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FREE-FLOW ELECTROMIGRATION SEPARATIONS

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SUMMARY

A continuous free-flow apparatus for thin-layer zone electrophoresis and adaptations for separations by isotachophoresis and by isoelectric focusing are described. The isotachophoresis and the isoelectric fractionation of an amylase concentrate is shown as an example. The concentration profiles of the leading electrolyte, KCl, and of the terminator, Tris, under conditions of continuous isotachophoresis are demonstrated.

INTRODUCTION

Zone electrophoresis, isoelectric focusing and isotachophoresis are methods which can be exploited in preparative-scale experiments. The losses of the products to be isolated, caused by non-specific adsorption, are reduced to a minimum in the continuous carrier-free process. Continuous zone electrophoresis in a thin electrolyte layer as originally designed by Hannig[1] is the best developed of these methods. The instrument built by us operates at a potential gradient of 60 V cm^{-1}, thermal losses being up to 0.36 W cm^{-2}. The ions of the products to be separated are deflected by an electric field at right angles to the electrolyte flow in a planar slit, 0.5 mm thick and 500 mm wide, with an effective length of 440 mm. The method was found to be useful especially in the purification of labile naturally occurring basic polypeptides with disulphide bonds[2]. The separation capacity varies around 100 μmole/h of octa- to decapeptides and the yields of the purified products are roughly twice those obtained with countercurrent distribution or column procedures.

EXPERIMENTAL AND RESULTS

For use in the focusing methods the individual circulation circuits of electrode electrolytes in the instrument were separated both hydraulically and electrically. This instrument, modified for free-flow isoelectric fractionation[3,4], is shown schematically in Fig. 1. Inlets 2a–2f are fed a solution or prefractionated mixture of low-molecular-weight ampholytes of LKB-type Ampholine (LKB, Stockholm, Sweden). As can be seen from Fig. 2, a stable pH gradient is obtained at a terminal potential gradient of 3000 V and at an electrolyte shift of 0.2–0.3 cm min^{-1} at the outlet. By

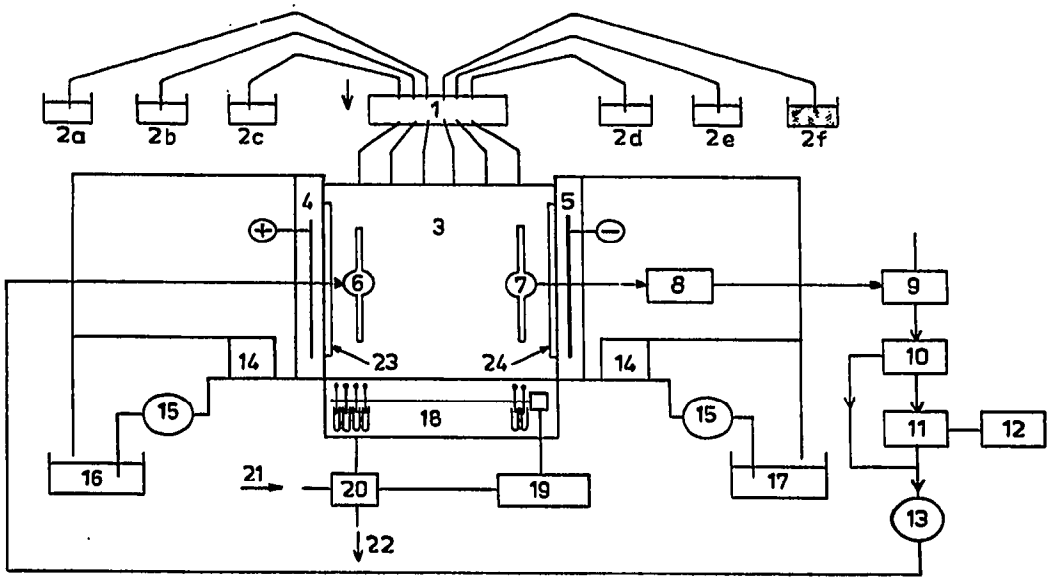

Fig. 1. Schematic diagram of continuous isoelectric focusing with prefractionated ampholyte carrier. 1 = Pump for electrolyte carrier; 2 = reservoirs of prefractionated electrolyte (Ampholine); 2f = solution of Ampholine fraction containing the protein solution; 3 = separating slit chamber; 4 = anodic electrode chamber; 5 = cathodic electrode chamber; 6 = air-cooling inlet; 7 = air-cooling outlet; 8 = temperature detector; 9 = pneumatic amplifier; 10 = proportional mixer; 11 = heat exchanger; 12 = cooling unit; 13 = fan; 14 = constant-level device; 15 = centrifugal pump; 16 = acid reservoir; 17 = base reservoir; 18 = fraction collector; 19 = high-frequency-level detector; 20 = air valve; 21 = atmospheric air inlet; 22 = vacuum; 23, 24 = ion-exchange membranes.

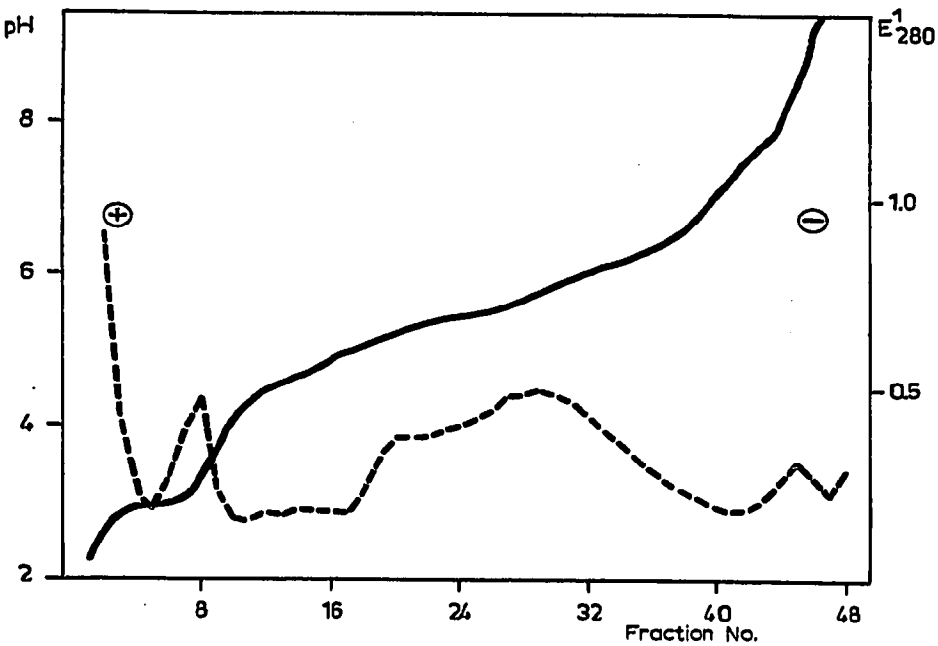

Fig. 2. Isoelectric free-flow fractionation of pancreatic amylase extract from pig. Terminal voltage, 3000 V; steady current, 8 mA; temperature, 5°; Ampholine fractions, pH 5–8, at a concentration of 0.3% (w/v). Electrolyte shift, 0.3 cm min^{-1}. ———, pH; — — —, UV absorbance.

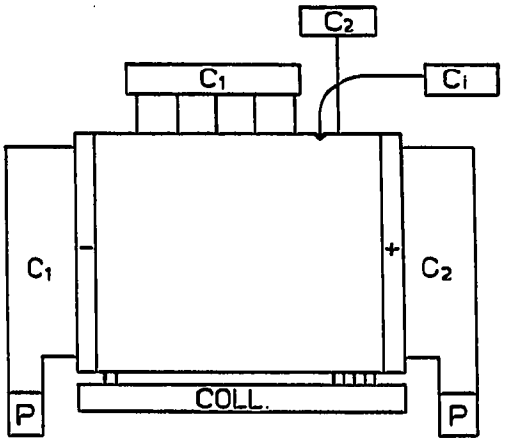

Fig. 3. Scheme of arrangement for sample injection in boundary of leading and terminating cations. C_1 = Leading ion; C_i = sample; C_2 = terminating ion. Polarity adjusted for cationic separation.

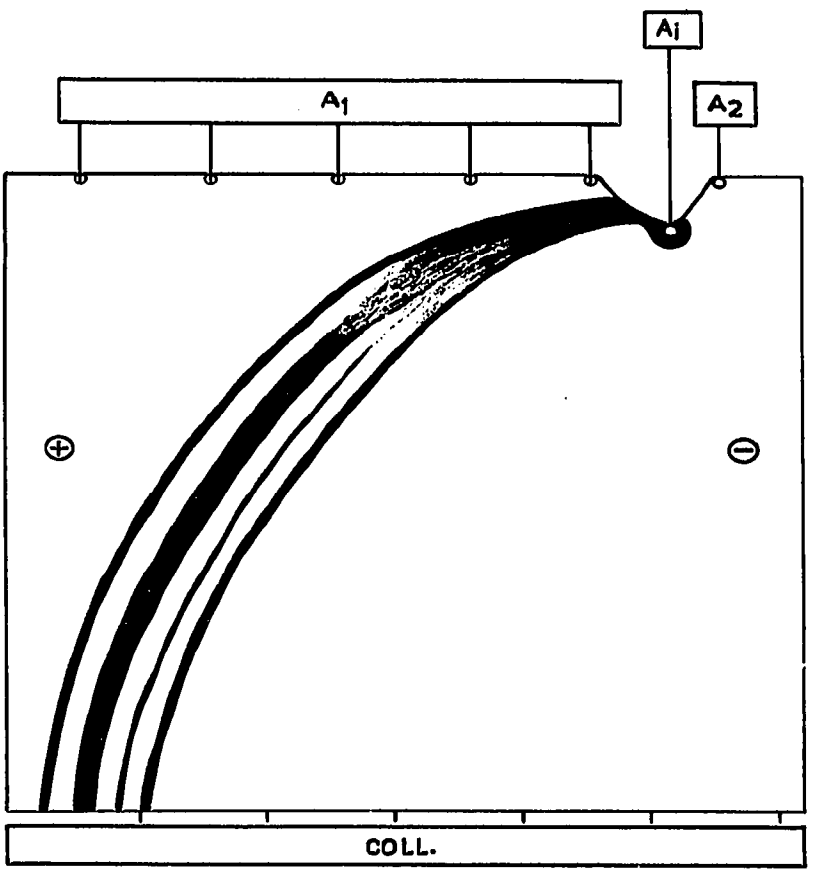

Fig. 4. Scheme of zone curving during non-compensated free-flow isotachophoresis. Mixture of anionically migrating dyes in the system chloride (A_i)–diethylbarbiturate + glycinate (A_2) with ethylenediamine as counter-ion. A_1 = leading ion; A_2 = terminating ion; A_i = sample.

this procedure, fractions of very narrow pH range, corresponding to the distribution of the gradient over 48 fractions have been prepared from Ampholine at pH 5–8. Fractionation of proteins by this method is unfortunately limited by the low solubility of certain proteins at the isoelectric point. The separation of an extract of pancreatic amylase is shown in Fig. 2 as an example of separation by the free-flow procedure (Fig. 3).

If the apparatus is used for continuous free-flow isotachophoresis, the instrument must be located horizontally, as in the case of isoelectric focusing, in order that thermoconvection caused by uneven development of Joule heat be limited. The electrode vessels remain separated by ion-exchange membranes, unlike Preetz's instrument[5] with asbestos feed wicks. The sample solution is injected in the boundary between the leading and terminating electrolytes. A special spacer is therefore placed at the slit where the sample is injected, as seen in Fig. 3. The separation process itself resembles in time distribution the separation process of capillary isotachophoresis at a constant terminal potential gradient. The process near the sample inlet resembles the theoretical model of Brouwer and Postema[6], describing the time profile of the isotachophoretic process in a capillary tube when zone migration is countercurrent compensated. This difference manifests itself in the neighbourhood of the ion-exchange membranes where concentration changes occur. The current load in the thin layer decreases in the direction of the outlet and simultaneously decreases the potential gradient in the zone of the leading ion. This results in the curving of zones as shown in Fig. 4 by an idealized model experiment with an anionically migrating mixture of dyes in the system chloride–diethylbarbiturate, glycinate with ethylenediamine as

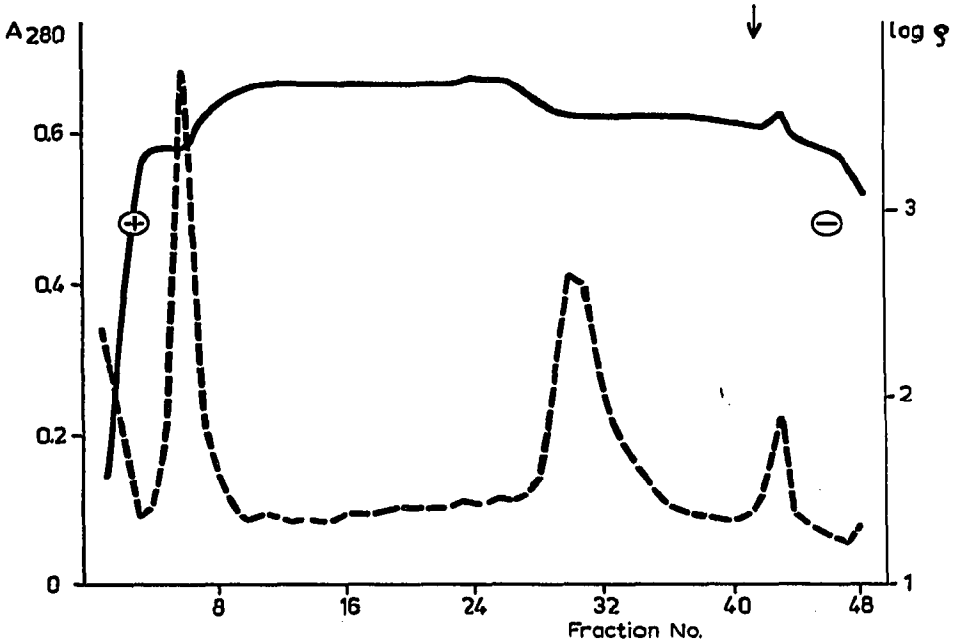

Fig. 5. Separation of a pancreatic amylase extract of pig in a system with a leading chloride ion in the form of ethylenediamine dihydrochloride and with ethylenediamine diethylbarbiturate as terminator. ———, Logarithm of specific resistance; — — —, UV absorbance.

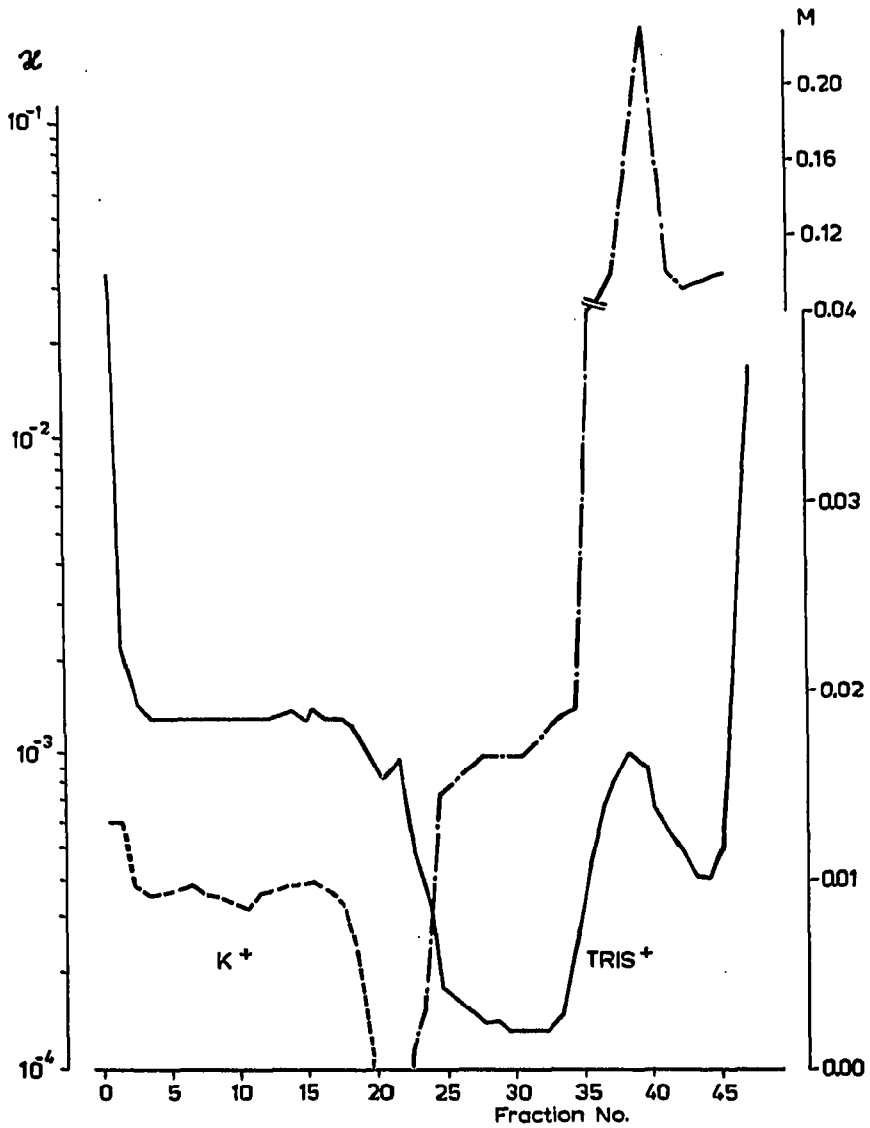

Fig. 6. Free-flow isotachophoresis in the system 0.01 *M* KCl with 0.02 *M* Tris as terminator. A solution of 0.01 *M* NaCl and 0.01 *M* LiCl was injected between the leading and terminating electrolytes at a rate of 4 ml/h. Terminal voltage, 1950 V; steady current, 29 mA; temperature, 4.5°; electrolyte shift, 0.75 cm min^{-1}. *x*, Electrolytic conductivity (———); *M*, concentration moles·l^{-1}.

counter-ion*. After a steady state had been achieved the zone width did not change substantially. If the width of the zone is small, position is critical from the viewpoint of the isolation. Therefore not only the potential gradient but also the flow, especially of the sample, and its composition as well as the temperature must be maintained with higher accuracy than in the case of zone electrophoresis. As the thermal losses are un-

* The ratio of the mixture is: 0.0075 *M* ethylenediamine added to pH 7.0; 0.015 *M* diethylbarbituric acid + 0.015 *M* glycine + ethylenediamine to pH 7.0.

evenly distributed among the individual zones, the temperature stabilization is derived from the mean temperature of the coolant transported from the chamber. Fig. 5 shows an experiment with isotachophoretic separation of an amylase extract in a system with a leading chloride ion in the form of ethylenediamine dihydrochloride and with ethylenediamine diethylbarbiturate as terminator. The specific resistance of the individual fractions shows that the isotachophoretic conditions have been established in the anodic part of the chamber.

The concentration relations in the zone of the leading electrolyte, and of the terminator in the continuous uncompensated arrangement, can be seen in a model experiment in the system K^+, Na^+, Li^+, $Tris^+$ with 0.01 *M* KCl as leading electrolyte and 0.02 *M* Tris as terminator. The fractions were subjected to flame spectrophotometry in the region of the leading electrolyte. The concentration of the terminator was determined by means of capillary isotachophoresis at a constant current of 70 μA. The system of 0.01 *M* KCl was used for the determination of Tris concentration and the very slow tetrabutylammonium ion (0.02 *M* tetrabutylammonium bromide) was employed as a terminator. The data measured show that a slight increase in potassium ion concentration can be observed in the cathodic fractions 1 and 2. The concentration of K^+ in the remaining fractions attains a value corresponding roughly to that of the inlet electrolyte. The zones of Na^+ and Li^+, which were dosed at a rate of 40 μmoles/h, are distributed over four fractions. Next follows the terminating $Tris^+$ at a concentration corresponding to isotachophoretic conditions up to fraction 33. Next comes a concentration zone with a maximum in fraction 40. The terminal voltage was 1950 V and the flow through the chamber lasted 58 min (Fig. 6).

CONCLUSION

The experiment shows that the capacity of continuous isotachophoresis without compensation of the zone boundary of the leading ion is at least comparable to the capacity of free-flow zone electrophoresis. The capacity of the separation will be increased further after the compensation of zone motion by countercurrent. The obtainable capacity depends on the percentage difference in electrophoretic mobilities of the components to be separated[7] and on the potential gradient in the zone of the leading ion[8].

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