Journal of Chromatography, 75 (1973) 165–168

Note

CHROM. 6362

LAURELL electrophoresis on cellulose acetate

Antigen–antibody crossed electrophoresis[1] is one of the most powerful methods available for the analysis of complex protein mixtures. In the form of the technique described by CLARKE AND FREEMAN[2], the sample is first subjected to a short zone electrophoresis in a thin layer of agarose gel (first dimension). A second agarose gel, impregnated with an appropriate antiserum, is then poured alongside the original layer and the partially separated proteins are driven into it by a second electrophoresis at right angles to the first (second dimension). Each antigenically distinct constituent of the sample thus generates a precipitin peak whose position reflects the mobility of the protein in the first dimension and whose area may, in standard conditions, be related to the protein concentration.

Some 60 human serum proteins have been separated using LAURELL electrophoresis in conjunction with gel filtration[3], a number of authors have used the method in quantitative studies of serum protein concentrations[4,5], and its uses in forensic science have been discussed[6]. The routine use of the method in these and other applications may, however, be delayed by a number of disadvantages inherent in the use of agarose as the supporting medium. Considerable skill and practice are required to achieve adequate results, and an experiment may take up to 24 h excluding the preliminary preparation of the gels and the final staining and evaluation procedures. Recently described modifications of the technique[7], which also permit economies in materials, represent only a partial improvement.

Recent work in our laboratory[8,9] has shown that a number of electrophoretic separations normally performed using gel media can also be obtained using the far more convenient cellulose acetate technique, and KROLL[10] and WATKINS *et al.*[11] have shown that individual proteins can be determined by a single electro-immunodiffusion step on various types of cellulose acetate. The present paper describes the use of cellulose acetate membranes in the performance of LAURELL electrophoresis.

Experimental

Mylar-backed layers of cellulose acetate approximately 100 μ thick were used, 11 × 5.5 cm rectangles being cut from the sheets supplied (Millipore U.K. Ltd., Wembley). This rigid form of cellulose acetate was easier to handle than flexible membranes. All experiments were carried out in covered electrophoresis chambers cooled by melting ice or a continuous flow of cold water. Several buffer systems were investigated but the electrolyte finally chosen for routine use was a Tris–barbiturate buffer, pH 8.9 ("High Resolution Buffer", Gelman-Hawksley Ltd., Lancing), diluted to an ionic strength of 0.03.

Pooled normal human blood serum containing a trace of bromophenol blue (B.D.H., Ltd.) was used as the sample in all experiments. Antisera to human serum proteins were obtained from Paines and Byrne, Ltd., Greenford, the Wellcome Laboratories, Beckenham, and the Central Blood Transfusion Laboratory, Amsterdam.

Antisera to individual proteins were obtained from Behringwerke Ltd., (Hoechst Pharmaceuticals) London, and from the Central Blood Transfusion Laboratory, Amsterdam. All other reagents were AnalaR or equivalent grade.

The following procedure was developed for the analysis of serum proteins. The antiserum to be used was diluted 1:9 (v/v) with buffer and one 11-cm edge of the cellulose acetate sheet was immersed in it until antiserum had moved by capillary action to within 2 cm of the opposite edge. The sheet was then removed from the antiserum, lightly blotted and immersed at the other 11-cm edge in buffer. When the buffer had risen through the cellulose acetate to meet the antiserum boundary the sheet was again removed and blotted lightly. It was then placed horizontally in the electrophoresis chamber, connection with the electrolyte being made with two filter-paper wicks, 0.4 × 4 cm. The wicks were placed near the edge of the cellulose acetate that was antibody-free and overlapped the sheet by 1 cm at each end. 0.5 μl of the serum sample was then applied to the antibody-free portion of the cellulose acetate using a microsyringe. The point of application was at least 2 cm from the cathode wick. When the sample had soaked into the cellulose acetate, the power supply was switched on and the first dimension electrophoresis allowed to proceed at 250 V (constant) until the bromophenol blue-stained albumin zone had moved about 3 cm (approx. $1^1/_4$ h).

The power was then switched off, the polarity of the electrophoresis chamber reversed, and the cellulose acetate sheet turned through 90°. The first dimension wicks were discarded and new wicks, 10 × 6 cm, were laid on to the sheet, overlapping it by 1 cm along each long edge. The second dimension electrophoresis was then allowed to proceed for $2^1/_2$ h at 150 V (higher voltages were permissible with an ice-cooled chamber). At the end of the second dimension electrophoresis, the cellulose acetate was washed in several changes of warm saline (40–50°) for 30 min, stained in amido-black (0.2% w/v in methanol–acetic acid 9:1), washed in dilute acetic acid, and air-dried. Some sheets were rendered transparent by, a 30-sec immersion in ethyl acetate–acetic acid (3:7), and subsequent drying. Precipitin peak areas were obtained by planimeter measurements on a magnified image of the stained sheet.

Results and discussion

Fig. 1 shows a typical pattern obtained using a polyvalent antiserum to human serum proteins. The total number of precipitin peaks normally visible on the stained cellulose acetate was approximately thirty, but some of the fainter peaks were not detectable by photography. The serum protein pattern is similar in general appearance to those obtained on agarose gels: proteins of low mobility, *e.g.* γ-globulins, cannot be estimated, although the agarose technique has been modified to overcome this problem[12]. During development of the present method the artefacts described by CLARKE AND FREEMAN[2] were occasionally observed, but the experimental technique described minimised their occurrence.

In a study of the reproducibility of the method, ten separations of the same sample were performed in identical conditions and the areas of two prominent precipitin peaks (labelled A an B in Fig. 1) were measured. The coefficients of variation of these areas were found to be 14.5% and 13.5%, respectively. The reproducibility of the technique is thus rather inferior to that of the agarose method (coefficients of variation approximately 10 %) but might well be improved by the use of an internal

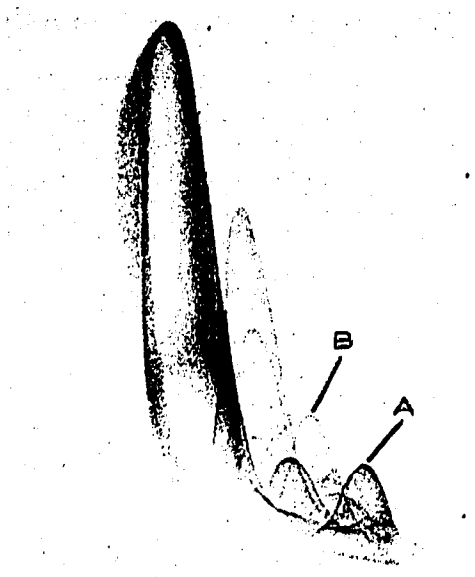

Fig. 1. LAURELL electrophoresis of normal human blood serum, using sheep anti-human serum. The areas of the peaks labelled A and B were determined (see text). The small spot near the bottom of the plate is the position of application of the sample.

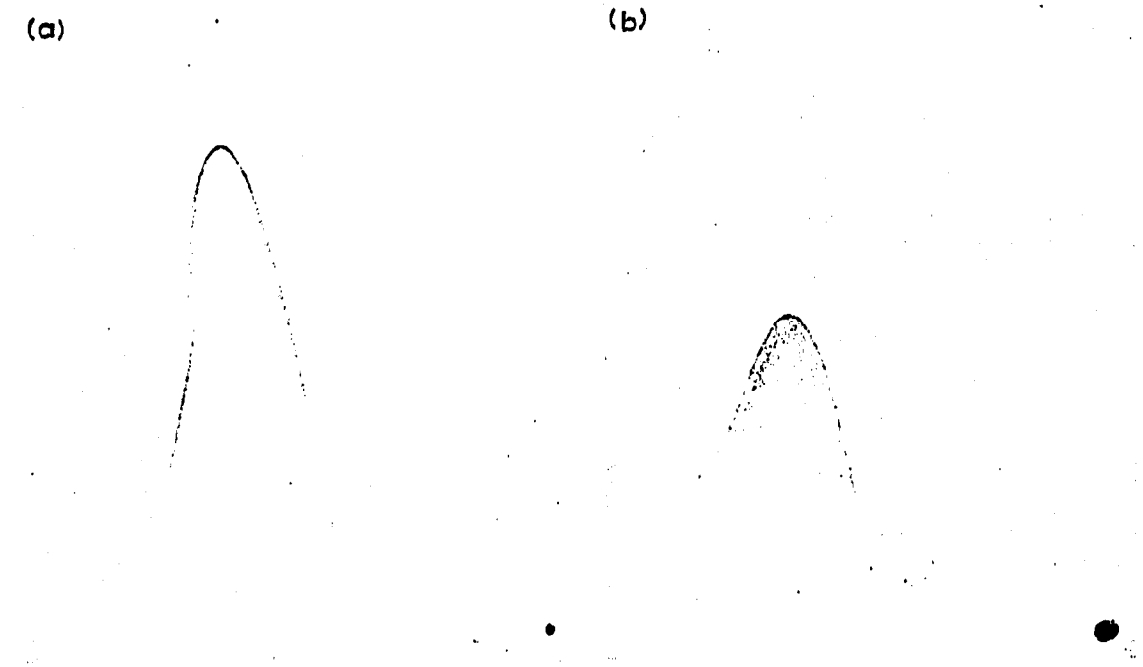

Fig. 2. LAURELL electrophoresis of normal human blood serum, using monospecific antisera to (a) transferrin, and (b) haptoglobin. The dark spot is the position of application of the sample.

standard[13] which should correct for variations in the volumes of sample and antiserum applied.

Notwithstanding this possible drawback, the advantages of the cellulose acetate method are considerable. It is a very simple and convenient technique, requiring little preliminary work. The separations are completed much more rapidly than when agarose is used, and the stained plates are more easily stored. Antiserum consumption is minimal, and the very small sample volume may be advantageous in some applications *e.g.* in forensic studies.

The availability of a simple, routinely-available form of the LAURELL method may permit its application in fields where it is not used at present. Fig. 2 shows the precipitin peaks obtained when monospecific antisera to individual plasma proteins were used, and indicates that the method will be of value in testing the specificity of antisera, and in studying the homogeneity of purified protein fractions.

It was concluded that, except perhaps where the highest resolution and precision are required, the LAURELL technique can be performed satisfactorily on cellulose acetate.

We are grateful to Dr. A. P. PHILLIPS, of the Home Office Central Research Establishment, Aldermaston, for gifts of antisera and for many stimulating discussions. We also thank Dr. P. ALLEN, of Paines and Byrne Ltd., for gifts of antisera. We valued the support and encouragement of Dr. D. THORBURN BURNS throughout the work.

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Received September 18th, 1972