**RADIOLABELLING TECHNIQUES**

When researchers contemplate using a radioactive compound there are several things they have to consider. First and foremost, they must ask the questions: is a radioisotope necessary is there another way to achieve our objectives? The reason for this is that radioisotope use is governed by very strict legislation. The rules are based on the premise that radioactivity is potentially unsafe (if handled incorrectly) and should therefore only be used if there are no alternatives. Then, once it is decided that there is no alternative, the safest way of carrying out the work needs to be planned. Essentially this means using the safest isotope and the smallest amount possible.

But why do we use radioisotopes in the first place? There are very good reasons; here are some of them.

* Firstly, it is possible to detect radioactivity with exquisite sensitivity. This means that, for example, the progress of a chemical through a metabolic pathway or in the body of a plant or animal can be followed relatively easily. In short, much less of the chemical is needed, and the detection methods are simple.
* Secondly, it is possible to follow what happens in time. Imagine a metabolic pathway such as carbon dioxide fixation (the Calvin cycle). All the metabolites in the cycle are present simultaneously so a good way to establish the order of the metabolism is to add a radioactive molecule (e.g. 14C-labelled sodium bicarbonate) and see what happens to it.
* Thirdly, it is possible to trace what happens to individual atoms in a pathway. This is done for example by creating compounds with 14C in specific locations on the molecule.
* Fourthly, we can identify a part or end of a molecule, and follow reactions very precisely. This has been very useful in molecular biology, where it is often necessary to label one end of a DNA molecule (e.g. for techniques such as DNA footprinting, a method for investigating sequence-specific DNA-binding proteins).

**DETECTION AND MEASUREMENT OF RADIOACTIVITY**

There are three commonly used methods of detecting and quantifying radioactivity. These are based on the ionisation of gases, on the excitation of solids or solutions, and the ability of radioactivity to expose photographic emulsions (i.e. autoradiography).

**Methods based upon gas ionisation**

If a charged particle passes through a gas, its electrostatic field dislodges orbital electrons from atoms sufficiently close to its path and causes ionisation. The ability to induce ionisation decreases in the order

α > β > γ (10 000 : 100 : 1)

If ionisation occurs between a pair of electrodes enclosed in a suitable chamber a pulse (current) flows. Ionisation counters like those shown in figure below are sometimes called proportional counters (‘proportional’ because small voltage changes can affect the count rate). 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.



Figure: Detection based on ionization

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.

**Methods based upon excitation**

Radioactive isotopes interact with matter in two ways, ionisation and excitation. The latter effect leads an excited atom or compound (known as a fluor) to emit photons of light. The process is known as scintillation. When the light is detected by a photomultiplier, it forms the basis of scintillation counting.

***Types of scintillation counting***

There are two types of scintillation counting, which are illustrated diagrammatically in the figure below:



Figure: (a) solid and (b) liquid scintillation counter

In solid scintillation counting the sample is placed adjacent to a solid fluor (e.g. sodium iodide). Solid scintillation counting is particularly useful for γ-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.

In liquid scintillation counting, the sample is mixed with a scintillation fluid containing a solvent and one or more dissolved fluors. This method is particularly useful in quantifying weak β-emitters such as 3H, 14C and 35S, which are frequently used in biological work. Scintillation fluids are called ‘cocktails’ because there are different formulations, made of a solvent (such as toluene or diisopropylnaphthalene) plus fluors such as 2,5-diphenyloxazole (PPO), 1,4-bis(5-phenyloxazol-2-yl)benzene (nicknamed POPOP, pronounced as it reads: ‘pop op’) or 2-(4’-*t*-butylphenyl)-5-(4’’-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 b-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**

No technique is without disadvantages, so the following have to be considered or overcome in the design of the instruments:

* 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.

**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.

**Suitable isotopes:** In general, weak β-emitting isotopes (e.g. 3H, 14C and 35S) are most suitable for autoradiography, particularly for cell and tissue localisation experiments. This is because the energy of the radiation is low. The sample must be close to the film, the radiation does not spread out very far and so a clear image results. Radiation with higher energy (e.g. 32P) give faster results but poorer resolution because the higher energy negatrons produce much longer track lengths, exposing a greater surface area of the film, and result in less discrete images.

**Choice of emulsion and film:** 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. Films differ in sensitivity; advice on what to use is provided by the manufacturers.

**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, 14C 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.

**SAFETY GUIDELINES:** When handling radioisotopes the rules are to:

* Wear protective clothing, gloves and glasses
* Use the smallest amount possible
* Keep radioactive materials safe, secure and well labelled
* Work in defined areas in a spill tray
* Monitor your working area frequently
* Have no foods or drinks in the laboratory
* Wash and monitor hands after the work is done
* Follow all local rules such as for the dispensing of stock and the disposal of waste
* Do not create radioactive aerosols or dust
* Maximise the distance between yourself and the source
* Minimise the time of exposure
* Maintain shielding at all times.