alternative to starch gels [3, 4], thus developing polyacrylamide gel electrophoresis or PAGE. The inclusion of ionic detergent Sodium Dodecyl Sulphate (SDS) to the gel and the sample was an important addition to this work. Shapiro et al. were one of the first to make use of this approach [5]. Laemmli showed that proteins could be reliably fractionated by SDS-PAGE, which he described in a figure legend in a Nature paper [2].

SDS-polyacrylamide gel electrophoresis involves the separation of protein based on their size. By heating the sample under denaturing and reducing condition, protein become unfolded and coated with SDS detergent molecules, acquiring a high negative charge that is proportional to the length of the polypeptide chain. When loaded onto a gel matrix and placed in an electric field, the negatively charge protein molecules migrate towards the positively charge electrode and are separated by a molecular sieving effect. After visualization by a protein specific staining technique, the size of protein can b estimated by comparison of its migration distance, with that of a standard of known molecular weight. It is possible to blot the separated protein onto a positively charge membrane and to probe with protein specific antibodies in a procedure termed western blotting.

Western blotting is a technique by which protein can be transferred from a polyacrylamide gel to a sheet of nitrocellulose in such a way that a faithful replica of the original gel pattern is obtained. A wide variety of analytical procedure can then be applied to immobilized protein, which makes western blotting a powerful tool for diagnosing various pathological conditions. In this technique a sheet of nitrocellulose is placed against the surface of a SDS-PAGE protein fractionation gel and a current applied across the gel and into the nitrocellulose where they bind finally by non-covalent forces. The technique involved three step; protein separating by SDS-PAGE, blotting and immune assay.

5. Materials Required

1. **Acrylamide(30% stock):-** dissolved 29.2 g acrylamide and 0.8 bis-acrylamide in distilled water and make upto 100ml. Store under dark in amber colour bottle at 4°c(can use upto 3 month)
2. **Resolving gel/ separating gel buffer pH 8.8, 1.5M tris-HCl:** - dissolved 18.17g tris in 75 ml distilled water. Adjust to pH 8.8 with 6 N HCl. Adjust the total volume to 100ml with distilled water and store at 4°c.
3. **Stacking gel buffer, pH-6.8, and 1.0Mtris-HCl:-** dissolved 3g tris in 40 ml distilled water, adjust to pH 6.8 with 6N HCl. Adjust the total volume to 50ml with distilled water. Store at 4°C.
4. **Electrophoresis buffer pH 8.3**:- dissolved 3g tris, 14.4 g glycine and 1g SDS in 100ml of distilled water. Store at 4°C.
5. **Ammonium per sulphate-initiator 10%:-** dissolved 0.1 g APS in 1 ml distilled water.
6. **TEMED(NNN'N' Tetramethylenediamine):-** catalyst.
7. **Sample buffer:-** 7.25 ml distilled water + 1.25 ml stacking gel buffer + 1ml glycerol + 0.5 ml β-mercaptoethanol + 150 mg SDS and a pinch of bromophenol blue.
8. **Staining solution:** -dissolved 200mgcoomassie brilliant blue R 250 in 50ml methanol/ethanol, 7ml acetic acid and 43ml distilled water & filter it.
9. **De-staining solution:-** add 7ml acetic acid to 30ml methanol/ethanol and 63ml distilled water
10. **Vertical slab-gel electrophoresis equipment.**
11. **Acrylamide mixture (10%) for 25 ml of resolving gel:-9.9 ml**, distilled water + 8.3ml, 0% acrylamide + 6.3ml, 0.5 M tris-HCl + .25ml, 10% SDS + .25ml, 10% APS +.01 ml, TEMED.
12. **5% stacking gel for 5 ml:-** 3.04ml, distilled water + 0.83ml, 30% acrylamide + .63ml, 0.5 M Tris – HCl(pH-6.8) + 0.05ml, 1% SDS +0.05ml, APS + 0.005ml, TEMED.

Tip: gel buffer and self-prepared acrylamide/bis-acrylamide stock solution should be filtered, degassed and stored at 4°C.

Sample Preparation

Samples may be any material containing proteins or nucleic acids. These may be biologically derived, for example from prokaryotic or eukaryotic cells, tissues, viruses, environmental samples, or purified proteins. In the case of solid tissues or cells, these are often first broken down mechanically using a blender (for larger sample volumes), using a homogenizer (smaller volumes), by sonicator or by using cycling of high pressure, and a combination of biochemical and mechanical techniques – including various types of filtration and centrifugation – may be used to separate different cell compartments and organelles prior to electrophoresis. Synthetic biomolecules such as oligonucleotides may also be used as analytes.

The sample to analyze is optionally mixed with a chemical denaturant if so desired, usually SDS for proteins or urea for nucleic acids. SDS is an anionic detergent that denatures secondary and non–disulphide linked tertiary structures, and additionally applies a negative charge to each protein in proportion to its mass. Urea breaks the hydrogen bonds between the base pairs of the nucleic acid, causing the constituent strands to separate. Heating the samples to at least 60 °C further promotes denaturation [6, 7, 8, 9].

In addition to SDS, proteins may optionally be briefly heated to near boiling in the presence of a reducing agent, such as dithiothreitol (DTT) or 2-mercaptoethanol (beta-mercaptoethanol/BME), which further denatures the proteins by reducing disulfide linkages, thus overcoming some forms of tertiary protein folding, and breaking up quaternary protein structure (oligomeric subunits). This is known as reducing SDS-PAGE.A tracking dye may be added to the solution. This typically has a higher electrophoretic mobility than the analytes to allow the experimenter to track the progress of the solution through the gel during the electrophoretic run.

1. Take 1ml of culture solution of E.coli strain DH5α, in 1.5 ml of eppendorf's tube.

2. Then centrifuge at 12000 rpm for 5 min.
3. Then discard the supernatant.
4. Then add 500 µl of TE in the tube and dissolve the tube by gentle shaking.
5. Then add same volume of sample buffer.
6. Finally heat the sample on boiling bath for 5 minutes and then immediately keep on ice.

Preparing acrylamide gels

The gels typically consist of acrylamide, bisacrylamide, the optional denaturant (SDS or urea), and a buffer with an adjusted pH. The solution may be degassed under a vacuum to prevent the formation of air bubbles during polymerization. Alternatively, butanol may be added to the resolving gel (for proteins) after it is poured, as butanol removes bubbles and makes the surface smooth [10]. A source of free radicals and a stabilizer, such as ammonium persulfate and TEMED are added to initiate polymerization [11]. The polymerization reaction creates a gel because of the added bisacrylamide, which can form cross-links between two polyacrylamide molecules. The ratio of bisacrylamide to acrylamide can be varied for special purposes, but is generally about 1 part in 35. The acrylamide concentration of the gel can also be varied, generally in the range from 5% to 25%. Lower percentage gels are better for resolving very high molecular weight molecules, while much higher percentages are needed to resolve smaller proteins.

6. Procedures

Gel Casting Tray

1. Assemble vertical slab gel apparatus using 1mm spacer.

Tip:-the plate should be thoroughly cleaned and dried before use.

2. Seal the glass plates on 3 sides with 1% agarose.
3. Pour the separating gel/ resolving gel mixture to a level of approximately 2.5 cm below the glass plates, gently layer 250µl of TDW over the gel surface. Allow to polymerize.

Tips: prepare the solution freshly each time it is required. As soon as ammonium persulphate is added, the gel should be poured quickly before the acrylamide polymerizes.

4. After polymerization remove water from the top.
5. Pour the prepared 5% stacking gel over the resolving gel/ separating gel.
6. Immediately insert a comb and allow polymerizing the gel.

Sample Loading

1. Prepared the required volume of sample (100mg protein per lane) + equal volume of sample buffer.
2. Heat the sample in boiling water bath for 5 min to denature the protein. Immediately keep them on ice to retain the denature stage.
3. Remove the comb from the mould; wash the well with distilled water.

Tip: with a marker pen mark the number or the position of the wells before removing the comb. This aids easy loading of sample.

4. Mount the gel on electrophoretic apertures'.
5. Add electrophoresis buffer to the top and bottom reservoir of the electrophoretic apparatus.
6. Load the sample along with marker protein into the wells(20 µl)

Electrophoresis

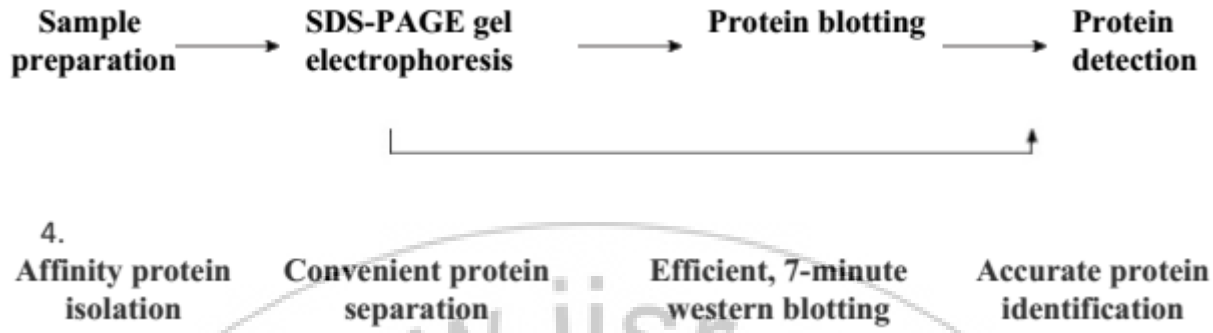
Various buffer systems are used in PAGE depending on the nature of the sample and the experimental objective. The buffers used at the anode and cathode may be the same or different [12, 13, 14]. An electric field is applied across the gel, causing the negatively charged proteins or nucleic acids to migrate across the gel from the negative electrode (the cathode) towards the positive electrode (the anode). Depending on their size, each biomolecule moves differently through the gel matrix: small molecules more easily fit through the pores in the gel, while larger ones have more difficulty. The gel is run usually for a few hours, though this depends on the voltage applied across the gel; migration occurs more quickly at higher voltages, but these results are typically less accurate than at those at lower voltages. After the set amount of time, the biomolecules have migrated different distances based on their size. Smaller biomolecules travel farther down the gel, while larger ones remain closer to the point of origin. Biomolecules may therefore be separated roughly according to size, which depends mainly on molecular weight under denaturing conditions, but also depends on higher-order conformation under native conditions. However, certain glycoproteins behave anomalously on SDS gels.

1. Attach the apparatus to the power supply unit and apply 8 V/Cm for stocking gel (70V) and 15 V/Cm for resolving gel(150-200V)
2. Electrophoresis is continued until bromophenol blue reaches the bottom of the gel.
3. Dismantles apparatus and remove gel from between the plates and place in a tray containing distilled water cut a small corner of the gel to indicate the direction of loading.

Coomassie Staining

1. Immersethe gel in 5volume of staining solution and stain for 4 hrs.at room temperature with gentle shacking. Coomassie brilliant blue R reacts non-specifically with proteins.
2. Gently agitate the stained gel in destining solution until the background becomes clear.
3. Store in 7 % acetic acid. Visualize the band in an illuminator.

SDS-PAGE workflow and illustration of an apparatus (Figure 5)



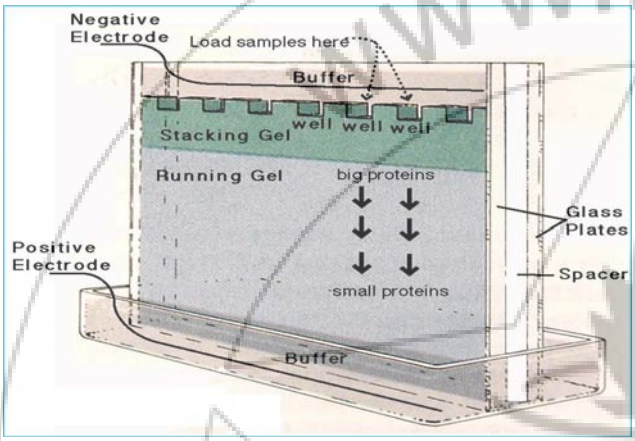


Figure 5: Illustration of an apparatus used for SDS PAGE

7. Result

The sample used in experiment are overnight culture of E.coli strain DH5α.we successfully separated the protein on SDS-PAGE and the protein can be separated on basis of its molecular weight separately by the blotting on a nitrocellulose paper.

Chemical ingredients and their roles

Polyacrylamide gel (PAG) had been known as a potential embedding medium for sectioning tissues as early as 1964, and two independent groups employed PAG in electrophoresis in 1959 [15, 16]. It possesses several electrophoretically desirable features that make it a versatile medium. It is a synthetic, thermo-stable, transparent, strong, chemically relatively inert gel, and can be prepared with a wide range of average pore sizes [17]. The pore size of a gel is determined by two factors, the total amount of acrylamide present (%T) (T = Total concentration of acrylamide and bisacrylamide monomer) and the amount of cross-linker (%C) (C = bisacrylamide concentration). Pore size decreases with increasing %T; with cross-linking, 5%C gives the smallest pore size. Any increase or decrease in %C from 5% increases the pore size, as pore size with respect to %C is a parabolic function with vertex as 5%C. This appears to be because of non-homogeneous bundling of polymer strands within the gel. This gel material can also withstand high voltage gradients, is amenable to various staining and de-staining procedures, and can be digested to extract separated fractions or dried for autoradiography and permanent recording.

Advantage of SDS PAGE

PAGE has a high loading capacity; up to 10 micrograms of DNA can be loaded into a single well (1 cm x 1 mm) without significant loss of resolution. Polyacrylamide contains few inhibitors of enzymatic reactions. PAGE is an ideal gel system from which to isolate DNA fragments for subcloning and other molecular biological techniques.

Disadvantage of SDS PAGE

The mobility of the fragments can be affected by base composition making accurate sizing of bands a problem. Polyacrylamide quenches fluorescence, making bands containing less than 25 ng difficult to visualize with ethidium bromide staining.

8. Conclusion

SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis, is a technique widely used in biochemistry, forensics, genetics and molecular biology to separate proteins according to their electrophoretic mobility (a function of length of polypeptide chain or molecular weight as well as higher order protein folding, posttranslational modifications and other factors).The SDS gel electrophoresis of samples having identical charge to mass ratios results in fractionation by size and is probably the world's most widely used biochemical method.

Proteins are amphoteric compounds; their net charge therefore is determined by the pH of the medium in which they are suspended. In a solution with a pH above its isoelectric point, a protein has a net negative charge and migrates towards the anode in an electrical field. Below its isoelectric point, the protein is positively charged and migrates towards the cathode. The net charge carried by a protein is in addition independent of its size - i.e. the charge carried per unit mass (or length, given proteins and nucleic acids are linear macromolecules) of molecule differs from protein to protein. At a given pH therefore, and under non-denaturing conditions, the electrophoretic separation of proteins is determined by both size and charge of the molecules. Nucleic acids however, remain negative at any pH used for electrophoresis and in addition

carry a fixed negative charge per unit length of molecule, provided by the PO_4 group of each nucleotide of the nucleic acid. Electrophoretic separation of nucleic acids therefore is strictly according to size.

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