

Radioisotope techniques

The nature of radioactivity

Atomic structure

An atom is composed of a positively charged nucleus that is surrounded by a cloud of negatively charged electrons. The mass of an atom is concentrated in the nucleus, even though the nucleus makes only a small fraction of the total size of the atom.

Atomic nuclei are made up of two major particles: protons and neutrons.

Protons: are positively-charged particles with a mass of $1.67262158 \times 10^{-27}$ kilograms, approximately 1,850 times greater than that of an orbital electron. The number of protons found in the nucleus of an atom, that is, the atomic number (Z), defines the type of atom.

Neutrons are the uncharged particles in the nucleus with mass approximately equal to the mass of a proton. Because protons are unable to bind with each other due to their mutual electromagnetic repulsion being stronger than the attraction of the nuclear force, the presence of a neutron is required within an atomic nucleus as they bind with protons via the nuclear force.

Electrons: negatively-charged with a mass of $9.10938188 \times 10^{-31}$ kilograms, which is about 1/1850 times the mass of a proton.

The number of orbital electrons in an atom must equal the number of protons present in the nucleus, since the atom as a whole is electrically neutral.

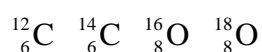
The number of neutrons in the nucleus is given a notation N . The number of neutrons determines the isotope of an element. For example, the abundant ^{12}C isotope has 6 protons and 6 neutrons, while the very rare radioactive ^{14}C isotope has 6 protons and 8 neutrons.

The sum of protons and neutrons in a nucleus is the mass number (A).

Thus, $A = Z + N$

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. Such atoms, having same number of protons but different mass numbers (i.e. different numbers of neutrons) are called isotopes.

By convention, an atom/element and its isotope are represented by a subscript for atomic number, and a superscript for mass number, followed by the symbol of the element. For example, the isotopes of carbon are represented below:



However, in practice it is more conventional just to cite the mass number (e.g., ${}^{14}\text{C}$). The number of isotopes of a given element varies: there are 3 isotopes of hydrogen, ${}^1\text{H}$, ${}^2\text{H}$, and ${}^3\text{H}$, and 7 isotopes of carbon, ${}^{10}\text{C}$ to ${}^{16}\text{C}$, and 20 or more isotopes of some of the elements of high atomic number.

Atomic stability and radiation

In general, the ratio of neutrons to protons in the nucleus will determine whether an isotope of a given element is stable enough to exist in nature. Stable isotopes for elements with low atomic numbers have neutron:proton ratio of 1, whereas stability for elements of higher atomic numbers is associated with a neutron:proton ratio in excess of 1. Unstable isotopes, also known as radioisotopes are often produced artificially, but many occur in nature.

Radioisotopes emit particles and/or electromagnetic radiation which arise from changes in the composition of the atomic nucleus. The process is known as radioactive decay and may occur either directly or as a result of a decay series in the production a stable isotope. Radioactive decay is the process by which an unstable isotope emits energy to reach a more stable state. Radioactive material decays until only stable substance is left and the decay of a substance is fixed and measurable.

Types of radioactive decay

There are several types of radioactive decay; only those relevant to biological scientists are considered below. A summary of these decay events and their properties is given in Table 9.1.

Table 9.1. Properties of different types of radiation

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

Decay by negatron emission (also called beta 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 \rightarrow Proton + Negatron

A negatron is an electron, but the term negatron is preferred, although not always used, since this particle originates from the nucleus and not from the orbit. Negatron emission causes the nucleus to lose a neutron but gain a proton. The N/Z (number of neutrons/no of protons) ratio therefore decreases because Z increases by 1 and A (mass no.) remains constant. An isotope frequently used in biological work that decays by negatron emission is ^{14}C .



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

Decay by positron emission

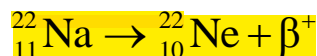
Some isotopes decay by emitting positively-charged β -particles referred to as positrons (β^+). This occurs when a proton is converted to a neutron:

Proton \rightarrow Neutron + Positron

Positrons are extremely unstable and have only a transient existence. Once they have dissipated their energy they interact with electrons and are completely destroyed. 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 but gains a neutron, **the N/Z ratio increases, Z decreases by 1 and A remains constant**. An example of an isotope which decays by positron emission is $^{22}_{11}\text{Na}$:



Positron emitters are detected by the same instruments used to detect γ -radiation. They are used in a clinical technique known as **Positron Emission Tomography (PET scanning)** to scan the brain to identify **active and inactive areas**.

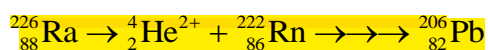
Decay by alpha particle emission

Isotopes of elements with high atomic numbers frequently decay by emitting alpha (α) particles. **An α -particle is a helium nucleus without electrons; it consists of two protons and two neutrons ($^4\text{He}^{2+}$)**.

Emission of α -particles results in the following:

- **A considerable lightening of the nucleus**
- **A decrease in atomic number of 2**
- **A decrease in the mass number of 4.**

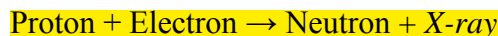
Isotopes that decay by α -emission are not frequently encountered in biological work. Radium-226 (^{226}Ra) decays by α -emission to radon-222 (^{222}Rn), which is itself radioactive, thus, beginning a complex decay series which culminates in the formation of ^{206}Pb :



Alpha emitters are extremely toxic if ingested, due to their large mass and ionizing power.

Electron capture

In this decay process, a proton captures an electron orbiting in the innermost K shell:



The proton becomes a neutron and electromagnetic radiation (X-ray) is given out. Example



Decay by emission of γ -rays

In contrast to emission of α - and β -particles, γ -emission involves electromagnetic radiation similar to, but with a shorter wavelength than X-rays. These γ -rays result from a transformation in the nucleus of an atom (in contrast to X-rays, which are emitted as a consequence of excitation involving the orbital electrons of an atom) and frequently accompany α - and β -particle emission. Emission of γ -radiation only, leads to no change in atomic number or mass.

Gamma radiation has low ionizing power but high penetration. For example, the γ -radiation from ^{60}Co will penetrate 15 cm of steel. The toxicity of γ -radiation is similar to that of X-rays.

Example:



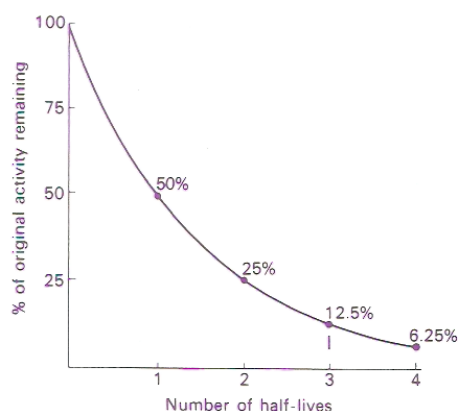
Radioactive decay energy

The usual unit used to express energy levels associated with radioactive decay is the **electron volt**. One electron volt (eV) is the energy required by one electron in accelerating through a potential difference of 1 V and is equivalent to $1.6 \times 10^{-19}\text{J}$. For the majority of isotopes, the term million or mega electron volts (MeV) is more applicable. Isotopes emitting α -particles are normally the most energetic, falling in the range of 4.0 – 8.0 MeV, whereas β - and γ -emitters generally have decay energies of less than 3.0 MeV.

Rate of radioactive decay

Radioactive decay is a spontaneous process and it occurs at a definite/fixed rate characteristic of the source. This rate always follows an exponential law. Thus the number of atoms disintegrating at any time is proportional to the number of atoms of the isotope (N) present at that time (t). When we express this mathematically, the exponential curve in Fig. 9.1 gives the equation below.

Fig. 9.1. The exponential nature of radioactive



$$-\frac{dN}{dt} \propto N \quad \text{or} \quad -\frac{dN}{dt} = \lambda N \dots (1)$$

where λ is the decay constant, a characteristic of a given isotope. Decay constant is defined as the fraction of an isotope decaying per unit time (t^{-1}). By integrating equation (1), it can be converted to a logarithmic form:

$$\ln \frac{N_t}{N_0} = -\lambda t \dots (2)$$

where N_t is the number of radioactive atoms present at time t , and N_0 is the number of radioactive atoms originally present. In practice, it is more convenient to express the decay constant in terms of half-life ($t_{1/2}$).

The half-life of a radioactive isotope is defined as the time taken for the activity to fall from any value to half that value (See Fig. 9.1). **Half life** - the time it takes for a product to decay sufficiently to convert half of its mass to a stable form. The half life of an isotope is fixed.

If N_t in equation (2) is equal to half of N_0 , then t will equal the half-life of the isotope. Thus

$$\ln \frac{1}{2} = -\lambda t_{1/2} \dots (3)$$

or

$$2.303 \log \left(\frac{1}{2} \right) = -\lambda t_{1/2} \dots (4)$$

So that

$$t_{1/2} = 0.693/\lambda \dots(5)$$

The values of $t_{1/2}$ vary widely from 3×10^{-7} s for polonium-212 (^{212}Po) to over 10^{19} years for lead-204 (^{204}Pb). The half-lives of some isotopes frequently used in biological work are shown in Table 9.2.

Table 9.2. Half-lives of some isotopes used in biological studies

Isotope	Half-life
^3H	12.26 years
^{14}C	5760 years
^{22}Na	2.58 years
^{32}P	14.20 days
^{33}P	25.4 days
^{35}S	87.20 days
^{42}K	12.40 hours
^{45}Ca	165 days
^{59}Fe	45 days
^{125}I	60 days
^{131}I	8.05 days
^{135}I	9.7 hours

In this table, we do not have the two important elements, oxygen and nitrogen. This is because the half-lives of the radioactive isotopes of these elements are too short for most biological studies. ^{15}O has a $t_{1/2}$ of 2.03 min, and ^{13}N has a $t_{1/2}$ of 10.00 min.

The advantages and disadvantages of working with isotopes of short half-lives are given in the table below.

Advantages and disadvantages of working with isotopes of short half-lives

Advantages	Disadvantages
High specific activity	Experimental design must be appropriate as isotope decays during time of experiment
Lower doses likely (e.g., in diagnostic testing of human subjects)	Cost of replacement for further experiments

Worked example

Given $\ln(N/N_0) = -\lambda t$ and that the half-life of ^{32}P is 14.2 days, how long would it take a solution containing 42,000 d.p.m. of ^{32}P to decay to 500 d.p.m.?

Solution

Use equation (5) to calculate the value of λ . This gives a value of 0.0488 days^{-1} . Then use equation (2) to calculate the time taken for the counts to decrease (both equations are presented below). In this equation, $N_0 = 42,000$ and $N_t = 500$. This gives a value of 90.8 days.

$$t_{1/2} = 0.693 / \lambda \dots (5)$$

$$\ln \frac{N_t}{N_0} = -\lambda t \dots (2)$$

Units of radioactivity

The Système International d'Unités (SI system) uses **Becquerel (Bq)** as the unit of radioactivity.

Becquerel is defined as one disintegration per second (1 d.p.s.), however, an older unit not in the SI system and still often used is the curie (Ci).

Curie 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} d.p.s. (or 37 GBq).

For biological purposes, this unit is too large and the microcurie (μCi) and millicurie (mCi) are used. It is important to realize that the curie refers to the number of disintegrations actually occurring in a sample and not to the disintegrations detected by the radiation counter, which will generally be only a fraction of the disintegrations occurring and are referred to as counts per second (c.p.s.).

Normally, in experiments with radioisotopes, a carrier of the stable isotope of the element is added. It therefore becomes necessary to **express the amount of radioisotope present per unit mass**. This is the **specific activity**. Specific activity may be **expressed in a number of ways including disintegration rate (d.p.s. or d.p.m.), count rate (c.p.s. or c.p.m.) or curies (mCi or μCi) per unit of mass or quantity of mixture (mass/quantity may be expressed in either moles or grams). An alternative method of expressing specific activity, which is not frequently used is **atom percentage excess**. This is defined as the number of radioactive atoms per total of 100 atoms of the compound.**

Interaction of radioactivity with matter

α -particles

Alpha-particles have quite an amount of energy (4 -8 MeV) and all the particles from a given isotope have the same amount of energy. Alpha particles react with matter in two ways:

1. **They may cause excitation:** in this case, the energy associated with the decay event is transferred from the α -particle to the orbital electrons of neighboring atoms and the electrons are elevated to higher orbitals. The α -particle continues on its path with its energy reduced by a little more than the amount transferred to the orbital electron. The excited electron eventually falls back to its original orbital, emitting the energy it picked up as **photons of light** in the visible or near visible range.
2. **α -particles may cause ionization** (i.e., an electron is ejected) of atoms in their path. When this occurs, the elevated orbital electron is removed completely. Thus, the atom becomes ionized and forms an ion-pair, consisting of a positively charged ion and an electron. Because of their size, their slow movement, and double positive charge (${}^4\text{He}^{2+}$), α -particles frequently collide with atoms in their path. Therefore, they cause **intense ionization and excitation** and **their energy is rapidly dissipated**. **Because of this, though they have initial high energy, α -particles are not very penetrating.**

Negatrons

Negatrons are very small, rapidly moving particles, which carry a single negative charge, compared to α -particles. They interact with matter to cause ionization and excitation in the same way as α -particles do. **However, due to their speed and size, they are less likely than α -particles to interact with matter and therefore are less ionizing but more penetrating than α - radiation.**

Another difference between negatrons and α -particles is that, whereas for a given α -emitter all the particles have the same energy, negatrons are emitted over a range of energies so that negatron emitters have a characteristic energy spectrum. The maximum energy level (E_{max}) varies from one isotope to the other, and ranges from 0.018 MeV for ${}^3\text{H}$ to 4.81 MeV for ${}^{38}\text{Cl}$. **The difference in E_{max} affects the penetration of the radiation.**

For example:

β -particles from ${}^3\text{H}$ can travel only a few millimeters in air

β -particles from ${}^{32}\text{P}$ can penetrate over 1 m of air.

W. Pauli in 1931 gave an explanation for the reason why negatrons of a given isotope are emitted within an energy range. He postulated that each radioactive event occurs with an energy equivalent to E_{max} but

that the energy is shared between a negatron and a **neutrino**. A neutrino is an electrically neutral particle with negligible mass. It is produced in many nuclear reactions such as in beta decay and do not interact with matter. The proportion of the total energy taken by the negatron and the neutrino varies for each disintegration.

γ -Rays and X-rays

For simplicity, we will refer to these rays collectively as γ -rays. These rays are electromagnetic radiation and therefore have no charge or mass. They have the following properties:

- They rarely collide with neighboring atoms and travel great distances before dissipating all their energy, that is, **they are highly penetrating**.
- If they collide with neighboring atoms, they react with matter in many ways. The three most important ways by which they react with matter lead to the **production of secondary electrons**, which in turn cause excitation and ionization. These are
 - a. **Photoelectric absorption**: here, **low energy γ -rays interact with orbital electrons, transferring all their energy to the electron which is then ejected as a photoelectron. The photoelectron subsequently behaves as a negatron.**
 - b. **Compton scattering**: this is caused by **medium energy γ -rays** which interact with orbital electrons, which gain energy and are ejected. **Part of the energy of the γ -ray is transferred to the target electron and this electron is ejected.** The γ -ray is deflected and moves on with reduced energy. Again the ejected electron **behaves as a negatron or a beta particle.**
 - c. **Pair production**: this results when **very high energy γ -rays (highly penetrative) react with the nucleus of an atom and all the energy they carry is used to convert nuclear particles into positron and a negatron.**

When high atomic number materials absorb high energy β -particles, the absorber gives out a secondary radiation, an X-ray called Bremsstrahlung. They are X-rays produced when energetic electrons are deflected in the field of an atomic nucleus. Thus, Bremsstrahlung radiation is present during all beta decay processes as the emitted beta particles (both negatrons and positrons) slow down in matter. For this reason, shields of ^{32}P use low atomic number materials such as Perspex.

Detection and measurement of radioactivity

There are three commonly used methods for detecting and quantifying radioactivity. The methods are based on:

1. Ionization of gases
2. Excitation of solids or solutions
3. The ability of radioactivity to expose photographic emulsion, i.e. autoradiography.

Methods based on gas ionization

A radioactive substance produces charged particle (${}^4_2\text{He}^{2+}$, β^+ , β^-). As a charged particle passes through gas, its electrostatic field dislodges orbital electrons from atoms of the gas which are sufficiently close to its path and causes ionization (Fig. 9.2).

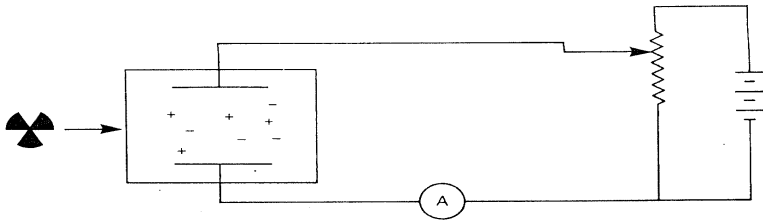


Fig. 9.2. Detection based on ionization.

The ability to induce ionization decreases in the order $\alpha > \beta > \gamma$ (10,000 : 100 : 1). Because of this, α - and β -particles may be detected by gas ionization methods, but these methods are poor for detecting γ -radiation. If ionization occurs between a pair of electrodes enclosed in a suitable chamber filled with gas, ionization of the gas creates surge in the current (called a pulse), whose magnitude depends on:

1. The applied potential, that is, the voltage across the electrodes
2. The number of radiation particles entering the chamber.

Fig. 9.3 shows the effect of voltage on pulse flow.

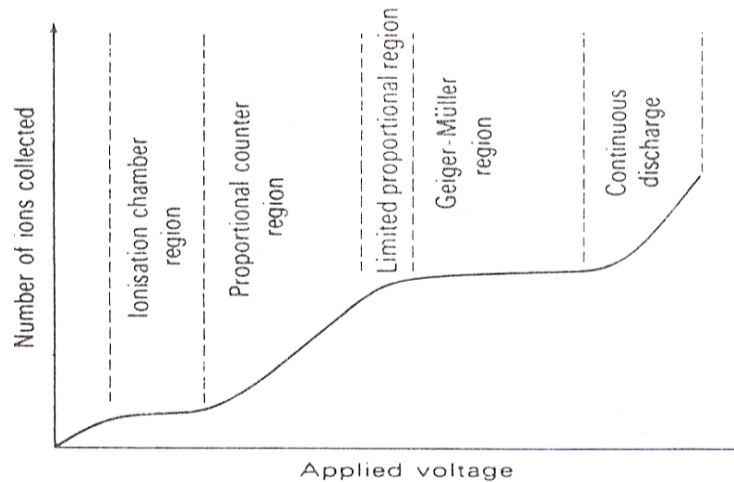


Fig. 9.3. Effect of voltage on pulse flow.

Comments from the curve:

1. An overall rise in the curve- demonstrates that as voltage across the electrodes increases, the number of ions produced from the passage of charged particles through the chamber increases, hence increase in pulse to create five characteristic regions.
 - a) Ionization chamber region: The voltage in this region is low. Each radioactive particle produces only one ion-pair per collision. The corresponding current is low and one needs very sensitive measuring device to detect them. This range of voltage is little used in quantitative work, but various types of electroscopes, which operate on this principle, are useful in demonstrating the properties of radioactivity.
 - b) Proportional counter region: At a higher voltage level than those present in simple ionization chambers, electrons resulting from ionization move toward the anode much more rapidly. In their movement, they cause secondary ionization of gas in the chamber, resulting in the production of secondary ionization electrons, whose fast movement lead to further ionization, and so on. Hence, from the original event, a stream of electrons reaches the anode. This is the principle of gas amplification and is known as the **Townsend Avalanche Effect**, named after its discoverer. **As a consequence of this gas amplification, current flow is much greater and more pulses are created. In the proportional counter region, we can see that the number of ion-pairs collected is directly proportional to the applied voltage.**

- c) Limited proportional region: as voltage is increased and more ion-pairs are produced, a point is reached where **increase in voltage does not result in further ionization** - a plateau occurs. Before the plateau is reached, there is a region known as the **limited proportion region**, which is not often used for detection and quantification of radioactivity. The main drawback of counters that are manufactured to operate in the proportional region is that they require very stable voltage supply because small fluctuations in voltage result in significant changes in amplification. Proportional counters are useful for detecting and quantifying α -emitting isotopes, but relatively few such isotopes are used in biological work.
- d) In the Geiger-Müller region: at this voltage level, **all radiation particles, including weak β -particles, induce complete ionization of the gas in the chamber**. Thus, the size of the current is no longer dependent on the primary ions produced. Since maximal gas amplification is produced in this region, the size of the output pulse from the detector will remain the same over a considerable voltage range (the Geiger- Müller plateau). Therefore, **it is not possible to distinguish between different isotopes using this type of counter**.

Since it takes a finite time for the ion-pair to travel to the electrodes, other ionizing particles entering the tube during this time fail to produce ionization and hence are not detected, thereby reducing the counting efficiency. This is referred to as the **dead time** of the tube and is normally 100 to 200 μ s.

- e) Continuous discharge region: when the ions reach the electrode, they are neutralized. Inevitably, **some escape and produce their own ionization avalanche**. Because of this, if unchecked, a Geiger-Müller tube would tend to give continuous discharge. To overcome this, the tube is **quenched** by the addition of a suitable gas, which reduces the energy of the ions. Common quenching agents are ethanol, ethyl formate and the halogens.

Instrumentation

Counters based on gas ionization were previously the main methods employed in quantification of radioisotopes in biological samples. Currently, scintillation counting is commonly used. However, all labs use small hand-held radioactivity monitors based on gas ionization, the end-window design being the most popular.

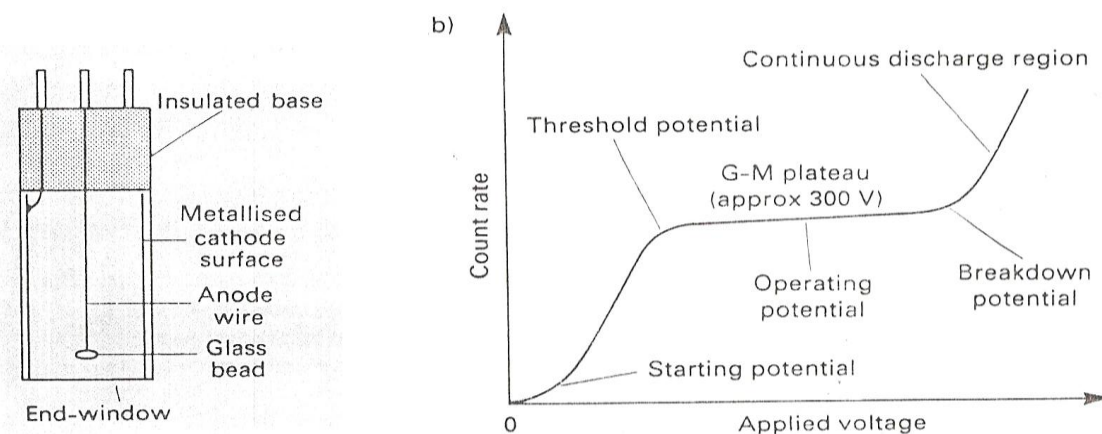


Fig. 9.4. (a) The Geiger-Müller (G-M) tube. (b) Effect of applied voltage on count rate.

The Geiger counter

A Geiger counter is the most common radiation detector based on gas ionization. This device uses a Geiger-Müller tube as the sensor. This tube is filled with an inert gas, usually argon, helium or neon with halogens added as quencher, and voltage in the chamber is maintained over 100 V. When a radioactive particle passes the tube, the gas becomes conductive and produces pulse which can be amplified by a cascade effect and outputs a magnified current or pulse, which is displayed visibly as glows of light or as audible clicks.



Fig. 9.5. A hand-held Geiger counter.

A large amount of radiation passing through the tube produces a higher reading and more clicks because of the greater amount of electric current generated inside the tube.

Geiger counters usually contain a sealed, gas-filled cylinder and a fine axial wire. The cylinder is operated at ground potential and the wire at a positive potential that is higher than that in the proportional counter. In this region, the discharge due to ionization spreads along the whole length of the wire. Total number of electrons produced does not depend on the amount of primary ionization, and all of the discharge pulses become equal in amplitude. In other words, each particle

crossing the cylinder produces a gas ionization that is roughly independent of the particle's nature and energy.

The number of pulses (clicks) over a certain time (count rate) is a quantitative measure of radiation.

The count rate is referred to as counts per minute (c.p.m.).

Geiger counters are useful for detecting the ionizing radiations: alpha, beta and gamma rays. With gamma radiation, sensitivity can be lower, so rarely used. Most hand-held Geiger counters are at their best with alpha and beta rays. The density of the gas within the tube is usually sufficient for these two rays but not for high-energy gamma rays.

These counters have a thin end-window made of aluminum and can detect β -radiation from high energy (^{32}P) and weak emitters (^{14}C) but are incapable of detecting weaker ^3H because the radiation cannot penetrate the end-window. For the same reason, they are not very efficient detectors of α -radiation.

End-window ionization counters are used for routine monitoring of the radioactive laboratory to check for contamination. They are also useful in experimental situations where the presence or absence of radioactivity needs to be known rather than the absolute quantity, for example, quick screening of radioactive gels prior to autoradiography, or checking of chromatographic fractions for labeled components.

The inability of end-window counters to detect β -emitters presents a problem in biosciences because ^3H is a commonly used radioisotope. This problem can be overcome by using a windowless counter where a gas flow is used. These instruments are cumbersome and need to be conveyed by trolleys. They are useful for mass screening of premises for ^3H contamination but are rarely used routinely. Most labs monitor for ^3H by doing a **wipe test** regularly. This test uses wet paper towels or cotton wool to take swabs for scintillation counting.

Methods based on excitation

Radioactive isotopes interact with matter in two ways - they cause (1) ionization, which forms the basis of Geiger-Müller counting, and (2) excitation, which governs scintillation counting.

Excitation of a fluorescent compound (fluor or phosphor) leads to emission of photons of light in the visible region of the electromagnetic spectrum which can be detected and counted. This process is known as **scintillation counting**.

Scintillators (fluor or phosphor) are materials which **fluoresce** (or phosphoresce) in the **presence of ionizing radiation**. Scintillation counters combine this fluorescence with a well-known phenomenon — the photoelectric effect (emission of electrons when light shines on the surface of a material), **to convert incident radiation from a radioisotope into an electrical signal and quantify it by means of a photomultiplier tube**. **Albert Einstein (1879-1955) was known for the theory of relativity and the law of photoelectric effect. A photomultiplier tube is a photoemissive device in which the absorption of a photon results in the emission of an electron.** It is useful for light detection of very weak signals.

TYPES OF SCINTILLATION COUNTING

There are two types of scintillation counting illustrated in Fig. 9.6.

1. Solid scintillation counting
2. Liquid scintillation counting

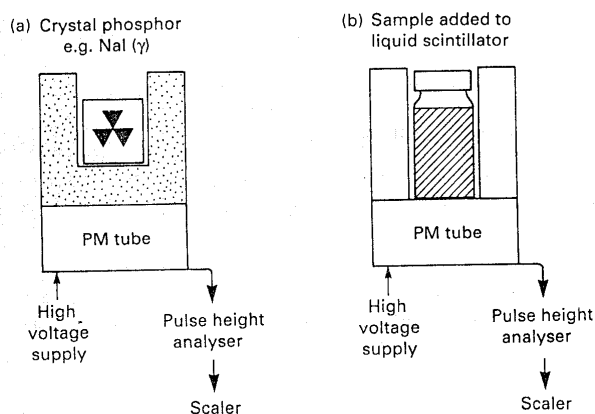


Fig. 9.6. Diagrammatic illustration of A) Solid scintillation counter. B) Liquid scintillation counter

When a radioactive particle hits the scintillator, its energy is absorbed and immediately reemitted as a photon of light of a given wavelength. The emitted photons are gathered and focused toward the photocathode, where they are absorbed and their energy is used to emit electrons via the photoelectric effect. **The photoelectrons are in turn focused toward a series of metal electrodes known as dynodes (A dynode is an electrode in an electron tube that produces electrons through secondary emission).**

Photocathodes are made of materials such as silver oxide/cesium (Ag-O-Cs), and temperature-resilient sodium-potassium-antimony (Na-K-Sb), cesium iodide, cesium terrulide)

When a photoelectron hits the first dynode, its kinetic energy is sufficient to knock several more electrons off the dynode surface via the **Auger effect**. These Auger electrons proceed through the rest of the dynode series, producing more and more electrons with each impact. Finally, the amplified electron signal is absorbed at the anode, measured, and quantified. The electrical pulse that results from the conversion of light energy to electrical energy in the photomultiplier is **directly proportional to the energy of the original radioactive event**. The advantage of this method is that two or 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. 9.6. **The source of light for the Photomultiplier is the light emitted by the scintillator. This light is directed through a glass or quartz window toward a photosensitive surface, known as a photocathode. The photocathode then releases electrons that are multiplied by electrodes known as metal channel dynodes.** At the end of the dynode chain is an anode or collection electrode. The current flowing from the anode to the scaler is directly proportional to the photoelectron flux generated by the photocathode and proportional to the energy of the original radioactive event.

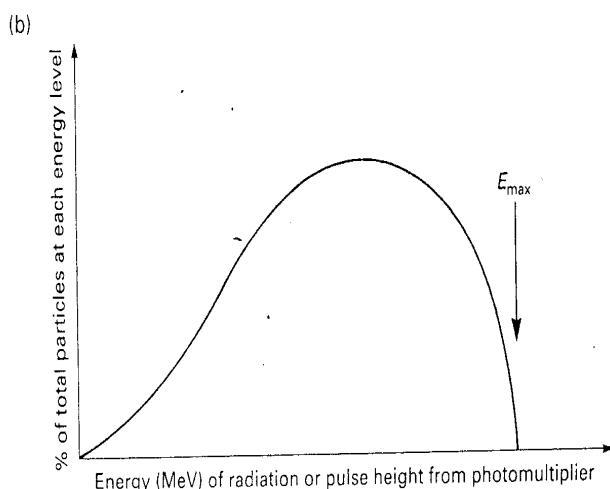
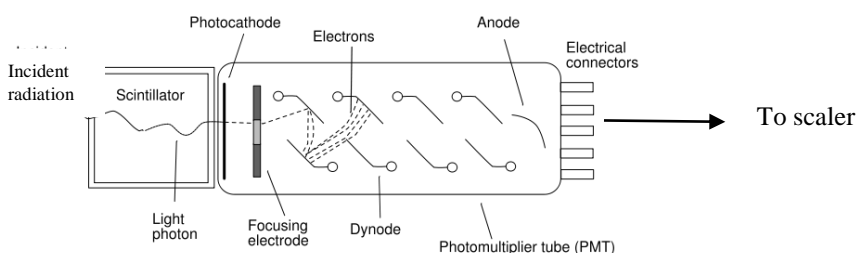


Fig. 9.6. (a) The mode of action of a photomultiplier and (b) the energy spectrum of a typical β -emitter.

Scintillation counting provides two kinds of information:

1. Quantitative: the number of scintillations is proportional to the rate of decay of the sample, i.e. the amount of radioactivity
2. Qualitative: the intensity of light given out and therefore signal from the photomultiplier is proportional to the energy of radiation

Solid scintillation counting

In solid scintillation counting, the radioactive sample is placed adjacent to a crystal of fluorescent material called a **scintillation phosphor** which is coupled to a suitable light-sensitive photomultiplier tube which in turn is connected to a high voltage supply and a scaler (Fig. 9.6). As radiation interacts with the scintillation crystal, energy is transferred to bound electrons of the crystal's atoms. These emit light, which can be amplified in a photomultiplier. The following crystals are used:

For γ -emitters - sodium iodide

For α -emitters – zinc sulphide

For β -emitters – organic scintillators such as anthracene

Two conditions are required of a scintillator: 1) must be able to absorb radiation, 2) must interact with the radiation. Medium- and low-energy beta particles cannot penetrate the crystal and, therefore, cannot interact. A thin scintillator is an excellent choice for low-energy gamma rays (in the range of 100keV) and high-energy beta particles. The ray or particle will be absorbed within the thin scintillator, interact with it to produce light, then travel through the remaining crystal thickness for onward detection. A high-energy gamma ray is likely to pass right through the thin scintillator without interacting.

A thick scintillator is the choice for radionuclides emitting high-energy gamma rays. This scintillator is thick enough to absorb the gamma ray but not too thick to prevent the light that is produced from being detected. A thick scintillator is not very good for low-energy gamma rays; they will interact but the scintillator is too thick and will absorb the light that is produced before it can be detected.

Solid scintillation counting is particularly useful for γ -emitters because they produce electromagnetic radiation and collide only rarely with neighboring atoms to cause ionization or excitation. In a crystal, the atoms are densely packed, making collisions (i.e., interactions) more likely. Conversely, solid scintillation counting is generally unsuitable for weak β -emitters such as ^3H and ^{14}C , because even the

highest energy negatrons emitted by these isotopes would have hardly sufficient energy to penetrate the walls of the counting vials in which the samples are placed for counting.

Because many of the isotopes used in radioimmunoassay are γ -emitters, solid scintillation counting is frequently used in biological work.

Liquid scintillation counting

Liquid scintillation counting (Fig. 9.5) detects radioactivity via the same type of light emission events which are used in solid scintillation. The key difference is that in liquid scintillation counting the scintillation takes place in a solution of scintillator, rather than in a solid crystal. This allows close contact between the isotope atoms and the scintillator, which is not possible with solid scintillation. With liquid scintillation counting, the short path length of soft β - emissions is not an obstacle to detection.

In this method, the sample is mixed with a scintillation cocktail containing a solvent and one or more fluors. Liquid scintillation cocktails absorb the energy emitted by radioisotopes and re-emit it as flashes of light. To accomplish these two actions of absorption and re-emission, cocktails contain two basic components, the solvent and the fluor(s). The solvent carries out the bulk of the energy absorption. The fluor, which is dissolved in the solvent, converts the absorbed energy into light. Many cocktails contain additional materials to extend their range of use to other sample compositions but the solvent and the fluor provide the scintillation of the mixture. Liquid scintillation is particularly useful in quantifying weak β -emitters such as ^3H , ^{14}C and ^{35}S , which are frequently used in biological work.