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Note

Sample applicator for granular gel slabs: effect of sample orientation on the sensitivity of detection in isoelectric focusing

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Numerous methods have been described for sample application on gel slabs of the continuous matrix type (*e.g.* polyacrylamide)[1]. These methods are generally not suitable on slabs of the granular type (*e.g.* Sephadex), because the gels are comparatively fragile. In analytical focusing on granular layers, the sample is usually applied to the slab on the edge of a cover-glass. This is done either freehand or with the hand steadied on a platform over the gel plate[2]; either way, the procedure is tedious because the gel is easily smeared. The requirement for a steady hand becomes increasingly important when contact with the slab must be prolonged while the sample soaks in.

The applicator described in Figs. 1–3 reduces much of the tedium of this procedure and provides a steady sample application. The applicator will accomodate standard 20 × 20 cm, and 10 × 20 cm plates. In practice, the gel plate is placed under the applicator, and then the latter is shifted along the *y*-axis of the plate to the desired position. The sample rod is detached from the magnetic surface of the slide bar, and the cover-glass is loaded with sample. The sample rod is returned to the slide bar and the latter shifted along the bridge to the desired position on the *x*-axis of the gel plate.

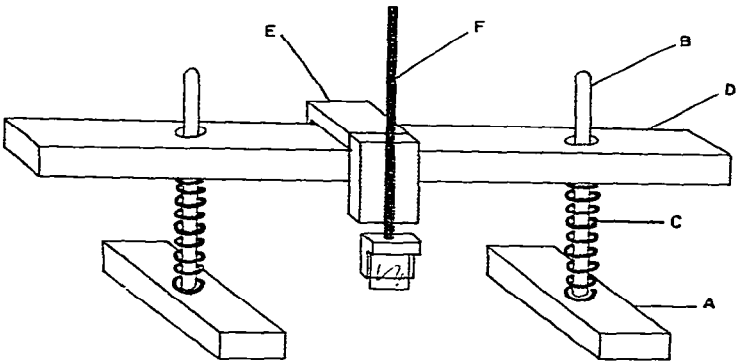

Fig. 1. Diagram of sample applicator. A, Base: lucite, or wood, 11.5 × 4.5 × 1.9 cm. A hole, ‡ in. in diameter and 1.5 cm deep, is drilled in the center of each base to receive the spindles (B): ‡ in. aluminum rods, 14 cm long. A little Pliobond cement (GC Electronics, Rockford, IL, U.S.A.) is introduced into each hole, and the spindles are tapped into position. C, Compression springs: medium strength, 2 3/4 in. long, 5/16 in. I.D., 3/8 in. O.D. D, Bridge: lucite, 36.5 × 2.0 × 2.0 cm. A hole, 5/16 in. in diameter, is drilled 5 cm from each end. E, Slide-bar (see Fig. 2). F, Sample rod (see Fig. 3).

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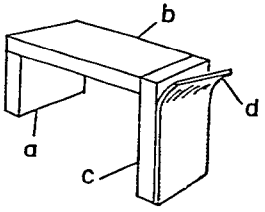
The sample rod is lowered to about 1 cm above the gel plate and then adjusted from side to side until the cover-glass is coplanar with the gel surface. As the bridge is depressed, the cover-glass is observed at eye level to insure that coplanarity is maintained. There is sufficient clearance between the moving parts of the applicator to permit small adjustments in each plane as the bridge is depressed.



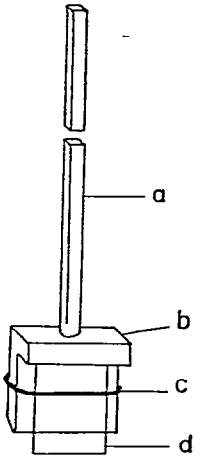


Fig. 2. Diagram of slide-bar. The following components are cut from ¼ in. lucite sheet, and glued together with lucite cement. a, 2.0 × 2.0 cm; b, 3.0 × 2.0 cm; c, 5.8 × 2.0 cm. After the components are assembled, a piece of adhesive magnetic strip d (Walbead, New York, NY, U.S.A.) is cut to size and fixed to component c, as shown in the figure.

Fig. 3. Diagram of sample rod. a, Adjusting rod: 1/8-in. steel rod, square cross-section, 14 cm long. b, Cover-glass holder: lucite, ¼-in. sheet, 2.2 × 1.9 cm. The lower 1.0 cm of one face is recessed to a depth of 2 mm, as shown in the figure. A hole, 1.8 in. in diameter and 6 mm deep, is drilled in the top. Six mm of one end of the ajusting rod is filed round and inserted into the hole with a little Pliobond cement; one face of (b) should be parallel with one face of the rod. A rubber band (c) holds a cover-glass (d) to the cover-glass holder. Cover-glasses (18 × 18 mm, or 20 × 20 mm) are easily slipped under the rubber band, as required. The sample rod is held firmly to the slide-bar by the magnetic strip, and can be raised and lowered, rotated around its axis, and pivoted from side to side, without danger of falling.

The sample rod is capable of rotation around its long axis, which permits sample application in either of two directions perpendicular to each other. This is of some advantage in the electrofocusing of dilute samples, owing to the concentrating effect achieved when the sample is applied in a line parallel with the electric field (y-axis).

Fig. 4 shows the effect of sample orientation on protein detectability. The protein used in this study was β-lactoglobulin, 1% stock solution in water. Decreasing concentrations of protein were electrofocused on 10 × 20 cm plates containing 16 g of gel slurry of the following composition: Sephadex G-75 (superfine) 5%, and carrier ampholyte (Servalyt, pH 2 to 11) 3% (w/v). Samples were applied with 18 × 18 mm cover-glasses oriented either perpendicular or parallel to the electric field. Plates were run at constant voltage at 4°C for 16 h at 200 V, followed by 4 h at 850 V. Paper prints of focused plates were made as described by Radola[3]; they were stained with Coomassie brilliant blue G-250. The photograph shows three paper prints, each with four sample tracks. With perpendicular application the smallest

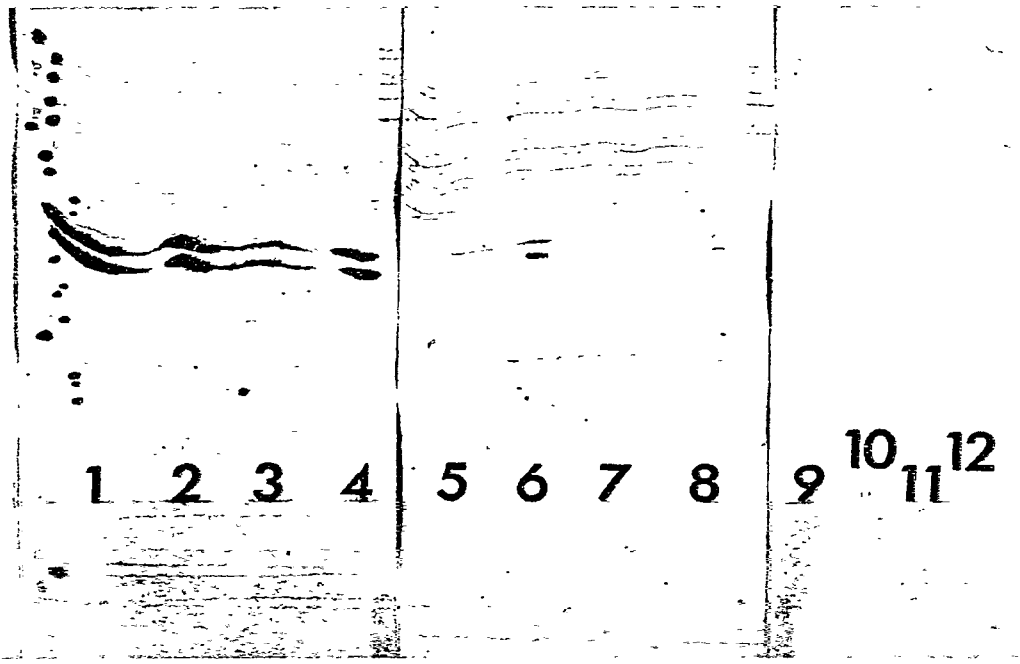


Fig. 4. Effect of sample orientation on protein detection ability. See text for details. Numbers refer to sample track. After each number is listed, in order: the sample concentration (g%), the sample volume, and the direction of application. 1 = 1%, 5 μl, perpendicular (perp); 2 = 1%, 5 μl, parallel (para); 3 = 0.5%, 5 μl, perp; 4 = 0.5%, 5 μl, para; 5 = 0.1%, 10 μl, perp; 6 = 0.1%, 10 μl, para; 7 = 0.1%, 5 μl, perp; 8 = 0.1%, 5 μl, para; 9 = 0.1%, 5 μl, para; 10 = 0.05%, 10 μl, para; 11 = 0.05%, 5 μl, para; 12 = 0.01%, 10 μl, para.

amount of protein detectable is 10 μg, on track 5. With parallel application the smallest amount is 2.5 μg, in track 11. Horizontal pencil lines on the prints, and dark spots on the first print (left), are location guides which were utilized in unrelated experiments and should be disregarded.

As seen in Fig. 4, a four-fold increase in sensitivity of sample detection is achieved with parallel compared with perpendicular application. In the former arrangement the sample is concentrated into a narrower area than in the latter arrangement.

A further advantage of parallel sample application is that a larger number of samples can be run on a given plate: twice as many samples may be accomodated with parallel compared with perpendicular application.

In parallel application, samples should be placed as far away as possible from the expected focusing site, because the application slit sometimes distorts the focused sample bands.

ACKNOWLEDGEMENTS

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