Chapter-6

Electrophoresis

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Introduction

Based on their size and electrical charge, charged molecules such as DNA, RNA, and proteins can be separated and analyzed in a lab setting using an approach called electrophoresis.It functions by subjecting a solution containing the combination of molecules to an electric field, which causes the molecules to migrate across a gel matrix at varying rates.

Imagine a race where the runners (molecules) are carrying different weights (size) and wearing shoes with varying degrees of grip (charge). The electric field acts like a downhill slope, urging the runners forward. Smaller molecules with less weight and a strong grip will move faster through the gel, while larger molecules with more weight and a weaker grip will move slower.

History

The groundwork for electrophoresis was laid in the early 19th century. Building on the principles of electrochemistry established by Michael Faraday, Russian scientists Franz Ferdinand Reuss and Peter IvanovichStrakhov, in 1807, saw how an electric field affected the movement of clay particles suspended in water. The basis for comprehending the motion of charged particles in an electric field was established by this first observation.

Arne Tiselius received the Nobel Prize in 1948 for his groundbreaking work in the field of electrophoresis, which he completed in 1931. Tiselius performed separation of protein molecules on a free liquid medium. His technique at primitive stage had so many problems like adverse effect of diffusion and convection current. Further improvement on the technique was done on the various parts of the electrophoretic unit and in 1937 he also developed Tiselius apparatus for moving boundary electrophoresis.

Electrophoresis has been a vital tool in molecular biology and biochemistry since the 1970s.It has been essential to the advancement of several methods, including:DNA fingerprinting,Gel electrophoresis for Southern blot and Northern blot analysis, DNA sequencing, Protein analysis and purification.

Principle

The term electrophoresis is made of two words, Elecro (*Eng.- Electricity*) + phorēsis (*Greek- being carried*). Migration of a charged particle or molecule under the influence of a spatially homogeneous electrical field is known as electrophoresis.All biomolecules—peptides, proteins, nucleotides, and nucleic acids—have ionisable groups, which means that they are electrically charged species that exist in solution at all pH values as either cations (+) or anions (-). This property is used to separate DNA, RNA, and proteins based on their mass and net charge.Depending on the nature of their net charge, these charged particles will migrate to either the cathode or the anode when subjected to an electric field.

Nucleic acids and proteins, which are charged molecules, migrate towards the electrode that has the opposite charge when an electric field is applied across a medium. The rate at which a molecule migrates depends on its charge-to-mass ratio. Higher charge and lower mass result in faster migration. Samples are loaded to a medium. Commonly used loading medium include Nitrocellulose membrane, agarose gel (for nucleic acids) and polyacrylamide (for proteins and smaller nucleic acids). Molecules are separated according to size using the gel as a kind of filter. The size-based separation is further aided by the fact that smaller molecules can pass through the pores more readily than larger ones.

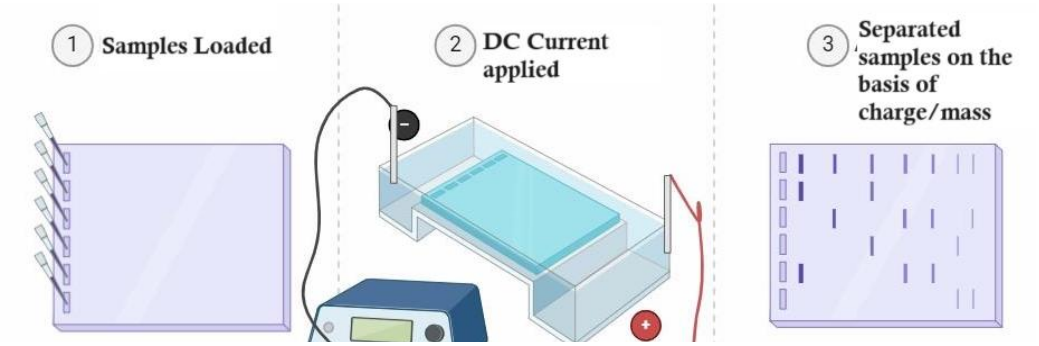

Figure 1: An Illustration of Basic Steps of Electrophoresis

Types of Electrophoresis

There are many modification in electrophoresis setups on the basis of which, its types has been categorised. Following types have been discussed here.

Paper Electrophoresis

A straightforward and conventional method of electrophoresis for separating and analyzing small charged molecules like peptides, short nucleotides, and amino acids is paper electrophoresis. In this approach, molecules migrate across a supporting medium—a sheet of filter paper saturated in a buffer solution—when they are subjected to an electric field.

Principle

Based on the fundamentals of electrophoresis, which involves the migration of charged particles through a medium in the presence of an electric field, paper electrophoresis works. The migration and separation of molecules according to their size and charge are facilitated by the paper.

Materials and Setup

1. **Paper:** Filter paper or chromatography paper is used. The paper must be of uniform thickness and free of impurities to ensure consistent results.

2. **Buffer Solution:** The paper is soaked in a buffer solution that maintains a constant pH and provides the necessary ions for conductivity.

3. **Electrophoresis Apparatus:** consists of an anode and a cathode that are coupled to a power source.

4. **Sample Application:** Samples are applied as small spots or lines onto the paper.

Procedure

1. **Preparation of Electrophoretic Paper:** The filter paper of appropriate quality is cut into the desired shape and size and dipped in the buffer solution to maintain a stable pH through out the experiment.

2. **Application of the Sample:** At some distance away from one end of the paper a line is drawn, very small amount of the sample is loaded on to that line.

3. **Arrangement of Electrophoretic Chamber:** The two chambers at the ends of the electrophoretic setup are filled with electrophoretic buffer. Each chamber has an electrode of opposite charges, the filter paper is put in such a way that the ends of the paper can touch both the buffer. In this way filter paper acts to maintain the continuity of the oppositely charged tanks of buffer.

4. **Turning on the Electricity:** When the power supply is turned on the electric field is generated along the paper strip and molecules charge molecules in the sample starts migrating towards electrodes of the opposite charge and gets separated forming some bands.

5. **Visualisation of the Samples:** The separated samples on the paper strips are visualised using various techniques like nynhydrin, auto radiography etc.

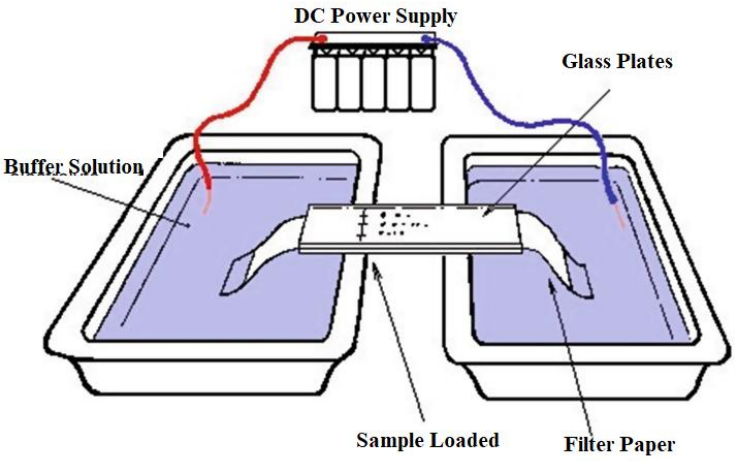

Figure 2: A Diagrammatic Illustration of Paper Electrophoresis

(Basha, M. (2020). Electrophoresis. In: Analytical Techniques in Biochemistry. Springer Protocols Handbooks. Humana, New York, NY. https://doi.org/10.1007/978-1-0716-0134-1_6**)**

Advantages of Paper Electrophoresis

1. **Cost Efficiency:** For the setup of this type of electrophoresis very less cost is required.

2. **Simple Setup:** The setup of the apparatus is very simple and doesn't involve sophistication.

3. **Training Purpose:** The simplicity of the setup provides understanding of basic principle related to electrophoresis and that is why it is basically used for learning purpose.

Disadvantages

1. **Less Sample:** Very less amount of the sample can be loaded at a time.

2. **Integrity of Material:** The loading material of the electrophoresis is a filter paper which has very low integrity and very fragile certificate difficult to handle the wet paper which can break.

3. **Low Resolution:** The resolving power of the setup is very low bands after separation get dissolved easily so the bands are not very fine and separable.

Applications

- The analysis of egg white protein, milk proteins, muscle proteins, insect and snake venoms is mainly done by paper electrophoresis
- For the routine analysis of serum for diagnosis purpose is carried out by this technique.

Starch Gel Electrophoresis

A laboratory method called starch gel electrophoresis is used to separate proteins according to their size, shape, and charge. This technique makes use of the starch gel's polysaccharide matrix to help proteins migrate when exposed to an electric field, enabling scientists to examine and distinguish between different protein components in a sample.

Principles

1. **Basis of Separation:** Starch gel electrophoresis separates proteins through their differential migration in an electric field applied across a starch gel matrix. The proteins move according to their net charge, size, and shape. The gel matrix provides a medium that impedes the movement of larger molecules more than smaller ones, enabling size-based separation.

2. **Starch Gel Composition:** Starch gels are composed of a mixture of amylose and amylopectin, two polysaccharides that form a semi-solid matrix when hydrated and cooked. The concentration of starch can be adjusted to optimize the resolution of protein separation, with typical concentrations ranging from 5% to 15%.

3. **Electrophoretic Mobility:** The electrophoretic mobility of a protein in starch gel is influenced by its charge at the given pH of the buffer system. Higher net charge proteins will migrate more quickly than lower net charge proteins. To improve separation efficiency, the buffer's pH and ionic strength can be changed.

Steps Involved

1. **Preparation of Starch Gel**

    - **Starch Solution Preparation:** The appropriate amount of starch powder in an electrophoresis buffer is dissolved. The solution is heated while stirring until the starch is completely dissolved and forms a clear, viscous solution.

    - **Gel Casting:** The hot starch solution is poured into a mold or gel casting tray. The gel is allowed to cool and solidify, forming a firm matrix suitable for electrophoresis.

    - **Gel Loading:** Wells are created in the gel using a comb or other tool. These wells will hold the protein samples to be analyzed.

2. **Sample Preparation**

    - **Protein Extraction:** The proteins are isolated from the sample using suitable extraction methods, such as homogenization or sonication, followed by centrifugation to remove debris.

- **Sample Buffer Addition:** The protein sample is mixed with a sample buffer that may contain tracking dyes and reducing agents to facilitate loading and tracking during electrophoresis.

3. Electrophoresis Setup

- **Buffer System:** The appropriate running buffer is selected which can facilitate protein migration through the gel. Some commonly used buffers include Tris-glycine and citrate-phosphate.

- **Assembly:** The gel is placed in an electrophoresis apparatus, ensuring that the gel is submerged in the running buffer.

- **Sample Loading:** The already-prepared samples of proteins are carefully transferred to the gel's wells.

4. Running the Gel

- **Power Supply:** The gel apparatus is coupled to the power supply unit. The appropriate voltage is applied to initiate protein migration through the starch gel.

- **Monitoring:** The progress of electrophoresis is observed by tracking the movement of the dye front, which indicates the progression of protein separation.

5. Staining and Visualization

- **Gel Fixation:** After electrophoresis, the proteins are fixed in the gel using an appropriate fixative, such as trichloroacetic acid (TCA) or methanol-acetic acid solution.

- **Staining:** To see the separated protein bands, the gel is stained with a protein-specific stain, like silver stain or Coomassie Brilliant Blue stain.

- **Destaining:** This gel is destainedto remove excess stain, enhancing the contrast of the protein bands for better visualization.

Applications of Starch Gel Electrophoresis

1. **Protein Analysis**

    - Identifying and characterizing proteins based on their electrophoretic mobility.
    - Comparing protein profiles across different samples or species.
    - Detecting protein modifications such as phosphorylation or glycosylation.

2. **Enzyme Assays:** Enzymes can be separated and identified using starch gel electrophoresis, followed by specific activity staining to locate enzyme activity zones within the gel.

3. **Genetic Studies:** This technique is utilized in genetic studies to analyze variations in protein expression and to map genetic loci associated with specific proteins or enzymes.

4. **Medical Diagnostics:** Starch gel electrophoresis aids in diagnosing certain diseases by analyzing protein patterns in biological samples, such as serum or urine.

Agarose Gel Electrophoresis

This commonly used molecular biology approach uses an agarose gel matrix to separate and analyze DNA and RNA fragments according to size. It is especially helpful for identifying the size of DNA fragments, assessing the quantity and quality of nucleic acids, and preparing DNA for additional research.

Principle

Under the influence of an electric field, negatively charged DNA or RNA molecules migrate across an agarose matrix in order to perform agarose gel electrophoresis. Size-based separation is possible because smaller fragments go through the gel matrix more quickly and extensively than bigger ones.

Smaller molecules are able to pass through the pores of the gel more readily than bigger ones, acting as a sieve. This difference in movement leads to separation based on size.

Materials and Setup

1. **Agarose:** A polysaccharide obtained from seaweed, agarose melts in a solution and cools to form a porous gel.

2. **Buffer:** Tris-acetate-EDTA (TAE) and Tris-borate-EDTA (TBE) are two often used buffers that supply the ions required to conduct electricity and keep a steady pH.

3. **Gel Casting Tray and Comb:** Used to cast the agarose gel and create wells for loading samples.

4. **Electrophoresis Apparatus:** Consists of a gel box (chamber) having electrodes with a power supply.

5. **DNA Ladder/Marker:** A mixture of known-sized DNA fragments used as a guide to gauge the sizes of unknown DNA pieces is called a DNA ladder or marker.

6. **Loading Buffer:** Contains a tracking dye (e.g., bromophenol blue) and glycerol to increase sample density for easier loading.

7. **Staining Dye:** Ethidium bromide or SYBR Safe is used to stain the DNA and visualize it under UV light

Procedure

1. **Preparation of the Gel**

    - **Gel Concentration:** Agarose concentration typically ranges from 0.5% to 2%, depending on the molecular mass of DNA fragments which is subjected to be resolved. Lower concentrations are used for larger fragments, while higher concentrations are for smaller fragments.

    - **Melting Agarose:** Agarose powder is dissolved in the chosen buffer by heating until completely melted.

    - **Casting the Gel:** A comb is put into a casting tray along with the melted agarose to create wells. The gel is left to solidify in a refrigerator or at room temperature.

2. **Loading the Samples**

    - **Preparing Samples:** DNA samples are mixed with loading buffer.

    - **Loading Wells:** The gel's wells are properly pipetted with samples and a DNA ladder.

3. **Running the Gel**

    - **Buffer in Gel Box:** The same buffer used to produce the gel is poured into the electrophoresis chamber along with the formed gel.

    - **Applying Voltage:** The negatively charged DNA pieces move toward the positive electrode (anode) when an electric field is introduced.

4. **Separation**

    - **Migration:** Smaller DNA fragments migrate quicker and further than larger ones, causing them to segregate based on size.

5. **Staining and Visualization**

    - **Staining:** A DNA-binding dye, such as ethidium bromide, which intercalates between DNA bases and fluoresces under UV light, is used to stain the gel after the run.

    - **Visualization:** The stained gel is placed on a UV transilluminator or gel documentation system to visualize and photograph the DNA bands.

6. **Analysis**

    - **Comparing Bands:** To determine the sizes of the DNA fragments, the migration distances of the fragments in the sample lanes are compared to those of the DNA ladder.

    - **Documentation:** Images of the gel are captured for further analysis and record-keeping.

Applications

1. **DNA Fragment Analysis:** Assessing the size of PCR products, restriction enzyme digests, and other DNA fragments.

2. **Quality Control:** Checking the integrity and purity of DNA and RNA samples.

3. **Cloning and Sequencing:** Preparing DNA fragments for cloning, sequencing, or other downstream applications.

4. **Genotyping and Mutation Detection:** Analyzing genetic variations and mutations.

Advantages

1. **Simplicity:** Easy to perform with basic laboratory equipment.

2. **Cost-Effective:** Relatively inexpensive materials and reagents.

3. **Versatility:** Suitable for a wide range of DNA fragment sizes.

Limitations

1. **Resolution:** Limited resolution for very small or very large DNA fragments compared to other techniques like polyacrylamide gel electrophoresis (PAGE).

2. **Staining Sensitivity:** Some dyes like ethidium bromide are mutagenic and require careful handling and disposal.

Isoelectric Focusing (IEF)

Proteins can be separated according to their isoelectric points (pI), or the pH at which they have no net electrical charge, using the extremely accurate and efficient method of isoelectric focusing (IEF). This essay delves into the principles, methodology, and applications of IEF, underscoring its importance in protein analysis and proteomics.

A protein is formed by the addition of so many amino acids having various natures. The side chains of amino acids are having acidic, basic, polar or non-polar characteristics depending upon the type of amino acid. These side chains get ionised on different pH of the solution conferring charge on that particular amino acid. In this way a protein has differently charged sites on it which decide the net charge on protein. So the pH of solution in which the protein has net zero charge is called isoelectric point (pI). At pI the protein theoretically should not move towards or away from any electrode.

Principles of Isoelectric Focusing

The movement of proteins in an electric field until they arrive at a pH gradient location where their net charge is zero is the fundamental idea behind IEF.

1. **Isoelectric Point (pI):** Every protein has a certain pH at which it has no net charge, known as its isoelectric point. The protein has a positive charge below this pH and a negative charge above this pH.

2. **pH Gradient:** IEF utilizes a stable pH gradient within a gel matrix. This gradient is typically created using a mixture of ampholytes, molecules that can carry both positive and negative charges depending on the pH.

3. **Electric Field:** Proteins move over a pH gradient when an electric field is applied. Proteins that are negatively charged travel toward the anode (positive electrode) and positively charged proteins move toward the cathode (negative electrode). Migration continues until the proteins reach their pI and stop moving because they have no net charge.

4. **Electrophoresis:** An electric field is applied after the sample has been put to the gel or IPG strip. Proteins follow the gradient until they get to their proper pI.

5. **Stabilization:** After focusing, the pH gradient is stabilized, and the proteins are fixed in place to prevent diffusion.

6. **Detection:** Coomassie Brilliant Blue and other staining techniques can be used to visualize the separated proteins.

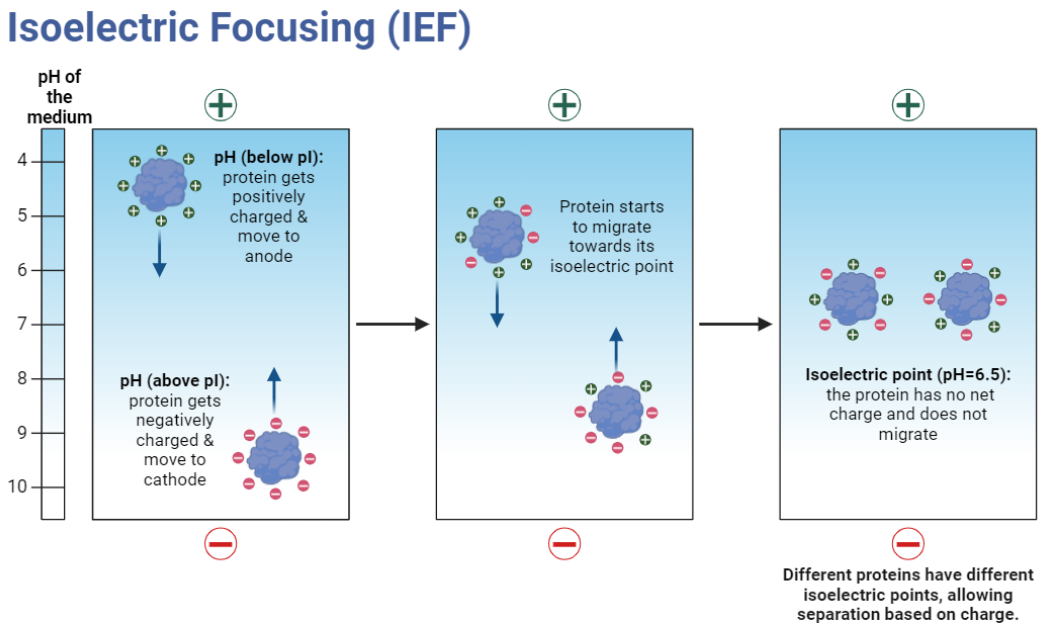

Figure 3: Movement of the Proteins in pH Gradient Gel Due to Influence of Electric Field

Applications of Isoelectric Focusing

IEF is widely used in various fields of biological research and biotechnology due to its precision and effectiveness. Key applications include:

1. **Protein Purification:** IEF is employed to purify proteins from complex mixtures, allowing researchers to isolate proteins of interest based on their pI.

2. **Characterization of Protein Isoforms:** IEF is particularly useful for identifying and characterizing protein isoforms and post-translational modifications, which can result in changes to the pI.

3. **Comparative Proteomics:** IEF is used in comparative proteomics to analyze differences in protein expression profiles between different samples, such as healthy and diseased tissues.

4. **Pharmaceuticals and Biotechnology:** IEF is used in quality control and analysis of pharmaceutical products, including the characterization of therapeutic proteins and antibodies.

5. **Clinical Diagnostics:** IEF can help diagnose diseases by identifying specific protein biomarkers in biological fluids like blood or urine.

Advantages

1. **High Resolution:** It can resolve proteins that differ by very small pI differences, providing high-resolution separation.

2. **Quantitative:** IEF allows for the quantification of proteins based on the intensity of the stained bands or spots.

3. **Reproducibility:** The use of IPG strips provides consistent and reproducible results.

Limitations

1. **Complexity:** The technique can be complex and requires careful preparation and handling.

2. **Protein Solubility:** Some proteins, particularly hydrophobic ones, may be difficult to solubilize and focus effectively.

3. **Sample Size:** IEF generally requires relatively large amounts of protein sample for effective separation and detection.

SDS-PAGE

In molecular biology and biochemistry, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) is a vital procedure. It enables scientists to divide intricate protein combinations according to nothing more than their molecular weight (size). This powerful tool finds numerous applications, from determining protein size to monitoring protein expression and purity.

Principle

The separation principle of SDS-PAGE hinges on two key strategies:

1. **Standardizing Protein Charge:** Proteins possess inherent charges because of the presence of acidic and basic amino acids. These charges influence their movement in an electric field. SDS-PAGE addresses this variability by introducing Sodium Dodecyl Sulfate (SDS). SDS, a detergent, unfolds and binds to proteins along their entire length, coating them with a uniform negative charge. This effectively masks the protein's original charge, ensuring all proteins migrate towards the positive electrode (anode) during electrophoresis.

   The application of beta mercaptoethanol is very essential in linearizing of the protein. It causes breaking of disulphide bonds present in the protein.

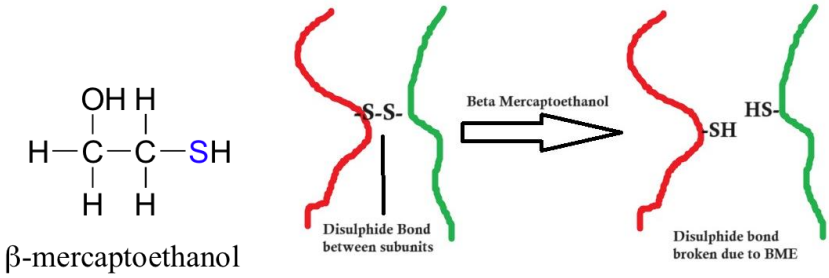

Figure 4:

2. **Size-Based Separation in a Gel Matrix:** Protein samples that have been treated with SDS are put onto polyacrylamide gel, a separation medium.This gel is made up by the polymerization of two monomers i.e. Acrylamide and Bis-Acrylamide.

Acrylamide forms linear polymer to which bis acrylamide makes cross link (branching). The higher the proportion of bis-acrylamide, the more cross-linking occurs, which results in a smaller pore size in the gel. Hence the pore size can be determined for the molecule to be separated by determining the ratio of acrylamide to Bis acrylamide. The polymerisation of Acrylamide and bis acrylamide takes place by free radical formation which is initiated by addition of TEMED (N,N,N',N'-tetramethylenediamine) and APS (ammonium persulphate). Smaller proteins navigate these pores with greater ease compared to larger proteins. Smaller proteins pass through the narrower pores more quickly and with less resistance as the negatively charged protein-SDS complexes flow through the gel under the influence of the electric field. Consequently, the proteins separate based solely on their size (length of the polypeptide chain).

Steps Involved in SDS-PAGE

1. **Gel Preparation:** Polyacrylamide gels are prepared with varying acrylamide percentages to achieve different pore sizes for optimal separation based on the expected protein size range.

2. **Sample Preparation:** Protein samples are combined with an SDS and β mercaptoethanol reducing agent solution. By breaking down disulfide links in the protein, the reducing agent guarantees full unfolding and stops interactions between protein molecules.

3. **Electrophoresis:** Samples and protein standards (proteins with known molecular weights) are loaded into the wells of the gel. The negatively charged protein-SDS complexes move through the gel matrix and toward the anode when an electric current is introduced.

4. **Protein Visualization:** After separation, proteins are not directly visible. Staining techniques, such as Coomassie Brilliant Blue, are employed to visualize the protein bands. Alternatively, Western blotting can be used to identify specific proteins of interest.

5. **Analysis:** The positions of the protein bands in the gel are compared to protein standards to estimate their molecular weight. The band pattern provides valuable information about the protein composition of the sample.

Applications of SDS-PAGE

1. **Determining Protein Molecular Weight:** Researchers can determine an unknown protein's size by comparing its migration with standards of known molecular weight proteins.

2. **Monitoring Protein Purity:** SDS-PAGE reveals the presence of multiple protein bands, indicating the purity of the sample. A single band signifies a purified protein.

3. **Analyzing Protein Expression Levels:** Researchers can compare protein band intensity in gels loaded with samples from different conditions to assess changes in protein expression.

4. **Identifying Specific Proteins:** Western blotting, a technique often coupled with SDS-PAGE, allows researchers to identify specific proteins of interest within a complex mixture using specific antibodies.

5. **Structural studies:** The SDS and beta mercaptoethanol applied in this technique disintegrates the bonds between multi-subunit proteins. The numbers of various subunit the protein can be determined by application of this technique.

Advantages of SDS-PAGE

1. **High Resolution:** SDS-PAGE offers excellent resolution, allowing separation of proteins with very similar sizes.

2. **Relatively Simple Procedure:** While SDS-PAGE requires specific equipment and careful handling, the basic principles and steps are readily understandable.

3. **Versatility:** The technique can be adapted for various protein samples and research objectives.

4. **Wide Applications:** SDS-PAGE is a cornerstone technique in numerous areas of biological research.

Limitations of SDS-PAGE

1. **Limited to Proteins:** SDS-PAGE is specific to proteins and cannot be used for separating other biomolecules like nucleic acids or lipids.

2. **Denaturation of Proteins:** SDS and the reducing agent denature proteins, meaning they lose their native structure and function. This may limit downstream applications requiring functional proteins.

3. **Inability to Separate Proteins with Similar Sizes and Charges:** SDS-PAGE might not be able to completely resolve proteins with very similar sizes or those with residual positive charges after SDS binding.

2D Gel Electrophoresis

2D gel electrophoresis (2DE) is a useful analytical technique used to separate and analyze complex mixtures of proteins. This technique divides proteins according to their molecular weight (MW) and isoelectric point (pI), two unique characteristics. This method provides high-resolution separation and is widely employed in proteomics for protein identification, characterization, and comparative analysis.

Principles of 2D Gel Electrophoresis

The foundation of 2D gel electrophoresis lies in its ability to resolve proteins more effectively than one-dimensional methods. The technique comprises two sequential separation processes:

1. **Isoelectric Focusing (IEF):** Proteins are categorized according to their isoelectric points in the first dimension. This entails dousing a gel with a pH gradient with a protein mixture. Proteins with various pIs can be successfully separated by migrating to the pH point (isoelectric point) under the influence of an electric field.

2. **SDS-PAGE (Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis):** In the second dimension, proteins previously separated by IEF are further separated by molecular weight. This step involves placing the IEF-resolved proteins onto an SDS-PAGE gel. Denatured proteins receive a consistent negative charge proportional to their length from SDS, a detergent. Smaller proteins move more quickly across the gel matrix when an electric field is introduced than larger ones.

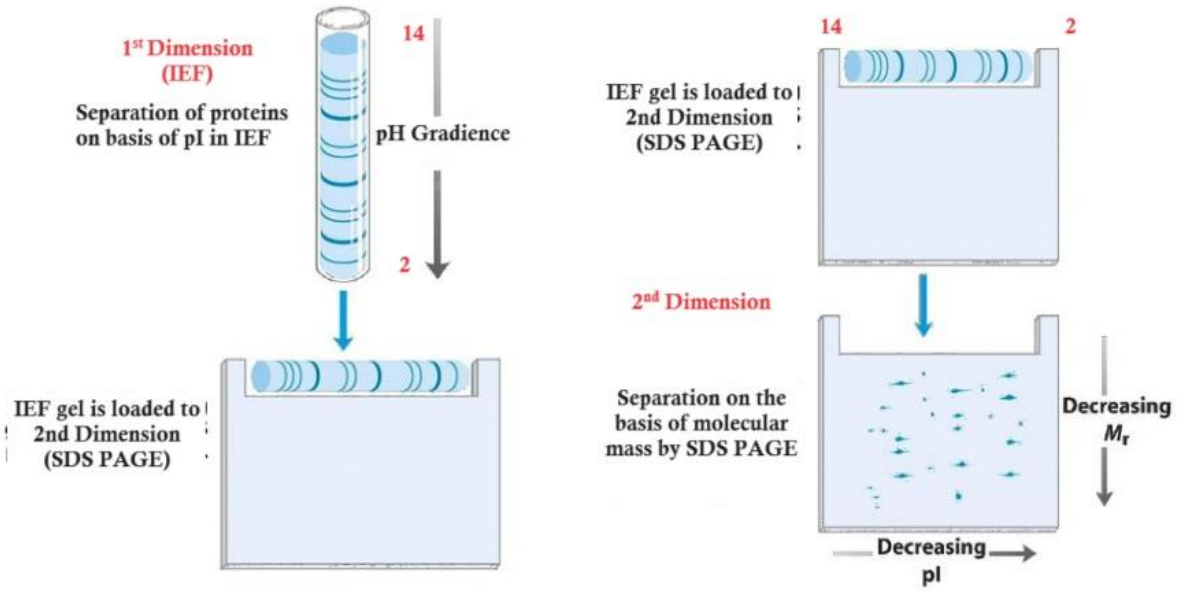

Figure 5: Representation of Both Dimensions of 2D Gel Electrophoresis

The Process of 2D Gel Electrophoresis Involves Several Key Steps

1. **Sample Preparation:** After extracting the proteins from the biological material, the proteins are quantified and dissolved in a buffer that contains ampholytes, reducing agents, and chaperones.

2. **First-Dimension Isoelectric Focusing:** An immobilized pH gradient (IPG) strip is coated with the protein sample. Proteins concentrate into distinct bands over a few hours as they travel to their corresponding isoelectric points.

3. **Equilibration:** The IPG strip is equilibrated in a buffer containing SDS and reducing agents, which prepares the proteins for the second-dimension separation.

4. **Second-Dimension SDS-PAG**E: An SDS-PAGE gel is positioned on top of the equilibrated strip. The proteins are separated according to their molecular weights using an electric field.

5. **Visualization:** Dyes like silver stain or Coomassie Brilliant Blue are used to stain the isolated proteins. The stained gel is then analyzed, and protein spots are identified.

Applications of 2D Gel Electrophoresis

2D gel electrophoresis is widely used in various fields of biological research and biotechnology due to its high resolution and ability to analyze complex protein mixtures. Key applications include:

1. **Proteomics:** 2DE is a basic technique in proteomics, enabling the identification and characterization of proteins, their isoforms, and post-translational modifications. It is instrumental in comparing protein expression profiles under different conditions, such as healthy vs. diseased states.

2. **Biomarker Discovery:** By comparing protein patterns between samples, researchers can identify potential biomarkers for diseases, aiding in early diagnosis and therapeutic interventions.

3. **Functional Genomics:** 2DE helps in understanding gene function by analyzing the expression of proteins encoded by genes. It complements genomic studies by providing information on the actual protein products.

4. **Comparative Analysis:** It is used to compare proteomes of different species or strains, providing insights into evolutionary relationships and functional diversity.

Advantages

1. **High Resolution:** It can resolve thousands of proteins simultaneously, providing a comprehensive protein profile.

2. **Post-Translational Modifications:** The technique allows the detection of protein isoforms and post-translational modifications, which are crucial for understanding protein function.Compatibility with downstream analytical techniques like mass spectrometry.

Limitations

- Labor-intensive and time-consuming.
- Limited ability to resolve very high or low molecular weight proteins.
- Hydrophobic and membrane proteins are often underrepresented.

Immunoelectrophoresis

This is a laboratory technique combining electrophoresis and immunodiffusion to analyze and characterize proteins, especially those in complex mixtures such as serum. Using the specificity of antigen-antibody interactions, this technique allows for the identification and measurement of individual proteins.

Principles of Immunoelectrophoresis

The underlying principle of immunoelectrophoresis involves two main steps:

1. **Electrophoresis:** Proteins are first separated based on their charge and size by electrophoresis. This step involves applying an electric field to a gel containing the protein mixture, causing proteins to migrate and separate into distinct bands.

2. **Immuno Diffusion:** Antibodies that are particular to the target proteins are added to the gel following electrophoresis. When these antibodies come into contact with their matching antigens (proteins), they diffuse across the gel and produce lines of precipitin that are visible. Based on antigen-antibody interactions, the creation of these lines both confirms the existence of particular proteins and makes their identification possible.

Methodology of Immuno Electrophoresis

1. **Sample Preparation:** The protein mixture (e.g., serum) is prepared and applied to a gel matrix, usually agarose or cellulose acetate.

2. **Electrophoresis:** The application of an electric field induces protein migration through the gel. The size and charge of the proteins affect migration direction and rate.

3. **Application of Antibodies:** After electrophoresis, a trough is cut parallel to the direction of protein migration, and antibodies are applied to this trough. Alternatively, antibodies can be placed in wells perpendicular to the migration direction.

4. **Immuno Diffusion:** The antibodies and proteins diffuse through the gel. Where the antibodies meet their specific antigens, they form visible precipitin lines, indicating the presence of specific proteins.

5. **Visualization:** The precipitin lines are visualized and analyzed. The identification and concentration of the proteins in the sample can be inferred from the position, quantity, and intensity of these lines.

Applications of Immuno Electrophoresis

1. **Clinical Diagnostics:** Immunoelectrophoresis is widely used to diagnose and monitor diseases by detecting abnormalities in protein composition. It is particularly valuable for identifying monoclonal and polyclonal gammopathies, such as multiple myeloma and other immunoglobulin disorders.

2. **Characterization of Serum Proteins:** This technique helps in the qualitative and semi-quantitative analysis of serum proteins, enabling the identification of specific proteins like immunoglobulins, complement components, and acute-phase reactants.

3. **Protein Purification and Analysis:** Immunoelectrophoresis can be used to assess the purity and identity of proteins in research and biotechnology, ensuring the quality of protein preparations.

4. **Research in Immunology:** The method is employed to study antigen-antibody interactions, providing insights into immune responses and the specificity of antibody binding.

Advantages

1. **Specificity:** The use of specific antibodies allows for the precise identification of proteins.

2. **Versatility:** It can analyze complex protein mixtures and is applicable to various biological fluids.

3. **Visualization:** The precipitin lines provide a clear visual representation of antigen-antibody interactions.

Limitations

1. **Resolution:** The technique may not separate proteins with similar electrophoretic properties effectively.

2. **Sensitivity:** It may need relatively large quantities of proteins and antibodies and is less sensitive than other methods like western blotting or the enzyme-linked immunosorbent assay (ELISA).

3. **Quantification:** While providing qualitative and semi-quantitative information, immunoelectrophoresis is not as precise in quantifying proteins as some other methods.