Basics and Theory of Electrophoresis

Jeff Prischmann
Diagnostic Lab Manager
North Dakota State Seed Department

North Dakota State
NDSSD
Seed Department

Basics and Theory of Electrophoresis

- Basic principles
- History of Electrophoresis
- Types of Electrophoresis
- Gel Electrophoresis
- Sample types
- Equipment
- Applications

North Dakota State
NDSSD
Seed Department

Basics and Theory of Electrophoresis

- Separation Science has become a very important tool for diagnostic and clinical applications.
- Separation scientists work in a variety of areas including: biochemistry, biotechnology, food science, clinical science, etc.

North Dakota State
NDSSD
Seed Department

Basics and Theory of Electrophoresis

- Separation scientists deal with different sample types and sample matrices and require different techniques to separate and quantify these samples.

North Dakota State
NDSSD
Seed Department

Basics and Theory of Electrophoresis

- The two most commonly used methods of sample separation are chromatography and electrophoresis.
- Chromatography: ideal for small molecules and has high selectivity.
- Electrophoresis: most often used for proteins and other small biological molecules like DNA.

North Dakota State
NDSSD
Seed Department

Basic Principles

What is Electrophoresis?

- A technique whereby charged molecules are separated by the use of an electric field.
- During electrophoresis, charged molecules will migrate towards an opposite charge.

North Dakota State
NDSSD
Seed Department

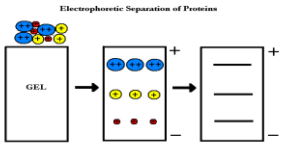
Basic Principles

What is Electrophoresis?

- A mixture of molecules of various sizes will migrate at different velocities and will be separated.
- Electrophoresis is usually carried out in an aqueous solution.
- Commonly used in many areas.

North Dakota State NDSSD Seed Department

Basic Principles



North Dakota State NDSSD Seed Department

History of Electrophoresis

- 1939 Zone electrophoresis developed
- 1950 Agar gel electrophoresis
- 1955 Starch gel (Smithies)
- 1957 Cellulose acetate (Kohn)
- 1959 Acrylamide gels first used (Raymond and Winstraub)
- 1961 IEF (Svensson)
- 1964 Disc Gel Electrophoresis (Ornstein and Davis)

North Dakota State NDSSD Seed Department

History of Electrophoresis

- 1969 SDS electrophoresis (Beber and Osborn)
- 1971 SDS electrophoresis (Laemmli)
- 1971 Cellulose acetate gels (Meera)
- 1975 2-D electrophoresis
- 1977 Sequencing gels first used
- 1979 Agarose gel electrophoresis
- 1983 Pulsed field electrophoresis
- 1983 Capillary electrophoresis

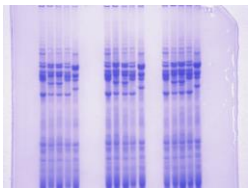
North Dakota State NDSSD Seed Department

Types of Electrophoresis

- Free solution electrophoresis (Capillary Electrophoresis)
- Supporting medium electrophoresis (paper, film, various gels)

North Dakota State NDSSD Seed Department

Gel Electrophoresis



North Dakota State NDSSD Seed Department

Gel Electrophoresis

- Electrophoresis that involves the use of a gelatinous material such as agarose, acrylamide, starch or cellulose acetate as the matrix.
- The gel acts as a support medium for the sample.
- Commonly used to separate samples containing proteins or DNA.

North Dakota State NDSSD Seed Department

Gel Electrophoresis

- An important purpose of a gel matrix is to introduce a sieving action which allows separations of molecules based on molecular size.
- Gel matrix viscosity, density, and pore size are all factors in determining the 'speed' of separation.

North Dakota State NDSSD Seed Department

Gel Electrophoresis

2 Main Types of Gels

- Slab gels
- Tube gels

North Dakota State NDSSD Seed Department

Gel Electrophoresis

Types of Separation

- Native: separation by size and charge (charge/mass)
- Denaturing: separation by size
- Others (IEF, 2-D)

North Dakota State NDSSD Seed Department

Gel Electrophoresis

Native

- continuous system--gel and tank buffers are the same, single phase gel; examples are PAGE, agarose, and starch gels.
- discontinuous system--gel and tank buffers are different, two-phase gel (stacking gel); example is PAGE.

North Dakota State NDSSD Seed Department

Gel Electrophoresis

Denaturing

- SDS (sodium dodecyl sulphate) used to denature proteins (discontinuous system).
- urea or formamide used to denature DNA or RNA.

North Dakota State NDSSD Seed Department

Gel Electrophoresis

Other types

- Isoelectric focusing: protein separation based on isolectric points in a pH gradient.
- 2-D electrophoresis: combination of IEF and SDS-PAGE.

North Dakota State NDSSD Seed Department

Gel Electrophoresis

Gel Types

- Starch
- Acrylamide
- Agarose
- Cellulose acetate
- IEF

North Dakota State NDSSD Seed Department

Gel Electrophoresis

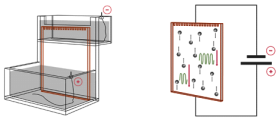
Main factors that effect separation:

- Resistance (pore size)
- Buffer strength
- Gel Temperature
- Sample
- Gel type

North Dakota State NDSSD Seed Department

Gel Electrophoresis

Resistance-Pore Size



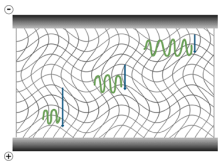
Gel electrophoresis units are simple DC circuits.

Source: National Diagnostics

North Dakota State NDSSD Seed Department

Gel Electrophoresis

Resistance-Pore Size



Source: National Diagnostics

North Dakota State NDSSD Seed Department

Gel Electrophoresis

Buffers

- Common buffer components include: acetic acid, boric acid, citric acid, glycine, phosphoric acid, tris, tricine.
- Ionic strength of buffers are important.
- Homogenous buffers vs multiphasic buffers

North Dakota State NDSSD Seed Department

Gel Electrophoresis

Buffers

Commonly Used Electrophoresis Buffers

Buffer	Molecular Weight	pK_a	$\Delta pKa/°C$
Acetic Acid	60.05	4.8	-0.0002
Boric Acid	61.83	9.23	-0.002
Citric Acid	192.1	6.4 (pK3)	0
Glycine	75.07	9.8	-0.03
MOPS	209.26	7.2	-0.006
Phosphoric Acid	98	7.2 (pK2)	-0.003
Taurine	125.1	9.1	-
Tricine	179.18	8.15	-0.021
Tris	121.1	8.06	-0.028

Source: National Diagnostics

North Dakota State NDSSD Seed Department

Gel Electrophoresis

Temperature

- Temperature management is critical to achieve good results.
- Some applications require maintaining a high temperature (denaturing PAGE of DNA/RNA).
- Other applications require a cool temperature to prevent sample degradation or gel melting.

North Dakota State NDSSD Seed Department

Sample Types

- Proteins
- Nucleic Acids

North Dakota State NDSSD Seed Department

Sample Types

Proteins

- Comprised of amino acids
- Protein structure is described as primary, secondary, tertiary, and quaternary based on amino acid sequence, bends/spirals, shape, and multiple peptide chains.
- A protein's structure affects how it is separated during electrophoresis.

North Dakota State
NDSSD
Seed Department

Sample Types

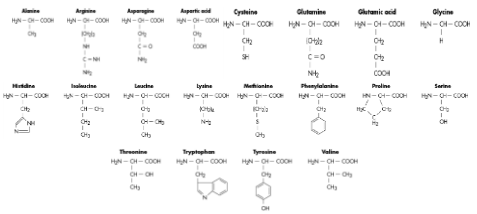
Types of Proteins

- Enzymes (albumins)
- Regulatory proteins
- Transport proteins
- Storage proteins (prolamins, globulins)
- Structural proteins (glutelins)

North Dakota State
NDSSD
Seed Department

Sample Types

Proteins-Amino Acids



North Dakota State
NDSSD
Seed Department

Sample Types

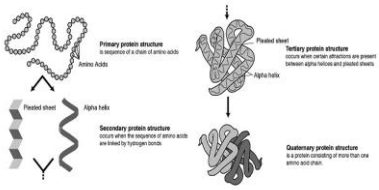
Proteins-Amino Acids

- Aspartic acid and glutamic acid impart acidic properties to proteins.
- Lysine, Arginine, and Histidine impart basic properties to proteins.

North Dakota State
NDSSD
Seed Department

Sample Types

Protein structure



Source: www.umass.edu/molvis/workshop/prot1234.htm

North Dakota State
NDSSD
Seed Department

Sample Types

Proteins and Electrophoresis

- Proteins are charged molecules and at neutral pH are either basic or acidic depending upon their AA composition.
- Most proteins placed into basic conditions become negatively charged. Acidic conditions cause most proteins to develop a positive charge.

North Dakota State
NDSSD
Seed Department

Sample Types

Proteins and Electrophoresis

- Native electrophoresis of proteins generally occurs in basic conditions.
- Denaturing protein electrophoresis causes proteins to become negatively charged (SDS-PAGE).
- Acidic running conditions can also be used.

North Dakota State NDSSD Seed Department

Sample Types

Proteins and Electrophoresis

- Isozymes (isoenzymes): enzymes that differ in amino acid sequence but catalyze the same chemical reaction.
- Isozymes have different molecular weights and chemical properties and thus can be separated from each other during electrophoresis.
- Isozymes are coded by genes on different loci.

North Dakota State NDSSD Seed Department

Sample Types

Proteins and Electrophoresis

- Allozymes: a variant form of an enzyme that is coded for by different alleles at the same locus.
- Allozymes also have different molecular weights and chemical properties and can be separated using electrophoresis.

North Dakota State NDSSD Seed Department

Sample Types

Proteins and Electrophoresis

- Monomeric enzymes: consists of a single polypeptide unit, each band represents a different polypeptide coded by a different allele.
- Dimeric enzymes: quaternery structure consisting of 2 polypeptide units, different gel bands represent different combinations of polypeptides.

North Dakota State
NDSSD
Seed Department

Sample Types

Source: Allozyme Electrophoresis and Population Structure in the Snowy Campion (*Silene latifolia* ssp. Alba). Vanderbilt University. (www.cas.vanderbilt.edu/bsci111b/index.htm).

North Dakota State
NDSSD
Seed Department

Sample Types

Proteins and Electrophoresis

- In Summary: The electrophoretic mobility of a protein is dependent upon its charge, size, and shape.
- The type of electrophoresis that is used affects a protein's separation.

North Dakota State
NDSSD
Seed Department

Sample Types

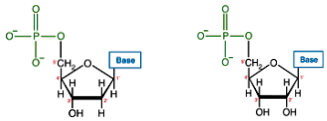
Nucleic Acids and Electrophoresis

- DNA is negatively charged (due to phosphate backbone).
- Electrophoresis of double stranded DNA occurs under native gel conditions.
- Electrophoresis of single stranded DNA occurs under denaturing conditions (ensures single stranded molecules and prevents base pairing).

North Dakota State NDSSD Seed Department

Sample Types

Nucleotide Structure



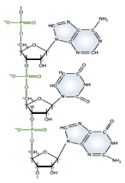
DNA RNA

Source: National Diagnostics

North Dakota State NDSSD Seed Department

Sample Types

RNA Structure

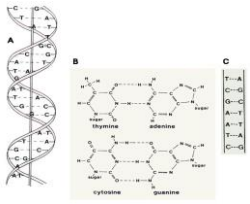


Source: National Diagnostics

North Dakota State NDSSD Seed Department

Sample Types

DNA Structure



Source: J. L. Schumann

Gel Electrophoresis

Main Steps

- Prepare samples
- Prepare gel and buffers
- Load samples onto gel
- Run gel
- Stain gel
- Interpret/analysis of gel
- Archive (photograph, dry gel)

Gel Electrophoresis

Results

- Electrophoretogram: The results of an electrophoresis test.
- Zymogram: banding pattern of enzymes or isozymes after electrophoresis.

Gel Electrophoresis

Results

- Unique bands and or banding patterns.
- Molecular weight standards can be ran with samples to estimate sizes.
- Band naming systems have been developed for some types of electrophoresis.
- Migration or separation of a sample can be measured (Rf values).

North Dakota State NDSSD Seed Department

Gel Electrophoresis

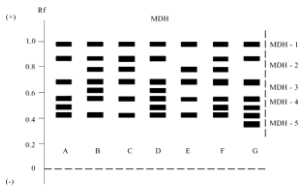


Figure 1. Zymogram pattern (A to G) for malate dehydrogenase isozymes in wild pepper

Source: Schuelter et al. 1999. Inheritance of Malate Dehydrogenase in Wild Pepper. Bragantia 58(1).

North Dakota State NDSSD Seed Department

Equipment

- Power supply
- Cooling Apparatus
- Electrophoresis gel apparatus
- White Light/UV Light Box
- Digital Camera/Gel Documentation System
- Reagents: Gel staining chemicals, pre-made gel or gel chemicals, buffers, etc.
- General lab equipment: pH meter, pipettors, scale, stir plates, etc.

North Dakota State NDSSD Seed Department

Equipment

Power Supplies

- Power supplies vary based on type of electrophoresis and type of gel.
- PAGE usually uses higher voltage.
- Agarose gel electrophoresis generally uses lower voltage.
- Some applications may use either constant current, constant watts, or constant volts.

North Dakota State NDSSD Seed Department

Equipment

Power Supplies

- Constant voltage generally used for SDS-PAGE of proteins.
- Constant power or constant current used for many types of denaturing PAGE.
- Effective way to manage heat during electrophoresis is to use constant current or constant power.

North Dakota State NDSSD Seed Department

Equipment

Electrophoresis Gel Apparatus

- Vertical apparatus: used for PAGE
- Horizontal apparatus: used for agarose gels, starch gels, IEF.

North Dakota State NDSSD Seed Department

Equipment

Gel Electrophoresis Apparatus Manufacturers

- C.B.S. Scientific (www.cbsscientific.com)
- Bio-Rad (www.bio-rad.com)
- Hoefer (www.hoeferinc.com)
- Owl Separation Systems (www.owlsci.com)

North Dakota State NDSSD Seed Department

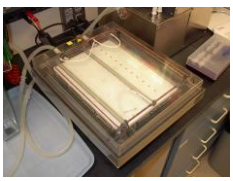
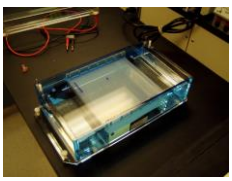
Equipment

Vertical Electrophoresis Gel Apparatus


North Dakota State NDSSD Seed Department

Equipment

Horizontal Electrophoresis Gel Apparatus

North Dakota State NDSSD Seed Department

Equipment

Digital Camera/Gel Documentation System

North Dakota State NDSSD Seed Department

Applications

- DNA Sequencing
- Blotting (DNA Hybridization /Southern Blot)
- Medical Research
- Protein research/purification
- Agricultural testing
- Many others

North Dakota State NDSSD Seed Department

Applications

Seed Testing

- Variety Identification
- Varietal purity (identify mixtures, off-types, etc.)
- GMO/Adventitious presence Testing

North Dakota State NDSSD Seed Department

Applications

Seed Testing-cont.

- Measurement of hybrid purity
- Identify genetic markers used by plant breeders
- Seed production/certification (monitor varietal purity and identity)

North Dakota State NDSSD Seed Department

Applications

Seed Testing-cont.

- Quality control in processing and other industries.
- Variety registration (PVP applications)
- Documentation of genetic resources in germplasm collections, etc.

North Dakota State NDSSD Seed Department

References

- Westermeier, R. 2001 *Electrophoresis in Practice*.
- Chrambach, A. 1992 *Advances in Electrophoresis*.
- Martin, 1996. *Gel Electrophoresis: nucleic acids*.
- Hames, B. 1998 *Gel Electrophoresis of Proteins: A Practical Approach*.

North Dakota State NDSSD Seed Department

References

- Electrophoresis journals: Journal of Separation Science (JSS), Electrophoresis.
- Societies: The American Electrophoresis Society (www.aesociety.org)

North Dakota State
NDSSD
Seed Department

Questions

North Dakota State
NDSSD
Seed Department