# ISOELECTRIC FOCUSING(IEF)

## PROTEIN FINGERPRINT

TUBA ÇAĞIRTEKIN

DAMLA YAY

# CONTENT

- Introduction
- Principle
- Procedure
- Applications

THERMINOLOGY

- Isoelectric point
- Amphoteric matters
- Electric field
- Ampholytes
- pH gradients
- Polyacrylamide gel
- Agarose gel

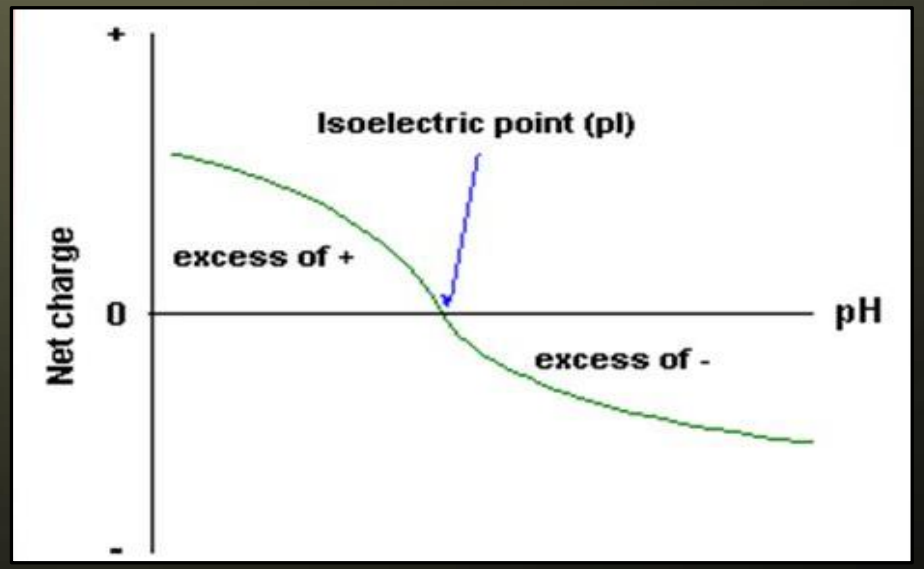
# INTRODUCTION

## Isoelectric focusing uses protein pI

Isoelectric point ph of a solution where the protein has a net charge of zero(ziwitterion form). Reached only when ph=pI as shown in graph.

pI dependant on which type of residues are present and how many.

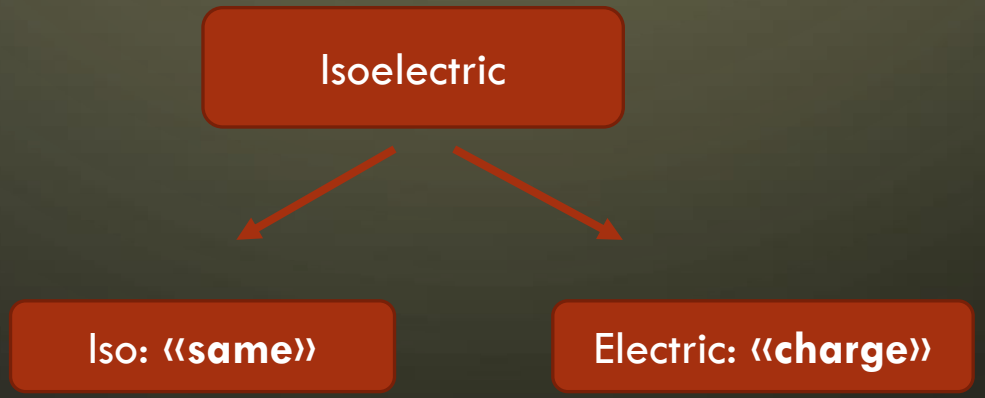
Specific for each protein.



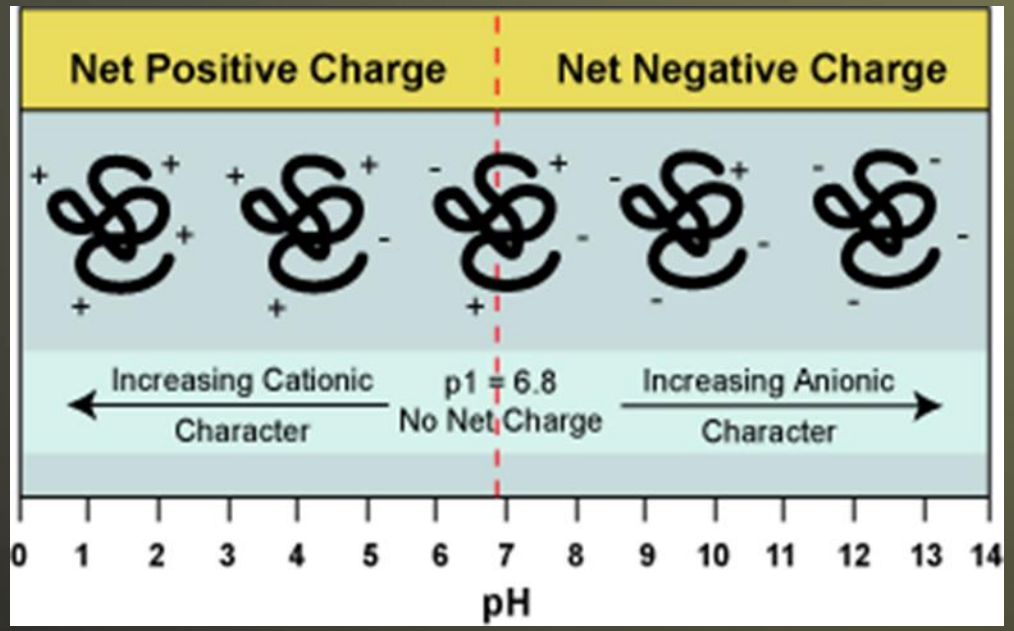
# What is isoelectric focusing (IEF)?

IEF, also known simply as electrofocusing, is a technique for separating charged molecules, usually proteins or peptides, on the basis of their pIs.

IEF began in 1964 when, Olaf Vesterberg filed a Swedish patent on the synthesis of new chemicals called carrier ampholytes. This technique popularized by H.Svensson in Sweden.



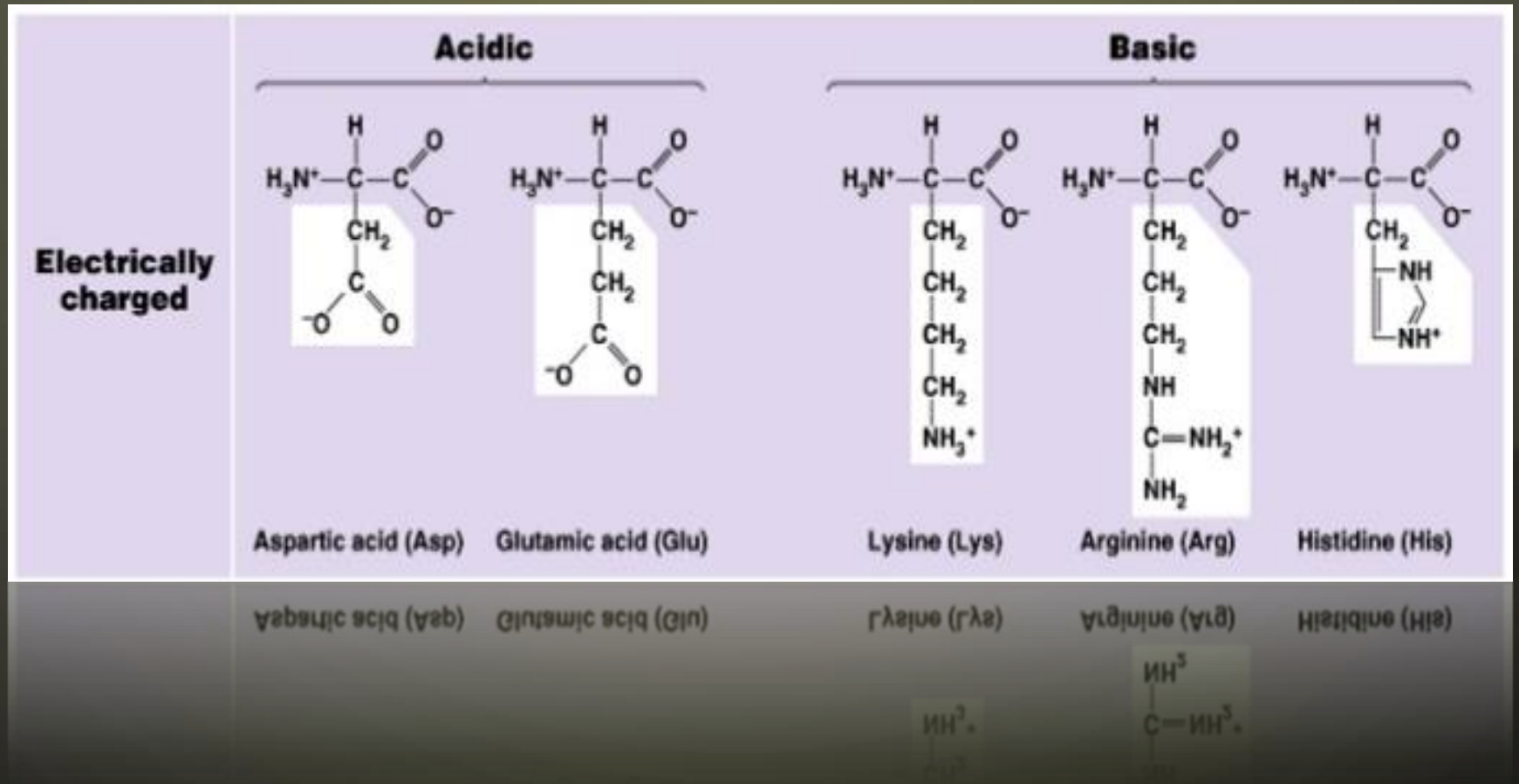
# PRINCIPLES



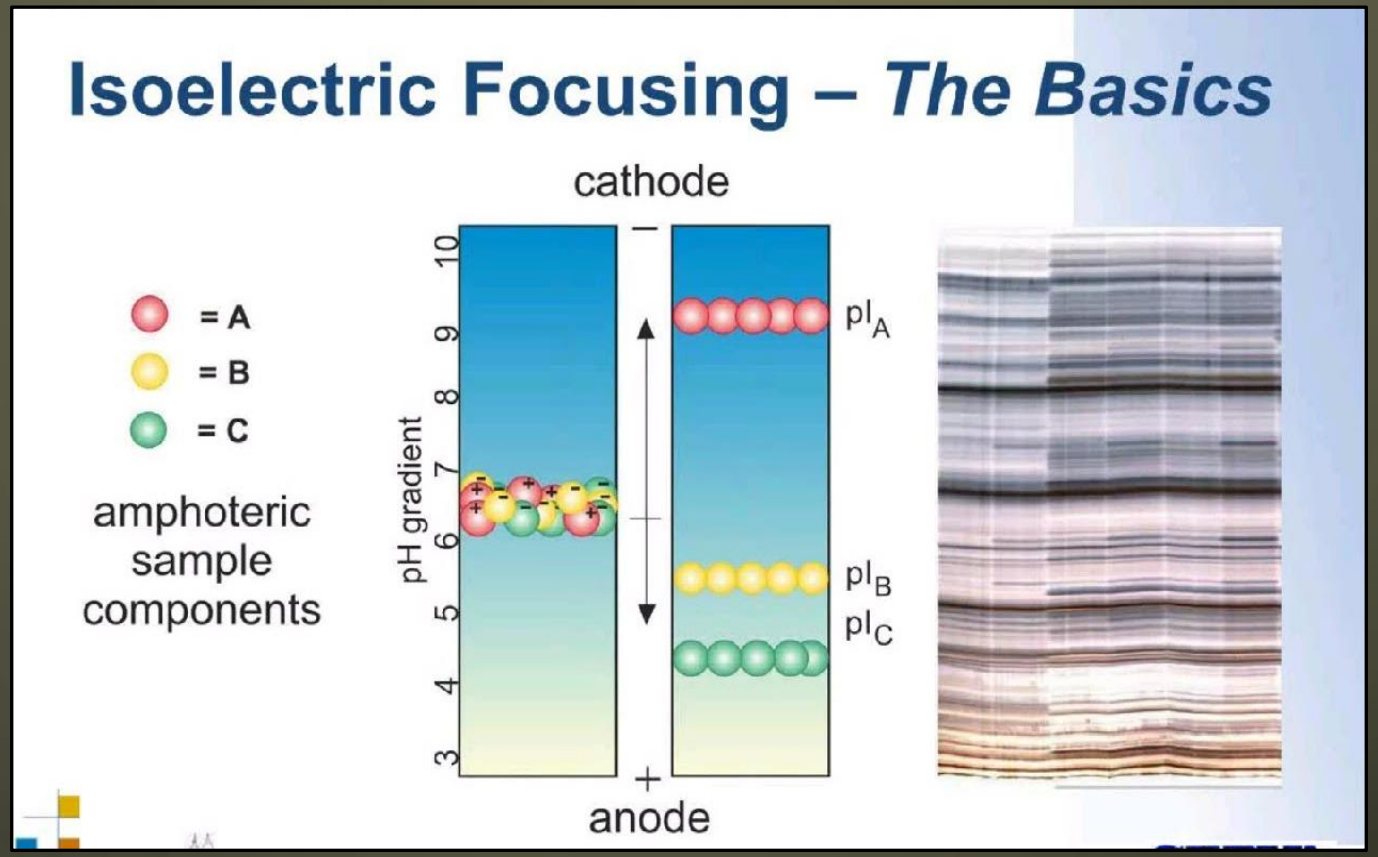

In acidic environment-more Hydrogen ions- positively charged protein

At PI- equal number of positive and negative charges on protein

In basic environment- little Hydrogen ions- negatively charged protein



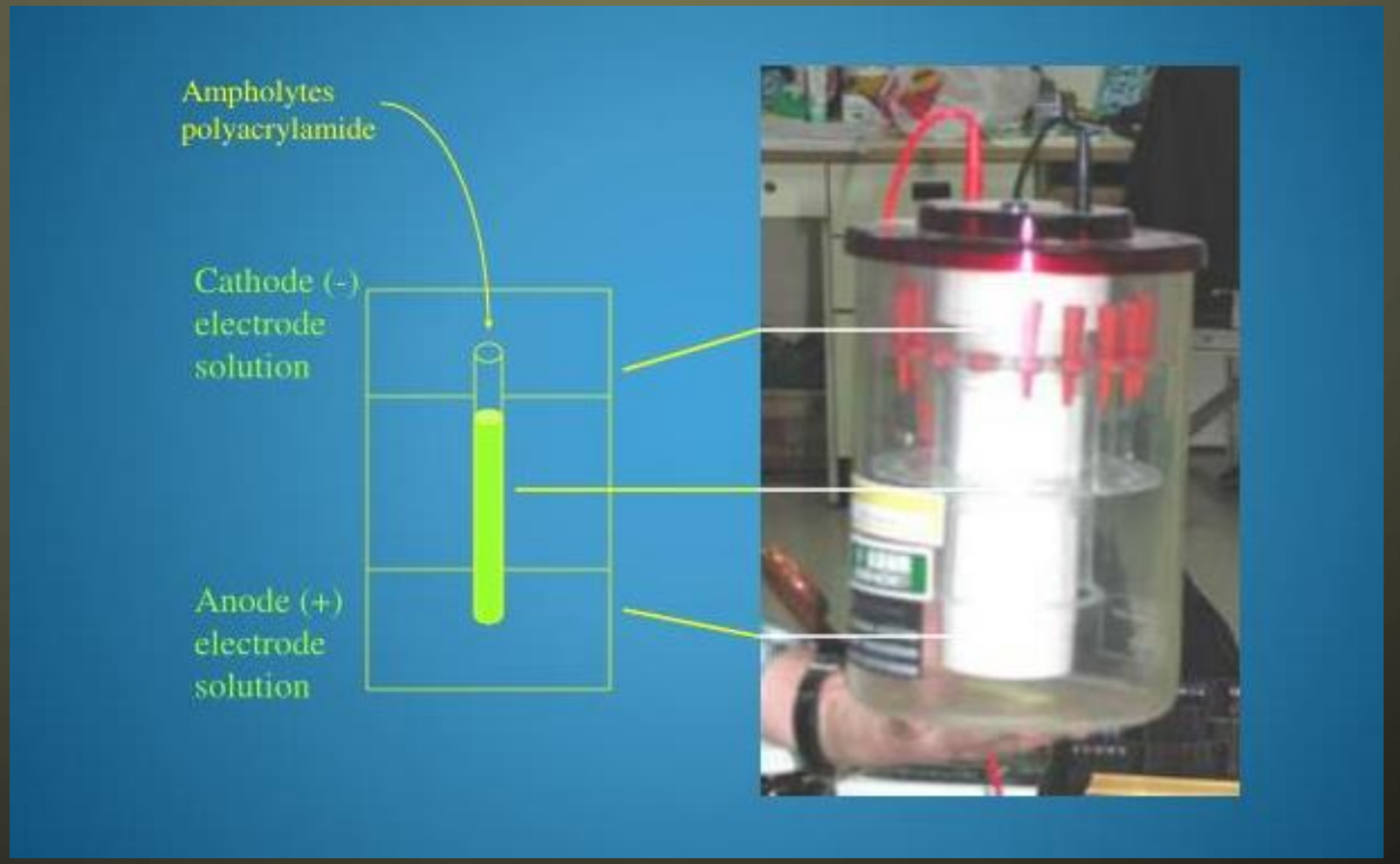

MOLEKULCE.COM/DAMLA_YAY-TUĞBA_ÇAĞıRTEKIN



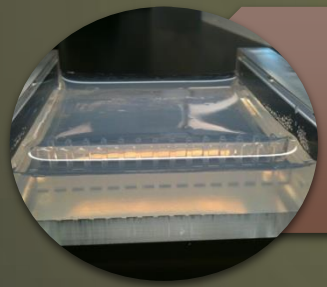

The sample is loaded and voltage is applied. The proteins will migrate to their isoelectric pH, the location at which they have no net charge.

Direction of proteins migration depends only from their charge.

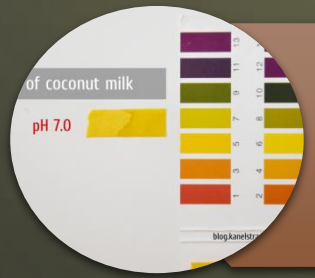
# TRADITIONAL EQUIPMENT FOR IEF



# PROCEDURE



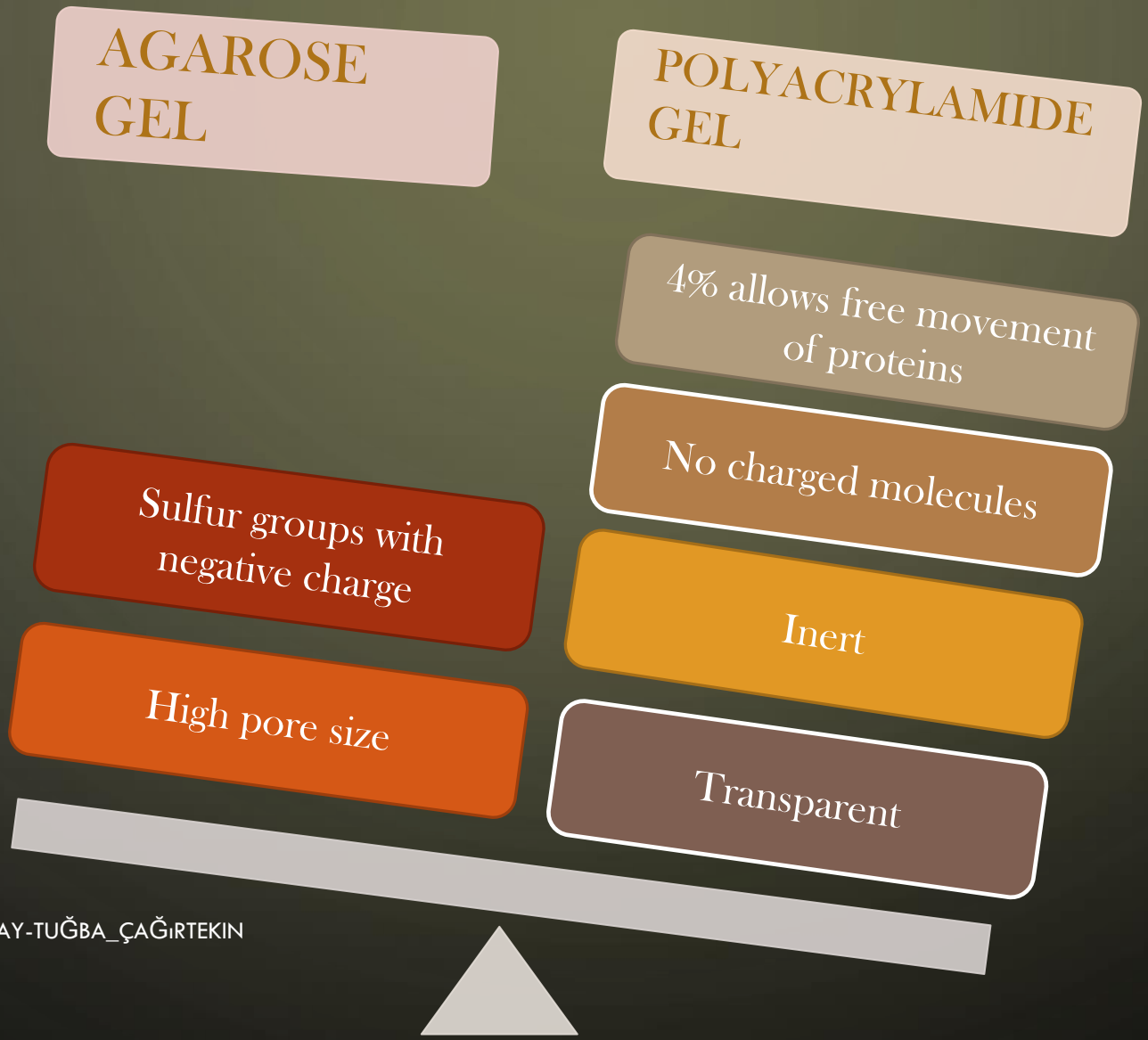
Gels for IEF




Establishing pH ingredient

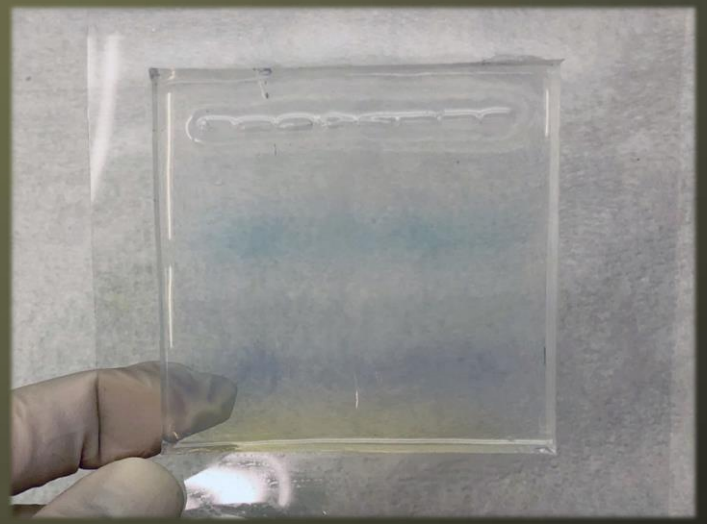
Staining

# GELS FOR IEF


AGAROSE GEL
POLYACRYLAMIDE GEL
4% allows free movement of proteins
No charged molecules
Sulfur groups with negative charge
Inert
High pore size
Transparent

# Polyacrylamide gel

IEF is carried out in large-pore polyacrylamide gels which serves mainly as anticonnective matrices and have high mechanical and chemical stability. Chemical polymerisation with the catalyst system ammonium persulfate and TEMED is preferably used instead of photopolymerization with riboflavin.



# ESTABLiSHiNG PH GRADiENT

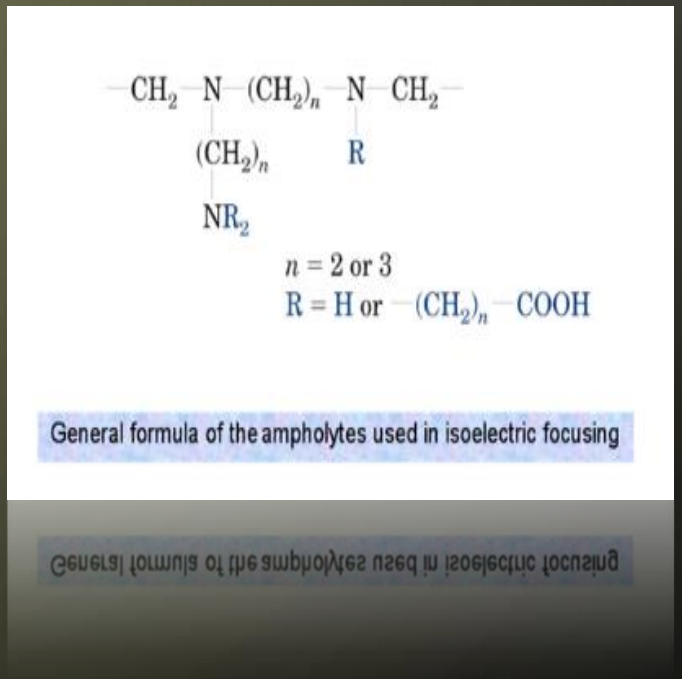
Stable, linear pH gradinets are the keys  to successful IEF. Establishment of such gradinents accomplish in two ways with two different molecules. Concentrating effects called focusing.

AMPHOLYTES (Amphoteric elctrolytes)

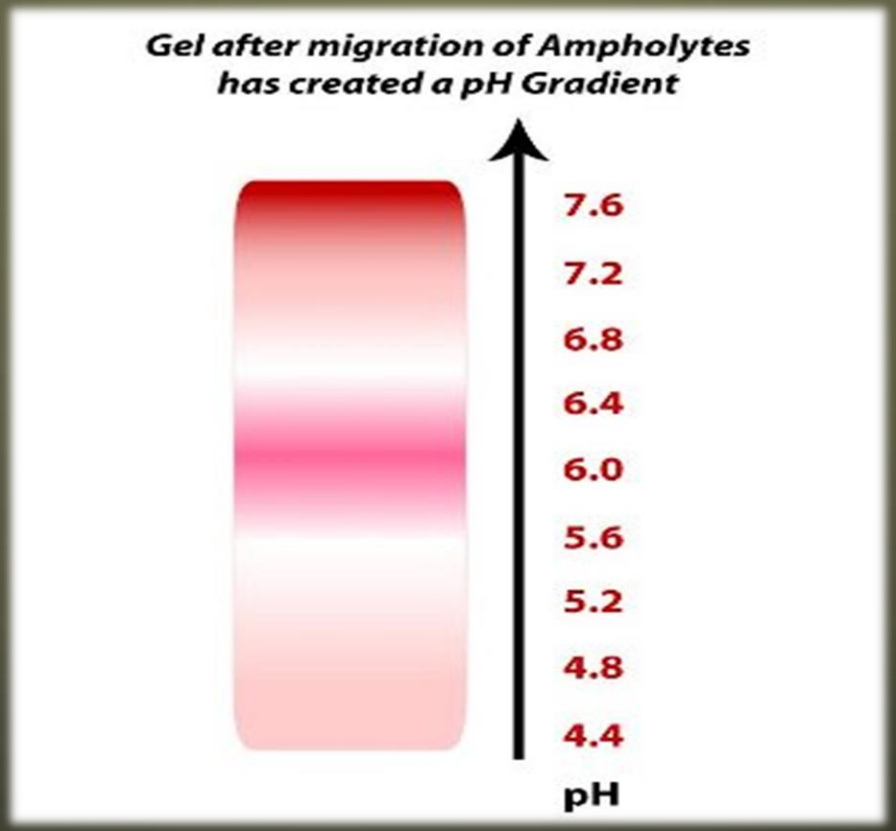
ACRYLAMIO BUFFERS

# AMPHOLYTES

- Carrier ampholytes are mixtures of molecules containing multiple aliphatic amino and carboxylate group
- They are small multi charged organic buffer molecules with closely spaced pI values
- Most common and simplest for forming pH gradient
- Included directly in IEF gel
- High conductivity



General formula of the ampholytes used in isoelectric focusing




MOLEKULCE.COM/DAMLA_YAY-TUĞBA_ÇAĞıRTEKIN

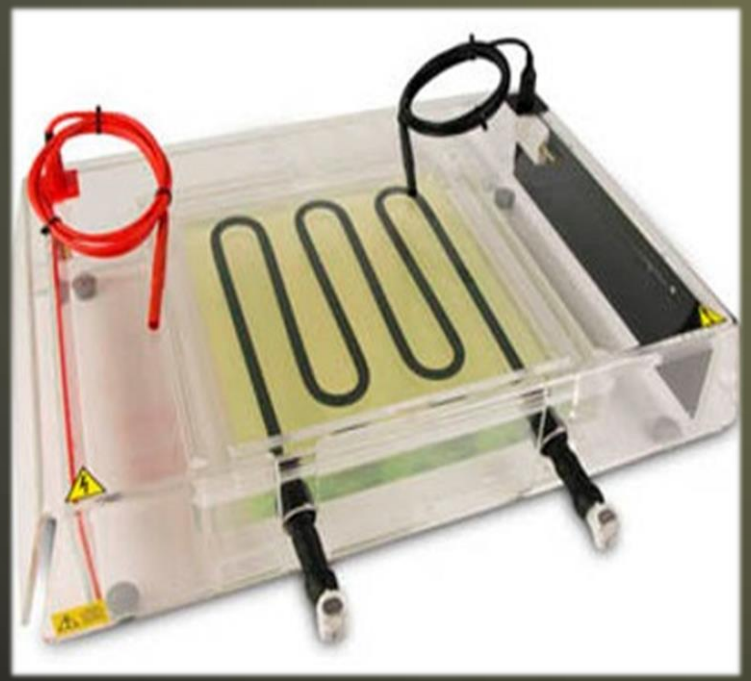
# OVERVIEW OF EXPRERIMENT

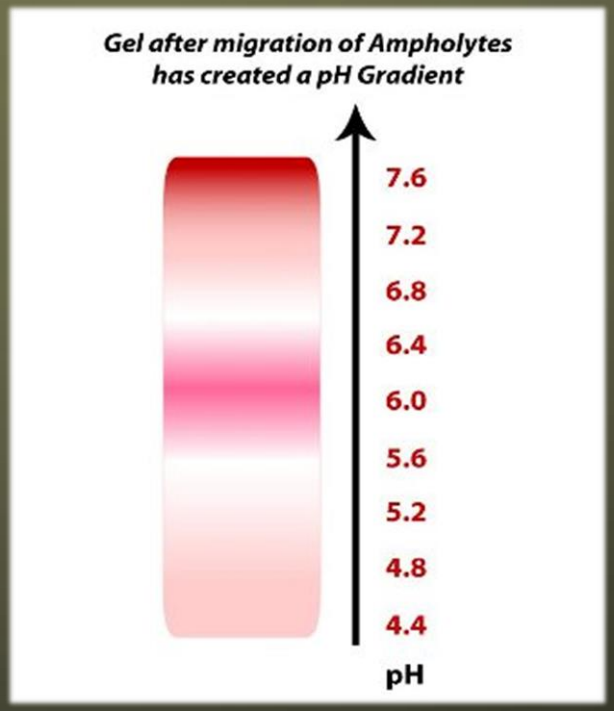
- Carrier ampholytes(suitable pH) and riboflavin mixed with acrylamide solution.
- Mixture is porured over a glass plate which contain spacer.

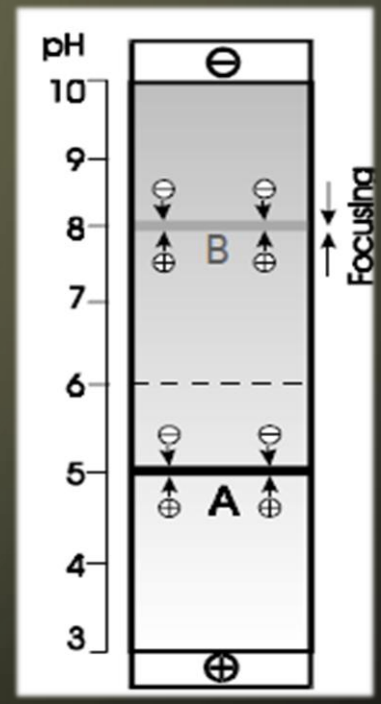
- Gel is polymerised.
- Electrode wicks are laid along each side of the gel.

- Potential difference is applied.
- Ampholytes form a pH gradient between anode and cathode.

# Isoelectric focusing system





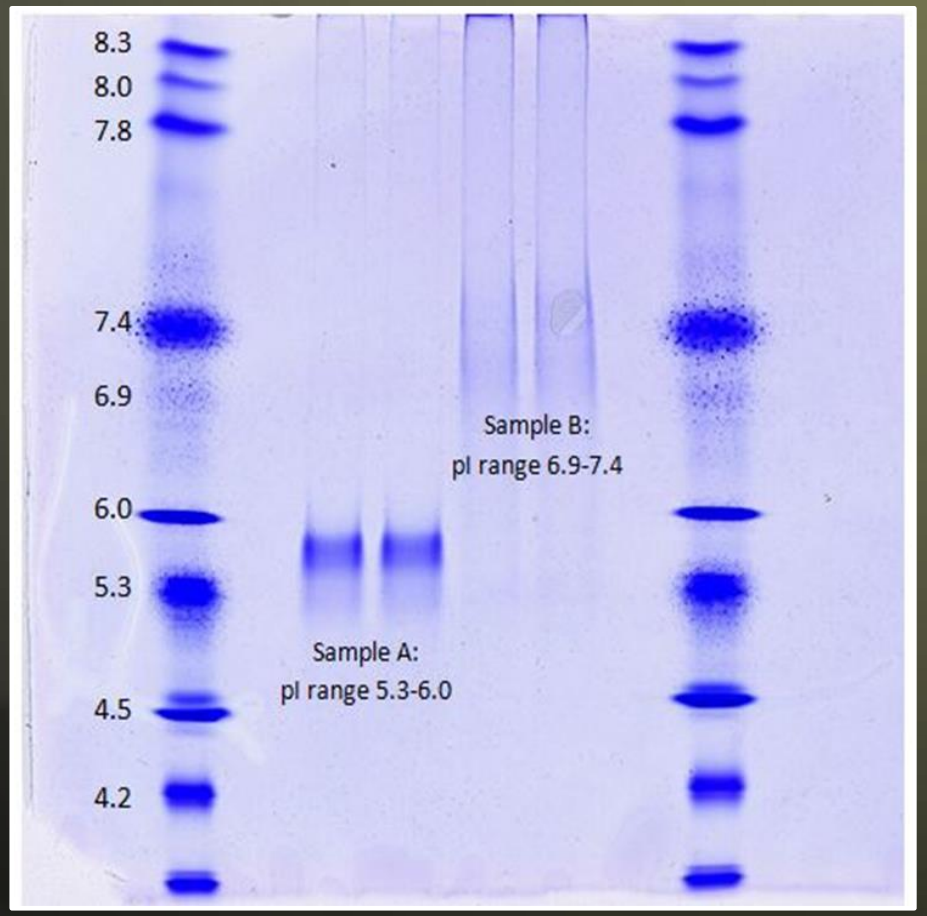

MOLEKULCE.COM/DAMLA_YAY-TUĞBA_ÇAĞıRTEKIN

- Power is then turned off.
- Sample applied by laying on gel filter paper soaked in the sample.
- Voltage is applied again.

- Proteins having positive charge will migrates towards the cathode, negatively charged protein will migrates towards anode.
- Become stationary when they  reaches ısoelectric point.

- The gel is washed with trichloroacetic acid, this precipitates the proteins and allows smaller ampholytes to be washed out.
- Gel is stained with dye.
- Destained.

# STAINING




MOLEKULCE.COM/DAMLA_YAY-TUĞBA_ÇAĞıRTEKIN

For staining proteins in IEF polyacrylamide gel; Fast green staining, coomassie brilliant blue R250 and G250 can be used. In agar gel, silver staining can be used.

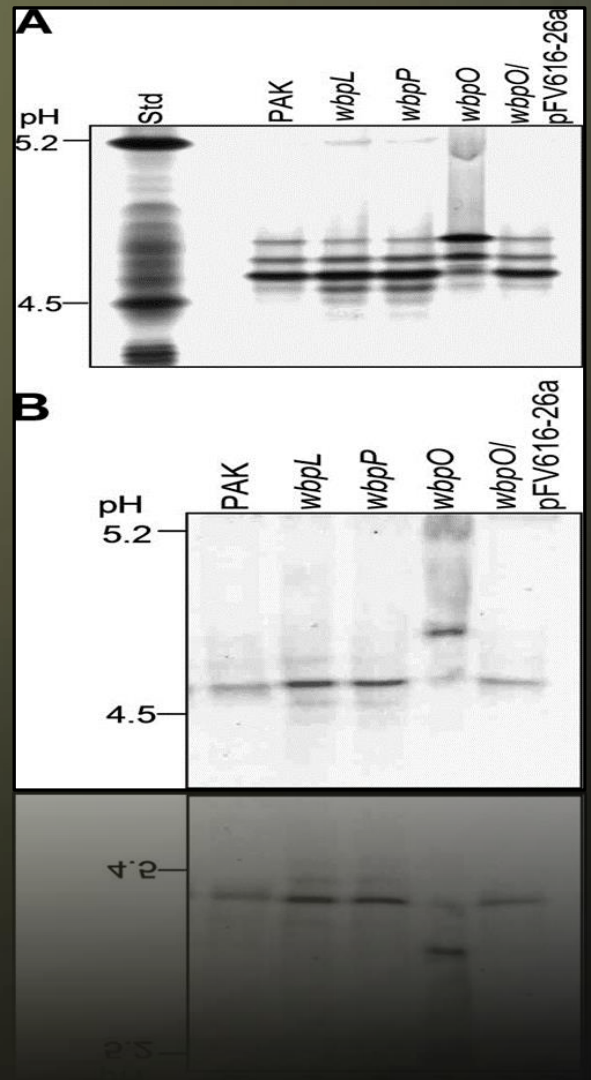
Coomassie blue:  G250 form dye exhibits a color change in dilute perchloric acid. After staining, an optional water-wash step further enhances staining sensitivity and yields a clear background.

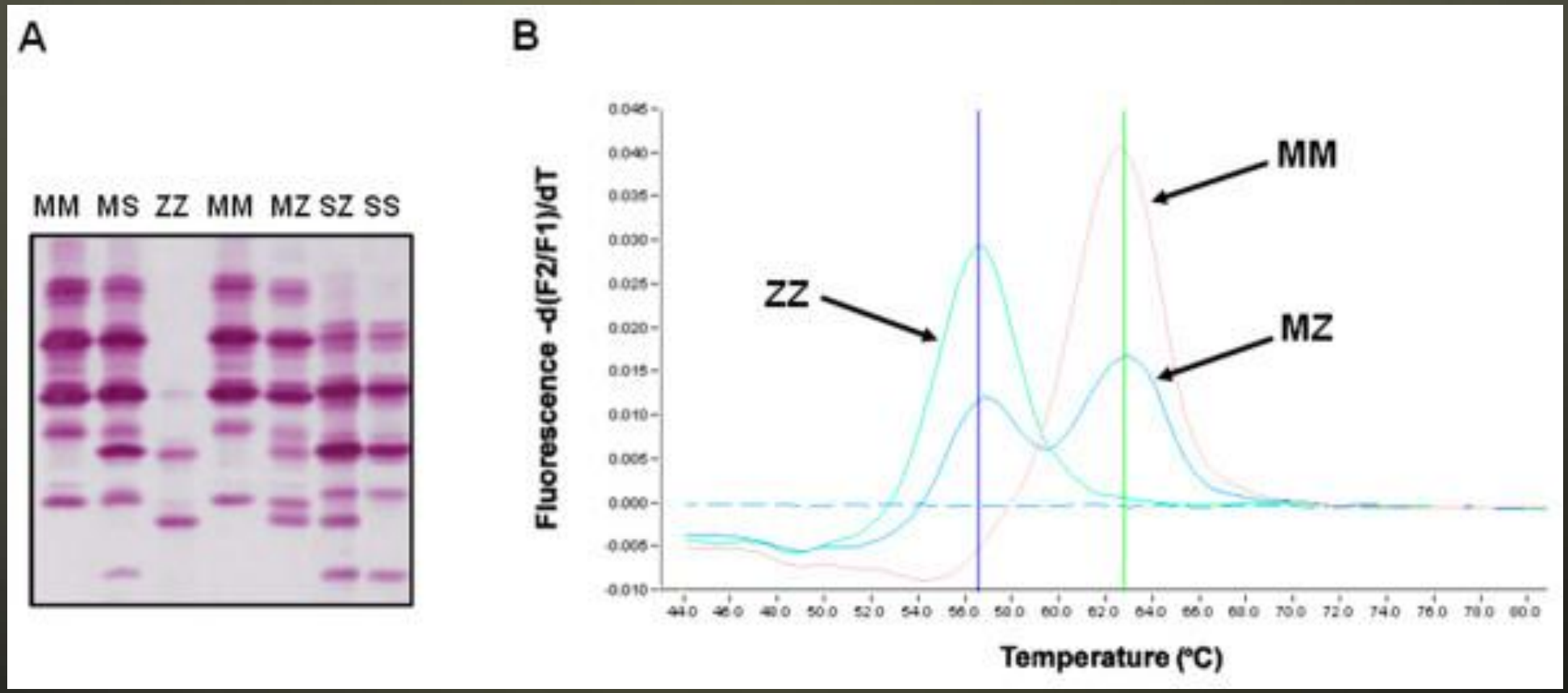
# RESOLUTION

IEF technique gives good separation with a high resolution compared to any other method.

Resolution depends on ;

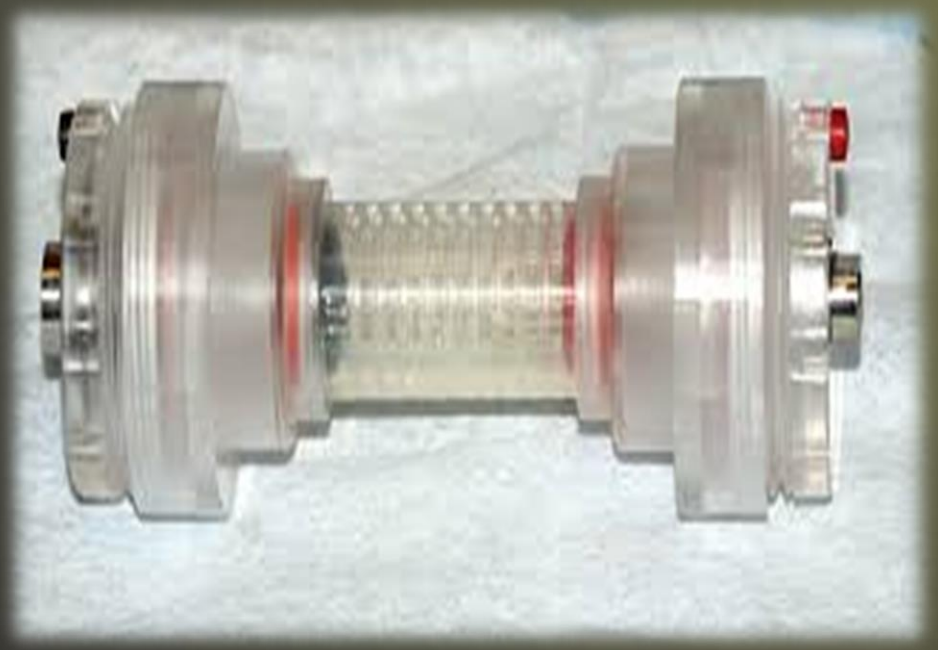
- ✓ The PH gradient
- ✓ Thickness of the gel
- ✓ Time of electropheresis
- ✓ The applied voltage
- ✓ Diffusion of the protein into gel

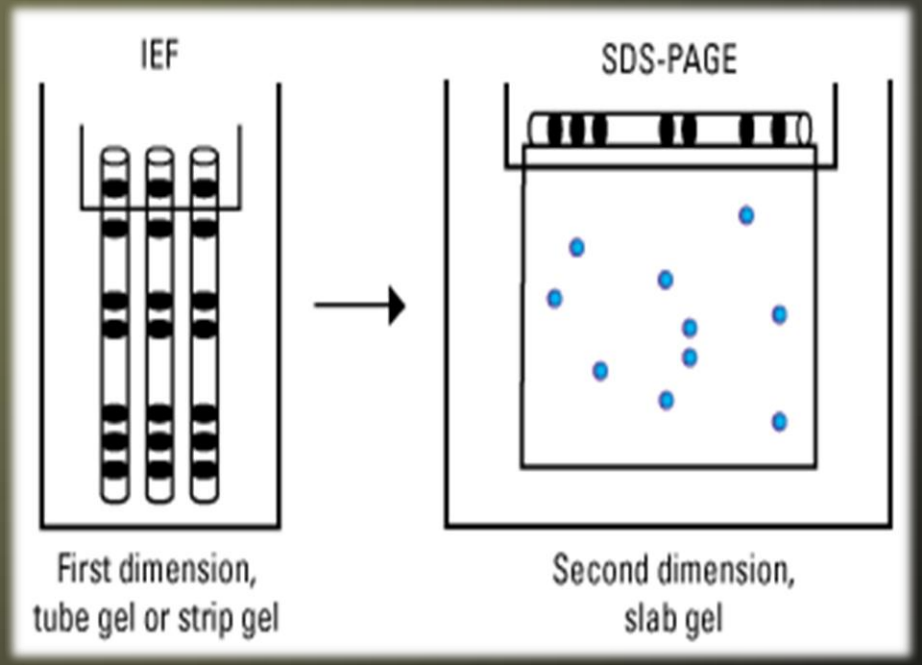





This figure is about a study of identification of phenotype by using IEF method.

# TECHNIQUES



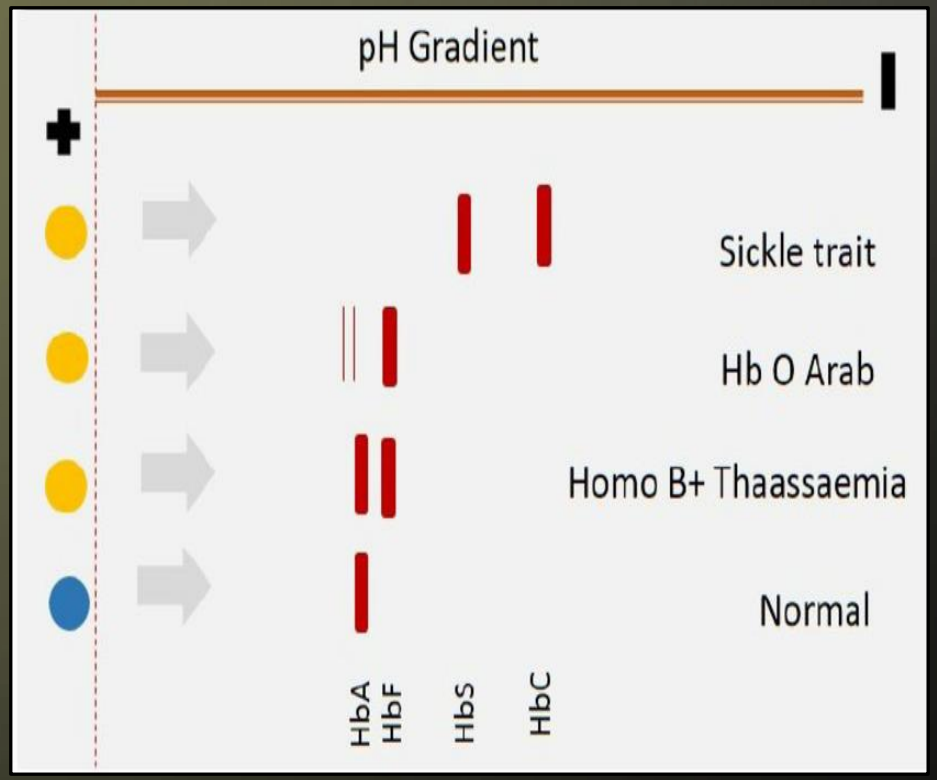



Bio-rad rotofor can be used for determination of PIs of proteins. IEF in the Rotofor has the added advantage that the proteins can be easily recovered once they are focused.

To increase the resolution of separation, we can couple the IEF technique with SDS-PAGE which would separate the proteins with similar isoelectric point based on their mass.

# APPLICATIONS

- ✓ Analyzing and characterizing proteins, peptides, glycoproteins or lipoproteins, cell membranes proteins, isoenzymes, etc.
- ✓ Accurate analysis of erytropoietin.
- ✓ Applications of isoelectric focusing in forensic serology.
- ✓ For research in taxonomy, cytology and immunology etc.
- ✓ Isoelectric focusing technique is also used for disease diagnosis.



# REFERENCES


- https://vdocuments.mx/isoelectric-focusing-guide-copy.html
- http://www.authorstream.com/Presentation/niraj517-1719870-isoelectric-focusing-electrophoresis/
- https://vdocuments.mx/education/isoelectric-focusing.html
- https://www.youtube.com/watch?v=YV4gR4vucwY
- http://www.aesociety.org/areas/pdfs/Garfin_IEF_WebArticle9-07.pdf
- https://www.quora.com/What-are-the-uses-of-isoelectric-focusing
- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5372225/
- https://www.ncbi.nlm.nih.gov/pubmed/849455
- http://clinchem.aaccjnls.org/content/clinchem/35/12/2297.full.pdf
- https://www.researchgate.net/publication/51488656_The_prevalence_of_alpha-1_antitrypsin_deficiency_in_Ireland

# THANK YOU..!

MOLEKULCE.COM/DAMLA_YAY-TUĞBA_ÇAĞıRTEKIN