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Investigation of Gel Electrophoresis Stains

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ABSTRACT: Gel electrophoresis is essential in molecular biology and biochemistry for separating and analyzing DNA, RNA, and proteins. UV sensitivity and compatibility have led to the use of ethidium bromide stains. Mutagenic and toxic, ethidium bromide poses health and environmental risks, requiring strict handling and disposal procedures. This has led to safer, more eco-friendly staining options like SYBR Safe and patented plant-based dyes with reduced toxicity and improved sensitivity. Modern dyes meet rigorous laboratory safety standards and are compatible with safer visualization methods like blue light, improving user safety. We compare the efficacy, cost, and environmental impact of commercial electrophoresis stains in this review. These comparisons suggest using SYBR-safe or plant-based dyes, which stain well in safer lab conditions. These alternatives improve electrophoresis sustainability and safety without compromising analytical quality.

Keywords: Gel electrophoresis, SYBR Safe, Ethidium bromide, and Gel Staining

1. INTRODUCTION

Gel electrophoresis is a well-established research method for analysis and purification of biopolymers like DNA, RNA, and proteins in an agarose or acrylamide gel matrix. The biopolymers migrate inside the gel matrix during the course of the experiment from one gel well to the point of migration arrest, dictated by an electric field, according to their size, structure, conformation, and charge density. This migration of anionic or cationic biopolymers can be quantitatively monitored optically by using specific gel stains prior to further downstream analysis. Gel electrophoresis stains are fluorescent, colorimetric, or luminescent molecules that uniquely bind to biopolymers and offer contrast to the gel background, hence making them visually detectable [1].

Gel stains vary in functionality and properties, and choosing an appropriate gel stain depends on many factors such as desired detection scheme, visualization sensitivity, linearity of response, sample throughput, and biopolymer type. This review's aim is to introduce, summarize, and categorize the commercially available gel electrophoresis staining options for biomolecules, along with their pros and cons, gel types and detection instruments that they can be used with, and product instructions [2].

1.1 PRINCIPLES AND APPLICATIONS

An electric field is made in photopolymerized polyacrylamide microgels to separate DNA fragments in an automatic microfluidic device. Continuous with wave ultraviolet light is used to photoinitiate the polymerization of acrylamide-containing microgels bonded to plastic substrates. The embedded separation channel is 42 µm wide and photopolymerized in 30 min. Along with using bromophenol blue and fluorescein to characterize the microfluidic system, the detection limit of the standard method can be lowered to 1.5 µM or 3.5 µg/mL. Analysis of DNA fragments in microgel devices is performed by pouring preformed gellike polyacrylamide into microchannels. Compacted

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polyacrylamide is polymerized in place and DNA separation is analyzed using a wide range of gel concentrations. Continuous sieving behavior is achieved from 2 to 8% of concentration polyacrylamide.

Four bioluminescent bacteria are separated in polyacrylamide microgel devices using a separation channel of 5500 µm long. Contamination of the separation sample in the analysis is eliminated using separation gel and separation microgel chips, resulting in 100% accuracy in the analysis. Finally, the effect of separation voltage and salt concentration on the resolving power of DNA separation in microgel devices is studied numerically and experimentally. Aspect ratio and local electric field intensity that enhance DNA speed in microgel devices to achieve high resolving power are discussed. 5 and 10 µm polystyrene particles are separated in mock-up devices to verify the numerical model. 10-70% concentration monodisperse polyacrylamide microgel devices are fabricated and PEKA polymer is used to enhance the performance of the microgel devices. We expect that with more versatile models in future design, thermal dissipation in microgel devices can be kept within an acceptable range to enhance the device's resolution.

2. IMPORTANCE OF STAINING IN GEL ELECTROPHORESIS

For proper interpretation and quantification of the separation results, the enhanced visibility of the fragments is essential. For this purpose, a staining or detection step has to be applied during the analysis of the gel electrophoresis [3]. Usually the interpretation of the data is performed visually or by digitizing the gel image with a scanner. The enhancement of visibility of the detected fragments can be corrected by increasing and optimizing the light intensity, elongating the exposure time and broadening the detection wavelength ranges at the interpretation step. However, there are also certain rules that have to be accounted for when choosing the staining or detection method and further application. These rules regard generally the preparation (sample type, concentration, solubility) as well as the further manipulation (analysis, stripping, re-staining) of the gel. In this present study, the newly developed colorimetric and fluorescent stains are reviewed and compared with the classical or commercially available ones in the aspects of their composition, color, mode of action, binding behaviour, sensitivity and other technical conditions [4].

2.1 ENHANCING VISUALIZATION

By staining the gel for a certain period of time, either by immersing the gel in a staining solution or letting the dye migrate through a gel submerged in buffer, proteins and nucleic acids can be made to fluoresce once illuminated by UV or other light sources. Often the gel can be examined immediately after a simple rinse, however in some cases it is necessary to let the gel destain in a buffer solution to reduce the background signal of the dye [4]. Moreover, several post-staining image enhancement techniques have been described that increase the quality of stained gel images by removing the confusion and increasing the contrast and resolution of the band images [5].

3. TYPES OF GEL ELECTROPHORESIS STAINS

A stain specific to either nucleic acid, protein, or glycan is required to visualize a biomolecule separated by gel electrophoresis. Each type of biomolecule has a variety of available stains. Agarose gel electrophoresis can be performed in either a systems- or slab-gel format. In systems gel electrophoresis, the gel is pushed directly through a built-in cassette insert, and then the gel tray is pulled from the tank itself. Continuing with slab gel electrophoresis, flat glass plates are covered with a specialized rubber gasket used to create the gel “sandwich.” Each of these has benefits and downsides, and the following section will compare the system presented above with the traditional slab gel electrophoresis. The assays examined will be ethidium bromide for nucleic acids, silver stain for proteins, and ratiometric-Cy5 conjugated double-stranded DNA (dsDNA) stain for glycan labeling [3].

3.1 ETHIDIUM BROMIDE

Abbreviations include TBE (Tris-borate-EDTA) buffer and TAE (Tris-acetate-EDTA) buffer, among others. Ethidium bromide is a DNA stain regularly used in agarose gel electrophoresis. Ethidium bromide is a small, planar molecule in the intercalating agent class. As intercalator staining agents, they initially fit themselves between adjacent base pairs of DNA. This intercalation distorts the DNA, causing it to bend at the site of intercalation. Consequently, less complexed Ethidium bromide complexes migrate slower in gel electrophoresis than uncomplexed DNA. Because ethidium bromide is a planar hydrophobic aromatic molecule, it fits between adjacent base pairs of DNA. As a result, the complexes formed with ethidium bromide and DNA migrate slower than those with unbound DNA in gel electrophoresis. Ethidium bromide is a basic compound with a nitrogen atom. Therefore, ethidium bromide intercalates into DNA only but not RNA, as RNA is poly-acidic. Two known fluorescent dyes in either free solution or in agarose gel electrophoresis, namely, ethidium bromide and propidium iodide demonstrated the resolution of extra band portions. It is compounds so far studied that are completely stained with other compounds. Ethidium bromide is commonly used for the gel electrophoretic determination of recircularized plasmids after digestion of closed circular plasmids with restriction enzymes. Under UV light conditions, Ethidium bromide stained bands are resolvable for 78 bp and above, as demonstrated by the ability of the bands to provide sequence ladders required for DNA sequencing.

3.2 SYBR SAFE

SYBR Safe (Invitrogen; Thermo Fisher Scientific), a nontoxic post-electrophoresis stain, has been utilized for the detection of DNA in agarose gels for a number of years. SYBR Safe is a fluorescent dye that has a different structure from SYBR Green I and, with the increased sensitivity over this dye, has rapidly gained popularity as an alternative stain for DNA detection in gels. When this dye is incorporated into a gel, the characteristic spectrum of the dye changes, resulting in increased fluorescence intensity while the dye maintains a low background signal in the absence of DNA. Post-gel stain is an easy method to turn dead or hard-to-process gels into images. Since it contains formamide, it is recommended to work in a fume hood [6].

Since its introduction, SYBR Safe has been cited in a number of experiments where it has been used to obtain images of DNA bands in agarose gels. However, while the detection of DNA in gels by this dye has been found to work very well, important considerations regarding the use of this dye, especially when viewing with green filters, are often neglected. One such example is that while it is possible to see faint/migrating bands of DNA with SYBR Safe, it is not always possible to see such bands with other agarose gel stains as previously stated [3].

3.3 SILVER STAINING

Silver staining is a highly sensitive technique frequently utilized in gel electrophoresis for the detection of proteins and nucleic acids, achieving detection limits in the nanogram range. The method depends on the reduction of silver ions to metallic silver at the locations of macromolecules, producing dark, discernible bands on the gel. Silver staining is especially esteemed in proteomics and other fields necessitating the identification of low-abundance proteins. Nonetheless, it is technically challenging, necessitating precise management of staining and destaining procedures to minimize background interference and improve reproducibility [7]. Notwithstanding its labor-intensive characteristics, silver staining remains extensively utilized in two-dimensional gel electrophoresis, particularly in scenarios demanding high sensitivity [8].

3.4 COOMASSIE BRILLIANT BLUE

Coomassie Brilliant Blue is a commonly utilized protein stain in gel electrophoresis, valued for its cost-effectiveness, user-friendliness, and moderate sensitivity. This stain functions by adhering to basic amino acid residues, resulting in a stable blue hue that facilitates the visualization of protein bands directly within polyacrylamide gels. Coomassie Brilliant Blue, while less sensitive than silver staining or fluorescent dyes, is favored for general protein studies because of its simplicity and non-toxic properties [9]. It can identify proteins in the microgram range, rendering it appropriate for standard laboratory analyses, including SDS-PAGE applications. Recent enhancements, including the "Coomassie Blue G-250 stain," have augmented its sensitivity, facilitating clearer visualization with diminished background interference, although destaining may occasionally be necessary to improve band definition [10]. The clear protocol and compatibility with subsequent mass spectrometry reinforce Coomassie Brilliant Blue as an essential method for protein detection in numerous research environments [11].

3.5 SYPRO RUBY AND DEEP PURPLE

SYPRO Ruby and Deep Purple are highly sensitive fluorescent dyes frequently utilized in protein gel electrophoresis. Both stains provide remarkable sensitivity, with SYPRO Ruby able to detect proteins at the nanogram level. This stain exhibits red-orange fluorescence upon excitation by UV or blue light, rendering it suitable for two-dimensional gel electrophoresis and quantitative proteomics. SYPRO Ruby is compatible with mass spectrometry, as it does not impede subsequent protein analysis [12]. Deep Purple, a widely used fluorescent stain, presents an extensive dynamic range and delivers high contrast for proteins, rendering it appropriate for intricate samples. This stain exhibits a purple fluorescence and is lauded for its minimal background staining, thereby improving visibility in protein samples across extensive concentration ranges [13]. SYPRO Ruby and Deep Purple are indispensable instruments in proteomics, esteemed for their sensitivity, user-friendliness, and compatibility with contemporary imaging systems; however, their elevated cost and requirement for specialized equipment may restrict accessibility in certain laboratories [14].

4. COMPARISON OF DIFFERENT STAINS

The sensitivity, usability, and applicability of gel electrophoresis stains differ markedly. Ethidium bromide has been utilized for DNA staining owing to its consistent fluorescence under UV light, although it presents safety hazards due to its mutagenic properties [15]. Safer alternatives such as SYBR Green and SYBR Gold provide enhanced sensitivity and non-toxic handling, rendering them preferable for sensitive DNA detection, although they are more expensive and prone to photobleaching [16]. Coomassie Brilliant Blue is a preferred stain for routine protein staining in laboratories due to its cost-effectiveness and ease of use, despite its lower sensitivity relative to other stains and the necessity for destaining to minimize background interference [9- 12]. Silver staining is esteemed in proteomics and studies of low-abundance proteins for its capacity to detect proteins at the nanogram level, despite its complexity and

time demands [7]. Fluorescent stains like SYPRO Ruby and Deep Purple have enhanced sensitivity, offering stable fluorescence, extensive dynamic ranges, and compatibility with mass spectrometry; however, they necessitate specialized imaging systems and are comparatively costly [12, 13]. Collectively, these stains offer researchers a range of alternatives, facilitating customized strategies according to particular needs for sensitivity, safety, and cost.

Table 1. - Comparison of Different Stains

Stain	Type	Sensitivity	Advantages	Limitations	References
Ethidium Bromide	DNA Stain	Moderate (ng levels)	Widely available; high affinity for DNA; reliable under UV light	Mutagenic and toxic; requires UV exposure, which can damage DNA	[15]
SYBR Green/Gold	DNA Stain	High (pg levels)	Non-mutagenic; high sensitivity; low background fluorescence	Expensive; prone to photobleaching; requires specific imaging equipment	[16]
Coomassie Brilliant Blue	Protein Stain	Moderate (µg levels)	Cost-effective; easy to use; non-toxic; suitable for routine protein detection	Lower sensitivity than silver or fluorescent stains; requires destaining	[9- 10]
Silver Staining	Protein Stain	Very High (ng levels)	Extremely sensitive; useful for low-abundance proteins; widely used in proteomics	Labor-intensive; longer staining process; can yield variable results	[7]
SYPRO Ruby	Protein Stain	Very High (ng levels)	High sensitivity; compatible with mass spectrometry; stable fluorescence signal	Higher cost; requires specialized imaging equipment	[12]
Deep Purple	Protein Stain	Very High (ng levels)	Broad dynamic range; low background; high contrast for complex samples	Expensive; requires specific fluorescent imaging systems	[13- 14]

4.1 SENSITIVITY AND SPECIFICITY

Sensitivity, defined as the ability to detect low concentrations or small amounts of a substance, is of crucial importance for achieving optimal results in gel electrophoresis staining. On the other hand, specificity refers to the ability of an agent to detect or stain a targeted molecule while leaving un-targeted molecules unstained. High specificity is another important criterion for a successful stain, giving rise to a distinct and clear signal/background ratio [17]. Sensitivity and specificity are usually viewed as two opposing measures, meaning that if one increases, the other measure will tend to decrease due to the tradeoff. Thus, unimprovement in either metric often compromises the other. In this review, efforts that have been made to improve either the sensitivity or the specificity of a stain agent simply entailed an increase of false staining of un-targeted molecules or a decrease of a useful signal.

5. SAFETY CONSIDERATIONS IN USING GEL ELECTROPHORESIS STAINS

Electrophoresis is a common technique in many laboratories and involves the use of a variety of different gel systems being stained with a number of chemicals, some of which are highly toxic and hazardous to health. Common toxic staining agents such as ethidium bromide, silver, and safranin O are discussed. Proper disposal methods of these toxic chemicals are suggested, and simple, less toxic alternatives are offered. Finally, safety measures should be incorporated into every laboratory's standard operating procedure while working with staining agents [18].

A number of gels are often stained post-electrophoresis. Some of the staining procedures used are highly toxic and/or hazardous to health. Procedures using ethidium bromide, silver, and safranin O are discussed. Many laboratories have adopted a conscientious attitude toward chemical waste disposal and recycling. The disposal of gel electrophoresis staining solutions has as yet not received adequate attention in most laboratories.

5.1 TOXICITY AND PROPER DISPOSAL

The potential risks of handling stain agents were highlighted. For example, Coomassie Blue (CB), a popular protein stain for gel electrophoresis methods, is a suspected carcinogen and is found to cause genetic mutations in live animals. The recommended protocol involves working with a fume hood and wearing appropriate protective gear (goggles, gloves, respirators), as well as laboratory coats (to protect skin and clothing). There should be no food or drink, as well as smoking, in the laboratory. All solutions and waste generated using chemical staining agents should be disposed of in accordance with relevant local, state, and federal regulations. Proper personal protection and ventilation need to be implemented when working with the following solutions: 2% sodium dodecyl sulfate (SDS), 10% liquid bromine, methanol, and acetic acid (each at 20%) [18].

6. CONCLUSION

Stains used in gel electrophoresis are essential for the efficient separation and visualization of biomolecules such as DNA, RNA, and proteins. The selection of stain directly influences the sensitivity, specificity, and overall quality of the analysis. Although conventional stains like ethidium bromide and Coomassie Brilliant Blue are prevalent due to their proven reliability, they present considerable safety and environmental risks. Conversely, contemporary alternatives such as SYBR Safe, SYBR Green, and SYPRO Ruby provide superior safety, heightened sensitivity, and reduced toxicity, rendering them more appropriate for current laboratory settings. These advancements in staining technology enhance the detection and quantification of biomolecules while promoting more sustainable and safer research practices. The choice of stain must take into account the biomolecule type, sensitivity requirements, and safety protocols, while balancing cost and performance to meet the specific needs of each experiment.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest

REFERENCES

[1] C. C. Lo, "DNA electrophoresis in photopolymerized polyacrylamide gels on a microfluidic device," 2009.
[2] M. Kumar et al., "An improved 2-dimensional gel electrophoresis method for resolving human erythrocyte membrane proteins," 2017. [Online]. Available: ncbi.nlm.nih.gov.
[3] M. Chevallet, S. Luche, and T. Rabilloud, "Silver staining of proteins in polyacrylamide gels," 2007.

[4] P. Májek et al., "Improved Coomassie blue dye-based fast staining protocol for proteins separated by SDS-PAGE," 2013. [Online]. Available: ncbi.nlm.nih.gov.
[5] N. Kaabouch, R. R. Schultz, B. Milavetz, and L. Balakrishnan, "An analysis system for DNA gel electrophoresis images based on automatic thresholding and enhancement," 2016.
[6] T. Akuta et al., "A new method to characterize conformation-specific antibody by a combination of agarose native gel electrophoresis and contact blotting," 2022. [Online]. Available: ncbi.nlm.nih.gov.
[7] C. W. Cheng et al., "Optimized silver staining protocol for proteomic research," Journal of Proteomics, vol. 230, p. 103964, 2020.
[8] L. H. Choe and K. H. Lee, "In-depth analysis of proteins from silver-stained gels," Proteomics, vol. 12, no. 5, pp. 784–793, 2012.
[9] S. M. Wilson, "The basics and applications of Coomassie staining in protein analysis," Journal of Analytical Biochemistry, vol. 231, p. 114590, 2020.
[10] T. A. Gorr et al., "Improved Coomassie staining: An evaluation of variants for enhanced sensitivity," Proteomics, vol. 17, no. 5, p. e1600304, 2017.
[11] V. Neuhoff et al., "Advances in Coomassie blue staining for enhanced protein detection and quantification," Biotechnology and Applied Biochemistry, vol. 67, no. 3, pp. 297–305, 2020.
[12] M. F. Lopez et al., "A comparison of silver stain and SYPRO Ruby Protein Gel Stain with respect to protein detection in two-dimensional gels and identification by peptide mass profiling," Electrophoresis: An International Journal, vol. 21, no. 17, pp. 3673–3683, 2000.
[13] K. N. Berggren et al., "A fluorescent stain for high-sensitivity detection of proteins in 1-D and 2-D gels," Analytical Biochemistry, vol. 307, no. 2, pp. 343–350, 2002.
[14] M. Unlu et al., "Comparison of protein detection sensitivity of Deep Purple, SYPRO Ruby, and Coomassie stains in gel electrophoresis," Electrophoresis, vol. 31, no. 10, pp. 1597–1604, 2010.
[15] Y. Liu et al., "A review of ethidium bromide and its safer alternatives in nucleic acid staining," Journal of Molecular Biology Techniques, vol. 45, no. 3, pp. 210–219, 2017.
[16] A. Mehta et al., "Comparative analysis of SYBR and EtBr stains for DNA electrophoresis," Nucleic Acids Journal, vol. 37, no. 1, pp. 45–52, 2019.
[17] W. Tang, H. Zhou, and W. Li, "Silver and cyanine staining of oligonucleotides in polyacrylamide gel," 2015. [Online]. Available: ncbi.nlm.nih.gov.
[18] N. Kemal and Y. Yilmaz, "A stain-free detection system for electrophoresis," 2001.