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REVIEW: AN APPROACH TO POLYACRYLAMIDE GEL ELECTROPHORESIS (PAGE)

Divyanshi Kothari*; Prem Kumar Bichala[1]; Neha Batra[2]; Deeksha Jain[3]

*School of Pharmaceutical Sciences, Pratap University, Jaipur, Rajasthan, divyanshikothari6@gmail.com
[1]School of Pharmaceutical Sciences, Pratap University, Jaipur, Rajasthan, prem.kumar@pratapuniversity.in
[2]LBR College of Pharmacy, Panchkula, Haryana, nehabatrapharma@gmail.com
[3]Babulal Tarabai Institute of Pharmaceutical Science, Sironja, Sagar, Madhya Pradesh, deekshajain2801@gmail.com

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Abstract:

Polyacrylamide gel electrophoresis (PAGE) provides a versatile, gentle, high resolution method for fractionation and physical-chemical characterization of molecules on the basis of size, conformation, and net charge. It is a technique used almost universally in life science laboratories. The goal of this technique is to separate a mixed sample of proteins, to identify and quantify single proteins from the mixture. It is also used to separate DNA and RNA, although proteins remain the most common sample type. It can also be used to resolve RNA protein complexes and to detect RNA complex formation by analyzing changes in the electrophoretic mobility of the RNA.

Major advantage of PAGE is its high resolving power (sometimes 1 in 500 bp).

Keywords: Native PAGE, Denaturing PAGE, SDS-PAGE, BIS, Resolving gel, Stacking gel, Ammonium per sulphate, TEMED, Cleaver Scientific, omniPAGE, tris-Glycine, TBE, Western blot, Coomassie brilliant blue, EMSA, UPEP, SPEP.

1. Introduction

Electrophoretic techniques separate charged molecules in an electric field. The mobility of a molecule is inversely proportional to its size and directly proportional to its charge. During electrophoresis, proteins move towards an oppositely charged electrode in an electric field.

The rate of their movement in an electrophoretic system is governed by several factors such as temperature, pH, and buffer concentration in addition to intrinsic properties such as the size, charge and shape of the proteins.

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Electrophoretic separation of proteins strictly on the basis of their molecular weight is possible only if the charge of all the protein molecules can be manipulated to the same sign. In such a case, the mobility of the protein molecules will be solely reliant on their size.

Polyacrylamide gel electrophoresis (PAGE) is a technique based on this idea and is used to separate proteins on the basis of their size.

PAGE is a technique that separates macromolecules such as proteins based on their electrophoretic mobility, that is, the ability of analytes to move towards an electrode of the opposite charge. In PAGE, this is determined by the charge, size (molecular weight) and shape of the molecule. Analytes move through pores formed in polyacrylamide gel. Unlike DNA and RNA, proteins vary in charge according to the amino acids incorporated, which can influence how they run. Amino acid strings may also form secondary structures that impact their apparent size and consequently how they are able to move through the pores. It may therefore sometimes be desirable to denature proteins prior to electrophoresis to linearize them if a more accurate estimate of size is required.

There are two types of PAGE commonly used for various purposes and they are **non-denaturing** or **native-PAGE** and **denaturing PAGE**. In native PAGE, double-stranded DNA migrates according to its sizes and the voltage applied in the gel is low (1–8 V/cm). In the case of denaturing PAGE, denaturing agents like urea, formamide, etc., are used to denature DNA. The movement of DNA is based on the composition and sequence of the fragment. This sort of gel is used for DNA sequencing.

1.1 SDS PAGE vs Native PAGE

PAGE can be run under denaturing or non-denaturing conditions, depending on the purpose of the analysis. The anionic detergent, sodium dodecyl sulphate (SDS), in combination with heat and sometimes a reducing agent is used to denature proteins prior to electrophoretic separation in a process known as SDS PAGE. The heat disrupts the hydrogen bonds that hold secondary and tertiary structures while a reducing agent, such as β-mercaptoethanol, cleaves disulfide bridges. Proteins are linearized and complex with the SDS so that all have a similar mass-to-charge ratio. This eliminates the influence of structure and charge, and proteins are separated solely on the basis of differences in their molecular weight (Figure 1.1.1). This system was developed by Ulrich K. Laemmli and is typically used to separate proteins of 5–250 kDa.

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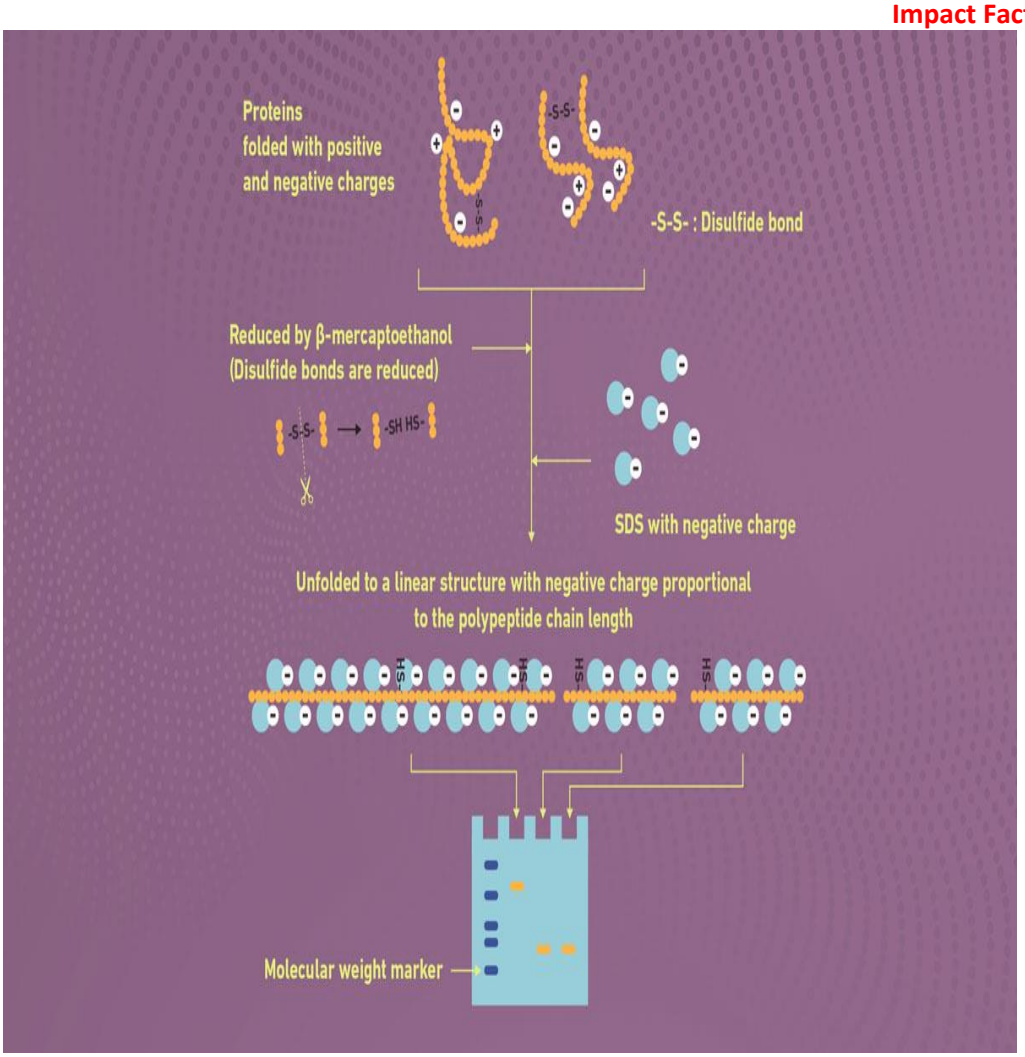

Figure 1.1.1: Linearization of proteins for SDS PAGE. Disulfide bonds are reduced by β-mercaptoethanol while SDS negates differences in mass-to-charge ratios so that proteins are separated on the basis of molecular weight.

In native PAGE these bonds are left intact, preserving the protein's higher order structure. Consequently, the distribution of proteins through the gel is mainly influenced by the protein's charge (determined by its amino acid sequence and post-translational modifications) and the pH of the separation rather than its size in kDa. However, it allows researchers to analyze proteins in their natural or "native" state. This may be desirable when analyzing bound proteins or complexes, for example, when it is important their biological activity remains intact. As the aim here is to preserve the natural state of the protein, SDS, reducing agents and heat are not used in sample preparation and lower voltages may also be used for separation.

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2. PRINCIPLE OF PAGE

In PAGE, an anionic detergent called sodium dodecyl sulfate (SDS) is used to bind to proteins and give them a negative charge. Proteins are then separated electrophoretically according to their size using a gel matrix made of polyacrylamide in an electric field.

Polyacrylamide is produced as a result of the polymerization reaction between acrylamide and N,N'-methylene-bis-acrylamide (BIS) using a catalyst. The degree of polymerization or cross-linking can be controlled by adjusting the concentration of acrylamide and BIS.

The more the cross-linking, the harder the gel. Hardness of the gel, in turn, modulates the friction experienced by macromolecules when they travel through the gel during PAGE, thus affecting the resolution of separation.

Loose gels (4-8% acrylamide) allow higher molecular weight molecules to migrate faster through the gel while hard gels (12-20% acrylamide) restrict the migration of large molecules and selectively allow small ones to move through the gel.

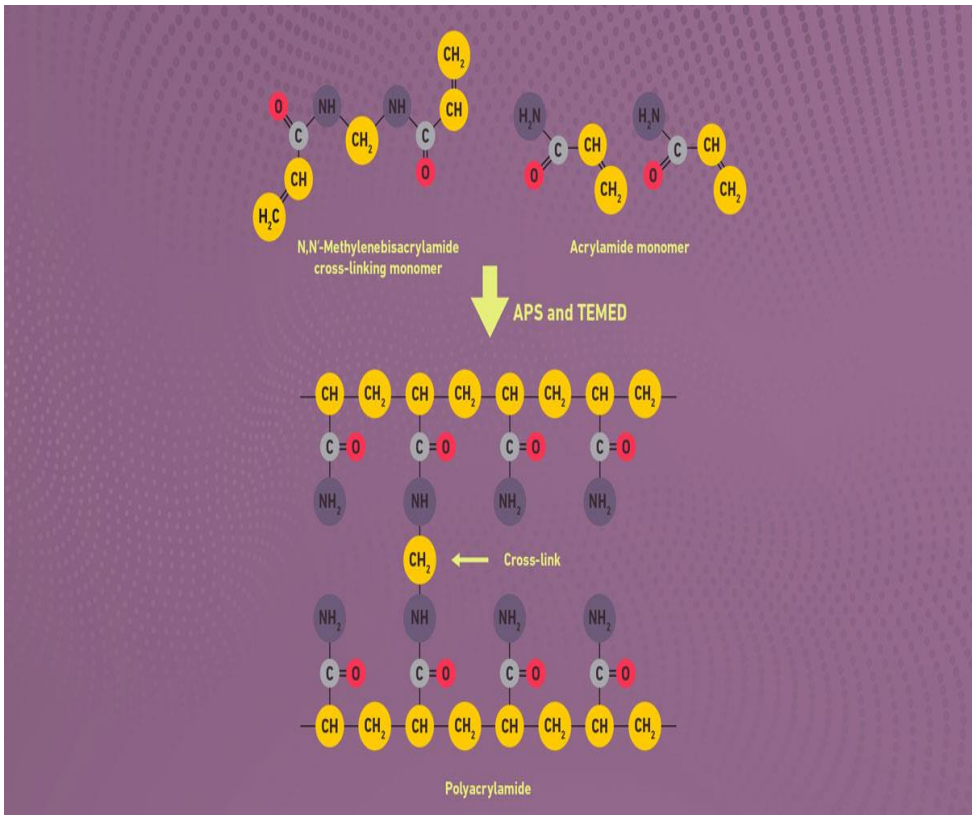

Figure 2.1: Polymerization and crosslinking of acrylamide. APS catalyzed by TEMED leads to the polymerization and crosslinking of acrylamide. The total concentration of acrylamide components and ratio of acrylamide to bisacrylamide affects the gel's pore size and therefore the range of protein sizes that can be resolved.

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3. WORKING MECHANISM

There are several types of PAGE technique that are used, but the most common is called SDS-PAGE. In SDS-PAGE the detergent - sodium dodecyl sulfate - is used to denature the proteins and normalise their mass-to-charge ratio. Without SDS, both the molecular weight and the charge of the protein would affect its separation in the gel. With SDS, only the molecular weight affects the migration speed and so samples separate according to this. PAGE without SDS is called native PAGE, as the proteins stay in their native conformation.

3.1 Equipment for Polyacrylamide Gel Electrophoresis

Irrespective of the type of PAGE gel being run, the equipment set up is the same. However, if you are switching between running SDS PAGE and native PAGE, make sure that all equipment is cleaned thoroughly, or have a separate set for each type, if possible, to avoid cross contamination of denaturing agents into the native analysis. Glass plates, spacers, a comb (used to create the sample wells) and casting frame are required to make the gel. The size of the spacers and comb will depend on the volume and number of samples you wish to run. It is important that the glass plates are cleaned and dried thoroughly prior to assembly to prevent poor quality gels or leaks when the gel is poured. Protein-based residues that are not removed may otherwise mar your gel when it is stained.

To run the gel, an electrophoresis tank, power pack and electrophoresis frame (which carries the current through the gel) will also be required.

a) Gel Tank/Gel Box

The electrophoretic gel usually has several components including acrylamide, BIS, and a buffer. The mixture is degassed to prevent bubble formation during polymerization of the gel. Ammonium persulfate, a free radical source, and a stabilizer are added to start polymerization. BIS is also added to form cross-links between acrylamide molecules until a gel is ultimately formed.

The gel is prepared by polymerizing acrylamide with the cross-linking agent *N*,*N*′-methylenebisacrylamide (bis-acrylamide). The polymerization process is accelerated by ammonium persulfate and is stabilized by *N*,*N*,*N*′,*N*′-tetramethylethylenediamine.

As with agarose gel electrophoresis, the samples are separated using an electrical field, and pass through a gel matrix which influences the migration of the proteins. In PAGE, rather than agarose, we use a chemical called polyacrylamide. Varying the percentage of polyacrylamide in the gel change the size of the pores in the gel, which means different sizes of protein in different percentage gels can be separated. Typical gel percentages are shown in the table below:

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Acrylamide Percentage	Separating Resolution
5 %	60 – 220 Kd
7.5 %	30 – 120 Kd
10 %	20 – 75 Kd
12%	17 – 65 Kd
15 %	15 -45 Kd
17.5%	12 – 30 Kd

Acrylamide is normally sold in a liquid form, as the powder form is neurotoxic and dangerous to handle. Polymerisation is achieved by mixing acrylamide with bis-acrylamide, which allows cross-links to form between the acrylamide molecules. Additional chemicals are added to initiate the polymerisation, usually ammonium persulphate as a source of free radicals and TEMED as a stabiliser. Once the polymerisation begins the gel is poured between 2 glass plates and allowed to completely polymerise.

The gel mixture is made up not in water but in electrophoresis buffer (Tris-HCl) that provides ions for electrophoresis. Often, the gel is poured in 2 parts. The first part is a resolving gel, with a pH around 8.8 which slows the migration of the proteins. Above the resolving gel, a stacking gel is poured with a pH of 6.8 having large pore size. This stacking gel works to compress the protein samples into a thin migration front, so that all the proteins in the sample arrive at the resolving gel at the same time, leading to an accurate relative migration. The proteins within the sample will then be separated so they can be "resolved" in the resolving gel. The optimal gel percentage will depend upon the sizes of the proteins to be separated. The lower the percentage, the larger the proteins can pass through. A lower percentage gel (often around 4% total acrylamide) is used for the stacking gel irrespective of analyte size as it does not perform the separation. It is normally at a lower pH (around 6.8 compared to 8.8) than the resolving gel and has a different ionic content to help focus the sample analytes into a tight band to enter the resolving gel. The percentage of the resolving gel is varied depending on the size of the proteins to be separated, typically falling between 7 and 12%. Gradient gels may also be created that have a low percentage of polyacrylamide at the top where the sample enters, increasing along the sample's path so that a broader range of protein sizes can be separated. For SDS PAGE, the buffer used for the gel includes SDS, but for native PAGE this is omitted.

The resolving gel is poured first to a level just below the comb. Following the addition of the polymerizing agents, one needs to work fast before the gel starts to set. The gel should be pipetted between the glass plates, avoiding the introduction of bubbles, and a layer of hydrated isopropyl alcohol (IPA) is then poured on top to give a crisp edge. Once the resolving gel is set, pour off the IPA and rinse repeatedly with water. The stacking gel should

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now be pipetted onto the top and the comb put in place, ensuring that there are no air bubbles. Once set, gels can be used immediately or stored in the fridge in an airtight bag with a little water to avoid dehydration until required. They can be stored successfully for a few days, even as long as a couple of weeks, but the longer they are stored for, the more diffusion will occur between the two gel layers and the resultant separation may suffer.

Precast gels can be purchased; however, they are typically more expensive than making your own.

The gel tanks used in vertical electrophoresis/SDS-PAGE differ from agarose gel tanks in a number of ways. As polyacrylamide gels are run in a vertical orientation, the gel tank includes a module to hold the glass plates upright. Most modern PAGE tanks will use the glass plates to create the inner buffer chamber, by clamping them against a U-shaped gasket opposite on another, thereby creating a section between the two plates that is separated from the rest of the chamber by the gel. The inner running module sits inside the gel tank and a lid is then connected which joins the tank to the power supply via the electrodes.

Cleaver Scientific manufactures a range of sizes of polyacrylamide gel tanks, all which compatible casting systems and western blotting modules. Most popular omniPAGE mini tank is compatible with all commercially available precast gels, making it ideal for fast routine protein separations.

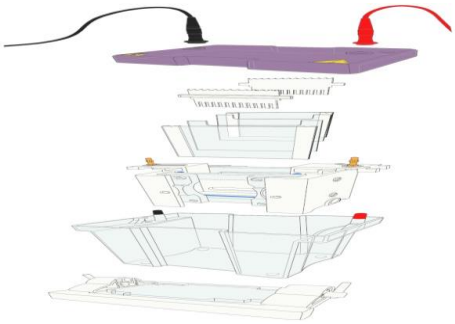

Figure 3.1.1: Components of the omni-PAGE mini polyacrylamide gel electrophoresis tank

b) Power Supply

To apply an electrical field to the gel, you will need an electrophoresis power supply. These power supplies are specifically manufactured for electrophoresis applications and features very stable voltage and current outputs to prevent fluctuations in migrations speed. A good power supply with allow you to set either constant current or voltage depending on the requirement of the experiment, and more advanced supplies will allow programming of individual steps at different parameter values.

The PowerPRO series of power supplies is a versatile range designed to power both multi-SUB horizontal and omniPAGE vertical electrophoresis tanks. Each power supply has a 2.4″ LCD display. Constant voltage, current and power options are available as well as pre-

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programmed or customer programmed conditions allow users to save and repeat their experiments for exceptional reproducibility.

c) Gel Documentation System

For the final stage of the technique - gel imaging, a gel documentation system is needed. Cleaver Scientific have a whole range of gel documentation systems to suite any budget or requirement.

4. REAGENTS FOR POLYACRYLMIDE GEL ELECTROPHORESIS

To run a gel electrophoresis experiment both the equipment and the reagents are required. The basic reagents required for polyacrylamide gel electrophoresis are:

- Acrylamide, TEMED and APS for making gels
- Buffer stocks to make the running buffer
- Loading dye to mix with protein samples
- Protein ladders to compare protein size and quantity
- Protein stain for visualising protein.

4.1 Buffers

Three categories of buffer are necessary for PAGE:

- Gel casting buffer (used to make the gel)
- Sample buffer
- Running buffer (fills the gel tank where electrophoresis takes place).

Electrophoresis may utilize a **continuous or discontinuous buffer system**. A continuous buffer system has only one buffer used for the sample, gel and gel tank and is rarely used for protein analysis as separations tend to diffuse and poorly resolved. A discontinuous buffer system, most often used for protein separation, uses different buffers for gel and running buffer.

The gels used also incorporate two layers **(stacking and resolving gels)** with different pore sizes and different buffer compositions.

The discontinuous buffer system tends to produce higher resolution separations.

Tris-based buffers are used for PAGE. Tris-glycine, bis-tris, tris-acetate and tris-tricine, all added with SDS, are used for SDS PAGE, where **tris-glycine** being the most commonly used. For native PAGE, tris-borate-ethylenediaminetetraacetic acid (TBE) with no SDS included is most frequently used.

Differences in pH and ionic strength, in addition to differing gel percentages, contribute to the discontinuity of the buffering.

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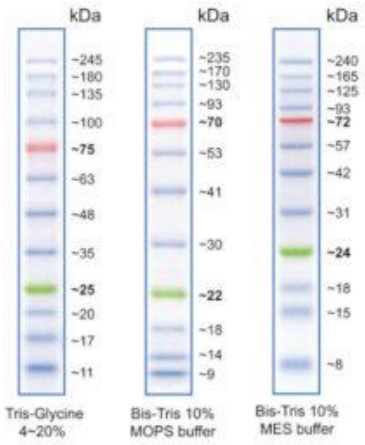

Figure 4.1: Vertical Protein Electrophoresis

5. PAGE PROTOCOL

i. Sample Preparation:

Sample preparation will vary depending on whether SDS PAGE or native PAGE experiment is being performed. For SDS PAGE, samples, such as lysed cells, tissues or bacteria are mixed with loading dye that contains a number of important ingredients. A dye, such as bromophenol blue, allows the samples to be seen during loading and running. Glycerol helps to weigh the sample down and prevent it floating out of the well during loading. SDS and β-mercaptoethanol linearize the proteins present and negate differences in charge. The mixture is heated, 100 °C for 3 mins is often sufficient, which also helps to denature the proteins.

Protein samples are denatured by heating them with a detergent SDS and mercaptoethanol. The former binds strongly to the proteins and gives them a high negative charge whilst the latter frees sulfhydryl groups, thus yielding polypeptide chains carrying an excess negative charge and similar charge to mass ratio. This helps in the resolution of proteins strictly based on their size during gel electrophoresis.

For native PAGE, SDS and β-mercaptoethanol are not included in the loading dye and no heating step is performed.

Prior to loading the gel, samples are centrifuged (16100 x g for 2 mins should be sufficient) to remove insoluble debris. Only the supernatant should be loaded as particulates can interfere with the running of the samples down the gel.

ii. Controls

Size markers are normally incorporated on either end of a sample row to enable size estimates for any bands detected. Reference proteins as well as positive and negative controls

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should be included where possible to verify observations in unknown samples. Lysate from a strain or cell known to produce the protein of interest or purified target protein may provide a suitable positive control. Whereas a strain or cell line the same as that of the unknown sample but in which the gene encoding the target protein has been removed could provide a suitable negative control. Controls that mimic the unknown samples as closely as possible can be particularly useful when examining samples that aren't purified proteins as it helps to differentiate background bands from the ones of interest.

iii. Electrophoresis/Running the gel

Fill the electrophoresis tank with an appropriate running buffer, often tris-glycine-SDS for SDS PAGE and TBE for native PAGE.

Remove the gel (still between the glass plates) from the casting frame and insert it into the electrophoresis frame. Lower this carefully into the electrophoresis tank and top up with running buffer so that the tops of the wells are submerged. Leave the comb in place until you are ready to run the gel as this helps to maintain the integrity of the wells. Unlike in agarose gel electrophoresis, where the gels are cast in trays are run horizontally, SDS-PAGE gels are cast vertically using a casting apparatus so that the stacking and resolving gels form a continuous gel (which would be much more difficult in a horizontal gel). It also allows a much greater protein amount to be loaded onto the gel.

After the comb is removed, samples should be loaded with a very fine pipette tip or needle. Be careful not to damage the edge of the wells or overload them otherwise samples may be cross contaminated between the lanes. The loading dye combined with the sample helps to visually guide this process and also pulls the sample mix into the bottom of the wells.

Place the lid on the tank. The gel tank is divided into 2 sections. Depending on the manufacturer the tank will have an inner (or upper) buffer chamber, and an outer (or lower) buffer chamber. These two chambers are linked by the gel to create a continuous circuit. Each chamber contains an electrode, negative in the inner chamber and positive in the outer chamber. The inner chamber contacts the top of the gel. Before applying electrical field one must ensure that the electrodes are connected in the correct way around (black to black and red to red). The voltage used and run time varies depending on the percentage of resolving gel being used, analyte size and whether an SDS or native analysis is being run. Typically, 200 V for 35 mins is a good starting point for SDS PAGE and 100 V for 40 mins for native PAGE (Figure 3). Prechilling the running buffer for native PAGE can help to prevent the samples from heating up, limiting denaturation and damage.

When an electrical field is applied, the proteins will move towards the positive electrode in the outer buffer chamber, due to the negative charges of the SDS molecules. The speed of movement through the gel is then determined by the voltage gradient, i.e. the voltage between the electrodes. The required field strength is related to the size of the gel tank being used and

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the required voltage can be calculated using the simple equation E = V/d where E is the field strength, V the voltage and d the distance in cm between electrodes. Vertical gel tanks are generally run at 5 – 10 V / cm so if your tank has an electrode distance of 10 cm, you would run the gel at 50 – 100V. The exact value depends on your samples and should be determined empirically.

To apply this electrical field, we use a DC power supply. Most electrophoresis power supplies can be set to provide either a constant current or a constant voltage, with each having advantages and disadvantages. One potential issue is the production of heat due to the flow of current through the system which can be especially high with larger tanks that require higher voltage. For this reason, it is advisable to use some form of cooling, either passive in the form of a cooling block, or active such as a recirculating chiller, for larger electrophoresis systems.

As an electric current is applied proteins migrate through the gel to the positive electrode as they have a negative charge. Each molecule moves at a different rate based on its molecular weight - small molecules move more rapidly through the gel than larger ones. Migration is usually faster at higher voltages. After a few hours, the protein molecules are all separated by size.

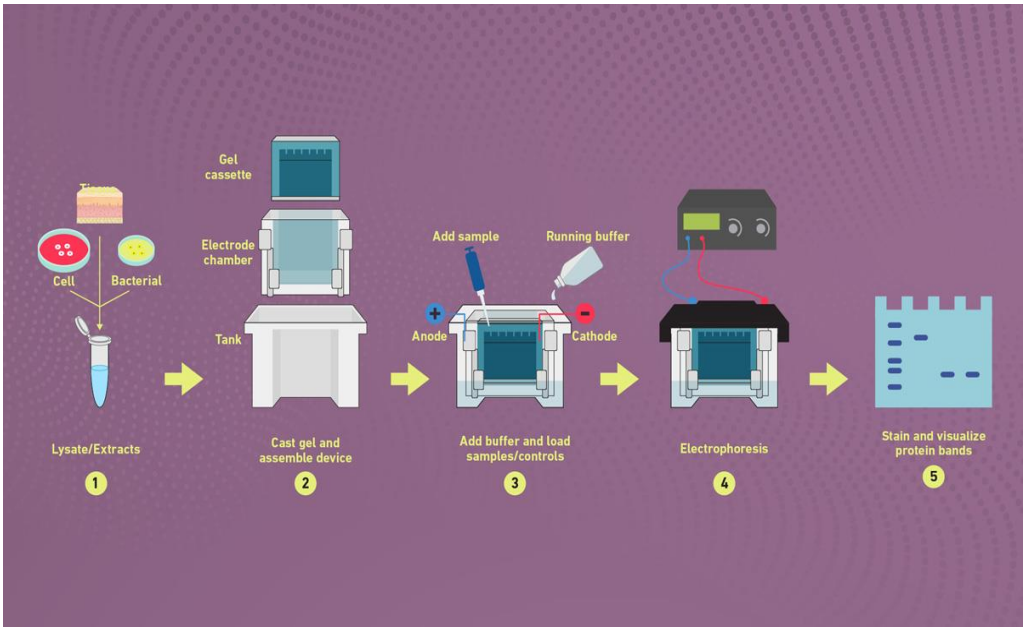

Figure 5.1: Sample preparation to protein electrophoresis in PAGE. 1) Samples are prepared for analysis, 2) gels are cast and the equipment prepared, 3) buffer is added to the gel tank and samples/controls are added to the gel, 4) current is applied to the samples to separate the proteins, 5) gels are stained and visualized.

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iv. Staining and Visualization:

There are several methods commonly used to visualise proteins that are either specific or non specific. Non specific protein visualisation targets all proteins, using dyes that bind to common regions of the proteins such as the amino groups. Examples of non-specific protein stains include Coomassie Brilliant Blue and Ruby Pro. These stains are often non-reversible and can (but don't always) interfere with downstream applications. Non-specific staining can be useful for quickly quantifying samples in a gel, or for ensuring a sample of interest is present.

To specifically visualise certain proteins, we need to use antibodies. Antibodies recognise unique 3 dimensional structures in the protein to distinguish them from others. By conjugating the antibody with a dye or enzyme, we can visualise just the protein that it recognises. The process of moving proteins from a gel to a membrane that can be probed with antibodies is called western blotting. Whether one is western blotting or performing non-specific staining, he has to visualise the proteins using the gel documentation system.

Normally once the samples have migrated a sufficient distance down the gel, (which can be seen by the dye front position), the gel frame is removed from the tank and the gel is carefully removed from the glass plates. The stacking gel has done its job and can be carefully cut off and discarded, leaving just the resolving gel.

The proteins are then stained using Coomassie brilliant blue or ethidium bromide. Gels are submerged in a staining solution normally consisting of 20% (v/v) methanol, 10% (v/v) glacial acetic acid and 0.1% (w/v) Coomassie brilliant blue made up with water for approximately 1 h (or until sufficiently stained) with gentle stirring (be careful not to tear the gel). Heating can expedite this process but do not boil the solution. Then wash off excess stain.

In case of proteins, the dye can be removed from the protein-free parts of the gel using a similar solvent of stain from which the dye is omitted as the dye binds more tightly to the proteins than the gel. A typical destain uses 50% (v/v) methanol in water with 10% (v/v) acetic acid. Gels can be left overnight in destain with gentle stirring to obtain a clear background with stained protein bands, but as with staining, destaining can be expedited by heating. Gels are then rinsed in water and can be visualized immediately; they can be stored in a little water to prevent dehydration.

Solutions that stain proteins without the need for destaining are available and can be particularly useful if a rapid result is required. However, they are typically more expensive than the standard staining protocol. Other stains, such as silver staining, can also be used for specific purposes but Coomassie staining is the most common.

Bands of radioactive proteins can be detected by autoradiography. The proteins can also be quantified as the protein content is directly proportional to the quantity of the bound dye.

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Some gel systems introduce a tracking dye such as bromophenol blue along with the protein sample – the visible distance travelled by the dye on the gel helps in deciding the required duration of electrophoresis. Bromophenol blue travels along with the sample molecules until it eventually reaches the bottom of the gel. Electrophoresis needs to stop at this point to ensure no protein molecules electrophorese out of the gel and into the buffer.

v. Interpreting Protein Gels

As PAGE gels are typically stained with Coomassie brilliant blue for detection, they can be visualized and interrogated with the naked eye, unlike agarose DNA gels, the images can be captured with a camera to provide a long-term record. Marker ladders are typically run alongside protein samples to allow their size (normally in kDa) to be estimated (Figure 5.2).

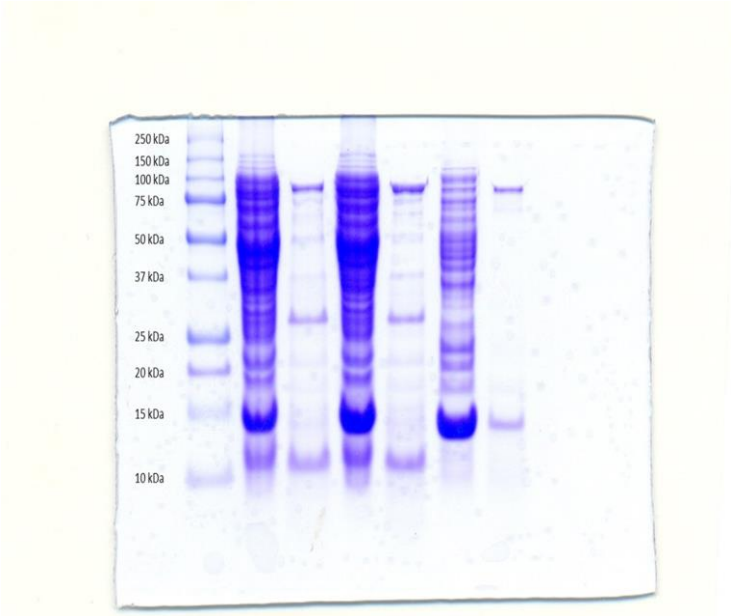

Figure 5.2: SDS PAGE with Taq DNA Polymerase. SDS PAGE is a useful technique to separate proteins according to their electrophoretic mobility. The marker (left hand lane) is the Precision Plus Protein Standard all Blue. This SDS PAGE was performed to determine the molecular weight of Taq DNA Polymerase.

It is important to make a note of which sample is loaded into which lane to enable accurate interpretation of results.

In native PAGE gels, sizes may not be accurate due to secondary structures that affect their passage through the gel. In these cases, reference proteins may be included to give the user an idea of how and where their target may be expected to appear. Native PAGE is also very useful when samples on the same gel are being compared, e.g., with different treatments,

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conditions or binding partners, to look for differences. The strength of the band can indicate the amount of protein present, the more protein there is, the stronger the band. If too much sample has been loaded, bands may appear as strong dark smears that are hard or impossible to interpret. In these cases, repeat the analysis with less sample.

6. 2D GEL ELECTROPHORESIS

Sometimes, separation of proteins in a single dimension is not enough to resolve similar species. In such cases, separation in two dimensions can add the required resolving power as it is less likely that two molecules will be very similar in two distinct properties. Two-dimensional polyacrylamide gel electrophoresis, or 2D PAGE, was introduced in 1975 concurrently by Joachim Klose and Patrick H. O'Farrel.

In the first dimension, proteins are separated linearly according to their isoelectric point (which relates to their charge and pH). In the second dimension, the molecules are then separated at 90° to the first separation according to molecular mass to produce an electropherogram (Figure 6.1).

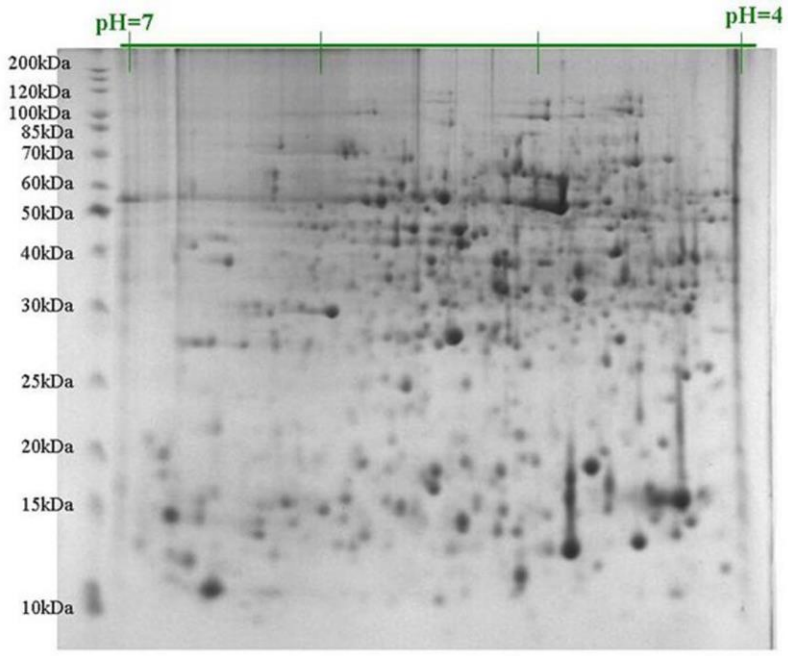

Figure 6.1: Example of 2D PAGE. Horizontal protein separation (X-axis) is according to the isoelectric point, vertical separation (Y-axis) is according to molecular weight. The sample is from cucumber plants populated with a pathogenic fungus.

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7. APPLICATIONS OF PAGE

SDS-PAGE and other forms of polyacrylamide gel electrophoresis are widely used in academic research into cellular and molecular biology. The ability to separate, identify and quantify the levels of proteins in certain cells and environments is essential for understanding how cellular processes work. Because of the ubiquity of these techniques any workflow that involves proteins is likely to include them, from a simple check of total protein content, all the way to whole proteome quantification using mass spectrometry.

PAGE is also used in many biological disciplines from molecular biology and forensics to biochemistry and genomics, providing an important analytical and diagnostic tool.

Protein Analysis

Examining samples directly by PAGE can provide a number of helpful indicators, including:

- if an expected protein is present
- what size (or apparent size) it is
- approximately how much there is
- how pure the protein sample is
- if cleavage (e.g., from a tag) has been successful
- which fraction a protein is present in (e.g., from cell lysate fractions)
- protein solubility

Recombinant proteins are a key for vaccine and biopharmaceutical development, as well as diagnostic assays and research. When producing them, it is important to check that the protein of interest has not been lost at multiple stages of purification and that it appears in the expected size. Depending on the fraction in which it is present, it can also indicate if there may be issues with protein solubility, which may impact its function or binding abilities or preclude its purification in the conditions used. Extraneous bands at the end of the purification process can also indicate the presence of contaminants.

Protein analyses are also important for many disciplines including food and beverage development, quality control, safety and fraud detection and the analysis of environmental samples. However, as technologies are advancing and becoming more affordable and accessible, some analyses in these areas are being replaced by techniques such as mass spectrometry (MS).

Electrophoretic Mobility Shift Assay (EMSAs)

EMSAs are an important experimental tool in identifying nucleic acid–protein complexes which further help in identifying the binding sites such as those used by transcription factors. While agarose gels are frequently used to achieve this as they permit easier migration of larger DNA–protein complexes, PAGE can offer greater separation resolution and greater stability for some complexes.

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- **Western Blot**

  Separation of sample proteins is an essential first step in any western blot experiment and PAGE is typically the technique employed to achieve this. While PAGE gels are blotted onto membranes for the blot itself, duplicate PAGE gels may also be stained with Coomassie brilliant blue to serve as loading controls when it comes to interpreting western blot results too. These may be used to diagnose infectious and non-infectious diseases, assess the efficacy of therapeutic interventions, inform fundamental research, check recombinant protein purification and provide functional or validative information to omics studies.

- **Extraction for Mass Spectrometry**

  Once separated, the proteins in gel bands may be excised and purified for further analysis. Techniques such as MS can be used to derive depth information about the proteins in the sample.

- **Urine Protein Electrophoresis and Immuno-fixation Electrophoresis**

  PAGE can provide a useful diagnostic tool to detect the amounts of certain proteins in bodily fluids such as urine or blood. Agarose gel electrophoresis and capillary electrophoresis may be used as alternatives for these analyses too.

  Ordinarily, there should be no or very little protein in urine so the **urine protein electrophoresis (UPEP) test**, which typically measures albumin and globulins, can be used as an indicator of pathological changes. High levels of protein in urine can be an indicator for numerous conditions including inflammation, kidney disease infection and some types of cancer (e.g., myeloma) and helps to guide further investigation or treatment.

  Albumin and immunoglobulins can also be analyzed in serum samples (**serum protein electrophoresis (SPEP)**) to diagnose a range of conditions including cancers such as multiple myeloma, lymphoma and leukemia, kidney disease, liver disease, malnutritional conditions, as well as some neurological and autoimmune conditions.

  Immunofixation can be used in these tests to identify certain subtypes of a protein, e.g., immunoglobulin A (IgA) lambda or the heavy and light chain type of the M protein.

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