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## HIGH RESOLUTION ISOTACHOPHORESIS BY MEANS OF DIRECT CONDUCTIVITY MEASUREMENTS WITH MINIATURE SENSING ELECTRODES

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### SUMMARY

A new detection system for analytical isotachophoresis, the conductivity detector, is described. This detector excels in its high resolving power, by means of which the time required for analysis is reduced enormously and the detectable amount of sample is decreased by a factor of at least 10.

A possible explanation is given for most artifacts that are connected with this method of detection.

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### INTRODUCTION

As already shown in a series of papers, isotachophoresis is a technique for qualitatively and quantitatively separating ionic species and charged particles. The theory of isotachophoresis has been dealt with in several papers<sup>1-3</sup>.

If the steady state has been reached, the sharpness of the zone boundaries is maintained by self-correction, an effect that is characteristic of all displacement methods. The zones will not broaden further. The fronts as finally recorded were less sharp than they were expected to be on the inside of the capillary tube, which was made of PTFE, because the boundaries were measured by means of micro-thermocouples on the outside of the capillary tube. The longitudinal conduction of heat, in both the liquid and the PTFE, distributes the temperature change along the tube and a period of time is required in order to heat up the part that is immediately behind the front to obtain dynamic equilibrium of the temperature step. A zone boundary passes the measuring thermocouple by a distance of about 1 cm, depending on the increase in temperature, before it is registered completely by the micro-thermocouple. For quantitative analyses, only the position of the boundary is required, whereas for qualitative analyses the step must be registered entirely.

Comparison of UV and thermometric detection of isotachophoretic zones shows that the boundaries are much sharper than the thermometric registration indicates.

Sometimes, differences in effective mobilities make separations impossible. Different buffers (separation by  $pK$  values) or different solvents (separation by solvation) must be investigated. Even greater differences in temperature between

adjacent zones or correspondingly greater differences in the effective mobilities are required if thermometric detection is used.

Possible mixed zones can be formed, if the ions in the sample are present in concentrations that are too different from each other. On the one hand, relatively long zones (5 mm) must be available when using thermometric detection, but on the other hand the possible formation of mixed zones indicates that as little sample as possible should be used, which is a serious limitation to the method.

While UV detection is not as general as thermometric detection, another method of sensing all types of zone boundaries was developed.

The measurement of the conductivity of the electrolyte inside the capillary has a higher resolving power than has thermometric detection, and is as universal. Experiments are described in this paper in which both the A.C. and the D.C. methods, explained below, are used.

Electrodes of several noble metals, of different shapes and sizes, were tested both in direct contact with the electrolyte and coated with a thin film of an inhibitor. As will be explained, coated electrodes, very thin (of the order of microns) electrodes or the addition of special inhibitors, to suppress redox reactions, can be applied.

The evolution of hydrogen and oxygen, due to the polarization of the miniature sensing electrodes by the direct driving electrical current, can make analyses irreproducible or even impossible. Besides evolution of hydrogen and oxygen, other reduction or oxidation reactions may disturb the analysis if non-coated electrodes are applied (even very thin electrodes, of diameter  $0.5 \mu\text{m}$ , sometimes cause difficulties of this type). Micro-sensing electrodes act as bipolar electrodes.

#### MATERIALS

The analyses described below were carried out in the equipment shown in Fig. 1.

The PTFE capillary, with an O.D. of 0.75 mm and an I.D. of 0.45 mm, was cooled directly with kerosene\*. Direct cooling with a non-conductive organic solvent has several advantages: the efficiency of this type of cooling is much greater, so that higher current densities can be applied, which results in both a shorter time for analysis and a higher resolving power; the electrolyte inside the capillary is uniformly thermostatted, and also at the position where the detection of the boundary passages is carried out\*\*; capillaries made of PTFE are porous, especially for gases, if the walls are very thin: difficulties may be expected if the pH of the electrolyte inside the capillary tube is too high and the capillary is in direct contact with its environment ( $\text{CO}_2$  absorption); a gas or vapour chemically related to PTFE would normally show a higher penetration rate than a dissimilar material, but PTFE can act as a semi-permeable membrane for ionic species, especially if water is present on both sides of the PTFE wall; our experiments showed that if the thin-walled capillary is directly cooled with water, the PTFE shows signs of deterioration even after a short time: the PTFE changed colour and became stiff, and the dielectric strength also decreased,

\* Previously, the capillary was mounted in a groove of an aluminium block in the form of a helix<sup>4</sup>. To ensure good thermal contact between the capillary and the aluminium block, a heat-sink compound ( $\text{Al}_2\text{O}_3$  and silicone oil) has been applied. The aluminium block was thermostatted.

\*\* For thermometric detection, special compartments for assembling the thermocouples or thermistors were constructed, so as to enable the boundary passages to be registered.

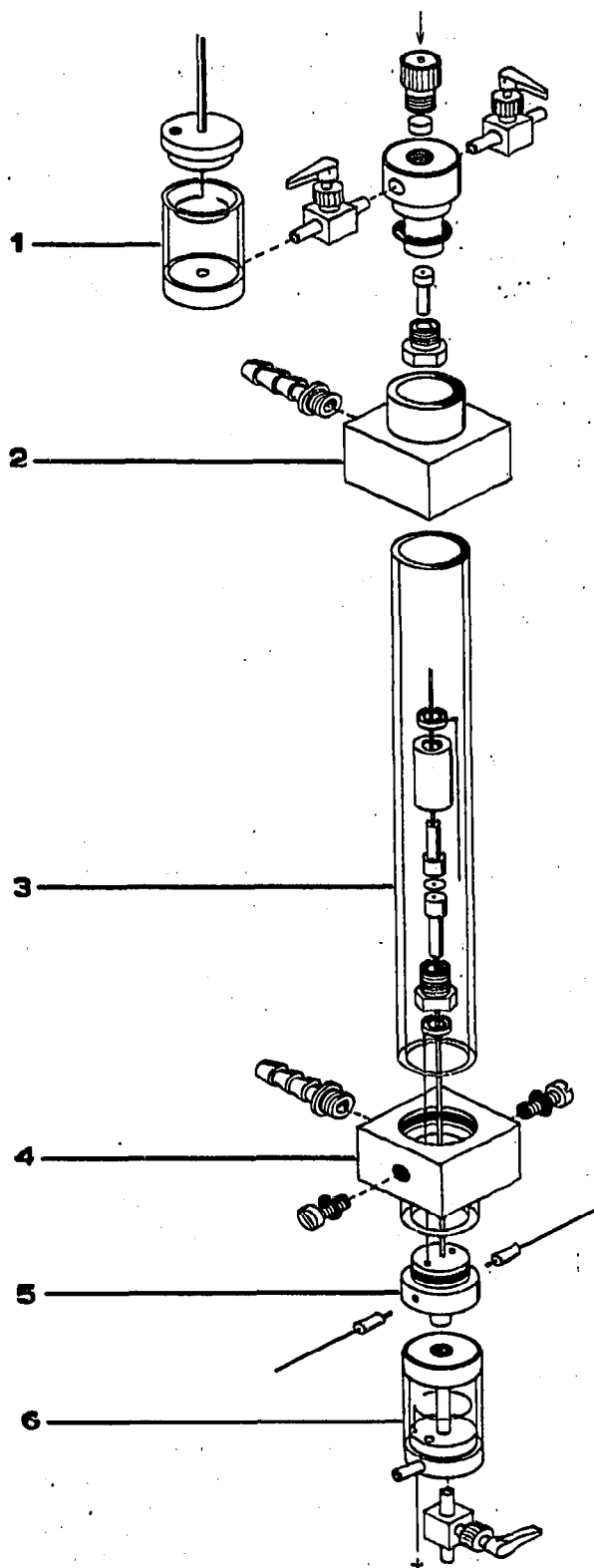


Fig. 1. Isotachophoretic equipment with conductivity detection of the zone boundaries. The cooling of the PTFE capillary tube is achieved directly, with a non-conductive liquid. 1 = Compartment for terminating electrolyte and sampling port; 2 = thermostatted water (out); 3 = micro-sensing electrodes; 4 = thermostatted water (in); place at which the detector is mounted; 5 = electrical connections; 6 = counter-electrode compartment with semipermeable membrane; connection towards reservoir with leading electrolyte.

possibly because of the presence of absorbed ionic material. The presence of A.C. components on top of the D.C. driving current makes this deterioration process worse.

It need not be explained further that conductive solvents cannot be used for cooling.

The reservoir for the terminating electrolyte and the injection block is made of Kel-F. Non-aqueous solvents such as methanol can therefore be applied.

To rinse the capillary and to refill it with leading electrolyte, the tap between the reservoir filled with the terminating electrolyte and the compartment for injection is closed. The tap on the connection to the drain is then closed.

The injection of the sample is now carried out with a Hamilton micro-syringe. The tap at the bottom is kept open while the needle of the syringe penetrates the septum; this needle enters the capillary bore of the injection block and the injection is made. The tap at the bottom is then closed and the tap between the reservoir filled with the terminating electrolyte and the injection block is opened, after which the syringe may be withdrawn. This is the most reliable method of injection\*.

The cathode compartment and the anode compartment are electrically connected and the analysis can then be carried out. The electrode compartment (Fig. 1; No. 6) consists of a semipermeable membrane (cellulose acetate) to prevent hydrodynamic flow during the analysis, due to the difference in levels between the two electrode compartments, and to reduce the electroosmotic flow.

Particular attention is required in the design of the detector. The miniature sensing electrodes are a part of the wall of the capillary tube<sup>5</sup>.

The electrodes, made of thin platinum foil (0.1 mm), are separated only by a sheet of insulating material (0.05 mm). The holes drilled in these electrodes are adapted to the diameter of the capillary tube. To minimize the dimensions of the sensing electrodes, experiments were carried out with several noble metals vaporized on both sides of a sheet of insulating material (Terylene, 0.05 mm) at  $10^{-5}$  Torr.

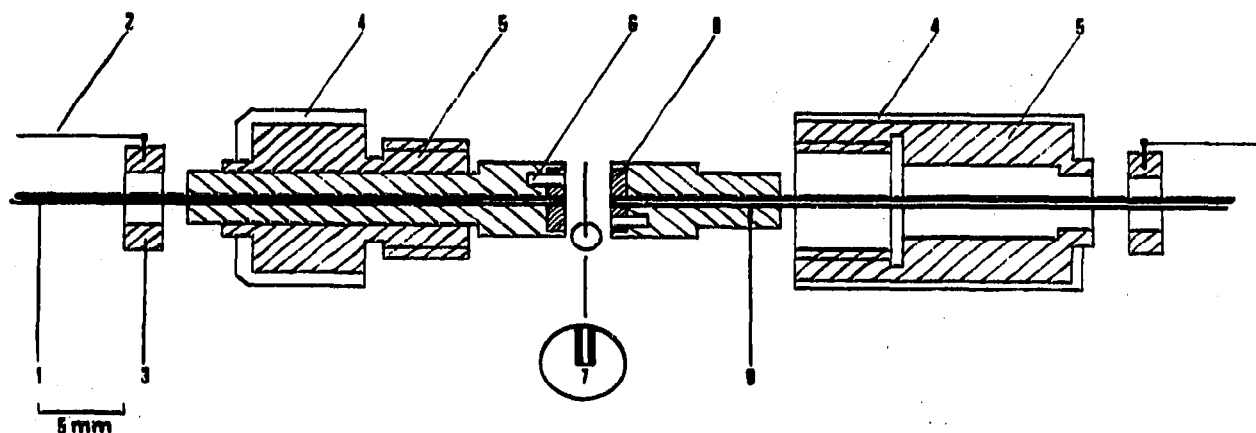


Fig. 2. Conductivity detector with micro-sensing electrodes. 1 = Capillary tube of PTFE (0.45 mm I.D., 0.7 mm O.D.); 2 = electrical connection with the sensing electrodes; 3 = electrical connection from lead with piece of brass, fitting the capillary tube; 4 = screen of stainless steel; 5 = insulator disc; 6 = piece of copper serving as the electrical contact with the sensing electrodes; 7 = sensing electrodes: a disc of platinum, a disc of insulating material and another disc of platinum; 8 = piece of plastic material, serving as a liquid-tight connection; 9 = piece of brass fitting the capillary tube.

\* The syringe must not remain inside the capillary bore of the injection block, because the metallic needle will act as a bipolar electrode, owing to the potential gradient over the capillary bore, and hydrogen and oxygen will be produced.

The construction of the sensing cell is shown in Fig. 2. The components (9) that are necessary for fitting the capillary tube are made of brass in order to prevent an excessive temperature increase just at that point where the conductivity has to be measured. The pieces of Kel-F (8) serve as a liquid-tight mechanical connection between the brass fitting (9) and the sensing electrodes made of platinum. The electrical contact between the brass fitting and the sensing electrodes is provided by a copper pin (6). Part of the miniature sensing electrodes and the insulation material in between are shown enlarged so as to demonstrate the construction (7).

For the measurements of the resistance of the liquid between the electrodes, a D.C. method and an A.C. method were used in the case of direct contact between the sensing electrodes and the electrolyte inside the narrow-hole tube.

In the D.C. method for the determination of the conductivity, the driving current was used, the resulting potential between the sensing electrodes being a direct measure of the resistance. A differential operational amplifier (Analog Devices, Type 272 J) was used. This amplifier could be connected directly to the sensing electrodes, being at a high tension. The D.C. potential at the sensing electrodes does not exceed 5000 V.

The input impedance of this amplifier was sufficiently high to prevent the formation of disturbing gas bubbles at the sensing electrodes due to the measuring current. The eventual evolution of hydrogen by the polarisation of the micro-sensing electrodes caused by the driving current is discussed below.

The circuit for measuring the resistance between the sensing electrodes by the A.C. method is shown in Fig. 3. The capacitors (Fig. 3; No. 1) that were used for the separation of the circuit from the high D.C. potential, were sufficiently large

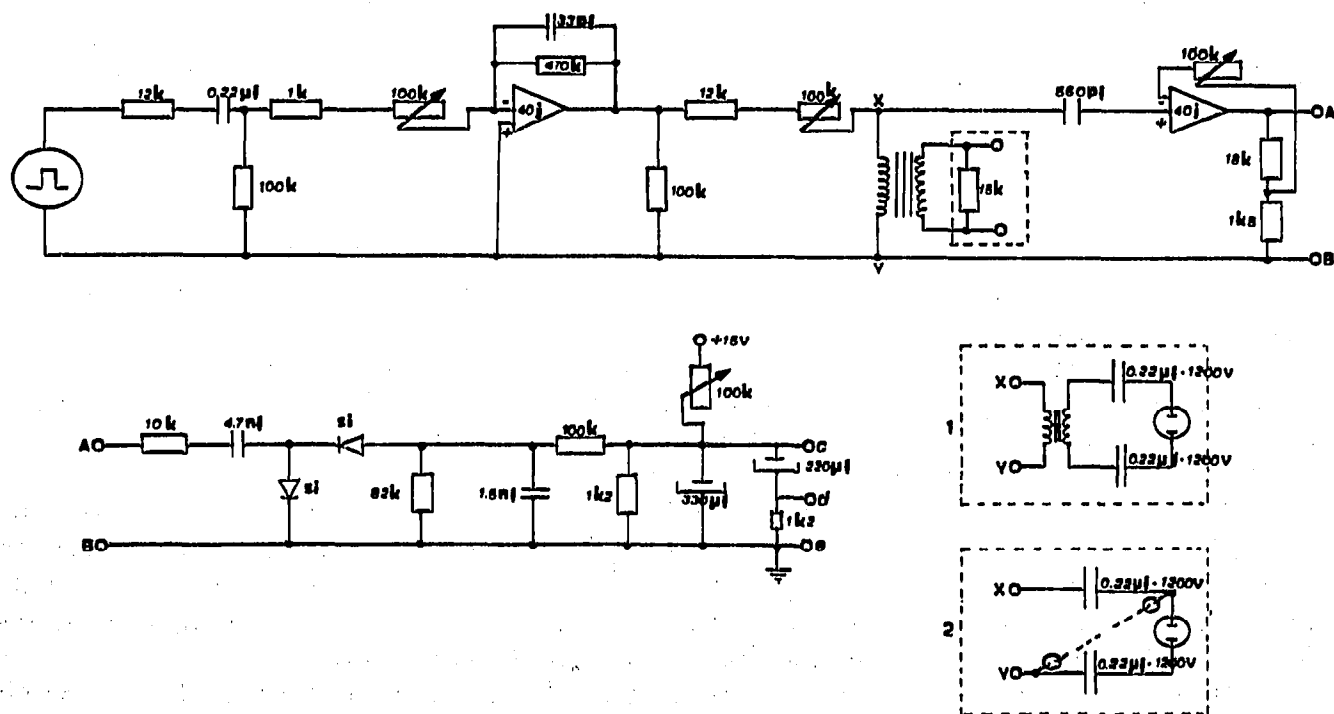


Fig. 3. Circuit diagram of the measuring equipment, with a transformer coupling (1) and a capacitor coupling (2). For liquid chromatography, a similar device can be applied. The measuring cell can be connected in this instance directly on the points X, Y.

( $0.2 \mu\text{F}$ ,  $1200 \text{ V}$ ) to allow their A.C. impedance to be neglected. Also, the potential measured is a direct measure of the A.C. impedance between the sensing electrodes. The response, however, is not linear with the circuit given for low measuring currents ( $i_{\text{A.C.}} > 50 \mu\text{A}$ ).

Excellent coupling of the measuring circuitry with the sensing electrodes at high D.C. potential was obtained with a transformer coupling (Fig. 3; No. 2). A transformer with both a primary coil of 500 turns and a secondary coil of 500 turns ( $0.1 \text{ mm}$  copper wire) was used. The capacitors ( $0.2 \mu\text{F}$ ,  $1200 \text{ V}$ ) were used to prevent a short-circuit for the D.C. current via the secondary coil. The D.C. resistance of the coil mentioned is about  $500 \Omega$ , while the D.C. resistance of the electrolyte between the sensing electrodes is approximately  $15 \text{ k}\Omega$ , *i.e.*, the electrolyte between the sensing electrodes consists of histidine ( $0.01 \text{ M}$ ) and histidine-HCl ( $0.01 \text{ M}$ ). With this type of coupling, high D.C. potentials can be applied even at the position where the sensing cell is mounted; this is in contrast to the transformer coupling. The sensing cell need not be mounted at the end of the capillary tube, where the driving D.C. potential approaches earth potential.

The possible disturbance of the analysis by a current leak through the capacitors, resulting in the production of gas by the sensing electrodes, is, of course, also much less.

Owing to the construction of the cell as part of the electronic measuring circuit, the frequency must be found at which the highest response is obtained. The A.C. generator (Hewlett-Packard, Type 3300 A, function generator) enables several frequencies to be used in order to find the optimal response for the circuit shown (Fig. 4). Fig. 4 shows the relation between the frequency of the A.C. generator and the final response measured by a recorder. Maximum response is obtained between 4 and 4.5 kHz. The influence of small changes in the frequency can be neglected if the working frequency equals the frequency at this maximum. Similar curves are measured directly during an isotachophoretic analysis using several ionic species as the terminating ions.

## EXPERIMENTAL AND RESULTS

The physical chemist usually distinguishes between two extreme types of ideal electrodes<sup>6,7</sup>.

The first is the reversible type, in which ions from the solution are actually charged and discharged, so that a steady current is possible. The D.C. potential of the electrodes has a well defined value, which depends on the current and the composition of the solution.

The second type is the polarized electrode, in which no transformation of ions take place, no steady current can pass and any that does pass represents the charging and discharging of a double layer made up of the electrode and the ions very close to its surface. The double layer is a structure that acts, as is well known, as a capacitance, the value of which is dependent on the potential across it.

This second type of electrode has no well defined D.C. potential. It may vary greatly under apparently identical circumstances and is enormously influenced by trace amounts of substances or impurities, as shown in this paper. Metallic electrodes are, in fact, always combinations of both types, and their impedance as a function

of frequency shows the extent to which one or the other mechanism dominates their behaviour. For instance, a bright platinum electrode in a fluid that is rich in adsorbable compounds has an impedance that is very nearly proportional to  $\omega^{-1}$  over the frequency range from 1 to 20 kHz. Hence it is nearly an ideal polarized electrode.

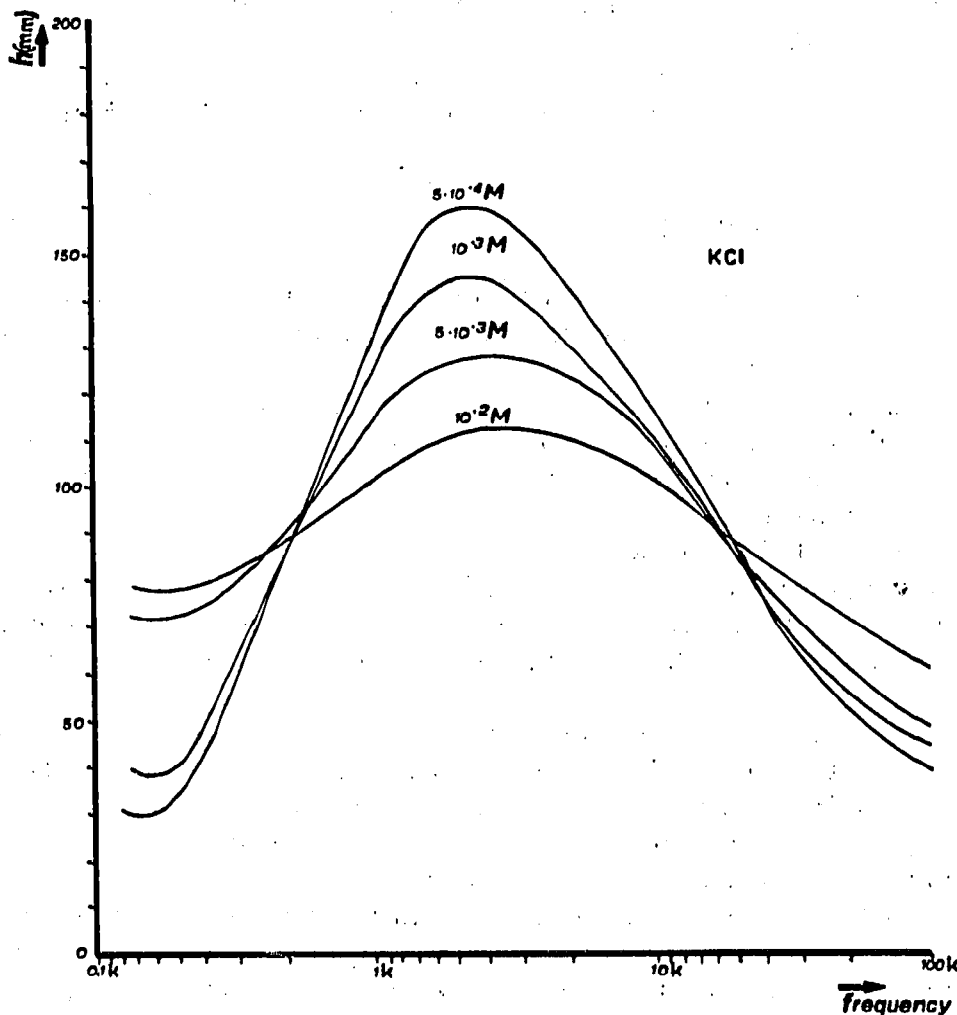


Fig. 4. The relation between the frequency of the measuring signal and the step-response as finally registered by a recorder, for the circuitry shown in Fig. 3.

For the same electrode in a saline solution, more complicated behaviour is observed. By the addition of Triton X-100 to this saline solution the relation mentioned above is obtained again. Without the addition of an inhibitor for redox reactions, the electrode reaction is rapid and the current is limited by diffusion of the reacting ions and the products between the surface of the electrode and the bulk of the solution. The impedance will decrease proportionally to  $\omega^{-1/2}$ . If the electrochemical reaction is itself slow, the impedance will be lower for small  $\omega$  and higher for large  $\omega$ , than for the case in which diffusion predominates. In practical cases, inhomogeneities in the electrode material will spread out the band of frequencies for which the impedance decreases only slowly with frequency, as the rate of the reaction is strongly dependent on the D.C. potential of the electrode, which will vary over the surface.

In instances in which an insoluble reaction product covers the electrode surface, the multiplicity of diffusion paths may have a similar effect. This is the case with a silver-silver chloride electrode in either a saline solution or a saline plus gelatin solution. It has an impedance that decreases approximately proportionally to  $\omega^{-1/2}$  over a wide range of frequencies. The D.C. potential of the electrode can make a considerable difference in the impedance function by changing the rate or even the nature of the reaction carrying current. Therefore, the balance between this potential and diffusion is responsible for the impedance. The real and imaginary components of the impedance of a particular electrode decrease as approximately the same function of  $\omega$ .

A fluid-filled micro-electrode can be compared with a low-pass filter that is D.C.-stable. They must be used when signals are large and for which D.C. and low potentials are of interest.

The metallic electrode is a high-pass filter, which is D.C.-unstable. The optimum use can be found when rapidly varying signals are of interest and the amplitude of the signals may be close to the noise level.

It should be remembered that the micro-sensing electrodes, as described in this paper, are also a combination of both types. Owing to the driving current, polarization of the micro-electrodes occurs, *i.e.*, these electrodes may act as charge-transfer electrodes. This depends on the potential gradient across the electrode caused by the direct driving current and the composition of the electrolyte.

When the direct driving current itself was used for measuring the conductivity difficulties similar to those found by several workers who used metallic electrodes were observed. Partial polarization of the metallic electrode made the registration of the boundaries obscure.

To make a study of the interaction of the electrolyte, the driving current and the micro-sensing electrodes, the electrodes were constructed such that the electrolyte inside the capillary remained surrounded by an uninterrupted cylindrical wall (Fig. 2). The distribution of the measuring current is much more linear, especially if this construction is compared with the type of cell with so-called pin-electrodes, which are mounted opposite to each other. Moreover, an advantage of measuring the conductivity between disc electrodes of platinum is the simple construction of the sensing cell. The capacitance of the sensing cell, shown in Fig. 2, is about 3 pF, if electrodes with a diameter of 5 mm and a thickness of 0.05 mm are used.

In the experiments described, the  $R_{A.C.}$  value varies from approximately 15 k $\Omega$  at the beginning to 50 k $\Omega$  at the end of the experiment. While the driving current is kept constant, the potential varies during the experiment from 4 to 20 kV.

If the metal electrodes are applied directly in contact with the electrolyte, the driving current causes a polarization as shown in Fig. 5.

Although the platinum electrode has the same potential all over its surface, it acts as a bipolar electrode with the cathode directed towards the anode compartment of the electrophoretic equipment and the anode towards the opposite side.

Dependent on the potential gradient and the composition of the electrolyte, the micro-sensing electrodes can be polarized or act as a charge-transfer electrode, as discussed above.

From the above discussion it is clear that normally the electrodes, under the conditions used, are located between the two extremes.



The moment at which oxidation and/or reduction starts depends on various factors: the roughness of the electrode surface<sup>8</sup>; the composition of the electrolyte<sup>9</sup>; the configuration of the electrodes<sup>10</sup>. Minor effects are: the influence of the temperature; the pretreatment of the electrode surface; the pressure; the current density.

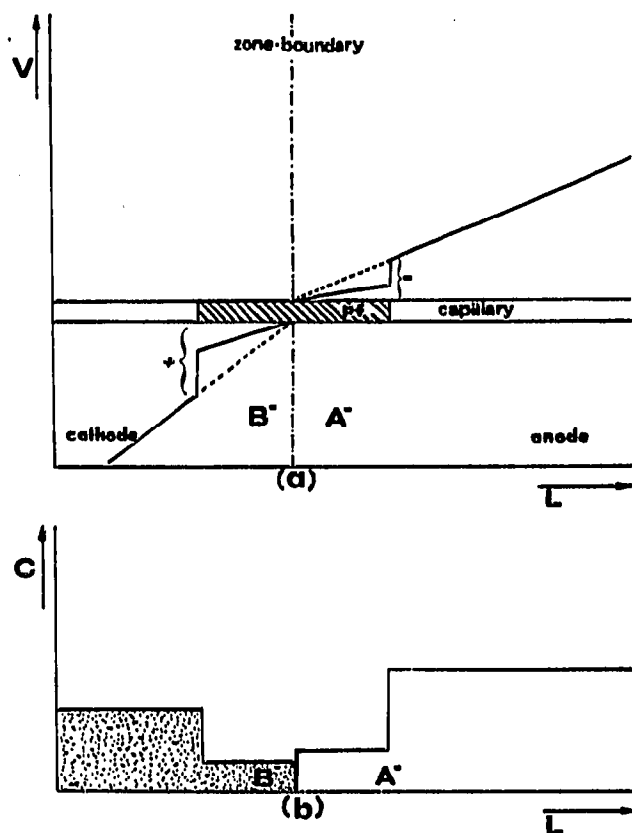


Fig. 5. The potential gradient, inside the capillary tube at the place where the electrode is mounted, decreases if the micro-sensing electrode changes its character from an ideal polarized electrode into a charge-transfer electrode, owing to the negligible resistance of the platinum electrode itself. Schematically, it is shown that a piece of metal, in general, acts as a bipolar electrode. Owing to this effect, the concentration of the electrolyte, inside the capillary at the place where the electrode is mounted, (b), will drop if the electrode changes its character in order to fulfill the isotachophoretic condition.

Data from the literature<sup>11</sup> show that under the conditions chosen, an overpotential of about 70 mV is adequate for the evolution of hydrogen, while the evolution of oxygen requires at least 700 mV (bright platinum electrodes).

Of course, difficulties can sometimes be expected if the micro-sensing electrodes are directly in contact with the electrolyte. If ions are present that can be oxidized more easily than the hydroxyl ion, or reduced more easily than the proton, *e.g.*, the equilibrium  $\text{Fe}^{3+} \rightleftharpoons \text{Fe}^{2+}$ , particularly obscure results are obtained. Although under normal conditions some hydrogen may be produced at the beginning of the experiment, the evolution stops, because the difference in potential between the anodic side of the bipolar sensing electrode and the electrolyte, which is surrounded by this electrode, is insufficiently great to start the evolution of oxygen (provided that no anion is present that can be oxidized more easily than the hydroxyl ion).

If a sensing electrode changes, for any reason, into a charge-transfer electrode, the zone length, as actually measured, of the ionic species present in that zone is longer than expected according to the concentration in the sample.

Particularly if thin electrodes made of palladium, and also of other noble metals, are used, considerable amounts of gaseous hydrogen will dissolve in the metallic layer of the sensing electrode. The metal does not lose its metallic character, but with palladium  $\text{PdH}_2$  is formed. The resistance of the electrode increases by this effect. Thin electrodes sometimes bend owing to the increase in volume caused by the dissolved hydrogen, if no precautions are taken.

If the overpotential against the formation of oxygen is exceeded, the bipolar electrodes start to produce hydrogen and oxygen. The zone boundary passage, which

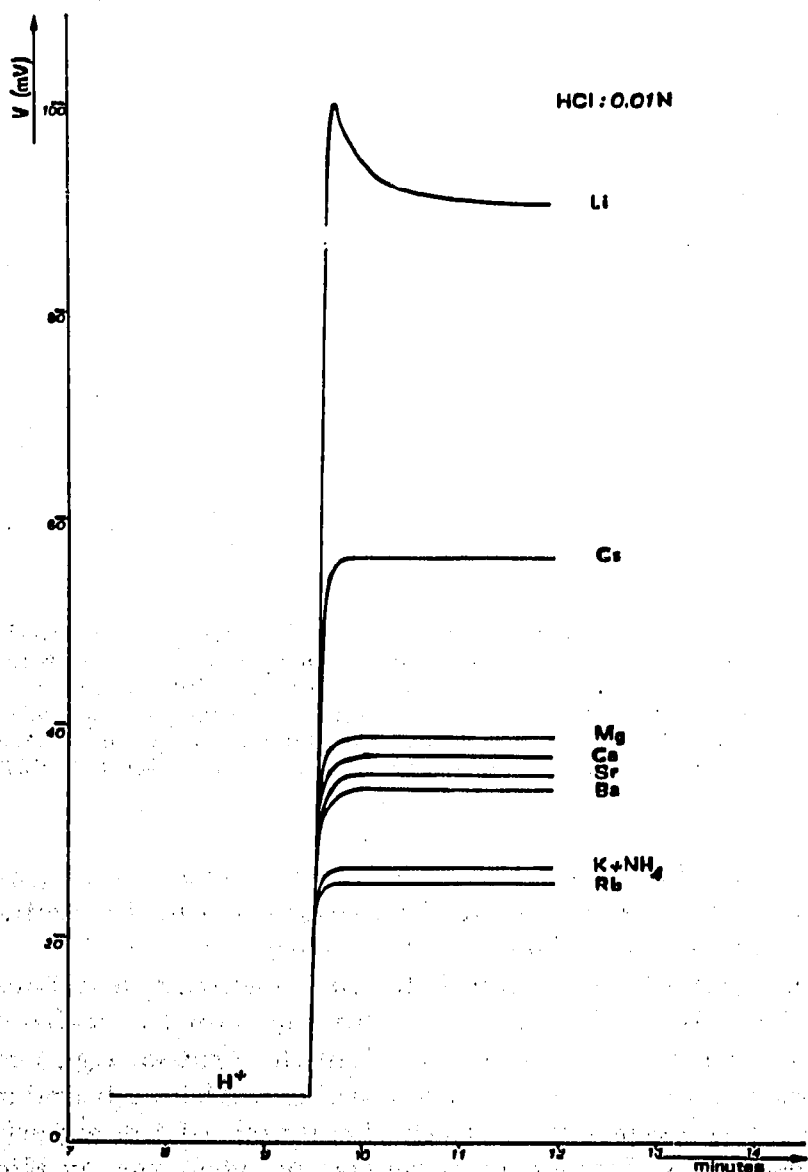


Fig. 6. Step-responses of several zones after the leading electrolyte  $\text{HCl}$  ( $0.01\text{ M}$ ). A current  $i = 50\ \mu\text{A}$  is applied. Clearly visible is the decrease in the concentration ( $\text{Li}^+$  ion) due to the change in character of the micro-sensing electrodes. The increment in potential gradient is not so great that the analysis is disturbed, and equilibrium is reached again.

causes the electrodes to start the production of gas because a higher potential gradient is present, is registered with an overshoot (Fig. 6).

Fig. 6 shows a series of boundary passages. The leading electrolyte consists of HCl (0.02 *M*), unbuffered system, while the cations themselves were used each time as the terminating ions. The current was stabilized at 50  $\mu\text{A}$ . The registration was made with platinum electrodes of 0.1 mm thickness and a circuitry shown in Fig. 3.

If an ion that is slower than the  $\text{Li}^+$  ion, *e.g.*, the  $\text{Fe}^{3+}$  ion, is present the evolution of gas is such that it cuts off the electrical current.

A possible explanation of the overshoot is as follows (Fig. 5b). The more the zone is situated towards the terminating zone, *i.e.*, the smaller is the mobility, the higher is the temperature and consequently the Joule heat produced by the driving and measuring current. The conductivity increases with temperature, so an overshoot may be expected if a boundary passage is measured with fairly high differences in conductivity. This, however, is not the only effect.

At the moment the electrodes start to conduct electricity, *i.e.*, the electrodes change from polarized electrodes to charge-transfer electrodes, the potential gradient over the electrolyte, surrounded by the charge-transfer electrode, decreases immediately. Owing to the electroneutrality principle, the ions in this zone must follow the equally charged ions of the same species in front of it. The only possible way in which this condition can be fulfilled is for the concentration to decrease. The electrodes are slowly coated with a layer of gas, and less of the driving current then passes through the sensing electrodes. The potential gradient adjusts itself again and so does the concentration.

If the potential gradient over the zone considered is not too great, the sensing electrodes change again into polarized electrodes at the moment the electrode surface is entirely covered with gas. The evolution of gas cannot be maintained and stops automatically. Of course, the noise will increase because a layer of gas is highly sensitive to many kinds of variations.

If the surface area of the bright platinum electrode is increased by covering it with platinum black, the overpotential against the formation of hydrogen and oxygen is decreased. If the boundary passage is such that a new steady state can be formed, equilibrium is attained much quicker with platinum black sensing electrodes (Figs. 7B and 7C).

The electropherogram in Fig. 7A, showing only the boundary passage  $\text{H}^+ - \text{Ca}^{2+}$ , was obtained with a bright platinum electrode. The electropherogram in Fig. 7B was obtained with electrodes covered with platinum black; a current of 50  $\mu\text{A}$  was passed for 5 min, using 2 g of  $\text{H}_2\text{PtCl}_6$  and 30 mg of  $\text{Pb}(\text{Ac})_2$  in 100 ml of water. The electropherogram in Fig. 7C was obtained with electrodes covered for 10 min (50  $\mu\text{A}$ ) with the same electrolyte. During the electrolysis, fresh electrolyte was pumped through the capillary tube. During the analysis, the current was stabilized at 80  $\mu\text{A}$ .

If the electropherograms in Fig. 7 are compared with those shown in Fig. 6 ( $i = 50 \mu\text{A}$ ), it can be seen that at higher current densities the overshoot can be expected to appear sooner. If the current is stabilized at 50  $\mu\text{A}$ , the potential gradient across the sensing electrodes is not sufficiently high to start the redox reactions. With bright platinum electrodes, analyses could be carried out without problems,

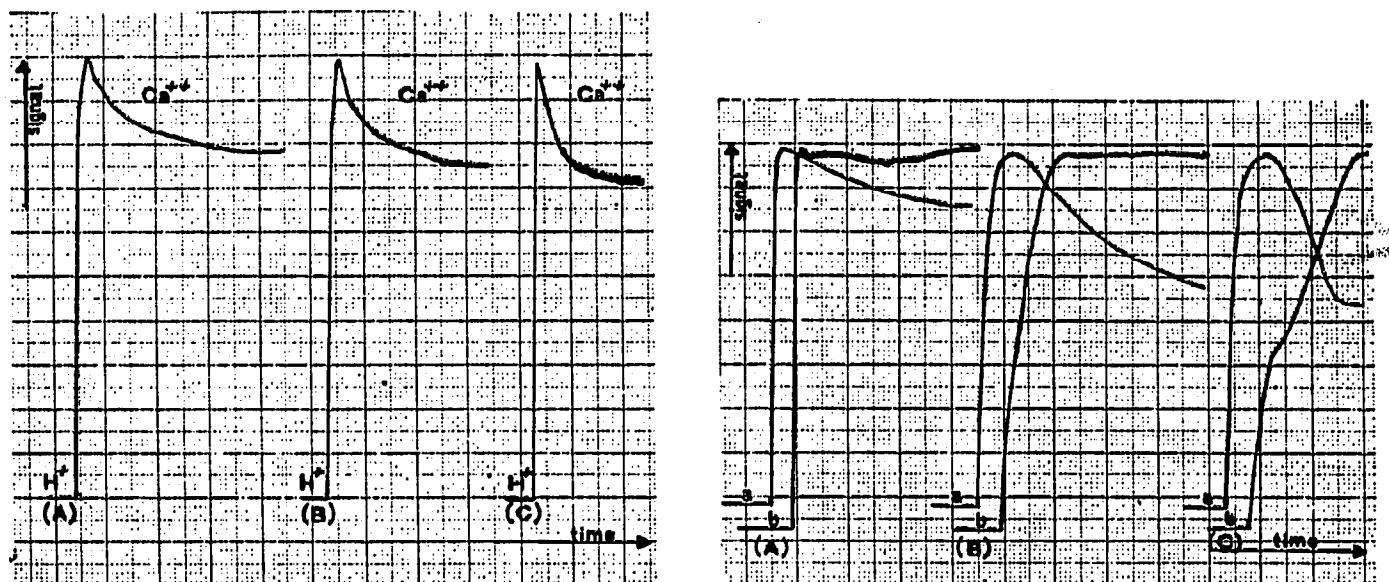


Fig. 7. The influence of a layer of platinum black, deposited on the sensing electrode, on the electrolytic equilibrium. The increment in the potential gradient is such that the electrode changes from an ideal polarized electrode into a charge-transfer electrode. (A) A step-response as detected by a bright platinum electrode. (B) A step-response as detected by a platinum electrode covered with a thin layer of platinum black. (C) As (B), with a thicker layer of platinum black.

Fig. 8. The influence of bathotonic components on the detection of zone boundaries in isotachopheresis by bright platinum electrodes.

while the evolution of gas was catastrophic if the platinum sensing electrodes were covered with a layer of platinum black. A few minutes after the zone of the ionic species with a low mobility had passed the sensing electrodes, the driving current decreased rapidly and finally the electrical current was cut off by the gas produced. The addition of bathotonic components greatly influences the equilibrium between the sensing electrodes and the electrolyte used (Fig. 8).

The conductivity of different zones was measured during an isotachopheretic run by the A.C. method (Fig. 8, curve b) and the D.C. method (Fig. 8, curve a). The same boundary passage,  $H^+-Ca^{2+}$ , as shown in Fig. 7, was studied, and the results are shown in Fig. 8A without any addition; in Fig. 8B with the addition of 0.2 ml of a solution containing 4 g/l of Triton X-100 to 100 ml of leading electrolyte; and in Fig. 8C with the addition of 0.5 ml of the same solution of Triton X-100. Fig. 8 shows that components which affect the surface tension also influence the degree of polarization of the sensing electrodes and hence the registration of the boundary passage. This had already been reported in the literature, because all kinds of easily absorbed organic "impurities" affect the capacitance of the double layer. The addition of Triton X-100 decreases the overpotential against evolution of oxygen, but makes the registration of boundary passages and conductivity differences more confused, in the case of cationic separation.

The adsorption of ions and uncharged substances can be distinguished in the treatment of isotherms. Accurate measurement needs to be carried out. It appears that, at present, the dependence of capacity-potential curves on the bulk activity of the adsorbate provides the best criterion.

The dependence of the standard free energy of adsorption on the electrode potential (or charge) is different for charged and uncharged species. While ionic adsorption (specific) leads to a linear relationship, uncharged particles cause a quadratic dependence.

It needs no explanation that the addition of Triton X-100 affects the D.C. measurement of the conductivity more than the A.C. measurement. This is shown very clearly in the electropherogram of the separation of oxalic acid, citric acid and acetic acid (Fig. 9).

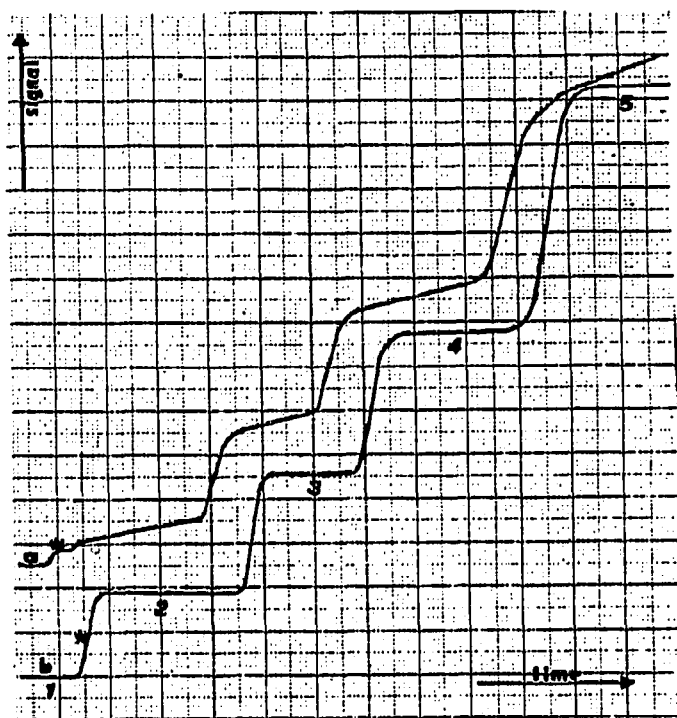


Fig. 9. The detection of zone boundaries in isotachophoresis by D.C. measurement (a) and A.C. measurement (b) as discussed in the text. At the points marked with asterisks, the evolution of both hydrogen and oxygen starts, which is more visible in the step-curves of the D.C. measurements because A.C. measurements are less affected by the formation of a capacitor by the thin layer of gas produced as the surface of the micro-sensing electrode. 1 = chloride; 2 = oxalate; 3 = citrate; 4 = acetate; 5 = glutamate.

As in the experiment shown in Fig. 8, the conductivity was measured by the A.C. method (curve b) and by the D.C. method (curve a). A mixture of histidine (0.01 *M*) and histidine-HCl (0.01 *M*) was used as the leading electrolyte. The terminating electrolyte was glutamic acid (0.01 *M*). The current was stabilized at 40  $\mu$ A. The mobilities of the anions are low in comparison with those of the cations. If the current is not stabilized at a low value, then even at the beginning of the sample zones the redox reaction disturbs the analysis.

It can be seen in Fig. 9 that the potential gradient from the oxalate zone is sufficiently great to start the production of oxygen and hydrogen or to initiate redox reactions\*.

The D.C. measurement of the conductivity of the different zones indicates

\* The potential gradient is constant in each zone, but increases from zone to zone towards the rear.

that the resistance increases slowly in each zone. In the zones of the ions with lower mobility, the increase in resistance is more pronounced. Because the electrolyte concentration is constant over all the zone, the evolution of gas makes the resistance increase to a greater extent per unit of time for D.C. measurements than for A.C. measurements, as shown in Fig. 9. The layer of gas produced by the electrode has a relatively low resistance for the A.C. measuring signals (4 kHz). The moment at which the evolution of gas starts, however, can be observed in both the electropherograms (marked with asterisks). To demonstrate that the addition of bathotonic components lowers the overpotential against redox reactions, two electropherograms of the separation of weak and strong acids are shown in Figs. 10 and 11.

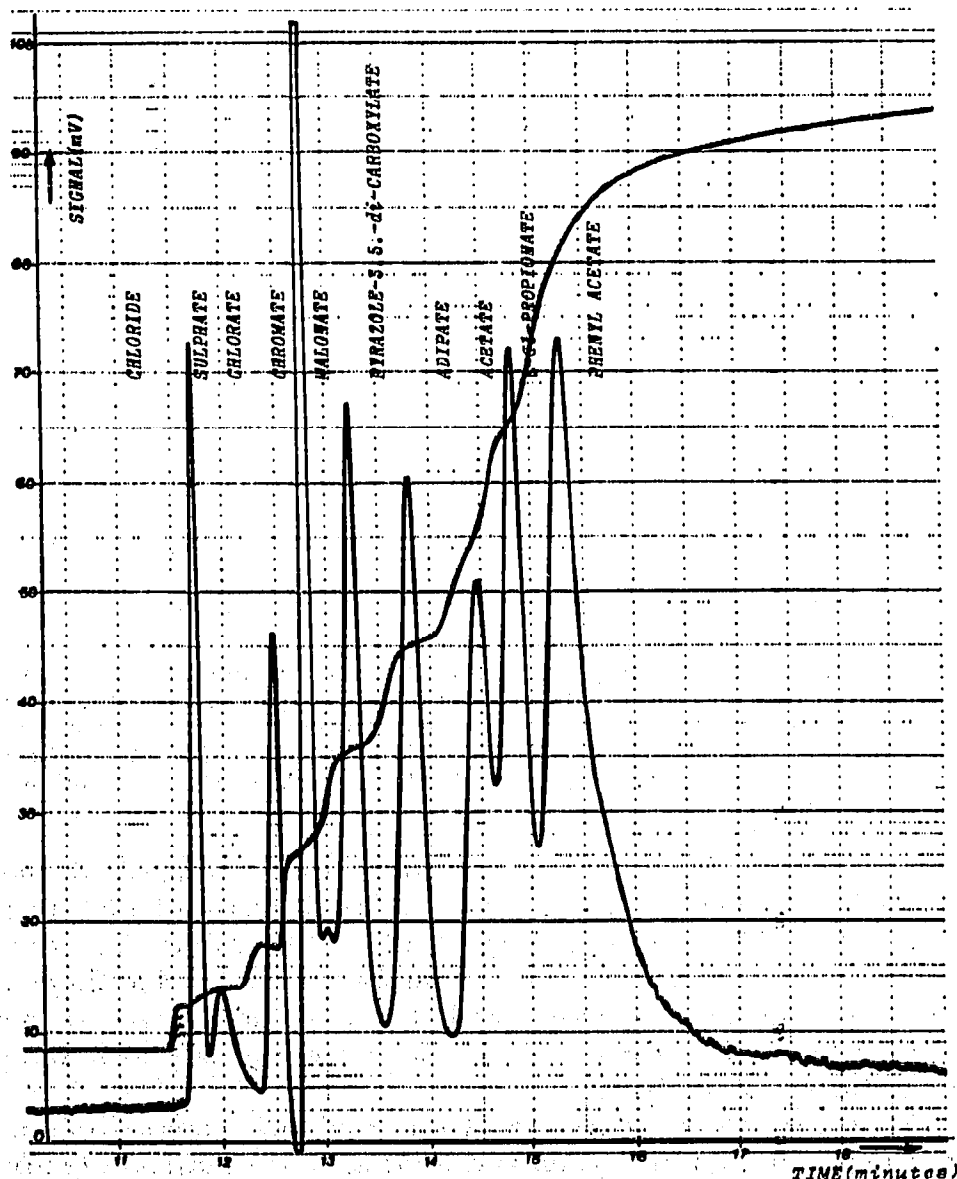


Fig. 10. The separation of a mixture of acids in the electrolytic system histidine (0.01 *M*) and histidine-HCl (0.01 *M*) at pH 6.02. The terminating electrolyte is phenylacetic acid (0.01 *M*). Clearly visible is the change in character of the measuring electrodes from polarized into charge-transfer, owing to the increment in potential gradient over the electrodes by the different sample zones. It is suggested that the boundaries are diffuse.

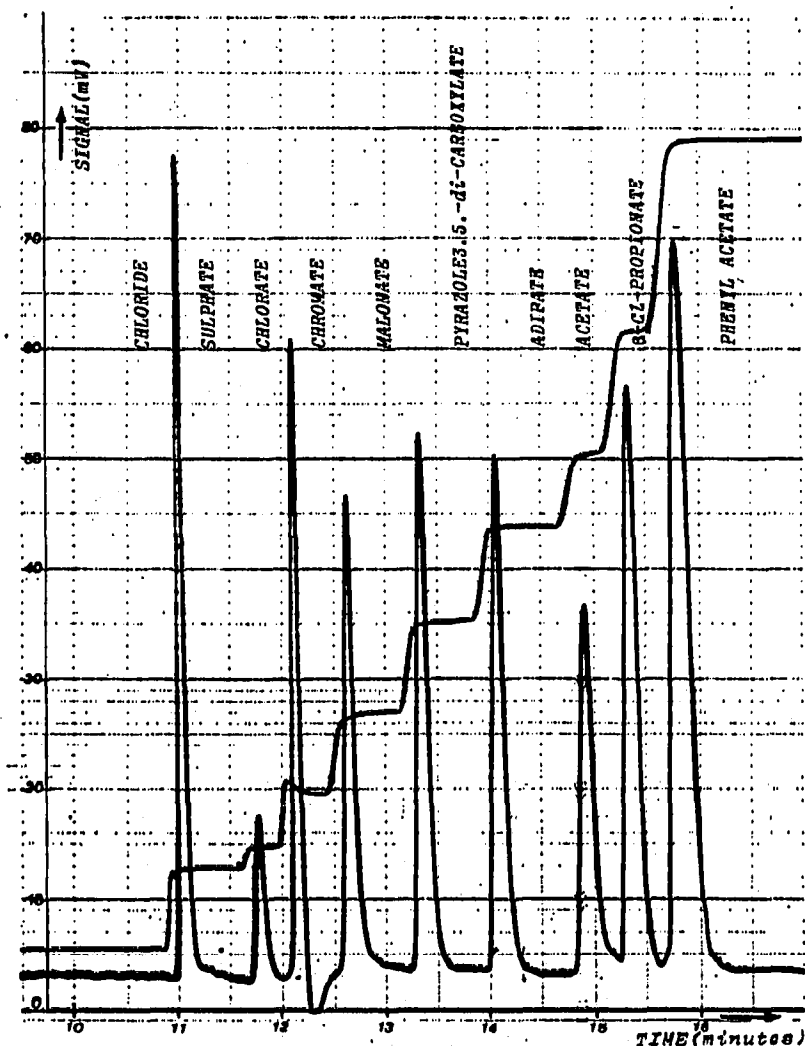


Fig. 11. Effect of the addition of a few drops (see text) of a dilute solution of Triton X-100 to the leading electrolyte. The overpotential against the electrode reactions is decreased. This isotachopherogram must be compared with the one shown in Fig. 10. The production of both hydrogen and oxygen starts in the chromate zone, at a higher potential gradient than that in Fig. 10.

The separation of sulphate, chlorate, chromate, malonate, pyrazole-3,5-dicarboxylate, adipate, acetate and  $\beta$ -chloropropionate was carried out. The leading electrolyte in both instances was histidine (0.01 M) and histidine-HCl (0.01 M). The terminating electrolyte was phenyl acetate (0.01 M). The current was stabilized at 40  $\mu$ A.

It can be seen in Fig. 10 that the evolution of gas starts in the first zone, behind the leading zone of chloride (*i.e.*, the sulphate zone).

The addition of a few drops of Triton X-100, in the diluted form as before, to the leading electrolyte gave a much more reliable separation (Fig. 11). All other conditions used in the separations shown in Figs. 10 and 11 were similar. It was also shown that higher current densities could be applied if the Triton X-100 was added to the leading electrolyte.

Fig. 11 shows that the evolution of gas starts in the chlorate zone. Increasing the electrical current shifts this point towards the zones with higher conductivities.

A decrease in the electrical current shifts this point towards the zones of ionic species with low mobilities. The derivation of the step-curves shows that the first steps are registered much more sharply and that all the peaks in Fig. 11 are sharper than the corresponding peaks in Fig. 10. This could indicate that the zone boundaries of the ions with high mobilities are much sharper\*. As already discussed, the zones seem to lack sharpness owing to the fact that part of the electricity (from the driving current) is transported via the electrodes\*\*.

The addition of  $\text{Na}_2\text{AsO}_4$  ( $10^{-4} M$ ) to the leading electrolyte increases the overpotential against the formation of hydrogen. Because the oxygen production, in the instances discussed, was the critical point at which the polarized sensing electrode changes into a charge-transfer electrode, similar results were obtained both with and without the addition of  $\text{Na}_2\text{AsO}_4$ . The contribution of the  $\text{Na}_2\text{AsO}_4$  ( $0.0001 M$ ) to the conductivity of the electrolytic systems can be neglected. Three methods can be recommended to prevent redox reactions, especially the production of gas: the micro-sensing electrodes can be made very thin, by vaporizing a thin film of a suitable metal on to an insulator; the micro-sensing electrodes can be coated by a thin film of an insulating material; and a component that suppresses redox reactions can be added to the electrolytes.

The use of thin electrodes, made by vaporizing metal on to an insulator, makes all types of redox reactions on the sensing electrodes still possible, but gas cannot be produced.

Under the conditions mentioned in this paper, potential gradients of the order of 50 mV can be expected, which are too great to be neglected. Our experiments with vaporized gold and palladium electrodes ( $0.5\text{-}\mu\text{m}$  thick) showed that the registration of the zone boundaries was still obscure, but the evolution of hydrogen and oxygen could not be observed.

The actual resistance between the two sensing electrodes was ten-fold with vaporised electrodes compared with the bright platinum electrodes of 0.05-mm thickness. This must be ascribed to contact between the metallic surface of the sensing electrode and the electrolyte. After some analyses, however, the contact between the sensing electrode and the electrolyte was even worse, possibly owing to the fact that hydrogen dissolves in the metallic layer of the sensing electrode. Under the microscope, it could be observed that the film was no longer smooth, but in some places the metallic film had loosened the insulator. Fig. 12 shows an isotachopherogram, again of a similar mixture as in Figs. 10 and 11.

The addition of Triton X-100 was made in the correct manner (2 ml of a solution containing 4 g/l of Triton X-100 is added to 1 l of leading electrolyte) and the dimensions of the electrodes are reduced ( $400\text{-}\mu\text{m}$  thickness) in order to suppress redox reactions.

A mixed step of chloride with the first sample zone, sulphate, is shown in Fig. 12, caused by the method of sampling.

If the sample is injected too low in the capillary bore of the injection port (Fig. 1, No. 1), too much of the leading electrolyte remains behind the sample and

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\* This cannot be checked by thermometric measurement of boundary passages.

\*\* It should be remembered that at the moment the evolution of gas starts, a certain amount of counterflow of electrolyte occurs, as the counter-electrode compartment is mechanically closed by a semipermeable membrane.



considerably longer times are needed to separate the chloride from the sample mixtures.

Although the resolving power is very high, some artifacts still cause problems because of the reliability of the conductivity detector. Electrodes coated with a thin film of a suitable insulator seem to give similar results, but the homogeneity in the coating-layer was not satisfactory. Research is being continued, especially with coated electrodes (*e.g.*, propargyl alcohol<sup>12</sup>). The concentrations of the acids, used in the sample of the electropherograms of Figs. 10, 11 and 12, were chosen arbitrarily.

For comparison, the mixture of acids, which was separated as described in this paper, was finally separated again in an equipment as described in ref. 4. The isotachopherogram is shown in Fig. 13. This isotachopherogram, registered by the

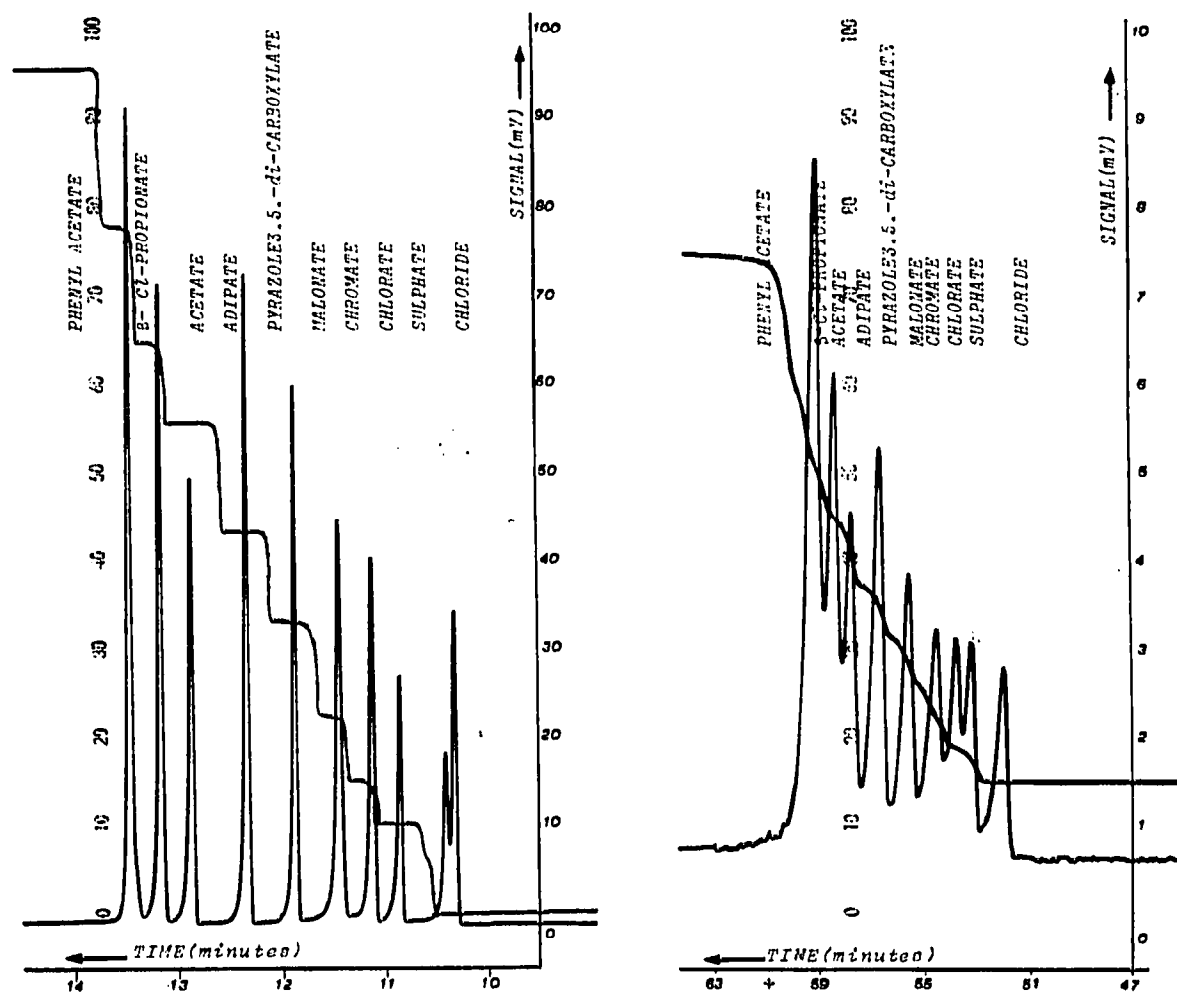


Fig. 12. An isotachopherogram, obtained with a conductometric detector, from a mixture of acids as in Figs. 10 and 11. The addition of Triton X-100 to the leading electrolyte was such that the electrodes remained polarized throughout the analysis.

Fig. 13. Isotachopherogram of a separation of a mixture of acids similar to that in Figs. 10, 11 and 12, in order to compare the results from a conductometric detector with those obtained with a thermometric detector. Special attention should be given to the speed of the recorder paper and the time for analysis. Because a thermometric detector requires a zone length of 5 mm to give qualitative and quantitative information, much more sample must be injected, resulting in considerably longer times for analysis to give optimal resolution.

thermometric detector, is identical with that deduced from signals from the conductometric detector.

The necessary length for resolving zones, with a thermometric detector, of the consecutive serried zones is 5 mm.

The injection of more sample, to resolve the integral curves\*, causes mixed zones between the adjacent zones, especially of those with a high mobility. To resolve these mixed zones, a longer capillary tube, or equipment that produces a counterflow of electrolyte, must be applied. This, on the other hand, results in appreciably longer times for analysis.

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\* A measure of the necessary qualitative information.