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ISOTACHOPHORESIS

EXPERIMENTS WITH ELECTROLYTE COUNTERFLOW

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SUMMARY

Regulated counterflow is necessary if differences in mobility between ion-species need an excessively long capillary which would result in the voltage being too high for practical reasons. Alternatively if a current stabilized power supply is available, it is possible to carry out experiments in short capillaries only. The aim of this paper is to give more details about equipment suitable for electrophoretic experiments with regulated counterflow of the electrolyte. The counterflow technique as described here is used for isotachophoretic analysis in capillaries.

INTRODUCTION

Two main principles characterize regulated counterflow, as used in the analytical isotachophoretic equipment.

(1) The electric current is constant during the analysis. The hydrodynamic counterflow of the electrolyte is regulated and controlled by signals derived from the electrophoretic apparatus.

(2) The hydrodynamic counterflow of electrolyte is constant during the time the counterflow is taking place. The electric current is adjusted to this counterflow by means of signals derived from the electrophoretic equipment. During the detection the current is stabilized again.

Some possibilities for regulating the counterflow of the electrolyte, if the current is stabilized for the duration of the analysis, are discussed in refs. 1 and 2. The regulation of the electric current during the counterflow of the electrolyte will be discussed in this paper. Some of the most important possibilities will be pointed out. Again several possibilities are available. The regulation can be carried out, for instance, by:

Electro-mechanical systems
Photoresistors
Electronic tubes or semi-conductors.

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By using one of these principles a stabilized current can easily be obtained, if a constant voltage power supply is available. This can also be applied even if no counterflow is wanted.

The counterflow can be used under optimal conditions if the isotachophoretic technique is used. Theoretical treatments have been given by several authors. The principle of this technique is to separate ions into consecutive zones by a potential gradient applied over an electrolytic system. The concentration of the electrolytes in these zones are adjusted to the concentration of the first zone.

MATERIALS AND METHODS

The experiments were carried out on an instrument as described in ref. 3. Basically the equipment consists of a PTFE-(teflon) capillary tube with an outside diameter of 0.7 mm and an inside diameter of 0.45 mm. The total length is 1 m. Four thermocouples are mounted around the capillary tube, fixed by cement, to follow the separation. Fig. 1 shows a block diagram of the equipment used. A light dependant resistor (LDR) is attached in series with the capillary tube. A constant potential gradient is applied over the capillary tube and the LDR.

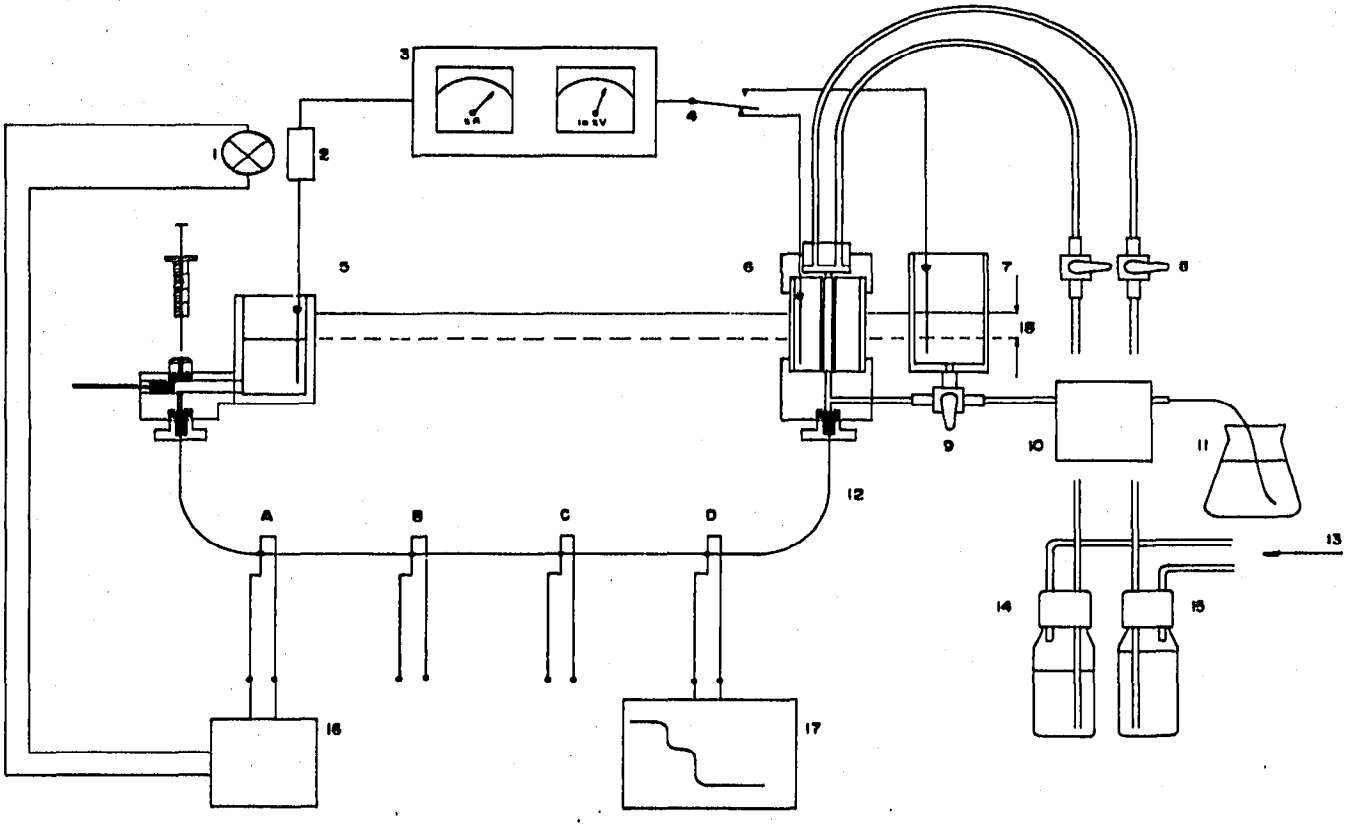

Fig. 1. Block diagram of the electrophoretic equipment used. 1 = lamp (6 V, 0.6 W); 2 = LDR; 3 = voltage stabilized power supply; 4 = switch; 5 = injection block annex cathode compartment; 6 = anode compartment with a membrane; 7 = anode compartment without a membrane; 8 = teflon lined Hamilton valves (180° connections); 9 = teflon lined Hamilton valve (T-connection); 10 = pump; 11 = reservoir with leading electrolyte; 12 = capillary tube; 13 = air-pressure; 14 = reservoir with leading electrolyte; 15 = reservoir with water for rinsing; 16 = regulator (see Fig. 2); 17 = recorder; A,B,C,D = thermocouples; 18 = difference in level.

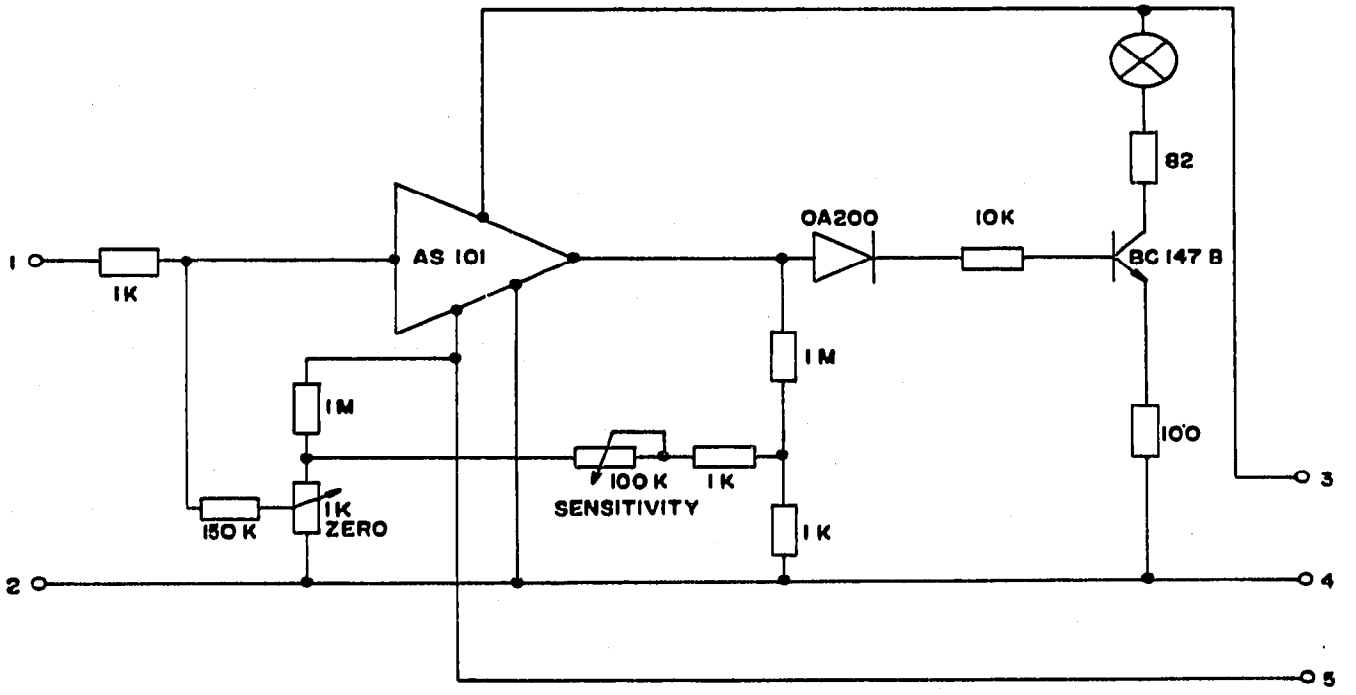

Fig. 2. Electric circuit for stabilizing the electric current by signals derived from a thermocouple mounted around the capillary. 1,2 = connection for the thermocouple; 3 = + 15 V; 4 = common terminal, note: AS 101 see ref. 4; 5 = −15 V.

the LDR, is switched off. The LDR can be seen as a voltage source. The amount of light given by the lamp is regulated by a thermocouple, fixed on the capillary tube (Fig. 2). A change in temperature of the capillary tube, will automatically involve a change in the electric current through the capillary tube.

The increment in the resistance of the capillary tube during the analysis will decrease the current, if a constant voltage is applied over the capillary. The decrease in current will result in a decrease of the temperature of the capillary tube. This decrease is registered by the thermocouple mentioned above. The resistance of the LDR and indirectly the voltage drop over the capillary tube is controlled by this and a stabilized current will be the result. A still more stable means of regulation can be found for current stabilization, if a resistor in series with the capillary tube is used. The potential drop over this resistor may be used for current stabilization. Slowly moving concentration fronts, such as pH disturbances, will not influence the regulation of the current.

With the arrangement mentioned above experiments with regulated counterflow can easily be carried out. The counterflow can be maintained by a pump, the stability of which does not need to be very high, or by a difference in level of the electrolytes in the anode and cathode compartment (Fig. 3). If the pump is used, the first electrode compartment (Fig. 3.1) will be disconnected. The pH drop over the membrane, as discussed in ref. 2, will build up during the analysis.

By making use of the differences in the levels of the electrolytes in the anode and the cathode compartment, this problem can be overcome. During the counterflow of electrolyte the open connection between the electrode compartment and the capillary tube (Fig. 3.1) ensures a constant pH by the absence of any membrane.

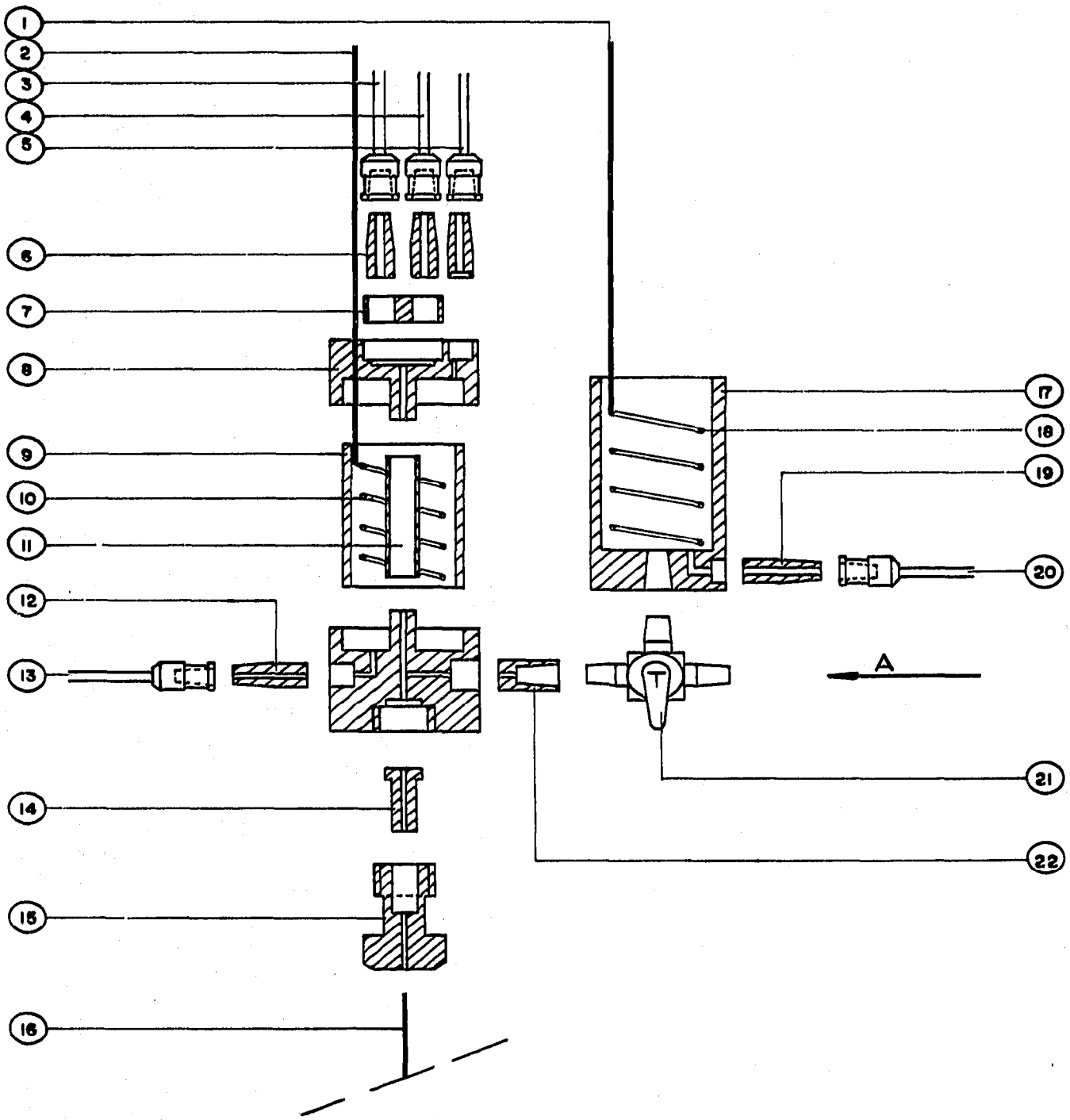

Fig. 3. Electrode compartment for electrophoretic experiments with counterflow of electrolyte. 1,2 = high tension; 3, 5, 13, 20 = connection to electrolyte reservoir; 4 = connection to reservoir with water for rinsing; 6, 7, 12, 19, 22 = pieces of perspex used for connection; 8 = cover of electrode compartment; 9 = electrode compartment; 10 = Pt-electrode; 11 = membrane made of cellulose acetate; 14 = piece of perspex used for fitting the capillary tube; 15 = bolt for fitting piece 14 and capillary tube; 16 = capillary tube; 17 = electrode compartment; 18 = electrode; 22 = teflon lined Hamilton valve (T-type). A = connection to pump.

If the counterflow is stopped the other electrode compartment (Fig. 3.2) will be used and a pH shift will again build up across the membrane.

The time for this pH shift to reach the detector will normally be longer than the time the zones need to move from the regulation thermocouple towards the detection thermocouple. Other problems also occur if the difference-in-levels method is used for the counterflow experiments.

One has to find the relation between the degree of counterflow obtained and the difference in levels between the electrolytes in the anode and the cathode compartment.

The counterflow changes with time. As will be discussed below there are two limits for the counterflow to be stated. At a certain moment the lower limit will be exceeded and the counterflow of electrolyte is not any longer able to stop the zones at the regulation thermocouple. Therefore, for counterflow over a long period of time, the electrode-compartment (Fig. 3.1) must be made very large or the level in this compartment must be controlled.

Conditions for regulating the counterflow of the electrolyte

Equations stating the conditions for the regulated counterflow of an electrolyte can easily be derived.

Equilibrium—all zones standing still at the regulation thermocouple in the capillary tube—will be found, if the transport of the mass of a zone in one direction, due to the electric field, equalizes the transport of the mass of a zone in the opposite direction, due to the mechanical counterflow of the electrolyte. At the beginning, however, the velocity of a zone, due to the electric field, must be greater than the velocity in the opposite direction, to enable the zones to reach the regulation thermocouple (upper limit).

On the other hand the potential gradient, applied over the capillary, must decrease to find, finally, the steady-state by the increase in temperature, when a zone reaches the regulation thermocouple. The velocity given to the zone by the electric field must now be greater than the velocity in the opposite direction, due to the counterflow, if the temperature of the terminating zone is to be used as the reference for the current stabilization (lower limit). If the velocity, due to the electric field, is still greater at this moment, a still higher temperature is needed to decrease the potential gradient. This is impossible because the temperature of the terminating zone is the highest one of the system and the counterflow now is unable to stop the zones. If the steady-state has been reached:

$$G_1 \sum_{i=0}^{\alpha} C_{A1i}\, m_{A1i} = C_{A1}^{*}\, \frac{Q}{\omega}$$

where:

G_1 = the potential gradient of the leading zone (V/cm)
C_{A1i} = the concentration of the ion A in zone 1 with a charge i (moles/l)
C_{A1}^{*} = the total concentration of the compound A in zone 1 (moles/l)
m_{A1i} = the mobility of the ion A in zone 1 with a charge i ($cm^2/V \cdot sec$)
Q = the amount of the counterflow given (cm^3/sec)
ω = the area of the capillary tube (cm^2)

An example will now be given: If the capillary is filled initially with a solution

of histidine (0.01 mole) and histidine·HCl (0.01 mole) the following data can be derived:

$\varrho = 1.12 \cdot 10^3$ cm
$m_{Cl^-} = 78 \cdot 10^{-5}$ cm^2/Vsec
$Q = 4.45 \cdot 10^{-5}$ cm^3/sec
$C_{A1i} = C_{A_1}{}^* = 0.01$ mole
$V_{cap.} = 160$ μl
$\omega_{cap.} = 15.9 \cdot 10^{-4}$ cm^2

Substitution of these values in the equation gives:

$G_1 = 35.8$ V/cm and $i_{equilibrium} = 51$ μA

If the leading ion and the terminating ion are known the upper-limit and the lower-limit for the counterflow can be found.

It comes to mind immediately that adjustment of the electric current to the mechanical counterflow, as described in this paper, will automatically result in an oscillation of the zone around the regulation thermocouple.

The zone has, however, passed the thermocouple already, before the current starts to decrease in order to adjust itself to the mechanical counterflow. In electrophoretic analysis, without counterflow, the zone has passed the thermocouple about 1.5 cm before the equilibrium-temperature of the zone is registered. With counterflow of the electrolyte of course this is less. The same thing will happen if the zone is driven back by the counterflow of electrolyte.

Regulation with the LDR like regulation with electronic tubes shows a negligible oscillation. The reason for this can easily be found. Although the time-constant for the regulation is very different from the time-constant for the detection, the proportional band of the regulation system is great. At the beginning of the experiment an oscillation of the current can be seen, if the temperature of the capillary tube rises from 18° towards 23°. The temperature difference between the zones is much smaller and owing to the sensitivity of the regulation system it can easily and quickly follow any signal derived from the thermocouple.

Adjustment of the current with electronic tubes presents the practical difficulty of finding electronic tubes suitable for work at high voltages. Experiments are carried out with this type of stabilization, with applied voltages with a maximum of 3.5 kV, in short capillaries.

No experiments were carried out with electro-mechanical systems.

EXPERIMENTAL

Fig. 4 shows two electropherograms of the separation of formate and acetate, A without and B with counterflow of the electrolyte. The leading ion is chloride, the terminating ion is glutamate. The aluminum block, at which the capillary is mounted, is thermostatted at 18°. The capillary is filled with a solution of histidine (0.01 mole) and histidine·HCl (0.01 mole). 2 μl of a solution of sodium acetate (0.02 mole) and sodium formate (0.02 mole) is injected. The current is stabilized by the thermocouple mounted at a short distance from the injection point (thermocouple A in Fig. 1). A thermocouple at the end of the capillary tube (thermocouple D in Fig. 1) is used as the detector. The time for analysis is about 1 h 10 min. The analysis is started with a current stabilized at about 92 μA. The temperature of the zone of the leading

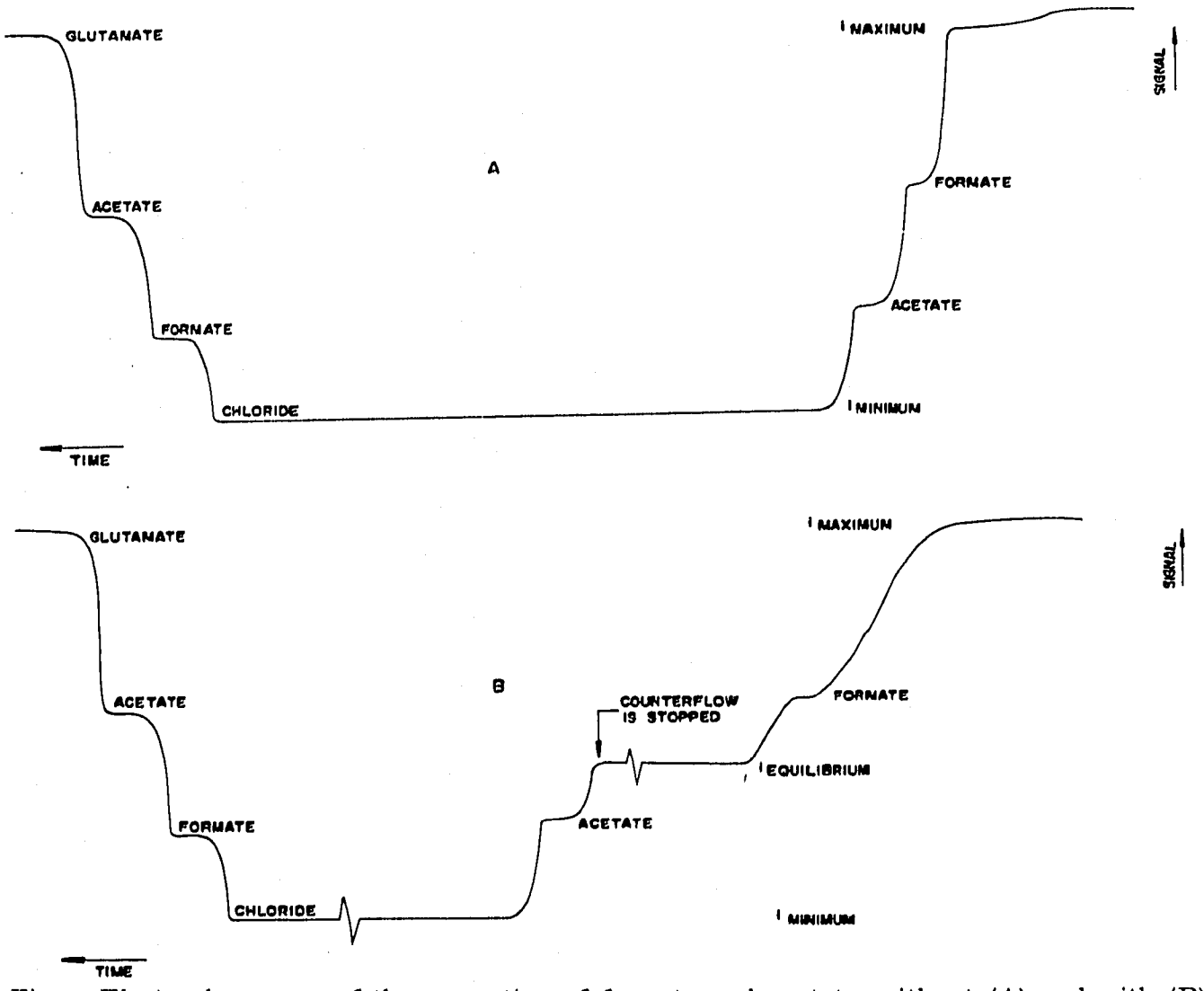

Fig. 4. Electropherogram of the separation of formate and acetate, without (A) and with (B) counterflow of electrolyte. Thermocouple A of Fig. 1 is used for regulation and stabilization of the current. Thermocouple D of Fig. 1 is used for the detection of the zones. For further explanation see text.

electrolyte is used as a reference for current stabilization (about 23°). Fig. 4 shows that the base-line here is not stable, because ions move into the capillary tube from the terminator side, not yet separated. These ions increase the conductivity of the electrolyte inside the capillary. The zones then reach the regulation thermocouple. Fig. 4 shows that the current drops from $i_{max.}$ (92 μA) towards $i_{min.}$ (52 μA).

If counterflow of the electrolyte is used, these two values are the limit values for the current. The minimum and maximum rate for counterflow can be calculated with the aid of these two values.

Fig. 4 shows that regulation and stabilization of the current, controlled by the temperature of the zone of the terminating electrolyte (about 28°) is more stable. The concentration of this zone seems to adjust to the concentration of the leading electrolyte zone about a quarter of an hour before the detection.

Fig. 4B shows the same analysis with counterflow of the electrolyte, at about

200 μl/h. The time for the zones to reach the regulation thermocouple is considerably longer. The current, $i_{equilibrium}$, at which all zones stand still in the capillary tube is reached very slowly. If the counterflow is stopped, an electropherogram is obtained as described for Fig. 4A. The ions chosen are not difficult to separate and so without any counterflow of the electrolyte a steady state would already have been reached.

The detector mounted around the capillary tube must be placed as far away from the regulation thermocouple as possible. Otherwise a zone can already have passed the detection thermocouple, due to an ion-species present in the sample in a high concentration. This is all because the regulation has not been achieved by the first zone.

By using the thermocouple at the end (Fig. 1D) as a regulation thermocouple for current stabilization and one of the thermocouples in the middle of the capillary tube (Fig. 1B or C) as a detector electropherograms can also be prepared.

The current stabilization is now carried out with the temperature of the leading electrolyte zone as a reference. The base-line from the beginning is constant because of the constant composition of the leading electrolyte. In this case no regulated counterflow of electrolyte, as described in this paper, is possible.

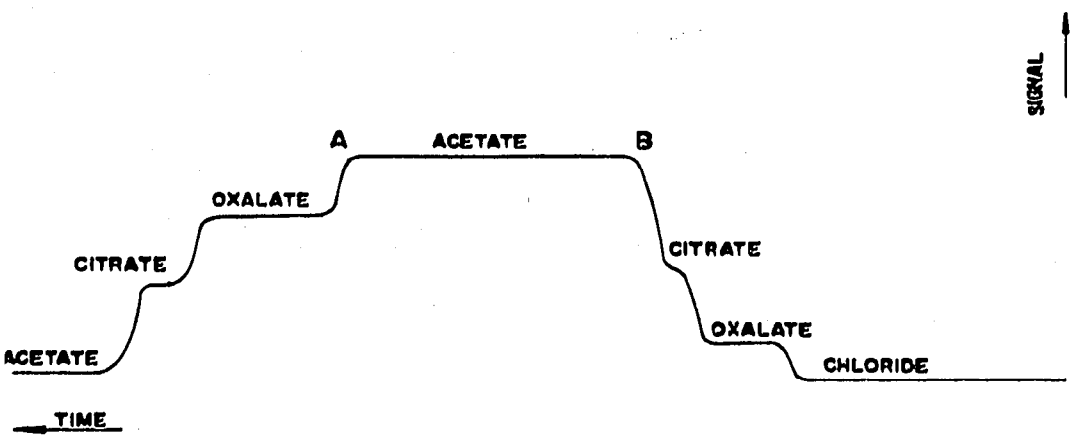

Fig. 5. Electropherogram of the separation of oxalate and citrate. Thermocouple D of Fig. 1 is used for regulation and stabilization of the current. Thermocouple C of Fig. 1 is used for the detection of the zones. For further explanation see text.

Too much sample must not be introduced, otherwise the regulation thermocouple will disturb the electropherogram. The amount of sample introduced may not exceed, in this case, the distance from the regulation thermocouple to the detection thermocouple, if the ion species from the sample are separated into consecutive zones and the steady state has been reached.

Fig. 5 shows an electropherogram of this type. The right-hand side shows an electropherogram as derived normally with a current stabilized power supply. At point A the zones reach the regulation thermocouple and a drop of the current, discussed above, will follow. The distance from A to B in Fig. 5 may still be occupied by the sample, before the regulation thermocouple will disturb the electropherogram.

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