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Note

Versatile modular microelectrophoresis system and the simultaneous preparation of large numbers of polyacrylamide gels[a]

J. SUTTNAR, J. E. DYR*, J. PRISTACH and K. HOLADA

Institute of Hematology and Blood Transfusion, Prague (Czechoslovakia)

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Many laboratories are equipped with conventional instruments for one- and two-dimensional polyacrylamide gel electrophoresis (PAGE), which utilize slab gels of *ca.* 10 × 10 cm and guarantee high-resolution separations. Modern chromatographic systems can separate proteins in 1 h or less, and provide numerous separated fractions [1,2]. The use of conventional electrophoretic apparatus to analyse these fractions is time-consuming and expensive. By using small gels (50 × 43 mm) and the highly sophisticated PhastSystem (Pharmacia, Uppsala, Sweden) with programmed running conditions and automated staining procedures, the results can be obtained within 25–75 min depending on the gel type and staining technique [3].

This paper describes an adaptation of the commercial Pharmacia electrophoretic system for miniature electrophoretic applications.

EXPERIMENTAL

Pharmacia apparatus GE-2/4 (Uppsala, Sweden) was adapted to accommodate up to eight gels measuring 75 × 25 × 0.5 mm (Fig. 1). The electrophoresis power supply LKB 2301 Macrodrive 1 (Bromma, Sweden) was used. The cassettes were prepared according to instructions for assembling cassettes provided with the Pharmacia gel cassette kits, using microscopic slides (75 × 25 × 1 mm) and spacers (25 × 5 × 0.5 mm) made from polystyrene sheet.

The cassettes were placed in the gel slab casting apparatus (Fig. 2), and the exponential acrylamide gradient was created using LKB 2001-500 gradient gel former. The solutions for the preparation of the gel and the buffers for electro-

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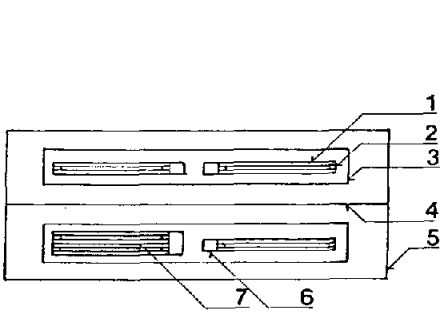

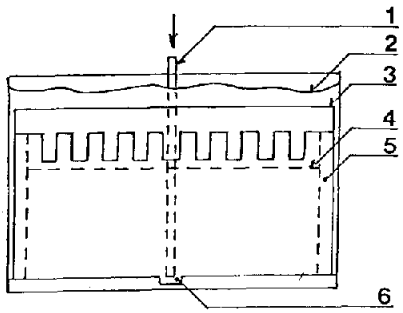

Fig. 1. Upper buffer vessel of the gel electrophoresis apparatus GE-2/4 with inserted microcassettes (top view): 1 = microcassette; 2 = spacer (0.5-mm polystyrene sheet); 3 = gasket; 4 = platinum electrode; 5 = upper buffer vessel; 6 = sealing of microcassette (Pharmacia spacer, 2.7 mm thick); 7 = polyester sheet (0.05 mm thick).

Fig. 2. Gel slab casting apparatus (front view): 1 = inlet of gradient of acrylamide; 2 = level of stacking gel; 3 = comb (0.5-mm polystyrene sheet); 4 = level of resolving gel; 5 = spacer (0.5-mm polystyrene sheet); 6 = groove.

phoresis were prepared according to LKB Application Note 320 [4]. A polyacrylamide gel of concentration 3% in buffer of pH 6.8 was used as the stacking gel. Combs made from polystyrene sheet (0.5 mm) were used as well formers (twelve positions) for sample application (1–5 μl).

Electrophoresis was carried out at 12°C in a discontinuous buffer system of pH 8.8 according to Laemmli [5], using a 40-mA constant current for 30–40 min for four gels. The gels were stained at 80°C for 10 min in 0.25% of Coomassie Brilliant Blue R-250 (Serva, Heidelberg, F.R.G.) in ethanol–10% acetic acid (1:1). Destaining was performed in 5% acetic acid with decreasing amounts of ethanol (40–20%, v/v) for *ca.* 1 h.

Destained gels, together with two cellophane sheets, were immersed in 2.5% glycerol with 25% methanol in water for 1 h. The gel, wrapped in the two cellophane sheets, was put into a frame and allowed to dry overnight at room temperature.

RESULTS AND DISCUSSION

Fig. 3A shows the result of microelectrophoretic examination of the fractions of prothrombin complex proteins separated on Sephacryle S-200. An electropherogram of a complex mixture of proteins of human and rat blood platelets is shown at Fig. 3B.

The lower limit for sample concentration depends on the volume of the sample applied (maximum 5 μl) and on the sensitivity of the detection technique used to develop the gel. Generally, the sample must contain at least 50–100 ng of each protein for Coomassie Blue staining and at least 1–5 ng of each protein for silver

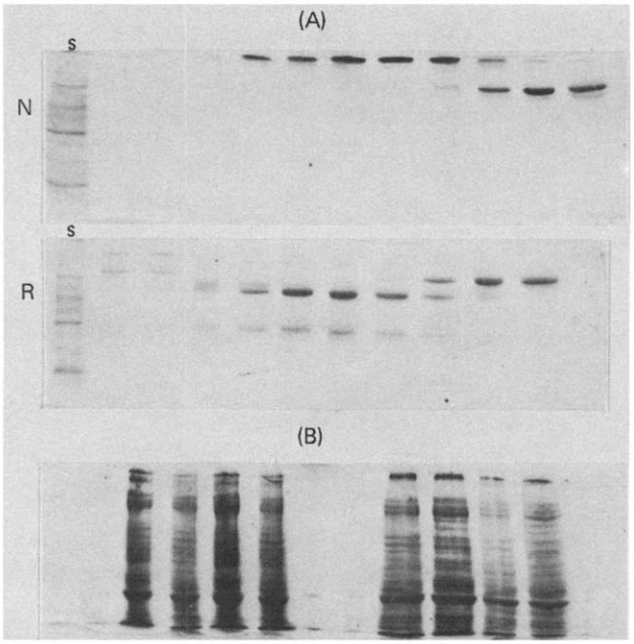

Fig. 3. Micro-sodium dodecyl sulphate PAGE. (A) Chromatographic fractions of prothrombin complex proteins separated on Sephacryl S-200: N = non-reduced samples; R = reduced samples; S = calibration proteins of M_r (kDa) 94.0, 67.0, 43.0, 30.0, 20.1 and 14.4; gradient, 10–15% of acrylamide. (B) Proteins of human and rat blood platelets; gradient, 5–10% of acrylamide.

staining (according to Oakley *et al.*) [6]. Fast protein staining is achieved by the combination of thin gels (0.5 mm) and elevated temperature.

For semi-dry electrophoretic blotting to a nitrocellulose membrane, the following conditions were found to be satisfactory: 1 h; 15 V/cm; 0.025 *M* Tris (pH 10.4); 20% methanol.

Gels can be stored for two days in a wet chamber at 4°C prior to electrophoresis without any change in the separation efficiency.

In conclusion, the modified system allows complete analysis of up to 96 samples with nanogram sensitivity in only 3–4 h. The microgel technique is compatible with most acrylamide-based systems and staining protocols in common use. Furthermore, microgels are more resistant to tearing when wet and to cracking while drying. Gels wrapped in sheets of cellophane and handled as described are stable for a long time, and may be used directly as transparencies for an overhead projector. The procedure is inexpensive and reliable, and requires only slight modifications of standard apparatus.

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