# ELECTRIC FOCUSING IN A HORIZONTAL APPARATUS

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A design for a horizontal apparatus for preparative electric focusing in a stable pH gradient and with many advantages over vertical columns for electric focusing in a sucrose density gradient is described. Isoelectric spectra of the IgG of some animals are examined.

In recent years isoelectric fractionation in a stable pH gradient has come to acquire a leading place in protein biochemistry [2, 4]. Columns in which a pH gradient is created in a vertical density gradient formed by the addition of sucrose are usually used for electric focusing.

As well as high resolution during the fractionation of proteins differing only slightly in their isoelectric points, apparatuses of this type also have certain disadvantages. The most important of these are the need to remove the sucrose, the concentration of which may reach 40%, in order to isolate the pure protein, and the impossibility of separating large quantities of protein.

The principle described previously [3] was used to design the separate units and to prepare a horizontal apparatus for electric focusing, the main advantage of which would be that considerable quantities of protein could be fractionated without the use of a density gradient. The consumption of ampholin is reduced by 33-50% compared with electric focusing in a vertical column.

The horizontal apparatus for electric focusing is shown schematically in Fig. 1. The apparatus is made of transparent plastic and consists of a tray (1) with corrugated base (2), the angle between the sides of the corrugations being 60°. A series of transverse gutters is formed between the corrugations in the base. Platinum electrodes (3) are placed at the ends of the tray and separated from its working volume by partitions made from No. 4 glass filters (4). A corrugated lid (5) fits into the tray, its projections corresponding to the hollows in the base of the tray. The tip of each hollow communicates through a vertical channel in the lid with its top surface. The base of the tray and the lid are of double thickness. By passing water or antifreeze cooled to 0.5°C through the apparatus the temperature in the working volume of the chamber during electric focusing is kept at about 2°C.

The apparatus is prepared for electric focusing as follows: 35-40 ml of 1% ampholin solution of the required pH range is poured into the bottom of the tray, and by gentle agitation efforts are made to ensure that the liquid is distributed uniformly in the hollows of the base and that the ridges between them prevented it from mixing. The ampholin solution is then removed from one or several adjacent central hollows and replaced by the solution of protein in 1% ampholin. To remove salts, the protein should be first dialyzed

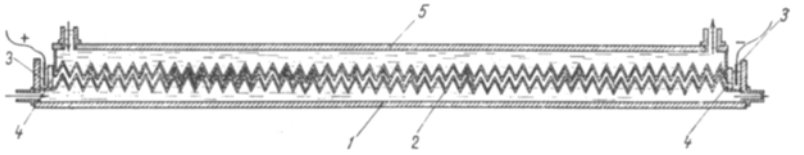


Fig. 1. Scheme of the horizontal apparatus for electric focusing (explanation in text).

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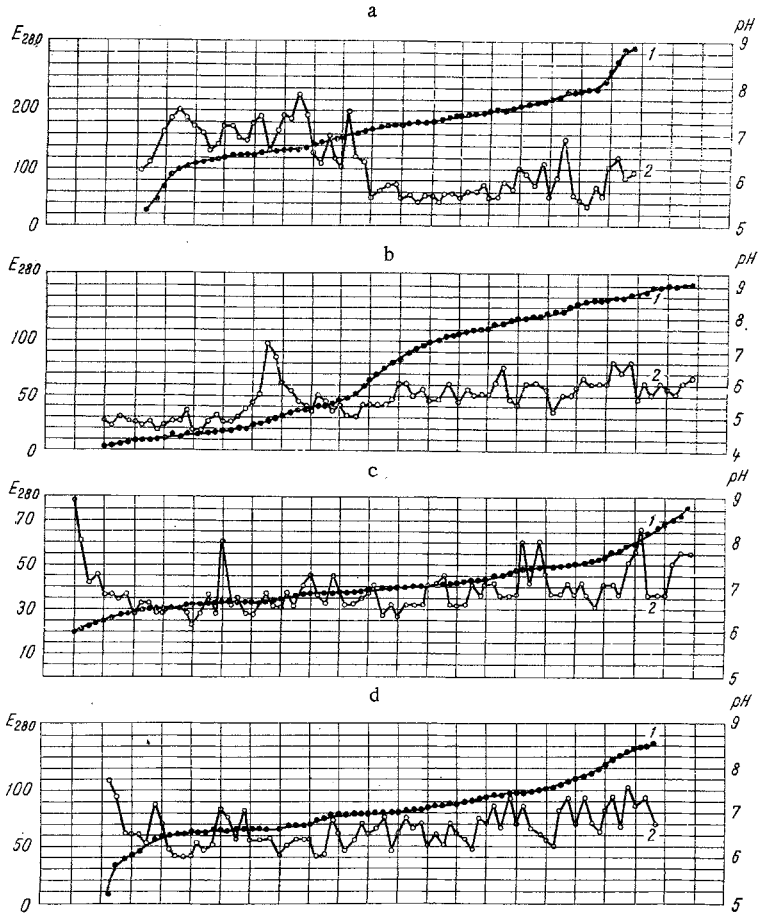


Fig. 2. Isoelectric spectra of bovine (a) and rabbit (b) IgG, horse anti-anthrax γ-globulin (c), and IgG from bovin lymph gland tissues (d): 1) pH-gradient curve; 2) protein distribution curve ($E_{280}$ for the cell 1.35 mm).

against distilled water. Good results are also obtained during electric focusing of proteins containing small quantities of salts as impurities, stabilizing them in solution. For example, isoelectric spectra of blood serum proteins can be studied without preliminary dialysis.

Up to 3 ml blood serum can be fractionated at one time in the apparatus of this design using ampholins for the pH region 3-10. For fractionation of human albumin in a gradient of pH 4-6, up to 100 mg protein was used for preliminary fractionation. To study the isoelectric spectra and preparative isolation of fractions of γG-globulin, batches of 70 mg were fractionated simultaneously.

After addition of the sample for fractionation, the lid is lowered on to the tray. The projections of its corrugated base displace some of the solution from the hollows in the floor of the tray. This creates a series of junctions between the V-shaped cells filled with ampholin solution. The lid must be lowered slowly, keeping watch that air bubbles escape from the tips of the hollows in the lid through the appropriate holes.

The electrode compartments, with a capacity of 0.3-0.5 ml, are filled with solutions of acid and alkali. For the pH gradient 5-8, 0.02 M $H_2SO_4$ solution was used as the anodal electrolyte and 0.05 mM NaOH solution as the cathodal electrolyte. It is also possible to use $H_3PO_4$ and ethylenediamine. With the cooling system turned on, a constant voltage of the order of 1200-1500 V is applied to the electrodes. The current grows for some time and then falls to 0.5-1 mA. At this time a stable pH gradient is formed in the steady electric field, in which the protein is concentrated around the zones of its isoelectric points. A vertical density gradient is created and the protein settles on the floor of the V-shaped section in which it was focused. After stabilization of the current, the experiment continues for a further 15-20 h.

Protein can also be added to the system after creation of the pH gradient. For this purpose, a certain quantity of ampholin is removed through the hole in the lid and replaced by protein solution which is con-

centrated at the isoelectric points and partially sedimented to the floor of the hollows. In the case of preparative fractionation, further small quantities of protein can be added, and the experiment ended after its fractionation. This method of addition of the specimen in small quantities at a time allows more protein to be fractionated than by a single addition without any increase in the concentration of ampholins.

At the end of fractionation the lid is carefully removed. The surface of the fluid in the tray falls and the tips of its corrugated base separate the individual portions of solution collected in the hollows. After determination of the pH of each sample and its protein concentration, a curve of protein distribution with pH is plotted.

An apparatus of this design, with a tray having 90 hollows, was used to study the isoelectric spectra of human and bovine albumin and of immuno-gamma-globulins (IgG) of certain animals, followed by a study of the physicochemical properties of the individual fractions. Isoelectric spectra of IgG from the blood serum of several animals are given in Fig. 2A, B, C, and the spectra -of IgG from a tissue homogenate of bovine lymph glands in Fig. 2D. The IgG from rabbit and bovine serum and also from bovine lymph gland homogenate were obtained by preliminary precipitation with ammonium sulfate (30:100) followed by purification on DÉAÉ-cellulose by the batch method in 0.01 M phosphate buffer, pH 7.2 + 0.015 M NaCl. Horse anti-anthrax $\gamma$ -globulin for medical use, obtained by alcoholic fractionation, was purified on Sephadex G-200 (column 80 × 3.0 cm, boric-borate buffer, pH 8.0). In the case of purification of IgG on DÉAÉ-cellulose, no fractions with isoelectric points below 6.5-6.8 were found. In preparations obtained by the alcohol method, the boundary of the acid region of the isoelectric spectrum extended to pH 5.0-5.5.

This last effect was demonstrated earlier by the writer during fractionation of human IgG by electric decantation [1], and also by other workers using electric focusing [2].

A great advantage of the apparatus of this design is that proteins forming precipitates at the isoelectric point can be fractionated. In particular, this is how some IgG fractions behave. The precipitates collect in the bottom of the corresponding hollows and do not spread into the adjacent zones, as is observed in vertical columns with a sucrose density gradient.

## LITERATURE CITED


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