Detection and measurment of different type of isotopes used in biology

Course Code –BOTY 4204

Course Title- Techniques in plant sciences , biostatistics and bioinformatics

Department of Botany

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Unit 3- Radiolabeling techniques

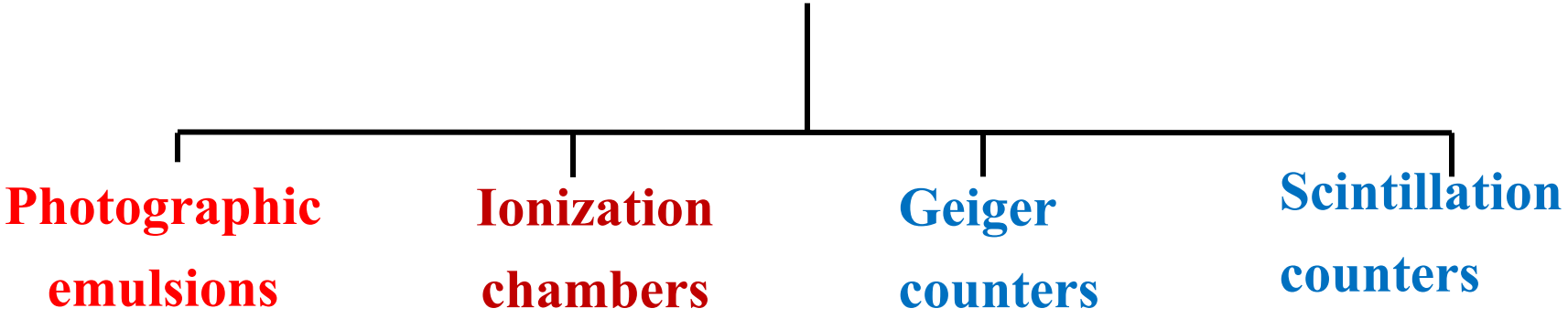
Detection and measurement of different types of radioisotopes used in biology, incorporation of radioisotopes in biological tissues and cells, molecular imaging of radioactive material, safety guidelines.

Detection and measurement of radioactivity

Radioactive isotopes interact with matter in two ways, ionisation and excitation. There are three commonly used methods of detecting and quantifying radioactivity. These are based on-

- ✓ Ionisation of gases (**Geiger- Muller counters**)
- ✓ On the excitation of solids or solutions (**Scintillation counting**)
- ✓ The ability of radioactivity to expose photographic emulsions(**Autoradiography**)

Basics means of detecting and measuring the characteristic radiations



Methods based on gas ionisation

- ✓ If a charged particle passes through a gas, causes ionisation. The ionisation ability is decreases as-

  $\alpha > \beta > \gamma$ (10000:100:1)

- ✓ If ionisation occurs between a pair of electrodes enclosed in a suitable chamber a pulse flows.
- ✓ Ionisation counters sometimes called proportional counters because small voltage changes can affect the count rate.
- ✓ The GM counter has a cylindrical –shaped gas chamber and it operates at a very high voltage which makes it 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 (dead time) are not detected and this reduces the counting efficiency.

Applications of ionisation counter

- Routine monitoring of laboratory to check for contamination.
- Quick screening of radioactive gel prior to autoradiography.
- Checking chromatography fraction for labelled components.

Methods based upon excitation

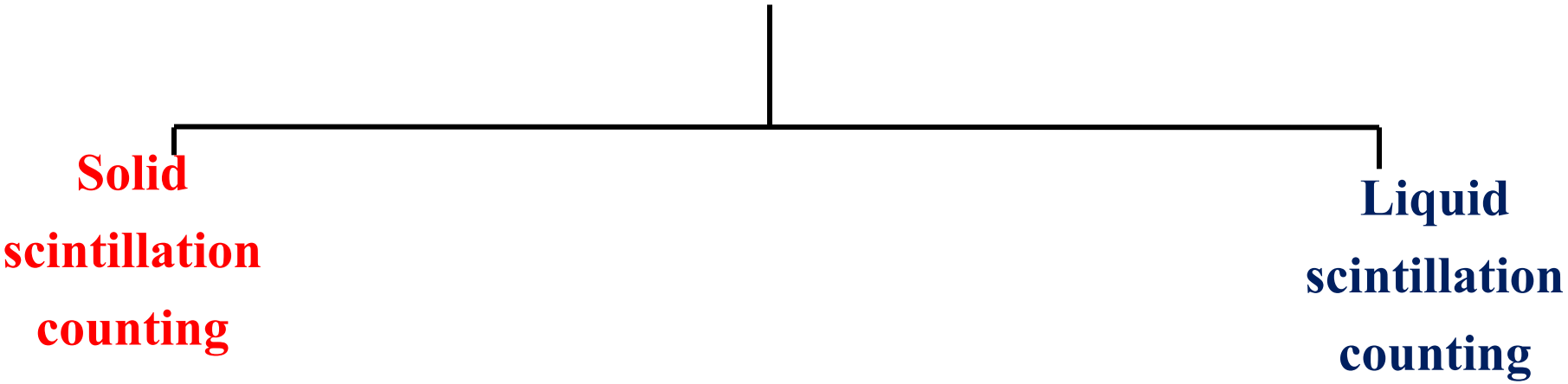
- ✓ In this method excited atom or compound (known as fluor) emit photon of light and this process is called scintillation.
- ✓ This light is detected by a photomultiplier and forms a basis of scintillation.
- ✓ Photomultiplier converts the energy of radiation into an electrical signal and the strength of electric pulse that results is directly proportional to the energy of the original radioactive event.

- ✓ By this method two or even more , isotopes can be separately detected and measured in the same sample , provided they have sufficiently different emission energy spectra.

Scintillation counter

- A scintillation counter is an instrument for detecting and measuring ionizing radiation by using the excitation effect of incident radiation on a scintillating material , and detecting the resultant light pulses.

Types of scintillation counting



Solid scintillation counting

- ✓ In solid scintillation counting the sample is placed adjacent to a solid fluor (e.g., NaI).
- ✓ It is useful for **γ–emitting** isotopes because they can penetrate the fluor.
- ✓ The counter can be small hand-held devices with the fluor attached to the photomultiplier tube or larger bench-top machines with a well-shaped fluor designed to automatically count many sample.

Liquid scintillation counting

- In this sample is mixed with a scintillation fluid containing a solvent and one or more dissolved fluors.

- This method is useful for quantifying weak β-emitter such as ^{3}H, ^{14}C and ^{35}S which are frequently used in biological work.

- Scintillation fluid (cocktails) contain solvent (such as toluene or diisopropylnaphthalene) and fluors such as 2,5- diphenyloxazole (PPO), 1,4-bis (5-phenyloxazol-2-yl) benzene (POPOP) or 2-(4'-t-butylphenyl)-5-(4"-bi-phenyl)-1,3,4-oxydiazole (butyl-PBD).

- Cocktails can be designed for counting organic samples, or many contain detergent to facilitate counting of aqueous samples.

Advantages of scintillation counting

- ✓ Fluorescence is very fast so there is effectively no dead time.
- ✓ Dead time is the time after each event during which system is unable to record another event.
- ✓ Counting efficiencies are high.
- ✓ The ability to count samples of many types, including liquids, solids, suspensions and gels.
- ✓ The ability to count separately different isotopes in the same sample (used in dual-labelling experiments).
- ✓ Easy to sample preparation.
- ✓ Highly automated.

Disadvantages of scintillation counting

- Cost of the instrument and cost per sample is high.

- High background count due to photomultiplier noise but can be compensated for by using more than one tube.

- Quenching: this is the name for reduction in counting efficiency caused by coloured compounds that's absorb the scintillated light, or chemical that interfere with transfer of energy from the radiation to the photomultiplier.

Correction: 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.

  Correction- modern instruments can detect chemiluminescence and subtract it from the result automatically.

- Phospholuminescence: this results from pigments in the sample absorbing light and re-emitting it.

  Correction – To keep the samples in the dark prior to counting.

Methods based on exposure of photographic emulsion

- Ionising radiation acts upon a photographic emulsion or film to produce a latent image much as does visible light. This is called autoradiography.
- Niepse de Saint Victor first described the phenomenon of autoradiography.
- An autoradiograph is an image on an X-ray film or nuclear emulsion produced by the pattern of decay emissions from a distribution of a radioactive substance.
- It is very sensitive technique.
- Used in a variety of biological experiments

- The emulsion or film contains silver halide crystals and 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 shows these silver grains as a blackening of the film , then fixer are used to remove any remaining silver halide and a permanent image results.
- Weak β-emitter such as ^{3}H, ^{14}C and ^{35}S which are suitable for autoradiography .

- Sample must be close to film , so that the radiation does not spread out very far and give clear image.

- Radiation with higher energy give faster results but proper resolution because the higher energy(^{32}P) negatrons produce much longer track lengths, exposing a greater surface area of the film and result in less discrete images.

- Autoradiography emulsions are solutions of silver halide that can be made to set solid by the inclusion of materials such as gelatine. This can be used for example for autoradiography of microscope slides. X-ray film is the alternative and is used for gels.

Direct autoradiography

- ✓ In direct autoradiography , the X- ray film or emulsion is placed as close as possible to the sample and exposed at any convenient temperature.
- ✓ Quantitative images are produced until saturation is reached.
- ✓ The shades of grey in the image are related to a combination of levels of radiation and length of exposure until a black or nearly black image results.
- ✓ Isotopes with an energy of radiation equal to or higher than ^{14}C (Emax= 0.156MeV) are required.
- ✓ The higher the energy the quicker the results.

Fluorography

- If low energy β-emitters are used it is possible to enhance the sensitivity several orders of magnitude by using fluorography.
- A fluor (e.g., PPO or sodium silicate) can be used to enhance the image.
- The β-particles emitted from the isotope will cause the fluor to become excited and emit light, which will react with the film.
- This has been used for example for detecting radioactive nucleic acids in gels.
- The fluor is infiltrated into the gel following electrophoresis; the gel is dried and then placed in contact with a preflashed film.

Preflashing

- The response of a photographic emulsion to radiation is not linear and usually involves a slow initial phase(lag) followed by linear phase.

- Sensitivity of films may be increased by preflashing.

- Preflashing involves a millisecond light flash prior to the sample being brought into juxtaposition with the film.

- It is often used where high sensitivity is needed or if results are to be quantified.

Intensifying screens

- Intensifying screens are used when obtaining fast result is more important than high resolution.

- It is useful for example in gel electrophoresis or analysis of membrane filters where high energy β-emitters(^{32}P- labelled DNA) or **γ–emitting isotopes (**^{125}I- labelled protein**) are used.**

- The intensifying screen consists of a solid phosphor, and it is placed on the other side of the film from the sample.

- When high energy radiation passes through the film, causes the phosphor to fluoresce and emit light, which in turn superimposes its image on the film.

Low temperature exposure

- When intensifying screens or fluorography are used the exposure should be done at low temperature because the kinetics of the films response are affected.

- The light is of low intensity and a back reaction occurs that cancels the latent image.

- Exposure at low temperature (-70 ^{0}C)slows this back reaction and will therefore provide higher sensitivity.

- There is no point in doing direct autoradiography at low temperature as the kinetic basis of the film's response is different.

Quantification

- Autoradiography is usually used to locate rather than to quantify radioactivity.
- However it is possible to obtain quantitative data directly from autoradiograph by using digital image analysis.
- Quantification is not reliable at low or high levels of exposure because of the lag phase or saturation, respectively.
- Preflashing combined with fluorography or intensifying screens create the best conditions for quantitative working.

Self absorption

- Self -absorption is primarily a problem with low energy β-emitters: radiation is absorbed by the sample itself.
- Self- absorption can be a serious problem in the counting of low energy radioactivity by scintillation counting if the sample is particulate or is, for instance, stuck to a membrane filter.
- Automated methods for calculating counting efficiency in a scintillation counter will not correct for self absorption effects.
- Particulate samples should be digested or otherwise solubilised prior to counting if quench correction is required.

Specific activity

- ✓ Specific activity of radioisotope is defined as the amount of radioactivity or the decay rate of a particular radionuclide per unit mass of radionuclide.

- ✓ Specific activity expressed by units such as Bq/mol, Ci/mol or d.p.m./mol.

- ✓ The higher the specific activity the more sensitive the experiment. Because the higher the specific activity the smaller the quantities of labelled substance that can be detected.

Specific activity of isotopes inversely related to half-lives. Because half-life inversely proportional to rate of decay per unit mass (or mol).

Formula to calculate specific activity

$$W = Ma(1/A' - 1/A)$$

W= mass of the carrier required in mg

M= amount of radioactivity present (MBq)

a= molecular weight of compound

A= original specific activity(MBq/mol)

A'= required specific activity(MBq/mol)

Statistics

- Emission of radioactivity is a random process and the spread of results forms a normal distribution. The standard deviation can be calculated by taking a square root of the counts.

- The more counts we take the smaller the standard deviation is as a proportion of the mean count rate.

- The more counts measured the more accurate the data.

Choice of radionuclide

The key factors in the decision are often based on-

- Safety
- The type of detection to be used
- The sensitivity required
- Cost

Isotope effect

- ^{33}P may be chosen for work with DNA because it has high enough energy to be detected easily, it is safer than ^{32}P and its half-life is short enough to give specific activity but long enough to be convenient to use.
- Although they undergo the same reactions, different isotopes may do so at different rates. This is known as isotope effect.
- The different rates are approximately proportional to the differences in mass between the isotopes.
- This can be a problem in case of ^{1}H and ^{3}H, but the effect is small for ^{12}C and ^{14}C, and almost insignificant for ^{32}P and ^{33}P.
- The isotope effect may be taken into account when choosing which part of a molecule to label with ^{3}H.

Thank you