Biochem. J. (1970) **116**, 147–149
Printed in Great Britain

Short Communications

A Rapid System for Preparative Electrophoresis Depending on Isoelectric Buffers of Low Conductivity

By J. R. FULLARTON and A. J. KENNY
Department of Biochemistry, University of Leeds, Leeds LS2 9LS, U.K.

(*Received* 21 *October* 1969)

One of the major disadvantages of preparative electrophoretic techniques is the time taken to achieve protein separations, particularly where labile enzymes are concerned. Schenkein, Levy & Weis (1968) have suggested that a decrease of the ionic strength of the buffer would enable the application of higher voltage gradients with lower current flow; but if this is taken too far with conventional systems the buffering capacity is low. The use of ampholytes at their isoelectric points provides an alternative approach whereby the conductivity can be decreased without undermining the buffering capacity. Ampholine carrier ampholytes (LKB-Producter A.B., Stockholm, Sweden) possess the required properties. Although these were developed for electrofocussing (Vesterberg & Svensson, 1966), it is also possible to utilize them in a dynamic electrophoresis system. Rapid separation of protein mixtures is possible and the pH gradient established by the heterogeneous carrier ampholytes helps to sharpen the protein zones. We have found that a simple ampholyte is also suitable for electrophoretic separations. Svensson (1962) lists a number of possible carrier ampholytes covering a range of isoelectric points from 2.77 to 10.76. Among those with pI values near neutrality are histidylglycine (6.81), histidylhistidine (7.30), histidine (7.47) and 1-methylhistidine (7.67). Of these histidylhistidine has the advantage that the pI and pK_a values differ by only 0.5. Histidine is less ideal in this respect, but has been used here (as the free base) because of its ready availability.

In this paper we present two examples of electrophoretic separation of an artificial mixture of proteins in a column stabilized with a sucrose density gradient. In the first Ampholine carrier ampholytes were present; in the second histidine was used. The specific conductances were determined at 18°C with a conductivity bridge standardized with 10mM-KCl. The values, in $ohm^{-1}cm^{-1}$, were as follows: 50mM-tris–HCl, pH 7.5, 2.09×10^{-3}; 1% (w/v) Ampholine pH 5–8 carrier ampholyte, 1.20×10^{-4}; 1% (w/v) histidine (free base), 5.98×10^{-5}. The isoelectric buffers possessed conductances that were lower, by at least tenfold, than a 'conventional' buffer system.

Electrophoresis of a mixture containing haemoglobin, serum albumin and an impure fungal glucose oxidase (*EC* 1.1.3.4) *in a sucrose density gradient.* (*a*) Ampholine carrier ampholytes. The apparatus consisted of a water-cooled electrofocussing column (LKB model 8101 electrofocussing apparatus) in which 120ml of a 0–45% (w/v) sucrose density gradient containing 1g of Ampholine pH 5–8 carrier ampholytes was set up over an anode solution containing 1% (v/v) sulphuric acid in 55% (w/v) sucrose. A two-chamber linear-gradient mixing device was used to set up the gradient (Haglund, 1967). A discontinuity was established near the top of the column by stopping the flow from the mixer about 20ml from completion (at which point the concentration of sucrose was 10%, w/v). The column was completed by layering on 20ml of Ampholine carrier ampholytes in 2% (w/v) sucrose. Finally, 10ml of aq. 2% (v/v) ethanolamine was layered on to cover the cathode. The effective height of the column was 24cm.

The protein sample was introduced through a side arm of the apparatus, by using a length of flexible tubing, to the point of discontinuity in the sucrose density gradient near the top of the column. By twice rotating the inner water jacket relative to the outer jacket, with the apparatus absolutely vertical, the sample was distributed as a narrow disc within the annular electrophoresis chamber. The criteria for zone stability in sucrose-density-gradient systems, as outlined by Svensson (1960, pp. 197–206), were taken into account.

The run was started at 200V and the voltage was increased stepwise as the conductivity decreased, ensuring that at no time did the power exceed 1W. The final running voltage of 600V was reached after 1h, and after 3.75h, when the unbound Bromophenol Blue had migrated 20cm towards the lower limit of the buffered zone, the current was switched off. Although the apparatus used was designed for electrofocussing, it should be noted that electrofocussing as such was not carried out, since the proteins never reached their isoelectric points. The positions of the components of the mixture at the end of the run are shown in Fig. 1(*a*).

The lower anode and upper cathode solutions absorbed strongly at 280nm owing to the presence of artifacts of electrolytic origin. The two main protein

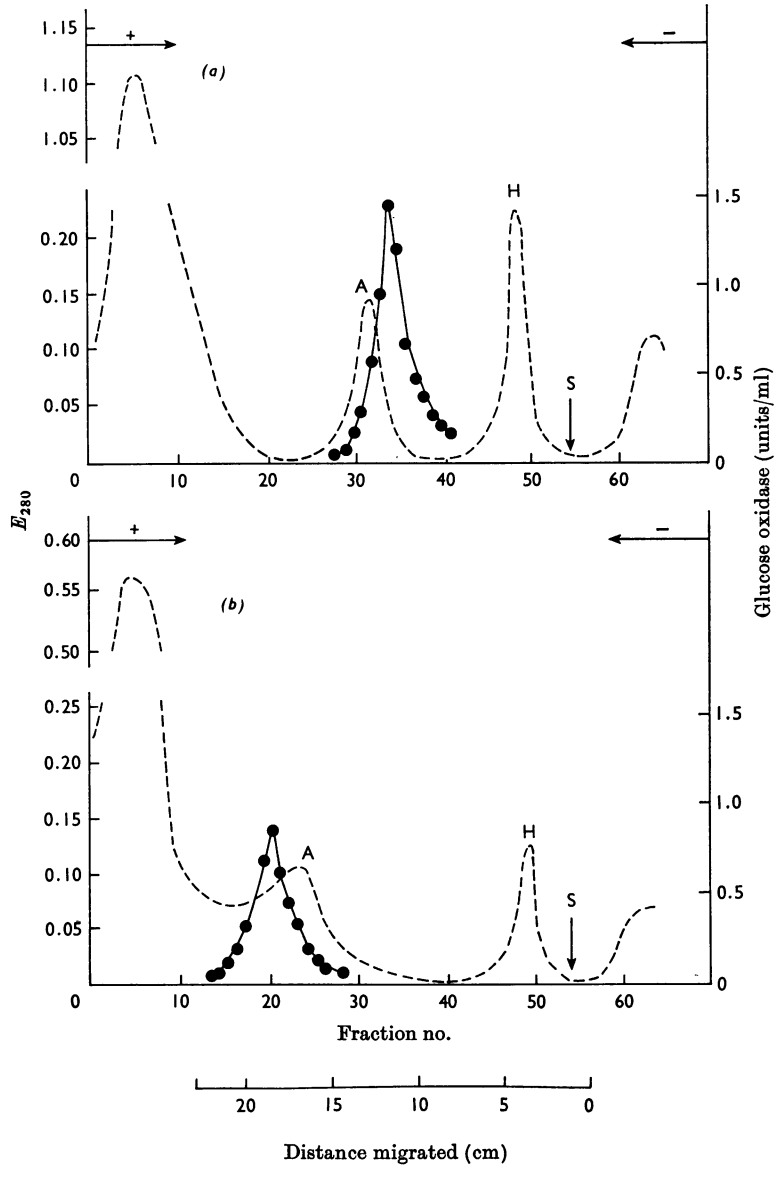

Fig. 1. Electrophoresis of a mixture of albumin, haemoglobin and a fungal glucose oxidase preparation in isoelectric buffer systems stabilized by sucrose density gradients. (*a*) Ampholine pH 5–8 carrier ampholytes, set up as described in the text. The sample (volume 1 ml) contained 1.4 mg of crystalline bovine serum albumin (BDH Chemicals Ltd., Poole, Dorset, U.K.), 1.9 mg of crystalline bovine haemoglobin [Sigma (London) Chemical Co. Ltd., London S.W.6, U.K.)] and 4 mg (16 units) of fungal glucose oxidase [Sigma (London) Chemical Co. Ltd.] dissolved in 5% (w/v) sucrose containing 1 drop of 0.01% Bromophenol Blue. The duration of the run was 3.75 h. Fractions (2.25 ml) were collected by pumping out the chamber with a peristaltic pump at the rate of 2 ml/min. (*b*) Histidine gradient (1–2%, w/v). The duration of the run was 3.0 h. Other details were as for (*a*), except that the sample contained 1.1 mg of haemoglobin and 3.4 mg (13.5 units) of glucose oxidase. The extinction at 280 nm (-----) of fractions was measured. Glucose oxidase (●—●) was assayed by a modification of the method of Saloman & Johnson (1959): the incubation mixture (volume 3 ml) contained 30 mg of D(+)-glucose, 300 μg of *o*-tolidine and 30 μg of peroxidase in 100 mM-sodium acetate buffer, pH 5. The extinction at 630 nm was recorded at 20°C by a recording spectrophotometer and initial velocities were calculated. One enzyme unit is defined as the amount that will catalyse the oxidation of 1 μmol of glucose/min. Haemoglobin (H) was identified spectroscopically and albumin (A) by the adsorbed Bromophenol Blue. $\overset{+}{\rightarrow}$ and $\overset{-}{\leftarrow}$ indicate the extent of the anode and cathode solutions. S indicates the point at which the sample was loaded at the start of the run.

components, bovine serum albumin and haemoglobin, were completely resolved and appear on the trace as sharp symmetrical peaks. The albumin had migrated about half the length of the column in less than 4h. The glucose oxidase activity migrated just behind the albumin band. Haemoglobin migrated a relatively short distance from the starting region. The recoveries were: glucose oxidase activity, 91%; albumin, 86%; haemoglobin, 74%.

(*b*) Histidine. A similar run was performed with a mixture of the same components but with histidine as the isoelectric buffer. In this case the dense and less dense sucrose solutions used to prepare the gradient contained 2% and 1% (w/v) of histidine free base respectively. This established a conductivity gradient down the column, which helped to concentrate the protein zones during electrophoresis. The starting voltage was 300V and the final running voltage (600V) was reached in 1h, giving a final potential gradient of 26V/cm. The run was terminated in 3h. The pH of the histidine medium was stable during the run: all the fractions were within the range pH7.40–7.60.

As in the previous experiment, sharpening of the sample zone occurred during the first few minutes, because the conductivity of the sample in distilled water is less than that of the histidine medium.

The results of this experiment are shown in Fig. 1(*b*). The position of the glucose oxidase peak shows that it has migrated slightly in advance of the albumin and both bands have migrated further than in the Ampholine experiment. The bands also appear to be less compact than those observed in the Ampholine system, but this difference has not been observed consistently with histidine systems. The recovery of glucose oxidase activity was 81%, and of haemoglobin 77%. The recovery of albumin was uncertain, because the peak was not fully resolved from the anodal products.

In both these experiments substantial electrophoretic migration and satisfactory resolution have been accomplished in a relatively simple apparatus in periods of less than 4h. Conventional buffer systems require an apparatus of greater complexity in order to combat the high heat output and accumulation of electrolytic products. We have also attempted to separate the same sample mixture by using 50mM-tris–HCl, pH7.5, as the buffer in the same apparatus (except for the substitution of remote electrodes in large buffer vessels). In this attempt the maximal potential difference achieved between the top and bottom of the sucrose density gradient was initially 100V and it fell to 10V by 18h. The sample mixture migrated less than 1cm during this period, and none of the components was resolved.

The resolution achieved here in 3½–4h in the isoelectric buffers appears to be comparable with that obtained in 18–40h by sucrose-density-gradient electrophoresis with conventional buffer systems (Svensson, 1960, pp. 237–242). Electrofocussing usually requires 24–72h for completion (Haglund, 1967; Bloemendal & Schoenmakers, 1968), and may be attended by precipitation of proteins at their isoelectric points, a complication that impairs the resolution and lowers the recovery of labile enzymes.

Isoelectric buffers may also be employed in setting up preparative polyacrylamide-gel electrophoretic systems. In our experience the time required for separations on polyacrylamide gels is also greatly shortened, but some of the technical problems inherent in this method may make it, at present, less attractive as a rapid preparative technique.

J.R.F. is in receipt of a Science Research Council Studentship.

Bloemendal, H. & Schoenmakers, J. G. G. (1968). *Sci. Tools*, **15**, 6.

Haglund, H. (1967). *Sci. Tools*, **14**, 17.

Saloman, L. L. & Johnson, J. E. (1959). *Analyt. Chem.* **31**, 453.

Schenkein, I., Levy, M. & Weis, P. (1968). *Analyt. Biochem.* **25**, 387.

Svensson, H. (1960). *A Laboratory Manual of Analytical Methods in Protein Chemistry (including Polypeptides)*, vol. 1. Ed. by Alexander, P. & Block, R. J. London: Pergamon Press Ltd.

Svensson, H. (1962). *Acta chem. scand.* **16**, 456.

Vesterberg, O. & Svensson, H. (1966). *Acta chem. scand.* **20**, 820.