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Short Communication

# Fast reversible single-step method for enhanced band contrast of polyacrylamide gels for automated detection

Staining SDS-PAGE is commonly used in protein analysis for many downstream characterization processes. Although staining and destaining protocols can be adjusted, they can be laborious, and faint bands often become false negatives. Similarly, these faint bands hinder automated software band detections that are necessary for quantitative analyses. To overcome these problems, we describe a single-step rapid and reversible method to increase (up to 500%) band contrast in stained gels. Through the use of alcohols, we improved band detection and facilitated gel storage by drying the gels into compact white sheets. This method is suitable for all stained SDS-PAGE gels, including gradient gels and is shown to improve automated band detection by enhanced band contrast.

**Keywords:**
Automated gel band detection / Band contrast / Gel analysis / Gel drying / Polyacrylamide gel electrophoresis (PAGE) DOI 10.1002/elps.201500094

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Raymond and Weintraub [1] discovered polyacrylamide gel with the use of SDS to separate proteins based on molecular weight (PAGE) [2]. Downstream applications of SDS-PAGE are many, and routinely include gel staining, image analysis, and gel drying for storage. For staining, the commonly used Coomassie blue staining claims a detection range of between 30 and 100 ng of protein [3]; however protein bands at 500 ng often remain undetectable or faint. This leads to false negatives, requiring high amounts of precious protein to be loaded for accurate visualization. While careful analysis may reveal faint smudges in the right light, faint bands pose a challenge for software-driven automated band detections that are used for certain computerized quantitative analysis.

Similarly, in the area of storage, the commonly used drying method of placing the gel between cellophane sheets [4] often with heating and vacuum, is time consuming, irreversible, and risks cracking the gel. As such, there is a demand for better methods that allow for better treatment of the polyacrylamide gels for visualization and storage.

To tackle these problems, we optimized and investigated a relatively obscure yet novel method of gel treatment that turns polyacrylamide gel opaque. Fadouloglou et al. first described a two-step incubation to shrink and dry polyacrylamide gels using ethanol [5], developing this method further, others found the reaction to be reversible with acetic acid [6]. Nonetheless, the method was not fully characterized and the results were not quantitatively analyzed.

Systematically, we studied this method and made the following improvements. First, we shortened the method into a single step of incubation with alcohol; second, we determined the parameters involved in the rate of reaction: the type of alcohols, and the percentage of gels; third, we quantified the improved contrast and automated detection for image analysis software.

In our experiments, we found that the treatment of the polyacrylamide gel (see Supporting Information for details of chemicals used) involved three reactions: (i) shrinkage (typically 60–75% of the original size), (ii) drying, and (iii) enhanced contrast of the stained bands (Fig. 1A). These reactions only occur on polyacrylamide gels (agarose data not shown) and are completely reversible by soaking the dried gel in water (rather than the hazardous acetic acid [6]).

Investigating the rate of the above reactions while standardizing the polyacrylamide gels (83 × 63 × 1 mm, Bio-Rad mini-PROTEAN Tetra Cell, see Supporting Information), we found the following parameters: type of alcohol, strength of alcohol solution, and the polyacrylamide percentage to be the factors. Investigating the type of alcohol, we used methanol, ethanol, and isopropanol on unused 10% polyacrylamide gels.

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**Colour Online**: See the article online to view Fig. 2 in colour.

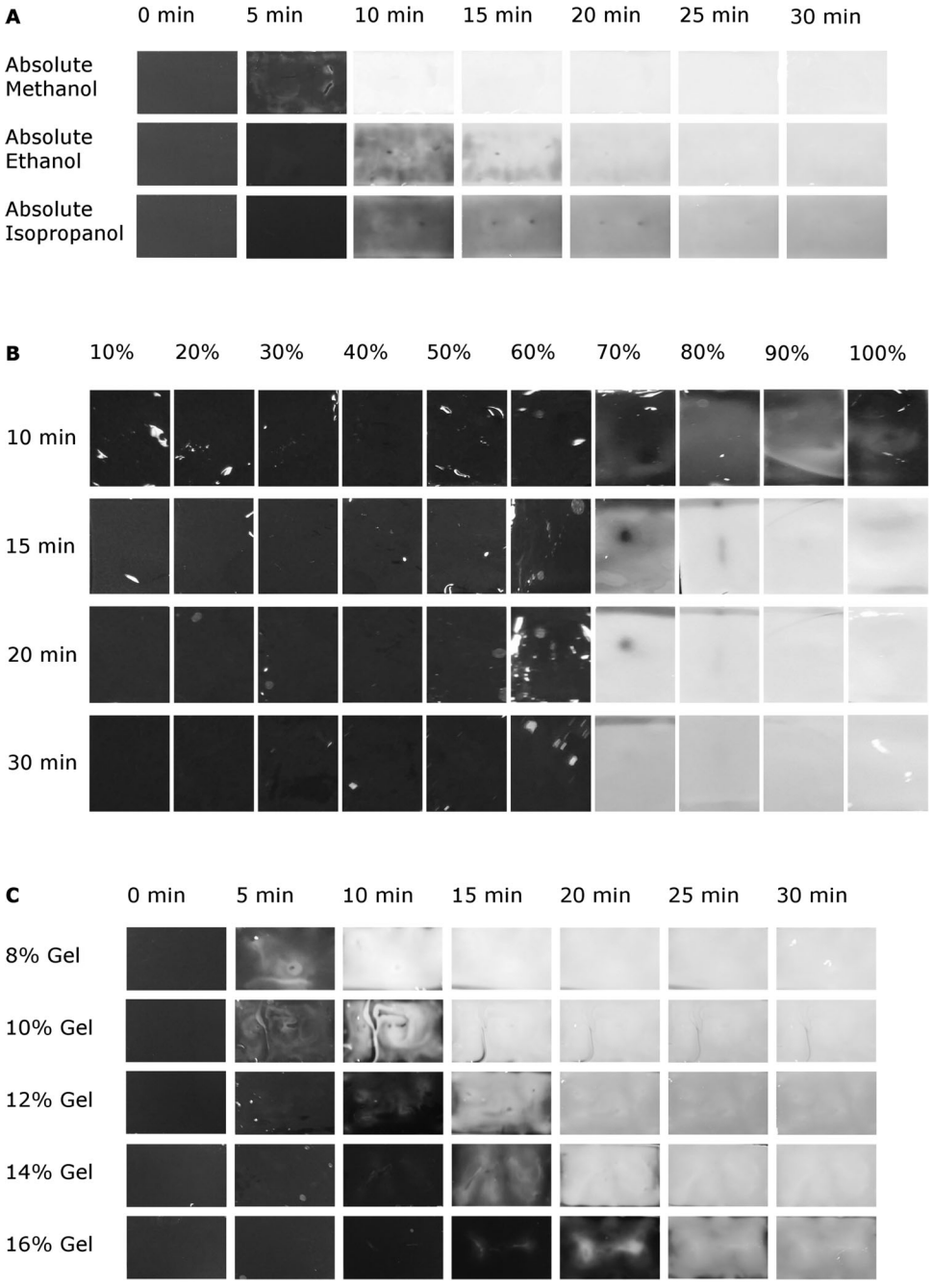
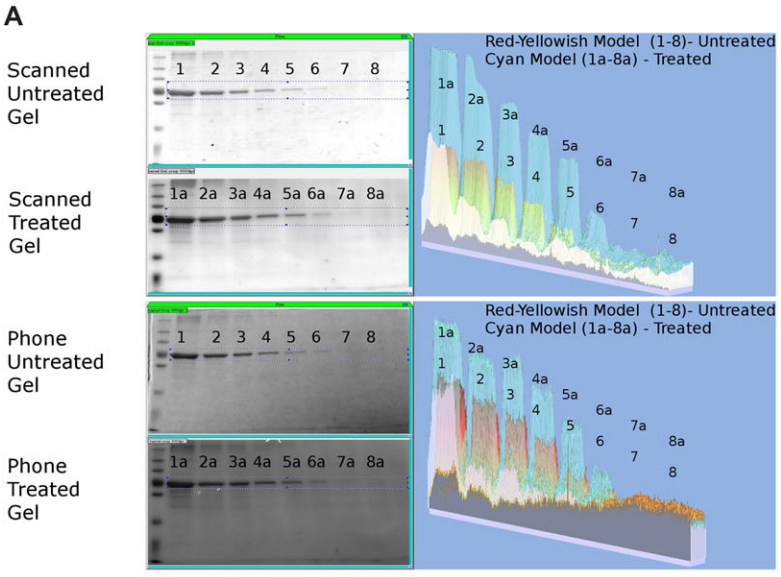
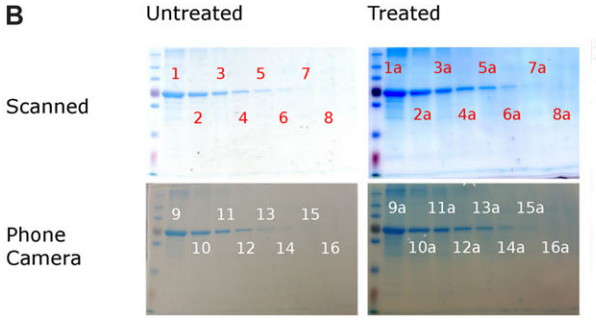



**Figure 1.** Factors that affect the alcohol treatment of SDS-PAGE on unused polyacrylamide gel (83 × 63 × 1 mm). The pictures were taken with a black background to show contrast with opacity after treatment. (A) Analysis of the rate of opacity reaction using methanol, ethanol and isopropanol. Methanol treated the gel at the fastest rate, with the gel gaining opacity at 5 min postincubation and turning completely opaque at 10 min. Both ethanol and isopropanol gels started gaining opacity at the 10 min time point. Full opacity was achieved with ethanol by 20 min, whereas isopropanol treatment took 25 min. (B) Analysis of the rate of opacity reaction using 10–100% ethanol solutions. Ten to hundred percent of (10% intervals) ethanol solutions were used to soak the polyacrylamide gels. No opacity was observed on gels soaked in 10–50% ethanol. Sixty percent of ethanol elicited slight translucency by the end of 30 min interval. Seventy percent of ethanol treated the gel to full opacity by 30 min interval, with higher concentrations doing so in shorter incubation times. (C) Analysis of the rate of reaction using polyacrylamide gels of varying percentages. Commonly used gel percentage formulations: 8, 10, 12, 14, and 16% were treated with absolute ethanol. The various gels : 8, 10, 12, 14, and 16%, turned opaque at the respective time points: 15, 20, 20, 25, and 30 min.

Methanol treated the gel at the fastest rate (full opacity by the 10$^{th}$ min), whereas ethanol and isopropanol were slower (full opacity achieved at the 20$^{th}$ min for ethanol, and an additional 5 min for isopropanol). Since chemically smaller alcohols diffused faster, they were able to hasten the drying, shrinking, and opacity reactions (Fig. 1A). Even so, availability and safety reasons advocated for ethanol in subsequent investigations. Further optimizations found that a single alcohol incubation step was sufficient to treat the gel, thus shortening the original protocol of two-step incubations [5].

Next, we investigated the strength of the alcohol solutions. Expectedly, we found higher percentage of ethanol solutions to directly increase the rate of the gel reactions. Using a range of ethanol solutions (10–100% ethanol at intervals of 10%), we found no effect for the unused gels treated with 10–50% ethanol (Fig. 1B). Sixty percent of ethanol elicited only slight translucency by the end of 30 min, whereas 70% ethanol treated gels were opaque by the 30$^{th}$ min. Higher ethanol concentrations reached opacity in shorter time spans, concluding that 60% ethanol was the lowest alcohol strength necessary




C

| Lane | Bands Intensity | Band intensity percentage increase after treament | Lane | Bands Intensity | Band intensity percentage increase after treament |
|---|---|---|---|---|---|
| 1 | 9046.3 | 121% | 9 | 5,720.4 | 122% |
| 1a | 10978.0 | | 9a | 6,996.3 | |
| 2 | 3920.8 | 123% | 10 | 2,896.5 | 116% |
| 2a | 4827.9 | | 10a | 3,373.5 | |
| 3 | 2680.6 | 149% | 11 | 1,811.7 | 134% |
| 3a | 3990.9 | | 11a | 2,436.7 | |
| 4 | 1767.2 | 204% | 12 | 1,044.1 | 173% |
| 4a | 3604.3 | | 12a | 1,806.9 | |
| 5 | 764.7 | 298% | 13 | 336.0 | 301% |
| 5a | 2282.4 | | 13a | 1,011.9 | |
| 6 | 414.5 | 208% | 14 | 200.7 | 245% |
| 6a | 863.2 | | 14a | 492.4 | |
| 7 | 105.1 | 116% | 15 | 22.8 | 534% |
| 7a | 122.4 | | 15a | 121.8 | |
| 8 | 79.5 | 217% | 16 | 24.5 | 350% |
| 8a | 172.3 | | 16a | 85.7 | |

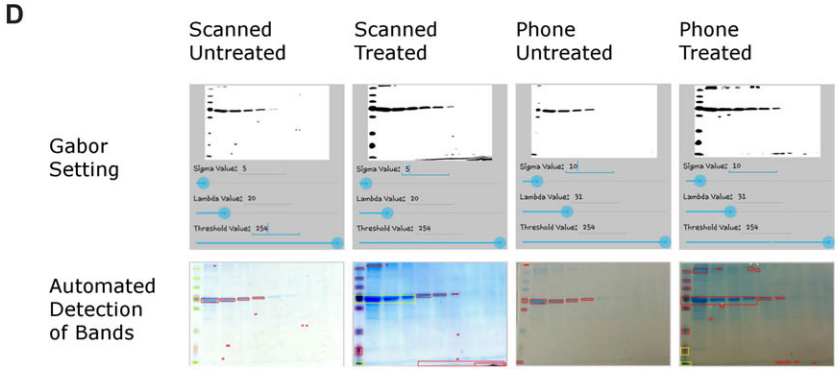



**Figure 2.** Quantitative analysis of pre- and posttreated images of the same gel in the same lighting. (A) Intensity analysis of protein bands using Melanie Viewer [7]. The 3D model showed the protein bands intensities of both scanned and phone camera images from the same gel. Untreated gels are shown in red–yellow color while treated gels are shown in cyan. Regardless of the capturing device, band contrast of the treated gel images increased across all six lanes when compared to their corresponding untreated gel images. Band contrast was most pronounced in lanes 5 and 6 of the phone images. (B) Scanned and camera taken images of treated and untreated gels (C) Readings of band intensity with GelQuant [8]. High concentration protein bands (bands 2 and 8) intensity were similar after treatment (bands 2a and 8a). Moderate concentration (bands 4 and 10) and low concentration (bands 6 and 12) protein bands showed the greatest increase in intensities after treatment. (D) Automated protein band detection using Gelapp [9]. Treatment of the gels facilitated detection of lower BSA levels (boxed up bands on the treated gel) despite using the same image processing settings. For untreated gels, detection of both scanned and phone camera images (first and third column) were only up to lane 4 – 2.81 μg. After treatment, detection extended to lane 6 – 0.70 μg for both scanned and phone camera images (second and fourth column).

for this gel treatment (Fig. 1B). Since typical staining and destaining solutions contains low concentrations of alcohol (usually up to 40%), it is not surprising that this phenomenon escaped observation despite their routine use.

The other factor that affected the speed of the gel treatment was the gel percentage. Observing the reaction on gradient gels (4–20%), lower acrylamide percentage regions reacted prior to higher acrylamide percentage regions in a linear fashion (see Supporting Information video where the left side of the gel is the lower percentage region). To confirm this finding, we treated commonly used gel percentage formulations (ranging from 8 to 16%) and found that opacity was reached within 30 min (at 15 (8% gel), 20 (10% gel), 20 (12% gel), 25 (14% gel), and 30 min (16% gel), respectively) in an inverse relationship between gel percentage and the time taken (Fig. 1C). As it is with alcohol type and concentration, the diffusion rate is hindered by the compactness of the gel matrix.

To quantify the increased band intensity, prestained gels loaded with BSA of decreasing concentrations (22.5-0.17 μg) were stained using Bio-Safe Coomassive G-250 stain. The pre- and posttreatment images were captured by scanner and mobile phone camera. Using Melanie Viewer 7.0 [7], a software that displays image intensities in 3D, increased band intensities were observed for both scanned and phone camera pictures after treatment (Fig. 2A). Since phone cameras are increasingly being used and are affected by environmental lighting (we standardized our lighting), the reproducible increased contrast in both capture devices validated the treatment results.

To quantify the improvement in band intensity of the same stained gels before and after treatment (Fig. 2B), we used GelQuant [8] and measured the band contrast to increase on an average of 240% and up to 534% (Fig. 2C, lanes 4a–6a). When subjected to a mobile phone automated band detection app: Gelapp [9], protein bands as low as 0.7 μg (shown in lane 6 of Fig. 2D) were automatically detected for the treated gel; whereas untreated images of the same gel had the lowest detection limit at 2.8 μg (lane 4 of the original gel, see Fig. 2D). In the treatment of the gels, we observed that background stains may become more pronounced, however, given that the aim was to detect faint bands, the method clearly increased band contrast, complementing the use of automated image processing software.

Treated gels shrank and hardened to a dimension of approximately 4 by 6 cm within 30 min (dehydration) with no risk of cracking and are suitable for easy storage. Since the treatment is easily reversed by soaking it in water, many downstream experiments can continue after reconstitution. We timed the rehydration process to take approximately 20–30 min, working in a reverse manner of the dehydration (data not shown). Therefore this method is faster than other drying methods which require extended hydration periods [4, 10].

In this work, we characterized and improved upon a method for drying gels that increased band intensities without changing the staining and de-staining procedures. We show that alcohols (specifically ethanol) are effective treatment agents for the proposed one-step rapid and reversible method. Since the method improved the contrast of faint bands, it complemented image processing software tools, working toward quantitative biology and improved protein analysis.

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## References

[1] Raymond, S., Weintraub, L., *Science* 1959, *130*, 711.

[2] Laemmli, U. K., *Nature* 1970, *227*, 680–685.

[3] Simspon, R. J. *Cold Spring Harb. Protoc.* 2010, doi: 10.1101/pdb.prot5413.

[4] Wallevik, K., Jensenius, J. C., *J. Biochem. Biophys. Methods* 1982, *6*, 17–21.

[5] Fadouloglou, V. E., Glykos, N. M., Kokkinidis, M., *Anal. Biochem.* 2000, *287*, 185–186.

[6] Ali, M., *Proceedings of the 4th International Iran & Russia Conference is in Shahrekord, Iran,* September 8-10, 2004, 258–260.

[7] Melanie Viewer 7.0 [software]. Available from: http://www.world2dpage.expasy.org/melanie/.

[8] GelQuant.NET (version 1.7.8) [software]. Available from: http://biochemlabsolutions.com/GelQuantNET.html.

[9] Sim, J.Z., Nguyen, P.V., Lee, H.K., Gan, S.K.E., GelApp: Mobile gel electrophoresis analyzer. *Nature Methods/Application Notes*. 2015, doi:10.1038/an9643

[10] Krishnan, M., Nguyen, H. T., *Anal. Biochem.* 1990, *187*, 51–53.