Agri Articles
(e-Magazine for Agricultural Articles)
Volume: 06, Issue: 03 (MAY-JUNE, 2026)
Available online at http://www.agriarticles.com
©Agri Articles, ISSN: 2582-9882

A Comparative Review of Agarose Gel Electrophoresis and Polyacrylamide Gel Electrophoresis (PAGE) in Agricultural Biotechnology

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Agarose gel electrophoresis (AGE) and polyacrylamide gel electrophoresis (PAGE) are two widely used techniques for the separation and analysis of biological macromolecules. Electrophoresis is a method used to separate and, in some cases, purify nucleic acids and proteins based on differences in their size, charge or conformation. Agarose gel electrophoresis is a simple, reliable and highly effective technique for separating, identifying and purifying large DNA and RNA fragments, typically using agarose concentrations ranging from 0.5% to 2%. In contrast, PAGE is primarily employed for the separation and analysis of proteins and small nucleic acid fragments due to its higher resolving power. Polyacrylamide gels are chemically cross-linked matrices formed by the polymerization of acrylamide with a cross-linking agent, usually N, N′-methylene bis-acrylamide, resulting in smaller and more uniform pores than those of agarose gels. This review provides a comparative overview of agarose and polyacrylamide gel electrophoresis, highlighting their structural characteristics, separation mechanisms, applications, advantages, limitations and significance in molecular biology, biotechnology and agricultural research.

Introduction

Electrophoresis is one of the most widely used techniques in molecular biology for the separation, identification and characterization of biological macromolecules such as nucleic acids and proteins. The technique is based on the movement of charged molecules through a supporting medium under the influence of an electric field (Mesapogu *et al.*, 2012). Depending on their size, charge and conformation, molecules migrate at different rates, enabling their separation and analysis. Electrophoresis is typically performed in buffered solutions to maintain a stable pH and ensure consistent migration of biomolecules during the process (Nelson and Cox, 2021).

Among the various electrophoretic methods, agarose gel electrophoresis (AGE) and polyacrylamide gel electrophoresis (PAGE) are the most commonly employed techniques. AGE utilizes a porous agarose matrix and is particularly suitable for the separation of large DNA and RNA fragments. In contrast, PAGE employs a chemically cross-linked polyacrylamide matrix with smaller and more uniform pores, providing higher resolution for the separation of proteins and small nucleic acid fragments. Due to their reliability, simplicity and versatility, both techniques have become indispensable tools in molecular biology, biotechnology, genomics and agricultural research.

The choice between AGE and PAGE depends on the size and nature of the molecules being analysed as well as the resolution required. Understanding the structural characteristics, separation mechanisms, applications, advantages and limitations of these techniques is essential for selecting the most appropriate method for a given research objective. Therefore, this review aims to provide a comparative overview of agarose gel electrophoresis and polyacrylamide gel electrophoresis, highlighting their principles, characteristics, applications and significance in modern biological research.

General Characteristics of Agarose and Polyacrylamide Matrices

Agarose Gel Electrophoresis (AGE)

Agarose is a natural polymer extracted from seaweed that forms a porous matrix through hydrogen bonding when heated in buffer and allowed to cool (Barril and Nates, 2012). The pore size of the matrix can be adjusted by altering the concentration of agarose. This property makes agarose gels particularly suitable for the separation of large molecules such as DNA fragments, ranging from 100 base pairs to several megabases in size. Agarose is the most widely used medium for nucleic acid separation; however, it possesses relatively low resolving power, resulting in broader and less distinct bands. This limitation is primarily due to its large and less uniform pore size, which cannot be precisely controlled. Agarose gels are electrically neutral and do not interact significantly with nucleic acids during electrophoresis. Therefore, separation occurs mainly on the basis of molecular size. The migration rate of DNA fragments is inversely proportional to the logarithm of their molecular weight, allowing estimation of fragment size by comparison with a DNA ladder (Nelson and Cox, 2021). Agarose gels typically possess relatively large and less uniform pores ranging from approximately 100 to 500 nm in diameter. Despite their porous structure, agarose gels maintain sufficient mechanical strength even at low concentrations (0.5-2.0% w/v), making them easy to prepare, handle and use in routine laboratory applications.

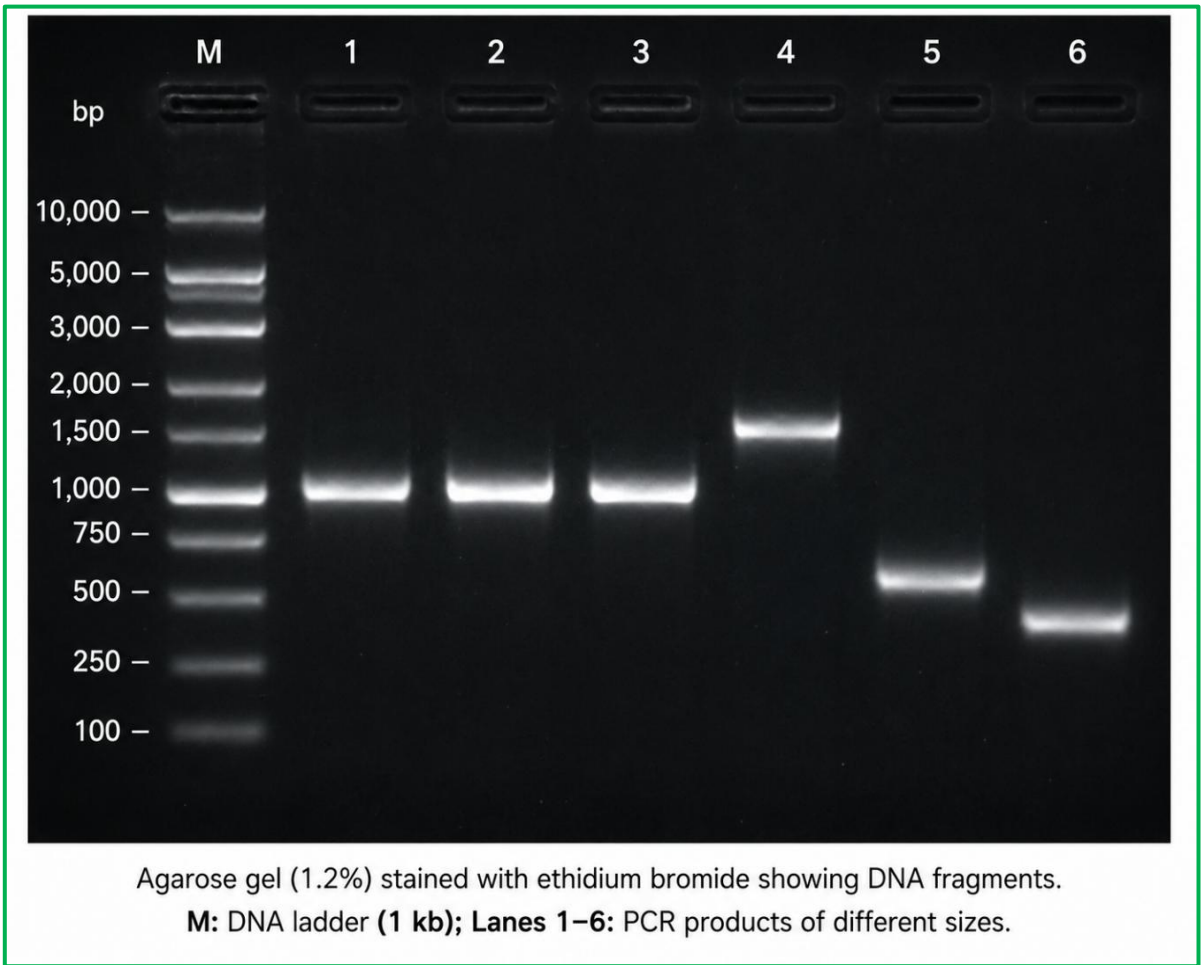

Fig 1. Visualization of DNA Fragments by Agarose Gel Electrophoresis

Polyacrylamide Gel Electrophoresis (PAGE)

Polyacrylamide gels are synthetic, chemically cross-linked matrices formed through the polymerization of acrylamide with a cross-linking agent, usually N, N′-methylene bis-acrylamide, resulting in a gel with small and highly uniform pores (Barril and Nates, 2012). The polymerization reaction is a free-radical process, typically initiated by ammonium persulfate (APS) and catalysed by tetramethylethylenediamine (TEMED) (Mesapogu *et al*., 2012). Compared with agarose gels, polyacrylamide gels possess a more uniform pore structure and offer superior resolving power for the separation of small molecules. Consequently, PAGE is widely used for protein electrophoresis and for the analysis of small DNA and RNA fragments. The pore size of polyacrylamide gels can be precisely controlled by adjusting the concentrations of acrylamide and bis-acrylamide, producing pore diameters typically ranging from 10 to 200 nm. This enables optimization of the gel matrix for specific molecular size ranges and facilitates high-resolution separation of molecules that differ only slightly in molecular weight or size. In addition, PAGE gels are chemically stable and inert; however, they are relatively thin and fragile and therefore require support between glass plates during casting and electrophoresis.

PAGE can be performed under either native or denaturing conditions (Green and Sambrook, 2020). In Native PAGE, proteins retain their natural conformation and biological activity, allowing separation based on size, shape and charge. In contrast, SDS-PAGE employs the anionic detergent sodium dodecyl sulphate (SDS) to denature proteins and impart a uniform negative charge, resulting in separation primarily according to molecular weight (Green and Sambrook, 2020). SDS-PAGE is the most widely used form of PAGE for protein characterization and molecular weight determination.

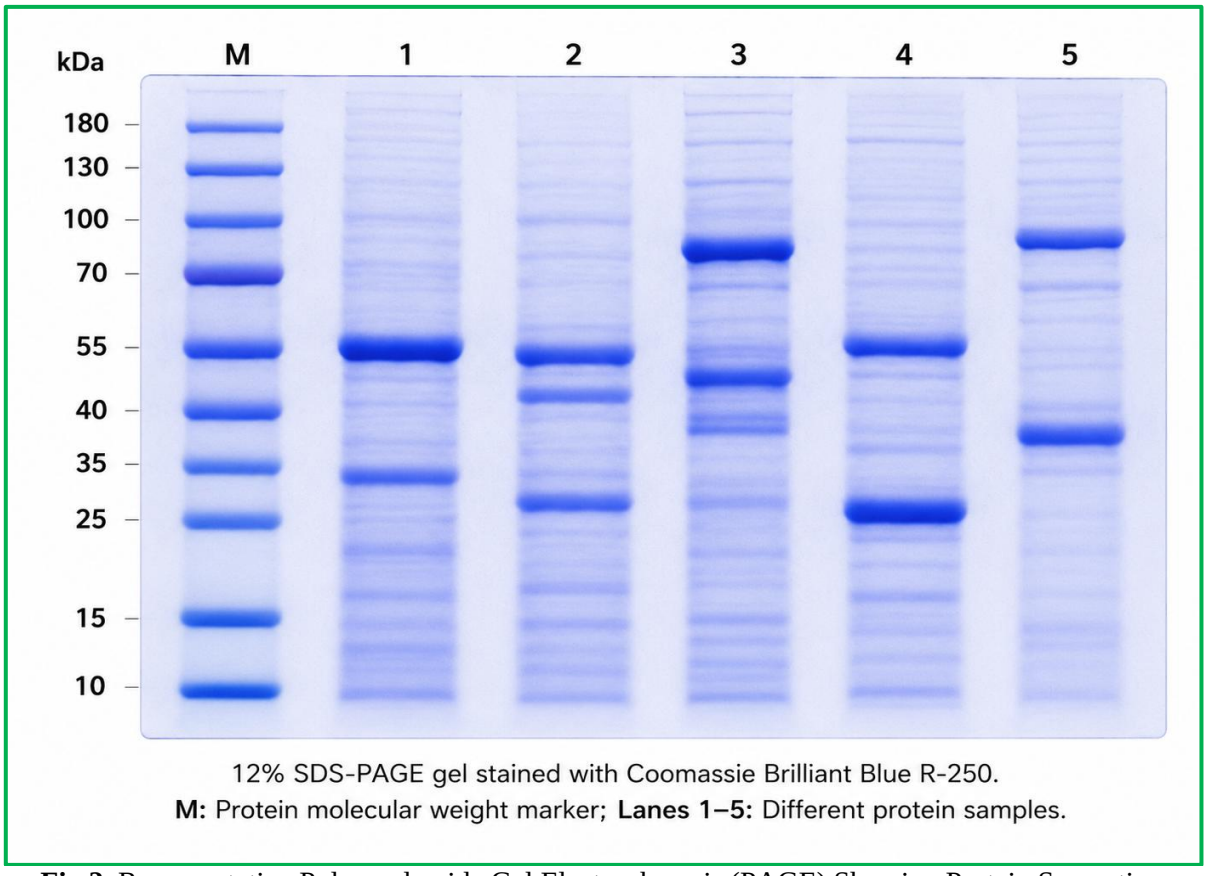

Fig 2. Representative Polyacrylamide Gel Electrophoresis (PAGE) Showing Protein Separation

Methods of Separation

Size-based Separation

The fundamental principle behind the use of agarose and polyacrylamide gels is size-based separation. In agarose gels, larger DNA fragments move more slowly through the matrix due to physical hindrance, whereas smaller fragments navigate the pores more easily.

Polyacrylamide gels operate on a similar principle but are capable of resolving molecules that are much closer in size due to their finer pore structure.

Molecular Sieving

Polyacrylamide gels excel in molecular sieving, a process where the gel's pore size plays a critical role in the separation of molecules (Barril and Nates, 2012). This characteristic is particularly beneficial in SDS-PAGE (Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis), where proteins are denatured and coated with SDS to give them a uniform charge-to-mass ratio. The polyacrylamide gel then separates these proteins based solely on size, allowing for precise analysis of protein composition. The efficiency of separation in both AGE and PAGE is influenced by factors such as gel concentration, applied voltage, buffer composition, molecular size and electrophoresis running time.

Distinguishing Features of AGE and PAGE

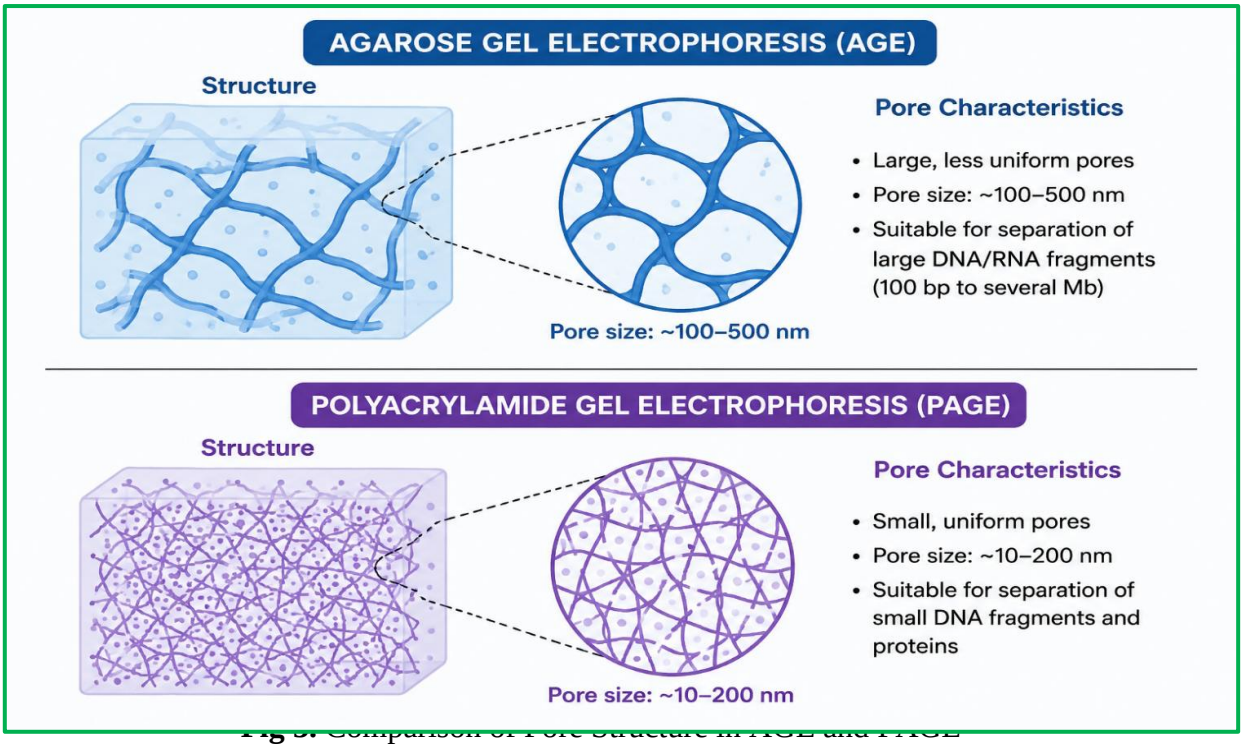

Fig 3. Comparison of Pore Structure in AGE and PAGE

Agarose Gel Electrophoresis (AGE)	Parameter	Polyacrylamide Gel Electrophoresis (PAGE)
Natural polymer (agarose) forms a porous network	Matrix / Structure	Synthetic polymer (acrylamide) forms a tightly cross-linked network
Large, less uniform pores (~100–500 nm)	Pore Size	Small, uniform pores (~10–200 nm)
Low resolution (Fuzzy bands)	Resolution	High resolution (Sharp bands)
Best for large DNA/RNA fragments (100 bp to several Mb)	Separation Range	Best for small DNA fragments and proteins (<10 bp to ~200 kDa)
PCR products, restriction digests, plasmid analysis, genomic DNA/RNA	Common Applications	Protein analysis (SDS-PAGE, Native PAGE), small DNA/RNA analysis, sequencing
Horizontal	Gel Casting Orientation	Vertical
Easy and quick to prepare	Preparation	Tedious and time-consuming
Gel can be melted, reheated and recast	Reusability	Irreversible polymerization, cannot be recast
Relatively low cost	Cost	Relatively high cost
Non-toxic	Toxicity	Acrylamide monomer is toxic (neurotoxic)

Fig 4. Comparative Analysis of AGE and PAGE

Applications of Agarose and Polyacrylamide Gel Electrophoresis

Agarose Gel Electrophoresis (AGE)

Agarose gel electrophoresis is widely used for the separation, analysis and purification of DNA and RNA fragments. Due to its simplicity, cost-effectiveness and ability to resolve large nucleic acid molecules, it is one of the most commonly employed techniques in molecular biology and agricultural biotechnology laboratories.

1. **Marker-Assisted Selection (MAS):** AGE is used to visualize PCR-amplified molecular markers such as SSR, RAPD and ISSR markers linked to desirable agronomic traits. This enables rapid selection of plants carrying genes for disease resistance, stress tolerance and improved yield.
2. **DNA Fingerprinting and Variety Identification:** DNA fragments generated during fingerprinting studies are separated using agarose gels to identify and distinguish crop varieties. This technique is useful for germplasm characterization, cultivar authentication and protection of plant breeders' rights.
3. **Genetic Diversity Analysis:** AGE is extensively employed in the analysis of molecular marker profiles to assess genetic variability among cultivars, breeding lines and wild relatives. Such studies aid in the conservation and utilization of genetic resources.
4. **Disease Diagnostics:** PCR-based detection of plant pathogens is commonly verified through agarose gel electrophoresis. The appearance of pathogen-specific DNA bands enables rapid and reliable diagnosis of bacterial, fungal and viral diseases.
5. **Seed Purity Testing and Hybrid Verification:** Molecular markers analysed through AGE help distinguish pure seed lots from contaminants and confirm the genetic identity of hybrids. This application is important in seed certification and quality assurance programs.
6. **Transgenic Crop Analysis:** AGE is used to verify the presence of inserted transgenes by analysing PCR-amplified DNA fragments from genetically modified plants. It assists in the molecular characterization and preliminary screening of transgenic crops.
7. **Crop Improvement Programs:** The technique plays a significant role in molecular breeding by supporting marker analysis, gene mapping and screening of breeding populations. These applications contribute to the development of improved crop varieties with desirable traits.

Polyacrylamide Gel Electrophoresis (PAGE)

Due to its high resolving power and ability to separate molecules differing only slightly in size, PAGE is extensively used for the analysis of proteins and small nucleic acid fragments.

1. **Protein Characterization and Profiling:** PAGE, particularly SDS-PAGE, is widely used to determine protein molecular weight, assess protein purity and analyse expression patterns. It plays an important role in studying plant proteins associated with growth, stress tolerance and disease resistance.
2. **Western Blotting:** Proteins separated by PAGE can be transferred onto membranes for immunological detection using specific antibodies. This technique is widely employed for identifying target proteins and studying their expression under different physiological or stress conditions.
3. **High-Resolution Oligonucleotide Separation:** PAGE provides excellent resolution for small nucleic acid fragments and is therefore used in DNA sequencing, miRNA studies and analysis of synthetic oligonucleotides. It is particularly useful when precise separation of fragments differing by only a few nucleotides is required.
4. **Genotyping and SNP Analysis:** PAGE is frequently used for detecting microsatellite markers, small sequence variations and SNPs. These markers are valuable in genetic mapping, germplasm characterization and marker-assisted breeding programs.
5. **Agricultural Biotechnology Applications:** PAGE is widely used for the analysis of isozymes, protein polymorphisms and molecular markers in crop plants. These applications support crop improvement, seed purity testing, genetic diversity assessment and identification of superior breeding lines.

Future Prospects

Advances in molecular biology, genomics and biotechnology continue to expand the applications of gel electrophoresis. Although automated sequencing technologies and high-throughput analytical platforms have become increasingly popular, agarose gel electrophoresis and polyacrylamide gel electrophoresis remain fundamental laboratory techniques due to their simplicity, reliability and cost-effectiveness. Modern electrophoretic systems are becoming more efficient through the integration of digital imaging, automated gel documentation systems and advanced staining methods that improve sensitivity and accuracy.

In agricultural biotechnology, these techniques continue to support crop improvement programs, genetic diversity studies, molecular marker analysis and disease diagnostics. Agarose gel electrophoresis remains a preferred method for routine DNA analysis and PCR verification, while PAGE is increasingly employed in proteomics, protein characterization and high-resolution nucleic acid studies. Future developments in electrophoretic technologies are expected to enhance analytical precision, increase automation, reduce processing time and contribute significantly to molecular breeding, genomics research and sustainable agricultural development.

Conclusion

Agarose gel electrophoresis (AGE) and polyacrylamide gel electrophoresis (PAGE) are fundamental techniques in molecular biology and biotechnology for the separation and analysis of nucleic acids and proteins. AGE is particularly suitable for the separation of large DNA and RNA fragments due to its simple preparation, large pore size and cost-effectiveness, whereas PAGE provides superior resolution for small nucleic acid fragments and proteins because of its uniform pore structure. Both techniques have wide-ranging applications in molecular diagnostics, genetic diversity studies, marker-assisted selection, crop improvement, transgenic crop analysis and proteomics research. Despite the emergence of advanced analytical technologies, AGE and PAGE continue to remain indispensable tools in research and teaching laboratories due to their reliability, versatility and ease of use. The choice between the two techniques ultimately depends on the size, nature and analytical requirements of the molecules being studied.

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