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S.Y.B.Sc. SEM-4

Subject: Biochemistry

Paper-401: BIOPHYSICAL & BIOCHEMICAL TECHNIQUES

Unit -3

Radio isotopic Techniques

Unit – 3

► THE NATURE OF RADIOACTIVITY

Atomic Structure

- An atom is composed of a positively charged central nucleus inside a much larger cloud of negatively charged electrons. The mass of an atom is concentrated in the nucleus, even though it accounts for only a small fraction of the total size of the atom. Atomic nuclei are composed of two major particles, protons and neutrons. Protons are positively charged with a mass approximately 1850 times greater than that of an electron.
- The number of protons present in the nucleus is known as the atomic number (Z), and it determines what the element is, for example six protons is carbon. Neutrons are uncharged particles with a mass approximately equal to that of a proton. The sum of protons and neutrons in a given nucleus is the mass number (A). Thus

  - **A = Z + N**

- Where, N is the number of neutrons present.
- Since the number of neutrons in a nucleus is not related to the atomic number, it does not affect the chemical properties of the atom.
- Atoms of a given element may not necessarily contain the same number of neutrons. Atoms of a given element with different mass numbers (i.e. different numbers of neutrons) are called isotopes.
- Symbolically, a specific nuclear species is represented by a subscript number for the atomic number, and a superscript number for the mass number, followed by the symbol of the element. For example:
- $_{6}C^{12}$ $_{6}C^{14}$ $_{8}O^{16}$ $_{8}O^{18}$
- However, in practice it is more conventional just to cite the mass number (e.g. ^{14}C). The number of isotopes of a given element varies: there are three isotopes of hydrogen (^{1}H, ^{2}H and ^{3}H), seven of carbon (^{10}C to ^{16}C inclusive) and 20 or more of some of the elements of high atomic number.

► Atomic Stability and Radiation

In general, the ratio of neutrons to protons will determine whether an isotope of an element is stable enough to exist in nature. Stable isotopes for elements with low atomic numbers tend to have an equal number of neutrons and protons, whereas stability for elements of higher atomic numbers requires more neutrons. Unstable isotopes are called radioisotopes. They become stable isotopes by the process of radioactive decay: changes occur in the atomic nucleus, and particles and/or electromagnetic radiation are emitted.

Table 1.1 Properties of different types of radiation

Alpha	Beta	Gamma, X-rays and Bremsstrahlung
Heavy charged particle	Light charged particle	Electromagnetic radiation (em)
More toxic than other forms of radiation	Toxicity same as em radiation per unit of energy	Toxicity same as beta radiation per unit of energy
Not penetrating	Penetration varies with source	Highly penetrating

Types of Radioactive Decay

There are several types of radioactive decay; only those most relevant to biochemists are considered below. A summary of properties is given in Table 1.1.

Decay by Negatron Emission

In this case a neutron is converted to a proton by the ejection of a negatively charged beta (β) particle called a negatron (β^-):

Neutron → Proton + Negatron

To all intents and purposes a negatron is an electron, but the term negatron is preferred, although not always used, since it serves to emphasise the nuclear origin of the particle. As a result of negatron emission, the nucleus loses a neutron but gains a proton. The mass number, *A*, remains constant. An isotope frequently used in biological work that decays by negatron emission is ^{14}C.

$$_{6}C^{14} \rightarrow {}_{7}N^{14} + \beta^-$$

Negatron emission is very important to biochemists because many of the commonly used radionuclides decay by this mechanism. Examples are: ^{3}H and ^{14}C, which can be used to label any organic compound; ^{35}S used to label methionine, for example to study protein synthesis; and ^{33}P or ^{32}P, powerful tools in molecular biology when used as nucleic acid labels.

Decay by Positron Emission

Some isotopes decay by emitting positively charged β-particles referred to as positrons (β^+). This occurs when a proton is converted to a neutron:

Proton → Neutron + Positron

Positrons are extremely unstable and have only a transient existence. Once they have dissipated their energy they interact with electrons and are annihilated. The mass and energy of the two particles are converted to two γ-rays emitted at 180° to each other. This phenomenon is frequently described as back-to-back emission. As a result of positron emission the nucleus loses a proton and gains a neutron, the mass number stays the same. An example of an isotope decaying by positron emission is ^{22}Na:

$_{11}Na^{22} \rightarrow {}_{10}Ne^{22} + \beta^+$

Positron emitters are detected by the same instruments used to detect γ-radiation. They are used in biological sciences to spectacular effect in brain scanning with the technique positron emission tomography (PET scanning) used to identify active and inactive areas of the brain.

Decay by Alpha Particle Emission

Isotopes of elements with high atomic numbers frequently decay by emitting alpha (α) particles. An α-particle is a helium nucleus; it consists of two protons and two neutrons ($4He_2$þ). Emission of α-particles results in a considerable lightening of the nucleus, a decrease in atomic number of 2 and a decrease in the mass number of 4. Isotopes that decay by α-emission are not frequently encountered in biological work although they can be found in instruments such as scintillation counters and smoke alarms. Radium-226 (^{226}Ra) decays by α-emission to radon-222 (^{222}Rn), which is itself radioactive. Thus begins a complex decay series, which culminates in the formation of ^{206}Pb:

$$_{88}Ra^{226} \rightarrow {}_{2}He^{4\ 2+} + {}_{86}Rn^{222} \rightarrow\rightarrow\rightarrow {}_{82}Pb^{206}$$

Alpha emitters are extremely toxic if ingested, due to the large mass and the ionising power of the α-particle.

Decay by Emission of γ -Rays

In some cases α and β particle emission also give rise to γ-rays (electromagnetic radiation similar to, but with a shorter wavelength than, X-rays). The γ-radiation has low ionising power but high penetration. For example, the radiation from ^{60}Co will penetrate 15 cm of steel. The toxicity of γ-radiation is similar to that of X-rays.

Example: $_{53}I^{131} \rightarrow {}_{54}Xe^{131} + \beta^- + \gamma$

One word Question

Sr. No.	Question	Answer
1	The number of protons present in the nucleus is known as the	Atomic Number
2	The number of Proton & Neutron present in the nucleus is known as the	Atomic mass
3	Unstable isotopes are called______	radioisotopes
4	Neutron is converted to a proton by the ejection of a negatively charged beta (β) particle called a	Negatron
5	Proton is converted to a Neutron by the ejection of a negatively charged beta (β) particle called a	Positron
6	An α-particle is a__________nucleus	Helium
7	The______Radiation has low ionising power but high penetration	γ-radiation

Radioactive Decay Energy

The usual unit used in expressing energy levels associated with radioactive decay is the electron volt. One electron volt (eV) is the energy acquired by one electron in accelerating through a potential difference of 1 V and is equivalent to 1.6×10^{-19} J. For the majority of isotopes, the term million or mega electron volts (MeV) are more applicable.

Isotopes emitting α-particles are normally the most energetic, falling in the range 4.0 to 8.0 MeV, whereas β and γ-emitters generally have decay energies of less than 3.0 MeV. The higher the energy of radiation the more it can penetrate matter and the more hazardous it becomes.

Rate of Radioactive Decay

Radioactive decay (measured as disintegrations per minute, d.p.m.) is a spontaneous process and it occurs at a rate characteristic of the source, defined by the rate constant (λ, the fraction of an isotope decaying in unit time, t^{-1}). Decay is a nuclear event so λ is not affected by temperature or pressure.

The number of atoms disintegrating at any time is proportional to the number of atoms of the isotope (N) present at that time (t). Clearly, the number of atoms N, is always falling (as atoms decay) and so the rate of decay (d.p.m.) falls with time. Also, the slope of the graph of number of unstable atoms present, or rate of decay (d.p.m.) against time, similarly falls. This means that a graph of radioactivity against time shows a curve, called an exponential decay curve (Fig. 1.1).

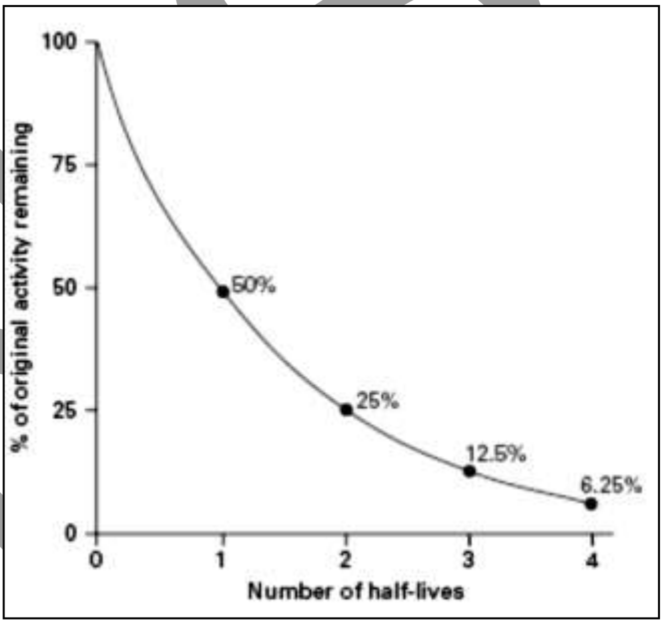

Fig. 1.1 ***Demonstration of the exponential nature of radioactive decay.***

The mathematical equation that underpins the graph shown is as follows:

$\ln Nt/N0 = -\lambda t$ **Eq. 1.1**

Where λ is the decay constant for an isotope, Nt is the number of radioactive atoms present at time t, and N0 is the number of radioactive atoms originally present. You will notice the natural logarithm (ln) in the equation; this means if we were to plot log d.p.m. against time we would get a graph with a straight line and a negative slope (gradient determined by the value of λ).

In practice it is more convenient to express the decay constant in terms of half-life (t½). This is defined as the time taken for the activity to fall from any value to half that value (see Fig. 1.1). When Nt in equation 1.1 is equal to one-half of N0 then t will equal the half-life of the isotope. Thus

$\ln 1/2 = -\lambda t_{1/2}$ **Eq. 1.2**

or $t_{1/2} = 0.693\lambda$ Eq. 1.3

The values of t½ vary widely from over 1019 years for lead-204 (^{204}Pb) to 3×10^{-7} seconds for polonium-212 (^{212}Po). The half-lives of some isotopes frequently used in biological work are given in Table 1.2. The advantages and disadvantages of working with isotopes of differing half-lives are given in Table 1.3.

Table 1.2 Properties of radioisotopes commonly used in the biological sciences

Property	^{3}H	^{14}C	^{35}S	^{32}P	^{33}P	^{125}I	^{131}I
$t_{1/2}$	12.3 years	5730 years	87.4 days	14.3 days	25.4 days	59.6 days	8.04 days
Mode of decay	β	β	β	β	β	X (EC) and Auger electrons	γ and β
Max β energy (MeV)	0.019	0.156	0.167	1.709	0.249	Auger electrons 0.035	0.806
ALI[a]	480 (Mbq)[b]	34 (Mbq)	15 (Mbq)	6.3 (Mbq)	14 (Mbq)	1.3 (Mbq)[c]	0.9 (Mbq)[c]
Maximum range in air	6 mm	24 cm	26 cm	790 cm	49 cm	>10 m	>10 cm
Shielding required	None	1 cm acrylic	1 cm acrylic	1 cm acrylic	1 cm acrylic	Lead 0.25 m or lead-impregnated acrylic	Lead 13 mm
γ dose rate ($\mu Sv\ h^{-1}$ from 1 GBq at 1 m)	-	-	-	(β dose rate 760 µSv, 10 cm from 1 MBq)	-	41	51
Čerenkov counting	-	-	-	Yes	-	-	-

Notes: [a]Annual limit on intake, based on a dose limit of 20 mSv using the most restrictive dose coefficients for inhalation or ingestion.
[b]Bound ^{3}H.
[c]Based on dose equivalent limit of 500 mSv to thyroid.

One word question

Sr.No.	Question	Answer
1	The atomic number is not changed by which type of radioactive decay?	gamma
2	Three types of radioactive elements are emitted when unstable nuclei undergo radioactive decay. Which of the following is not one of them	Delta
3	Helium nuclei particles are called	alpha particles
4	When two atomic nuclei combine it is called as	Nucleus fusion
5	The half life of a radioisotope is ___________	time taken for half the decay

► **Units of Radioactivity**

The Systeme International d'Unite´s (SI system) uses the becquerel (Bq) as the unit of radioactivity. This is defined as one disintegration per second (1 d.p.s.). However, an older unit, not in the SI system and still frequently used, is the curie (Ci). This is defined as the quantity of radioactive material in which the number of nuclear disintegrations per second is the same as that in 1 g of radium, namely 3.7×10^{10} (or 37 GBq). For biological purposes this unit is too large and the microcurie (mCi) and millicurie (mCi) are used. It is important to realise that the units Bq and Ci refer to the number of disintegrations actually occurring in a sample not to the disintegrations detected, which generally will be only a proportion of the disintegrations occurring. Detected decays are referred to as counts (i.e. counts per second or c.p.s.).

Table 1.3 The advantages and disadvantages of working with a short-half-life isotope

Advantages	Disadvantages
High specific activity (see Section 14.3.3) makes the experiment more sensitive	Experimental design; isotope decays during time of experiment
Easier and cheaper to dispose of	Cost of replacement for further experiments
Lower doses likely (e.g. in diagnostic testing of human subjects)	Frequently need to calculate amount of activity remaining

For quick reference, a list of units and definitions frequently used in radioisotope work is provided in Table 1.4.

Table 1.4 Units commonly used to describe radioactivity

Unit	Abbreviation	Definition
Counts per minute or second	c.p.m.	The *recorded* rate of decay
	c.p.s.	
Disintegrations per minute or second	d.p.m.	The *actual* rate of decay
	d.p.s.	
Curie	Ci	The number of d.p.s. equivalent to 1 g of radium (3.7×10^{10} d.p.s.)
Millicurie	mCi	$Ci \times 10^{-3}$ or 2.22×10^{9} d.p.m.
Microcurie	µCi	$Ci \times 10^{-6}$ or 2.22×10^{6} d.p.m.
Becquerel (SI unit)	Bq	1 d.p.s.
Terabecquerel (SI unit)	TBq	10^{12} Bq or 27.027 Ci

► **DETECTION AND MEASUREMENT OF RADIOACTIVITY**

There are three commonly used methods of detecting and quantifying radioactivity. These are based on the ionization of gases, on the excitation of

Gigabecquerel (SI unit)	GBq	10^{9} Bq or 27.027 m Ci
Megabecquerel (SI unit)	MBq	10^{6} Bq or 27.027 µCi
Electron volt	eV	The energy attained by an electron accelerated through a potential difference of 1 volt. Equivalent to 1.6×10^{-19} J
Roentgen	R	The amount of radiation that produces 1.61×10^{15} ion-pairs kg^{-1}
Rad	rad	The dose that gives an energy absorption of 0.01 J kg^{-1}
Gray	Gy	The dose that gives an energy absorption of 1 J kg^{-1}. Thus 1 Gy=100 rad
Rem	rem	The amount of radiation that gives a dose in humans equivalent to 1 rad of X-rays
Sievert	Sv	The amount of radiation that gives a dose in humans equivalent to 1 Gy of X-rays. Thus 1 Sv=100 rem

solids or solutions, and the ability of radioactivity to expose photographic emulsions (i.e. autoradiography).

One word Question

Sr.No.	Question	Answer
1	__________the unit of radioactivity	becquerel (Bq)
2	SI Units of Radioactivity	Curie
3	The Recorded rate of decay	c.p.m
4	The actual rate of decay	d.p.m
5	1 Ci	3.7 x10^{10} d.p.s

❖ **Methods Based Upon Gas Ionization**

- If a charged particle passes through a gas, its electrostatic field dislodges orbital electrons from atoms sufficiently close to its path and causes ionisation (Fig. 1.2).
- The ability to induce ionisation decreases in the order
- $\alpha > \beta > \gamma$ (10000: 100: 1)
- If ionisation occurs between a pair of electrodes enclosed in a suitable chamber (Fig. 1.2) a pulse (current) flows. Ionisation counters like those shown in Fig. 1.2 are sometimes called proportional counters ('proportional' because small voltage changes can affect the count rate).
- The Geiger–Muller counter (Figs. 1.3, 1.4a) has a cylindrical-shaped gas chamber and it operates at a high voltage. This makes the instrument less dependent on a stable voltage, so the counter is cheaper and lighter.
- In ionisation counters, the ions have to travel to their respective electrodes; other ionising particles entering the tube during this time (the so-called 'dead time') are not detected and this reduces the counting efficiency.

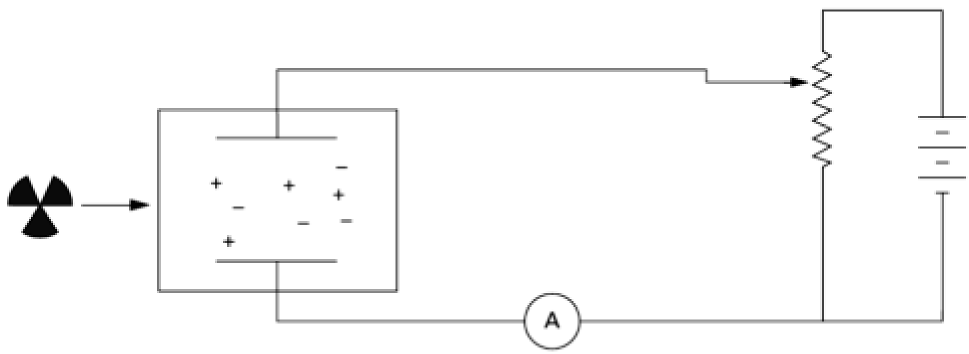

Fig. 1.2 Detection based on ionization.

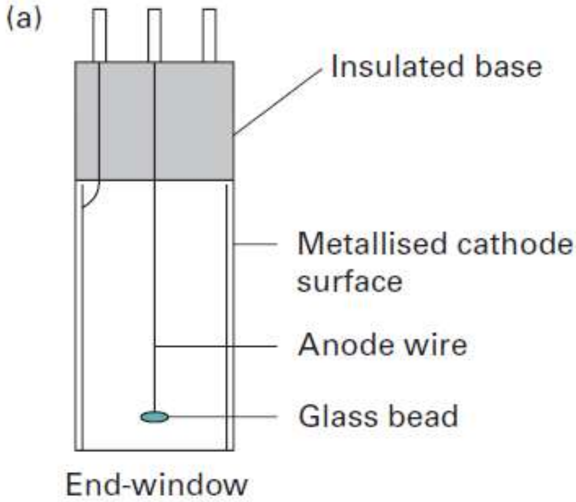

Fig. 1.3 (a) The Geiger–Muller (G–M) tube

► **Methods Based Upon Excitation**

Radioactive isotopes interact with matter in two ways, ionisation and excitation. The latter effect leads an excited atom or compound (known as a fluor) to emit photons of light. The process is known as scintillation. When the light is detected by a photomultiplier, it forms the basis of scintillation counting.

Essentially, a photomultiplier converts the energy of radiation into an electrical signal, and the strength of the electric pulse that results is directly proportional to the energy of the original radioactive event. This means that two, or even more, isotopes can be separately detected and measured in the same sample, provided they have sufficiently different emission energy spectra. The mode of action of a photomultiplier is shown in Fig. 1.5a, and the energy spectrum of a β-particle emitter in Fig. 1.5b.

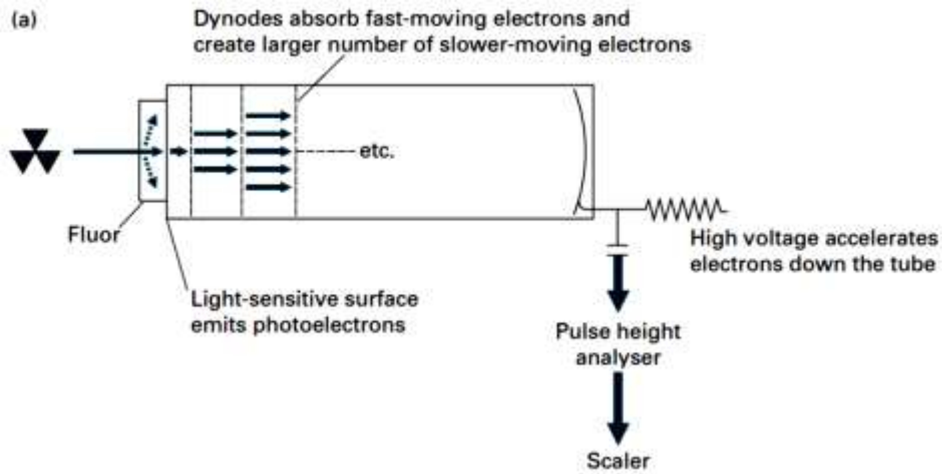

Fig. 1.5 (a) The mode of action of a photomultiplier

Types of scintillation counting

- There are two types of scintillation counting, which are illustrated diagrammatically in Fig. 1.6. In solid scintillation counting the sample is placed adjacent to a solid fluor (e.g. sodium iodide).
- Solid scintillation counting is particularly useful for g-emitting isotopes. This is because they can penetrate the fluor. The counters can be small handheld devices with the fluor attached to the photomultiplier tube (Fig. 1.5.a), or larger bench-top machines with a well-shaped fluor designed to automatically count many samples (Fig. 1.6.a).
- In liquid scintillation counting (Fig. 1.6b), the sample is mixed with a scintillation fluid containing a solvent and one or more dissolved fluors. This method is particularly useful in quantifying weak b-emitters such as ^{3}H, ^{14}C and ^{35}S, which are frequently used in biological work.
- Scintillation fluids are called 'cocktails' because there are different formulations, made of a solvent (such as toluene or diisopropylnaphthalene) plus fluors such as 2,5-diphenyloxazole (PPO), 1,4-bis(5-phenyloxazol-2-yl)benzene (nicknamed POPOP, pronounced as it reads: 'pop op') or 2-(4'-t-butylphenyl)-5-(4"-bi-phenyl)-1,3,4-oxydiazole (butyl-PBD).
- Cocktails can be designed for counting organic samples, or may contain detergent to facilitate counting of aqueous samples.

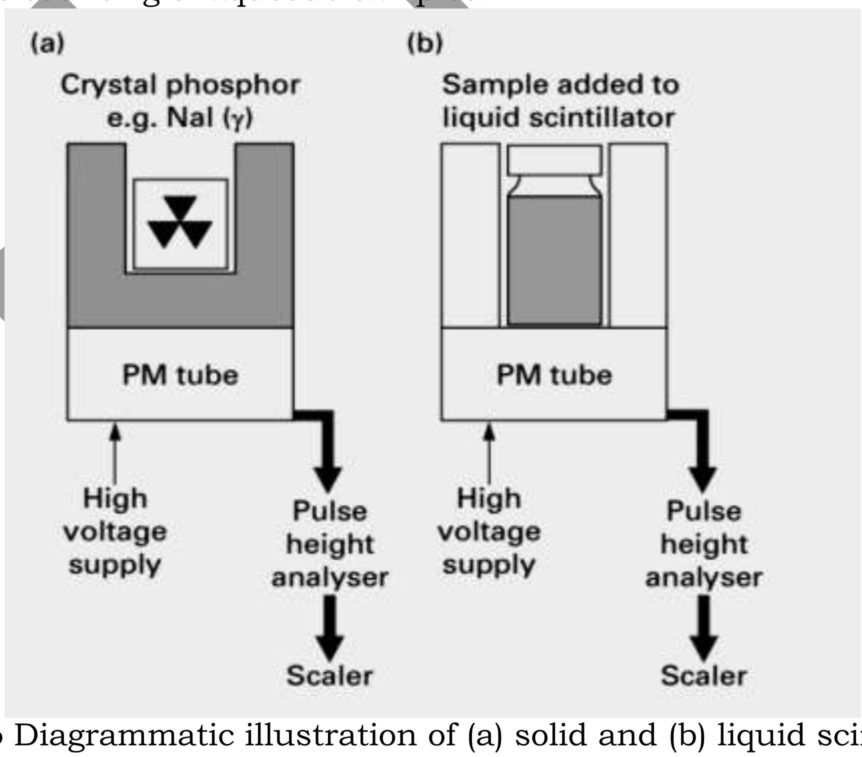

Fig. 1.6 Diagrammatic illustration of (a) solid and (b) liquid scintillation counting methods.

► **Advantages of Scintillation Counting**

- ❖ Scintillation counting is widely used in biological work and it has several advantages over gas ionisation counting:
- ❖ Fluorescence is very fast so there is effectively no dead time
- ❖ Counting efficiencies are high (from about 50% for low-energy β-emitters to 90% for high-energy emitters)
- ❖ The ability to count samples of many types, including liquids, solids, suspensions and gels
- ❖ The general ease of sample preparation
- ❖ The ability to count separately different isotopes in the same sample (used in dual-labelling experiments)
- ❖ Highly automated (hundreds of samples can be counted automatically and built-in computer facilities carry out many forms of data analysis, such as efficiency correction, graph plotting, radioimmunoassay calculations, etc.).

► **Disadvantages of Scintillation Counting**

No technique is without disadvantages, so the following have to be considered or overcome in the design of the instruments:

- ♣ Cost of the instrument and cost per sample (for scintillation fluid, the counting vials and disposal of the organic waste)
- ♣ Potentially high background counts; this is due to photomultiplier noise but can be compensated for by using more than one tube (noise is random, but counts from a radioactive decay are simultaneous, the coincident counts only are recorded)
- ♣ 'quenching': this is the name for reduction in counting efficiency caused by coloured compounds that absorb the scintillated light, or chemicals that interfere with the transfer of energy from the radiation to the photomultiplier (correcting for quenching contributes significantly to the cost of scintillation counting)
- ♣ Chemiluminescence: this is when chemical reactions between components of the samples to be counted and the scintillation cocktail produce scintillations that are unrelated to the radioactivity; modern instruments can detect chemiluminescence and subtract it from the results automatically

- ♣ Phospholuminescence: this results from pigments in the sample absorbing light and re-emitting it; the solution is to keep the samples in the dark prior to counting.

One word Question

Sr.No.	Question	Answer
1	Liquid scintillation spectrometry is a method of detecting ______________________	β-emitters
2	A Geiger-Muller tube is a _____ .	Gas ionization detector
3	Which of the following acts as ionising gas in Geiger Muller counter?	Argon
4	Scintillation detector is a large flat crystal of which of the following materials?	Sodium chloride
5	Liquid samples must be counted using ionization chamber by placing them in which of the following?	Ampoules
6	Which type of Scintillation detector are use?	Photomultiplier tube

► **SAFETY ASPECTS**

The greatest practical disadvantage of using radioisotopes is the toxicity: they produce ionising radiations. When absorbed, radiation causes ionisation and free radicals form that interact with the cell's macromolecules, causing mutation of DNA and hydrolysis of proteins. The toxicity of radiation is dependent not simply on the amount present but on the amount absorbed by the body, the energy of the absorbed radiation and its biological effect. There are, therefore, a series of additional units used to describe these parameters.

A key aspect of determining toxicity is to know how much energy might be absorbed, just as too much sun gives you sunburn and potentially skin cancer. The higher the energy of the radiation the greater the potential hazard. The gray (Gy), an SI unit, is the unit used to describe this; 1 Gy is an absorption of 1 J kg^{-1} of absorber. The gray (Gy) is a useful unit, but it still does not adequately describe the hazard to living organisms. This is because different types of radiation are associated with differing degrees of biological hazard.

► **When handling radioisotopes the rules are to:**

- Wear protective clothing, gloves and glasses
- Use the smallest amount possible
- Keep radioactive materials safe, secure and well labelled
- Work in defined areas in a spill tray

- Monitor your working area frequently

- Have no foods or drinks in the laboratory
- Wash and monitor hands after the work is done
- Follow all local rules such as for the dispensing of stock and the disposal of waste
- Do not create radioactive aerosols or dust and for penetrating radiations (e.g. ^{32}P and λ-emitters):
- Maximise the distance between yourself and the source
- Minimise the time of exposure
- Maintain shielding at all times.

► **Application of Radioisotopes in various Field:**

Field	**Selected Applications**
Food and agriculture	♣ Improve nutritional status and health of plants and animals ♣ Maximize optimal crop production ♣ Reduce food-borne diseases and increase food preservation
Biochemistry, biology, biotechnology, chemistry, physics, physiology	♣ Molecular studies ♣ Metabolic and biological tracers
Cosmology	♣ Exploration and understanding of the universe
Earth sciences: geochemistry, geology, geophysics, hydrology, and marine, sciences	♣ Exploration and preservation of natural resources ♣ Study of water resources and maintaining a safe and abundant water supply
Ecological and environmental research	♣ Environmental chemistry and measurements ♣ Environmental pollution studies: occurrence, cause, and remedy
Health care	♣ Diagnostic nuclear medicine such as cardiological diagnosis ♣ PET research and applications ♣ Radionuclide treatment of disease such as cancer ♣ Radiopharmaceuticals ♣ Drug research (uptake, binding, metabolism, clearance)
Industrial manufacturing and research	♣ Materials sciences ♣ Radioisotope thickness gauges for steel plate or paper production

	♣ Computer chip production
Nutrition	♣ Disease prevention and health promotion research (cancer, heart disease, obesity, osteoporosis, etc.) ♣ Energy metabolism in humans and animals ♣ Tracer techniques to determine nutrition requirements
Toxicology	♣ Risk assessment ♣ Soil and water exposure studies

One word question

Sr.no	Question	Answer
1	Radioactive isotopes are useful for establishing the ages of various objects by	Radio dating
2	rock is analyzed and is found to contain a certain amount of	Urenium-235
3	Which Radioactive isotopes have numerous medical applications—diagnosing and treating illness and diseases	iodine-131
4	PET research and applications	Radioisotope
	diagnosis and treatment of thyroid function	iodine-131
	Major research tool. Helps in research to ensure that potential new drugs are metabolized	Carbon-14