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CONDUCTIVITY DETECTION OF ZONES IN ISOTACHOPHORESIS WITH A HIGH-FREQUENCY BRIDGE

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SUMMARY

The conductivity method of detection of zones in isotachophoresis with the aid of a high-frequency bridge (operating frequency 500 kHz), built especially for this purpose, is described. A higher resolving power than in the thermometric method was achieved. Constructional aspects of the measuring cell and the electronic equipment are discussed. A simple construction of a measuring cell for application in isotachophoresis is described.

INTRODUCTION

The resolving power of isotachophoresis in a capillary tube is greater than that of other electrophoretic techniques and is characterized by the sharpness of the boundaries of two adjacent zones in the steady state. Everaerts¹ and Martin and Everaerts² showed by approximate theoretical analysis that the width of the boundary is *ca.* 1 mm. Later, Arlinger and Routs³ concluded that the real width of the boundary was less than 1 mm and Van de Wiel⁴ gave a value of 0.2 mm or less.

Using the thermometric method of detection of zones, as described, for example, by Everaerts and Verheggen⁵, as a consequence of longitudinal heat transfer and the time required to reach thermal equilibrium, there is no possibility of achieving a high resolving power. This fact led to the development of detection methods adequate for the required resolving power of the separation process.

Arlinger and Routs³ used UV absorption for the detection of the components separated from a mixture of anions and proteins. The only disadvantage of this method is its specificity, such that there is a risk that some components will not be recorded. Van de Wiel⁴ measured conductivity by non-polarizable electrodes for the

detection of zones in isotachopheresis, but the output plot showed anomalies due to contamination of the zones.

Everaerts and Verheggen⁶ developed a method for the direct measurement of conductivity in a capillary tube with the aid of miniature electrodes made from a noble metal. They also gave a possible explanation for the presence of artifacts due to the electrochemical interaction of the metallic electrode with the electrolyte.

A study of the phenomena that occur on measuring electrodes was described in detail by Everaerts and Rommers⁷. Mulder and Zuska⁸ developed an instrument involving damping of a resonant circuit containing a measuring cell for the detection of zones. Haruki and Akiyama⁹ used an unearthened d.c. transistor electrometer with signal transmission by optical means into an amplifier.

The aim of our work was to develop equipment for the conductimetric detection of zones and suitable for the recording of zones several tenths of a millimetre in length. Two basic factors were taken into account:

(a) with frequencies lower than 10^5 – 10^6 Hz, the application of a contactless method of measurement of conductivity is much less satisfactory than a method using direct contacts with the electrolyte, as the sensitivity of a contactless measuring cell in the conductivity range measured is appreciably lower than that of a direct contact cell;

(b) the application of a low-frequency conductimeter (80 Hz–3 kHz) with direct contact electrodes gave poor results when connected with the stabilized current source on the same capillary tube. The resistance of the electrolyte column in the capillary tube increases with time, and the current source must increase the output voltage so as to keep the current constant. The Fourier components of this varying sawtooth voltage also affect the input of the conductimeter and cause a large zero instability, even at frequencies 2–3 decadic orders higher than the reciprocal of the output time constant of the current source.

EXPERIMENTAL AND RESULTS

The equipment that we constructed is the same in principle as that described by Everaerts and Verheggen⁶. A schematic diagram is illustrated in Fig. 1.

We used two types of measuring cell (Fig. 2), with disc measuring electrodes and with electrodes made from platinum wire. PTFE capillaries of 0.4 mm I.D. and 0.7 mm O.D. in the first instance and 0.4 mm I.D. and 1.0 mm O.D. in the second were used. The reservoirs for the leading and terminating electrolytes were made of PTFE. The leading electrolyte in the reservoir was separated from the solution in the capillary by a 0.01 mm thick PTFE membrane (Radiometer, Copenhagen, Denmark).

In constructing the injection block (Fig. 3), special attention was paid to obtaining the sharpest possible boundary between the leading and terminating electrolytes after filling the capillary tube with electrolytes. The sample was injected with a Hamilton (Reno, Nev., U.S.A.) micro-syringe through a septum. Because of the necessity to locate the analyzed mixture exactly in the boundary of the leading and terminating electrolytes, we carried out preliminary experiments with a mixture of anions in which the location of injection was varied while the other operating conditions (length of capillary tube, volume of sample, composition of the leading and the terminating electrolytes and current) remained constant. When the sample was injected

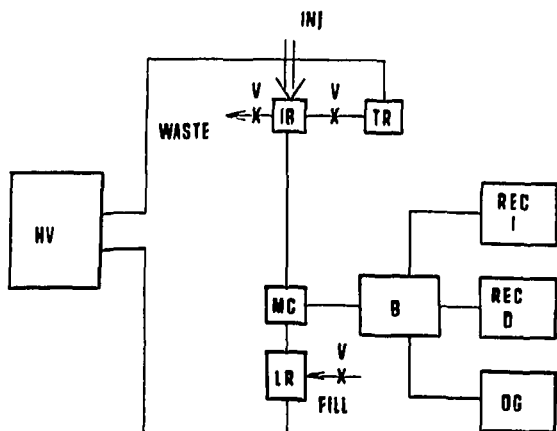


Fig. 1. Schematic diagram of isotachophoretic equipment with conductivity detection of the zone boundaries by means of a high-frequency bridge. HV = current-stabilised power supply; V = Hamilton valves; IB = injection block; INJ = position of injection of sample; TR = terminating reservoir; LR = leading reservoir; MC = measuring cell; B = high-frequency bridge; REC I and REC D = recorders for integral and differential records, respectively; OG = cathode oscillograph.

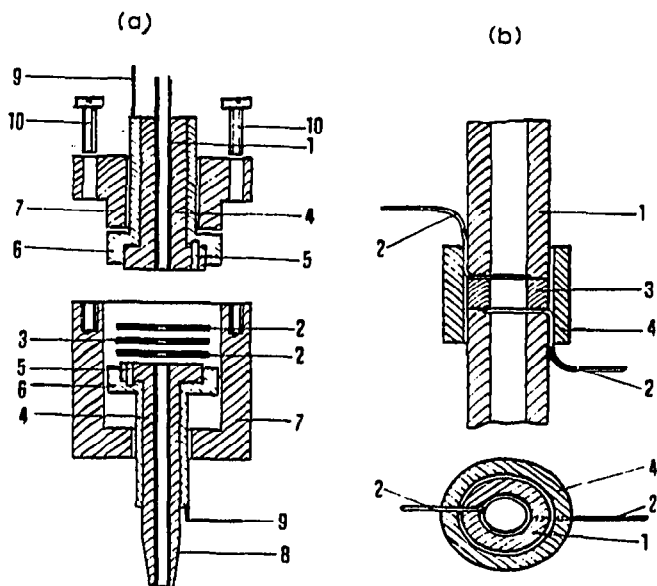


Fig. 2. (a) Modified Everaerts and Verheggen measuring cell. 1 = PTFE capillary; 2 = platinum disc electrodes; 3 = isolating foil; 4 = piece of PTFE for fitting the capillary tube; 5 = copper pin; 6 = piece of brass for cooling and electrical contact; 7 = piece of Perspex for fitting of measuring electrodes; 8 = connection to the leading reservoir; 9 = connection to the measuring instruments; 10 = screws for tight connection. (b) Measuring cell welded from PTFE capillary tubes. 1 = Capillary tube; 2 = platinum wire electrodes; 3 = insulator; 4 = glass tube.

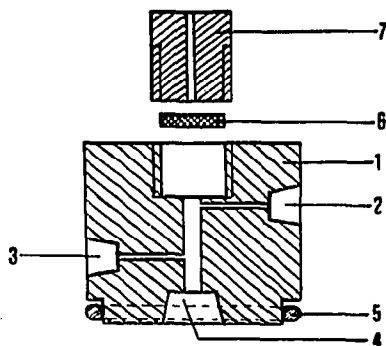


Fig. 3. Injection block. 1 = Piece of Perspex; 2 = connection to the reservoir of terminating electrolyte; 3 = hole for rinsing the capillary; 4 = position for connection of capillary tube; 5 = O-ring fitting; 6 = septum; 7 = bolt for fitting of septum.

ted about 1–2 mm below the boundary, the separation was imperfect and a mixed zone containing the leading ion (chloride) and the most mobile ion (sulphate) appeared. It was necessary to take this fact into account so that the length of the capillary tube was effectively utilized.

Special attention was paid to construction and investigation of the measuring cell. The best results were obtained by using the two types shown in Fig. 2. Fig. 2a shows a modification of Everaerts and Verheggen's measuring cell^{6,10}. The modification was necessary because at the connection between the two sections of the capillary tube, at the position where the measuring electrodes and the insulating foil were located, we were not able to obtain precise fitting of the capillary tubes in all instances so that the inner profile of the capillary was locally perturbed. After switching the current on, small bubbles were formed and the measuring cell could not operate correctly. Using the modified measuring cell illustrated in Fig. 2a, these effects were substantially reduced. An important quantity was also the thickness of the electrode material (a thickness of 0.02–0.05 mm seems to be the optimum). For the insulating disc, 0.15-mm PTFE foil was used.

As materials for the electrodes, platinum, gold and graphite were tested. Using our equipment, we did not find any substantial differences in the quality of recording, the lower sensitivity of the graphite electrodes being the only exception. The graphite electrodes were made as a 0.05-mm thick graphite film, cemented on both sides of 0.15 mm thick polyethylene insulating foil by a solution of polystyrene in chloroform. By covering the measuring electrodes with a layer of platinum black, the character of recording remained unchanged. The platinum black layer was made by a common procedure recommended in the literature¹¹.

A very simple and sufficiently sensitive measuring cell, with the possibility of keeping the temperature constant along the whole length of the capillary tube and visual observation of the area near the electrodes, is illustrated in Fig. 2b. The sensitive parts consist of two loops made from platinum wire 0.03 mm in diameter, separated by a 0.1 mm thick ring made from the PTFE capillary tube. The long separating part of capillary, the measuring part with the insulating ring and the part for the coupling to the leading electrolyte reservoir were welded together by heating, as follows. On a molybdenum wire, 0.4 mm in diameter, were placed successively the

capillary tube for connection to the leading reservoir, the insulating ring and the separating part of the capillary. Simple loop electrodes made from platinum wire 0.03 mm in diameter were attached on both sides of the insulating ring. At the position of welding was placed a short glass tube with an I.D. greater than the O.D. of the PTFE capillary. The welding was carried out by means of a heating element consisting of several loops of high-resistivity wire, placed on the glass tube and heated. During the welding, the PTFE tubes were pressed together gently. After welding, the molybdenum wire was removed and the quality of the weld was tested by water pressure. The measuring cell was then tested in the measuring equipment with a mixture of anions.

Taking into account the difficulties involved with a low-frequency detection system, as mentioned above, we decided to use a conductimeter working at a frequency separated sufficiently from the highest Fourier components of the sawtooth-like high-voltage source; we chose the frequency 500 kHz. The time constant of the high-voltage source was about 1 sec, so that the frequency of 500 kHz is higher than necessary (100 kHz should be completely satisfactory), but at this frequency commercially available resonant circuits can be used. The ohmic conductivity of the electrolytes at high frequencies is lower than the d.c. conductivity, but this effect was not of great importance in this work. Of the possibilities available for the conductimeter circuit, such as the Ohm's law, Q-meter and bridge systems, we preferred the bridge scheme, taking into account its principal merits of sensitivity and the reduction of the influence of generator instabilities on the stability of the zero and measured signals. We chose a bridge with inductively coupled arms, which retains good features at the frequencies used¹².

The circuit diagram of the bridge is illustrated in Fig. 4. Two of the arms of the bridge were represented by the symmetrical secondary winding of the high-frequency transformer with a ferrite core. The third arm was the conductivity measuring cell, its electrodes being d.c. separated by two high-voltage capacitors (100 pF, 10–20 kV). The balancing arm was a series of nine 50-k Ω resistors with a switch and a 68-k Ω potentiometer for resistive balance (coarse and fine), and a 100-pF trimmer for capacitive balance.

The feeding oscillator (500 kHz) was not coupled directly to the bridge, but through the buffer stage, the output voltage being about 1.5 V. Great attention had to be paid to the amplitude and frequency stability of the oscillator, as the quality of the resonance circuit should not be decreased by inappropriate connection of the load (bridge transformer). A quartz-crystal oscillator and silicon transistors may conveniently be used for the stability of the equipment.

The output signal from the bridge was led via the coupling transformer (tuned to resonance at 500 kHz) to the input stage of the receiver. The alignment of this transformer is very important for decoupling of the receiver from the d.c. voltage variations of the high-voltage power supply in order to obtain stabilization of current in the capillary. Through its primary winding, the separating capacitor (100 pF) of the measuring cell is stored when the high-voltage source is switched on. The 500-kHz transformer transfers this impulse to the base of the input transistor with only a very low efficiency. By means of RC decoupling, damage to the input transistor can occur.

The two-stage amplifier of the receiver gives a voltage amplification factor of about 200, with a symmetrical output transformer, tuned to 500 kHz, feeding the phase-

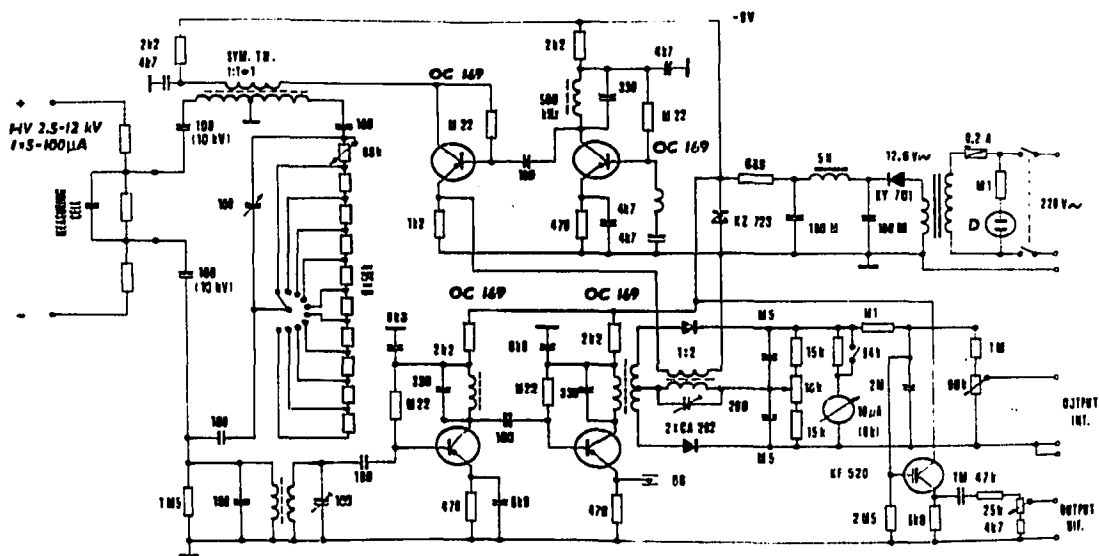


Fig. 4. Circuit diagram of the measuring instrument with high-frequency bridge.

sensitive lock-in detector. The reference signal (500 kHz, 3 V) is led from the oscillator via the buffer stage. The relative phase shift of this reference signal and of the signal from the bridge should be approximately zero on the lock-in detector, which is possible by tuning the transformer. This arrangement is satisfactory, but it is only a substitution for the common phase shifter, which should be aligned in the channel of the reference signal. We used this arrangement because of its simplicity, although the stability of the output signal will be more influenced by the frequency instabilities of the oscillator when the transformer is not tuned exactly to resonance.

On the output of detector the properly chosen integrating time constant was about 0.2 sec. The balance of the bridge was inspected by means of a cathode oscillograph connected to the second stage of the amplifier.

The leadings from the bridge to the measuring cell were located in an adjustable brass tube so as to prevent time instabilities of this bridge arm. The conductors to the cell were insulated with 20-kV polystyrene in order to hold out the full voltage of the high-voltage source.

The output voltage of the power supply for the transistor was stabilized by a Zener diode. This stabilization is unavoidable if the bridge is to operate reliably.

The bridge carried the integral and derivative outputs for the recorders. On passage of the boundary between the leading electrolyte ($10^{-2} M$) and the terminating electrolyte ($10^{-2} M$) through the measuring cell, the bridge gave an output voltage of 0.15 V.

For feeding the electrolyte column in the capillary tube, a supply of stabilized current of own construction was used. The current range was 5–100 μA and the voltage range 2.5–12 kV.

Figs. 5a and 5b show integral and differential records for separated mixtures of

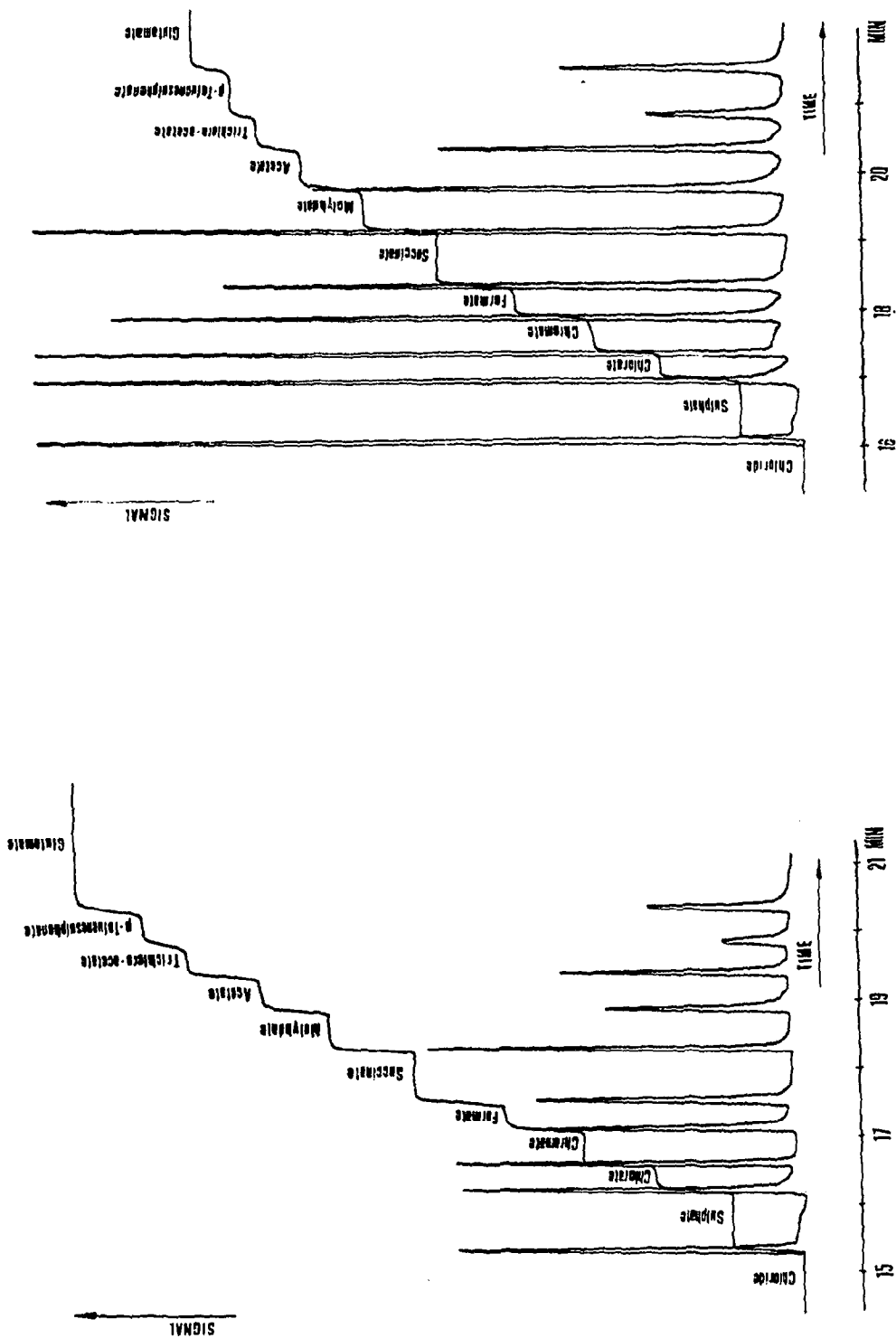


Fig. 5. (a) Isotachopherogram of separated mixture of anions. Amount of sample, 0.5 μ l (with concentration of each component 10^{-2} M). Recorded at 30 μ A (separated at 100 μ A). The measuring cell illustrated in Fig. 2a was used. (b) Isotachopherogram of separated mixture of anions. Amount of sample, 0.6 μ l (with concentration of each component 10^{-2} M). Recorded at 30 μ A (separated at 100 μ A). The measuring cell illustrated in Fig. 2b was used.

anions obtained by the above detection equipment. In the first case (Fig. 5a), we used the modified Everaerts-Verheggen measuring cell, and in the second case (Fig. 5b), we used the measuring cell with the sensitive parts consisting of two loops made from thin platinum wire.

A TZ-21 S line recorder (Laboratorní Přístroje, Prague, Czechoslovakia) was used for integral records and an EZ-11 line recorder (Laboratorní Přístroje) for differential records.

CONCLUSIONS

The high resolving power obtained with the high-frequency bridge and proposed conductivity detector for isotachopheresis enables one to detect and utilize the sharp boundaries between successive zones, as follows from the principle of the separation process. As we have stated, by using this detection system it is possible to detect reliably amounts of the order of 10^{-10} – 10^{-11} g-equiv. of a substance (10^{-4} M in 10^{-7} l of sample).

The use of the high-frequency bridge permits several disturbing influences to be avoided, which is unusual with direct current or low frequencies. The high stability of the zero and measured signals, and also the high sensitivity to conductivity changes, are the main advantages the application of high frequency.

The most important component of the measuring equipment is the measuring cell. In its construction, it is necessary to ensure precise machining of the holes in the electrodes and insulating disc, smoothness of the electrode surface that is in contact with the electrolyte, and that the inner profile of the capillary in the measuring section is maintained constant.

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