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Alizarin, Juglone, and Lawson: Novel Dyes for Protein Gel Electrophoresis

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Abstract

In this study, it was investigated whether new dying matters are able to stain electrophoresis gels or not. For this purpose juglone, alizarin and lawson were used for gel staining. Blood proteins such as albumin, globulin, hemoglobin, and fibrinogen were used as SDS PAGE samples and after running, the polyacrylamide gels were stained with these dye solutions and compared with the coomassie blue stained gels.
In conclusion, some of these dyes can be used for staining protein bands on polyacrylamide electrophoresis gels.

Keywords: Protein, Electrophoresis, SDS-PAGE, dying matters, juglone, alizarin

Introduction

Polyacrylamide gel electrophoresis is a simple, rapid, and inexpensive method used to separate mixtures of proteins based on their molecular weight and charge. The principle of electrophoresis is to consider the differences in molecular weight and the distance the electrical field in the molecule travels from one charge to another through the gel. Enzymes or proteins can be separated according to these differences (1). Polyacrylamide gels are formed by the polymerization of acrylamide monomers with a crosslinking agent (2). One of the most appropriate tools that can be used to separate proteins by size is Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). SDS is a detergent that denatures the

secondary and tertiary structures on the proteins while coating them with a negative charge associated with their length, which enables the estimation of molecular weights. (1).

A range of dyes are used for visualization of the separated proteins in SDS-PAGE gels and this is a well-established method. There are many protein visualization techniques. Coomassie brilliant blue R250 is the most common staining method used for SDS-PAGE, with some alternatives to Coomassie including Amido black and direct red 81 stains. Protein staining methods are popular due to their simplicity, however, they require lengthy stain-removal steps (3). Moreover, colloidal dyes such as Silver are very sensitive but impractical, requiring multiple incubations and stain removal steps (4).

Coomassie brilliant blue R250 dye was first used in 1963 to stain proteins on cellulose acetate sheets (5). Polyacrylamide gel staining with Coomassie brilliant blue R250 can be time-consuming and requires relatively long staining and destaining times mainly due to the slow diffusion of the dye into the gel matrix and slow binding to the proteins (6). Various methods have been developed to reduce the time and environmental effects of methanol in the dye formulation and to increase the sensitivity of dyeing protocols. Depending on the method, Coomassie brilliant blue R250 staining has a detection limit of 10-100 ng of protein in polyacrylamide gels (7,8).

The dyes produced by traditional methods from some plants in Turkey have been used for dyeing wool and leather throughout history. Juglone in walnut, alizarin in Rubia tinctorum, and lawson in Rubia tinctorum are the most commonly used dyes. In this study, the potential use of conventional dyestuffs in SDS-PAGE gel electrophoresis was investigated.

Material and Method

Chemicals used in the study; TEMED was obtained from Bio-Rad, acrylamide, and APS were obtained from Sigma, methanol, and acetic acid were obtained from Merck (Main, Germany). Furthermore, “Bio-Rad” (Richmond, USA) power source, “Mighty Small” (Hoefer) electrophoresis device, and “Arçelik” brand microwave oven were used to perform SDS-PAGE electrophoresis. Dyestuffs; juglone, lawson, and alizarin, were obtained from Merck and Sigma.

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-Gels, Solutions, and buffers

- **Lower buffer:** pH was adjusted to 8.8 using 1.5 M Tris solution and 0.4% SDS solution.
- **Upper buffer:** pH was adjusted to 6.6 using 0.5 M Tris solution and 0.4% SDS solution.
- **Resolution jel:** 2.1 mL of 40% acrylamide solution, 2.25 mL of lower buffer solution, and 3.98 mL of deionized water has been mixed, and at the last stage, 50 µL of 10% APS and 20 µL TEMED were added.
- **Stacking jel:** 0,35 mL of 40% acrylamide solution, 1 mL of upper buffer solution, and 2,59 mL of deionized water has been mixed, and at the last stage, 50 µL of 10% APS and 10 µL TEMED were added.
- **Running buffer (5x):** A solution of 1 M glycines, 0.12 M Tris, and 0.5% SDS was used to prepare the running buffer.
- **Stain solutions:** 250 mL of methanol, 50 mL of glacial acetic acid, and 0.5 g of stain have been mixed and made up to 500 mL with water.
- **Destain solution:** 250 mL of Methanol and 50 mL of Glacial acetic acid have been mixed and made up to 500 mL with water.

- Preparation of gel and sample loading

Spacers were placed between the glass sheets and fixed. After checking the leakage with deionized water, a Resolution gel (bottom gel) solution was prepared and APS and TEMED were added in the last step. The solution was poured quickly but carefully between the glass sheets, 1-2 drops of butanol were added to smooth the surface and prevent the formation of air bubbles and left until polymerization was completed. Polymerization time varies depending on the concentration of the substances in the gel and factors such as the temperature of the environment. After the polymerization was completed, the butanol on the surface was washed away. A stacking gel (top gel) solution was prepared and APS and TEMED were added in the last step. It was quickly poured onto the resolution gel, a comb was placed, and allowed to polymerize. The polymerization process could be followed by following the polymerization of the remaining solution in the container in which we prepared the gel mixture. After the removal of the comb, SDS-PAGE gel was ready for loading and running (9).

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- Electrophoresis conditions

A drop (10 µl) of 0.1% bromophenol blue is placed on the samples before the application to the gel. By connecting the system to the power supply, the device is adjusted so that a current of 16 mA is passed, when the band of the dye, which allows monitoring of the movement of proteins in the gel, comes out of the loading gel and enters the separation gel, the current is increased to 30 mA and this current is continued until the end of the process. The temperature should not exceed 35–40 °C to prevent the gels from heating up. Towards the end of the run, the voltage can be increased gradually to shorten the electrophoresis time. Fast runs give better results than overnight runs, especially with 10% acrylamide gels. Alternatively, constant power of ~10 W per gel might be set to ensure an even distribution of heat (10).

- Coomassie staining

Coomassie "brilliant blue"; is a dye that randomly binds to proteins. The regions where the proteins precipitate on the gel are stained blue with dye. The regions where the proteins precipitate on the gel are stained blue with dye. After the excess paint on the subfloor is washed out with a destaining solution. This process is repeated by renewing the washing solution until the paint on the floor comes off and the bands become evident. If a sufficient level of staining is obtained in the desired bands in the gel, its photograph is taken. This gel can be stored by drying in a 7% acetic acid solution or in gel drying systems (11).

- Staining with Alizarin, Juglone, and Lawson

While preparing the dyes, the staining procedures in Coomassie blue were applied exactly. But the dyes were added until they formed a saturated solution.

Results

The bands appeared quite clearly in the gel we stained with Alizarin red. There is no need for a destaining step in staining with Alizarin. The gel was scanned after only washing it with water (Şekil 1).

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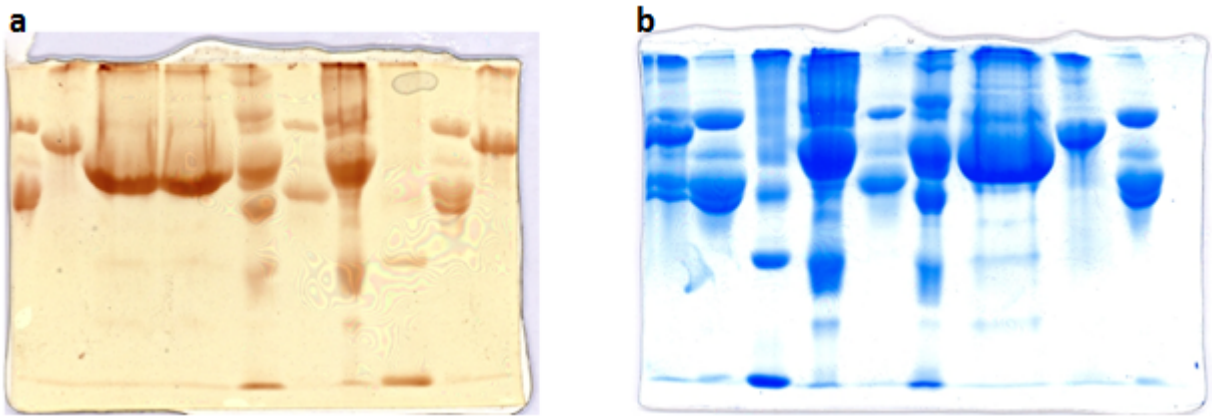

Figure 1. Compairing (a) Alizarin staining with (b) comassie staining

Lawson stained the proteins but did not highlight all the bands. Proteins with low concentration are not well seen. Making changes to the dye concentration may produce better results.

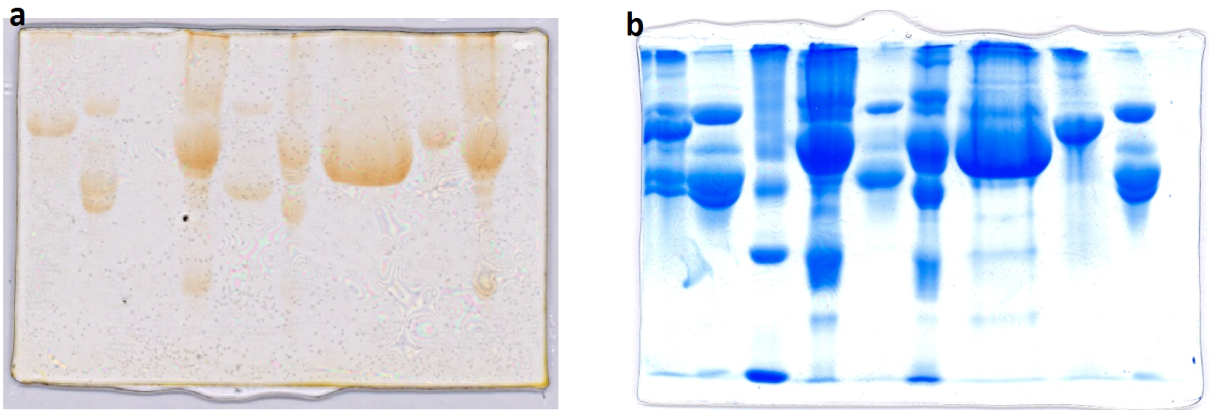

Figure 2. Compairing(a)Lawson staining with (b) comassie staining

Juglone stained proteins well. All bands are clearly visible. However, low-concentration proteins are not very clear. An alternative dye to coomassie blue can be obtained by further work on juglone.

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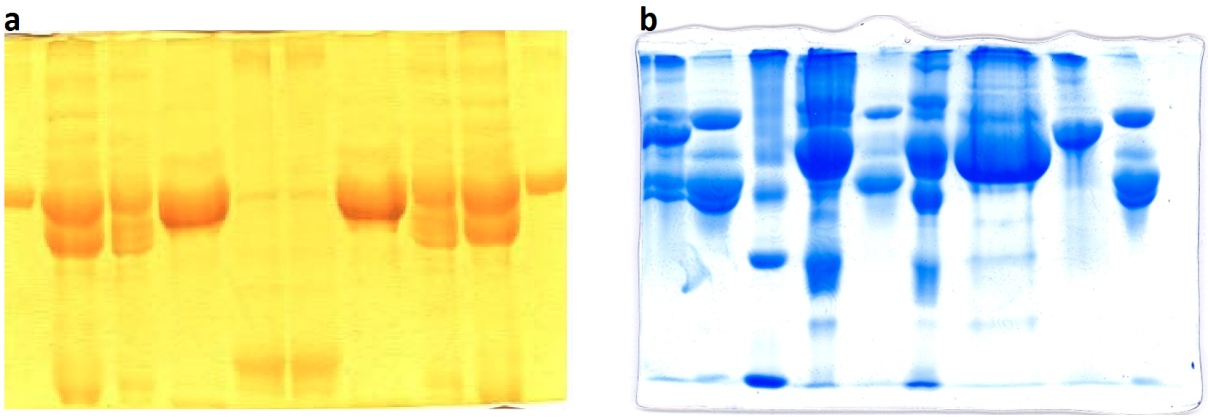

Figure 3. Comparing (a) juglone staining with (b) coomassie staining

Discussion

An acceptable level of protein staining was observed in the gels stained with Alizarin when compared with the coomassie blue. Moreover, providing visualization without a destaining stage is superior to coomassie blue. Compared to Coomassie dyeing, it is an advantage in that it is a one-step process. However, bands of low-concentration proteins are still not as clear as in Coomassie staining. Even though, this problem can be eliminated by changing the dye solution or extending the dyeing time. Studies on the dyeing technique may make alizarin an alternative dye to Coomassie blue. Gedikli et al (2015) have found similar results in their studies with Rubia tinctorum extract. Rubia tinctorum is a plant that contains abundant alizarin in its roots and is used in traditional dyeing (2).

Lawson is a dye found in the henna plant. The dye obtained from this plant has been traditionally used in tattooing and hair coloring since the past (12). Lawson stained the proteins but did not highlight all the bands. Proteins with low concentration are not well seen. Making changes to the dye concentration may produce better results.

Juglone is a traditional dye obtained from walnut wood. The reduced derivative of this substance, which is used in dyeing, is found in the form of glycosides in fresh leaves, root of the tree, stem bark and fruit (12). Juglone stained proteins well. All bands are clearly visible.

However, low-concentration proteins are not very clear. An alternative dye to coomassie blue can be obtained by further work on juglone.

If we make a ranking for the observation of the bands according to the data obtained; alizarin > juglone > lawson. As can be seen from the ranking, the best staining dye is alizarin red (Turkish red). As mentioned before, the dyeing process takes place in a single step and the process takes less time. In addition, alizarin red is a substance widely used in the textile industry and is very cheap. Even with a little work on it, it is possible to obtain an effective paint. juglon and lawson, on the other hand, although not as good as alizarin, are promising for the improvement of the study. Furthermore, by investigating the spectroscopic properties of the dyestuffs used, quantitative analysis of proteins or amino acids can be made with the spectroscopic method, as with the Bradford method. Proteins with different molecular weights can be stained with highly effective new dyestuffs in different colors to make a new and more useful prestained blotter for Western blotting.

Conclusion

In further studies, an alternative technique to coomassie dyeing can be developed by creating a protocol with dyestuffs by making experiments such as increasing the dye concentration, extending the dyeing time, or shortening the washing time. The use of natural dye sources will pave the way for electrophoresis studies with a technique that is both less costly and more easily available.

Conflict of interest: The authors have no conflict of interest in this paper

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