Journal of Chromatography, 238 (1982) 226–231
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 14,653

Note

Apparatus for the recovery of proteins from polyacrylamide gels during electrophoresis

M. FAUPEL*, M. DE GASPARO and E. VON ARX
Chemical and Biological Research Laboratories, Pharmaceuticals Division, Ciba-Geigy Limited, CH-4002 Basle (Switzerland)

(Received December 15th, 1981)

Disc electrophoresis is used extensively[1] as a sensitive technique for the analysis of biological materials. To overcome the major problem associated with this technique, *viz.*, the elution of separated compounds from the gel, we have devised a disc electrophoresis elution apparatus that permits the recovery, during electrophoresis, of materials which are then readily available for further characterization. We have demonstrated its efficiency in the separation of the two insulins of rat pancreas extracts.

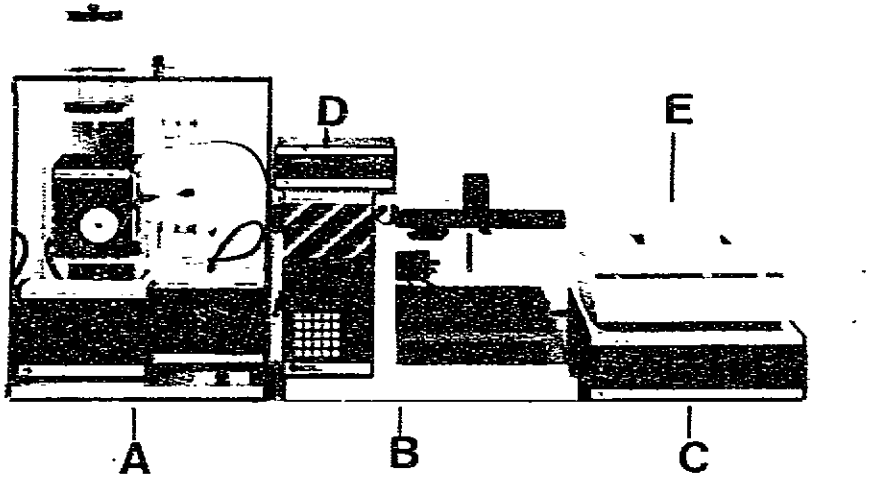

Fig. 1. (A) Separation unit detailed in Figs. 3 and 4; (B) fraction collector; (C) electrophoresis constant power supply; (D) volt-hour integrator; (E) Hewlett-Packard recorder.

0021-9673/82/0000-0000/$02.75

EXPERIMENTAL

Chemicals

All chemicals were of analytical-reagent grade from Fluka (Buchs, Switzerland). Rat insulins were obtained from the Novo Research Institute (Bagswerd, Denmark).

Apparatus

The disc electrophoresis apparatus (Fig. 1) consists of a glass tube (Fig. 2) inserted in two buffer chambers containing the electrodes (Fig. 3) and connected to a peristaltic pump (Pharmacia P.1), a buffer reservoir and a detection system consisting of an optical unit (Pharmacia UV-1 single path monitor, a control unit (Fig. 4) (Pharmacia single path monitor), a recorder (Hewlett-Packard 3390A integrator) and a fraction collector (Pharmacia FRAC-300). The electrophoresis constant-power

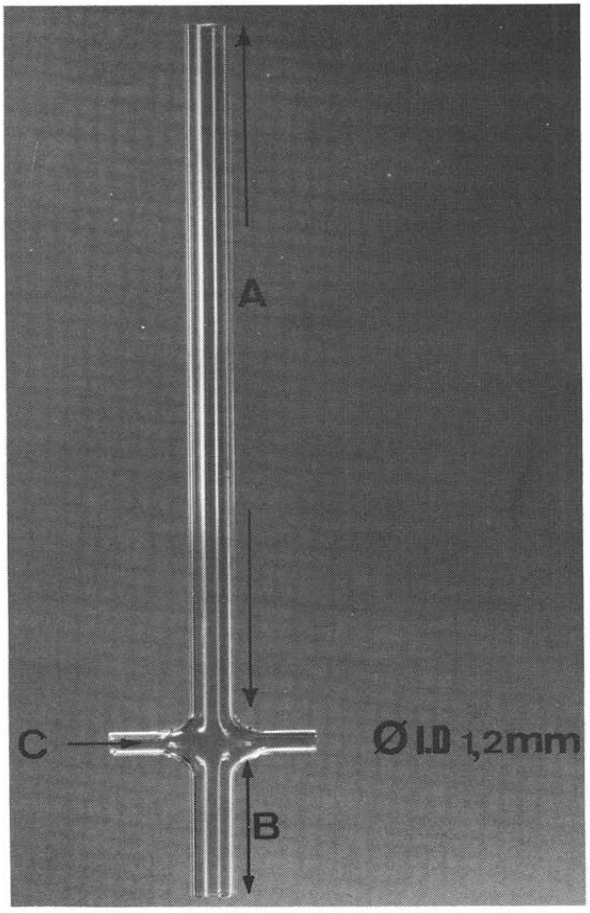

Fig. 2. Glass tube. (A) Upper part (containing the "separating gel"); (B) lower part (containing the "plug gel"); (C) elution channel.

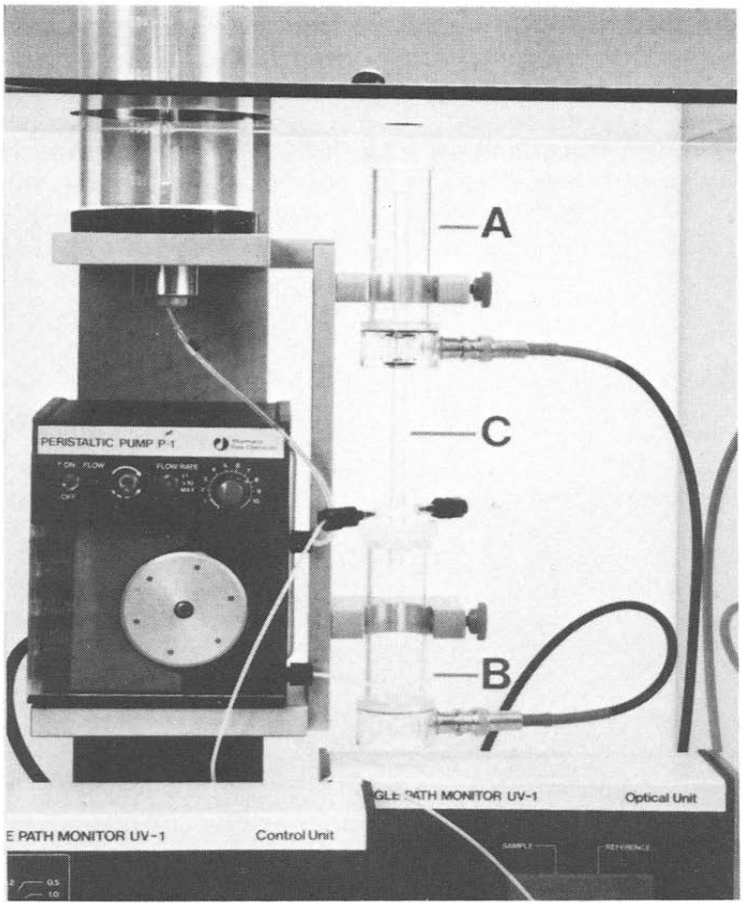

Fig. 3. (A) Upper electrode chamber; (B) lower electrode chamber; (C) glass tube.

supply (Pharmacia ECPS 3000 150) was connected to a volt–hour integrator (Pharmacia VH-1).

Polyacrylamide gels

The lower part of the tube was sealed with Parafilm foil and filled to a height of 2.5 cm with plug gel consisting of 40 % acrylamide, 0.02 % bisacrylamide and 0.375 *M* Tris–HCl (pH 8.3), which was polymerized with 9 μl of N,N,N′,N′-tetramethylethylenediamine (TEMED) and 30 μl of 10 % ammonium persulphate solution. The gel was overlayed with circulating buffer, which was removed after polymerization. The elution channel (Fig. 2A) was filled with a mixture of 50 % glycerol in circulating buffer. The separating gel, consisting of three gels as described by Rall *et al.*[2], was introduced and overlayed with pH 8.3 buffer during polymerization. The channel solution was finally washed out with elution buffer.

Electrophoresis conditions

A 100-μg amount of Novo rat insulin was dissolved in 50 μl of pH 8.3 buffer

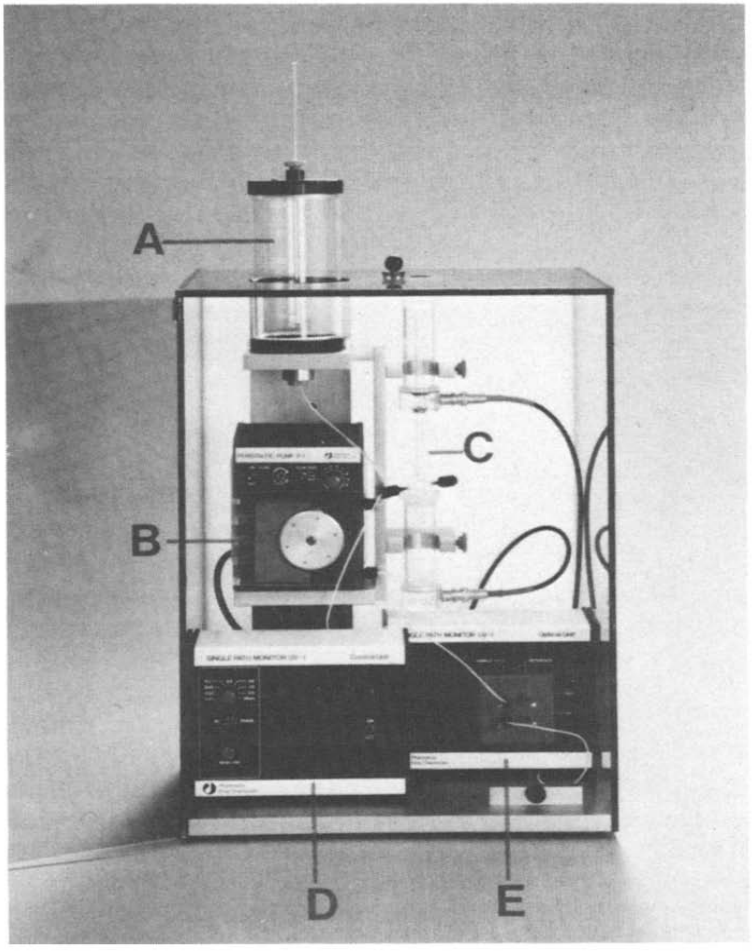

Fig. 4. (A) Buffer reservoir; (B) peristaltic pump; (C) glass tube; (D) control unit; (E) optical unit.

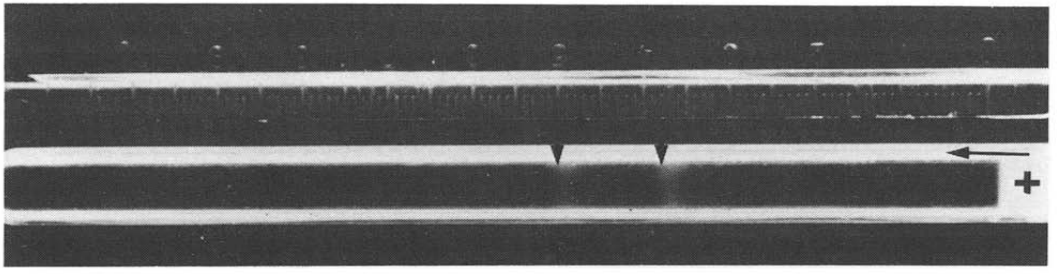

Fig. 5. Disc electrophoretic separation of the two insulins' bands precipitated with 12.5% TCA using the method of Rall *et al.*[2].

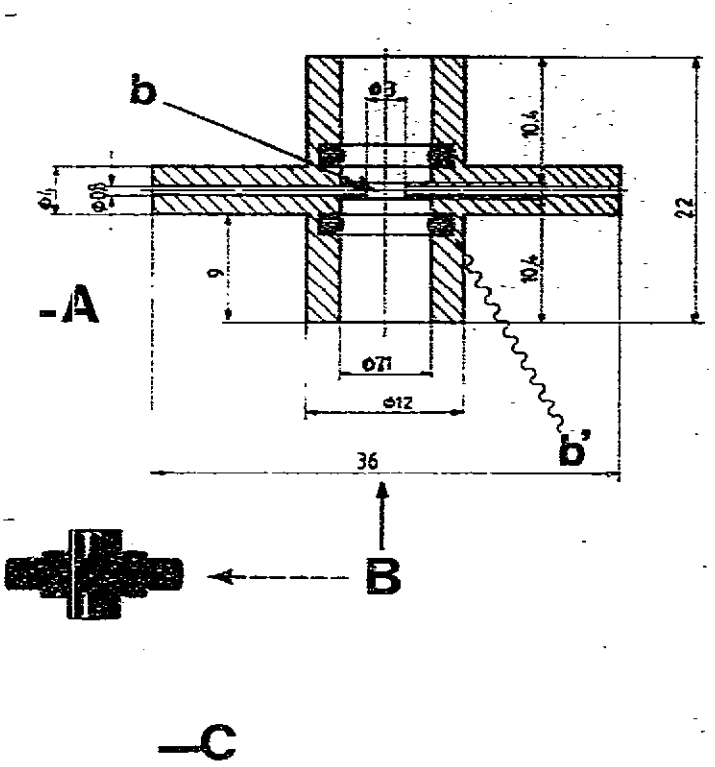

Fig. 6. Fine calibre cell. (A) Standard borosilicate glass tube (containing the separating gel); (B) detailed calibre cell, (b) elution channel and (b') O-ring; (C) borosilicate glass tube (containing the plug gel).

and applied with a Hamilton syringe on top of the gel (cathode side). This solution was overlayed with 0.01% bromophenol blue solution containing 6 *M* urea (50 µl). The degassed electrode buffer and the degassed circulating buffer respectively contained a 10% and 5% solution of 6.0 g of Tris and 28.8 g of glycine in 1 l of water. Electrophoresis was carried out at 2 mA for 10 h.

RESULTS AND DISCUSSION

Fig. 5 shows the disc electrophoretic separation of the two insulins' bands precipitated with 12.5% trichloroacetic acid (TCA) in a separated gel (4 h running time) and demonstrates the excellent reproducibility of the work of Rall *et al.*[2].

The blue band of bromophenol blue was eluted after 4 h. From experience, we know that the first band of insulin reaches the elution channel after 8 h. The UV photometer was then started and 1-ml fractions were collected at 254 nm. The second band was collected in the same way. The eluted fractions were analysed, after separation of buffer on Pharmacia PD-10 columns and concentration, by high-performance

thin-layer chromatography on cellulose (Merck) with 1-butanol–pyridine–water–acetic acid (34:25:12:30) as eluent. For detection, Pauly's reagent[3] and N,N,N′,N′-tetramethyl-4,4′-diaminodiphenylmethane[4] were used. This apparatus is of great potential value for the purification and recovery of proteins after isoelectric focusing[5]. A new narrow bore cell (Fig. 6) has been developed to optimize its use for establishing the biosynthesis and secretion of mutant (functionally defective) insulins responsible for insulinopathies[6–9].

ACKNOWLEDGEMENTS

We thank M. Vernier, K. Wagner for constructing the apparatus, J. Kieffer and G. Hostetter for technical assitance and K. Kuhn for making the glass tube.

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