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# HIGH-SPEED ISOTACHOPHORESIS: CURRENT SUPPLY AND DETECTION SYSTEM

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## SUMMARY

A stabilized d.c. power supply for high-speed analytical isotachophoresis, providing a stepless constant current adjustable up to 400 $\mu$A at a voltage up to 16 kV, and a detection system for the direct measurement of potential gradients in isotachophoretic zones are described. The design enables any of the power supply output terminals to be earthed. Hence the detection cell can be kept at a relatively low above-earth potential when analyzing both anions and cations.

It has been verified that the ionic species separated can be identified by determining the relative ratios of the potential gradients in their zones and by comparing these experimental data with the inverse values of the relative ratios of the tabulated mobilities. The relative deviations found did not exceed 2% for a model mixture of inorganic anions.

## INTRODUCTION AND ANALYSIS OF THE PROBLEM

It was shown in a previous paper[1] that the time of an isotachophoretic analysis can be shortened successfully by applying higher voltages across the column and higher driving currents. Limiting factors for employing a higher current and voltage are the dissipation homogeneity of the Joule heat produced, the homogeneity of the electric field in the column and the electric insulation of the column. The conditions for analytical quantitation of an isotachophoretic separation were shown earlier[2]. In this connection, it was necessary to devise both a suitable power supply and a universal detection system.

Several types of power supplies for isotachophoresis have already been described by Vacík *et al.*[3]. They proposed some designs that provide output voltages up to 12 kV d.c.; however, the maximum adjustable value of the constant current is too low (100 $\mu$A) and does not permit the full performance of present types of isotachophoretic columns to be achieved. Other workers[4–6] have merely reported the maximum d.c. voltage of the power supplies used without any detailed performance specifications.

The apparatus for high-speed isotachophoresis according to our design[1] requires a power supply that provides a constant stepless d.c. current adjustable up

to 400 $\mu$A at the maximum voltage of about 16 kV. The analytical quantitation of the isotachophoretic separation requires a very stable driving current, a sufficiently low ripple and the possibility of earthing any of the output terminals. In order to ensure adequate safety of the power supply, the amount of electricity accumulated in the supply must not be dangerous. Further, too high a no-load voltage of the power supply is undesirable because, if any interruption of the electrolyte line inside the isotachophoretic capillary occurs (*e.g.*, due to a gas bubble), the insulation of the capillary up to the point of the interruption is stressed by the full no-load voltage and can be damaged by a disruptive discharge. The power supply circuit should allow the d.c. current intensity to be controlled manually also.

Earthing of any of the output terminals is important with respect to the selection of the detection mode and of the position of the detection cell in the capillary and to the design of the electric circuits for processing the detector signal and for connection to the recorder.

Presently available detectors can be divided into contact and contactless types, depending on whether the sensor does or does not come into contact with the electrolyte. The contactless detectors are either thermal[7,8], based on detection of the differences in the Joule heat produced in individual zones, or optical[9], based on the differences in the absorption of UV light in individual zones. The thermal detectors are simple, but they have a low resolving power with respect to zone length, the shortest detectable zones being about 5 mm long[10]. Their dynamic properties are not acceptable for high-speed analysis. The optical detectors show better zone length resolution and satisfactory dynamic properties but they are not universal.

The presently available contact detectors employ sensing electrodes immersed inside the capillary and are essentially also of two types. The first type measures the conductivity of zones by using a.c. current[11–14]. Measuring with a.c. current simplifies considerably the transfer of the signal from the potential of the detection cell to the earth potential on which the recorder and the power supplies operate. The disadvantages of such devices stem from the fact that adaptations in the measuring circuits are necessary for large changes in the operating conditions (concentrations of the electrolytes used) and that difficulties arise from the connection of cables to the detection cell, as the admittance of the cables is part of the admittance measured. In addition, the results of the measurements are affected by the resistance of the sensing electrodes, which virtually eliminates the possibility of using non-polarizable electrodes connected to the solution in the capillary via liquid junctions. The second detection principle is represented by the direct measurement of potential gradients in isotachophoretic zones[4,6,15]. Detection is carried out by measuring the voltage drop between the two sensing electrodes that are incorporated in the column[1] and are placed a small distance apart in the direction of movement of the zone. As the measurement is currentless, the resistance of the electrodes does not play any role and even non-polarizable electrodes connected to the electrolyte in the column via salt bridges can be employed.

In analytical isotachophoresis, the detection system should detect the shortest possible zones, *i.e.*, it should possess a high resolving power. Further, it should detect different components by employing a universal property of the zones (*e.g.*, specific conductivity or electric gradient). In addition, the detection system should be capable of resolving zones for which the detected property differs only slightly. Moreover, in

high-speed analysis, the detection system should have dynamic properties that would provide the minimum distortion, *i.e.*, it should produce a sufficiently rapid and true response.

In addition to the above requirements, it is advantageous that the detector should not only resolve individual zones but also provide some information useful for the identification of the ions being separated.

## EXPERIMENTAL

*Power supply*

Fig. 1 shows the regulation loop used for constant current control. The current, $I$, through the column is transferred to the voltage, $U_M$, which is compared with the reference voltage, $U_{REF}$. The magnitude of $U_{REF}$ is proportional to the required value of the adjusted driving current. The difference between the two voltage values is processed in a regulator, REG. The output d.c. voltage, $U_{DC}$, of the regulator is converted by a transducer, $U_{DC}/U_{HV}$, into a d.c. high voltage, $U_{HV}$, necessary for the required value of the current through the column. The transducer, $U_{DC}/U_{HV}$, is an inductive d.c. converter with a rectified filter for damping the output voltage undulations. A controller with an integration channel is used, permitting a zero sustained deviation from the required current value through the column to be reached.

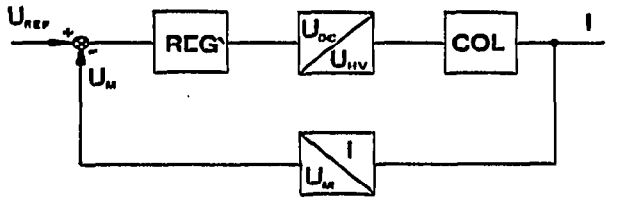


Fig. 1. Control loop for current stabilization. $I$, Current through the column; $U_M$, measured voltage; $U_{REF}$, reference voltage; REG, controller; $U_{DC}/U_{HV}$, voltage–high voltage converter; COL, column; $I/U_M$, current–voltage transducer.

Fig. 2 is a detailed diagram of the designed power supply. The input circuits of the controller enable the power supply to operate at both polarity modes of the output voltage. The polarity mode is changed by a change-over switch, $S_1$, in the controller circuits and by connecting power cables between the power supply and the column. A throw-switch, $S_2$, serves as a working regime selector (automatic constant current control or manual control). The set-point of the current is adjusted in both instances by means of a potentiometer, P. The specifications of the power supply are as follows: voltage of 16 kV at a current through load of 400 $\mu$A, and 17.4 kV at 12 $\mu$A; the ripple is less than 1.5 V at 400 $\mu$A and about 10 kV. The stability of the adjusted driving current is determined only by the stability of the reference voltage and by the drifts of the electronic components built in the controller input, as the control deviation is theoretically equal to zero. At room temperature, the deviation of the actual current value from the adjusted value cannot be measured practically.

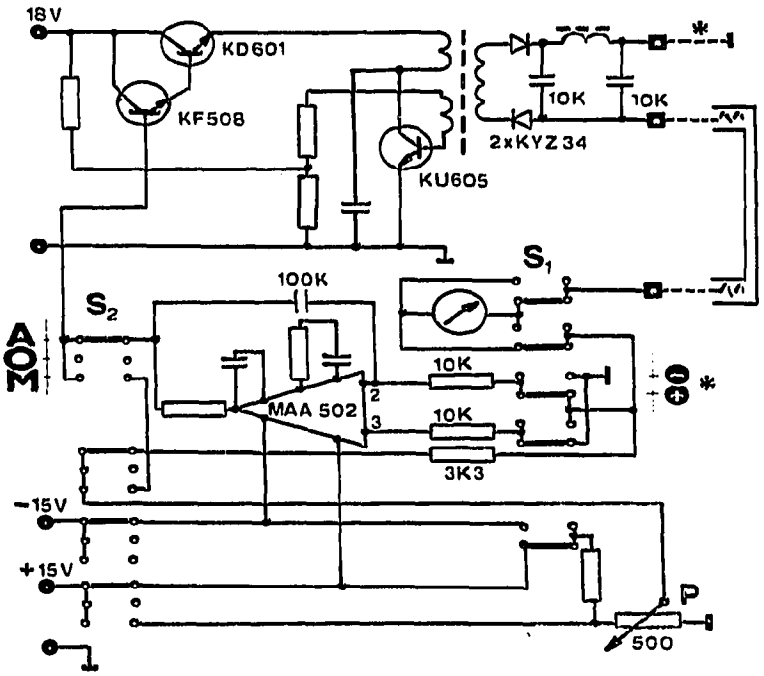


Fig. 2. Power supply. $S_1$, Change-over switch of polarity mode; $S_2$, throw-switch of the operation regime (A = automatic; 0 = zero; M = manual); P, potentiometer for current adjustment. All components are produced by Tesla, N.E., Czechoslovakia.

*Detection system*

The detection system consists of the detection cell (the part of the column in which the detection is carried out), circuits for processing the detector response, and the recorder. An arrangement[16] was used that combines the advantage of measuring the electric gradient with the simplicity of the a.c. current measurement. The input d.c. voltage is imposed from the sensing electrodes of the detection cell[1] across a Varicap and the capacitance of the latter is then measured by an a.c. method. Fig. 3 shows the principles of the detection system. The magnitude of the input current through the sensing electrodes is determined by the Varicap parameters and is less than $10^{-9}$ A. A compensating bridge connection including two Varicaps with identical parameters (Fig. 4) is used in order to make the dependence of the output voltage for the recorder upon the input voltage linear. If the bridge is balanced, the values of the d.c. voltages across both Varicaps must be equal. Provided that these voltages

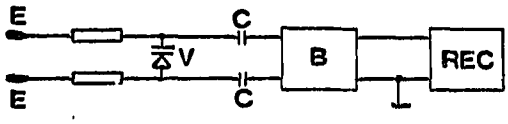


Fig. 3. Principle of the detection system. E, Sensing electrodes; V, Varicap; C, capacitors; B, measuring circuit; REC, recorder.

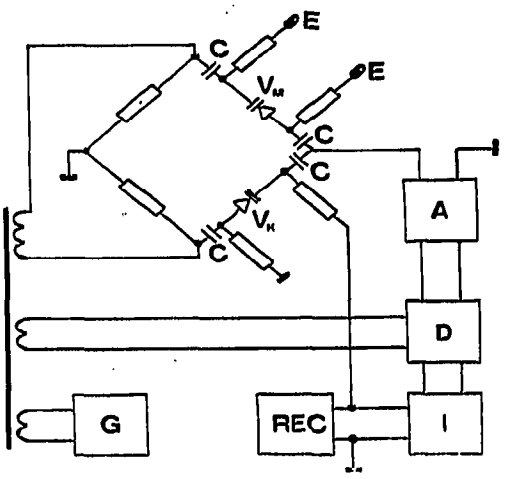


Fig. 4. Detection system. E, Sensing electrodes; $V_M$ and $V_K$, measuring and compensating Varicaps (Type KA 213, Tesla, N.E., Czechoslovakia); C, high-voltage capacitor (470 pF, 20 kV); A, amplifier; D, discriminator; I, integrator; REC, recorder; G, generator (500 kHz).

do not become equal, the bridge, fed from a high-frequency generator, is not balanced and a high-frequency voltage is produced at the input terminals of the amplifier. The magnitude and phase of this voltage depend on the difference in the magnitudes of the voltages across the measuring and the compensating Varicaps. After being amplified, the high-frequency voltage is rectified by a synchrodetector to a d.c. voltage, which is integrated. The integrator output voltage is amplified back to the bridge across the compensating Varicap and is changed automatically until the bridge is balanced, *i.e.*, until the integrator output voltage is equal to the voltage across the sensing electrodes. By this method, the voltage sensed in the detection cell is transferred to an earth potential and can easily be measured and recorded.

## DISCUSSION

The detection system described above, designed for sensing and measuring electric gradients in isotachophoretic zones, has been found to be both simple and reliable. The magnitude of the input current of the detection system is very small (less than $10^{-9}$ A) so that the measurement of electric gradients in zones is virtually currentless. A number of possible difficulties resulting from polarization and depolarization of the sensing metal electrodes[11] are thus eliminated to a considerable extent. The dynamic properties of this apparatus are suitable for application to high-speed analyses.

Direct measurement of the electric gradients in individual zones is advantageous as their magnitudes provide direct information on the mobilities of the ionic species separated[4]. It can be shown that

$$E_1u_1 = E_2u_2 = E_Xu_X = E_Ru_R = v = \text{constant}$$

where $E_1$, $E_2$, $E_X$ and $E_R$ are electric gradients in the zones containing ionic species 1, 2, X and R, and $u_1$, $u_2$, $u_X$ and $u_R$ are the mobilities of these species; $v$ represents the

velocity of migration of the zone and is constant and identical for all the zones under given operating conditions. For the identification of zones, it is advantageous to use relative data in which the magnitudes of the electric gradient (measured as heights of the steps in the record, $h_1$, $h_2$, $h_X$ and $h_R$) are related to the magnitude of the electric gradient in the zone of the reference species R. The inverse ratio of the step heights is then equal to the ratio of the mobilities, $h_R/h_X = u_X/u_R$, and can be compared with the values calculated from the mobilities tabulated for a given temperature.

In order to evaluate the extent to which the ionic species can be identified by using the relative values of the electric gradients (determined as the relative values of the step heights) and by comparing them with the relative values of the mobilities tabulated, the systematic measurement of a mixture containing chlorate, bromate, iodate, phosphate and arsenate ions was carried out. In Fig. 5, a high-speed isotachophoretic analysis of the above anions is demonstrated, SPADNS (trisodium salt of sulphanilazochromotropic acid) serving as an optical marker for the direct measurement of the velocity of migration of the zones[1]. The magnitudes of the electric gradients of the zones of the above species, measured as step heights, were related to the electric gradient of the leading ionic species, $Cl^-$, serving as a reference substance. The leading electrolyte consisted of 0.0066 *M* hydrochloric acid adjusted to pH 4.2 by the addition of aniline serving as the buffering counter-ion. At this pH, the species being separated are present in the form of the ions $ClO_3^-$, $BrO_3^-$, $IO_3^-$, $H_2PO_4^-$

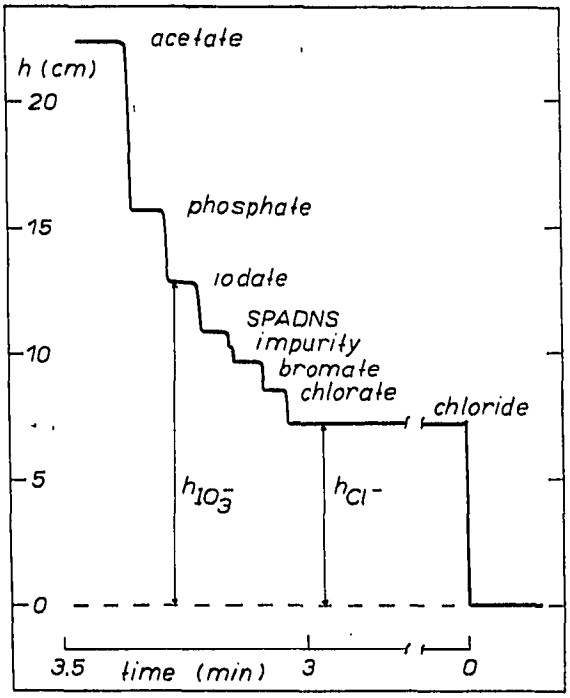


Fig. 5. High-speed isotachophoretic analysis of 4 µl of a model mixture. Composition: $6.2 \cdot 10^{-4}$ *M* $NaClO_3$, $6.7 \cdot 10^{-4}$ *M* $KBrO_3$, $6.6 \cdot 10^{-4}$ *M* $KIO_3$, $7.2 \cdot 10^{-4}$ *M* $NH_4H_2PO_4$, $4.0 \cdot 10^{-4}$ *M* SPADNS.

TABLE I

COMPARISON OF EXPERIMENTAL VALUES OF RELATIVE STEP HEIGHTS WITH VALUES OF THE INVERSE RELATIVE MOBILITIES TAKEN FROM THE LITERATURE

| *Ionic species, X* | $h_{Cl}/h_X$* | $u_X/u_{Cl}$ | $\Delta$ | $\Delta$(%) |
|---|---|---|---|---|
| $Cl^-$ | 1.000 | 1.000 | — | — |
| $ClO_3^-$ | 0.842 | 0.845 | −0.003 | −0.4 |
| $BrO_3^-$ | 0.740 | 0.735 | +0.005 | +0.7 |
| $IO_3^-$ | 0.547 | 0.535 | +0.012 | +2.2 |
| $H_2PO_4^-$ | 0.467 | 0.472 | −0.005 | −1.1 |
| $H_2AsO_4^-$ | 0.442 | 0.445 | −0.003 | −0.7 |

* Each value represents the arithmetic mean of five measurements.

and $H_2AsO_4^-$ in their zones and sufficiently accurate values of the mobilities of these ions have been published[17]. Acetic acid (0.012 *M*) was used as a terminator. Table I gives the relative step heights of the ionic species separated and compares them with the calculated relative mobilities. The agreement of the values measured with those from the literature is evidently very good, the maximum deviation being 2.2%. The possibility of identifying ionic species in zones by means of the gradient detection system is clearly evident.

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