ELECTROPHORESIS IN POLYACRYLAMIDE CONCENTRATION GRADIENT

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Separation of proteins by differential molecular sieving in polyacrylamide gel of continuously decreasing pore size promises to yield better resolution than conventional block or disc electrophoresis (Wright and Mallmann, 1966, and Margolis and Kenrick, 1967). Since the advancing edge of each migrating zone is retarded more than its trailing edge, the zones remain compact and eventually come to a dead stop if allowed to run far enough. Electrophoresis of serum across the gel concentration gradient was reported in a previous paper (Margolis and Kenrick, 1967). In this account electrophoresis in the direction of increasing gel concentration, that is decreasing pore size, is described.

PREPARATION OF GEL GRADIENTS

Table I shows the composition of solutions for 4-20% polyacrylamide block with 5% cross linkage. The solutions A and B are poured into two interconnected 250 ml beakers which serve as the compartments of a linear gradient mixer, the beaker containing solution A being equipped with a stirrer.

TABLE I

Solutions for 4-20% polyacrylamide gradient block.

Components 1-7 are dissolved at approx. 40°C, then degassed and cooled to 15°C. Ammonium persulfate is added immediately before pouring into the gradient mixer. (See Text)

		A (20%)	B (2%)
1.	Acrylamide*	42.75 g	4.3 g
2.	Methylene bisacrylamide*	2.25 g	0.2 g
3.	Sucrose	25 g	-
4.	Dimethylaminopropionitrile	1.0 ml	1.0 ml
5.	$K_3Fe(CN)_6$ (1%)	4.5 ml	3.0 ml
6.	Buffer pH 9.2**	112.5 ml	112.5 ml
7.	H_2O	to 213.7 ml	to 213.7 ml
8.	$(NH_4)_2S_2O_8$ (10%)	11.3 ml	11.3 ml
	Total volume***	225 ml	225 ml

* Eastman Organic Chemicals

** Tris, 22.31 g; disodium EDTA, 1.92 g; boric acid, 0.73 g; H_2O, to 1 l for gel buffer (double strength), 2 l for electrode vessel buffer.

*** The last 25 ml remaining in each compartment of the gradient mixer is discarded, so that the total volume is 400 ml and gradient stops at 4%.

The outflow is directed via a cotton thread onto the apex of a hollow conical float, covered with a layer of gauze and counter balanced by a weight suspended from the thread across a pulley.

The gel gradient is layered at a rate of 50-100 ml/min in a cubical plexiglass mould (78 mm i.d.) on top of 20% sucrose solution, cooled to 10°C (Margolis and Kenrick, 1967). The flow is stopped at exactly 400 ml and warm water is layered

on top.

Since acrylamide is relatively light, sucrose is included in solution A to stabilize the density gradient. It is also most important to select concentrations of $K_3(FeCN)_6$ in A and B so that the gel formation starts at the top and spreads downwards, otherwise convection currents are set up by the heat liberated during polymerisation. Adjustment of the temperatures of sucrose, gel and water to 10°, 15° and 30°C respectively is an added safeguard. Under these conditions the gel starts to set at the top in about 10 minutes and is complete in 20 minutes. The block is then expelled by pumping water into a port at the bottom of the mould and sliced across the gradient into slabs 6.5 mm thick.

Gel rods for disc electrophoresis are prepared by a modification of this method. A cluster of 96 glass tubes, 6 mm i.d. resting on a stainless steel mesh platform is lowered into a cylindrical mould, 95 mm i.d., and the gel components are fed in reverse order (i.e. water, gradient mixture and 20% sucrose) through a funnel-shaped inlet at the bottom. The tubes are allowed to fill with the gel gradient to the desired level. After setting, the gel block with the imbedded tubes is removed and the tubes extracted. With this apparatus 275 ml of acrylamide mixture produces gel rods 55 mm high.

ELECTROPHORESIS

In the present series the gel slabs were cemented with 10% polyacrylamide into a flat plexiglass chamber (90 x 80 x 6.5 mm), leaving the upper, large-pore edge free. The gel occupied the lower 70 mm of the cell and in the 20 mm remaining space vertical sample slots, 7 mm wide and 1½ mm thick were cast in 3½% polyacrylamide gel prepared as in Tab. I but without

the ferricyanide. The chamber was attached to a vertical electrophoresis cell which was filled with Tris-EDTA-boric acid buffer, pH 9.2 (Raymond, 1964; see Tab. I). The results in Fig. 1 were obtained with 0.020 ml samples of serum diluted 1:4 with buffer containing 5% sucrose. These were carefully layered in the sample slots under buffer. The cells were then connected to a current supply at 120 mA for 2½ hours, the temperature being maintained at 10°C.

The apparatus for disc electrophoresis was similar to that described by Davis (1964) except that the tubes were completely submerged in the buffer which was circulated and cooled. The serum samples were diluted 1:10 with sucrose-buffer and 0.050 ml was layered directly on top of the gradient gel. The current was maintained at 5 mA per tube for 2½ hours at 15°C.

The gels were stained overnight with 1% Amido-Black in acetic acid:methanol:water in proportion 10:30:60 by volume and were de-stained in 7% acetic acid by applying a current at 5-10V/cm from a 12V battery charger across the thickness of the gel slabs or rods (cf. Fitschen, 1964; Ferris, Esterling & Budd, 1962).

For better resolution some stained gel rods were split longitudinally by driving them through a grid of four nylon filaments threaded across the mouth of plastic tube, 6 mm i.d., as in the "noughts and crosses" game.

RESULTS AND DISCUSSION

Fig. 1 shows the degree of resolution attainable in a linear 4-20% gel gradient. In principle, still better separation can be achieved by extending the gradient beyond 20% and increasing the length of the run, but with the present techniques swelling and fractures of the gels made it difficult to reach the theoretical limits of migration. An added problem in disc

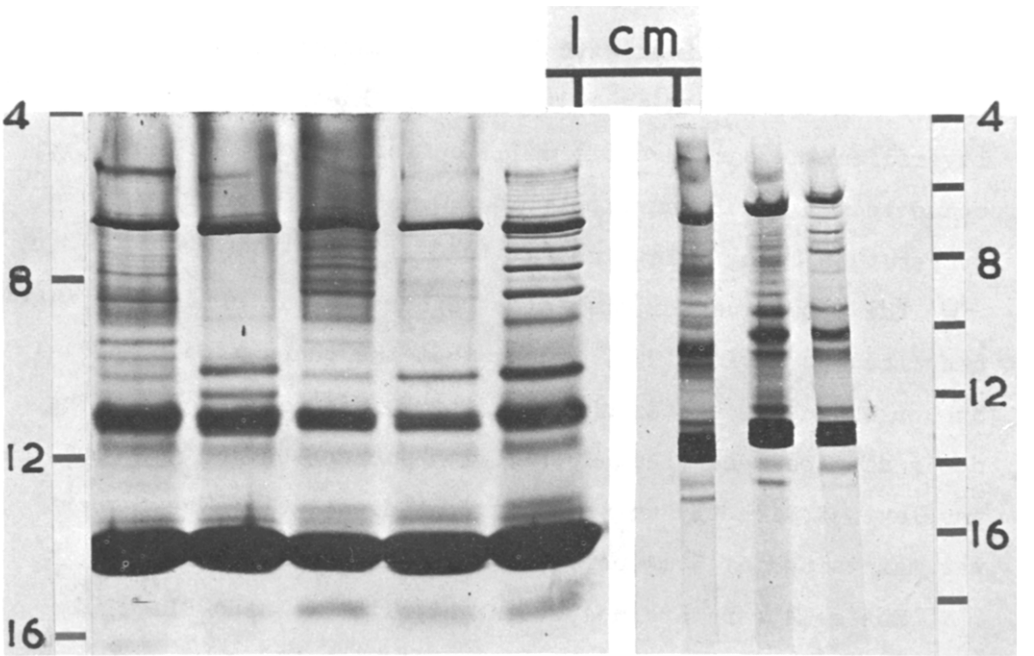

Fig. 1. Serum patterns after electrophoresis in 4-20% acrylamide gel gradient (see Text and Tab. I). Ordinate indicates acrylamide concentration (per cent).
A, vertical gel slab; samples from left to right are: 1 and 2, normal sera; 3, increased gamma globulin, 4 and 5, decreased gamma globulin.
B, disc electrophoresis; the photograph shows central cores of square cross section removed from the gel rods to minimise the effect of sagging, which is seen in the upper part of the gels (see Text).

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electrophoresis was sagging of the central part of the zones which gave rise to overlapping, U-shaped zones in the upper par

of the gel. At present this is being dealt with by splitting the gels as described (Fig. 1B).

When compared with the general advantages of the method these are minor faults which will probably be corrected when we learn more about this technique. Even now resolution can be further increased in a selected region of the spectrum, e.g. haptoglobins, transferrins or Group specific components (Gc), if the gel gradient is taylored for the specific purpose. The limits, slope and shape of the gradient can be easily determined after inspection of the overall pattern in a broad-spectrum linear gradient as in Fig. 1 or as obtained by electrophoresis across the gradient (Margolis and Kenrick, 1967).

Work along these lines is proceeding in our laboratory and the present indications are that the use of molecular sieve gradients will open new possibilities in analytical and perhaps preparative electrophoresis of proteins.

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