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Design of a high-resolution detection system for use in isotachophoresis

In the last few years, there has been a growing interest in isotachophoresis, notably in isotachophoretic separations carried out in capillary columns. In this field, apparatus has been developed with a wide range of applications[1]. The zones are normally detected by means of temperature measurements. This detection principle is very simple, and has the advantage that the electrophoretic process is not interfered with. Detection is universal and, at constant current, is related to an important isotachophoretic quantity, the field strength. However, thermometric detection cannot be relied upon in those cases where sharp transitions between the zones have to be measured.

The resolving power of the technique depends to a high degree on the design of the detector[2].

Since the introduction of the thermometric detection technique, several attempts have been made at constructing detectors that are more capable of visualizing the isotachophoretic separation.

FREDRIKSSON[3] measured the conductivity of a zone by means of metal electrodes immersed in the solution. However, little can be said as yet about the adequacy of this approach.

ARLINGER AND ROUTS[4] made use of the difference in UV absorption between the components. An advantage of their method is that the recorded transition between the zones is reduced to approximately 1 mm; the zones leave the detector undisturbed. A drawback, however, is that the detector has only limited applicability. Field-strength recording is another way of visualizing the separation. In the stationary state, the field strength is inversely proportional to the mobility and the response provides a direct qualitative indication. Determination of the field strength is achieved by measuring the potential difference between two electrodes placed a short distance apart. Optimum utilization of the resolving power of the isotachophoretic technique is ensured by having the measuring points very close together.

Potential measurements by means of metal electrodes involve the hazard of polarisation of the electrodes. Systems that use such polarizable electrodes are difficult to define electrochemically.

The present author used non-polarizable electrodes placed in separate compartments that were connected with the electrophoresis capillary via liquid junctions[5].

Design of the detection system

An illustration of the detector (made of Perspex) is shown in Fig. 1. The capillary passage, of 0.45-mm diameter, connects on one side to the electrophoresis capillary and on the other to the leading-ion compartment. In the wall of the capillary passage two diametrically opposed apertures are drilled, the centre-lines of which are staggered a distance of 0.3 mm. The diameter of the apertures is 0.15 mm and their length 0.5 mm. Each aperture forms a liquid junction with an electrode compartment.

The electrode compartment is filled with leading electrolyte. The concentration in the electrode compartment equals the concentration in the electrophoresis capillary.

In the course of the electrophoresis process, the most mobile sample ion will

migrate into the detection compartment. Since the electrode compartment and the detection department contain different types of ions, exchange by diffusion will take place. The sample zone will therefore be contaminated with the leading electrolyte; the degree to which this happens depends on the dimensions of the liquid junction. These are such that in the stationary state, contamination of the sample zone by leading electrolyte will always be less than 1 %, relative to the original concentration of the sample component.

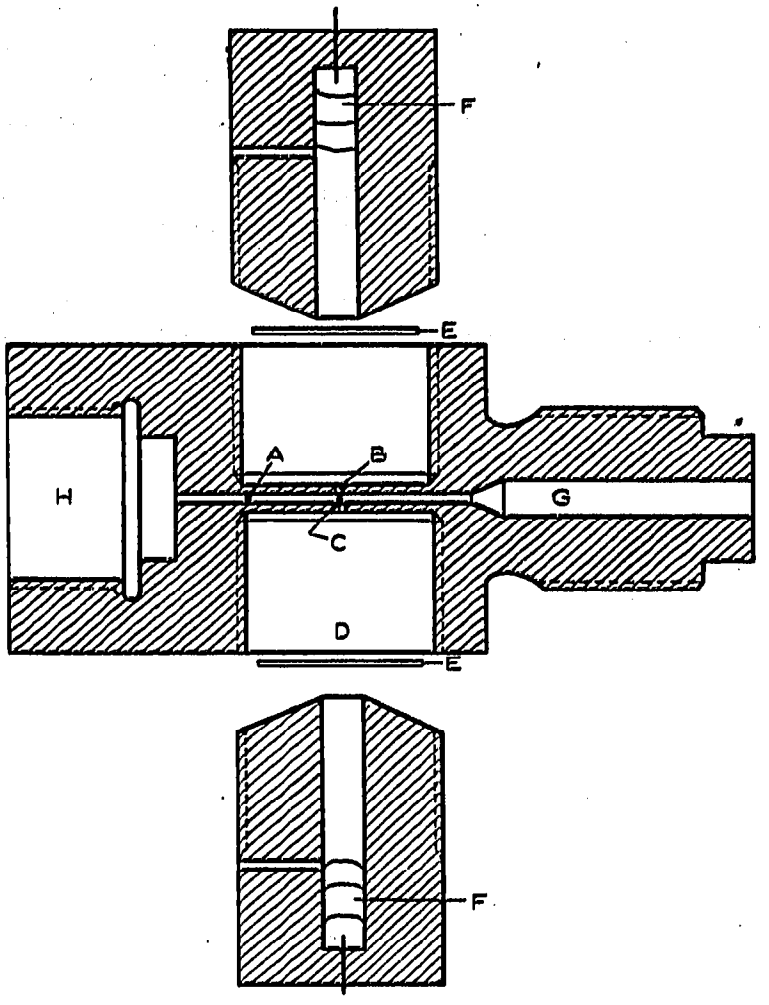

Fig. 1. Detector design. A = Capillary tube; B = liquid junction; C = detection compartment; D = electrode compartment; E = cellulose acetate membrane; F = calomel electrode; G = attachment to leading electrolyte compartment; H = attachment to electrophoresis capillary tube.

The electrophoresis capillary is closed to prevent both electro-osmotic flow as well as flow caused by hydrostatic pressure. For this reason, the liquid junction is sealed with a cellulose acetate membrane of low selective permeability. The potential difference in the capillary is measured via the liquid junctions by means of two calomel electrodes. The electrodes should be only slightly loaded so as not to disturb the electrophoresis process. The signal is measured by means of a Keithly 602 solid-state electrometer (input impedance greater than $10^{15}\ \Omega$) and recorded on a Servogor type RE 511 recorder. Neither instrument is earthed, in order to prevent current leakage.

Apparatus

The isotachophoresis apparatus used was that described by EVERAERTS AND VERHEGGEN[1].

The high-voltage power supply (Brandenburg type 800) was modified in a simple manner into a constant-current power supply. Deviations from the adjusted current are less than 0.2 %, and the maximum potential is 30 kV.

Results and discussion

The electrophoresis capillary was filled with leading electrolyte (10^{-3} *M* hydrochloric acid). As the field strength in the capillary is proportional to the current, we determined the relation between detector response and current. This was found to be linear.

The performance of the detection system as regards its precision in recording a separation was checked with reference to the separation of a known system (potassium, sodium and lithium chlorides).

To eliminate the influence of the inertia of the electrometer and recorder, the current was adjusted to a low value (long analysis time). The recorded detection signal is shown in Fig. 2.

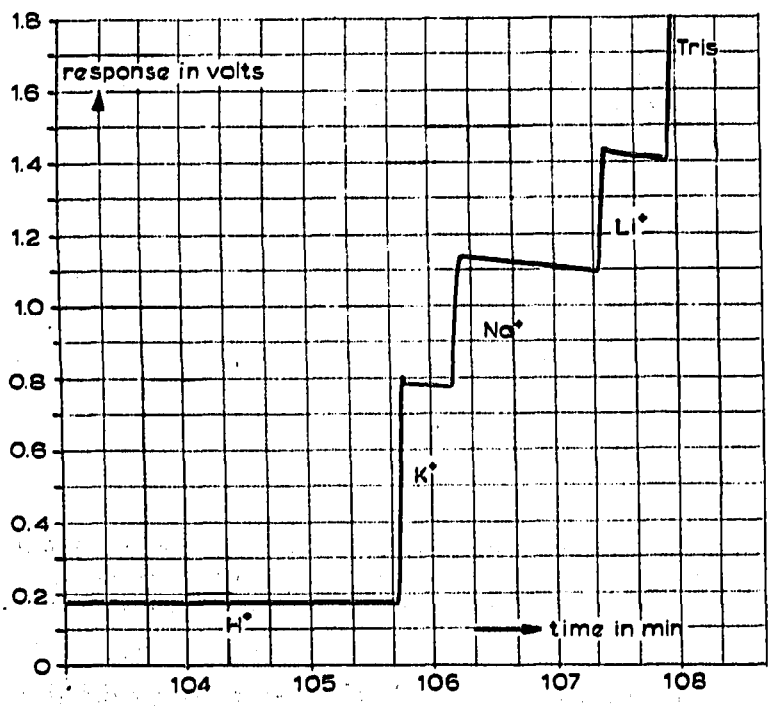

Fig. 2. Detection of separated metal ions. Leading electrolyte, 10^{-2} *M* HCl; trailing electrolyte, 10^{-2} *M* tris(hydroxymethyl)aminomethane; sample, mixture of potassium, sodium and lithium chlorides. Current, 35 μA.

The zone transitions calculated from the electropherogram have a length of 0.5 mm. If only the disturbing effect of the finite detection length of the detector

(minimum 0.3 mm) on the detection signal is taken into account, it can be concluded from this value that the actual length of the transition between two zones is 0.2 mm or less.

The degree to which the zone boundaries are disturbed in the detection compartment is difficult to establish. It is certain, however, that the detection device described here is unequalled as regards the sharpness with which the transitions are recorded.

An important aspect of qualitative analysis is the response, which is a measure of the field strength in the detection compartment. Calculation shows that the field strength in one zone is uniform at all points; this is indirectly corroborated by the results of a detection run performed with thermocouples. In Fig. 2 it can be seen that during detection of the sample zones, the field strength in the detection compartment decreases continuously. This effect cannot be ascribed to a difference in temperature or to the use of membranes. Further examination of the mass transport in the detection compartment shows the influence of the contamination on the field strength. If sample ions, such as K^+, migrate into the detection compartment, the zone will be contaminated with the leading H^+ ions, while a fraction of the K^+ ions will diffuse into the electrode compartment. The migration of K^+ ions into the detection compartment can be described by the following equation:

$$I = A \cdot F \cdot C_{K^+} \cdot V_{K^+} \cdot \frac{(M_{K^+} + M_{Cl^-})}{M_{K^+}} \quad (1)$$

where I = current (A); A = surface area of the column section (cm^2); F = Faraday constant (= 96,500 A s); C_{K^+} = K^+ concentration (g-equiv./cm^3); V_{K^+} = velocity of the K^+ ions (cm/s); M_{K^+}, M_{Cl^-} = mobilities of the K^+ and Cl^- ions (cm^2/V s). The equation for the ions leaving the detection compartment is:

$$I = A \cdot F \cdot C_{K^+} \cdot V_{K^+} \cdot \left(\frac{M_{K^+} + M_{Cl^-}}{M_{K^+}}\right) + A \cdot F \cdot C_{H^+} \cdot V_{H^+} \cdot \left(\frac{M_{H^+} + M_{Cl^-}}{M_{H^+}}\right) \quad (2)$$

The difference between mass input and mass output of K^+ per unit time is:

$$A \cdot \Delta(C_{K^+} \cdot V_{K^+}) = A \cdot C_{H^+} \cdot V_{H^+} \cdot \left(\frac{M_{H^+} + M_{Cl^-}}{M_{H^+}}\right)\left(\frac{M_{K^+}}{M_{K^+} + M_{Cl^-}}\right) \quad (3)$$

It can be seen from eqn. 3 that the K^+ concentration in the detection compartment will increase. Depending on the value of this concentration, a fraction of the K^+ ions will diffuse from there into the electrode compartment. The K^+ concentration continues to rise until the difference between mass input and mass output equals the number of K^+ ions diffusing into the electrode compartment. The increase of the K^+ concentration causes a decrease of the field strength *in situ*. This effect influences both the qualitative and the quantitative aspects of the analysis. The effect cannot be eliminated because liquid junctions are essential in this detection technique.

Reducing the diameter of the liquid junctions will diminish the influx of leading electrolyte and, in consequence, retard the rate of the disturbance process.

Further investigation is needed to establish whether reduction of the diffusion process via the liquid junctions will suppress the variation in field strength to the point where the effect ceases to have any practical significance.

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