MARUDHAR KESARI JAIN COLLEGE FOR WOMEN VANIYAMBADI

PG AND RESEARCH DEPARTMENT OF BIOCHEMISTRY

B.Sc. BIOCHEMISTRY

E-NOTES

SUBJECT NAME: BIOINSTRUMENTATION

SUBJECT CODE: GECBC31

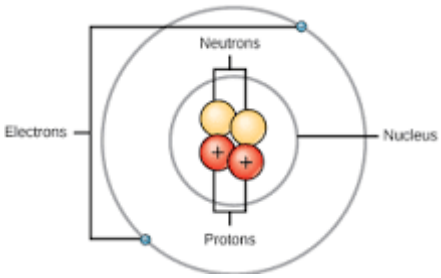
UNIT - V

RADIOACTIVITY

Atomic structure, radiation, types of radioactive decay, half life, units of radio activity. Detection and measurement of radioactivity - methods based upon ionization (GM counter), methods based upon excitation (Scintillation counter).Autoradiography. Applications of radioisotopes in the elucidation of metabolic pathways. Biological hazards of radiation and safety measures in handling radio isotopes.

Atomic structure

Atoms consist of three basic particles: protons, electrons, and neutrons. The nucleus (center) of the atom contains the protons (positively charged) and the neutrons (no charge). The outermost regions of the atom are called electron shells and contain the electrons (negatively charged).



Radioactivity

Radioactivity, property exhibited by certain types of matter of emitting energy and subatomic particles spontaneously. It is, in essence, an attribute of individual atomic nuclei.

An unstable nucleus will decompose spontaneously, or decay, into a more stable configuration but will do so only in a few specific ways by emitting certain particles or certain forms of electromagnetic energy. Radioactive decay is a property of several naturally occurring elements as well as of artificially produced isotopes of the elements. The rate at which a radioactive element decays is expressed in terms of its half-life; i.e., the time required for one-half of any given quantity of the isotope to decay. Half-lives range from more than 10^{24} years for some nuclei to less than 10^{-23} second. The product of a radioactive decay process—called the daughter of the parent isotope—may itself be unstable, in which case it, too, will decay. The process continues until a stable nuclide has been formed.

The nature of radioactive emissions

The emissions of the most common forms of spontaneous radioactive decay are the alpha (α) particle, the beta (β) particle, the gamma (γ) ray, and the neutrino. The alpha particle is actually the nucleus of a helium-4 atom, with two positive charges $^{4}/_{2}He$. Such charged atoms are called ions. The neutral helium atom has two electrons outside its nucleus balancing these two charges. Beta particles may be negatively charged (beta minus, symbol e^{-}), or positively charged (beta plus, symbol e^{+}). The beta minus [β^{-}] particle is actually an electron created in the nucleus during beta decay without any relationship to the orbital electron cloud of the atom. The beta plus particle, also called the positron, is the antiparticle of the electron; when brought together, two such particles will mutually annihilate each other. Gamma rays are electromagnetic radiations such as radio waves, light, and X-rays. Beta radioactivity also produces the neutrino and antineutrino, particles that have no charge and very little mass, symbolized by ν and ν, respectively.

In the less common forms of radioactivity, fission fragments, neutrons, or protons may be emitted. Fission fragments are themselves complex nuclei with usually between one-third and two-thirds the charge Z and mass A of the parent nucleus. Neutrons and protons are, of course, the basic building blocks of complex nuclei, having approximately unit mass on the atomic scale and having zero charge or unit positive charge, respectively. The neutron cannot long exist in the free state. It is rapidly captured by nuclei in matter; otherwise, in free space it will undergo beta-minus decay to a proton, an electron, and an antineutrino with a half-life of 12.8 minutes. The proton is the nucleus of ordinary hydrogen and is stable.

Types of radioactivity

The early work on natural radioactivity associated with uranium and thorium ores identified two distinct types of radioactivity: alpha and beta decay.

Alpha decay

In alpha decay, an energetic helium ion (alpha particle) is ejected, leaving a daughter nucleus of atomic number two less than the parent and of atomic mass number four less than the parent. An example is the decay (symbolized by an arrow) of the abundant isotope of uranium, ^{238}U, to a thorium daughter plus an alpha particle:

$$^{238}_{92}\mathrm{U} \longrightarrow {}^{234}_{90}\mathrm{Th} + {}^{4}_{2}\mathrm{He} \qquad Q_\alpha = 4.268 \text{ MeV} \qquad t_{1/2} = 4.51 \times 10^9 \text{ years}$$

Given for this and subsequent reactions are the energy released (Q) in millions of electron volts (MeV) and the half-life ($t_{1/2}$). It should be noted that in alpha decays the charges, or number of protons, shown in subscript are in balance on both sides of the arrow, as are the atomic masses, shown in superscript.

Beta-minus decay

In beta-minus decay, an energetic negative electron is emitted, producing a daughter nucleus of one higher atomic number and the same mass number. An example is the decay of the uranium daughter product thorium-234 into protactinium-234:

$$^{234}_{90}\mathrm{Th} \longrightarrow {}^{234}_{91}\mathrm{Pa} + \varepsilon^- + \bar{\nu} \qquad Q_{\beta^+} = .263 \text{ MeV} \qquad t_{1/2} = 24.1 \text{ days}$$

In the above reaction for beta decay, ν represents the antineutrino. Here, the number of protons is increased by one in the reaction, but the total charge remains the same, because an electron, with negative charge, is also created.

Gamma decay

A third type of radiation, gamma radiation, usually accompanies alpha or beta decay. Gamma rays are photons and are without rest mass or charge. Alpha or beta decay may simply proceed directly to the ground (lowest energy) state of the daughter nucleus without gamma emission, but the decay may also proceed wholly or partly to higher energy states (excited states) of the daughter. In the latter case, gamma emission may occur as the excited states transform to lower energy states of the same nucleus. (Alternatively to gamma emission, an excited nucleus may transform to a lower energy state by ejecting an electron from the cloud

surrounding the nucleus. This orbital electron ejection is known as internal conversion and gives rise to an energetic electron and often an X-ray as the atomic cloud fills in the empty orbital of the ejected electron. The ratio of internal conversion to the alternative gamma emission is called the internal-conversion coefficient.)

Isomeric transitions

There is a wide range of rates of half-lives for the gamma-emission process. Usually dipole transitions (see below Gamma transition), in which the gamma ray carries off one $\hbar$ unit of angular momentum, are fast, less than nanoseconds (one nanosecond equals 10^{-9} second). The law of conservation of angular momentum requires that the sum of angular momenta of the radiation and daughter nucleus is equal to the angular momentum (spin) of the parent. If the spins of initial and final states differ by more than one, dipole radiation is forbidden, and gamma emission must proceed more slowly by a higher multipole (quadrupole, octupole, etc.) gamma transition. If the gamma-emission half-life exceeds about one nanosecond, the excited nucleus is said to be in a metastable, or isomeric, state (the names for a long-lived excited state), and it is customary to classify the decay as another type of radioactivity, an isomeric transition. An example of isomerism is found in the protactinium-234 nucleus of the uranium-238 decay chain:

$$^{234m}_{91}\mathrm{Pa} \longrightarrow {}^{234}_{91}\mathrm{Pa} + \gamma \qquad Q_\gamma = 0.0698\ \mathrm{MeV},\quad t_{1/2} = 1.17\ \mathrm{min}$$

The letter m following the mass number stands for metastable and indicates a nuclear isomer.

Beta-plus decay

During the 1930s new types of radioactivity were found among the artificial products of nuclear reactions: beta-plus decay, or positron emission, and electron capture. In beta-plus decay an energetic positron is created and emitted, along with a neutrino, and the nucleus transforms to a daughter, lower by one in atomic number and the same in mass number. For instance, carbon-11 (Z = 6) decays to boron-11 (Z = 5), plus one positron and one neutrino:

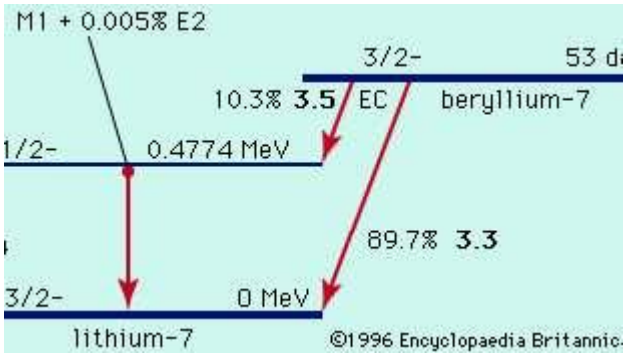
$$^{11}_{6}\mathrm{C} \longrightarrow {}^{11}_{5}\mathrm{B} + \varepsilon^+ + \nu \qquad Q_{\beta^+} = 0.97\ \mathrm{MeV},\quad t_{1/2} = 20.4\ \mathrm{min}$$

Electron capture

Electron capture (EC) is a process in which decay follows the capture by the nucleus of an orbital electron. It is similar to positron decay in that the nucleus transforms to a daughter of one lower atomic number. It differs in that an orbital electron from the cloud is captured by the nucleus with subsequent emission of an atomic X-ray as the orbital vacancy is filled by an electron from the cloud about the nucleus. An example is the nucleus of beryllium-7 capturing one of its inner electrons to give lithium-7:

$$^{7}_{4}\mathrm{Be} + e^{-} \longrightarrow {}^{7}_{3}\mathrm{Li} + \nu \qquad Q_{\mathrm{EC}} = 0.8616\ \mathrm{MeV} \qquad t_{1/2} = 53\ \mathrm{days}$$

The main features of radioactive decay of a nuclear species are often displayed in a decay scheme. Figure 1 shows the decay scheme of beryllium-7. Indicated are the half-life of the parent and that of the excited daughter state, as well as its energy 0.4774 MeV. The spins and parities of all three states are provided on the upper left-hand side of the level. The multipolarity of the gamma ray (magnetic dipole, M1, plus 0.005 percent electric quadrupole, E2) is indicated above the vertical arrow symbolizing the gamma transition. The slanted arrows symbolize the electron-capture decay with labels giving the percentage of decay directly to ground state (89.7 percent) and the percentage of EC decay going via the excited state (10.3 percent). The boldface numbers following the percentages are so-called log ft values, to be encountered below in connection with beta-decay rates. The overall energy release, Q_{EC}, is indicated below. The Q_{EC} is necessarily a calculated value because there is no general practical means of measuring the neutrino energies accompanying EC decay. With a few electron-capturing nuclides, it has been possible to measure directly the decay energy by measurement of a rare process called inner bremsstrahlung (braking radiation). In this process the energy release is shared between the neutrino and a gamma ray. The measured distribution of gamma-ray energies indicates the total energy release. Usually there is so much ordinary gamma radiation with radioactive decay that the inner bremsstrahlung is unobservable.



decay of beryllium-7

Figure 1: Radioactive decay of beryllium-7 to lithium-7 by electron capture (EC; see text). Encyclopædia Britannica, Inc.

Spontaneous fission

Yet another type of radioactivity is spontaneous fission. In this process the nucleus splits into two fragment nuclei of roughly half the mass of the parent. This process is only barely detectable in competition with the more prevalent alpha decay for uranium, but for some of the heaviest artificial nuclei, such as fermium-256, spontaneous fission becomes the predominant mode of radioactive decay. Kinetic-energy releases from 150 to 200 MeV may occur as the fragments are accelerated apart by the large electrical repulsion between their nuclear charges. The reaction is as follows:

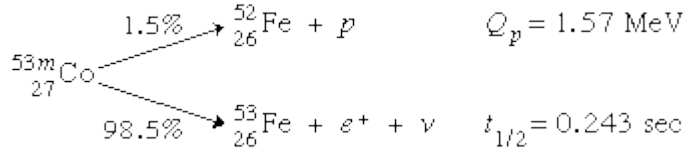
$$^{256}_{100}\mathrm{Fm} \longrightarrow {}^{140}_{54}\mathrm{Xe} + {}^{112}_{46}\mathrm{Pd} + 4n \qquad Q = 150\text{–}200\ \mathrm{MeV}$$

$$+ \text{ other fission products} \qquad t_{1/2} = 2.7\ \text{hours}$$

Only one of several product sets is shown. A few neutrons are always emitted in fission of this isotope, a feature essential to chain reactions. Spontaneous fission is not to be confused with induced fission, the process involved in nuclear reactors. Induced fisson is a property of uranium-235, plutonium-239, and other isotopes to undergo fission after absorption of a slow neutron. Other than the requirement of a neutron capture to initiate it, induced fission is quite similar to spontaneous fission regarding total energy release, numbers of secondary neutrons, and so on (see nuclear fission).

Proton radioactivity

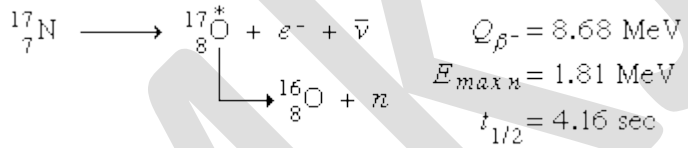
Proton radioactivity, discovered in 1970, is exhibited by an excited isomeric state of cobalt-53, ^{53m}Co, 1.5 percent of which emits protons:



Special beta-decay processes

In addition to the above types of radioactivity, there is a special class of rare beta-decay processes that gives rise to heavy-particle emission. In these processes the beta decay partly goes to a high excited state of the daughter nucleus, and this state rapidly emits a heavy particle.

One such process is beta-delayed neutron emission, which is exemplified by the following

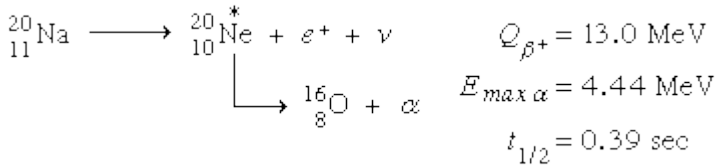


reaction:

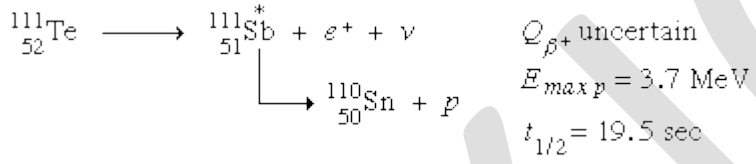
(Note: the asterisk denotes the short-lived intermediate excited states of oxygen-17, and $E_{max\ n}$ denotes the maximum energy observed for emitted neutrons.) There is a small production of delayed neutron emitters following nuclear fission, and these radioactivities are especially important in providing a reasonable response time to allow control of nuclear fission reactors by mechanically moved control rods.

Among the positron emitters in the light-element region, a number beta decay partly to excited states that are unstable with respect to emission of an alpha particle. Thus, these species exhibit alpha radiation with the half-life of the beta emission. Both the positron decay from boron-8 and electron decay from lithium-8 are beta-delayed alpha emission, because ground as well as excited states of beryllium-8 are unstable with respect to breakup into two alpha particles. Another example, sodium-20 (^{20}Na) to give successively neon-20 (^{20}Ne; the

asterisk again indicating the short-lived intermediate state) and finally oxygen-16 is listed below:



In a few cases, positron decay leads to an excited nuclear state not able to bind a proton. In these cases, proton radiation appears with the half-life of the beta transition. The combination of high positron-decay energy and low proton-binding energy in the daughter ground state is required. In the example given below, tellurium-111 (^{111}Te) yields antimony-111 (^{111}Sb) and then tin-110 (^{110}Sn) successively:



Heavy-ion radioactivity

In 1980 A. Sandulescu, D.N. Poenaru, and W. Greiner described calculations indicating the possibility of a new type of decay of heavy nuclei intermediate between alpha decay and spontaneous fission. The first observation of heavy-ion radioactivity was that of a 30-MeV, carbon-14 emission from radium-223 by H.J. Rose and G.A. Jones in 1984. The ratio of carbon-14 decay to alpha decay is about 5×10^{-10}. Observations also have been made of carbon-14 from radium-222, radium-224, and radium-226, as well as neon-24 from thorium-230, protactinium-231, and uranium-232. Such heavy-ion radioactivity, like alpha decay and spontaneous fission, involves quantum-mechanical tunneling through the potential-energy barrier. Shell effects play a major role in this phenomenon, and in all cases observed to date the heavy partner of carbon-14 or neon-24 is close to doubly magic lead-208 (see below Nuclear models).

Occurrence of radioactivity

Some species of radioactivity occur naturally on Earth. A few species have half-lives comparable to the age of the elements (about 6×10^9 years), so that they have not decayed away after their formation in stars. Notable among these are uranium-238, uranium-235, and

thorium-232. Also, there is potassium-40, the chief source of irradiation of the body through its presence in potassium of tissue. Of lesser significance are the beta emitters vanadium-50, rubidium-87, indium-115, tellurium-123, lanthanum-138, lutetium-176, and rhenium-187, and the alpha emitters cerium-142, neodymium-144, samarium-147, gadolinium-152, dysprosium-156, hafnium-174, platinum-190, and lead-204. Besides these approximately 10^9-year species, there are the shorter-lived daughter activities fed by one or another of the above species; e.g., by various nuclei of the elements between lead (Z = 82) and thorium (Z = 90).

Another category of natural radioactivity includes species produced in the upper atmosphere by cosmic ray bombardment. Notable are 5,720-year carbon-14 and 12.3-year tritium (hydrogen-3), 53-day beryllium-7, and 2,700,000-year beryllium-10. Meteorites are found to contain additional small amounts of radioactivity, the result of cosmic ray bombardments during their history outside the Earth's atmospheric shield. Activities as short-lived as 35-day argon-37 have been measured in fresh falls of meteorites. Nuclear explosions since 1945 have injected additional radioactivities into the environment, consisting of both nuclear fission products and secondary products formed by the action of neutrons from nuclear weapons on surrounding matter.

The fission products encompass most of the known beta emitters in the mass region 75–160. They are formed in varying yields, rising to maxima of about 7 percent per fission in the mass region 92–102 (light peak of the fission yield versus atomic mass curve) and 134–144 (heavy peak). Two kinds of delayed hazards caused by radioactivity are recognized. First, the general radiation level is raised by fallout settling to Earth. Protection can be provided by concrete or earth shielding until the activity has decayed to a sufficiently low level. Second, ingestion or inhalation of even low levels of certain radioactive species can pose a special hazard, depending on the half-life, nature of radiations, and chemical behaviour within the body. For a detailed discussion on the biological effects of radiation, see radiation: Biological effects of ionizing radiation.

Detection and measurement of radioactivity

Units of Radiation Measurement

Activity (Unit: Curie)

The Curie (Ci) is defined as the activity of that quantity of radioactive material in which the number of disintegrations per second is 3.7E10 (a number nearly the same as the number of disintegrations per second from 1 gram of radium).

Since a Curie is a large amount of radioactivity sub-units of a Curie, a millicurie (mCi, 1E-3 Curie) or microcurie (μCi, 1E-6 Curie), are commonly used to express the amount of activity.

Exposure (Unit: Roentgen)

The Roentgen (R) is defined as 2.58E-4 coulomb/kg air. This unit is special in that it is defined only for X or gamma radiation in air.

Absorbed Dose (Unit: rad)

The rad is the special unit of absorbed energy. It is defined as that amount of ionizing radiation that deposits 100 ergs/gram of material. For most applications, it can be assumed that
1 Roentgen = 1 rad.

Dose Equivalent (Unit: rem)

The rem is the unit of dose equivalent. The dose equivalent accounts for the difference in biological effectiveness of different types of radiation. It is the product of the absorbed dose (rad) times the quality factor (QF) of the radiation. The QF for x, gamma, and beta radiation is 1, for alpha radiation 20, and varies with energy from 2-11 for neutrons.

GM counter

Introduction

When radioactive isotopes are used in medical research work particularly in human subjects it is very important that the amount of radioactive material given is as small as possible, in

order that there should be minimum harmful radiations. Hence a very sensitive instrument is necessary to measure the radioactivity of materials.

Geiger and Muller developed a 'Particle detector' for measuring 'ionizing radiation' in 1928. They named it as 'Geiger Muller Counter'. Ever since then it has been one of the most widely used nuclear detectors in the developmental days of Nuclear physics. The particle detector developed by Geiger and Muller is a gas filled counter. The main difference between 'proportional counter' and 'Geiger-Muller Counter' is in the formation of the avalanche. In the proportional counter, the avalanche is formed only at a point whereas in Geiger-Muller Counter it is formed in the central wire. Therefore, in GM Counter amplification is independent of initial ionization produced by the ionizing particle.

Geiger counter is also called as Geiger tube. This instrument is actually used for detecting and measuring ionizing radiation like alpha particles, beta particles, and gamma rays. A Geiger-Müller counter can count individual particles at rates up to about 10,000 per second and is used widely in medicine and in prospecting for radioactive ores.

Construction of Geiger-Muller counter

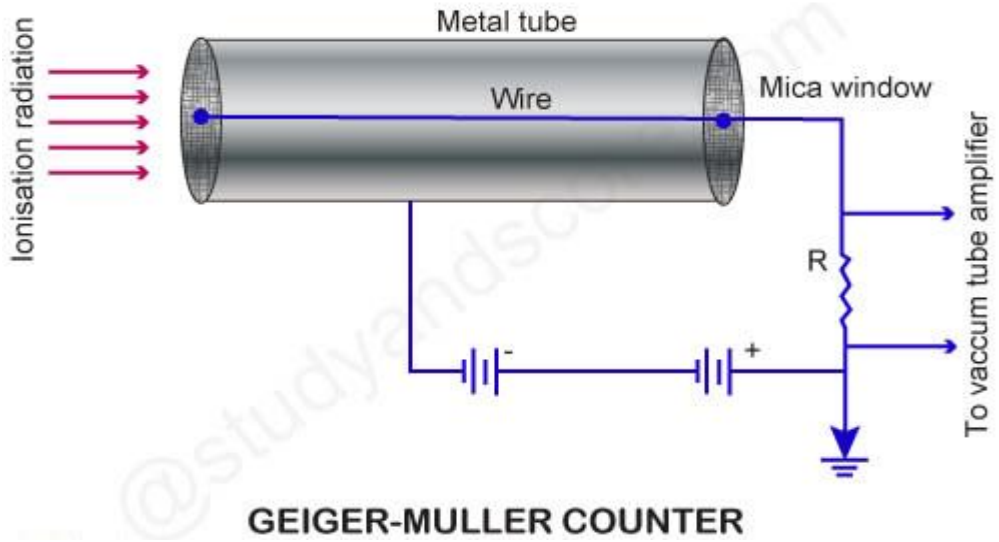
- It consists of a hollow metal case enclosed in a thin glass tube. This hollow metal case acts as a cathode.
- A fine tungsten wire is stretched along the axis of the tube and is insulated by ebonite plugs. This fine tungsten wire acts as anode.
- The tube is evacuated and then partially filled with a mixture of 90% argon at 10 cm pressure and 10% ethyl alcohol vapours at 1cm pressure. sten
- The fine tungsten wire is connected to positive terminal of a high tension battery through a resistance R and the negative terminal is connected to the metal tube.
- The direct current voltage is kept slightly less than that which will cause a discharge between the electrodes.
- At one end of the tube a thin window of mica is arranged to allow the entry of radiation into the tube.

Principle of Geiger-Muller counter

The basic principle of the Geiger Muller counter can be understood as follows. When an ionizing particle passes through the gas in an ionizing chamber, it produces a few ions. If the applied potential difference is strong enough, these ions will produce a secondary ion avalanche whose total effect will be proportional to the energy associated with the primary ionizing event.

If the applied potential difference is very high, the secondary ionization phenomenon becomes so dominant that the primary ionizing event loses its importance. In other words, the size of the final pulse produced depends only on the triggering off of ionization by an ionizing particle but independent of the energy of this particle.

A high energy particle entering through the mica window will cause one or more of the argon atoms to ionize. The electrons and ions of argon thus produced cause other argon atoms to ionize in a cascade effect. The result of this one event is sudden, massive electrical discharge that causes a current pulse. The current through R produces a voltage pulse of the order of 10μV. An electron pulse amplifier accepts the small pulse voltage and amplifies them to about 5 to 50 V. The amplified output is then applied to a counter. As each incoming particle produces a pulse, the number of incoming particles can be counted.



GEIGER-MULLER COUNTER

Working of Geiger-Muller counter

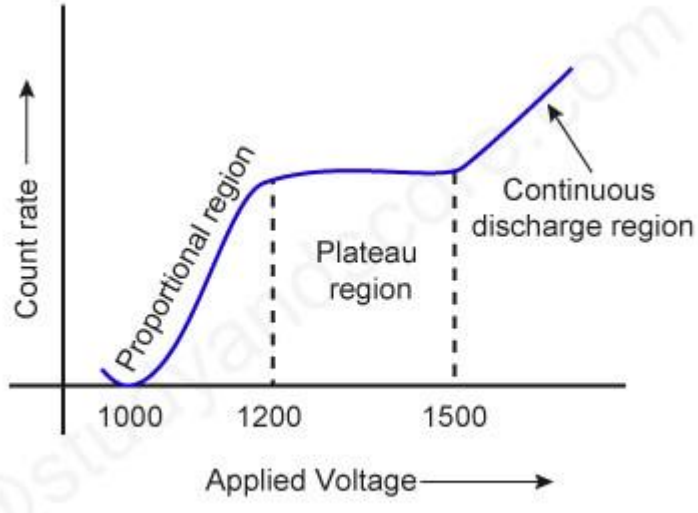
The tube is filled with Argon gas, and around voltage of +400 Volts is applied to the thin wire in the middle. When a particle arrives into the tube, it takes an electron from Argon atom. The electron is attracted to the central wire and as it rushes towards the wire, the electron will knock other electrons from Argon atoms, causing an "avalanche". Thus one single incoming particle will cause many electrons to arrive at the wire, creating a pulse which can be amplified and counted. This gives us a very sensitive detector.

Plateau graph of Geiger Muller counter

There is a threshold below which the tube doesn't work. This can be several hundred volts. After this, the number of pulses is proportional to the voltage. This region is known as proportional region.

If the applied voltage is increased further, then a point will be reached after which the count rate remains constant over a certain region. This region is known as plateau region or Geiger region. This region is used for Geiger Muller operation.

Beyond the plateau region the applied electric field is so high that a continuous discharge takes place in the tube and the count rate increases very rapidly. It does not require any ionization event to happen so that the tube must not be used in this region.



GEIGER-MULLER COUNTER: PLATEAU GRAPH

The Geiger Muller counter can account for about 500 particles per sec ond. The GM counter will not register those particles that pass through it in the dead time. Dead time refers to the time taken by the tube to recover between counts. It requires about 200 μs for the tube to recover. If lot of particles enter the GM tube at a rapid rate, the tube will not have time to recover and some particles may not be counted.

The efficiency of the counter is defined as the ration of the observed counts per second to the number of ionizing particles entering the counter per second. Counting efficiency is defined as the ability of counting of the GM counter.

Counting efficiency, $\eta = 1- \exp(spl)$

Where,

s = specific ionization at one atmosphere

p= pressure in atmosphere

l = path length of the ionization particle in the counter

Precautionary measures while operating

- The operating voltage must correspond to the midpoint of flat plateau region of plateau graph.
- If the continuous discharge is produced, the voltage should be lowered.
- The applied voltage must be relatively stabilized.
- Introduction of light should be prevented to avoid photo electric effect.

Applications of Geiger Muller counter

Geiger counters have many applications in radioactivity detection. Here are few of the examples:

1. To detect radioactive rocks and minerals in the course of mineral prospecting.

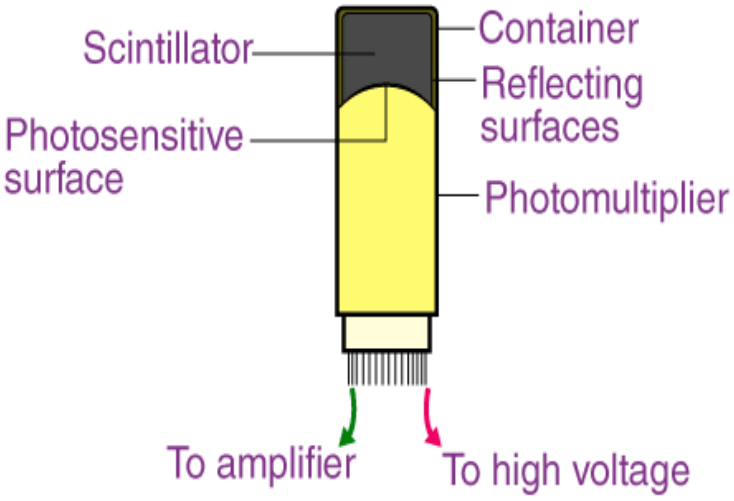
2. For Fire responders for making an initial determination of radiation risk.
3. For Hazard Management personnel in checking for radiation danger in an emergency situation.
4. To check for environmental levels of radioactivity near a nuclear power facility.
5. To test for danger amidst a nuclear accident or leakage of radioactive coolant.
6. To check for radioactive contamination of clothing and shoes in your workplace.
7. Radiation detection in the scrap metal processing business.
8. To check possible leakage or exposure to X-rays in a medical facility
9. To check for radiation in areas where depleted uranium ammunition shells have been used.
10. To check for irradiated gemstones in the jewellery trade.
11. To check the levels of iodine 131 in cancer patients undergoing radiation therapy.
12. You are in close proximity to a uranium mine and want to test the soil and environment for dangerous levels of radioactivity.
13. To test for radioactive contamination of food.
14. To check materials in your anthropology or archaeology field.
15. To check for radioactivity in metal objects in your home or office that could be made of recycled radioactive materials.

It is important to note that Geiger-Muller counter does not detect the following,

- Neutron radiation
- Microwave radiation
- Radon gas
- Laser energy

Scintillation Counter

Scintillation Counter is an instrument that is used for measuring ionizing radiation. “It comprises the scintillator that generates photons in response to incident radiation”, a PMT tube is used to convert an electronics and electric signal to process the signal.



A scintillation counter is used to detect gamma rays and the presence of a particle. It can also measure the radiation in the scintillating medium, the energy loss, or the energy gain. The medium can either be gaseous, liquid, or a solid. The scintillator counter is generally comprised of transparent crystalline material such as glasses, liquids, or plastics. One sector of the scintillators is placed (optical contact) with the pin code.

A charged particle loses energy when passing through the scintillator thus leaving the trail of excited molecules and atoms. A rapid interatomic transfer of electronic excitation energy follows, which leads to the burst of scintillator material luminescence characteristics. The scintillation response, when a particle stops leading to the light output. The energy loss of a particle is measured when a particle passes completely through a scintillator.

Applications of Scintillation Counter

1. Scintillation Counters are widely used in radioactive contamination, radiation survey meters, radiometric assay, nuclear plant safety, and medical imaging, that are used to measure radiation.
2. There are several counters of mounted on helicopters and some pickup trucks for rapid response in case of a security situation due to radioactive waste or dirty bombs.
3. Scintillation counters designed for weighbridge applications, freight terminals, scrap metal yards, border security, contamination monitoring of nuclear waste, and ports.

4. It is widely used in screening technologies, In vivo and ELISA alternative technologies, cancer research, epigenetics, and Cellular research.
5. It also has its applications in Protein interaction and detection, academic research, and Pharmaceutical.
6. Liquid Scintillation Counter is a type of scintillation counter that is used for measuring the beta emission from the nuclides.

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Autoradiography. Applications of radioisotopes

(A) Investigating Aspects of Metabolism:

1. Metabolic Pathways:

Radioisotopes are frequently used for tracing metabolic pathways. This usually involves adding a radioactive substrate, taking samples of the experimental material at various times, extracting and chromatographically or otherwise separating the products.

Radioactivity detectors can be attached to gas liquid chromatography or high performance liquid chromatography columns to monitor radioactivity coming off the column during separation.

Alternatively, radioactivity can be located on paper or thin-layer chromatography with either a Geiger-Muller chromatograph scanner or with autoradiography. If it is suspected that a particular compound is metabolized by a pathway, then radioisotope can also be used to confirm this. For instance, it is possible to predict the fate of individual carbon atoms of [^{14}C] acetate through the tricarboxylic cycle, or Krebs cycle. Methods have been developed whereby intermediates can be ascertained. This is the so-called specific label ling pattern. Should the actual pattern coincide with the theoretical pattern, then this is a very good evidence for the mode of operation of the Krebs cycle.

Another example of the use of radioisotopes to confirm the mode of operation, or other-wise, of a metabolic pathway is in studies carried out on glucose catabolism. There are numerous

ways whereby glucose can be oxidized, the two most important ones in aerobic organisms being glycolysis followed by Krebs cycle together with the pentose phosphate pathway.

Frequently, organisms or tissues posses the necessary enzymes for both pathways to occur and it is of interest to establish the relative contribution of each to glucose oxidation. Both pathways involve the complete oxidation of glucose to carbon dioxide, but the origin of the carbon dioxide in terms of the six carbon atoms of glucose is different (at least in the initial stages of respiration of exogenously added substrate), thus it is possible to trap the carbon dioxide evolved during the respiration of specifically labelled glucose (e.g., [$^{6\text{-}14}$C] glucose or [$^{6\text{-}14}$C] glucose in which only the C-6 atom is radioactive) and obtain an evaluation of the contribution of each pathway to glucose oxidation. The use of radioisotopes in studying the operation of Krebs cycle or in evaluating the pathway of glucose catabolism are just two examples of how such isotopes can be used to confirm metabolic pathways.

2. Metabolic Turnover Times:

Radioisotopes provide a convenient method of ascertaining turnover times for particular compounds. As an example, the turnover of proteins in rats will be considered. A group of rats is injected with a radioactive amino acid and left for 24 h, during which time most of the amino acid is assimilated into proteins.

The rats are then killed at suitable time intervals and radioactivity in organs or tissues of interest is determined. In this way it is possible to ascertain the rate of metabolic turnover of protein. Using this sort of method, it has been shown that liver protein is turned over in 7-14 days, while skin and muscle proteins are turned over every 8-12 weeks, and collagen is turned over at a rate of less than 10% per annum.

3. Studies of Absorption, Accumulation and Translocation:

Radioisotopes have been very widely used in this study of the mechanisms and rates of absorption, accumulation and translocation of inorganic and organic compounds by both plants and animal. Such experiments are generally simple to perform and can also yield evidence on the route of translocation and sites of accumulation of molecules of biological interest.

4. Pharmacological Studies:

Another field where radioisotopes are widely used is in the development of new drugs. This is a particularly complicated process, because, besides showing whether a drug has a desirable effect, much more must be ascertained before it can be used in the treatment of clinic al conditions. For instance, the site of drug accumulation, the rate of accumulation, the rate of metabolism and the metabolic products must all be determined.

In each of these areas of study, radiotracers are extreme v useful, if not indispensable. For instance, autoradiography on whole sections of experimental anima yields information on the site and rate of accumulation, while typical techniques used in metabolic studies can be used to follow the rate and products of metabolism.

(B) Analytical Applications:

1. Enzyme and Ligand Binding Studies:

Virtually any enzyme reaction can be assayed using radiotracer methods provided that a radioactive form of the substrate is available. Radiotracer-based enzyme assays are more expensive than other methods, but frequently have the advantage of a higher degree of sensitivity. Radioisotopes have also been used in the study of the mechanism of enzyme action and in the studies of ligand binding to membrane receptors.

2. Isotope Dilution Analysis:

There are many compounds present in living organisms that cannot be accurately assayed by conventional means because they are present in such low amounts and in mixtures of similar compounds. Isotope dilution analysis offers a convenient and accurate way of overcoming this problem and avoids the necessity of quantitative isolation.

For instance, if the amount of iron in a protein preparation is to be determined, this may be difficult using normal methods, but it can also be done if a source of ^{59}Fe is available. This is mixed with the protein and a sample of iron is subsequently isolated, assayed for total iron and the radioactivity is determined.

If the original specific activity was 10000 d.p.m. $(10\ mg)^{-1}$ and the specific activity of the isolated iron was 9000 d.p.m $(10\ mg)^{-1}$, then the difference is due to the iron in the protein (x), i.e.,

$9000/1 = 10000/10+x$

$x = 1.1$ mg.

This technique is widely used, for instance, in studies on trace elements.

3. Radioimmunoassay:

One of the most significant advances in biochemical technique in recent years has been the development of the radioimmunoassay.

4. Radio Dating:

A quite different analytical use for radioisotopes is in the dating (i.e., determining the age) of rocks, fossils and sediments. In this technique it is assumed that the proportion of an element that is naturally radioactive has been the same throughout time. From the time of fossilization or deposition the radioactive isotope will decay.

By determining the amount of radioisotope remaining (or by examining the amount of the decay product) and from the knowledge of the half-life, it is possible to date the sample. For instance, if the radioisotope normally comprises 1% of the element and it is found that the sample actually contains 0.25%, then two half-lives can be assumed to have elapsed since deposition. If the half-life is one million years then the sample can be dated as being two million years old.

(C) Other Applications:

1. Molecular Biology Techniques:

Recent advances in molecular biology that have led to advances in genetic manipulation have dependent heavily upon use of radioisotope in DNA and RNA sequencing, DNA replication, transcription, synthesis of complementary DNA, recombinant DNA technology and many

similar studies. Many of these techniques are more fully discussed in other chapters of the book.

2. Clinical Diagnosis:

Radioisotopes are very widely used in medicine, in particular for diagnostic tests. Lung function tests routinely made using xenon-133 (^{133}Xe) are particularly useful in diagnosis of malfunctions of lung ventilation. Kidney function tests using [133] iodohippuric acid are used in diagnoses of kidney infection, kidney blockages or imbalance of function between the two kidneys. Various aspects of hematology are also studied by using radioisotopes. These include such aspects as blood cell lifetimes, blood volumes and blood circulation times, all of which may vary in particular clinical conditions.

3. Ecological Studies:

The bulk of radiotracer work is carried out in biochemical, clinical or pharmacological laboratories; nevertheless, radiotracers are also of use to ecologists. In particular, migratory patterns and behaviour patterns of many animals can be monitored using radiotracers. Another ecological application is in the examination of food chains where the primary producers can be made radioactive and the path of radioactivity followed throughout the resulting food chain.

4. Sterilization of Food and Equipment:

Very strong y-emitters are now widely used in the food industry for sterilization of pre-packed foods such as milk and meats. Normally either ^{60}Co or ^{137}Ce is used, but care has to be taken in some cases to ensure that the food product itself is not affected in any way.

Thus doses often have to be reduced to an extent where sterilization is not complete but nevertheless food spoilage can be greatly reduced. ^{60}Co and ^{137}Ce are also used in sterilization of plastic disposable equipment such as Petridishes and syringes, and in sterilization of drugs that are administered by injection.

5. Mutagens:

Radioisotopes may cause mutations, particularly in micro-organisms. In various microbiological studies mutants are desirable, especially in industrial microbiology. For instance, developments of new strains of a micro-organism that produce higher yields of a desired microbial product frequently involve mutagenesis by radioisotopes.

Precautions for handling radioactive substances

General principle

The following guidelines provide information on the safe handling of radioactive substances. They are based on the relevant legislation and on the Code of Practice for Handling Radioactive Substances.

Practical procedure

- Each experiment with radioactive substances should be reported to the HSE Department in advance (see also "Procedures for handling ionizing radiation"). Once an overall description of the experiment has been provided, the HSE Department will provide specific guidelines for workplace safety, health protection and environmental protection. These guidelines should be strictly observed.
- The radioactive substances used should comply with the following characteristics:
    - radiotoxicity must be as low as possible.
    - short-living isotopes are preferred to long-living ones
    - the amounts used must be kept to a minimum.
- Never work alone in a radioactive lab, especially not outside normal working hours. Always make sure to have someone nearby in case of emergency.
- Take all precautions to prevent radioactive contamination:
    - always separate radioactive activities from non-radioactive activities.
    - as far as possible, limit the area where radioactive substances are used and mark the area, e.g. by using containers with absorbent paper.
    - Apply a radiation symbol to any containers and items that have come into contact with radioactive substances.
    - never bring documents such as notes into the radioactive zone.

- When handling radioactive materials, always wear the appropriate protective clothing:
    - wear a lab coat. If there is a risk of serious contamination, wear disposable clothing. Store your lab coat away from your regular clothes.
    - always wear gloves when handling radioactive substances. Regularly check the radiation level of these gloves . Never touch anything with potentially contaminated gloves; use paper tissues instead.
    - wear shoe covers in rooms where the floor may be contaminated.
    - keep personal items such as handbags, etc., outside the lab.
- Use appropriate radiation shields. Return the stock solution to storage immediately after removing the amount needed.
- To avoid internal contamination, strict hygiene is essential when handling radioactive materials
    - Eating, smoking, drinking, and applying cosmetics are prohibited in radioactive labs.
    - Never pipette by mouth. Use pipetting devices instead.
    - Wash your hands thoroughly when you leave the lab.
- Regularly check the radiation level of your working area and all objects used, or at least at the end of each working day. Replace contaminated absorption paper. Decontaminate contaminated objects.
- Dispose of all radioactive waste in the appropriate containers. Limit the amount of waste to a bare minimum. Separate short-living and long-living radioactive waste.
- In case of an incident involving radioactive materials (e.g. spills), always remain in the room unless injured. Alert your HSE special contact person radioprotection, who will in turn contact the HSE Department.

  Incidents outside normal working must be reported using the general emergency number (016 32) .22.22. Specify clearly that the incident involves radioactive materials.

  Clean up any radioactive spills as thoroughly as possible.