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A Review on- Electrophoresis Technique

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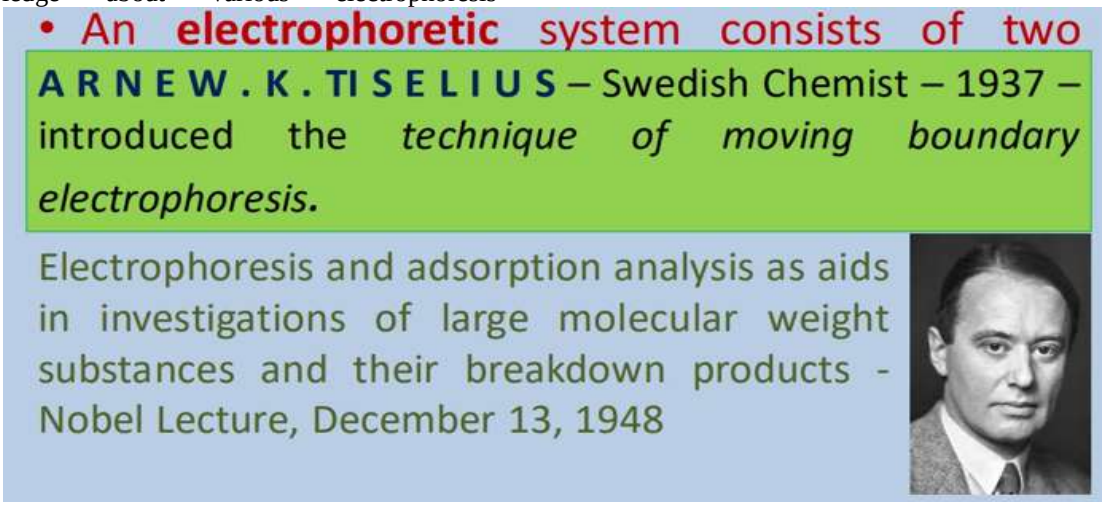
ABSTRACT
Proteins, nucleic acids, and other biological components can be separated from sample fluids using the straightforward but sensitive analytical process known as electrophoresis. In order to separate complex protein mixtures (such as those derived from cells, fractions, column fractions, subcellular components, or immunological precipitates), analyse subunit compositions, and verify protein homogeneity, electrophoresis is often used. When a mixture of charged molecules is subjected to electrophoresis, they are separated by size when exposed to an electric field. In order to employ proteins in various applications, they can also be purified via this method.In polyacrylamide gel electrophoresis, protein migrate through openings in a polyacrylamide gel matrix in response to an electrical field; pore size decreases with increasing acrylamide concentration. The protein migrates at a rate that depends on the pore size, protein charge, size, and shape. The primary goal of this review effort is to gain more knowledge about various electrophoresis techniques. Future electrophoresis techniques will produce results that are more accurate in many domains as required by their applications.

Key Words: Electrophoresis, Protein Charge, electrical Field, Separation

I. INTRODUCTION:

The capacity of liquid molecules to move through an electric field allows for the separation process called as electrophoresis. The most popular technique for analysing macromolecules in biochemical or molecular biology, including genetic components like DNA or RNA, proteins, and polysaccharides, is electrophoresis, which is utilised in a variety of ways. While submerged in a solution buffer, charged particles are separated using several types of electrophoresis. For all forms of electrophoresis, an electrophoresis unit, sometimes referred to as an electrophoresis chamber, is necessary.



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DEFINITION:

The movement of a free electron through a solution while being influenced by an external magnetic field is known as electrophoresis. Ions hanging between two electrodes have a tendency to go in the direction of the electrodes with the opposing charges.

PRINCIPLE:

- Due to their varied electrophoretic mobilities, molecules with different overall charges will start to separate when a potential difference is applied.
- Because differing molecular sizes will result in varying frictional forces, even molecules with comparable charges will start to separate.
- As a result, some types of electrophoresis rely almost entirely on the various charges on the molecules to separate them, while others take advantage of the molecules' differences in size (molecular size).
- Because the electric field is reduced before the molecules in the samples reach the electrode, electrophoresis is thought of as an incomplete type of electrolysis.
- However, the molecules will have have been divided based on their electrophoretic mobilities.
- The separated samples are then found using autoradiography or stained with a suitable dye.

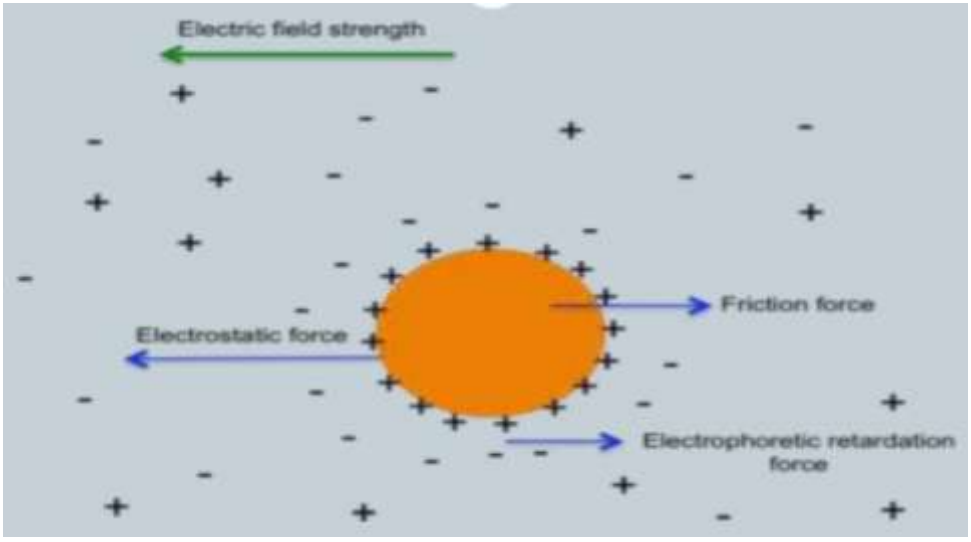

Figure 1: Electrophoresis

THEORY:

- Since the frictional coefficient—which in turn depends on the function of some of the physical characteristics of the molecules, such as weight, molecular shape, and size—determines how easily ions move or how mobile they are.
- The law of electrostaticsstates:

F electric = qE

- where F electric is electrical force on an ion, q is the charge on the ion and E is the electric field strength.

- The resulting electrophoretic migration of the ion though the solution is opposedby a frictionalforce.

friction = Vf

- where f is the ion's frictional coefficient and V is the ion's velocity (rate of migration).
- The frictional coefficient, which depends on the size, shape, and state of the ion as well as the viscosity of the solution, is a measure of the drag that the solution places on the moving ion.

WORKING OF ELECTROPHORESIS

- The way electrophoresis works is that it employs the force of an electric fieldto separate molecules, ions, or colloidal particles suspended in a matrix.
- Therefore, electrophoresis is the electrokinetic phenomena where the mobility of molecules happens under an electric field.
- The electric field permits the migration of the positively charged molecule towards the anode and the migration of the negatively charged

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molecule towards the cathode.

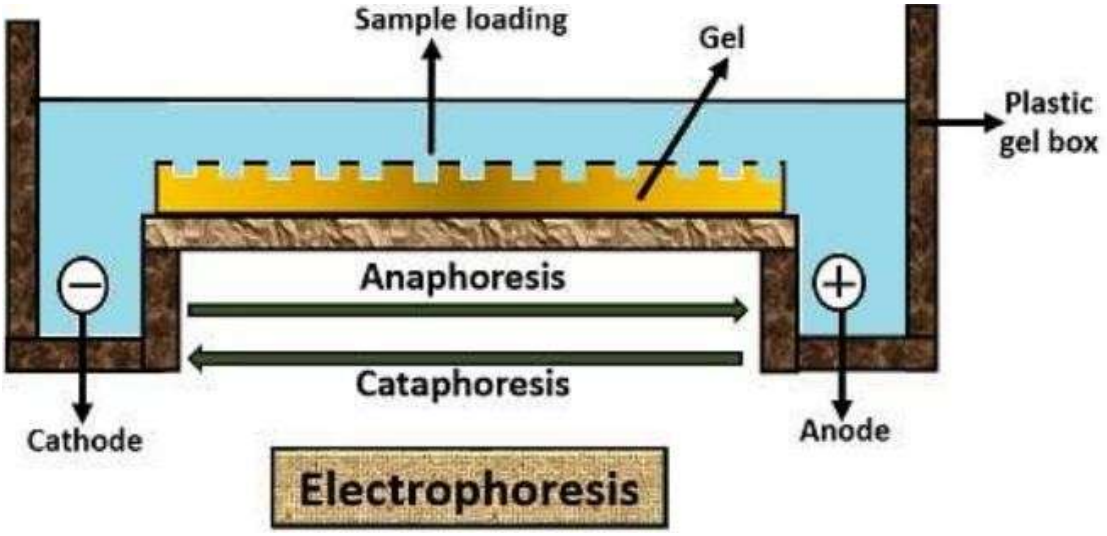

Figure 2: Working of Electrophoresis

FACTORS AFFECTING ELECTROPHORESIS

1. Nature ofcharge
2. Voltage
3. Frictionalforce
4. Electrophoreticmobility
5. Current
6. Heat
7. Elecrtroendosmosis

1. Nature ofcharge:
Under the influence of an electric field these charged particles will migrate either to cathode or anode depending on the nature of their net charge.

2. Voltage:
When a potential difference (voltage) is applied across the electrodes, it generates a potential gradient (E), which is the applied voltage (v) divided by the distance "d" between the two electrodes i.e.p.d.
(E) = V/d

When this potential gradient 'E' is applied, the force as the molecule bearing a charge of 'q' coulombs is 'Eq ' Newtons. It is this force that drives the molecule towards the electrodes.

3. Frictionalforce:
Thereisalsoafrictionalforcethatretardsthemovement ofthischargedmolecule.This frictional force is the measure of the hydrodynamic size of the molecule, the shape of the molecule, the pore size of the medium in which the electrophoresis is taking place and the viscosity of thebuffer.
The velocity 'v' of the charged molecule is an electric field is therefore given by the equation. U=Eq /f, where 'f'= frictional coefficient iv.

4. Electrophoreticmobility:
More commonly a term electrophoretic mobility ( ) of an ion is used, which is the ratio of the velocity of the ion and the field strength. i.e. =U/E.
Whenap.d.isapplied,themoleculewithdifferentovera llchargeswillbegintoseparate owing to their different electrophoreticmobility.
Even the molecule with similar charges will begin to separate if they have different molecular sizes, since they will experience different frictional forces.

5. Current:
Ohm's law: V/I=R
It therefore appears that it is possible to accelerate an electrophoretic separation by increasingtheappliedvoltage,whichultimatelyresults incorrespondingincreaseinthe current flowing. The distance migrated by the ions will be proportional to both current and time.

6. Heat:
One of the major problems for most forms of electrophoresis, that is the generation of heat. During electrophoresis, the power (W) generated in one supporting medium is given by W= I2R. Most of the power generated is dissipated as heat.
The following effects are seen on heating of the electrophoretic medium has:

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- An increased rate of diffusion of sample and buffer ions which leads to the broadening of the separatedsamples.
- Formation of convention currents, which leads to mixing of separatedsamples.
- Thermal instability of samples that are sensitive toheat.
- A decrease of buffer viscosity and hence reduction in the resistance of the medium.

If a constant voltage is applied, the current increases during electrophoresis owing to the decrease in resistance and this rise in current increases the heat output still further. For these reasons, often a stabilized power supply is used, which provides constant power and thus eliminates fluctuations in heating.
Constant heat generation is however a problem.
For which the electrophoresis is run at very low power (low current) to overcome any heatingproblems,butthiscanleadtopoorseparationasa resultoftheincreasedamount of diffusion due to long separationtime.
Compromise condition have to be found out with reasonable power settings, to give acceptableseparationtimeandanappropriatecoolings ystem,toremoveliberatedheat. While such system works fairly well, the effect of heating are not always totally eliminated.

7. Elecrtroendosmosis:
Thephenomenonofelectroendosmosis(aka-electro-osmoticflow)isafinalfactorthat can affect electrophoretic separation.
Thisphenomenonisduetothepresenceofchargedgrou psonthesurfaceofthesupport medium.
For instance, paper has some carboxyl group present, agarose contains sulfate groups depending on the purity grade and the surface of glass walls used in capillary electrophoresis contains silanol (Si-OH) groups. These groups, at appropriate pH, will ionize, generating charged sites.

Itisthesechargesthatgenerateelectroendosmosis.Inca seofcapillaryelectrophoresis, the ionized sianol groups creates an electrical double layer, or a region of charge separation, at the capillary wall/electrolyticinterface.
When voltage is applied cations in the electrolyte near the capillary walls migrate towards the cathode, pulling electrolyte solution with them.
This creates a net electroosmotic flow towards cathode.

The Rate of migration of charged molecules depends upon following factors •

a) The strength of electric field, size andshape.
b) Relative hydrophobicity of thesample.
c) Ionic strength and temperature of thebuffer.
d) Molecular size of the takenbiomolecule.
e) Net charge density of the taken biomolecule.
f) Shape of the takenbiomolecule.

Type of Electrophoresis

➢ Introduction:

Electrophoresis is a physical method of analysis which involves separation of the compounds that are capable of acquiring electric charge in conducting electrodes.

➢ Definition:

Electrophoresis may be defined as the migration of the charged particle through a solution under the influence of an external electrical field.
Ions that are suspended between two electrodes tends to travel towards the electrodes that bears opposite charges

❖ **Types ofelectrophoresis**

Zone electrophoresis

a) Paper electrophoresis
b) Gelelectrophoresis
c) Thin layer electrophoresis
d) Cellulose acetateelectrophoresis

Moving Boundary Electrophoresis

a) CapillaryElectrophoresis
b) Isotachophoresis
c) Isoelectric Focusing
d) ImmunoElectrophoresis

➢ Zoneelectrophoresis
➢ It involves the migration of the charged particle on the supporting media (Paper, cellulose acetate membrane, starch gel, polyacrylamide).
➢ The separated components are distributed into discrete zone on the supportmedia.
➢ Supporting media is saturated with buffer solution, small volume of the sample is applied as narrowband.

Advantages

➢ Useful in biochemicalinvestigations.
➢ Small quantity of sample can beanalyzed.
➢ Low cost and easymaintenance.

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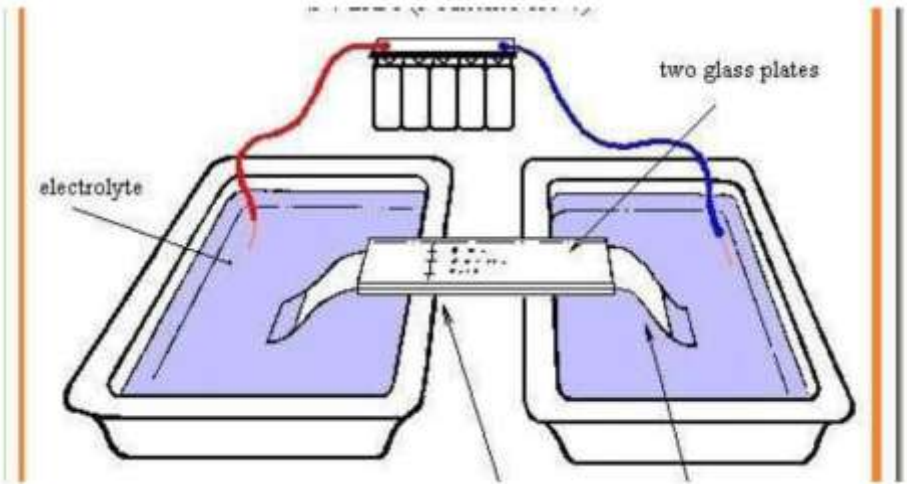
Disadvantages

- Unsuitable for accurate mobility and isoelectric pointdetermination.
- Due to the presence of supporting medium, technical complications such as capillary flow, electro osmosis, adsorption and molecular sieving areintroduced.

General method of operation

- Saturation of the medium with thebuffer.



- Sampleapplication.

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Figure 3: Paper Electrophoresis

- FilterpapersuchasWhatmannno1andno3instripo f3mmor5cmwidehavebeen used to goodeffect.
- Separation takes place in 12 to 14 hrs.
- Types:

1. Horizontal paper Electrophoresis

2. Vertical Paper Electrophoresis

Advantages

- It iseconomical
- Easy touse

Disadvantages

- Certain compounds such as proteins, hydrophilic molecules cannot be resolved due to the adsorptive and ionogenic properties of paper which results in tailing and distortion of componentbands.
- Electrophoreticseparation.
- Removal of the supportingmedia.

Instrumentation

- Electrophoresischamber.
- Electrodes.
- Diffusionbarriers.
- Supporting / stabilizing media. (inert to sample and to any developingreagents)

A. Paperelectrophoresis

- Electroosmosis

B. Gelelectrophoresis

- Separation is brought about through molecular sieving technique, based on the molecular size of thesubstances.
- Gel material acts as a "molecularsieve"
- Gel is a colloid in a solid form (99% iswater).
- It is important that the support media is electricallyneutral.
- Different types of gels which can be used are; Agar and Agarose gel, Starch, Sephadex, Polyacrylamidegels.

Aporousgelactsasasievebyretardingor,insomecases, bycompletelyobstructing the movement of macromolecules while allowing smaller molecules to migrate freely.

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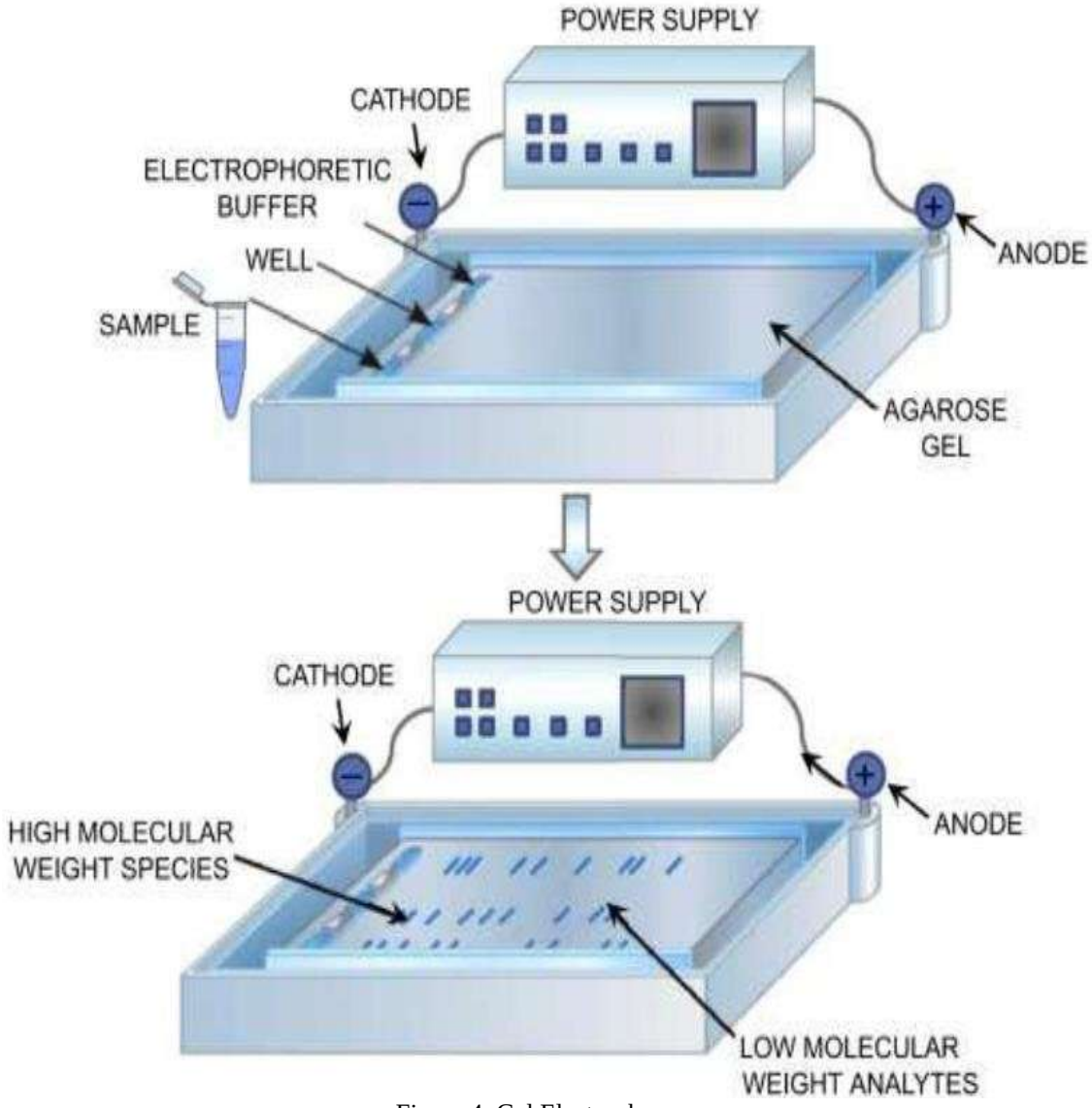

Figure 4: Gel Electrophores

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- **Agar and agarosegel**
- Agar is a mixture of polysaccharides extracted from seaweeds.
- Agarose is a highly purified uncharged polysaccharide derived fromagar.
- Agarose is chemically basic disaccharide repeating units of 3,6- anhydro-L- galactose.
- Agarose dissolves when added to boiling liquid. It remains in a liquid state until the temperature is lowered to about 40 °C at which point itgels.
- Theporesizemaybepredeterminedbyadjustingth econcentrationofagaroseinthe gel.
- Agarosegelsarefragile.Theyareactuallyhydroco lloids,andtheyareheldtogether by the formation of weak hydrogen and hydrophobic bonds.
- The pores of an agarose gel are large, agarose is used to separate macromolecules such as nucleic acids, large proteins and protein complexes.

Advantages
- Easy to prepare and small concentration of agar isrequired.
- esolution is superior to that of filterpaper.
- Large quantities of proteins can be separated andrecovered.
- Adsorption of negatively charged protein molecule isnegligible.
- It adsorbs proteins relatively less when compared to othermedium.
- Sharp zones are obtained due to lessadsorption.
- Recovery of protein is good, good method for preparativepurpose

Disadvantages
- Electro osmosis ishigh.
- Resolution is less compared to polyacrylamidegels.
- Different sources and batches of agar tend to give different results and purification is oftennecessary.

Application
- Widely used in immunoelectrophoresis.
- Polyacrylamide gel electrophoresis(PAGE)
- Itispreparedbypolymerizingacrylamidemonom ersinthepresenceofmethylene- bis-acrylamide to cross link themonomers.
- Structure of acrylamide(CH2=CH-CO-NH2)
- Polyacrylamide gel structure held together by covalentcross-links.
- Polyacrylamide gels are tougher than agarosegels.
- It is thermostable, transparent, strong and relatively chemicallyinert.
- Gels are uncharged and are prepared in a variety of poresizes.
- Proteins are separated on the basis of charge to mass ratio and molecular size, a phenomenon called Molecularsieving.

Advantage
- Gels are stable over wide range of pH andtemperature.
- Gels of different pore size can beformed.
- Simple and separation speed is goodcomparatively.

TYPE OF PAGE

NATIVE-PAGE
- Native gels are run in non-denaturing conditions, so that the analyte's natural structure ismaintained.
- Separation is based upon charge, size, and shape ofmacromolecules.
- Useful for separation or purification of mixture ofproteins.
- This was the original mode ofelectrophoresis

DENATURED-PAGE OR SDS-PAGE
- Separation is based upon the molecular weight ofproteins.
- The common method for determining MW ofproteins.
- Very useful for checking purity of proteinsamples.

PAGE-procedure
- Thegelofdifferentporesizesiscastintoacolumnin sideaverticaltube,oftenwith large pore gel at the top and small pore gel at thebottom.
- Microgram quantity of the sample is placed over the top of the gel column and covered by a buffer solution having such a pH so as to change sample components into anions.
- Thefootofthegelcolumnismadetodipinthesameb ufferinthebottomreservoir.
- Cathode and anode are kept above and below the column to impose an electricfield through thecolumn.
- Macromolecular anions move towards the anode down the gelcolumn.
- There is no external solvent space, all the

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migratory particles have to pass through the gelpores.

- Rate of migration depends on the charge to massratio.
- Different sample components get separated into discrete migratory bands along the gel column on the basis of electrophoretic mobility and gel filtrationeffect.

SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

- SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis, is a techniquewidelyusedinbiochemistry,forensics, geneticsandmolecularbiologyto separate proteins according to their electrophoreticmobility.
- When a detergent SDS added to PAGE the combined procedure is termed as SDS PAGE.
- SDS coats protein molecules giving all proteins a constant charge-massratio.
- Due to masking of charges of proteins by the large negative charge on SDS binding withthem,theproteinsmigratealongthegelinorde rofincreasingsizesormolecular weights.
- SDS is an anionic detergent which denatures secondary and non–disulfide– linked tertiary structures by wrapping around the polypeptide backbone. In so doing, SDS confers a net negative charge to the polypeptide in proportion to itslength.
- Molecules in solution with SDS have a net negative charge within a wide pHrange.
- A polypeptide chain binds amounts of SDS in proportion to its relative molecular mass.
- ThenegativechargesonSDSdestroymostoftheco mplexstructureofproteins,and are strongly attracted toward an anode in an electricfield.

Sodium dodecyl sulfate (SDS-PAGE)

- Native protein is unfolded by heating in the presence of mercaptoethanol andSDS.
- SDS binds to the protein so that it stays in solution anddenatures.
- LargepolypeptidesbindmoreSDSthansmallpoly peptides,soproteinsendupwith negative charge in relation to theirsize.
- When treated with SDS and a reducing agent, the polypeptides become rods of negative charges with equal “charge densities" or charge per unitlength.
- Thus, we can separate the proteins based on theirmass.

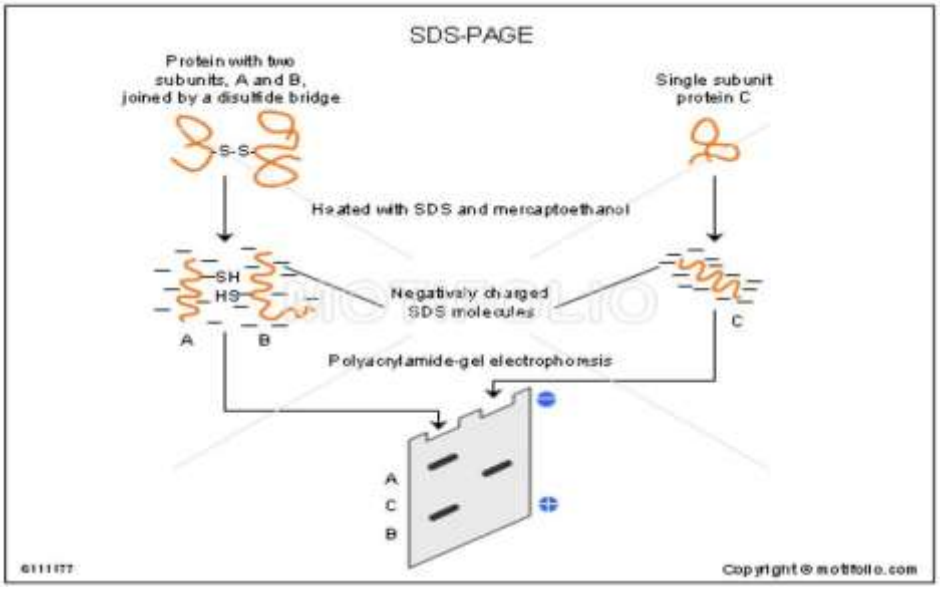

Figure 5: Sodium dodecyl sulfate (SDS-PAGE)

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➢ **Starch**

✓ A suspension of granular starch should be boiled in a buffer to give a clear colloidal suspension.

✓ The suspension on cooling sets as a semisolid gel due to intertwining of the branched chains ofamylopectin.

✓ In order to avoid swelling and shrinking petroleum jelly isused.

Advantages

✓ High resolving power and sharp zones are obtained.

✓ The components resolved can be recovered in reasonable yield especiallyproteins.

✓ Can be used for analytical as well as preparativeelectrophoresis.

Disadvantages

✓ Electro osmoticeffect.

✓ Variation in pore size from batch tobatch.

C. Thin Layer Electrophoresis

✓ Studies can be carried out in thin layer of silica,alumina.

Advantages

✓ Less time consuming and goodresolution.

Application

✓ Widelyusedincombinedelectrophoretic-chromatographystudiesintwodimensional study of proteins and nucleic acidhydrolysates.

C. Cellular acetateelectrophoresis

✓ It contains 2-3 acetyl groups per glucose unit and its adsorption capacity is less than that ofpaper.

✓ It gives sharperbands.

✓ Provides a good background for stainingglycoproteins.

Advantages

✓ No tailing of proteins or hydrophilic materials.

✓ Available in wide range of particle size and layerthickness.

✓ Give sharp bands and offer good resolution.

✓ High voltage can be applied which will enhance theresolution.

Disadvantages

✓ Expensive.

✓ Presence of sulphonic and carboxylic residue causes induced electro osmosis during electrophoresis.

Application

✓ Widely used in analysis of clinical and biological protein samples (albumin and globulins).

Alternative to paperelectrophoresis.

Moving boundaryelectrophoresis

PRINCIPLE:

The moving boundary method allows the charged species to migrate in a free movin.

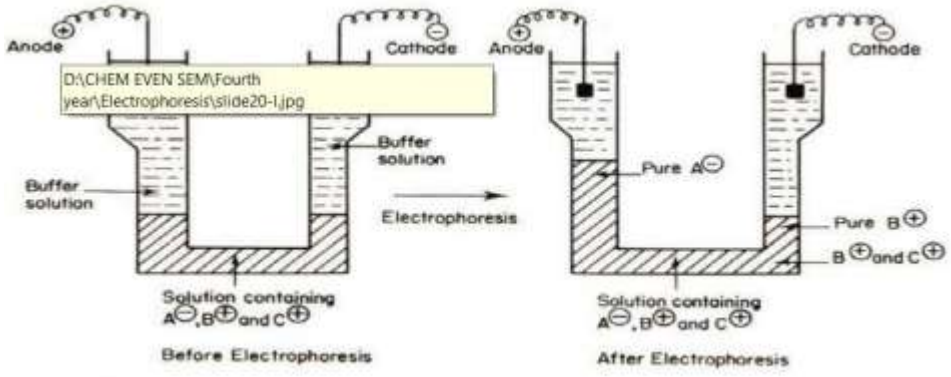

Figure 6: Moving boundary Electrophoresis

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Advantages

- ✓ Biologically active fractions can be recovered without the use of denaturingagents.
- ✓ A reference method for measuring electrophoreticmotilities.

Disadvantages

- ✓ Costly
- ✓ Elaborate optical system arerequired.

Application

- ✓ To study homogenecity of a macromolecularsystem.
- ✓ Analysis of complex biologicalmixtures.

A. Capillaryelectrophoresis

- ✓ The principle behind electrophoresis is that charged molecules will migrate toward the opposite pole and separate from each other based on physicalcharacteristics.
- ✓ Capillary electrophoresis has grown to become a collection of a range of separation techniques which involve the application of high voltages across buffer filled capillaries to achieve separations.
- ✓ Capillary electrophoresis, then, is the technique of performing electrophoresis in buffer filled, narrow-bore capillaries, normally from 25 to 100 mm in internal diameter(ID).
- ✓ A high voltage (typically 10-30 kV) isapplied.
- ✓ Capillaries are typically of 50 µm inner diameter and 0.5 to 1 m inlength.
- ✓ Due to electro osmotic flow, all sample components migrate towards the negative electrode.
- ✓ The capillary can also be filled with a gel, which eliminates the electro osmoticflow. Separation is accomplished as in conventional gel electrophoresis but the capillary allows higher resolution, greater sensitivity, and on-linedetection.
- ✓ The capillary is filled with electrolyte solution which conducts current through the inside of the capillary. The ends of the capillary are dipped into reservoirs filled with theelectrolyte.
- ✓ Electrodes (platinum) are inserted into the electrolyte reservoirs to complete the electricalcircuit.

Electroosmotic flow

- ✓ The surface of the silicate glass capillary contains negatively-charged functional groups that attract positively-charged counter ions. The positively-charged ions migrate towards the negative electrode and carry solvent molecules in the same direction. This overall solvent movement is called electro osmotic flow. During a separation, uncharged molecules move at the same velocity as the electro osmotic flow(withverylittleseparation).Positively-chargedionsmovefasterandnegatively- charged ions moveslower.
- ✓ Asmallvolumeofsampleismovedintooneendofthecapillary.Thecapillarypasses through a detector, (usually a UV absorbance detector), at the opposite end of the capillary.
- ✓ Application of a voltage causes movement of sample ions towards their appropriate electrode usually passing through thedetector.
- ✓ A plot of detector response with time is generated which is termed an electropherogram

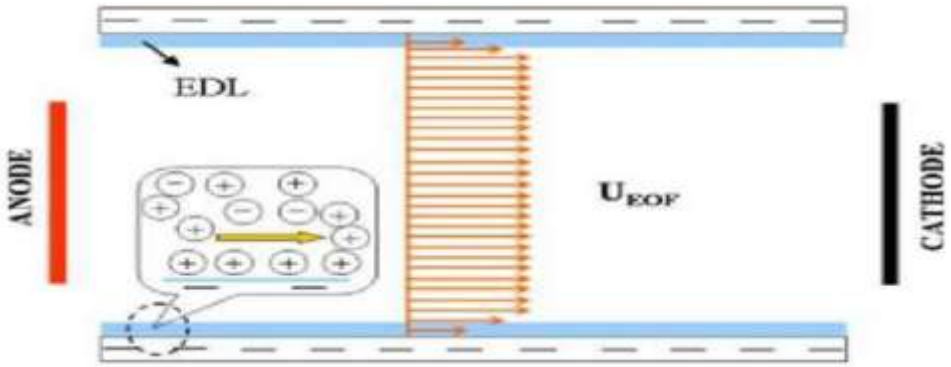

Figure 7: Electroosmatic flow

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B. Isotachophoresis

- ✓ The technique of isotachophoresis depends on the development of potentialgradient.
- ✓ Principle:
- ✓ Based on principle of moving boundary electrophoresis. A leading electrolyte(e.g. chloride) with a higher mobility than the analytes, and a trailing electrolyte(e.g. glycinate) with a lower mobility areused.
- ✓ Solution in which the separation takes place is normally an aqueous medium, which contains sucrose to provide a higher density to thesolution.
- ✓ WheretheseparationbyIsoelectricfocusingdepen dsontheexistenceofapHgradient in the system. The technique of Isotachophoresis depends on the development of a potentialgradient.
- ✓ Separation of the ionic components of the sample is achieved through stacking them into discrete zones in order of their mobilities , producing very highresolution.
- ✓ The analyte are positioned between the electrolytes and, when the voltage is applied, they migrate in order of decreasing mobility.
- ✓ This establishes the potential gradient; from that point on, all the analyte move at the samespeed.

❖ **Drugs determine byElectrophoresis**

TABLE 2: Drugs determine by Electrophoresis

Sr. No.	Various Drug	Determine by Electrophoresis
1.	Diptera: Oestridae: Oestrinae	polyacrylamide
2.	Protein Analysis	polyacrylamide gel electrophoresis
3.	infectious bursal disease virus	capillary zone electrophoresis
4.	capillary electrophoresis –mass spectrometry	successive multiple ionic-polymer layer (SMIL) and static linear polyacrylamide (LPA)
5.	Detection of buffalo milk adulteration with cowmilk	dairy industries because of the lower price and greater availability of cow milk throughout the year.
6.	sunflower using dual priming oligonucleotide system-based multiplex PCR	DPO-primer-based multiplex PCR system provides a rapid, reliable and cost-efficient solution for thediagnosis of fungal pathogens in sunflower.
7.	Automated Analysis of Arrayed Single-Cell Gel Electrophoresis	coefficient of quartile variation (CQV) of protein peak area under the curve (AUC)

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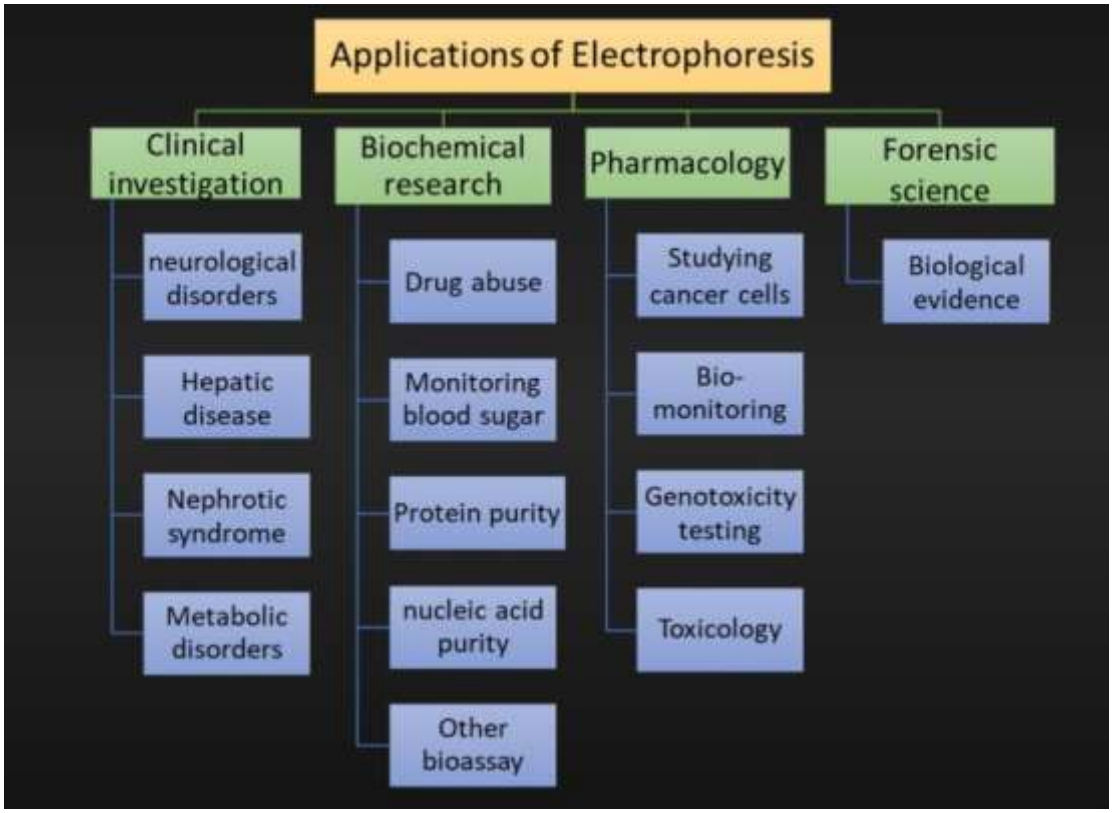
8.	Top-down proteomic analysis of monoclonal antibodies	capillary zone electrophoresis-mass spectrometry
9.	Cyclodextrins as modulators for separation of charged variants of mAbs	capillary zone electrophoresis
10.	Establishment of two-dimensional gel electrophoresis	soybean protein isolate and its application88

❖ **Application ofElectrophoresis**



Applications of Electrophoresis

- ✓ It is a tool of macromolecularseparation
- ✓ Many biological complex samples can be separated by using various methods ofelectrophoresis
- ✓ In some cases ,identification of molecules is also possible
- ✓ Gel method is more commonly used for routine laboratory experiments as well as research oriented separations andidentification
- ✓ Electrophoresis is handy tool for biologist and biochemist like the use for chromatographic techniques by organicchemist.
- ✓ Many handy instruments are available for conducting such separation experiments.

II. SUMMARY & CONCLUSION:

- ➢ Electrophoresisisaseparationtechniqueforliquid moleculesbasedontheirability to travel in an electricfield.
- ➢ Electrophoresis,initsnumerousformsandkinds,h asbecomethemostwidelyused method for analyzing biomolecules in biochemistry or

molecular biology, including genetic components like DNA and RNA, proteins, as well as polysaccharides. Electrophoresis is a simple yet sensitive analytical technique for separatingproteins,nucleicacids,orotherbiologicalcomponentsinsamplefluids.

- Themajorgoalofthisstudyistolearnmoreaboutvariouselectrophoreticmethods. Inthebiologicalsciences,electrophoresishasbecomeoneofthemostwidelyused analyticalmethods.
- Duetotheirsimplicityofuseandimproveddesign, manualelectrophoresissystems haveachievedahighdegreeofreliability,resolution,andaccuracyaftermorethan 30 years of research and improvement. Automation is the most recent step in this progression, and it offers unrivalled simplicity of use and repeatability, as well as less hands-on time and fasteroutcomes.
- Electrophoresis is a simple yet sensitive analytical technique for separating proteins, nucleic acids, or other biological components in sample fluids. Electrophoresisisaseparation techniqueforliquidmolecules basedontheirability to travel in an electricfield.
- Electrophoresis,initsnumerousformsandkinds,hasbecomethemostwidelyused method for analyzing biomolecules in biochemistry or molecular biology, including genetic components like DNA and RNA, proteins, as well as polysaccharides.

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