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A REVIEW ARTICIE ON ELECTROPHORESIS GUIDELINES

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ABSTRACT

Electrophoretic separation was first demonstrated in the year of 1807 and has since been a electrophoresis system, the development of electrophoresis systems have been driven by the advancement of technology such staple tool used by biologists and chemists for more than a century since its inception. From the initial crude paper electrophoresis system to today's modern automated electro miniaturization, precision engineering, biochemistry, electrical and electronics. These advancements were introduced to meet the requirement for faster and better resolution of results. This paper reviews the evolution of the electrophoresis technology over one century and provides an insight into the possible future development of electrophoresis. Various. Aspects of the electrophoresis system such as the performances, designs, usages, separation phases, and biochemistry were analysed. Them technological advancements for this field have been evidenced by the increasing complexity of the electrophoresis system. A peek into the possible future for the world of electrophoresis has been provided by drawing insights from the missing links of current technologies. It is both exciting and equally to explore the promises that this seeming simple separation technology holds for the future.

1. INTRODUCTION

Positive or negative electrical charges are frequently associated with biomolecules. When placed in an electric field, charged biomolecules move towards the electrode of opposite charge due to the phenomenon of electrostatic attraction. Electrophoresis is the separation of charged molecules in an applied electric field. The relative mobility of individual molecules depends on several factors. The most important of which are net charge, charge/mass ratio, molecular shape and the temperature, porosity and viscosity of the matrix through which the molecule migrates. Complex mixtures can be separated to very high resolution by this

process transport of particles through a solvent by application of an electric field is called as electrophoresis.

Most of the polymers (containing macromolecules) are electrically charged and will therefore move in an process of migration of charged particle through a solution under the influence of external electric field. Electrophoresis of positively charged particles is sometimes called cataphoresis, while electrophoresis of negatively charged particles is sometimes called an electrophoresis. Electrophoresis is used in laboratories to seprate macromolecules based on size. Electrophoresis is used extensively in DNA, RNA and Protein analysis. When a potential difference applied between the two electrodes in a colloidal solution, it has been observed that the colloidal particles are carried to either the positive or the negative electrode. In other words, they behave as if they have electric charge present within them with respect to the dispersion medium. The phenomenon is known as Electrophoresis and may be defined as the migration of the colloidal particles through a solution under the influence of electric field. 3 3electric field. Electrophoresis is useful in identification and structure determination of such big molecules.

Definition

Electro means Electricity means Separation of serum proteins by the effect of an electric current. Electrophoresis is a physical method of analysis which involves separation of the compounds that are capable of acquiring electric change in conducting electrodes.

Electrophoresis may be defined as the migration of the charged particle through a solution under the influence of an external field Ions that are suspended between two electrodes tends to travel towards the electrodes that bears opposite charges Macromolecules can be characterized by their rate of movement in an electric field. This property is used to determine protein molecular weights, to distinguish molecules by virtue of their net charge or their shape and to separate different molecular species quantitatively.

Rate of movement of macromolecules in an electric field is useful parameter to know any change in amino acid regarding its charge.

Electrophoresis is similar to chromatography. Electric field is used as a dragging force.

Technique is simple, very effective and clean.

Large number of samples can be separated, identified and quantitatively measured.

The term electrophoresis describes the migration of a charged particle under the influence of electric field (electrocharged particle and electrophoresis -movement).

Many important biological molecules such as amino acids, peptides, proteins, nucleotides, nucleic acids possess ionisable groups and, therefore, at any given pH, exists in solution as electrically charged species either as cations or anions.

Under the charge of an electric field these charged particles will migrate either to cathode or to anode, depending on the nature of their net charge.

This is one of the most fundamental processes used in all types of molecular biology and RDT experiments. Electrophoresis is migration of charged particles or molecules in a medium under the influence of an applied electric field.

The Rate of migration of charged molecules depends upon following factors

(a) The strength of electric field, size and shape.
(b) Relative hydrophobicity of the sample.
(c) Ionic strength and temperature of the buffer.
(d) Molecular size of the taken bio molecule.
(e) Net charge density of the taken bio molecule.
(f) Shape of the taken bio molecule.

In the process of electrophoresis large molecules have more difficulty in moving through the supporting medium (i.e., gel) whereas the smaller medium has more mobility through it.

The different components in a mixture will have different electrophoretic mobilities and hence they can be separated.

Mixtures of amino acids, proteins and nucleotides can be separated by their migration in an electric field.

Theory of electrophoresis

As movement of ions or their mobility depends upon the frictional coefficient, which in turn depends on the function of some of the physical properties of the molecules such as weight, molecular shape, size etc.

The resulting electrophoretic migration of the ion though the solution is opposed by a frictional for friction = V where V is velocity (rate of migration) of the ion and f is its 'frictional coefficient'.

The frictional coefficient is a measure of the drag that the solution exerts on the moving ion and is dependent on the size, shape and state of the ion as well as on the viscosity of the solution.

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.

PRINCIPLE OF ELECTROPHORESIS

When a potential difference is applied, the molecules with different overall charge will begin to separate owing to their different electrophoretic mobility.

Even the molecules with similar charge will begins to separate if they have different molecular sizes, since they will experience different frictional forces.

Therefore, some form of electrophoresis reply almost totally on the different charges on the molecules for separation while some other form exploits difference in size (molecular size) of molecules.

Electrophoresis is regarded as incomplete form of electrolysis because the electric field is removed before the molecules in the samples reaches the electrode.

But the molecules will have been already separated according to their electrophoretic mobilities.

The separated samples are then located by staining with an appropriate dye or by autoradiography, if the sample is radio labelled.

The fundamental principle of electrophoresis is the existence of charge separation between the surface of a particle and the fluid immediately surrounding it. An applied electric field acts on the resulting charge density, causing the particle to migrate and the fluid around the particle to flow.

It is the process of separation or purification of protein molecules, DNA, or RNA that differ in charge, size, and conformation.

The charged molecules are placed at one end of the field according to their charge, and an electric field is applied.

On passing electric current, depending upon the kind of charge the molecules carry, they move towards the opposite electrodes - either cathode (negative electrode) or anode (positive electrode).

The size, shape, and charge of the molecule remains constant during electrophoresis and determines ionic particle mobility. The rate of migration of an ion in a supporting medium under the influence of an electric field depends upon the following factors

*The net charge of the molecule
*The size and shape of the molecule
*The strength of the electric field
*The properties of the supporting medium
*The temperature of the procedure

When a potential difference is applied, the molecules with different overall charge will begin to separate owing to their different electrophoretic mobility. Even the molecules with similar charge will begins to separate if they have different molecular sizes, since they will experience different frictional forces. Therefore, some form of electrophoresis rely almost totally on the different charges on the molecules for separation while some other form exploits difference in size (molecular size) of molecules.

Electrophoresis is regarded as incomplete form of electrolysis because the electric field is removed before the molecules in the samples reaches the electrode but the molecules will have been already separated according to their electrophoretic mobilities.

The separated samples are then located by staining with an appropriate dye or by autoradiography, sample is radiolabelled.

FACTORS AFFECTING ELECTROPHORESIS

1. SAMPLE Charge: Rate of migration increases with increase in net charge. It depends on pH. Size: Rate of migration decreases for larger molecules.
2. It is due to increase frictional and electrostatics forces. Shape Molecular have similar charge but differ in shape exhibit different migration rate.
3. ELECTRIC FIELD According to ohms law I=V/R Current = Voltage / Resistance Voltage: Increase in voltage leads to increase in rate of migration Current. Increase in current leads to Increase in voltage, so the migration also Increases Resistance: If resistance increase migration decreases.
4. BUFFER determines & stabilizes pH of the supporting medium Also affects the migration rate of compounds in a number of ways Composition of Buffer Acetate Barbiturate Citrate EDTA For mate Phosphate Pyridine buffers commonly used.
5. IONIC STRENGTH As ionic strength of buffer increases Proportion of current carried by buffer increases Proportion of current carried by the sample decreases and hence showing decrease in sample rate of migration. High ionic strength
6. Also increases overall current and hence heat is produced as an ionic strength of buffer decrease Proportion of current carried by buffer decreases
7. Proportion of current carried by the sample increases and hence showing increase in sample rate of migration. Low ionic strength.
8. Also decrease in overall current and hence decrease in heat production.
9. pH determines the ionization, if ionization of organic acid increases as pH increases, ionization of organic acid decreases as pH decrease.
10. Therefore the degree of ionization is pH dependent. SUPPORTING MEDIUM Adsorption is the retention n of sample molecule by supporting medium.
11. Adsorption causes tailing of sample so that it moves in the shape of a ‘comet’ rather than a distinct compact band. Adsorption reduces both the rate of migration and resolution of separation of molecule.
12. Electro – endo-osmosis Electro – endo-osmosis due to the presence of charged groups on the surface of the supporting medium Eg. Paper - Carboxyl group (COO-) Agarose - Sulphate group (SO2-) Glass wall- Silanol (SiO-)
13. Above the pH value of three these charged groups will have ionize and generates

negatively charged sites. These ionized groups create an electrical double layer or region at supporting medium.

14. When voltage is applied, cation in electrolyte near supporting medium migrate towards cathode pulling electrolyte solution with them
15. This creates a net Electro – endo-osmotic flow towards the cathode.The Electro – endo-osmosis will accelerate the movement of cations, but retard anion movements.

Types of electrophoresis

1. Moving boundary electrophoresis
2. Zone electrophoresis

1. Moving boundary electrophoresis

First used by Sweet dish biochemist sellus, to separate proteins in 1937.In this method, the electrophoresis is carried in solution, without a supporting media.

The sample is dissolved the buffer and molecules move to their respective counter charge electrodes. Moving boundary electrophoresis is carried out in a U shape tube with platinum electrodes attached to the end of both arms.

At the respective ends, tube has refractometer to measure the change in refractive index of the buffer during electrophoresis due to presence of molecule is loaded in the middle of the U tube and then the apparatus is connected to the external power supply. Charged molecule moves to the opposite electrode as they passes through the refractometer, a change can be measured.

As the desirable molecule passes, sample can be taken out from the apparatus along with the buffer.

Disadvantages of Moving Boundary electrophoresis

The resolution of the technique is very low due to the mixing of the sample as well as over-lapping of the sample components. The electrophoresis technique is not good to separate and analyze the complex biological sample instead it can be used to study the behavior of the molecule in an electric field.

2. Zone electrophoresis

In this method, an inert polymeric supporting media is used between the electrodes to

separate and analyze the sample.

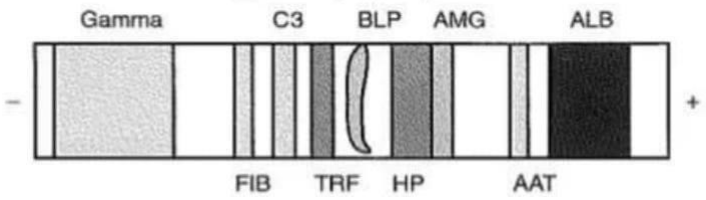

Fig. 1.1: Zone electrophoresis.

Supporting media used in zone electrophoresis are absorbent paper, gel of starch, agar and polyacrylamide. the major advantage of presence of supporting media is that it minimizes mixing of the sample and immobilization of the molecule after electrophoresis. It makes the analysis and purification of the molecule from the gel much easier than the moving boundary electrophoresis.

- It involves the migration of the charged particle on the supporting media. (Paper, Cellulose acetate membrane, Starch Gel, Poly acrylamide).

- Components separated are distributed into discrete zone on the support media. • Supporting media is saturated with buffer solution, small volume of the sample is applied as narrow band. • On application of PD at the ends of a strip components migrates at a rate determined by its electrophoretic mobility.

- **ADVANTAGES**
- Useful in biochemical investigations.
- Small quantity of sample can be analysed.
- Cost is low and easy maintenance.

DISADVANTAGES

Unsuitable for accurate mobility and isoelectric point determination.

Due to the presence of supporting medium, technical complications such as capillary flow, electro osmosis, adsorption and molecular sieving are introduced.

Methods of electrophoresis

1. Paper electrophoresis.
2. Gel electrophoresis.

3. Capillary electrophoresis.

1. Paper electrophoresis

It is separation technique, where ions of different charges are separated on a medium of paper (moistened with a buffer), by the application of a voltage between two electrodes, which are in contact with the paper.

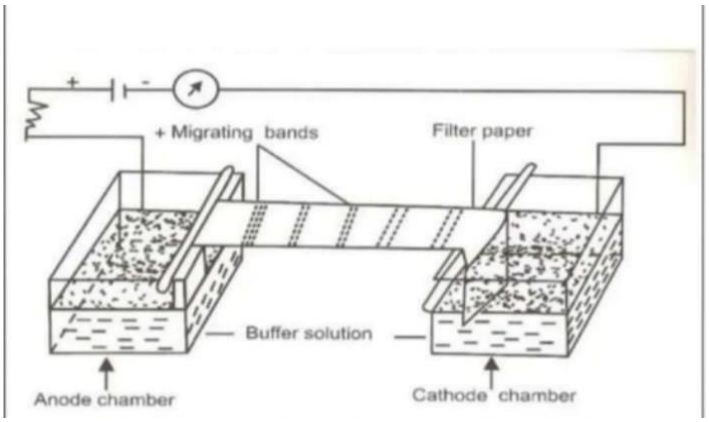

Fig. 1.2: Paper electrophoresis.

Principle of Separation

A mixture of ions or ionisable substances is applied on the centre of a paper, previously immersed in a buffer of known ionic strength. This paper is placed across two trays, filled with buffer, into which two electrodes are immersed. When a voltage is applied across these electrodes, the ions or ionisable substances migrate towards anode or cathode, based on their charge and other factors. Neutral or non-ionisable substances do not migrate. Anionic substances move towards anode and cations move towards cathode. Ultimately, there is separation of anionic, cationic and non-ionic / zwitterionic substances. The spots/bands which migrate can be detected bu using appropriate spray reagents or visualizing agents as in paper chromatofraphy and can be quantified by using densitometer. Both qualitative and quantitative analysis can be performed in paper electrophoresis.

Components of paper electrophoresis

Normally Whatman@ filter paper (Grade 3 MM or No. l) of suitable dimension (2.5cm to 5cm) with a length so that ends of the strip of paper touch the buffer solution, kept in the electrode vessels. The paper to be used is washed with double distilled water followed by 0.1 M HCI or 0.01 M EDTA to remove impurities.

Electrodes and voltage to be applied The electrode in the form of a thin wire is made up of carbon or platinum. A DC voltage of about 8-15 V/cm length of paper is normally applied. In low voltage electrophoresis, the voltage across two electrodes is about 100-300V, with a current of 0.4mAmp per cm width or 1.5mAmp/strip.

In high voltage electrophoresis, a potential of about 50-215V/cm(Total10,000V/strip) is applied across the electrodes.

Buffers used: The pH of buffer to be used depends upon the types of compounds to be separated. The following are some of the buffers used

l. Barbitone buffer (Veronal buffer) (0.07mole/litre, pH 8.6). Ionic Strength 0.05

2. Tris-acetate buffer (0.07 mole/litre, pH 7.6)

3. Citrate buffer (0.07 mole/litr, pH 3.0 or pH 6.8).

Other buffers of different pH and ionic strength can also be for separation, based on the type of compounds. Usually ionic strengths (IS) of 0.05-0.5 is used in most separations.

Types of paper electrophoresis (PE)

A. Horizontal /vertical Continuous Electrophoresis There are different types of electrophoresis instrument based on the design of the instrument. The diagrams of these 3 types of instruments are shown in.

B. Horizontal and vertical modes are used in analytical scale; whereas continuous electrophoresis is used on a preparative scale (i.e. large amount of sample mixture is used). The principles involved in all the modes are same, but the design of each instrument varies.

In Horizontal type / Vertical, buffer solution of known pH and ionic strength is filled into two troughs. Appropriate grade of Whatsman filter paper and suitable width and length of filter paper are immersed in buffer solution. 10-20pl of sample solution is applied at the centre of the paper and fixed in position.

The transparent lid is closed for safety as well as to prevent evaporation of buffer/solvent. A suitable potential (100-300V) is applied across two electrodes dipped in buffer solution.

When such potential is applied across the electrodes, migration of cations and anions take place towards cathode and anode respectively. Non-ionisable / neutral substances do not

move. Hence the separation of compound from the mixture takes place.

In this vertical mode, the migrations of ions are assisted by gravity and hence a typical separation takes place in about 6-8 hours. After sufficient migration, the paper is taken out and dried, to fix the spots / bands. Then the compounds / bands /spots can be visualized by using the visualizing agent. The quantitation of spots can be done by densitometer.

The horizontal mode is similar to the vertical mode, in principle. However, the paper is placed on a flat bed, as shown in the procedure to be followed is same as that of vertical type. In horizontal mode, it takes about 12-14 hours for separation.

Continuous electrophoresis is meant for preparative samples, where a predetermined sample volume through a valve device is applied continuously on the centre of paper. The application of voltage causes migration of samples and hence compounds are separated as bands. Thus each band is made to fall down and pure compounds are collected in separate containers. The solvent is evaporated and pure fractions are reused.

Various factors like charge of ions, size of the ions, viscosity of the medium, applied voltage, pH of buffer and ionic strength affect the migration of ions in paper electrophoresis.

There are two types of paper electrophoresis based on the voltage applied, i.e. Low voltage or High voltage Paper electrophoresis.

	Voltage/cm	**Votage/strip**
Low voltage PE.	8-15 V/ cm.	100-300
High voltage PE.	50-215V/ cm.	10,000v

High voltage paper Electrophoresis has the following advantages
Separation is faster, hence less time is required for separation Sharp bands are obtained, since there is less diffusion of bands.

As sharp bands are obtained, separation of closely related compounds can be achieved.

More number of samples can be analysed simultaneously. Disadvantages of High Voltage Paper Electrophoresis.

1. It is dangerous to the operator, since high voltage is applied.
2. More heating effects are seen because of high voltage and the paper becomes dry.

3. So in most of the laboratories, low voltage paper electrophoresis is used.

ADVANTAGES OF PAPER ELECTROPHORESIS

1. The technique is easy to follow
2. Less expenditure
3. Number of samples can be separated on a sample paper, at a time.
4. Wide variety of ionisable substances such as amino acids, proteins and peptides, antibiotics, alkaloids etc., can be separated.

DISADVANTAGES

1. The time required for separation is more, i.e. 6-8 hours in vertical mode and 1214hours in horizontal mode.
2. Use of high voltage may be dangerous, unless precautions are taken.

Application of Paper Electrophoresis

Paper electrophoresis is used mainly for the separation of ionizable substances, by using buffers of different pH and ionic strength. The following are some of the pharmaceutical applications of paper ele. ctrophoresis.

1. Separation of amino acids into acidic or basic or zwitterionic type.
2. Separation of proteins in serum (into albumin, al, O, 13 and gamma globulins). The type of protein and the percentage of each component can be estimated using densitometer.

a) Separation of lipoproteins in serum (in case of hyperlipidemia)
b) Separations of enzymes in blood.
c) Separation of alkaloids and antibiotics in different samples can be carried out.

A firmly adhering layer of gel of uniform thickness. The connection between the gel and the conducting solution is effected in various ways according to the type of apparatus used. Precautions are to be taken to avoid condensation of moisture or drying of the solid layer.

2. Gel electrophoresis

Vertical Gel Instrument- The schematic diagram of a vertical gel electrophoresis apparatus is given. It has two buffer chambers, upper chamber and a lower chamber. Both chambers are fitted with the platinum electrodes connected to the external power supply from a power pack which supplies a direct current or DC voltage. The upper and lower tank filled with the

running buffer is connected by the electrophoresis gel casted in between two glass plates (rectangular and notched). There are additional accessories needed for casting the polyacrylamide gel such as comb (to prepare different well), spacer, gel caster etc.

Casting of the gel: The acrylamide solution (a mixture of monomeric acrylamide and a bifunctional cross linker bis-acrylamide) is mixed with the TEMED and APS and poured in between the glass plate fitted into the gel caster. What is the mechanism of acrylamide polymerization? Ammoinum persulfate in the presence of TEMED forms oxygen free radicals and induces the polymerization of acrylamide monomer to form a linear polymer. These linear monomers are interconnected by the cross linking with bis-acrylamide monomer to form a 3-D mesh with pores. The size of pore is controlled by the concentration of acrylamide and amount of bis-acrylamide in the gel. In a vertical gel electrophoresis system, we cast two types of gels, stacking gel and resolving gel. First the resolving gel solution is prepared and poured into the gel cassette for polymerization. A thin layer of organic solvent (such as butanol or isopropanol) is layered to stop the entry of oxygen (oxygen neutralizes the free radical and slow down the polymerization) and make the top layer smooth. After polymerization of the resolving gel, a stacking gel is poured and comb is fitted intothe gel for construction of different lanes for the samples.

Running of the gel: The sample is prepared in the loading dye containing SDS, ß-mercaptoethanol in glycerol to denature the sample and presence of glycerol facilitates the loading of sample in the well.

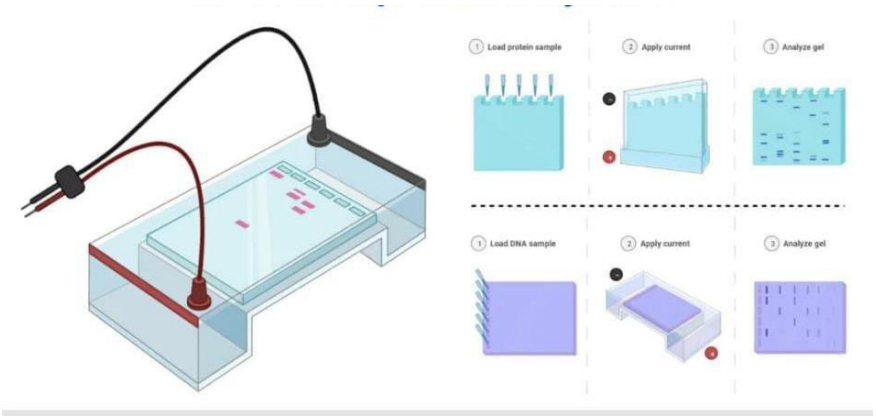

Fig. 1.3: Gel electrophoresis system.

Calculation of molecular weight of the unknown protein sample is a S step proces:

1. Resolve the protein sample on the SDS-PAGE along with the molecular weight markers.
2. Calculate the relative mobility (RD using the following formula: Rf=migration of protein from the lane /migration of tracking dye.
3. Plotlog molecular mass (y-axis) versus relative mobility (x-axis) of the standards.
4. Perform a linear regression using a calculator or using regression software such as Microsoft excall.
5. Use the linear regression equation (Y = mx + c) to estimate the mass of the unknown protein.

Log Molecular Weight = (slope) (mobility of the unknown) + Y intercept.

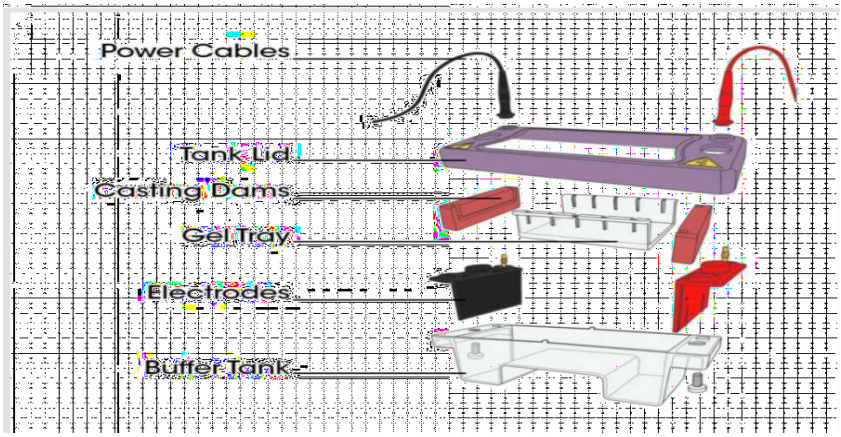

Fig. 1.4: Parts of gel electrophoresis.

Buffer and reagent for electrophoresis- The different buffer and reagents with their purpose for vertical gel electrophoresis is as follows-

N, N, N', N'-tetra methylethylene diamine (TEMED)-it catalyzes the acrylamide polymerization.

1. Ammonium Persulfate (APS)-it is an initiator for the acrylamide polymerization.
2. Tris-HCI- it is the component of running and gel casting buffer.
3. Glycine it is the component of running buffer
4. Bromophenol blue- it is the tracking dye to monitor the progress of gel electrophoresis.
5. Cooma«ie brilliant blue R250 it is used to stain the polyacrylamide gel.
6. Sodium dodecyl sulphate it is used to denature and provide negative charge to the protein.
7. Acrylamide monomeric unit used to prepare the gel.
8. Bis acrylamide cross linker for polymerization of acrylamide monomer to form gel.

3. Capillary Electrophoresis

Capillary electrophoresis employing a narrow bore fused quartz silica capillary tube usually 50-75cm long with an i.d. of 25-100gm (and an o.d. of 400 pm) containing an appropriate electrolyte using a direct current (DC) high voltage source, capable of producing a current of 25() PA at voltage ranging from 1000 to 30,000volts and on-line detector that similar to those HPLC are involved (high voltage electrophoresis).

A cross — sectional view of such a capillary .The capillary is protected with an outer layer of a polyimide (polymer of imide monomer).

The system's main components are a sample vial, source and destination vials, a capillary, electrodes, a high voltage power supply, a detector, and a data output and handling device.

The source vial, destination vial and capillary are filled with an electrolyte such as an aqueous buffer solution. To introduce the sample, the capillary inlet is placed into a vial containing the sample. Sample is introduced into the capillary via capillary action, pressure, siphoning, or electrokinetically, and the capillary is then returned to the source vial.

The migration of the analytes is initiated by an electric field that is applied between the source and destination vials and is supplied to the electrodes by the high voltage power supply. In the most common mode of CE, all ions, positive or negative, are pulled through the capillary in the same direction by electroosmotic flow (EOF) The analytes separate as they migrate due to their electrophoretic mobility, and are detected near the outlet end of the capillary. The output of the detector is sent to a data output and handling device such as an integrator or computer.

The data is then displayed as an electropherogram, which reports detector response as a function of time. Separated chemical compounds appear as peaks with different retention times in an electropherogram.

Detection in CE: The most commonly used detectors are a UV absorbance or a fluorescence monitor or a diode array spectrometer producing absorbance data at multiple wavelengths, on account of the very small volume (< of the separated analytes, the detection is carried out on column (or on-line detection).

For this a small outer part of the protective polyimide coating from the capillary surface is

removed either by burning, dissolving or scratching. Various events of migration of species and EOF in capillary electrophoresis The efficiency, N (Number of theoretical plates) may be expressed by the equation:

gEdN=1/2D

Where,

D- the diffusion coefficients of migration d - the distance travelled

= - electrophoretic mobility of the species, and E - the applied electric field

CE mechanism is entirely different from a chromatographic distribution mechanism, in that it is readily applicable smaller as well as macromolecules. Thus it is used for the separation of large biomolecules.

Representative Electropherogram showing the separation of a seized heroin sample byusing MEKC with short end injection

(1) Morphine,

(2) Heroin,

(3) Acetylcodeine,

ISTD = internal standard (N, N-dimethyl-5-methoxytryptamine). UV absorbance at 210 nm, uncoated fused silica capillary 50 cm x 50 pm I.D. O.D., effective separation length 8 cm, back- ground electrolyte: 15 mM sodium borate, 25 mM sodium dodecyl sulphate, 15% (v/v) acetonitrile, pH 9.5, 25 oc.—25 kV, hydro dynamic injection.

Advantage and disadvantage electrophoresis Advantages of Electrophoresis

Separation of Molecules: Electrophoresis is a powerful technique for separating molecules based on their size and charge, allowing for the analysis of DNA, RNA, proteins, and other biomolecules.

High Resolution: It provides high-resolution separation, allowing the identification of distinct bands or peaks corresponding to different molecules in a sample.

Versatility: Electrophoresis is versatile and can be applied to various types of molecules, making it a widely used technique in molecular biology and biochemistry.

Quantification: It enables the quantification of nucleic acids and proteins by analyzing the intensity of bands or peaks in the resulting electrophoretic pattern.

Speed: Depending on the type of electrophoresis, it can be a relatively quick method for separating molecules.

Disadvantages of Electrophoresis

Heat Generation: Electrophoresis can generate heat during the process, which may affect the stability of some molecules, particularly proteins.

Complexity: Certain types of electrophoresis, such as capillary electrophoresis, can be technically challenging and require specialized equipment.

Limited Separation Based on Charge: In traditional electrophoresis, separation is mainly based on charge, which may not be sufficient for highly similar molecules.

Sample Deterioration: The process can lead to sample deterioration, especially for delicate molecules, and may not preserve the native state of proteins or nucleic acids.

Application

Analytes	Matrix	Method
Coca alkaloids and sugars	Illicit cocaine	MEKC (Micellar electrokinetic chromatography) with indirect UV detection
Heroin, morphine, acetylcodeine, caffeine, paracetamol	Heroin seizures	MEKC with short-end injection, detection by UV absorbance
Methamphetamine, amphetamine, dimethylamph etamine, and phydroxymethamphetamine	Urine from subjects using methamphetamine	CZE using cyclodextrins for separation of enantiomers with
		MS detection

a. Capillary electrophoresis (CE) is the primary methodology used for separating and detecting short tandem repeat (STR) alleles in forensic DNA laboratories.
b. Capillary electrophoresis may be used for the simultaneous determination of the ions NH+4, Na+, K+, Mg+2 and Ca+2 in saliva.
c. Illicit Drug Analysis.

Applications of capillary electrophoresis to illicit drugs in seizures and toxicology

1. DNA Sequencing
2. Medical Research
3. Protein research/purification

4. Agricultural testing
5. Separation of organic acid, alkaloids, carbohydrates, amino acids, alcohols, phenols, nucleic acids, insulin.
6. In food industry
7. It is employed in biochemical and clinical fields i.e. in the study of protein mixtures such as blood serum, haemoglobins and in the study of antigen- antibody interactions.
8. Electrophoresis in combination with autoradiography is used to study the binding of iron to serum proteins.
9. Used for analysis of steroids and antibiotics.
10. For testing purity of thyroid hormones by zone electrophoresis.
11. Paper chromato-electrophoresis is used to separate free Insulin from plasma proteins.
12. It is used for diagnosis of various diseases of kidney, liver and CVS.
13. It is also used for separation of Scopolamine and Ephedrine using buffer at PH 4.2.
14. Electrophoresis is also used for separation of carbohydrates and vitamins.
15. Quantitative separation of all fractions of cellular entities, antibiotics, RBC, Enzymes etc is possible.

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