Indian Journal of Experimental Biology
Vol. 39, January 2001, pp. 95-97

Detergent-mediated destaining of Coomassie Brilliant Blue-stained SDS polyacrylamide gels

J.K. Pal, A. Sarkar & B. Katoch

Department of Biotechnology, University of Pune, Pune 411 007, India
FAX: 91-20-5655179. email: jkpal@unipune.ernet.in

Received 3 July 2000; revised 3 November 2000

A simple and one-step detergent-mediated destaining procedure for SDS Polyacrylamide gels for proteins is described. Suspension (5%, w/v) of a commercially available household detergent, Vim Ultra, has been found to be very efficient in destaining polyacrylamide gels without interfering with the resolution of proteins. As compared to the routinely used solvent (methanol-acetic acid-water)-mediated destaining procedure, the present method is economical and user-friendly.

Polyacrylamide gel electrophoresis for analysis and characterisation of proteins is a routine procedure employed by researchers in the field of protein biochemistry. From the beginning of development of this valuable method, efforts are being made to improve on its quality in various directions and the enthusiasm still continues. Among many such parameters, visualisation of proteins following electrophoretic separation has been given enough attention. Visualisation of proteins in polyacrylamide gels requires staining of the proteins and destaining of the gels to get a clear background[1]. For this purpose, various staining techniques have been used. However, the most popular techniques are Coomassie Brilliant Blue (CBB)[2] and silver[3] staining due to their proven reliability, simplicity and economy. The standard CBB staining procedure involves immersing the gel in a solution of methanol / acetic acid /water containing 0.1% (w/v) CBB followed by destaining with the same solution excluding the stain[4]. Traditionally, staining and destaining usually require 3-6 and 10-48 hr, respectively[1]. Though destaining can be shortened by use of absorbents (e.g., charcoal containing sponges), such procedures require more apparatus and / or chemicals and are not simple. At the same time these solvents / chemicals are also toxic for handling on routine basis. In the present communication, a simple and single-step destaining procedure using a commercially available household detergent, Vim Ultra has been described. This procedure is easy to use and the detergent is inexpensive and less toxic as compared to the solvents that are used in the conventional destaining procedures.

Protein sample used in these experiments was extracted from chickpea in lysis buffer containing the following: 20 mM Tris-HCl, pH 8.0; 1 mM EDTA; 1 mM PMSF; 0.1% Triton X-100. Extract was centrifuged at 15,000 rpm for 20 min and the soluble supernatant was used for analysis. Proteins were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS PAGE) according to Laemmli[5]. Immediately after electrophoresis gels were stained overnight in methanol/acetic acid/ water (5:1:4) containing 0.125% (w/v) CBB R-250. For destaining, various concentrations of different detergents, namely, Vim Ultra and Surf Excel (Hindustan Lever Ltd., Mumbai) and Ariel (Procter & Gamble Home Products Ltd., Mumbai) were used, either alone or as a mixture of two (1:1). The detergent suspensions (5%) were filtered through Whatman No. 1 filter paper to eliminate large particles, before their use. For each experiment, one gel was destained by the routine procedure (as control) using methanol/acetic acid/water (4:1:5) along with gels destained by detergents. Optimum results were obtained with 5% suspension of Vim Ultra. After partial destaining for about 3hr, the gels were transferred to 7.5% acetic acid overnight for obtaining a clear background.

The resolution of protein bands, in general, in terms of numbers and intensities on the gel destained by the detergent Vim Ultra (Fig. 1B) was very similar to that destained by the routine procedure (Fig. 1A). The background in both the gels was indistinguishable. The detergent-mediated destaining took almost an hour more as compared to that taken

Table 1—Summary of results on destaining with various detergents

Destaining method used	Time taken for partial destaining* (hr)	Results obtained
1. Traditional solvent- mediated (as control)	2	Clean background, good resolution
2. Detergent-mediated a) Vim Ultra (5% w/v)	3	Clean background, resolution similar to control
(b) Aerial (5% w/v)	3	Loss of resolution of less intense bands, colour of protein bands changed from blue to yellowish green
(c) Surf Excel (5% w/v)	3	Loss of protein bands of low intensity

* For complete destaining, gels were kept in 7.5% acetic acid overnight.

by the routine destaining procedure. However, the time taken could be shortened if the destaining is done with a warm (40°C) suspension of the detergent. When the cost of destaining per gel (mini gel) was calculated, detergent-mediated destaining was found to be about 6-times cheaper as compared to the routine destaining procedure (Rs. 2/- vs Rs. 12/-). These results, therefore, suggest that this new procedure of detergent-mediated destaining of polyacrylamide gels could be used as an alternative to the routine destaining procedure.

Two other household detergents, namely, Surf Excel and Aerial, either alone or as a mixture (1:1) of two in various combinations were also used (data not shown). The protein profile of the gel destained with Vim Ultra was the best as compared to those destained by the other two detergents, using either alone or as mixtures, in terms of resolution and status of the gel. In general, the other two detergents resulted in loss of stain in some minor protein bands with molecular weight of wide range all over the gel. Furthermore, Aeriel caused a change of colour of the protein bands from blue to yellowish green, which was found unsuitable for documentation (photographic) purpose. The summary of results obtained with all these detergents is presented in Table 1.

Household detergents are cheap and easily available as compared to organic solvents present in routine destainer. Beside this, the detergents at the concentration used are much less toxic as compared to methanol and acetic acid used in traditional destainer, so the use of the detergents as destainer is much more eco-friendly. In conclusion, Vim Ultra, a household detergent, could be used as a suitable alternative reagent for destaining polyacrylamide gels in laboratories engaged in protein research, and more so in schools and colleges where this technique is used for demonstration purpose.

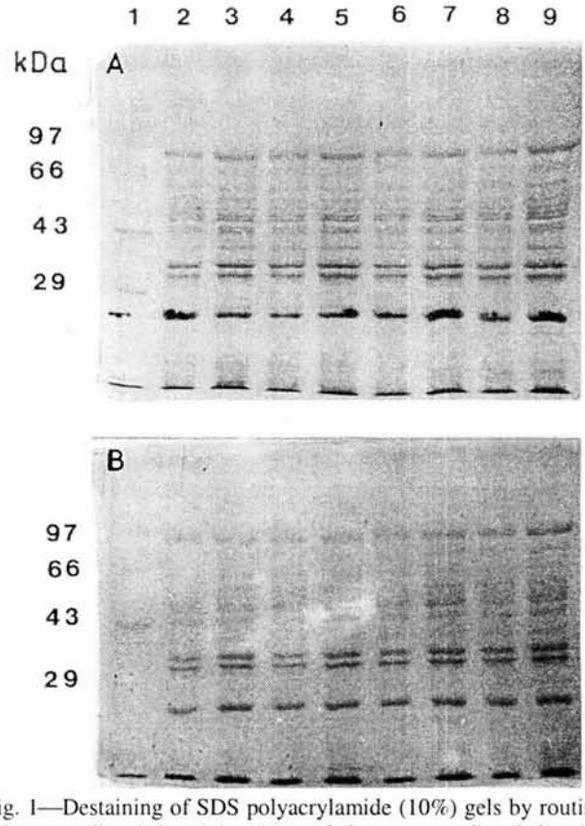

Fig. 1—Destaining of SDS polyacrylamide (10%) gels by routine solvent-mediated destaining (A) and detergent-mediated destaining (B) procedures. Lane1, Molecular weight marker proteins; lanes 2-9, same quantity (35 μg) of chickpea extract. Electrophoresis was carried out at 25 mA (constant current), gels were stained in 0.125% CBB overnight and destained by either Methanol: Acetic acid: H_2O (40:10:50) (Panel A) or by 5% Vim Ultra (Panel B) for 3hr and then transferred to 7.5% Acetic acid.

This work was supported by a Research Project from the Department of Biotechnology, Govt. of India, New Delhi. AS is a JRF appointed in the same project. BK was a postgraduate student of Modern College, Pune and she carried out part of this work as her M.Sc. research project. We thank Drs. Anita Kar and H.V. Ghate for suggestions and comments on the manuscript.

References

1 Wu W & Welsh M J, Rapid Coomassie Blue Staining and Destaining of Polyacrylamide Gels, *Biotechniques*, 20 (1996) 386.

2 Fazekas de St Groth S, Webster R G & Datyner A, Two new staining procedures for quantitative estimation of proteins on electrophoretic strips, *Biochim. Biophys. Acta*, 71 (1963) 377.

3 Merril C R, Goldman D, Sedman S A & Ebert M H, Ultrasensitive stain for proteins in polyacrylamide gels shows regional variation in cerebrospinal fluid proteins, *Science*, 21 (1981) 1437.

4 Zehr B D, Savin T J & Hall R E, A one-step low background coomassie staining procedure for polyacrylamide gels, *Anal Biochem*, 182 (1989) 157.

5 Laemmli U K, Cleavage of structural proteins during the assembly of the head of bacteriophage T4, *Nature* (London), 227 (1970) 680.