



Mastering the Art of Gel Preparation: A Step-by-Step Guide for Effective Gel Electrophoresis from Loading to Luminescence

(Vasanth V, *Ashwin Niranjan M, Navaneetha Krishnan S, Nitish G and Aqueala Carmel P T)

Forest College and Research Institute, Mettupalayam-641301

*Corresponding Author's email: ashwinniranjanm@gmail.com

Abstract

Gel electrophoresis is a fundamental technique in molecular biology, widely employed for the separation and analysis of DNA fragments. This review provides a detailed overview of the gel preparation process, gel apparatus setup, and the steps involved in the separation and observation of DNA fragments. The focus is on agarose gel electrophoresis, a versatile and commonly used method in laboratories.

Key words: Gel Electrophoresis, Agarose Preparation, DNA Fragment Separation, Electrophoresis Setup, Safety and Disposal.

Introduction

Gel electrophoresis is a fundamental technique in molecular biology and biochemistry that plays a crucial role in the analysis and separation of biological macromolecules, such as DNA, RNA, and proteins. This powerful and widely used method enables researchers to examine the size, charge, and quantity of biomolecules, providing valuable insights into various biological processes. The basic principle of gel electrophoresis involves the migration of charged particles through a gel matrix under the influence of an electric field.

The gel, usually made of agarose or polyacrylamide, acts as a molecular sieve, allowing the separation of molecules based on their size and charge. Smaller molecules move more quickly through the gel, while larger ones lag behind, resulting in distinct bands or patterns that can be visualized and analysed. In DNA electrophoresis, for example, the technique is commonly employed for tasks such as DNA fragment analysis, genotyping, and DNA purification. Agarose gel electrophoresis is particularly well-suited for separating large DNA fragments, while polyacrylamide gels offer higher resolution and are better suited for smaller fragments. The separated DNA bands can be visualized by staining the gel with a fluorescent dye or using other detection methods.

Protein electrophoresis is another vital application, facilitating the separation and analysis of proteins based on their size and charge. Polyacrylamide gels are typically used for protein electrophoresis due to their higher resolving power. This technique is widely applied in protein purification, characterization, and the study of protein-protein interactions. As an indispensable tool in molecular biology research and diagnostics, gel electrophoresis continues to evolve with advancements in technology, such as the introduction of capillary electrophoresis and next-generation sequencing. Its versatility and reliability make gel electrophoresis an essential method for scientists exploring the intricacies of biological macromolecules

Gel Preparation

Materials and Reagents

- Agarose powder
- TAE (Tris-Acetate-EDTA) or TBE (Tris-Borate-EDTA) buffer
- Ethidium bromide or alternative DNA stain
- Gel casting tray and comb
- Electrophoresis chamber
- Power supply
- DNA or protein samples
- Loading dye
- Microwavable flask or beaker
- Stir bar
- Microwave or hot plate
- Pipettes and tips
- Safety equipment (gloves, goggles)

Procedure

1. Prepare the Gel Casting Tray:

- Clean the gel casting tray and comb thoroughly to remove any contaminants.
- Place the comb in the gel casting tray, ensuring that it forms wells for sample loading.

2. Weigh Agarose:

- Weigh the appropriate amount of agarose powder based on the desired gel concentration. Typically, a 1% agarose gel is suitable for most applications.

3. Prepare Buffer:

- Measure the required volume of TAE or TBE buffer. The buffer serves as the electrolyte solution for the electrophoresis process.

4. Mix Agarose and Buffer:

- Add the weighed agarose to a microwavable flask or beaker.
- Pour the measured buffer into the flask containing agarose.
- Mix well to ensure the agarose is evenly distributed in the buffer.

5. Heat and Dissolve Agarose:

- Heat the agarose-buffer mixture using a microwave or hot plate until the agarose is completely dissolved. Stir the mixture intermittently to aid dissolution.

6. Cooling:

- Allow the agarose solution to cool to a temperature where it is still in a liquid state but can be handled safely. Pour the liquid agarose into the gel casting tray with the comb in place.

7. Adding Ethidium Bromide:

- If using ethidium bromide, add it to the agarose solution before pouring the gel. Follow recommended safety procedures for handling ethidium bromide.

8. Pour the Gel:

- Carefully pour the liquid agarose solution into the gel casting tray with the comb, ensuring that no air bubbles are trapped.

9. Solidification:

- Allow the gel to solidify by letting it sit undisturbed at room temperature or placing it in a gel caster with a cooling system.

10. Remove Comb:

- Once the gel has solidified, carefully remove the comb to create wells for sample loading.

11. Prepare Samples:

- Mix the DNA or protein samples with loading dye to make them denser than the surrounding gel, allowing them to sink into the wells during loading.

12. Load Samples:

- Load the prepared samples into the wells using a micropipette, being careful not to puncture the gel.

13. Electrophoresis:

- Place the gel in the electrophoresis chamber and fill it with the TAE or TBE buffer.
- Connect the electrodes to the power supply and run the electrophoresis at the desired voltage and time.

14. Visualization:

- After electrophoresis, visualize the separated DNA or proteins using UV light or another appropriate method.

Observing Separated DNA Fragments**a. Completing Electrophoresis:**

- Turning off the power supply and removing the lid.
- Draining excess buffer and handling the gel. *Manabe, T. (2000)*

b. Exposure to UV Light:

- Utilizing a gel documentation system for UV exposure. *Meka, V. S et.al., 2017*
- Visualization of DNA bands and capturing gel images.

c. Disposal and Safety:

- Emphasis on adhering to institution regulations for the proper disposal of gels and running buffer. *Meka, V. S et.al., 2017*
- Safety precautions, including the use of gloves when handling ethidium bromide.

Remember to follow safety guidelines and protocols when working with chemicals, especially ethidium bromide. Additionally, be sure to dispose of used materials properly, following institutional or local regulations.

Conclusion

Recap of the key steps in gel electrophoresis. Highlighting the importance of safety measures and proper disposal procedures. This comprehensive review aims to serve as a valuable resource for researchers and students involved in molecular biology, providing a step-by-step guide to the gel electrophoresis technique.

References

1. Hames, B. D. (Ed.). (1998). Gel electrophoresis of proteins: a practical approach (Vol. 197). OUP Oxford.
2. Manabe, T. (2000). Combination of electrophoretic techniques for comprehensive analysis of complex protein systems. *ELECTROPHORESIS: An International Journal*, 21(6), 1116-1122.
3. Meka, V. S., Sing, M. K., Pichika, M. R., Nali, S. R., Kolapalli, V. R., & Kesharwani, P. (2017). A comprehensive review on polyelectrolyte complexes. *Drug discovery today*, 22(11), 1697-1706.
4. Ragab, M. A., & El-Kimary, E. I. (2021). Recent advances and applications of microfluidic capillary electrophoresis: A comprehensive review (2017–Mid 2019). *Critical Reviews in Analytical Chemistry*, 51(8), 709-741.