Radioisotopes used in biological sciences and their properties and method of incorporation of radioisotopes in biological tissues and cells

Course Code –BOTY 4204

Course Title- Techniques in plant sciences , biostatistics and bioinformatics

Department of Botany

Mahatma Gandhi Central University, Motihari

Unit 3- Radiolabeling techniques

Detection and measurement of different types of radioisotopes used in biology, incorporation of radioisotopes in biological tissues and cells, molecular imaging of radioactive material, safety guidelines.

Scintillation counting for dual –labelled samples

- Different beta particle emitters have different energy spectra so it is possible to quantify two isotopes separately in a single sample, provided their energy spectra can be distinguished from each other.

- ^{3}H and ^{14}C, ^{3}H and ^{35}S, ^{3}H and ^{32}P, ^{14}C and ^{32}P, ^{35}S and ^{32}P are the pairs isotopes that can be used together.

- Spectra of two isotopes overlap only slightly which can be avoid by setting a pulse height analyzer to reject all pulses of an energy below threshold X and to reject all pulses of an energy above window Y and also to reject below a threshold of A and a window of B, it is possible to separately count the two isotopes.

- Modern counters operate with a so-called multichannel analyzer that records the entire energy spectrum simultaneously.

- This greatly facilitates multi isotope counting and in particular allows the effect of quenching on dual-label counting to be assessed adequately.

- Dual label counting has proved to be useful in many aspects of molecular biology (e.g., nucleic acid hybridization and transcription), metabolism(e.g., steroid synthesis) and drug development.

Determination of counting efficiency

- ✓ When we detect radioactivity we need to know the actual rate of decay (d.p.m.).To calculate d.p.m. we need to know the efficiency of counting.
- ✓ Due to quenching problem in liquid scintillation counter. Efficiency of counting needs to be determined for every sample.
- ✓ counting efficiency determined by using internal standard called spike.

Counting efficiency = (100(C-A)/ B)

Where

A- sample counted (c.p.m.) by using counter and take reading

B- Reading of standard material of known disintegration per minute

C- Reading after recounting sample (c.p.m.)

d.p.m.(sample) = c.p.m.(sample)/ counting efficiency

% counting efficiency = cpmx100/dpm

It is most accurate way of correcting quenching.

Sample preparation for scintillation

Selection of vial accordingly experiment, isotopes used and counter it may be-

- ✓ Glass vial (low potassium glass vial) for low level of ^{40}K that reduce background count
- ✓ Polythene- cheaper and not re-usable

Vials should be chemically resistant, have good light transmission and give low background count

- ✓ Selection of appropriate scintillation fluid according to aqueous or organic sample.

- ✓ If color quenching is a problem then bleach samples before counting.

- ✓ solid sample(plant or animal tissue) may be counted after solubilisation by quaternary amine such as NCS solubiliser or Soluene (highly toxic , great care required).

- ✓ Radioactive compound separated by HPLC and the output of HPLC connected to a flow cell system where scintillation fluid is added to the effluent prior to entering a detector.

Scintillation proximity assay

- It is a assay development and biochemical screening that allows the fast and sensitive measurement of a broad range of biological process in a homogenous system.
- It is highly suited to work such as screening for biological activity in new drugs.
- The bead for SPA is constructed from polystyrene (sometimes other materials) that combine a binding site for a molecule of interest with scintillant.
- Remember if you uses weak energy emitters radioisotopes such as ^{3}H and ^{14}C (emit beta radiation which do not travel far)in the solution with a suspension of SPA beads unable to stimulate the scintillant in the beads and can not be detected efficiently by a scintillation counter.

- It happen because the radiation is absorbed by the solution it does not reach to the scintillant.

- If radioisotope becomes bound to the bead, it is close enough to stimulate the scintillant in the bead, so light is given out and the isotope is detected.

Applications of SPA

- Enzyme assays
- Receptor binding
- Interaction between two molecules (protein and nucleic acid)

Advantages of SPA

✓Versatile : use with enzyme assays, receptors, any molecular interaction.

✓Work with a range of appropriate isotopes such as ^{3}H, ^{35}S, ^{14}C and ^{33}P.

✓No need for separation step(e.g., free from bound ligand).

✓Less manipulation therefore reduced toxicity.

✓Amenable to automation.

Cerenkov counting

- Some chemical and coloured samples can interfere with the counting process. This interference is known as quenching.
- It can be overcome through-

✓ data correction.

✓ through careful sample preparation.

High energy beta emitters (^{32}P), can also be counted in scintillation counter without the cocktail, instead using an aqueous solution. This technique known as Cerenkov counting.

- The Cerenkov effect occurs when a particle passes through a substance with a speed higher than that of the light passing through the same substance.
- If a beta-emitters has a decay energy excess of 0.5MeV, then this causes water to emit a bluish white light usually referred to as Cerenkov light.
- It is possible to detect this light using a typical liquid scintillation counter.
- There is no requirement for organic solvents and fluors.

Advantages of Cerenkov counting

- Cheap
- Easy sample preparation
- Eliminate the problem of chemical quenching.
- Quick and rough measurement

Interaction of radioactivity with matter

Alpha-particles

- These particle have a very considerable energy(3-8 MeV) and all the particles from a given isotope have the same amount of energy.
- They react with matter in two ways: they cause excitation and they ionise atoms in their path.
- In excitation the energy is transferred from the alpha particles to orbital electrons of neighbouring atoms, these electron being elevated to higher orbitals, but eventually fall back, emitting energy as photons of light.
- In ionisation the target orbital electron is removed, thus the atom becomes ionised and form an ion-pair consisting of a positively charged ion and an electron.

- Because of their size alpha particles have slow movement and double positive charge.

- They cause intense ionisation and excitation and their energy rapidly dissipated.

- Despite their initial high energy, alpha-particles frequently collide with atoms in their path and so the radiation is not very penetrating (a few centimetres through air).

Negatron

- It is very small and rapidly moving particles that carry a single negative charge.

- They interact with matter to cause ionisation and excitation exactly as with alpha-particles.

- Due to their speed and size, they are less likely than alpha-particles to interact with matter and therefore are less ionising and more penetrating.

- Negatrons are emitted over a range of energies

- Negatron emitters have a characteristic energy spectrum. The maximum energy (Emax) level varies from isotope to isotope ranging from 0.018 MeVfor ^{3}H to 4.81 MeV for ^{38}Cl.

- The difference of Emax affects the penetration of the radiation and therefore the safety measures that are required.

- Beta –particles from ^{3}H can travel only a few millimeters in air, whereas those from ^{32}P can travel over 1 meter of air.

- Therefore radiation shield are required when working with ^{32}P.

γ- rays and X-rays

These rays are electromagnetic radiation and therefore have no charge or mass. Te cause excitation and ionisation. They interact with matter to create secondary electrons that behave as per negatron emission.

Bremsstrahlung radiation

When high atomic number materials absorb high energy beta-particles, the absorber gives out a secondary radiation, an X-ray, called Bremsstrahlung radiation. For this reason, shields for ^{32}P use low atomic number materials such as acrylic.

Incorporation of radioisotopes in biological sample

- Inhalation, ingestion and wound contamination radioisotope particles may be transported via blood or lymphatics into cells, tissues and organs.
- Radioisotopes can be incorporated into one or more organs specific for that isotope resulting an exposure at that site.
- Medical countermeasures called decorporation agents or other procedures (e.g., diuresis) may be needed to remove radioisotopes that have been incorporated into tissues
- Toxic effects of radioisotopes may be due to their chemical or radiological properties.

Properties of radioisotopes used in biological sciences

(^{3}H , ^{32}P, ^{35}S , ^{14}C , ^{33}P and ^{125}I)

	^{3}H	^{32}P	^{35}S
Half-life-	12.3 years	14.3 days	87.4 days
Mode of decay-	β	β	β
Max β energy-	0.019MeV	1.709 MeV	0.167 MeV
ALI-	480(Mbq)	6.3(Mbq)	15(Mbq)
Maximum range in air-	6mm	790cm	26cm
Cerenkov counting-	no	yes	no
Shielding required-	no	1cm acrylic	1cm acrylic

	^{14}C	^{33}P	^{125}I
Half-life-	5730 years	25.4 days	59.6 days
Mode of decay-	β	β	X(EC) and Auger electron
Max β energy-	0.156MeV	0.249 MeV	0.035 MeV
ALI-	34(Mbq)	14(Mbq)	1.3(Mbq)
Maximum range in air-	24cm	49cm	>10m
Cerenkov counting-	no	no	no
Shielding required-	1cm acrylic	1cm acrylic	lead 13mm

Merits and demerits of radioisotopes

^{3}H merits

- It is relatively safe
- Have high specific activity
- Wide choice of positions in organic compounds
- High resolution in autoradiography

^{3}H demerits

- Low efficiency of detection
- Isotope exchange with environment
- Isotope effect

^{14}C merits

- It is relatively safe
- Wide choice of labelling positions in organic compounds
- Resolution is good in autoradiography

^{14}C demerits

- Low specificity

^{35}S merits

- Good resolution in autoradiography
- High specific activity

^{35}S demerits

- Short half-life
- Relatively long biological life

^{125}I merits

- Ease of detection
- Have high specific activity
- Good for labelling proteins

^{125}I demerits

- High penetration of radiation

^{127}I merits

- Ease of detection
- High specific activity

^{127}I demerits

- High penetration of radiation
- Short half-life

^{32}P merits

- Ease of detection
- Have high specific activity
- Short half-life simplifies disposal

^{32}P demerits

- Short half-life affects costs and experimental design
- High β energy so external radiation hazard

^{33}P merits

- Good resolution in autoradiography
- High specific activity
- Less hazardous than ^{32}P

^{33}P demerits

- Low specific activity ^{32}P
- Less sensitive than ^{32}P
- Cost

Units commonly used to describe radioactivity

- cpm and cps (counts per minute or counts per second)- the recorded rate of decay.

- dpm or dps (disintegrations per minute or disintegrations per second)- the actual rate of decay.

- Curie (Ci)- unit of ionizing radiation (radioactivity). Equal to 3.7×10^{10} dps. it is equivalent to radioactivity emitted by 1gm of radium 226. it is named after Pierre Curie.

$$1\text{millicurie} = 2.22 \times 10^{9} \text{ dpm or Ci} \times 10^{-3}$$

$$1\text{microcurie} = 2.22 \times 10^{6} \text{ dpm or Ci} \times 10^{-6}$$

- **Becquerel (Bq)-** SI derived unit of radioactivity. One becquerel is defined as the activity of a quantity of radioactive material in which one nucleus decays per second.

1Bq= 1dps

1TBq(terabecquerel)= 10^{12} Bq= 27.027Ci

1GBq (Gigabecuquerel)= 10^{9} Bq= 27.027mCi

1 MBq (megabecquerel)= 10^{6} Bθ=27.027μCi

Electron volt (eV)- The energy attained by an electron accelerated through a potential difference of 1 volt. Equivalent to 1.6 x 10^{-19} J.

Roentgen (R)- The amount of radiation that produces 1.61×10^{15} ion-pairs/kg.

Rad (rad)-The dose that gives an energy absorption of 0.01J/kg.

Gray (Gy)- The dose that gives an energy absorption of 1J/kg. thus 1Gy= 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 1Sv= 100 rem.

Thank you