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ISOTACHOPHORESIS

PHENOMENA THAT OCCUR WHEN CONDUCTOMETRIC DETECTION IS APPLIED

F. M. EVERAERTS and P. J. ROMMERS

Department of Instrumental Analysis, University of Technology, Eindhoven (The Netherlands)

SUMMARY

Some phenomena involved in conductometric detection during isotacho-phoretic analysis are discussed and particular attention is paid to the addition of surface-active compounds, which influence electroendosmosis, the electrode reaction and heat transfer from the electrolyte towards the solid wall. These effects sharpen the recording of the zone boundaries.

A method is described for making the measuring electrodes sensitive for singly and doubly charged ions. The application of coatings also sharpens the recording of the electrophoretic analysis.

INTRODUCTION

Studies on new detectors with high resolution in isotachophoresis have so far led to the development of a conductometric detector[1] and a UV detector[2]. These detectors have similar resolving powers and indeed give a resolution at least 50 times greater than that of the thermometric detector (thermocouple or thermistor, mounted on the outside of the capillary). While the UV detector does not disturb the electrophoretic pattern, some exceptional cases occur in which components are destroyed or changed by UV light, and the conductometric detector may disturb the electrophoretic pattern due to the polarization initiated by the driving current. Because the addition of surfactants not only decreases the overpotential of the measuring electrodes[1], but also sharpens the zone boundaries, especially when a conductometric or UV detector is used, a study has been made in order to elucidate some of the phenomena involved.

It is assumed that the sharpening of the zone boundaries by surfactants is the result of two effects: electroendosmosis may be suppressed by the increase in the viscosity near the capillary wall, and the heat transfer* coefficient is increased, so that

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*This effect was measured by the difference in time required for equilibrium of the thermal signal (15 μm copper–constantan thermocouple, mounted on the outside of the capillary wall). The time required when surfactants were added was considerably shorter.

the temperature difference between the centre of the capillary and the wall decreases, which flattens the parabolic profile that normally occurs. Both effects result in sharper zone boundaries, although the real zone boundaries may be as sharp as they were without any addition. For some materials we have found that no additions of surfactants were needed, e.g., Perspex (acrylic).

The aim of this paper is to show that the choice of the electrode material, as well as the addition of detergents, for instance, influence the final signal if conductometric detection is used. Also, we found that if a layer was deposited on the measuring electrodes, unusual phenomena were observed that depended on the measuring frequency of the electric current. Large differences were found if d.c. measurement of the conductivity was used instead of a.c. measurement[1].

A method is described for making the measuring electrodes sensitive to the presence of singly and doubly charged ions. Special attention is paid to the suppression of electrode reactions.

EXPERIMENTAL AND RESULTS

Two approaches can be used in order to suppress electrode reactions, both of which result in an increase in the overpotential against electrode reactions on the measuring electrodes: (a) the addition of components that inhibit the electrode reactions; and (b) coating of the detection electrodes homogeneously with an inert coating (this method limits the use of a.c. current for detection).

The method used for measuring the conductivity during isotachophoretic analyses is similar to that as discussed in a previous paper[1], i.e., both d.c. and a.c. methods are applied. From the driving current, a different potential gradient can be found for the different zones. With an electronic device, the conductivity can also be measured by means of an extra a.c. signal of which the frequency may be varied[3].
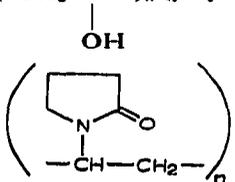
The use of additives

Components that inhibit electrode reactions are characterized by strong adsorption on the electrode surface. The electrode reactions may be inhibited in two ways[4]: (1) the transport of ions involved in the electrode reactions towards the electrodes is decreased as a result of an increase in viscosity in the vicinity of the measuring electrodes; and (2) the inhibitor, as it is adsorbed, inhibits the reaction by its presence. Generally, adsorption on the electrode surface is caused by the interaction of free-electron pairs (e.g., in oxygen, nitrogen and sulphur compounds) or by π-electrons (e.g., in aromatic compounds). Again, two groups can be distinguished: surface-active compounds, including detergents, and organic nitrogen or sulphur compounds, which are commonly used as corrosion inhibitors.

Because the additives are to be used in electrophoretic analyses, compounds must be selected that do not take part in electrophoretic transport (non-ionogenic compounds). Exceptions are those compounds that both inhibit the electrode reaction and can be applied as the buffering counter-ions, e.g., pyridine and related compounds.

The surface-active components studied were not only detergents, but also some soluble polymers. Detergents consist of a polar and an apolar part. The non-ionic part consisted mainly of 8–20 units of ethylene oxide, condensed with units of 8–20

carbon atoms, with or without functional groups. The apolar part can be formed by alkylphenols, alkyl alcohols, alkylamines, alkyl mercaptans or alkanes. Some possibilities are shown in Table I. Of these compounds, Triton X-100 and Mowiol 8-88 are especially useful. All of the compounds were purified on a mixed bed ion exchanger. The nitrogen and sulphur compounds were expected to be particularly useful, because they tend to show surface-active activity and are used commercially as corrosion inhibitors. However, these compounds adversely affect the analysis, possibly because they take part in the electrophoretic transport. The additives could not inhibit the interaction between the electrode and the chromate ions, which show the most pronounced electrode reaction, and this is discussed further later in the paper. Sharper boundaries were obtained than calculated by Van der Steen et al.[5].

TABLE I

SURFACE-ACTIVE COMPOUNDS USED IN ISOTACHOPHORETIC ANALYSIS

Compound	Structure	Commercial source
Triton X-100	$(C_2H_4O)_{9-10}$–monoisooctylphenol	Rohm & Haas, Philadelphia, Pa., U.S.A.
Ethomene T/20	$(C_2H_4O)_{20}$–talc amine	Armour Industrial Chem. Co., Chicago, Ill., U.S.A.
Priminox 32	$(C_2H_4O)_{32}$–tertiary amine (chain length unknown)	Rohm & Haas
Serdox ZCA-10	$(C_2H_4O)_{10}$–C_{12-18} amine	N.V. Servo, Delden, The Netherlands
Serdox NJAD-20	$(C_2H_4O)_{20}$–C_{16-18} amine	N.V. Servo
Nonic 218	$(C_2H_4O)_{9-10}$–tert-dodecylmercaptan	Pennsalt Chem. Corp.,
Mowiol 8-88	$(-CH_2-CH-)_n$ (polyvinyl alcohol) $\mid$ OH	Philadelphia, Pa., U.S.A. Hoechst A.G., Frankfurt, G.F.R.
PVP	(polyvinylpyrolidone)	Fluka, Buchs, Switzerland
PEG 200	$(-CH_2-CH_2-O-)_n$ (polyethylene oxide, molecular weight 200)	E. Merck, Darmstadt, G.F.R.

An improvement in the detection was found when either an a.c. or d.c. detector was used for the conductivity, although a difference between them was observed. In order to accentuate this difference, gold was used as the electrode material because it can easily be passivated and the influence of possible electrode reactions is greater. Unusual effects were obtained when gold was used.

In order to demonstrate the effects on various ionic species, the test mixture described earlier[1] was applied, consisting of sulphate, chlorate, chromate, malonate, pyrazole 3,5-dicarboxylate, adipate, acetate and β-chloropropionate. The leading electrolyte was histidine (0.01 M) and histidine·HCl (0.01 M) and the terminating electrolyte was phenyl acetate (ca. 0.01 M). The electric current was stabilized at 80 μA. Fig. 1 shows the isotachopherogram for the separation of this mixture when a.c. (left) and d.c. (right) detection was used.

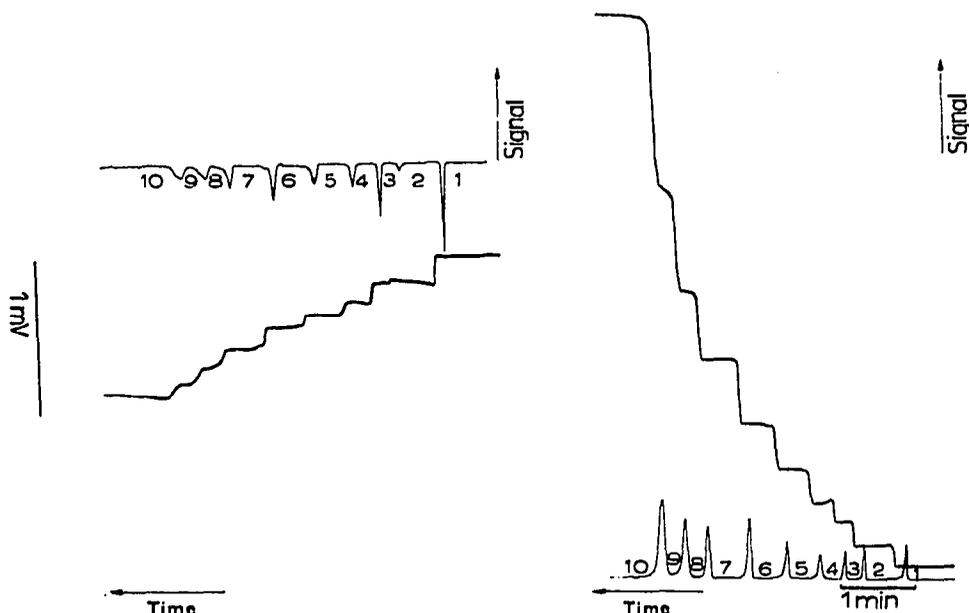The a.c. method gives the impression that the conductivity of the zones

Fig. 1. Isotachopherogram of the separation of sulphate (2), chlorate (3), chromate (4), malonate (5), pyrazole 3,5-dicarboxylate (6), adipate (7), acetate (8) and β-chloropropionate (9). The leading electrolyte was histidine plus histidine·HCl (1) and the terminating electrolyte was phenyl acetate (10). The electric current was stabilized at 80 μA. The left-hand trace shows an isotachopherogram when the a.c. method was applied in combination with gold measuring electrodes on which a layer of oxide was formed after many analyses. The right-hand trace shows a similar isotachopherogram when the d.c. method was applied.

decreases towards the terminator, whereas the d.c. method shows the normal isotachophoretic pattern. This effect must be ascribed to the dominating influence on the capacity of the passivated oxide layer of the gold surface, and this is discussed later.

Many coatings were tested with these gold electrodes, because they were easily fixed on gold. Sometimes the coatings were formed automatically during the electrophoretic process by the d.c. driving current, and the formation of such layers could easily be recognized during the recording of the sulphate step*. This effect is discussed later.

Some other unusual effects occurred when surface-active components were added. The amount of a surface-active component added does not influence the analyses strongly. The concentration can be varied from 2% to 0.05% without any recognizable difference in the recording of the zone boundaries. Of course, the material may not consist of ionic impurities.

Another phenomenon that occurs is the "memory" effect. When an analysis was carried out with Nonic 218 and the capillary was then rinsed, subsequent experiments without the addition of Nonic 218 gave a low resolution. However, when experiments with Mowiol were performed, many subsequent analyses could be made without the addition of Mowiol, as Mowiol is difficult to remove. It can therefore be

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* If a layer (coating) is present on the measuring electrode, equal conductivities may be recorded differently if the A.C. method is used and the zones consist of singly and doubly charged ions.

concluded that the adsorption of surface-active compounds is really important. Other workers have also reported[6] that polyvinyl alcohol (Mowiol) shows little desorption if adsorbed. From our experiments so far, Mowiol proved superior to the other compounds, even Triton X-100, especially if used for long times. Triton X-100 shows a kind of saturation effect after some time, which results in poor resolution.

Some corrosion inhibitors were also tested in two groups of experiments: (1) small amounts were added to the leading electrolyte, *e.g.*, thiourea and benzothiazole; and (2) compounds were used as the buffering counter-ion, *e.g.*, pyridine and β-picoline.

These compounds also sharpened the pattern, but not better than Mowiol. When gold electrodes were used, coatings were again formed during the analysis, as shown by the higher sensitivity for zones with doubly charged ions, *e.g.*, sulphate. The d.c. method of detection was used as before, without any difference. In this case also a layer is formed, possibly containing the sulphur component; it is well known that thiourea may easily form such layers.

In the experiments in which pyridine and β-picoline were used as buffering counter-ions, the pH of the leading electrolyte was about 6, containing 0.01 N hydrochloric acid ($pK_a = 5.25$ for pyridine and 5.69 for β-picoline). The analyses again showed very sharp zones. However, these compounds cannot be applied if UV detection is used because they show high absorption at 260 nm. It must be remembered that the effective mobilities of pyridine and β-picoline, however, are greater than the effective mobility of histidine. This results in a need for longer capillaries for the separation of similar mixtures, because mixed zones are formed much more easily as components with a higher effective mobility transport a higher proportion of the electricity.

Nevertheless, here also the disturbance in the detection of the chromate zone (electrode reaction) was found to be a function of the driving current, which illustrates further that an electrode reaction indeed occurs (Fig. 2, curves 1, 2, and 3). The electrode reaction of the chromate ions is shown to be part of the profile normally obtained if chromate is used as a terminating ion. The reaction time for this electrode reaction is of the order of seconds. All analyses, so far, in which chromate is present show this typical reaction, but at low pH (4) it is less pronounced.

If the chromate zone has passed the measuring electrodes, the electrodes are passivated. All other zones following the chromate zone are measured correctly if the chromate level instead of the leading electrolyte zone is taken as a reference. An extra resistance, reversible and stable, arises during the analysis, but if the capillary is rinsed after the experiment this resistance disappears again, which indicates that the layer does not adhere strongly to the surface.

If malonic acid is injected as a sample and the chromate zone is absent, a similar behaviour to that with the chromate is found (Fig. 2, curves 4 and 5). If both chromate and malonate are present, and even the chromate zone may be very small, only the typical behaviour of the electrodes can be recognized during the passage of the chromate zone (Fig. 2, curve 6).

For a.c. measurements, a real electric current (at least 10 μA) has to pass the boundary electrode electrolyte, while in d.c. detection this current is much lower (10 nA). The resistance build up by a layer of coating material is therefore more affected in the a.c. detection.
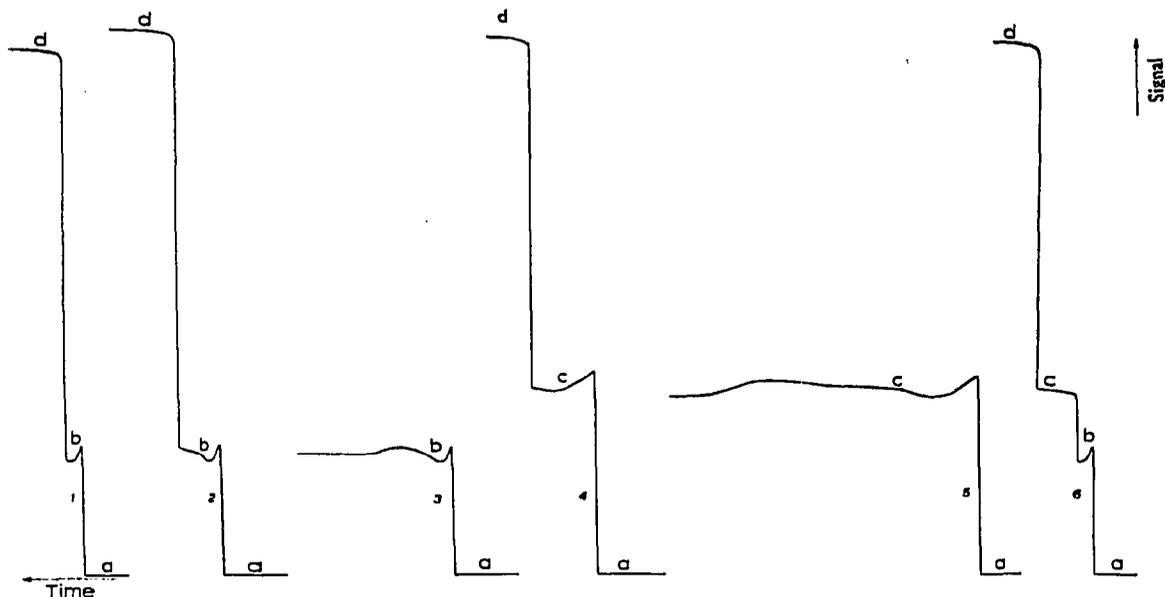
Fig. 2. Behaviour of chromate and malonate with conductometric detection in isotachophoretic analysis. a = Chloride; b = chromate; c = malonate; d = phenyl acetate.
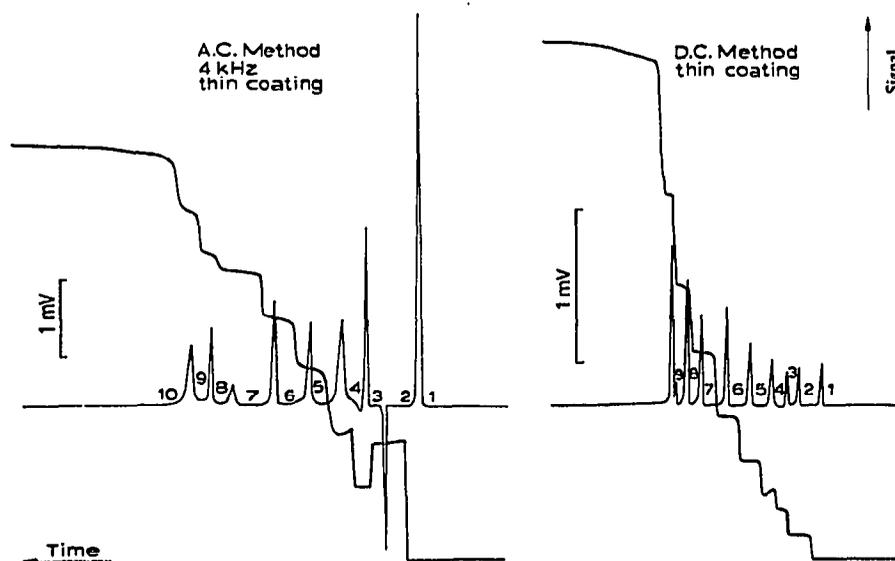

· Fig. 3. Isotachopherogram of the separation of the mixture of acids shown in Fig. 1. The left-hand trace shows an isotachopherogram with a thin coating of 1-aminoanthracene deposited on the electrodes. No further addition was made. The right-hand trace shows an isotachopherogram recorded by the d.c. method. Clearly visible is the impurity present between the zone of β-chloropropionate and the terminating phenyl acetate.

Coating of the measuring electrodes

The second means of preventing electrode processes is the application of a polymer coating on the measuring electrodes. The main problem is to find a method of coating that gives a uniform layer. Two different methods were tested: (1) the electrophoretic coating process and (2) the electrolytic coating process.

In the electrophoretic process for preparing coatings of polystyrene, acrylic and epoxy resins, both water and methanol were used as solvents. However, no means of forming a satisfactory uniform coating could be found. The platinum metal used as the electrode material is probably not suitable for these coatings, and it is known that the metal plays a very important role in the coating process. For this reason, only electrolytic coatings are considered below; in the electrolytic process, the electrode metal is less important.

Especially successful was the anodic polymerisation of aromatic amines. The more aromatic rings present in the compound, provided that sufficient could be dissolved, the more stable the coatings were found to be. 1-Aminonaphthalene dissolved in ethanol (saturated solution at room temperature) was employed in several experiments. A few drops of this solution were added to 10 ml of 1 M potassium chloride
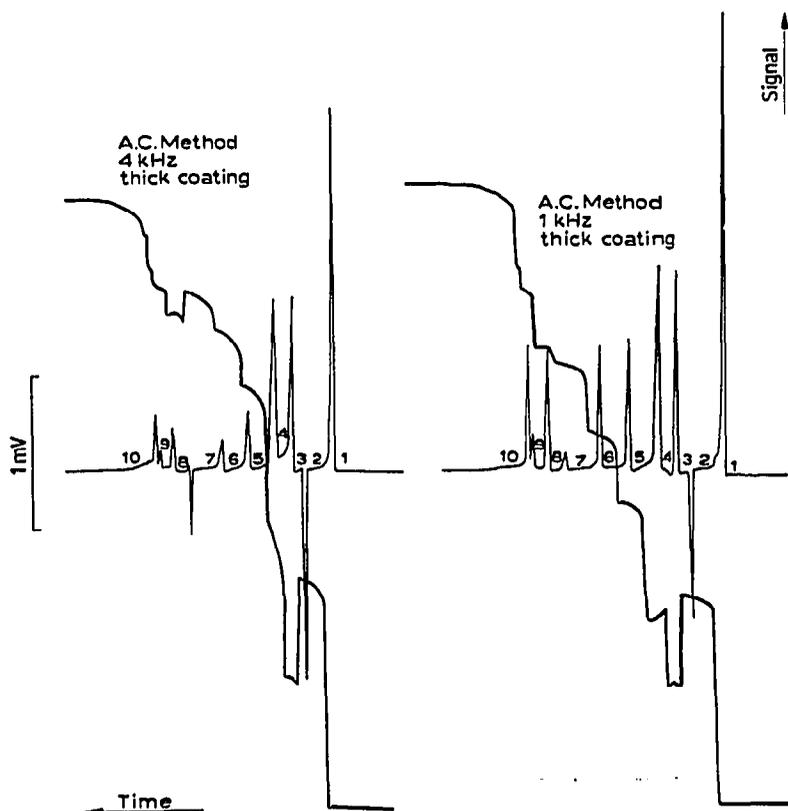

Fig. 4. Isotachopherogram of the separation of the mixture of acids shown in Figs. 1 and 3 with a thick layer of 1-aminoanthracene deposited on the platinum measuring electrodes. The left-hand trace shows a recording with the a.c. method at 4 kHz and the right-hand trace shows a recording with the a.c. method at 1 kHz.

solution and water was then added to a total volume of 100 ml. The solution was filtered in order to remove undissolved 1-aminonaphthalene. The filtrate was approximately 0.01 M in the aromatic amine.

During the anodic oxidation at 700 mV, a violet-coloured layer was formed. The electric current was kept at 0.1 mA for 5 min and an increase in the electrode potential up to 2000 mV was obtained. The cell constant increased from 0.68 to 2.5 cm^{-1}. The layer formed was cathodically very stable and, even after drying and heating (100°), the quality of the electrode improved. While the results with the coating of 1-aminonaphthalene were satisfactory, the results with 1-aminoanthracene were even better. The colour of the coating layer was yellow, and the cell constant increased from 0.68 to 3.5 cm^{-1}.

From these electrodes, current–voltage curves were obtained[7].

However, it is difficult to estimate the thickness of the layers, experiments in the isotachophoretic equipment are still carried out. In order to discriminate between electrodes, only a selection of thin and thick coatings was examined, depending mainly on the electric current applied during the coating procedure.
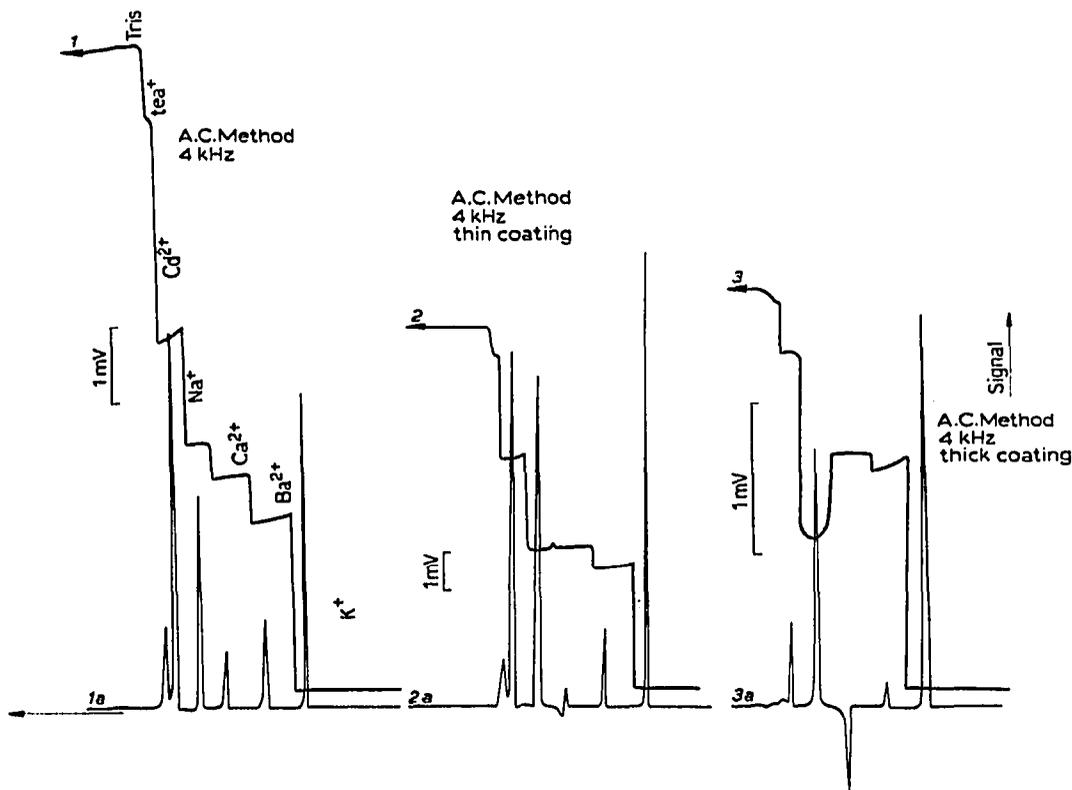

Fig. 5. Isotachopherogram of the separation of some cations. The leading electrolyte was 0.01 M potassium acetate plus acetic acid at a pH of 5.39. The terminating electrolyte was Tris. The electric current was stabilized at 80 μA. From left to right (1 and 1a, 2 and 2a, 3 and 3a): a recording under normal conditions; a recording with a thin coating layer deposited on the platinum measuring electrode; and an A.C. recording with a thick coating. The frequency in all experiments was 4 kHz. The coating was prepared with 1-aminoanthracene.

The a.c. method of detection gave an unusual electropherogram for the test mixture (Fig. 3, left), as before (Fig. 1). The sensitivity (selectivity) of the combination of the a.c. method with coated electrodes for the sulphate ion (doubly charged) was such that the next zone of chlorate was measured with a negative step, which suggests that this zone has a higher conductivity than the preceding zone. This is in contradiction to the isotachophoretic principle, if these ions are involved. If the thickness of the coating layer is increased, this effect also increases (Fig. 4, left).

Even the adipate–acetate transition was recorded with a negative step (in Fig. 3, left, the difference between adipate and acetate is smaller than expected). The difference between a.c. and d.c. detection of the conductivity, as already found in some instances when passivated electrodes (gold) were applied, must be ascribed to the capacity change of the conductivity cell. For this reason, the frequency of the measuring a.c. signal was lowered from 4 to 1 kHz. The electropherogram is shown in Fig. 4, right. It can be seen that the negative step of the adipate–acetate transition disappears, while the sulphate step is lowered. If d.c. detection for the conductivity is used (Fig. 3, right), the normal electropherogram is again obtained. Now the coating seems not to have any influence. Similar behaviour was found if cations were separated, as shown in Fig. 5.

Hence the coating is not selective only for the difference between singly and doubly charged anions, but cations can also be analyzed and specific and useful information can be obtained.

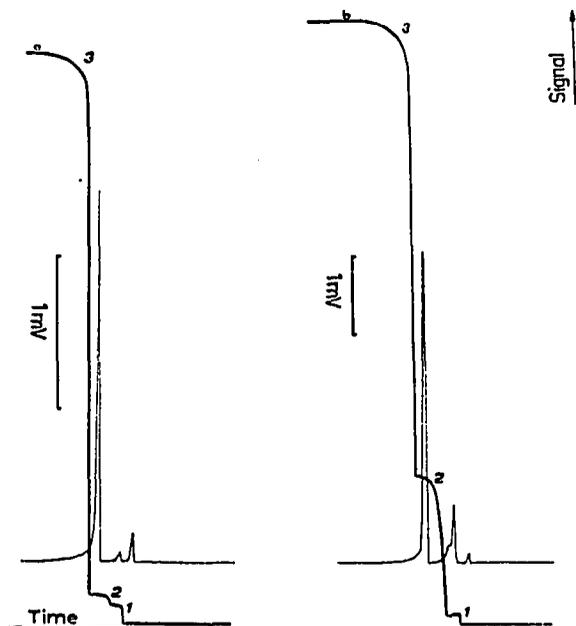In Fig. 6, two electropherograms are shown for the separation of nitrate and

Fig. 6. Isotachopherogram of the separation of nitrate and sulphate in the system histidine (0.01 M) plus histidine·HCl (0.01 M) at a pH of 6. The left-hand trace (a) shows the d.c. recording and the right-hand trace (b) shows the a.c. recording. The electrodes were covered with a layer of 1-amino-anthracene.

sulphate in the system histidine (0.01 M) plus histidine·HCl (0.01 M) at pH 6. Glutamic acid was used as the terminating electrolyte. The d.c. measurement showed a separation between the zones, but it was not pronounced. The a.c. measurement, however, showed a large difference between the singly charged nitrate ion and the doubly charged sulphate ion. With thermometric detection, these ions could hardly be detected. From the a.c. recording, the mixed zone that still exists between the real zones of nitrate and sulphate is clearly visible. In order to determine whether sulphate or nitrate (or both) was present, these electrodes were successfully applied in the separation of fruit juices.

A possible explanation of the behaviour of coated electrodes is discussed elsewhere[7].

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