min	0.51 (β^+)	5	10	90
Rubidium	23 min	0.239	5	10	1
Scandium	3.92 hr	0.51 (β^+)	5	10	20
Selenium	17.5 sec	0.162	1	2	20
Silicon	2.3 min	1.78	5	10	2
Sodium	40.2 sec	0.439	1	2	20
Strontium	2.80 hr	0.388	5	10	1
Tantalum	8.15 hr	0.093	5	10	20
Tellurium	1.2 hr	0.475	5	10	60
Tungsten	1.62 min	0.130, 0.165	5	10	20
Titanium	3.09 hr	0.51 (β^-)	5	10	90
Vanadium	5.79 min	0.323	5	10	7
Zinc	38.3 min	0.51 (β^-)	5	10	30
Zirconium	4.4 min	0.588	5	10	4

* Courtesy of Nuclear Chicago Corp.

NOTE: The above data was obtained at the General Atomic Division of General Dynamics Corporation, employing a Texas Nuclear Model 9900 neutron generator. The neutron flux was 10^8 n/cm^2-sec. The detection limit is based upon a minimum of 100 photopeak counts.

At least 6 ft of concrete shield must surround the accelerator to provide the necessary neutron shield. In spite of the above disadvantages, the Cockroft-Walton presents the highest neutron output consistent with simplicity. A most recent advance in the field was the design of a sealed-tube type of accelerator which has a lower neutron output but less tritium hazard.

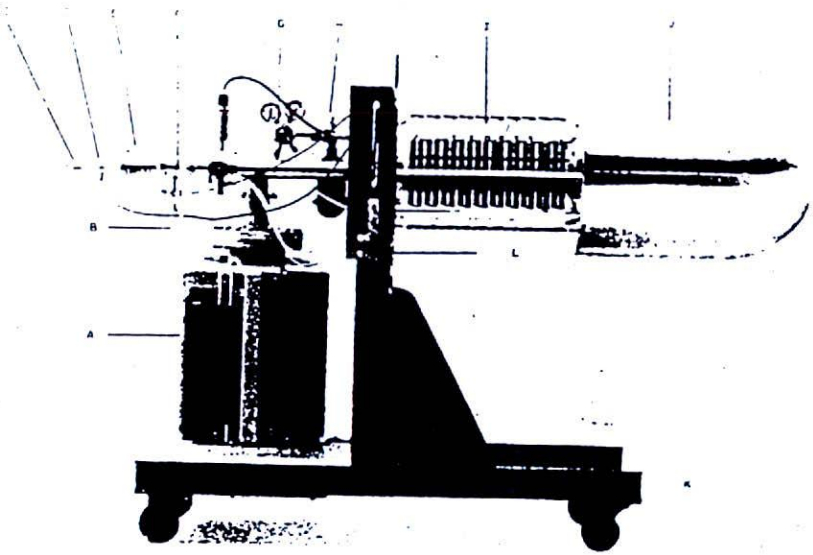

FIGURE 19.12: Cockroft-Walton positive ion accelerator (total yield 2.5×10^{11} n/sec). *A*, Vacion pump; *B*, pump-out valve; *C*, target; *D*, suppressor; *E*, bellows; *F*, chopping slit; *G*, deuterium supply gauge controls; *H*, deuterium supply bottle; *I*, accelerating tube and resistor rack; *J*, terminal dome; *K*, neutron generator; *L*, water manifold.

Isotopic sources of neutrons consist of a high concentration mixture of two or more isotopes which undergo a nuclear reaction, a result of which is the production of neutrons. Plutonium-beryllium, radium-beryllium, and antimony-beryllium are common isotopic sources. The yield and the energy of the neutrons produced are much lower than those of the accelerators and reactors. Because the neutrons are continuously produced, a permanent shield is necessary.

2. Induction of Radioactivity

There are at least four useful reactions by which radioactivity can be induced: (n, γ), (n, p), (n, α) and (n, $2n$) reaction. In each case the neutron is the bombarding particle, while the γ, p, α, and $2n$ are the emitted or ejected radiation, which is sometimes called prompt radiation. It is emitted

virtually instantaneously following the capture of the neutron by the nucleus. The type of reaction most likely to occur is dependent on the energy of the incoming neutron and the nuclear cross section of the particular nuclide. The cross section is a measure of the probability that a single incident neutron will hit within the nuclear circle of interaction. The circle of interaction, in turn, is equivalent to the sum of radii of the nucleus and neutron. The cross section is expressed in barns (1 barn $= 10^{-24}$ cm^2) and varies markedly as a function of the neutron energy. Examples of such interactions are

Reaction with slow neutrons:

$$^{23}\text{Na} + n \longrightarrow {}^{24}\text{Na} + \gamma \qquad \text{(prompt)} \qquad (19.36)$$

$$^{24}\text{Na} \xrightarrow[15\text{ hr}]{} {}^{24}\text{Mg} + \beta^- + \gamma \qquad \text{(delayed)} \qquad (19.37)$$

Reaction with fast neutrons:

$$^{16}\text{O} + n \rightarrow {}^{16}\text{N} + p \qquad (19.38)$$

$$^{16}\text{N} \rightarrow {}^{16}\text{O} + \beta^- + \gamma \qquad (19.39)$$

When the element of interest is bombarded, the number of radioactive atoms will increase with the time of bombardment. However, some of those radioactive atoms will decay at the same time according to Eq. (19.2). Both the decay and buildup process follow exponential relationships as outlined in Fig. (19.13). If N' represents the rate of buildup of a particular radioisotope per unit time and N represents the number of radioactive atoms after time t from the start of bombardment, then the net rate of change in the number of radioactive atoms having a decay constant, λ, is

$$\frac{dN}{dt} = N' - \lambda N \qquad (19.40)$$

Integration of Eq. (19.40) for $N = 0$, when $t = 0$, yields

$$N = \frac{N'}{\lambda}[1 - \exp(-\lambda t)] \qquad (19.41)$$

but

$$\lambda = \frac{0.693}{T}$$

Then, by substitution,

$$N = \frac{N'T}{0.693}\left[1 - \exp\left(-\frac{0.693t}{T}\right)\right] \qquad (19.42)$$

or

$$N = 1.44N'T\left[1 - \exp\left(-0.693\,\frac{t}{T}\right)\right] \qquad (19.43)$$

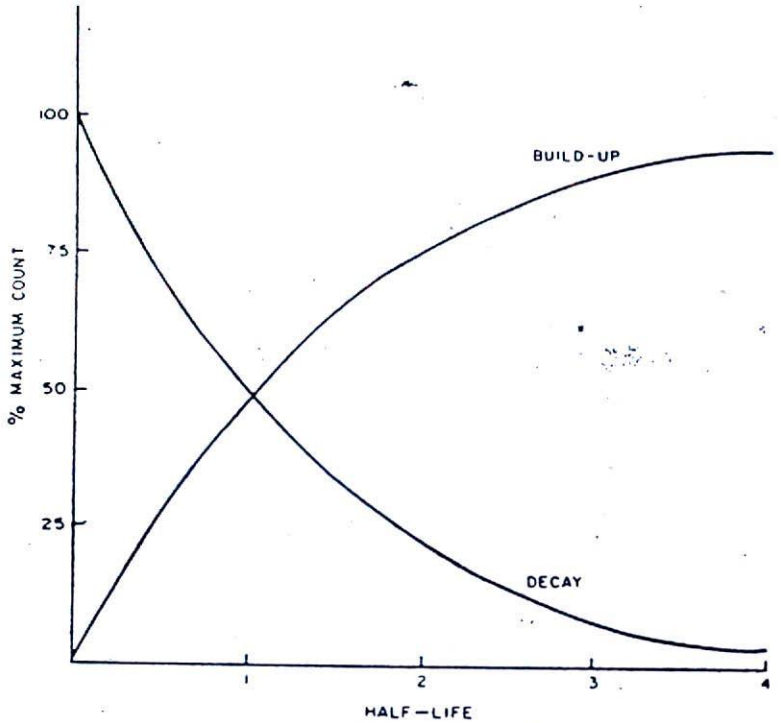

FIGURE 19.13: Relative decay and buildup of an activated sample.

When t is large, the above expressions represent maximum concentration of N, and thus Eq. (19.43) is reduced to

$$N = 1.44N'T \tag{19.44}$$

The rate of buildup, N', is in turn related to the incoming number of neutrons, the number of target nuclei, and nuclear cross section of such nuclei. This relationship is expressed as

$$N' = \sigma F N_t \tag{19.45}$$

where σ = nuclear cross section
F = flux of bombarding neutrons, expressed as number of neutrons per square centimeter per second
N_t = number of target nuclei

To determine the mass of an unknown element in a particular mixture, the

following parameters should also be known: the atomic weight of the unknown isotope, its per cent occurrence in nature, its method of decay, the efficiency of the detector, and the decay constant. The following equation describes the various relationships:

$$M = \frac{AN_c}{1.44N_0\sigma FEB\left[1 - \exp\left(-\frac{t}{\tau}\right)\right]\left[\exp\left(-\frac{t_1}{\tau}\right) - \exp\left(-\frac{t_2}{\tau}\right)\right]} \tag{19.46}$$

where M = mass of unknown in grams
A = atomic weight
N_c = number of counts recorded in the detector during the time interval $t_1 - t_2$
N_0 = Avogadro's number 6.025×10^{23} atoms per gram atom
σ = nuclear cross section of isotope
F = flux of neutrons (n/cm^2-sec)
E = efficiency of detector
B = branching ratio in the decay scheme
t = activation time
t_1 = time at beginning of count as measured from the end of bombardment
t_2 = time at the end of count as measured from the end of bombardment
τ = mean life of radioactive species formed ($1.44T$)

Equation (19.46) holds true only when uniformity of the neutron flux throughout the sample is assumed. Self-absorption corrections must be made in situations where the sample is large or the neutron energy is small.

3. Application of Activation Analysis

The technique is found to be most useful where it is desirable but impractical to use the standard radiotracer method. Of particular interest is the routine analysis of oxygen in steel and other metals. Silicon and silver are common elements detected and measured in geological formations by means of activation analysis. The applications in the field of crime investigation are many. Traces of arsenic in hair, gun powder residues, and comparison of paint samples are a few examples of the potential presented by this technique. In the biomedical field, activation analysis was used for the determination of cations such as manganese, copper, selenium, magnesium, zinc, cobalt, barium, sodium, and potassium in blood sera. The future of this method in pharmaceutical chemistry is limited only to the ingenuity of the analyst. However, it should not be considered as a solution to all analytical problems, but rather as another tool complementing already established instrumental techniques.

19.5 LABORATORY EXPERIMENTS

A. EXPERIMENT I: DETERMINATION OF THE OPERATING CHARACTERISTICS OF A GEIGER-MÜLLER TUBE

Procedure

1. Carefully pipette 100 μl of a radioactive ^{32}P solution onto the center of a filter paper 1 in. in diameter. The solution must have a specific activity of approximately 15,000 dpm/100 μl.
2. The spotted solution is dried under an infrared lamp for 5 min.
3. The filter paper is then positioned on a plexiglass holder and adjusted to a distance of 2 cm under the window of the GM tube.
4. The power control on the scaler is turned on, and after 2 min the high voltage is also activated. One minute later, the high voltage is increased to 500 V and 2-min counts are recorded on the scaler. This procedure is repeated at intervals of 50 V until 1200 V are reached. Background counts of 1 min are taken after each sample count.
5. Plot the net count rate versus the corresponding high voltage on graph paper. The operating voltage of this particular GM tube is selected close to the lower threshold observed on the curve. This particular voltage is recorded and maintained throughout the experiment.
6. Carefully divide the circular filter paper into two even portions marked 1 and 2 (the radioactive spot will also be divided into two portions). Count each portion for 2 min individually.
7. Calculate the slope of the GM plateau, the resolving time of the GM tube, and the true count rate of the spotted radioactive material. The following equations should be used for your calculations:

$$\text{a. Slope \% per 100 V} = \frac{(C_2 - C_1) \times 10^4}{(V_2 - V_1)C_1} \tag{19.47}$$

where C_2 = count rate at the upper threshold
C_1 = count rate at the lower threshold
V_2 = voltage of the upper threshold
V_1 = voltage of the lower threshold

$$\text{b. Resolving time} = T\ (\text{min}) = \frac{(C_1 + C_2) - C_{1,2}}{2C_1C_2} \tag{19.48}$$

where C_1 = count rate of portion 1
C_2 = count rate of portion 2
$C_{1,2}$ = count rate of portion 2 plus 1

$$\text{c. True count rate} = C = \frac{C_0}{1 - C_0T} \tag{19.49}$$

where C_o = observed count rate
T = resolving time

8. Decontaminate all the glassware with a nonlabeled sodium phosphate solution and monitor the working bench, clothes, and hands for any residual contamination.

B. EXPERIMENT II: DETERMINATION OF CHEMICAL QUENCH IN LIQUID SCINTILLATION COUNTING

Procedure

1. Prepare four vials each containing 10 ml of the following scintillator solution:

Dioxane	600 ml
Anisole	100 ml
Dimethoxyethane	100 ml
PPO	12 g
POPOP	500 mg

2. To each of the above vials carefully add 50 μl of a ^{14}C solution of toluene having a specific activity of approximately 20,000 dpm/50 μl and thoroughly mix the contents.

3. Using a liquid scintillation spectrometer having three individual channels and a proper external standard, set the respective two channels for ^{14}C as given in the operation manual of the particular instrument.

4. Count all samples, without the use of an external standard, and record the ratio of activity between channels B and A such that channel A represents the entire beta particles spectrum.

5. Using a set of known quenched standards and the same instrument settings, plot the efficiency of channel A versus the ratio of counts observed (B/A).

6. From the above curve determine the true count rate of the activity in the four prepared samples.

7. Using the same set of quenched standards, plot the efficiency versus channels ratio of the net activity due to the external standard.

8. Recount the four samples in the presence of the external standard, and from the curve obtained in step 7, determine the counting efficiency of each sample.

9. To samples number 2, 3, and 4 add the following chemical quenchers consecutively: 0.2 ml of water to sample 2, 0.1 ml of carbon tetrachloride to sample 3, and 0.2 ml of methyl alcohol to sample 4.

10. Recount each sample after adding the various quenchers and calculate the true count rate using both the channels ratio technique and the external standard method.

11. Add to each sample 50 μl of NBS standard ^{14}C-toluene, the specific activity of which is exactly known, and recount each sample without the use of external standard.

12. Calculate the true activity of each sample using the following formula:

$$\% \text{ efficiency} = \frac{A - B}{S} \times 100 \tag{19.50}$$

where A = total counts of channel A after the addition of internal standard
B = total counts of channel A before the addition of internal standard
S = dpm of internal standard per 50 μl volume.

The true counts are then equivalent to

$$C_0 = \frac{B \times 100}{E} \tag{19.51}$$

where C_0 = true counts in dpm
B = total counts of channel A before the addition of internal standard
E = per cent efficiency

Questions:

a. How do the three methods of determining the extent of chemical quench compare with each other?

b. Would the same relationships hold true under extreme quench conditions?

C. EXPERIMENT III: RADIOCHROMATOGRAPHY

Procedure

1. *Preparation of thin-layer chromatographic plates.* Two glass plates, 5 × 20 cm each, are coated with a layer 250 μ thick of silica gel G by means of a special applicator (thicker layers are useful for preparative work). The adsorbent layer is then dried and activated by heating the plates in an oven at 120°C for 30 min (cellulose and ion-exchange layers are generally air-dried). Apply 10 μl of a radioactive solution containing 5000 dpm in 100 μg of ^{14}C-sodium acetate on the starting point, which should be about 2 cm from the bottom of each plate. Air dry for 10 min and then develop each chromatogram in an equilibrated glass tank containing a solvent system consisting of

Ethanol	80 parts
Ammonium hydroxide	4 parts
Distilled water	16 parts

The plates are removed from the tank when the solvent reaches the marked front, which should be 10 cm from the point of application. Air dry for 15 min to remove most of the solvent from the plates.

2. *Detection of radioactive spot.* a. Use of Actigraph II radiochromatogram scanner: The mode of operation of the scanner is provided in the respective manuals. The instrument is connected to a linear recorder, and the speed of such recorder adjusted to be equal to that of the scanner. Using only one developed chromatographic plate, a marker spot of radioactivity is placed on the spot of origin. The chromatogram is then scanned at a low speed and narrow-slit aperture. The R_f value is then determined according to

$$R_f = \frac{\text{distance between the two peaks on the recorded graph}}{\text{distance traveled by the solvent front}}$$

After scanning the radiochromatogram, the plate is sprayed with a bromocresol purple indicator (0.04 g of bromocresol purple in 50% ethanol adjusted to pH 10 with sodium hydroxide). A bright yellow spot on a blue background is formed.

Compare the R_f value obtained after spraying with the indicator with that observed after use of the scanner.

b. Liquid scintillation spectrometry: The distance between the spot of origin and the solvent front of the second plate is accurately marked into 10 equal parts (1 cm each). Each part is scraped, by means of a sharp razor, into a marked liquid scintillation vial containing 3% Cab-O-Sil in a toluene fluor. Each vial is shaken vigorously to suspend the silica gel. The vials are then counted in a liquid scintillation spectrometer.

Calculate the R_f value of acetate using this method.

Questions:

a. How do the R_f values compare in the above three methods?

b. What are, in your opinion, the advantages and disadvantages in each of those methods?

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