ISSN (E): 2583 – 0821

Sun. Agri.:e- Newsletter, (2025) *5*(11), 52-55

Article ID: 475

Polyacrylamide Gel Electrophoresis (PAGE) : Procedure and Applications

Kolli Leela Bhavani[1], Yogendra Singh[2], Salvi Pandey[3], Esha Baberwal[4] and Anurag Muvel[4]

[1]M.Sc Research Scholar, Biotechnology Centre, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur (M.P)
[2]Assistant Professor (Senior Scale)-Biotechnology, Department of Genetics and Plant Breeding, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur (M.P)
[3]M.Sc Research Scholar, Department of Genetics and Plant Breeding, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur (M.P)
[4]M.Sc Research Scholar, Department of Horticulture (Vegetable science), Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur (M.P)

Open Access

*Corresponding Author
Kolli Leela Bhavani*

Available online at
www.sunshineagriculture.vitalbiotech.org

Article History
Received: 1. 11.2025
Revised: 5. 11.2025
Accepted: 10. 11.2025

INTRODUCTION

Understanding protein activity is critical in the current era of molecular biology and proteomics for unraveling the complexities of life. Proteins require a precise and reliable method for separation and analysis due to their diverse sizes, charges, and structural variations. Among the different instruments developed for this purpose, Polyacrylamide Gel Electrophoresis (PAGE) is regarded as a foundational technology that transformed biochemical research.

PAGE's origins can be traced back to Arne Tiselius' seminal study in 1937, when he demonstrated the mobility of charged biomolecules in an electric field, providing the groundwork for electrophoretic analysis. In the decades that followed, scientists improved this technology by including gel matrices as support media in the 1950s, which improved molecular resolution. A significant advancement took place in 1970 when Ulrich K. Laemmli introduced Sodium Dodecyl Sulfate (SDS) into the procedure. This innovation enabled the denaturation of proteins and facilitated their separation based on size, a technique now referred to as SDS-PAGE. In 1975, Patrick O'Farrell enhanced this method by combining isoelectric focusing with SDS-PAGE, resulting in the highly effective two-dimensional PAGE (2D-PAGE) technique that is widely utilized in proteomics (Issaq& Veenstra, 2008).

Polyacrylamide gel electrophoresis (PAGE) is an analytical technique employed to separate charged biomolecules, primarily proteins and peptides. This separation occurs based on their size and charge as they traverse a polyacrylamide gel matrix under the influence of an electric field. PAGE is recognized for its mechanical stability, customizable pore size, and inertness; those qualities that are difficult to achieve with agarose gels. (Green & Sambrook, 2020; Kothari *et al.*, 2023).

Principle:

The basic concept behind polyacrylamide gel electrophoresis (PAGE) revolves around the movement of charged biomolecules through a cross-linked polyacrylamide gel matrix when an electric field is applied. In this procedure, biomolecules like proteins or peptides migrate at varying speeds based on their size, charge, and structure. The gel functions as a molecular sieve, limiting the mobility of larger molecules while permitting smaller ones to migrate faster (Green & Sambrook, 2020).

The polyacrylamide gel is produced by the polymerization of acrylamide monomers along with a cross-linker, usually N, N′-methylene-bis-acrylamide, using ammonium persulfate (APS) and tetramethylethylenediamine (TEMED) as initiators. This polymerization results in a stable, transparent mesh whose pore size can be adjusted precisely by varying the concentrations of acrylamide and bis-acrylamide (Kothari *et al.*, 2023). Depending on the type of analysis being conducted, PAGE can take place under either native or denaturing conditions. In native PAGE, proteins maintain their original charge and structure, separating based on their size and inherent charge. In SDS-PAGE, the detergent Sodium Dodecyl Sulfate (SDS) denatures proteins and uniformly covers them with negative charges, ensuring that separation occurs solely based on molecular weight (Magdeldin, 2012).

Table 1: Characteristics of different types of PAGE

Type of PAGE	Basis of Separation	Denaturing Agent	Protein State
Native PAGE	Charge and size	None	Native (folded)
SDS-PAGE	Molecular weight	SDS	Denatured (linearized)
2D-PAGE	Isoelectric point (pI) and molecular weight	Isoelectric focusing + SDS	Denatured

Source: Kothari *et al*., 2023; Issaq& Veenstra, 2008)

Procedure:

The process of polyacrylamide gel electrophoresis (PAGE) consists of four key stages, each of these steps is designed to facilitate the precise separation of proteins and visualization of individual molecular bands.

1. Sample Preparation:

Protein samples are combined with a sample buffer that includes Tris–HCl, glycerol, SDS, bromophenol blue, and a reducing agent like β-mercaptoethanol. The buffer denatures the proteins and provides a consistent negative charge, allowing migration purely based on molecular weight under an electric field. Samples are briefly heated to ensure full denaturation before loading, generally at 100 °C for three minutes. Note that only the centrifuged protein sample (supernatant) is added to the gel, discarding the cell debris, to promote clear and accurate band formation (Kothari *et al.*, 2023).

2. Gel Casting:

A vertical gel system is created with two layers: the stacking gel and the resolving gel. The resolving gel (7–15% acrylamide) facilitates the separation of proteins based on their molecular size, whereas the stacking gel (approximately 4%) organizes all proteins into a condensed zone before they enter the resolving layer.

3. Electrophoresis:

The polymerized gel is assembled within an electrophoresis chamber that is filled with Tris–glycine–SDS buffer. Samples along with molecular weight markers are carefully placed into the wells, and an electric current (usually set at 100–150 V) is applied. Proteins, which carry a negative charge, migrate towards the anode, with smaller proteins moving more quickly through the gel's pores compared to larger ones.

4. Staining and Visualization:

After the electrophoresis process, gels are soaked in Coomassie Brilliant Blue R-250 or silver stain to reveal distinct protein bands. The resulting pattern gives insights into protein purity, relative abundance, and molecular weight. In proteomic studies, the separated protein bands may undergo additional analysis through Western blotting or mass spectrometry for identification and characterization.

ISSN (E): 2583 – 0821

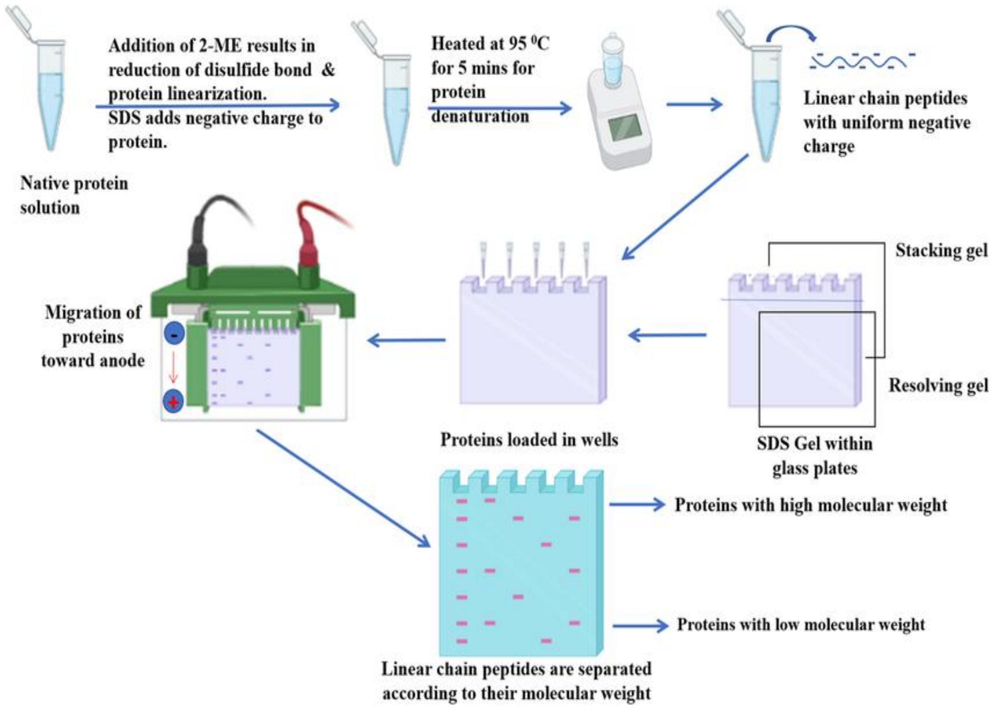

Figure 1: A schematic representation of different steps involved in the SDS gel electrophoresis (Manoswini*et al.*, 2024).

Applications:

Polyacrylamide gel electrophoresis (PAGE) is a versatile analytical method used for the separation, characterization, and quality evaluation of proteins.

Table 2: Applications of Polyacrylamide Gel Electrophoresis (PAGE)

Application Area	Type of PAGE Used	Purpose / Outcome	Supporting Reference(s)
Protein Purity and Molecular Weight Estimation	SDS-PAGE	Determines molecular weight and verifies protein homogeneity during purification and characterization.	Kothari *et al.*, 2023; Magdeldin, 2012
Proteomic Profiling and Comparative Analysis	2D-PAGE	Separates proteins by isoelectric point and molecular weight to study differential expression in complex samples.	Issaq& Veenstra, 2008; Magdeldin, 2012
Enzyme Activity and Oligomeric State Studies	Native PAGE	Maintains protein structure and activity for evaluating enzyme kinetics and multimeric complexes.	Kothari *et al.*, 2023; Magdeldin, 2012
Post-Translational Modification Detection	2D-PAGE / SDS-PAGE	Identifies modified proteins (e.g., phosphorylation, glycosylation) by mobility shifts and spot patterns.	Issaq& Veenstra, 2008
Clinical and Diagnostic Applications	SDS-PAGE	Used to analyze serum proteins, and immunoglobulin patterns for disease diagnosis.	Kothari *et al.*, 2023; Magdeldin, 2012
Western Blotting and Immunodetection	SDS-PAGE	Acts as a preparatory step for protein transfer and antibody-based detection in Western blot assays.	Magdeldin, 2012; Kothari *et al.*, 2023

CONCLUSION

Polyacrylamide gel electrophoresis (PAGE) is an important technique utilized in molecular biology and proteomics. It enables precise separation of proteins based on their size, charge, and conformation. This approach is beneficial for assessing protein purity, modifications, and levels of expression. Additionally, it is crucial in maintaining standards for therapeutic protein production. The versatility and dependability of PAGE make it vital for both research and clinical uses (Kothari *et al.*, 2023; Magdeldin, 2012; Issaq& Veenstra, 2008).

REFERENCES

Green, M. R., & Sambrook, J. (2020). Polyacrylamide gel electrophoresis. *Cold Spring Harbor Protocols*, 2020(12), pdb.prot100412. https://doi.org/10.1101/pdb.prot100412

Issaq, H. J., & Veenstra, T. D. (2008). Two-Dimensional Polyacrylamide Gel Electrophoresis (2D-PAGE): Advances and Perspectives. *BioTechniques*, 44(5), 697–700. https://doi.org/10.2144/000112823

Kothari, D., Bichala, P. K., Batra, N., & Jain, D. (2023). An approach to polyacrylamide gel electrophoresis (PAGE). *International Journal of Pharmaceutical Sciences & Medicine (IJPSM)*, 8(10), 117–133.

Magdeldin, S. (Ed.). (2012). Gel Electrophoresis - Principles and Basics. *InTech*. doi: 10.5772/2205

Manoswini, M., Mohanty, M., Majumdar, A., Sahu, B., & Mohanty, P. (2024). Extraction and characterizations of viral protein particles: A methodological study. BioNanoScience, 14, 1–17. https://doi.org/10.1007/s12668-023-01289-6