ANALYTICAL BIOCHEMISTRY 126, 422–424 (1982)

Agarose Drop Method for Loading Thin Polyacrylamide Gels

ROGER C. WIGGINS

Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, Michigan 48109

Received May 17, 1982

Thin gels (<1 mm) can be loaded conveniently, rapidly, and quantitatively by suspending the sample to be analyzed in a drop of agarose gel and simply placing the solidified drop on top of the stacking gel. With this method there is no lower limit to the size of the sample to be loaded or to the thinness of the gel to be employed. Using the silver stain quantitation of between 1 and 1000 ng/sample is easily achieved.

With the introduction of the silver stain (1–3) it became possible to detect nanogram amounts of protein on polyacrylamide gels. For maximum sensitivity and resolution, thin gels (<1 mm) are optimal. The conventional well technique for sample loading is unsatisfactory for very small sample volumes (1–2 μl) and thin gels. This report describes a simple, fast, and accurate method for loading small samples onto thin gels.

METHODS

Electrophoretic system. For optimal sensitivity small (6.5 × 3.5 cm), thin (0.4 mm) polyacrylamide gels were run using a Laemmli buffer system (4). The apparatus was similar to that previously described by Ogita and Markert (5) and made use of disposable glass 7.5 × 5-cm microscope slides (Fisher). Plastic 0.4-mm spacers were cut from the remains of children's toy packages. The running and stacking gels were poured in a conventional manner except that the stacking gel was poured right to the top of the gel mold.

Agarose preparation. Agarose was purified in the following manner to remove contaminating proteins. Agarose (Kallestad, 1000 Lake Hazeltine Avenue, Chaska, Minn.) was dissolved (2%) by heating in a buffer containing 8 M urea, 10% sodium dodecyl sulfate (SDS),[1] 5% beta-mercaptoethanol, and 0.5 M Tris buffer, pH 8.8. The melted agarose was poured into a large gel mold (12 × 9-cm glass plates separated by 3-mm spacers). After solidification by cooling, the "gel" was subjected to electrophoresis at 100 V using a Tris–glycine buffer system at pH 8.3. Electrophoresis was continued for 3 days, replacing the buffer twice each day. The agarose was then removed from the mold and soaked in 2 liters of distilled water with 6 changes for 2 days at 4°C. The agarose was then remelted and divided into 1-ml aliquots. To each 1-ml aliquot was added 100 μl of a mixture of 1 vol bromphenol blue (0.7 mg/ml in water) to 2 vol glycerol. The "blue agarose" was stored at 4°C.

Sample preparation. Samples were prepared in the center of appropriately labeled 1-cm diameter circles drawn on parafilm. The sample was usually in a solution of 8 M urea and 10% SDS with or without beta-mercaptoethanol. One microliter of the sample was placed at the center of the circle. Within a few moments the sample evaporated, leaving a small pile of crystals. When the gel was ready to be loaded, 1 μl of 1.5 M Tris buffer, pH 6.8, (Laemmli stacking gel buffer) was

[1] Abbreviations used: SDS, sodium dodecyl sulfate; BSA, bovine serum albumin.

0003-2697/82/160422-03$02.00/0

Copyright © 1982 by Academic Press, Inc.
All rights of reproduction in any form reserved.

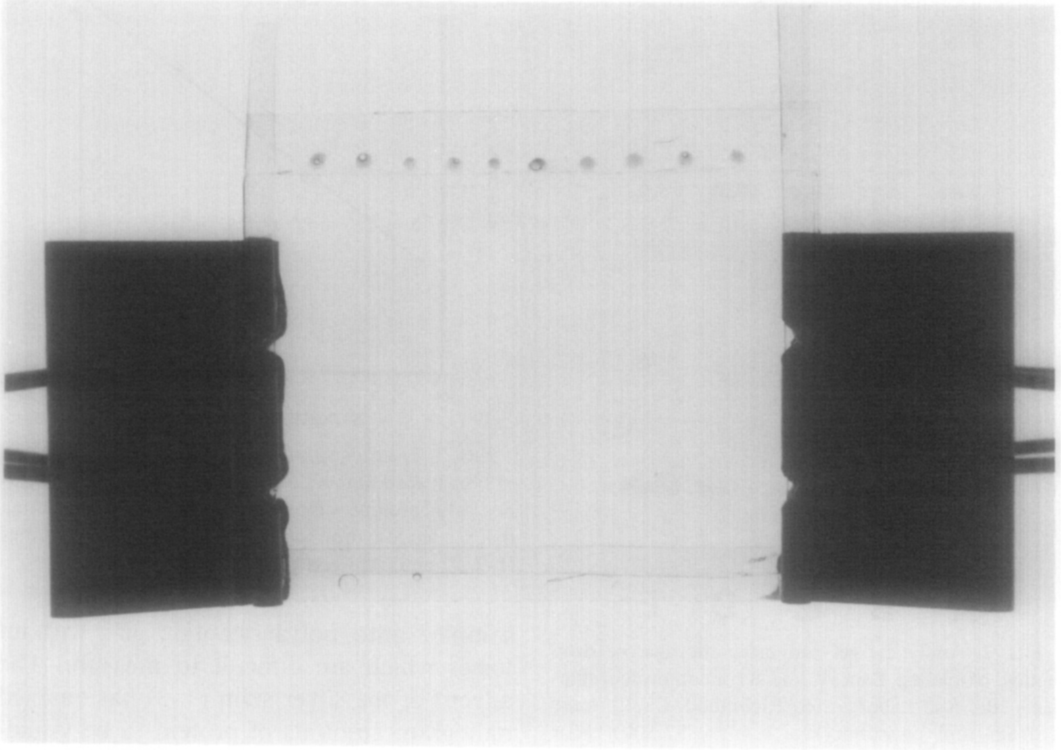

FIG. 1. Blue agarose drops placed on the back glass plate of the gel mold in contact with the stacking gel.

added to the crystalline sample. To this drop was added 1.5 μl of molten "blue agarose." The drops were allowed to solidify at room temperature. Any bubbles were popped. If a delay before sample loading occurred, the agarose drops on parafilm were stored in a moist box to prevent drying out. To facilitate loading, the gel circles were separated by cutting up the parafilm. Each sample (in a solid blue agarose drop) was slid onto the glass back plate of the horizontal gel mold. The drops were positioned 2–3 mm apart (edge to edge) on the glass back plate *in contact* with the polymerized stacking gel (Fig. 1). the gel mold was *then* attached to the electrophoretic apparatus. Tris–glycine buffer, pH 8.3, was added to the bottom reservoir. Finally, Tris–glycine buffer was gently added to the top reservoir to avoid dislodging the agarose drops. The power was immediately switched on. The samples were stacked at 4 mA and run at 9 mA. Total time for gel run was 45 min. During the run the bromphenol blue dye migrated out of the agarose drops and formed a tight dye-front band as in a conventional gel system. At the completion of a run the glass mold was removed and the plates were separated. The plate to which the gel remained attached was placed in 50% methanol, 12% acetic acid, and processed for silver staining according to Merril *et al.* (3).

RESULTS

As shown in Fig. 2, gels loaded by the agarose drop technique and subsequently stained by the silver method (3) resulted in lanes with even distribution of material across their widths. Resolution was good over a wide range of molecular weights. Furthermore, when gels were scanned by laser densitometry (Zeineh, soft laser scanning densitometer, LKB Instruments), the integrated areas under the curve bore a linear relation-

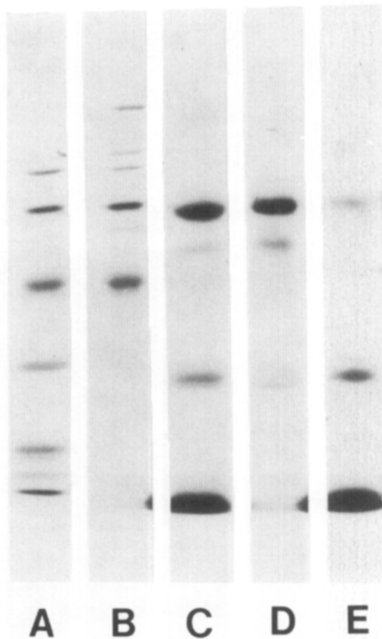

FIG. 2. Analytical gel run using the agarose drop method of loading. Lanes A and B contain protein standards (Bio-Rad Laboratories, Richmond, Calif.). Lane A from top: phosphorylase *b* (M_{-1} 92,500); BSA (66,200); ovalbumin (45,000); carbonic anhydrase (31,000); soybean trypsin inhibitor (21,500); lysozyme (14,400). Lane B from top: myosin (200,000); β-galactosidase (116,250); phosphorylase *b*; BSA; ovalbumin. Lane C, human blood (1 nl). Lane D, human plasma (1 nl). Lane E, human erythrocytes equivalent to 1 nl human blood.

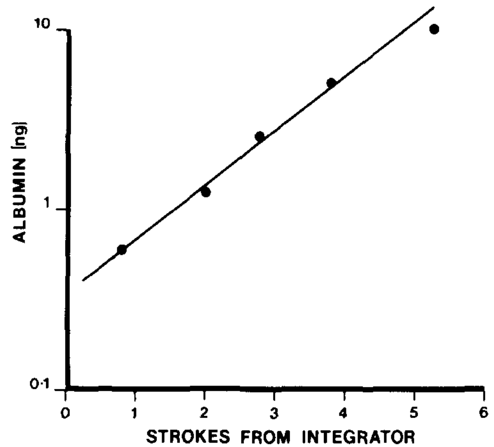

FIG. 3. Laser densitometric analysis of 0.6 to 10 ng of BSA loaded onto a thin gel as described under Methods. A linear slope was reproducibly obtained, indicating that the agarose drop method of loading small volumes (1-2 μl) onto thin gels was quantitative.

ship to the amount of protein loaded onto the gel (Fig. 3). However, the area under the curve was different for the same weight of different proteins (e.g., albumin, actin, and hemoglobin). Furthermore, the dose-response slope was different for different proteins, but was linear for each of the above proteins over the range 1-1000 ng/lane. Therefore, so long as a standard curve for an individual protein was included in the gel, it was possible to quantitate that protein in a complex mixture of proteins.

DISCUSSION

It is sometimes necessary to work quantitatively with small volumes (1-2 μl) of fluid containing nanogram amounts of protein. Technically, it is difficult to load such small samples onto polyacrylamide gels without losses which are difficult to measure. The advent of the silver stain (1-3) has enabled nanogram amounts of protein to be visualized. Optimal sensitivity is obtained using small thin gels. To load such gels quantitatively the agarose drop method has been devised. This proves to be simple, fast, and quantitative. Using this approach it is possible to quantitate between 1 and 1000 ng of a protein which is a major component of a complex protein mixture.

ACKNOWLEDGMENTS

The technical assistance of Ms. Bharati Kshirsagar is gratefully acknowledged. The efficient secretarial help of Ms. Catherine Corson is appreciated.

REFERENCES

1. Switzer, R. C., Merril, C. R., and Shifrin, S. (1979) *Anal. Biochem.* **98,** 231-237.
2. Merril, C. R., Switzer, R. C., and Van Keuren, M. L. (1979) *Proc. Nat. Acad. Sci. USA* **76,** 4335-4339.
3. Merril, C. R., Goldman, D., Sedman, S. A., and Ebert, M. H. (1981) *Science* **211,** 1437-1438.
4. Laemmli, U. K. (1970) *Nature (London)* **227,** 680-685.
5. Ogita, Z., and Markert, C. L. (1979) *Anal. Biochem.* **99,** 233-241.