Radioactive techniques

It is also known as Radioisotopic techniques
Radioactive is a spontaneous disintegration process of unstable isotopes nucleus. Isotopes are different forms of atom having identical no. of protons & different no. of neutrons. eg Hydrogen occurs threat of hydrogen forms deuterium & tritium consisting of identical no. of protons.

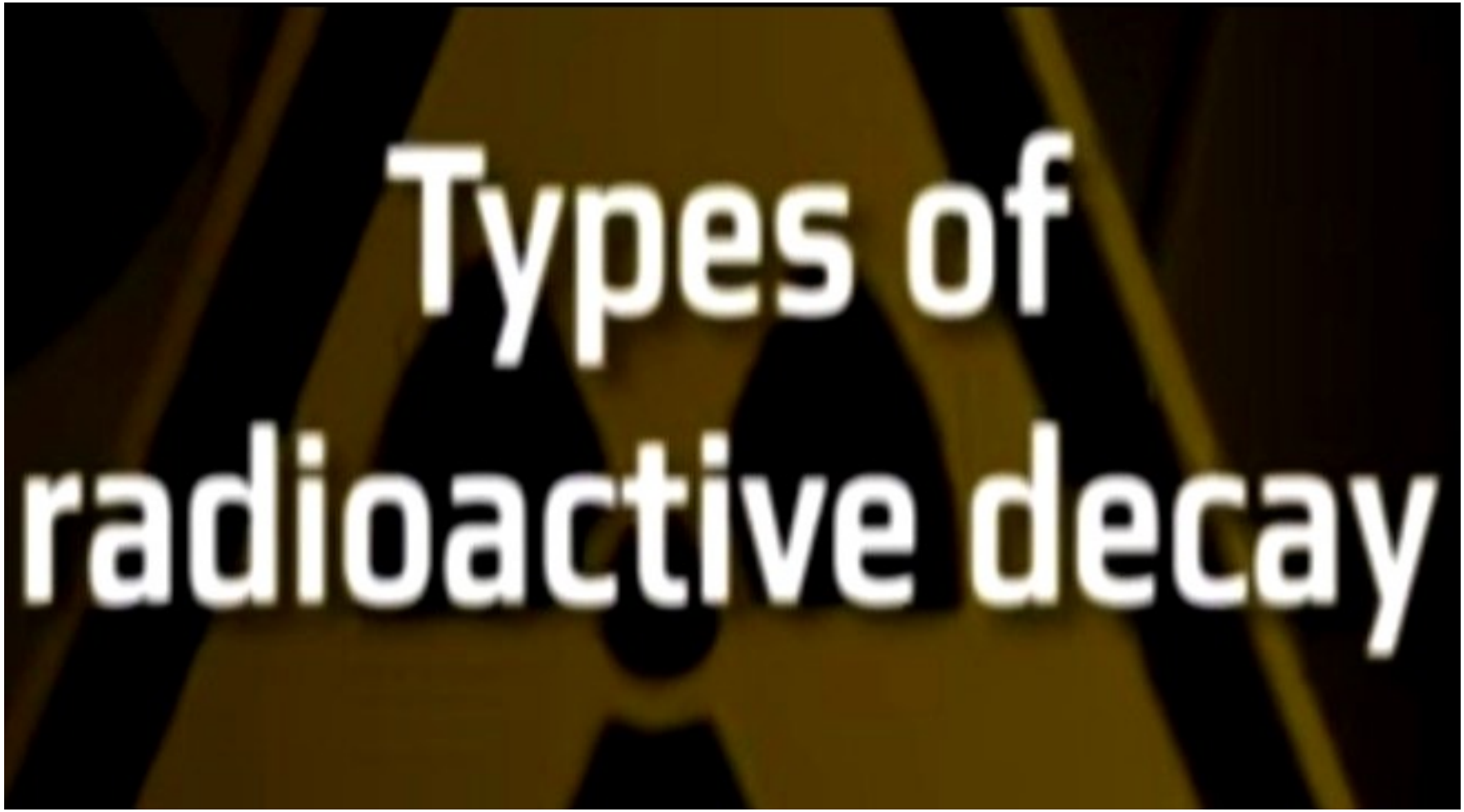
Radio isotopes are the unstable isotopes.

Types of radioactive decay

(I) Decay by negatron emission —

⇒ A neutron is converted to proton by ejection of negatively charged beta (β) - partical called negatron (β^-)

neutron → proton + negatron


Types of
radioactive decay

1. Decay by negatron emission

- A neutron is converted to a proton by the ejection of a negatively charged beta (β) particle called a **negatron** (β-)

$$\text{Neutron} \rightarrow \text{proton} + \text{negatron}$$

The nucleus loses a neutron but gains a proton and the mass number, A, remains constant.

• 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, are powerful tools in molecular biology when used as nucleic acid labels.

2. Decay by positron emission

- Positron emitters are detected by the same instruments used to detect γ-radiation.
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- 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°C 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.

3. 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 (4He2þ). 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.

3. Decay by alpha particle emission

- 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.

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

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

4. Electron capture

- In this form of decay a proton captures an **electron orbiting in the innermost K shell.**

$$\text{proton} + \text{electron} \rightarrow \text{neutron} + \text{X-ray}$$

4. Electron capture

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

$$^{125}_{53}\mathrm{I} \rightarrow {}^{125}_{52}\mathrm{Te} + \text{X-ray}$$

5. 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. The toxicity of γ -radiation is similar to that of X-rays.

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

Units of radioactivity

- **Becquerel (Bq):** This is defined as one disintegration per second (1 d.p.s.). It is the S.I. unit for radioactivity.

• **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 (μCi) 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.

1. Methods based upon gas ionisation

- The Geiger–Muller counter 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.

- Ionisation counters are used for routine monitoring of the 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, checking that a labelled DNA probe is where you think it is (and not down the sink!) or checking chromatographic fractions for labelled components.

2. Methods based upon excitation

- 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.

- Types of scintillation counting

i) Solid scintillation counting:

The sample is placed adjacent to a solid fluor (e.g. sodium iodide). Solid scintillation counting is particularly useful for -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, or larger bench-top machines with a well-shaped fluor designed to automatically count many samples.

1, 4-bis (5-phenyloxazol-2-yl) benzene (nicknamed POPOP, pronounced as it reads: 'pop op') or 2-(40-t-butylphenyl) -5-(400- 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.

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

- 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.

3. Methods based upon exposure of photographic emulsions

- Ionising radiation acts upon a photographic emulsion or film to produce a latent image much as does visible light. This is called autoradiography.

- The emulsion or film contains silver halide crystals. As energy from the radioactive material is dissipated the silver halide becomes negatively charged and is reduced to metallic silver, thus forming a particulate latent image. Photographic developers show these silver grains as a blackening of the film, then fixers are used to remove any remaining silver halide and a permanent image results.

- It is a very sensitive technique and has been used in a wide variety of biological experiments. A good example is autoradiography of nucleic acids separated by gel electrophoresis.