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Preparative Electrophoresis in a Concentrated Polymer Solution: Automated Procedure for Microsome Isolation

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A recently introduced automated gel electrophoresis apparatus with an intermittent scanning of the migration path, the HPGE 1000 apparatus (LabIntelligence, Belmont, CA) has a unique preparative feature: A recovery cup can be automatically positioned on the band of interest, which is then electroeluted with fluorescence monitoring of recovery. Electroelution time is extended stepwise until quantitative recovery is attained. This preparative procedure has been extended from electrophoresis in gels to that of subcellular-sized particles in concentrated polymer solutions (Chang *et al.*, *Electrophoresis* 17, 776–780, 1996). However, that application required manual rather than computer-directed positioning of the recovery chamber over the band. The present report details a modification of instrumentation and procedure by which automated operation was restored. The modified procedure extends the benefits of automation and known quantitative recovery to isolation of particles too large to enter into gels.

Electrophoresis in polymer solutions has extended to subcellular-sized particles the benefit of gel electrophoresis of being able to separate species on the basis of differences in size, shape, and conformation ("molecular sieving") (Table 1 of Ref. (1)). However, by contrast to relatively small macromolecules (with Stokes' radii less than 15–20 nm), the size of larger particles is not simply related to the decrease of mobility in polymer solution, but retardation becomes a function of shear rate (mobility × field strength/radius) (2). Preparative electrophoresis in polymer solutions by use of capillary apparatus under the conditions previously described (2) was not feasible in view of the essentially analytical nature of such apparatus. It became possible subsequently to a previous demonstration that an automated gel electrophoresis apparatus (HPGE 1000, LabIntelligence, Belmont, CA) (3) which has preparative capability (3, 4) was suitable for the electrophoresis in concentrated polymer solutions as well (5). Preparative electrophoresis in that apparatus has the unique and unprecedented advantage that the recovery of the isolated band can be monitored quantitatively, and therefore that quantitative recovery can be achieved in all applications by the requisite number of sequential recovery steps (3). However, the first preparative application of the HPGE 1000 apparatus to subcellular-sized particle electrophoresis in concentrated polymer solution sacrificed the automated location of the band to be isolated, reverting to a visual detection (6). The present report remedies that previous defect, restoring the automated detection of the band to be isolated by appropriate modifications of instrumentation and procedure. The isolation of the rat liver microsome was selected as a representative example for subcellular-sized particle isolations, since the microsome had been the particle originally isolated by electrophoresis in concentrated polymer solution, using the nonautomated HPGE 1000 procedure (6).

MATERIALS AND METHODS

Fluorescently labeled rat liver microsomes were obtained as described previously (6, 7). The sample volume was 5 μl providing a relative fluorescence intensity of 18,277 units, sufficient to attain a peak height of 90% of the scale of the HPGE 1000 graphic output (Fig. 3), and containing 7 μg protein determined as described previously and 10.4 μg of microsome (9). Polyvinylpyrrolidone (PVP)[1] was obtained and solutions were pre-

[1] Abbreviation used: PVP, polyvinylpyrrolidone.

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pared as described previously (5, 6). Hepes–Cl buffer, pH 7.0, was prepared as described (6) and used as the gel buffer, in the electrolyte reservoirs and in the gel bridges ("legs") connecting the polymer solution and those reservoirs.

The commercial automated gel electrophoresis apparatus with intermittent fluorescence scanning and preparative capacity, the HPGE 1000 apparatus (LabIntelligence), was obtained and applied to electrophoresis in PVP solution as described (6) except that the legs connecting the gel with the electrolyte reservoirs were filled with polyacrylamide of 10% *T*, 4% *C*(Bis).

Three instrumental modifications were made on the HPGE 1000 apparatus: (i) The sample cup insert holding a thin Teflon tube (Fig. 1A, 1 and 2) in the cup was constructed and the arms holding the sample cup in the apparatus (Fig. 1B, 7) were adjustably elongated so that the bottom edge of the cup reached 0.5 mm into the polymer solution of 3 mm height (Fig. 2). (ii) The syringe (Fig. 1D, 8) by which the band of interest is withdrawn was connected to the insert by a thin Teflon tube (Fig. 1C, 3 and 2) of sufficient length to allow the sample cup to occupy any position along the migration path. (iii) A hole was drilled into the cover of the HPGE 1000 apparatus to allow for the tube connection between syringe and sample cup; the hole was covered by mounting the syringe solidly in a frame on top of the instrument cover (Fig. 1D). These instrumental modifications allowed one to position the sample cup over the band of interest automatically while the instrument cover was closed, and to withdraw the band by syringe while the sample cup was automatically placed into the position above and in contact with the band.

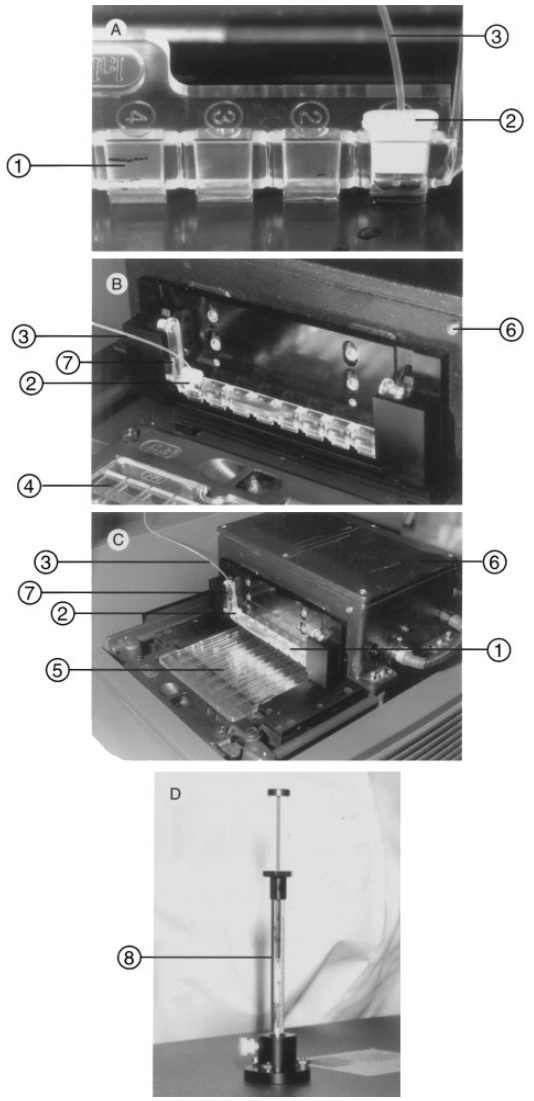

FIG. 1. Instrumental modifications of the HPGE 1000 apparatus for the purpose of its application to the isolation of bands from electrophoresis in concentrated polymer solution. (A) Tube connection between sample applicator (6) and 2.5-ml syringe. A Teflon sleeve holds the tube in the sample cup. (B) Shortened arm of the sample applicator array. (C) Sample cup positioned above the band of interest. (D) Syringe mounted on the apparatus cover. The syringe holder prevents light entry through the hole in the cover through which the tube connection between syringe and sample cup is made. Part designations: 1, sample applicator used as a cup for band recovery ("sample cup"); 2, sample cup insert made of silicone rubber, with a central hole through which tubing (part 3) passes from the syringe (part 8) into the 50 μl elution buffer held by capillary adhesion in the bottom flange of the sample cup (see schematic in Fig. 2); 3, Teflon tubing, 1 mm o.d.; 4, gel bridge between polymer solution and electrolyte reservoirs (leg (6)); 5, separation cell containing the polymer solution in 8 lanes; 6, mobile detector carriage; 7, support for array of 8 sample applicators; 8, 2.5-ml gas-tight syringe, connected to tubing (part 3).

RESULTS AND DISCUSSION

An exterior lane, and the lane adjacent to it, of the cell (Fig. 1C) of the HPGE 1000 apparatus were filled with 18% PVP solution in Hepes–Cl buffer to a height of 3 mm (9 ml). Fluorescently labeled rat liver microsome (5 μl in 12.5 μl Hepes–Cl buffer) was loaded by manual pipetting to a depth of 2 mm into the PVP solution in the position of the sample slot (6.5 mm from the cathodic end of the lane).

Electrophoresis was carried out at 15 V/cm, 5°C. A representative scan of fluorescence intensity after 25 min of electrophoresis is shown in Fig. 3A. Electrophoresis was interrupted, and the sample cup, filled with 50 μl Hepes–Cl buffer (held in the cup by capillary adhesion) and connected to the syringe by a narrow Teflon tube (Figs. 1A and 2), was lowered onto the position of the microsome band, steered by the software of the apparatus, and 60 μl was withdrawn into the tube by the syringe (Fig. 1D). The electrophoresis was resumed, and the scan shown in Fig. 3B was obtained immediately. The numerical output associated with Figs. 3A and 3B (not shown) states that the ratio of relative fluorescence units between peak 3 of Fig. 3A and peak 2 of Fig. 3B is 17,241/686, corresponding to a 96% recovery.

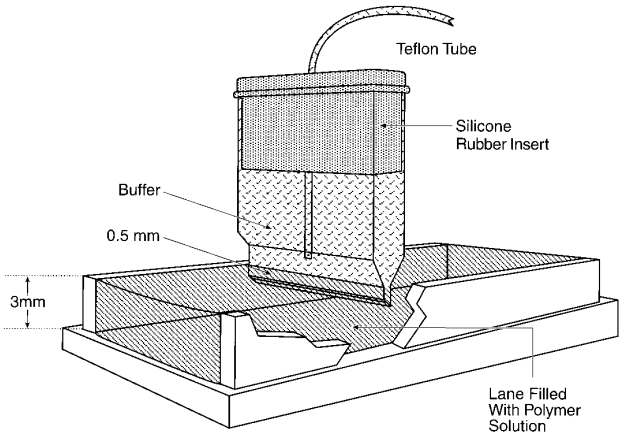

FIG. 2. Schematic diagram of the sample cup for volumetric withdrawal from concentrated polymer solutions. One of eight lanes filled with polymer solution to a height of 3 mm is shown in contact with a sample cup superimposed on the position of the band of interest under control of the HPGE 1000 apparatus software. The narrow bottom of the sample cup is filled with 50 μl of buffer through and from which the polymer solution containing the band is withdrawn by a syringe (Fig. 1D) connected to the sample cup by a 1-mm o.d. Teflon tubing.

The 96% reduction of relative fluorescence intensity after volumetric withdrawal of 60 μl from the position of the major microsome peak is interpreted as its isolation in view of the previous evidence for recovery of microsome fractions [weight of lyophilized product, its protein content, and SDS–PAGE pattern (9)].

The representative isolation of the microsome band in a single step with a 96% yield demonstrates the potential of the preparative method for the biochemistry and biology of subcellular-sized particles. However, the instrumental and procedural capacity to isolate a single band is not to be confused with the resolving capacity of a concentrated polymer solution, or specifically that of PVP. The available data (e.g., Fig. 5 of Ref. (6)) indicate that these solutions act as effective anticonvective media for separations based on mobility differences, but do not by themselves exert molecular sieving effects (concentration-dependent size separations) in contrast to dilute polymer solutions which are able to resolve microsome components on the basis of size differences (Fig. 2 of Ref. (7)). This suggested the application of the automated isolation procedure to electrophoretic systems in density stabilized dilute

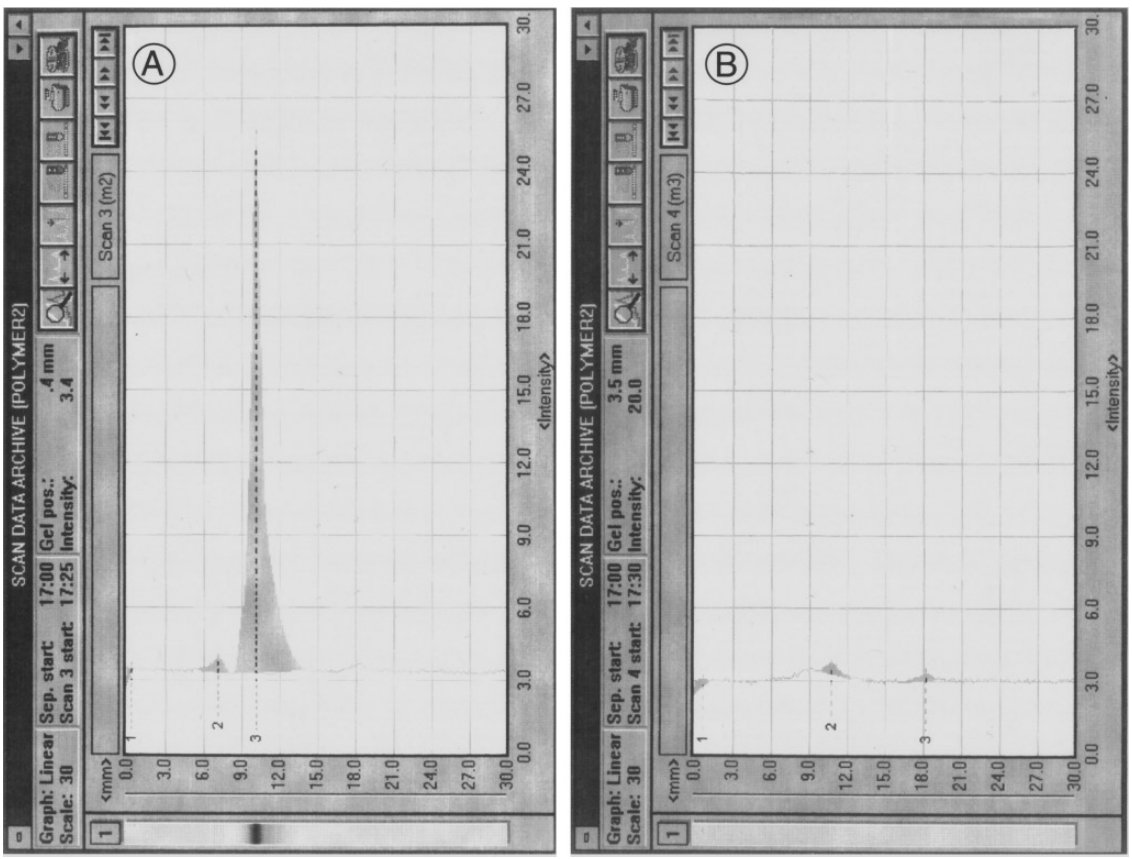

FIG. 3. Scan of fluorescence intensity along the migration path. (A) Microsome band scanned after 25 min of electrophoresis (15 V/cm, 5°C). (B) Scan of the migration path after withdrawal of 60 μl from the position of the microsome band. Peak 2 is the residual microsome band.

polymer solutions in order to achieve isolations of large particles by exploiting separations which rest predominantly on differences in size and shape. However, that application proved possible even in the absence of density stabilization by a rigid control of spatial relations between the surface of the dilute polymer solution, the degree of insertion of the elution cup into it, and the distance between the end of the elution tube and the bottom of the elution cup (10).

Since in dilute polymer solution the microsome preparation resolves into three components (7, 10), the preparative application (10) also answered the question in the affirmative whether a sequential isolation of a number of bands from a polymer solution is feasible, as it is in the isolation of proteins and DNA from mixtures by a similar procedure applied to gels, using electroelution in lieu of volumetric withdrawal (8).

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