Tracer techniques

Tracer techniques

For the study of biological pathway and mechanism **Tracer techniques** has its importance. Tracer techniques involve use of isotopically labelled molecules and detection of the isotopes for the study. An isotope is a form of an element which has the same atomic number of electrons as the common form of the element but it differs in atomic weight. The difference of atomic weight is due to difference of number of neutrons in its nucleus. An isotope may be stable or radioactive depending on the relative number of protons and neutrons in its nucleus.

Both stable and radioactive isotopes of an element are identical in chemical properties, and thus they undergo all the physical and chemical changes like the ordinary form of the element. Moreover, the can be detected at any time by atomic weight or radioactivity. By **Ginger-Muller counter** or other sensitive detectors the radioactive isotopes can be detected by their radioactivity even when it present in very small quantity. Detection of stable isotopes can be done by their atomic weight through a **mass spectrograph.** Stable or radioactive isotopes used for studying the fate of a molecule in physical, chemical or biological processes are called **tracer element** and the methods for such studies are called **tracer techniques**. Commonly used radioactive tracers in the study of biology are C^{14}, P^{12}, H^{3} etc. An important stable isotope used as tracer in biology is O^{18}.

At specific positions in their molecules inorganic and organic compounds can be prepared with isotopes. Such compound containing an isotope in its molecule is used as a tracer. In the tracer technique the isotope element in the molecule is said 'tagged 'or 'labelled'. When an isotopically labelled compound is administrated to an animal or a plant or incubated with tissue preparations, it undergoes same fate as the unlabelled form of the compound and the isotopically labelled products can be detected. In this way, this source, metabolic pathway and end products of bio-molecules can be studied with the use of isotopically tagged tracers.

In the process of Tracer techniques tracers are also used for determining the following:

i) Metabolic turnover of a substance.

ii) Relative proportion of a substance being catabolised through different pathways.

iii) Intestinal absorption of the nutrient.

iv) Volume of body fluids.

v) Blood level of a hormone.

vi) Mechanism and site of action of a hormone.

vii) Cardiac output.

viii) Flow of blood through an organ.

ix) Intracellular distribution, i.e. autoradiography.

The most common radioactive tracer used in metabolic studies is C^{14}. P^{32}, which is a radioactive tracer is mainly used to study the phosphorylation reactions. H^3 or tritium is also a radioactive isotope which is used as a tracer in the form of tritium oxide (THO) for determination of total body fluid volume. O^{18} is a stable isotope and it is used to trace the source of O_2 liberated in photosynthesis. When O^{18} labelled water (H_2O^{18}) is used in photosynthesis, $O^{18}{}_2$ is liberated. It is thus proved that the water is the source of oxygen liberated in photosynthesis.

A **radioactive tracer**, or **radioactive label**, is a chemical compound in which one or more atoms have been replaced by a radioisotope so by virtue of its radioactive decay it can be used to explore the mechanism of chemical reactions by tracing the path that the radioisotope follows from reactants to products. **Radiolabeling** is thus the radioactive form of isotopic labeling.

Radioisotopes of hydrogen, carbon, phosphorus, sulphur, and iodine have been used extensively to trace the path of biochemical reactions. A radioactive tracer can also be used to track the distribution of a substance within a natural system such as a cell or tissue,[1] or as a flow tracer to track fluid flow. Radioactive tracers are also used to determine the location of fractures created by hydraulic fracturing in natural gas production.[2] Radioactive tracers form the basis of a variety of imaging systems, such as, PET scans, SPECT scans and technetium scans. Radiocarbon dating uses the naturally occurring carbon-14 isotope as an isotopic label. There are two main ways in which radioactive tracers are used

1. When a labeled chemical compound undergoes chemical reactions one or more of the products will contain the radioactive label. Analysis of what happens to the radioactive isotope provides detailed information on the mechanism of the chemical reaction.
2. A radioactive compound is introduced into a living organism and the radio-isotope provides a means to construct an image showing the way in which that compound and its reaction products are distributed around the organism.

Autoradiography

Autoradiography is any technique used to produce an image of the 2D distribution of a radioactive substance. The first autoradiography was obtained accidently around 1867 when a blackening was produced on emulsions of silver chloride and iodide by uranium salts. Such studies and the work of the Curies in 1898 demonstrated autoradiography before, and contributed directly to, the discovery of radioactivity. The development of autoradiography as a biological technique really started to happen after World war II with the development of photographic emulsions and then stripping film made of silver halide.

An autoradiograph is an image on an x-ray film or nuclear emulsion produced by the pattern of decay emissions (e.g., beta particles orgamma rays) from a distribution of a radioactive substance. Alternatively, the autoradiograph is also available as a digital image (digital autoradiography), due to the recent development of scintillation gas detectors or rare earth phosphorimaging systems. The film or emulsion is apposed to the labeled tissue section to obtain the autoradiograph (also called an autoradiogram). The auto- prefix indicates that the radioactive substance is within the sample, as distinguished from the case of historadiography or microradiography, in which the sample is X-rayed using an external source. Some autoradiographs can be examined microscopically for localization of silver grains (such as on the interiors or exteriors of cells or organelles) in which the process is termed micro-autoradiography. For example, micro-autoradiography was used to examine whether atrazine was being metabolized by the hornwort plant or by epiphytic microorganisms in the biofilm layer surrounding the plant.

In biology, this technique may be used to determine the tissue (or cell) localization of a radioactive substance, either introduced into a metabolic pathway, bound to a receptor or enzyme, or hybridized to a nucleic acid.

The use of radiolabeled ligands to determine the tissue distributions of receptors is termed either in vivo or in vitro receptor autoradiography if the ligand is administered into the circulation (with subsequent tissue removal and sectioning) or applied to the tissue sections, respectively. The ligands are generally labeled with 3H (tritium) or 125I(radioiodine). The distribution of RNA transcripts in tissue sections by the use of radiolabeled, complementary oligonucleotides or ribonucleic acids ("riboprobes") is called in situ hybridization histochemistry. Radioactive precursors of DNA and RNA, [3H]-thymidine and [3H]-uridine respectively, may be introduced to living cells to determine the timing of several phases of the cell cycle. RNA or DNA viral sequences can also be located in this fashion. These probes are usually labeled with 32P, 33P, or 35S. In the realm of behavioral

endocrinology, autoradiography can be used to determine hormonal uptake and indicate receptor location; an animal can be injected with a radiolabeled hormone, or the study can be conducted in vitro.

This autoradiographic approach contrasts to techniques such as PET and SPECT where the exact 3-dimensional localization of the radiation source is provided by careful use of coincidence counting, gamma counters and other devices.

Krypton-85 is used to inspect aircraft components for small defects. Krypton-85 is allowed to penetrate small cracks, and then its presence is detected by autoradiography. The method is called "krypton gas penetrant imaging". The gas penetrates smaller openings than the liquids used in dye penetrant inspection and fluorescent penetrant inspection.

General Methods for Autoradiography Two General Types of Experiments:

1) In-vivo autoradiography - receptors are labeled in intact living tissue by systemic administration of the radioligand (like in PET). Tissue is removed, processed, and visualized.

2) In-vitro autoradiography - slide-mounted tissue sections are incubated with radioligand so that receptors are labeled under very controlled conditions

Radiography is the visualisation of the pattern of distribution of radiation. In general, the radiation consists of X-rays, gamma (g) or beta (b) rays, and the recording medium is a photographic film. For classical X-rays, the specimen to be examined is placed between the source of radiation and the film, and the absorption and scattering of radiation by the specimen produces its image on the film. In contrast, in **autoradiography** the specimen itself is the source of the radiation, which originates from radioactive material incorporated into it. The recording medium which makes visible the resultant image is usually, though not always, photographic emulsion.

History

The first autoradiography was obtained accidently around 1867 when a blackening was produced on emulsions of silver chloride and iodide by uranium salts. Such studies and the work of the Curies in 1898 demonstrated autoradiography before, and contributed directly to, the discovery of radioactivity. The development of autoradiography as a biological technique really started to happen after World war II with the development of photographic emulsions and then stripping film (see Rogers, 1979) made of silver halide. Radioactivity is now no longer the property of a few rare elements of minor biological interest (such as radium, thorium or uranium) as now any biological compound can be labelled with radioactive isotopes opening up many possibilities in the study of living systems.

Radioisotopes,

The mass of the atomic nuclei can vary slightly (=isotopes) for a particular element although the number of electrons remains constant and all the isotopes have the same chemical properties. The nuclei of radioactive isotopes are unstable and they disintegrate to produce new atoms and, at the same time, give off radiations such as electrons (b rays) or radiations (g rays). Naturally occurring radioisotopes are rare because of their instability, but radioactive atom can be produced in nuclear reactors by bombardment of stable atoms with high-energy particles. The disintegrations can be detected in 3 ways. **These detection methods are extremely sensitive and <u>every</u> radioactive atom that disintegrates can be detected.**

<u>Detection</u>

(i) *Electrical:* This depends on the production of ion pairs by the emitted radiation to give an electrical signal that can be amplified and registered: used in Geiger counter, ionisation counter and gas flow counter

(ii) *Scintillation:* Some materials have the property of absorbing energy from the radiation and re-emitting this in the form of visible light. In a *scintillation counter* these small flashes of light are converted into electrical impulses. Both of these techniques count the pulses of the disintegrating atoms. They are fast and quantitative.

(iii) **Autoradiography** differs from the pulse-counting techniques in several ways. Each crystal of silver halide in the photographic emulsion is an independent detector, insulated from the rest of the emulsion by a capsule of gelatin. Each crystal responds to the charged particle by the formation of a **latent** (hidden) image that is made permanent by the process of development. The record provided by the photographic emulsion is cumulative and spatially accurate. It provides information on the localisation and distribution of radioactivity within a sample (i & ii do not do this). Thus there is little point on doing autoradiography on a specimen that is homogeneously labelled. Although it can be quantitative, autoradiography is a much slower and more difficult approach.

Nuclear emulsions have a very high efficiency for b particles (electrons of nuclear origin), particularly those with low energies. Many of the isotopes of interest to biologists have suitable isotopes, e.g. tritium (= hydrogen-3), carbon-14, , sulphur-35 and iodine-125. The effective volume of the detector emulsion in the immediate vicinity of the source may be as little as 100 cubic microns.

<u>Autoradiography Method</u>

- Living cells are briefly exposed to a ‘pulse’ of a specific radioactive compound.
- The tissue is left for a variable time.
- Samples are taken, fixed, and processed for light or electron microscopy.

- Sections are cut and overlaid with a thin film of photographic emulsion.
- Left in the dark for days or weeks (while the radioisotope decays). This exposure time depends on the activity of the isotope, the temperature and the background radiation (this will produce with time a contaminating increase in 'background' silver grains in the film).
- The photographic emulsion is developed (as for conventional photography).
- Counterstaining e.g. with toluidine blue, shows the histological details of the tissue. The staining must be able to penetrate, but not have an adverse affect on the emulsion.
- Alternatively, pre-staining of the entire block of tissue can be done (e.g. with Osmium on plastic sections coated with stripping film [or dipping emulsion] as in papers by McGeachie and Grounds) before exposure to the photographic emulsion. This avoids the need for individually (post-) staining each slide.
- It is not necessary to coverslip these slides
- The position of the silver grains in the sample is observed by light or electron microscopy Note: the grains are in a different plane of focus in the emulsion overlying the tissue section. Often oil with a x100 objective is used for detailed observation with the light microscope.
- These autoradiographs provide a permanent record.
- Full details on the batch of emulsion used, dates, exposure time and conditions should be kept for each experiment.

Types of photograhic detection systems

Stripping film consists of an **even layer** of photographic emulsion on a supporting gelatin membrane (e.g. Kodak AR10), it is floated on water and then wrapped around the slide and forms very close contact as it dries (Rogers, Chap 15). This was once widely used but is now no longer made. It has the major advantage of uniform thickness but the disadvantage that the supporting membrane prevents counterstaining of the section and therefore the tissue block must be pre-stained before sections are coated.

Liquid photographic emulsion. This is the method routinely used today (see details below). It is simpler and much quicker to do, but the layer of liquid emulsion (e.g. Kodak NB2) can be **slightly uneven** in thickness as it flows down to the bottom of the slide as it is withdrawn: for most purposes this slight variation is not important, unless the number of grains are being strictly counted and compared across one slide (Rogers, Chap 16).

Method for coating and developing dipping emulsion

Coating the slides

- Wear gloves and work in the darkroom (using only a red safety light)
- Allow Kodak-NB2 emulsion (which comes as a thick white gel and is stored in the dark at 4°C) to come to room temperature for 2 hours.
- Mix equal volumes of Kodak-NB2 and double distilled water together (say 5ml of each), place in a water bath at 37°C and shake gently for about 15 mins.
- Dip slides vertically into a small amount of emulsion (about 2ml is all that is required) in a holder designed to take one slide at a time (economises on the amount of emulsion)
- Place horizontally for about 15 minutes to air dry. Then stand vertically for at least 2 hours to dry.
- Transfer to a black, light free box and store in the fridge (4°C) with dessicant.
- Allow exposure time as specified e.g. 2 weeks. Or remove test slides at various times to determine optimal exposure time for your particular situation.

Developing the film

- Use a dark room.
- Use Kodak D19 developer mixed 50:50 with water. Immerse slides for 4 mins.
- Wash in gently running tap water.
- Wash in double distilled water
- Use Ilford Hypan Rapid Fixer (leaflet T1812). Mix 40ml+160ml double distilled water +2ml Hypan hardener. Immerse slides for 5mins.
- Wash in gently running tap water.
- Note: once it is fixed can turn the lights on and tidy up. But make sure all sensitive film is put away before you do this.

X-ray film This is still widely used for macroscopic analysis of big specimens (not requiring a microscope). This film has much bigger crystal diameters and comes on hard sheets. It is traditionally used for analysing gels where the separated proteins or nucleic acids are labelled with radioisotopes.

Phosphoimager screen This is a new variation on detection of bands in radioactively labelled gels This has (i) very high sensitivity, (ii) a shorter development time and (iii) a major advantage is that the amount of signal gives a linear increase over a wide range of labelling intensities making quantitation very easy. Radioactive signal activates fluorescence in the screen (nothing is visible = latent image). The screen is scanned on a special densitometer,

hooked to a computer which produces a digital picture. Can enhance the image and quantify the intensity of the signal. Can easily clear the screen and re-use.

Radioisotopes are used to trace molecules in cells and organisms

Tracer studies: Radioisotope labelling is uniquely valuable as a way to distinguish between molecules that are chemically identical but have different histories - for example those that differ in their time of synthesis. The earliest uses of autoradiography were for tracer studies e.g. radioactivity was used to label various molecules such as amino acids and then the way they were assembled into proteins over time throughout the cell could be followed. This technique was essential to understand:

- oxidative respiration,
- photosynthesis,
- the control of protein synthesis by nucleic acids,
- the timing of events throughout the cell cycle (Alberts p730),
- the fate of populations of cells - i.e number of cell divisions, migration, relationship of stem cells to the final differentiated phenotypes (by tritiated thmyidine labelling of replicating cells - see lecture on Cell Replication).
- Comparison of experimental treatments on events such as above

Pulse chase is used to sharpen the resolution of timing in many of these experiments.

Analytical techniques: Radioactive labelling of various molecules enables the **binding of these molecules** (as markers of other molecules) to be accurately monitored by radioisotope cytochemistry e.g:

- enzyme inhibitors,
- antibodies
- nucleic acid probes

In molecular biology experiments , S^{35} P^{32} (and I^{125}) are widely used to label nucleic acid probes to detect mRNA by *in situ* hybridisation on tissue sections and also for quantitation by Northern analysis on gels. Radioisotope labelling has great sensitivity but the disadvantage that each time a hybridisation is performed, the probe has to be labelled with fresh radioisotope (since it decays rapidly) and this can be tedious and expensive. Furthermore radioisotopes are dangerous (especially I^{125}). For these reasons digoxygenin is now often favoured for labelling probes for *in situ* hybridisation studies (it is detected by an antibody and a colour reaction), particularly since digoxygenin-labelled probe is stable for many months.

Ingestion: Radioactive isotopes are also used to track the distribution and retention of ingested materials. Exotic radioisotopes with very short half-lives are used clinically

Dosimetry

Whilst **Dosimetry** in its original sense is the measurement of the absorbed dose delivered by ionizing radiation, the term is better known as a scientific sub-specialty in the fields of health physics and medical physics, where it is the calculation and assessment of the radiation dose received by the human body.

Internal dosimetry due to the ingestion or inhalation of radioactive materials relies on a variety of physiological or imaging techniques. External dosimetry, due to irradiation from an external source is based on measurements with a dosimeter, or inferred from other radiological protection instruments.

Dosimetry is used extensively for radiation protection and is routinely applied to occupational radiation workers, where irradiation is expected, but regulatory levels must not be exceeded. It is also used where radiation is unexpected, such as in the aftermath of the Three Mile Island, Chernobyl or Fukushima radiological release incidents, where the public irradiation is measured and calculated from a variety of indicators such as ambient measurements of radiation and radioactive contamination.

Other significant areas are medical dosimetry, where the required treatment absorbed dose and any collateral absorbed dose is monitored, and in environmental dosimetry, such as radon monitoring in buildings.

A **radiation dosimeter** is a device that measures exposure to ionizing radiation. It has two main uses: for human radiation protection and for measurement of dose in both medical and industrial processes

Measuring radiation dose

External dose

There are several ways of measuring absorbed doses from ionizing radiation. People in occupational contact with radioactive substances or who may be exposed to radiation routinely carry personal dosimeters. These are specifically designed to record and indicate the absorbed dose (or derived dosimetric index) received. Traditionally these were badges containing photographic film (film badge dosimeter), which would be chemically developed following exposure to indicate the total absorbed dose received. Film badges have now been

largely replaced with other devices such as the TLD badge which uses Thermoluminescent dosimetry or optically stimulated luminescence (OSL) badges.

A number of electronic devices known as Electronic Personal Dosimeters (EPDs) have come into general use using semiconductor detection and programmable processor technology. These are worn as badges, but can give an indication of instantaneous dose rate and an audible and visual alarm if a dose rate or a total integrated dose is exceeded. A good deal of information can be made immediately available to the wearer of the recorded dose and current dose rate via a local display. They can be used as the main stand-alone dosimeter, or as a supplement to such as a TLD badge. These devices are particularly useful for real-time monitoring of dose where a high dose rate is expected which will time-limit the wearer's exposure.

The ICRP states that if a personal dosimeter is worn on a position on the body representative of its exposure, assuming whole-body exposure, the value of ambient dose equivalent H(10) is sufficient to provide an effective dose value suitable for radiological protection.[1]

In certain circumstances dose can be inferred from readings taken by fixed instrumentation in an area in which the person concerned has been working. This would generally only be used if personal dosimetry had not been issued, or a personal dosimeter has been damaged or lost. Such calculations would take a pessimistic view of the likely received dose.

Internal Dose

Internal dosimetry is the science and art of internal ionising radiation dose assessment due to radionuclides incorporated inside the human body.[1]

Radionuclides deposited within a body will irradiate tissues and organs and give rise to committed dose until they are excreted from the body or the radionuclide is completely decayed.

The internal doses for workers or members of the public exposed to the intake of radioactive particulates can be estimated using bioassay data such as lung and body counter measurements, urine or faecal radioisotope concentration, etc. The International Commission on Radiological Protection (ICRP) biokinetic models are applied to establish a relationship between the individual intake and the bioassay measurements, and then to infer the internal dose.

The internal radiation dose due to ingested or inhaled radioactive substances is known as committed dose.

Medical dosimetry

Medical dosimetry is the calculation of absorbed dose and optimization of dose delivery in radiation therapy. It is often performed by a professional medical dosimetrist with specialized training in the field. In order to plan the delivery of radiation therapy, the radiation produced by the sources is usually characterized with percentage depth dose curvesand dose profiles measured by medical physicists.

In radiation therapy, three-dimensional dose distributions are often evaluated using the dosimetry technique known as gel dosimetry.

Environmental Dosimetry

Environmental Dosimetry is used where it is likely that the environment will generate a significant radiation dose. An example of this is radon monitoring. Radon is a radioactive gas generated by the decay of uranium, which is present in varying amounts in the earth's crust. Certain geographic areas, due to the underlying geology, continually generate radon which permeates its way to the earth's surface. In some cases the dose can be significant in buildings where the gas can accumulate. A number of specialised dosimetry techniques are used to evaluate the dose that a building's occupants may receive.

Measures of Dose

There are a number of different measures of radiation dose, including absorbed dose (*D*) measured in grays(Gy), Equivalent dose (*H*) measured in sieverts (Sv), Effective dose (*E*) (also measured in sieverts) andKerma (K) measured in grays, along with dose area product (DAP) and dose length product (DLP). Each measure is often simply described as 'dose', which can lead to confusion. Non-SI units are still used, particularly in the USA, where dose is often reported in rads and dose equivalent in rems. By definition, 1 Gy = 100 rad and 1 Sv = 100 rem.

The fundamental quantity is the absorbed dose (*D*), which is defined as the mean energy imparted [by ionising radiation] (dE) per unit mass (dm) of material (D = dE/dm)[3] The SI unit of absorbed dose is the gray (Gy) defined as one joule per kilogram. Absorbed dose, as a point measurement, is suitable for describing localised (i.e. partial organ) exposures such as tumour dose in radiotherapy. It may be used to estimate stochastic risk provided the amount and type of tissue involved is stated. Localised diagnostic dose levels are typically in the 0-50 mGy range. At a dose of 1 milligray (mGy) of photon radiation, each cell nucleus is crossed by an average of 1 liberated electron track

Equivalent dose

The absorbed dose required to produce a certain biological effect varies between different types of radiation, such as photons, neutrons or alpha particles. This is taken into account by the equivalent dose (H), which is defined as the mean dose to organ T by radiation type R ($D_{T,R}$), multiplied by a weighting factor W_R . This designed to take into account the biological effectiveness (RBE) of the radiation type,[3] For instance, for the same absorbed dose in Gy, alpha particles are 20 times as biologically potent as X or gamma rays. The measure of 'dose equivalent' is not organ averaged and now only used for "operational quantities". Equivalent dose is designed for estimation of stochastic risks from radiation exposures. Stochastic effect is defined for radiation dose assessment as the *probability* of cancer induction and genetic damage.[5]

As dose is averaged over the whole organ; equivalent dose is rarely suitable for evaluation of acute radiation effects or tumour dose in radiotherapy. In the case of estimation of stochastic effects, assuming a linear dose response, this averaging out should make no difference as the total energy imparted remains the same.

Effective dose

Effective dose is the central dose quantity for radiological protection used to specify exposure limits to ensure that the occurrence of stochastic health effects is kept below unacceptable levels and that tissue reactions are avoided.[7]

It is difficult to compare the stochastic risk from localised exposures of different parts of the body (e.g. a chest x-ray compared to a CT scan of the head), or to compare exposures of the same body part but with different exposure patterns (e.g. a cardiac CT scan with a cardiac nuclear medicine scan). One way to avoid this problem is to simply average out a localised dose over the whole body. The problem of this approach is that the stochastic risk of cancer induction varies from one tissue to another.

The effective dose E is designed to account for this variation by the application of specific weighting factors for each tissue (W_T). Effective dose provides the equivalent whole body dose that gives the same risk as the localised exposure. It is defined as the sum of equivalent doses to each organ (H_T), each multiplied by its respective tissue weighting factor (W_T).

Weighting factors are calculated by the International Commission for Radiological Protection (ICRP), based on the risk of cancer induction for each organ and adjusted for associated lethality, quality of life and years of life lost. Organs that are remote from the site of irradiation will only receive a small equivalent dose (mainly due to scattering) and therefore contribute little to the effective dose, even if the weighting factor for that organ is high.

Effective dose is used to estimate stochastic risks for a 'reference' person, which is an average of the population. It is not suitable for estimating stochastic risk for individual medical exposures, and is not used to assess acute radiation effects

Radio frequency (RF) dosimetry is the quantification of the magnitude and distribution of absorbed electromagnetic energy within biological objects that are exposed to RF fields. At RF, the dosimetric quantity, which is called the specific absorption rate (SAR), is defined as the rate at which energy is absorbed per unit mass. The SAR is determined not only by the incident electromagnetic waves but also by the electrical and geometric characteristics of the irradiated subject and nearby objects. It is related to the internal electric field strength (E) as well as to the electric conductivity and the density of tissues; therefore, it is a suitable dosimetric parameter, even when a mechanism is determined to be "athermal." SAR distributions are usually determined from measurements in human models, in animal tissues, or from calculations.

RADIOFREQUENCY DOSIMETRY: WIRELESS HANDSETS

R. C. Petersen

Dosimetry at radiofrequencies (RF) is the quantification of the distribution of energy absorbed within objects exposed to RF electromagnetic fields. At frequencies between about 100 kHz and 6 GHz, the dosimetric quantities of interest are specific absorption (SA) and specific absorption rate (SAR). Specific absorption, which is defined as the energy absorbed by an incremental mass of an exposed object divided by that mass, is expressed in units of joules per kilogram (J/kg). Specific absorption *rate*, which is defined as the rate at which energy is absorbed by an incremental mass of an exposed object divided by that mass, is expressed in units of watts per kilogram (W/kg) (NCRP 1981). An SAR of 1 W/kg corresponds to the conversion of one joule of energy per kilogram per second. Thus, specific absorption is analogous to "dose" and SAR is analogous to "dose rate." At frequencies below about 100 kHz, the current induced in an object is an important dosimetric parameter; at frequencies above about 6 GHz, where the energy is absorbed superficially (the depth of penetration is roughly equal to about one-tenth of a wavelength), the energy flux-density (J/cm^2) and the power density (W/m^2) incident on the object are important.

X-ray Powder Diffraction (XRD)

X-ray powder diffraction (XRD) is a rapid analytical technique primarily used for phase identification of a crystalline material and can provide information on unit cell dimensions. The analyzed material is finely ground, homogenized, and average bulk composition is determined.

Fundamental Principles of X-ray Powder Diffraction

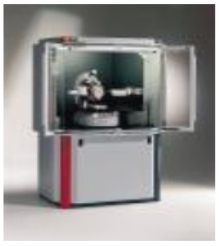
Max von Laue, in 1912, discovered that crystalline substances act as three-dimensional diffraction gratings for X-ray wavelengths similar to the spacing of planes in a crystal lattice. X-ray diffraction is now a common technique for the study of crystal structures and atomic spacing.

X-ray diffraction is based on constructive interference of monochromatic X-rays and a crystalline sample. These X-rays are generated by a cathode ray tube, filtered to produce monochromatic radiation, collimated to concentrate, and directed toward the sample. The interaction of the incident rays with the sample produces constructive interference (and a diffracted ray) when conditions satisfy Bragg's Law ($n\lambda=2d \sin\theta$). This law relates the wavelength of electromagnetic radiation to the diffraction angle and the lattice spacing in a crystalline sample. These diffracted X-rays are then detected, processed and counted. By scanning the sample through a range of 2θangles, all possible diffraction directions of the lattice should be attained due to the random orientation of the powdered material. Conversion of the diffraction peaks to d-spacings allows identification of the mineral because each mineral has a set of unique d-spacings. Typically, this is achieved by comparison of d-spacings with standard reference patterns.

All diffraction methods are based on generation of X-rays in an X-ray tube. These X-rays are directed at the sample, and the diffracted rays are collected. A key component of all diffraction is the angle between the incident and diffracted rays. Powder and single crystal diffraction vary in instrumentation beyond this.

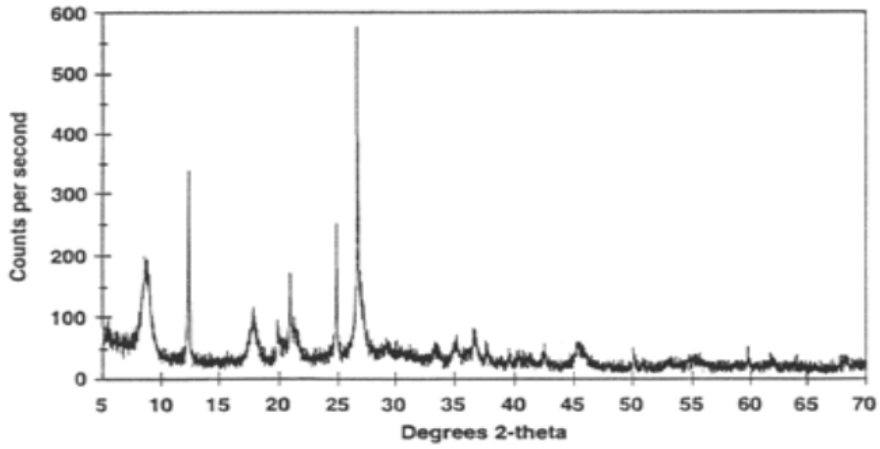
X-ray Powder Diffraction (XRD) Instrumentation

X-ray diffractometers consist of three basic elements: an X-ray tube, a sample holder, and an X-ray detector.



Bruker's X-ray Diffraction D8-Discover instrument.

X-rays are generated in a cathode ray tube by heating a filament to produce electrons, accelerating the electrons toward a target by applying a voltage, and bombarding the target material with electrons. When electrons have sufficient energy to dislodge inner shell electrons of the target material, characteristic X-ray spectra are produced. These spectra consist of several components, the most common being K_α and K_β. K_α consists, in part, of $K_{\alpha1}$ and $K_{\alpha2}$. $K_{\alpha1}$ has a slightly shorter wavelength and twice the intensity as $K_{\alpha2}$. The specific wavelengths are characteristic of the target material (Cu, Fe, Mo, Cr). Filtering, by foils or crystal monochrometers, is required to produce monochromatic X-rays needed for diffraction. $K_{\alpha1}$and $K_{\alpha2}$ are sufficiently close in wavelength such that a weighted average of the two is used. Copper is the most common target material for single-crystal diffraction, with CuK_α radiation = 1.5418Å. These X-rays are collimated and directed onto the sample. As the sample and detector are rotated, the intensity of the reflected X-rays is recorded. When the geometry of the incident X-rays impinging the sample satisfies the Bragg Equation, constructive interference occurs and a peak in intensity occurs. A detector records and processes this X-ray signal and converts the signal to a count rate which is then output to a device such as a printer or computer monitor.



The geometry of an X-ray diffractometer is such that the sample rotates in the path of the collimated X-ray beam at an angle θ while the X-ray detector is mounted on an arm to collect the diffracted X-rays and rotates at an angle of 2θ. The instrument used to maintain the angle and rotate the sample is termed a*goniometer*. For typical powder patterns, data is collected at 2θ from ~5° to 70°, angles that are preset in the X-ray scan.

Applications

X-ray powder diffraction is most widely used for the identification of unknown crystalline materials (e.g. minerals, inorganic compounds). Determination of unknown solids is critical to studies in geology, environmental science, material science, engineering and biology.

Other applications include:

- characterization of crystalline materials
- identification of fine-grained minerals such as clays and mixed layer clays that are difficult to determine optically
- determination of unit cell dimensions
- measurement of sample purity

With specialized techniques, XRD can be used to:

- determine crystal structures using Rietveld refinement
- determine of modal amounts of minerals (quantitative analysis)
- characterize thin films samples by:
    - determining lattice mismatch between film and substrate and to inferring stress and strain
    - determining dislocation density and quality of the film by rocking curve measurements
    - measuring superlattices in multilayered epitaxial structures
    - determining the thickness, roughness and density of the film using glancing incidence X-ray reflectivity measurements
- make textural measurements, such as the orientation of grains, in a polycrystalline sample

Strengths and Limitations of X-ray Powder Diffraction

Strengths

- Powerful and rapid (< 20 min) technique for identification of an unknown mineral
- In most cases, it provides an unambiguous mineral determination
- Minimal sample preparation is required
- XRD units are widely available
- Data interpretation is relatively straight forward

Limitations

- Homogeneous and single phase material is best for identification of an unknown
- Must have access to a standard reference file of inorganic compounds (d-spacings, *hkl*s)

- Requires tenths of a gram of material which must be ground into a powder
- For mixed materials, detection limit is ~ 2% of sample
- For unit cell determinations, indexing of patterns for non-isometric crystal systems is complicated
- Peak overlay may occur and worsens for high angle 'reflections'

User's Guide - Sample Collection and Preparation

Determination of an unknown requires: the material, an instrument for grinding, and a sample holder.

- Obtain a few tenths of a gram (or more) of the material, as pure as possible
- Grind the sample to a fine powder, typically in a fluid to minimize inducing extra strain (surface energy) that can offset peak positions, and to randomize orientation. Powder less than ~10 μm(or 200-mesh) in size is preferred
- Place into a sample holder or onto the sample surface:
    - smear uniformly onto a glass slide, assuring a flat upper surface
    - pack into a sample container
    - sprinkle on double sticky tape

Typically the substrate is amorphous to avoid interference

- Care must be taken to create a flat upper surface and to achieve a random distribution of lattice orientations unless creating an oriented smear.
- For analysis of clays which require a single orientation, specialized techniques for preparation of clay samples are given by USGS.

For unit cell determinations, a small amount of a standard with known peak positions (that do not interfere with the sample) can be added and used to correct peak positions.