Journal of Chromatography, 582 (1992) 249–252
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 6527

Short Communication

Improved procedure for the drying and storage of polyacrylamide slab gels

Ľudovít Škultéty and Rudolf Toman

Institute of Virology, Slovak Academy of Sciences, Dúbravská cesta 9, 842 46 Bratislava (Czechoslovakia)

(First received May 5th, 1992; revised manuscript received July 31st, 1992)

ABSTRACT

An improved procedure for the drying and storage of polyacrylamide (PAA) slab gels of various sizes and acrylamide contents is presented. A PAA slab gel is preferentially fixed in methanol–acetic acid–glycerol–water (1.0:1.0:0.1:2.0) and then dried under low pressure in an adapted photoprint dryer between Cellophane and polyethylene foils at 75–80°C for 50–90 min. Strong, transparent and flexible sheets are obtained that can be stored for extended periods. The potential of the procedure is exemplified following electrophoresis of both lipopolysaccharides and protein isolated from *Coxiella burnetii* bacterium.

INTRODUCTION

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) is widely applied in many fields of modern biochemistry. However, the method has some shortcomings connected with the procedures currently in use for drying and storage of slab gels. Most frequently, after the gel has been stained, *e.g.*, with Coomassie Brilliant Blue [1] or silver nitrate [2], a photograph is taken for record-keeping purposes. The gel is stored, if necessary, at room temperature in dilute acetic acid or at 4°C in distilled water or dried. Numerous procedures have been developed for drying polyacrylamide (PAA) slab gels. Many of them are complex and require, among other things, a special drying unit connected to a vacuum pump [3–6] or dry-ice [4,7]. Others [4,8,9] prefer drying of gels without any costly equipment. In our experience, drying without the use of a drying unit or dry-ice takes a minimum of 24 h. In the absence of a drying unit, gelatine is routinely used for the binding of gels to a solid support such as foil or glass. The gels are fragile and difficult to store. This is also true of gels dried in the presence of dry-ice alone. Although drying proceeds significantly faster in this case, the gels may deteriorate easily.

Better results are obtained by drying gels in a suitable drying unit. In the procedure presented here we have optimized both the fixing and drying conditions in order to obtain high-quality fixed gels that could be stored indefinitely. Moreover, it is shown that an inexpensive, widely used photoprint dryer can easily be adapted to serve as a simple and efficient drying unit.

Correspondence to: Dr. R. Toman, Institute of Virology, Slovak Academy of Sciences, Dúbravská cesta 9, 842 46 Bratislava, Czechoslovakia.

0378-4347/92/$05.00

EXPERIMENTAL

Reagents and chemicals

The following reagents and chemicals were used: tris(hydroxymethyl)aminomethane (Tris) (Sigma, St. Louis, MO, USA); acrylamide (AA), Coomassie Brillant Blue R-250, N,N′-methylenebisacrylamide, sodium dodecyl sulphate and N,N,N′,N′-tetramethylethylenediamine (Serva, Heidelberg, Germany), ammonium peroxodisulphate, 2-mercaptoethanol (Fluka, Buchs, Switzerland), periodic acid (Merck, Darmstadt, Germany) and acetic acid, bromophenol blue, citric acid, ethanol, formaldehyde, glycerol, hydrochloric acid, silver nitrate and sodium carbonate (Lachema, Brno, Czechoslovakia).

Apparatus

A photoprint dryer with temperature control (Kovos Teplice, Teplice, Czechoslovakia) was used for the construction of the drying unit (Fig. 1).

Procedures

SDS-PAGE was performed in slabs by the method of Laemmli [10]. The separating gels contained 10–18% PAA. *Coxiella burnetii* cells of the strains Nine Mile, S, Priscilla, 48, 1/IIA and IXO and their corresponding lipopolysaccharides (LPSs) were isolated as described elsewhere [11,12]. To each 0.2% aqueous solution of LPS in distilled water, an equal volume of 0.1 *M* Tris · HCl (pH 6.8) solubilization buffer containing 4% SDS, 12% glycerol, 10% 2-mercaptoethanol and 0.001% bromophenol blue was added. The solutions were heated at 100°C for 10 min, cooled and 5 μg of LPS were applied to each well. *C. burnetii* cells were lysed in the same buffer at 100°C for 10 min prior to electrophoresis. An amount of 150–300 μg of cell lysate was applied to each well.

For silver staining, a modified method of Tsai and Frasch [2] was used. Briefly, fixed polymacromolecular material was treated with 0.7% periodic acid in ethanol–acetic acid–water (8:1:11) for 10 min and washed with 30% methanol (15 min) and distilled water (2 × 15 min). The staining reagent containing 0.2% silver nitrate and 0.02% citric acid in water was allowed to stand over the gel for 20 min and the last traces of the reagent were removed by two washes with distilled water. Staining was developed in a 5.5% aqueous solution of sodium carbonate. Proteins were stained [1] with 0.05% Coomassie Brillant Blue R-250 in methanol–acetic acid–water (23:4:23). Destaining was accomplished with the same solvent system but without the dye. Gels were temporarily stored in water prior to drying.

The slab gel was fixed in (A) methanol–acetic acid–glycerol–water (1.0:1.0:0.1:2.0) or (B) acetic acid–glycerol–water (1.0:0.1:3.0) for 15 min and dried for 50–90 min under low pressure. The construction of the drying unit is depicted in Fig. 1. It consists of the following parts: a photoprint dryer with temperature control, rubber sheet (240

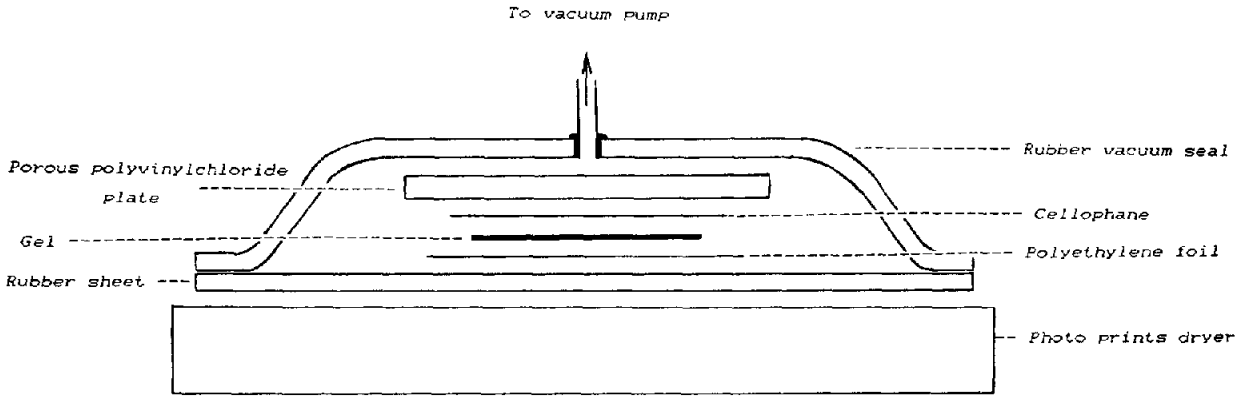

Fig. 1. Cross-sectional view of an adapted photoprint dryer for drying of slab gels.

× 300 × 3.5 mm), polyethylene (PE) foil (170 × 220 mm), porous poly(vinyl chloride) (PVC) plate (180 × 230 × 2.5 mm) and rubber vacuum seal (240 × 300 × 3.5 mm). The gel to be dried is sandwiched between the PE foil and Cellophane presoaked in the fixing solution. The Cellophane is always larger than the gel, so that there is direct adhesion of the Cellophane to the PE foil. Any air bubles present are carefully removed. The porous PVC plate is then put on the sandwiched gel and the rubber vacuum seal is connected directly to the water vacuum pump. The gel is left to dry at 75–80°C for 50–90 min. After drying, the gel is immediately stored between two flat surfaces until cool (*ca.* 10 min). Finally, the PE foil is peeled off.

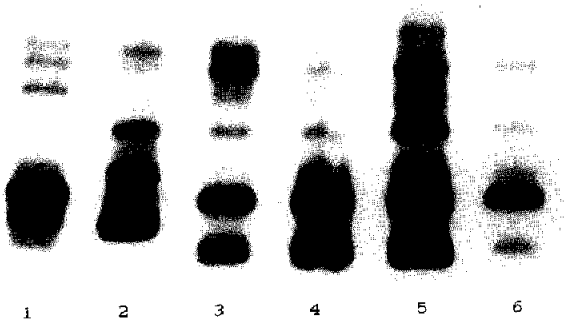

Fig. 2. SDS-PAGE slab gel run, silver-stained, dried and stored as described. Lanes 1–6 are LPSs of *C. burnetii* strains S, Priscilla, Nine Mile, 48, I/IIA and IXO, respectively.

RESULTS AND DISCUSSION

The procedure described is easily applicable in any laboratory. Nevertheless, the optimum conditions for drying may vary as a function of the composition of fixing solution, drying temperature, time, relative humidity, AA and bisacrylamide concentrations, thickness and surface area of the slab gel. Mixtures of methanol or ethanol, glycerol, acetic acid and water with various volume ratios (seventeen solvent systems in all) were tried used for fixing. Solvent systems A and B (see above) were the most suitable, and the former gave slightly better results. The use of fixing solutions in which the methanol or ethanol content was higher than 35% led to a loss of plasticity and an increase in fragility of the gel.

We also investigated the influence of temperature on the drying of gels following fixation with solution A. Temperatures of 50, 60, 70, 75, 80, 90 and 95°C were selected. It was found that a temperature in the range 70–80°C is the most suitable for drying of gels. For example, drying of a 12.5% PAA slab gel was accomplished in 50 min at 80°C. At temperatures lower than 60°C, drying of the gel is slow (more than 2 h), with a concomitant loss of plasticity, and the use of temperatures above *ca.* 90°C resulted in a rapid drying of gels but they often shattered.

As mentioned above, the drying time is primarily dependent on temperature, thickness and size of the slab gel and AA concentration. The drying time is shorter for thinner gels with small surface areas or low AA concentrations. The procedure presented has been routinely applied to gels of various size with 10–18% AA concentrations. The optimum drying time is 50–90 min. On prolonged drying, the gel loses its flexibility and plasticity. Therefore, in some instances, it is useful to terminate drying within the given time interval and then perform the final drying for a

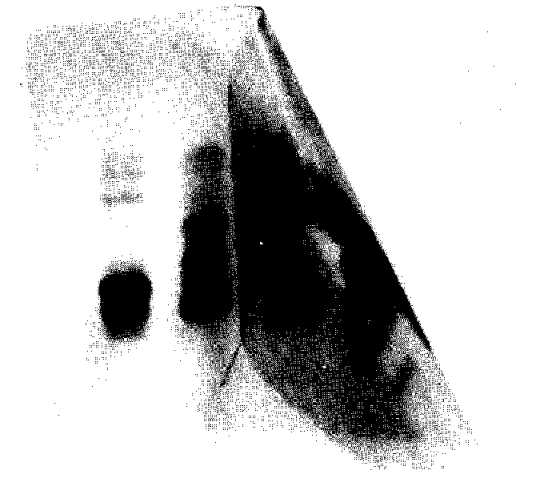

Fig. 3. Example of a strong, transparent and flexible SDS-PAGE slab gel.

short period of time. If necessary, the dried gel can be regenerated in the solvent system methanol–acetic acid–water (1:1:1) within 30–60 min and then dried again without any deterioriation of its surface area.

Fig. 2 is representative of a PAA slab gel that had been silver-stained, dried and stored according to the procedure described. Such gels have been found to be strong, transparent, smooth, flexible (Fig. 3) and easily handled. They can be stored in a binder without sticking to each other and used at any time for autoradiographic and densitometric applications.

REFERENCES

1 G. Fairbanks, T. L. Steck and D. F. H. Wallach, *Biochemistry*, 10 (1971) 2606.
2 C. M. Tsai and C. E. Frasch, *Anal. Biochem.*, 119 (1982) 115.
3 R. Lim, J. J. Huang and G. A. Davis, *Anal. Biochem.*, 29 (1969) 48.
4 Ö. Gaál, G. A. Medgyesi and L. Vereckzkey, *Electrophoresis in the Separation of Biological Macromolecules*, Akadémiai Kiadó, Budapest, 1980.
5 D. M. Gersten, E. J. Zapolski and R. S. Ledley, *Anal. Biochem.*, 129 (1983) 57.
6 R. H. Juang, Y. D. Chang, H. Y. Sung and J. C. Su, *Anal. Biochem.*, 141 (1984) 348.
7 H. Nishizawa, K. Suzuki and Y. Abe, *Electrophoresis*, 10 (1989) 498.
8 O. Popescu, *Electrophoresis*, 4 (1983) 432.
9 B. B. Samal, *Anal. Biochem.*, 163 (1987) 42.
10 U. K. Laemmli, *Nature (London)*, 227 (1970) 680.
11 R. Toman and J. Kazár, *Acta Virol.*, 35 (1991) 531.
12 R. Toman and L. Škultéty, *Infect. Immun.*, submitted for publication.