Electrophoresis Cheat Sheet

by nenei via cheatography.com/162536/cs/44903/

Electrophoresis

Electrophoresis
Separation technique
on the basis of charge
incomplete separation
"migration of a charged particle under the influence of an electric field"
separates- amino acids, peptides, proteins, nucleotides, nucleic acids; possess ionizable groups

Points to remember-

Ion	Migrates towards
Cation(+)	**Cathode**
Anion(-)	Anode

Purpose

Purpose
To determine number , amount, mobility of components in a given sample OR to separate them
To obtain information about the electrical double layers surrounding the particles

Factors affecting electrophoretic mobility-

Sample	Charge	Higher the charge greater is electr-oph-oretic mobility	Buffer	compos ition	Type of buffer depend upon what sample is to run
(charge to mass ratio)		Charge depends upon pH			e.g, formate, citrate, phosphate, EDTA, acetate pyridine, Tris, and barbitone etc
	Size	Bigger the molecule greater the frictional force		ionic strength	between 0.05-0.1M

Factors affecting electrophoretic mobility- (cont)

	Larger the particle slower the electroph-oretic mobility		Higher the strength of buffer more the current carried by buffer ions that affect electroph-oretic run
Shape	Rounded molecules has lesser frictional and electrostatic retardation compared to sharp molecules	pH	Determines degree of ionization of organic compounds
	Globular and Fibrous proteins		Amino acids shows charge based on surrou-nding pH

Factors affecting electrophoretic mobility- (cont)

Electric field	rate of migration under unit potential gradient is referred to as mobility of the ion	Medium	led to electr-o-o-smosis	ac
	increase in potential gradient increases the rate of migration			m si

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Factors affecting electrophoretic mobility- (cont)

current (total charge carried per second to the electrode) in the solution placed between two electrodes is carried mainly by the buffer ions, only a small proportion being carried by the sample ions. An increase in the potential-difference therefore increases the current.

Advantage over free electrophoresis

Microliters of sample are sufficient

Sample components during their migration split up into as many different zones as it contains differently migrating components. each zone consisting of a single component which can be easily isolated

stabilizing system does not allow the zones to disperse and spoil the separation

Many detection schemes are Available And elution helps in further analysis

Gel electrophoresis

Separation not only depends on the charge on the molecule but also on its size.

Gels are porous and the size of the pores relative to that of the molecule determines whether the molecule will enter the pore and be retarded or will bypass it.

Resolution of a sample is sharper and better in a gel than in any other type of medium.

Electrophoretic Mobility in Gels

Molecular sieving action of the gels and its effect on the mobility of a macromolecule.

The pore size thus the molecular sieving action and therefore the effect on electrophoretic mobility of a molecule are functions of gel concentration

$Kr = C (R + r)$

Kr is the retardation coefficient. C is constant. R is the mean radius of the macromolecule and r is the radius of the gel fibers.

Solubilizers

To study subunit composition of oligomeric proteins

Solubilizers destabilize native structure of proteins by destroying charges that associate subunits together

Urea - Disrupt hydrogen bonds At high concentration (3-12M). Also, dsDNA can be rendered into ssDNA by use of urea.

SDS - SDS is an anionic detergent and disrupts macromolecules whose structure has been stabilized by hydrophobic associations. Also imparts a large negative charge to the denatured polypeptides

beta mercaptoethanol - Disrupt Disulphide bridges. Separation of peptides in proteins Linked by disulphide bonds

Types of Gel

Starch Gel, Agarose Gel, Acrylamide Gel and Agarose-Acrylamide Gel

Starch Gel

High porosity starch gels are obtained by using 2% (w/v) starch and low porosity gels are obtained by adding 10-15% starch to the buffer.

The pore size in a starch gel cannot be controlled and this is the biggest drawback of these gels.

Difficult to prevent contamination of starch gels by microorganisms.

Another disadvantage of starch gels is that upon staining to detect the separated components, the starch gel turns opaque making direct photoelectric determination impossible.

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Starch Gel (cont)

the resolving power of starch gels is very high and can be matched only by polyacrylamide gels. One of their important applications is the analysis of isoenzyme patterns (zymograms).

Principle

Any charged ion or molecule migrates when placed in an electric field.

The rate of migration depend upon its net charge, size, shape and the applied electric current.

Formula

$$v = \frac{Eq}{F}$$

where **F** = frictional coefficient, which depends upon the mass and shape of the molecule.

E = electric field (V/ cm)

q = the net charge on molecule

v = velocity of the molecule.

Electrophoretic mobility (μ)

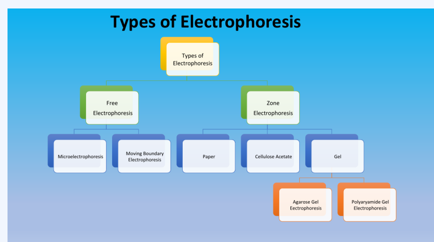
Electrophoretic mobility (μ) of an ion is used, which is the ratio of the velocity of the ion to field strength (v/E)

Electrophoretic mobility v is directly proportional to the charge and inversely proportional to the viscosity of the medium, size and shape of the molecule

Formula

$$u = \frac{Q}{6\Pi r\eta}$$

Types-



Free electrophoresis

carrier-free electrophoresis

matrix-free electrophoretic separation technique

used to quantitatively separate samples according to differences in charge or isoelectric point.

Two Main Techniques- Microelectrophoresis and Moving Boundary Electrophoresis.

Moving boundary electrophoresis

allows the charged species to migrate in a free moving solution in the absence of a supporting medium

Samples are fractioned in a U shaped tube that has been filled with buffer

An electrical field is applied by means of electrodes at the ends of the U tube and Separation takes place as a result of difference in mobilities

Method was very popular for quantitative analysis of complex mixtures of macromolecules, especially proteins,e.g., those in blood plasma.

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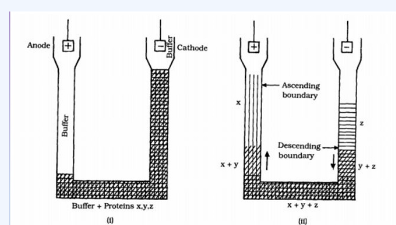
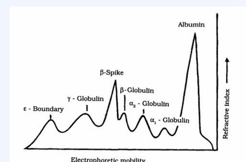


fig-3



Zone electrophoresis

separation technique employing stabilizing media

electrophoresis in stabilized media

Paper electrophoresis

Filter paper as a stabilizing medium is very popular for the study of normal and abnormal plasma proteins

Paper of good quality should contain at least 95% of cellulose and should have only a very slight adsorption capacity.

Chromatography paper is suitable for electrophoresis and needs no preparation other than to be cut to size.

Two arrangements of paper in paper electrophoresis are horizontal and vertical

Process

Filter paper

Apparatus - Power pack and Electrophoresis cell

Sample application

Electrophoretic run

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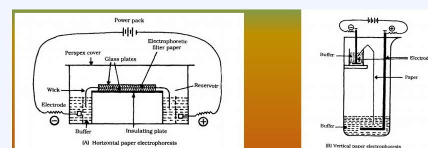

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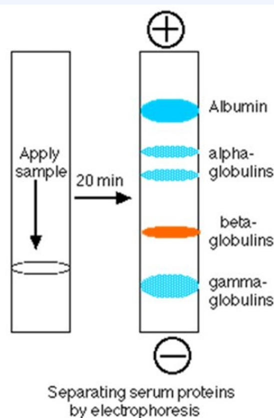


Detection and Quantitative Assay

Fluorescence
Ultraviolet absorption
Staining
Detection of enzymes in situ

Applications of paper E-

Separating amino acids into acidic and basic
Separation of enzymes in blood
Protein separation in serum
Studying SCA in blood

History

Time	Late 18th century	-	-	1930
Scientist	Faraday	Johann Wilhelm Hittorf, Walther Nernst, and Friedrich Kohlrausch	Friedrich Kohlrausch	Arne Tiselius

History (cont)

Experiment	Laws of electrolysis	To measure the properties and behavior of small ions moving through aqueous solutions under the influence of an electric field	created equations for varying concentrations of charged particles moving through solution, including sharp moving boundaries of migrating particles	

Problems

Generation of heat (of the electrophoretic medium) has following effects-
increased rate of diffusion of sample and buffer ions leading to broadening of the separated samples
thermal instability of samples that are rather sensitive to heat.
formation of convection currents, which leads to mixing of separated samples
decrease of buffer viscosity, and hence a reduction in the resistance of the medium

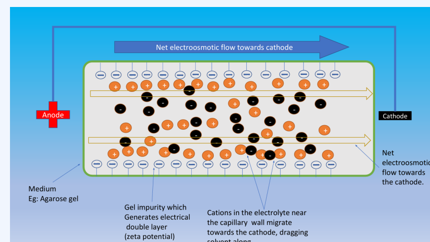
Electroendosmosis

electroosmotic flow	
Cause-	Due to the presence of charged groups on the surface of the support medium.

Electroendosmosis (cont)

Molecular separation and chemical	Eg: Eg: Carboxyl groups in paper, sulphate impurities in agarose, SiOH groups in capillary electrophoresis

Diagram



Points

1. **Electroosmotic Flow (EOF)**: Movement of liquid in response to an electric field, toward the cathode.
2. **Zeta Potential**: Surface charges on the gel form an electrical double layer, creating a zeta potential that drives ion flow.
3. **Cation Migration**: Cations near the capillary wall migrate toward the cathode, dragging the solvent with them.
4. **Electric Field Setup**: An anode (positive) and cathode (negative) are placed to create the electric field across the medium.
5. **Ion Separation**: EOF aids in separating analytes; positive ions move quickly to the cathode, while negative ions are slowed.
6. **Applications**: Used in capillary electrophoresis for separating molecules like DNA and proteins by charge and size.

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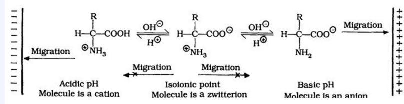
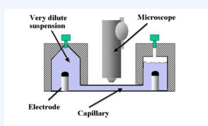
Fig-1



Microelectrophoresis

method for determination of zeta potentials
apparatus-capillary cell, two chambers that include electrodes, and a means of observing the motion of particles.
apparatus is filled with very dilute suspension and the chambers are closed.
direct-current voltage is applied between electrodes in the respective chambers.
One uses a microscope to determine the velocity of particles.
Zeta potential values near to zero indicate that the particles in the mixture are likely to stick together when they collide, unless they also are stabilized by non-electrical factors.
Particles having a negative zeta potential are expected to interact strongly with cationic additives
In modern days this technique is applied only for measuring the zeta potentials of cells such as R.B.Cs, neutrophils, bacteria etc.

fig-2



Cellulose Acetate Electrophoresis

Advantages
it is chemically pure
cellulose strips are translucent
very low content of glucose.
Cellulose acetate is not very hydrophilic and thus holds very little buffer.

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