IMMUNOLOGICAL TECHNIQUES
UNIT IV

Principle, procedure and application of immunodiffusion

Immunodiffusion is a technique used to detect and quantify the presence of specific antigens or antibodies in a sample. The principle of immunodiffusion is based on the diffusion of antigen and antibody molecules through a gel matrix, which allows the formation of visible precipitin lines where the antigens and antibodies interact.

The basic steps of immunodiffusion are:

- Preparation of antigen and antibody solutions: The antigen and antibody solutions are prepared in separate vials.
- Preparation of the gel: A gel containing a diffusible agent, such as agar, is prepared in a Petri dish.
- Formation of wells: Wells are made in the gel using a punch or another tool.
- Addition of antigen and antibody solutions: The antigen and antibody solutions are added to the separate wells in the gel.
- Diffusion: The gel is allowed to incubate for a period of time to allow diffusion of the antigen and antibody molecules through the gel.
- Detection: The formation of precipitin lines where the antigen and antibody have interacted is visualized and measured.

Immunodiffusion has a wide range of applications in both research and clinical settings. Some of the key applications include:

- Detection of specific antigens or antibodies in patient samples: Immunodiffusion can be used to detect the presence of specific antigens or antibodies in serum or other bodily fluids, which is useful for diagnosing infectious diseases or autoimmune disorders.
- Quantification of antigen-antibody interactions: The size and intensity of the precipitin lines in immunodiffusion can be used to quantify the strength of antigen-antibody interactions, which is useful for determining the relative concentration of specific antigens or antibodies in a sample.
- Characterization of antigenic properties: Immunodiffusion can be used to characterize the antigenic properties of different molecules, such as proteins, by determining their reactivity with specific antibodies.

- Purification of antigens or antibodies: Immunodiffusion can be used to purify specific antigens or antibodies from a mixture by using an antibody to trap the target antigen or antibody in the gel.
- Overall, immunodiffusion is a useful technique for detecting and quantifying specific antigens or antibodies in a variety of samples, and has numerous applications in research, medicine, and biotechnology.

Immuno-electrophoresis

Immunoelectrophoresis is a technique that combines immunodiffusion and electrophoresis to separate and identify individual proteins in a sample based on their antigenic properties. In this technique, an electric field is used to separate proteins in a sample based on their size and charge, while antibodies are used to identify and visualize the proteins of interest.

The basic steps of immunoelectrophoresis are:

- Preparation of the gel: A gel containing a diffusible agent, such as agarose, is prepared in a rectangular tray.
- Application of the sample: The protein sample is applied to the gel and allowed to migrate a short distance into the gel.
- Electrophoresis: An electric current is applied across the gel, which causes the proteins to migrate towards the oppositely charged electrode. As the proteins migrate, they separate based on their size and charge.
- Addition of antibodies: After electrophoresis, wells are cut into the gel and antibodies specific to the protein of interest are added to each well. The antibodies diffuse into the gel and bind to the corresponding protein, forming a visible precipitin line.
- Visualization: The location of the precipitin line indicates the position of the protein of interest in the gel. The size and intensity of the line can also provide information on the amount of protein present.

Immunoelectrophoresis has several applications in research and clinical settings, including:

- Analysis of serum proteins: Immunoelectrophoresis can be used to identify and quantify individual serum proteins in a patient's blood, which can be useful for diagnosing diseases and monitoring treatment.

- Characterization of proteins: Immunoelectrophoresis can be used to identify and characterize individual proteins based on their antigenic properties.
- Purification of proteins: Immunoelectrophoresis can be used to purify specific proteins from a mixture based on their antigenic properties, by using antibodies to trap the target protein in the gel.
- Overall, immunoelectrophoresis is a powerful tool for separating and identifying individual proteins based on their antigenic properties, and has numerous applications in research and clinical settings.

Enzyme linked immunosorbent assay (ELISA)

Enzyme-linked immunosorbent assay (ELISA) is a widely used laboratory technique that uses antibodies and enzymes to detect the presence and quantity of specific substances, such as proteins, in a sample. It is a highly sensitive and specific method that is commonly used in medical and research laboratories for diagnosing diseases, detecting viruses and bacteria, and measuring levels of hormones and other molecules in biological fluids.

The basic steps of an ELISA are:

- Coating the plate: A microtiter plate is coated with an antigen, which can be a protein or another molecule that is specific to the target substance being detected.
- Blocking the plate: The remaining protein binding sites on the plate are blocked with a protein such as bovine serum albumin (BSA) to prevent nonspecific binding.
- Adding the sample: The sample, which may contain the target substance, is added to the plate and incubated.
- Adding the primary antibody: A primary antibody specific to the target substance is added to the plate and incubated. If the target substance is present in the sample, it will bind to the antigen on the plate.
- Adding the secondary antibody: A secondary antibody, which is specific to the primary antibody and is conjugated to an enzyme such as horseradish peroxidase (HRP), is added to the plate and incubated. This antibody binds to the primary antibody and creates an enzyme-linked complex.

- Adding the substrate: A substrate specific to the enzyme is added to the plate, and if the enzyme is present, it will convert the substrate into a detectable product. The amount of product produced is directly proportional to the amount of target substance in the sample.
- Reading the results: The product is usually a color change that can be measured using a spectrophotometer, and the amount of product is used to determine the concentration of the target substance in the sample.

ELISA has several advantages over other methods of detecting target substances, such as its high sensitivity, specificity, and speed. ELISA can be used in a variety of applications, including:

- Diagnosis of diseases: ELISA is commonly used to diagnose infectious diseases, such as HIV, hepatitis, and Lyme disease, by detecting antibodies or antigens in patient samples.
- Detection of drugs and hormones: ELISA can be used to measure the concentration of drugs and hormones in blood or urine samples, which can be useful in therapeutic drug monitoring and drug testing.
- Research: ELISA can be used to measure the levels of specific proteins or other molecules in biological samples, which can be useful in basic research and drug development.
- Overall, ELISA is a powerful and versatile technique that has many applications in medicine, research, and industry.

Radioimmunoassay (RIA)

Immunological assays are analytical methods that use the interaction between antigens and antibodies to detect and quantify various biological molecules. Radioimmunoassay (RIA) is an immunological technique that uses radioactive isotopes to detect and quantify antigens or antibodies in a sample. RIA is used to detect and measure small amounts of substances in biological samples, such as hormones, drugs, and proteins.

The basic steps of RIA are:

- Antigen preparation: The antigen is labeled with a radioactive isotope, such as iodine-125.
- Antibody preparation: An antibody specific to the antigen is prepared and added to the sample.
- Adding the sample: The sample containing the antigen is added to the mixture of labeled antigen and antibody.

- Separation of the bound and unbound antigen: A separation step is carried out to separate the antigen-antibody complex (bound fraction) from the unbound antigen.
- Measuring the radioactivity: The radioactivity of the bound and unbound fractions is measured separately using a gamma counter.
- Calculating the concentration: The concentration of the antigen in the sample is calculated based on the ratio of the bound to unbound radioactivity.

RIA is a sensitive and specific method for detecting and quantifying antigens or antibodies in a sample, and it has many applications in medical research and clinical diagnosis. For example, RIA is used to measure the levels of hormones, such as thyroid hormone and insulin, in blood samples. RIA can also be used to detect the presence of drugs or toxins in body fluids and tissues.

Radioisotope techniques: Concept of radioisotopes

Radioisotopes are isotopes of an element that have unstable nuclei, which undergo radioactive decay to attain stability. In the process of decay, the radioactive isotopes emit radiation in the form of alpha, beta, or gamma particles. The use of radioisotopes in various fields is based on the property of radioactivity, which allows them to be detected and monitored using radiation detectors.

Radioisotope techniques involve the use of radioactive isotopes to study biological, chemical, and physical processes. The radioactive isotopes are usually introduced into a system or a sample in a specific way, and their behavior is monitored by measuring the radiation emitted. These techniques have applications in medical diagnosis and treatment, industrial processes, and scientific research.

In medicine, radioisotopes are used for diagnostic imaging, such as in PET (positron emission tomography) scans and SPECT (single-photon emission computed tomography) scans. They are also used for radiation therapy to treat cancer. In industry, radioisotopes are used for quality control and process monitoring. In scientific research, radioisotopes are used to trace metabolic pathways and study the kinetics of chemical reactions.

Types and properties of radioactive decay

Radioactive decay is the process by which an unstable atomic nucleus loses energy by emitting particles or radiation. There are several types of radioactive decay, including:

- Alpha decay: In alpha decay, an atomic nucleus emits an alpha particle, which consists of two protons and two neutrons. This process reduces the atomic number by 2 and the mass number by 4.
- Beta decay: In beta decay, a neutron in the atomic nucleus is converted into a proton and an electron (beta particle). The electron is emitted from the nucleus, while the proton remains. This process increases the atomic number by 1 and does not change the mass number.
- Gamma decay: Gamma decay is the emission of gamma radiation from an excited atomic nucleus. Gamma rays are high-energy electromagnetic radiation and do not change the atomic or mass number.
- Electron capture: In electron capture, an electron from the surrounding electron cloud is captured by the nucleus, combining with a proton to form a neutron. This process decreases the atomic number by 1 and does not change the mass number.
- Positron emission: In positron emission, a proton in the nucleus is converted into a neutron and a positron (a positively charged electron). The positron is emitted from the nucleus, while the neutron remains. This process decreases the atomic number by 1 and does not change the mass number.

The properties of radioactive decay depend on the type of decay. Alpha decay releases the most energy, followed by beta and gamma decay. Alpha particles have the lowest penetrating power and can be stopped by a sheet of paper, while beta particles can penetrate several millimeters of material and gamma rays can penetrate several centimeters of material. The half-life of a radioactive isotope is the time it takes for half of the atoms in a sample to decay, and it is characteristic of each isotope.

Units of radioactivity

Radioactivity is the property of certain materials to emit ionizing radiation due to the decay of unstable atomic nuclei. The activity of a radioactive material is measured in units of becquerel (Bq), which is defined as one decay per second. The becquerel is the SI unit of radioactivity.

Another unit of radioactivity commonly used is the curie (Ci), which is equal to 3.7 x 10^10 becquerels. The curie was originally defined as the activity of one gram of radium-226, a highly

radioactive element. However, this definition has been replaced by the becquerel as the international standard.

Radioactivity is also often expressed in terms of radiation dose. The absorbed dose is the amount of energy absorbed per unit mass of tissue, and is measured in units of gray (Gy). The equivalent dose takes into account the different biological effects of different types of ionizing radiation, and is measured in units of sievert (Sv). The sievert is a measure of the effective dose, which is the absorbed dose multiplied by a factor that depends on the type of radiation and the tissue being irradiated.

It is important to note that even small doses of ionizing radiation can have harmful effects on living organisms, and exposure should be minimized as much as possible.

Characteristics of radioisotopes commonly used in biology

Radioisotopes are used in a variety of biological applications, including tracer studies, radiolabeling, and imaging. The choice of radioisotope depends on the specific application, as different isotopes have different properties and decay characteristics. Some of the commonly used radioisotopes in biology and their characteristics are:

- Carbon-14 (14C): Carbon-14 is a beta emitter with a half-life of 5,730 years. It is commonly used in radiocarbon dating to determine the age of organic materials, and in tracer studies to track the movement of carbon in biological systems.
- Hydrogen-3 (3H or tritium): Tritium is a beta emitter with a half-life of 12.3 years. It is commonly used in radiolabeling studies to track the movement of hydrogen in biological systems.
- Phosphorus-32 (32P): Phosphorus-32 is a beta emitter with a half-life of 14.3 days. It is commonly used in radiolabeling studies to track the movement of phosphorus in biological systems.
- Iodine-125 (125I): Iodine-125 is a gamma emitter with a half-life of 59.4 days. It is commonly used in radioimmunoassays to detect the presence of specific antigens or antibodies.

- Technetium-99m (99mTc): Technetium-99m is a gamma emitter with a half-life of 6 hours. It is commonly used in medical imaging, particularly in single-photon emission computed tomography (SPECT).
- Fluorine-18 (18F): Fluorine-18 is a positron emitter with a half-life of 109.8 minutes. It is commonly used in positron emission tomography (PET) imaging to visualize metabolic activity in biological tissues.

These radioisotopes have different properties, such as half-life, emission type, and energy, which make them useful for different applications in biology. However, it is important to handle radioisotopes with care and follow appropriate safety protocols to minimize exposure and ensure the safety of researchers and the environment.

Measurement of radioactivity

Radioactivity is typically measured using a device called a radiation detector or radiation survey meter. These instruments can detect and measure the intensity of different types of radiation emitted by radioactive isotopes, such as alpha, beta, and gamma radiation.

One common method for measuring radioactivity is counting the number of radioactive decay events that occur in a given sample over a certain period of time. This can be done using a device called a Geiger counter or a scintillation detector. The Geiger counter detects ionizing radiation by measuring the electrical charge produced by the radiation in a gas-filled tube, while a scintillation detector detects radiation by measuring the light emitted when the radiation interacts with a scintillating material.

Another method for measuring radioactivity is to measure the amount of energy absorbed by a sample of material, which is proportional to the number of radioactive decay events that occur. This can be done using devices such as a gamma-ray spectrometer, which detects and measures the energy of gamma radiation emitted by radioactive isotopes.

Radioactivity is typically measured in units of becquerels (Bq) or curies (Ci), which indicate the number of radioactive decays per second. One becquerel is equal to one radioactive decay per second, while one curie is equal to 3.7 x 10^10 radioactive decays per second.

It is important to handle radioactive materials safely and follow appropriate protocols to minimize exposure and ensure the safety of researchers and the environment. Regulations and guidelines for handling radioactive materials vary by country and institution, but typically involve measures such as shielding, containment, and monitoring to minimize exposure to radiation.