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An Automatic Conductivity Bridge for Chromatographic Analyses

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A recording device for the separation in chromatographic columns is always time-sparing and useful, especially if it admits quantitative conclusions. In that case it is a useful analytical tool and moreover gives information about the shape of the elution curve. Conductivity as a principle for measurement has been used by Wickbold [1], James, Martin and Randall [2], and Drake [3].

Wickbold uses a measuring cell and a comparison cell, fed from two separate transformer windings. The currents from the cells are rectified in a Graetz coupling and compared with each other by a recording millivoltmeter. The apparatus constructed by James *et al.* is built around a conventionel A. C. Wheatstone bridge. The amplified, unbalanced voltage is fed to a phase-discriminating circuit, the output voltage of which controls a strip chart recorder. The slidewire in the recorder is a part of the bridge and thus automatically corrects the adjustment of the bridge. The measuring device described by Drake is of high precision compared with the other two and has a sensitivity corresponding to about 0.02 % of the total resistance (cell: 1 600 ohms; bridge potentiometer: 50 ohms). In his apparatus the voltage from the discriminator controls a reversible motor which rotates two potentiometers. One of these is the slidewire in the bridge. A D.C. voltage over the second potentiometer is applied to a recorder. The conductivity-measuring cells have been differently constructed. James *et al.* have the cell in the column but give no details. As mentioned above, Wickbold has both a measuring and a comparison cell. The comparison cell is filled with the solvent used as elutriant. Drake has a micro cell (volume about 0.1 ml) immersed in a thermostated oil bath.

In order to obtain a less complicated apparatus than the one described by Drake we tried to improve the construction of James *et al.* However, it was found difficult to get full balance of the bridge due to the great capacity of the conductivity cell and electrical asymmetry against earth, which resulted in unsteadiness and no sharp zero point. These disadvantages were eliminated by inserting a Wagner earth and a comparison cell of the same geometrical shape as the measuring cell. A more detailed description and some applications are given in the following.

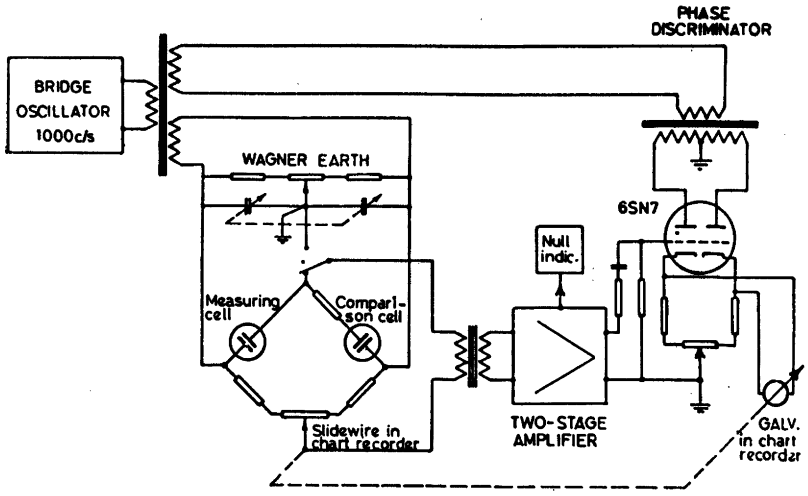

Fig. 1. Block scheme of the electronic device.

DESCRIPTION OF THE APPARATUS

Electronic equipment. A block scheme of the automatic conductivity bridge is shown in Fig. 1. Two opposite arms in the bridge contain the measuring cell and the comparison cell. By means of a switch a decade can be inserted in series with one of the cells. The regular slidewire in the chart recorder is also used as the continuously variable resistance in the bridge. It is mechanically coupled to the galvanometer. An unbalance in the bridge causes a deflection of the galvanometer pointer in one or the other direction. Then, by means of a special mechanism, this deflection is transformed to a winding movement which is such that it tends to restore the balance of the measuring circuit by moving the slidewire along the slider. The movement of the slidewire is also mechanically transferred to the pen, thus showing the difference in conductivity between measuring cell and comparison cell. The sensitivity of the bridge is determined by the resistance of the slidewire relative to the total resistance of the half of the bridge containing the slidewire. The sensitivity can consequently be chosen in arbitrary steps by giving suitable values to the resistors complementing the slidewire. A change of one ohm in the decade can be made to correspond to a certain fraction of the chart width. For instance with 500 ohms resistors in the arms containing the slidewire and a slidewire resistance of 2 ohms a change of 0.8 % of the total resistance in the cell corresponds to a deflection over the whole chart width. In order to compensate for the unbalance against earth the bridge is fed from the bridge oscillator over a Wagner earth. To eliminate the differences in capacity between the conductivity cells, the measuring device is supplied with a switch through which various condensers can be inserted in parallel with the measuring cell or the comparison cell. The voltage across the conductivity cells

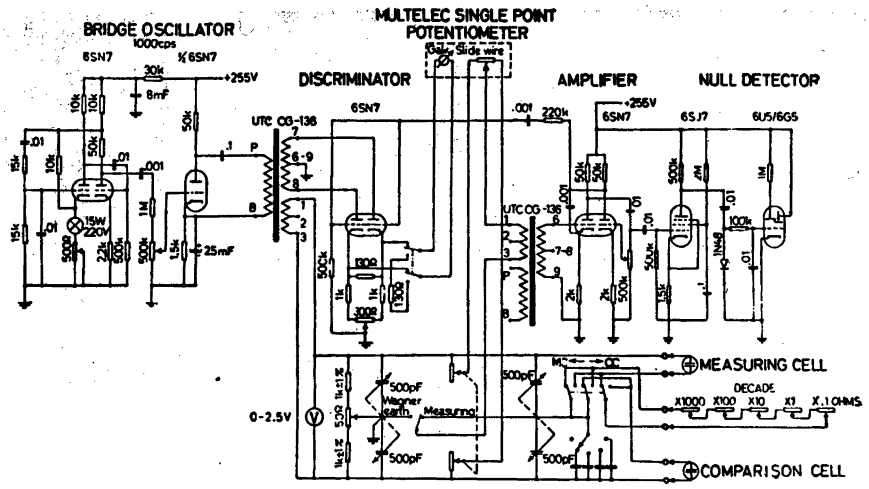

Fig. 2. Circuit diagram of the conductivity bridge.

can be varied between 0 and 1 volts in steps of 0.1 volts. The frequency of the oscillator is 1 000 c/s.

The unbalanced voltage from the bridge is amplified by a two-stage amplifier (gain about 350). The output voltage is then applied both to a phase discrimination circuit to which the galvanometer is connected and to a zero-point indicator. The purpose of this is to show when the bridge is sufficiently balanced so that the galvanometer circuit can be switched on without risk of ruining the galvanometer. The whole apparatus is fed from a voltage-stabilised rectifier. A more detailed circuit diagram is shown in Fig. 2.

The bridge oscillator is RC-coupled and is of conventional type. It is followed by a power amplifier with transformer output. The output-transformer has two secondary windings, one feeding the bridge, the other supplying the voltage of the discriminator anodes. The discriminator works as follows: when the bridge is in balance, the voltages over the cathode resistors are equal, *i.e.* the voltage to the galvanometer is zero. However, when the bridge is unbalanced the voltage is incoming over the grids of the discriminator. According to the phase relation of this voltage, the anode current rises in one of the triode-halves of the 6SN7. The result of this is a higher voltage over the corresponding cathode resistor and hence a voltage difference is indicated by the galvanometer. Of course, a rise of the anode current is only obtained when the phase relation is such that the grid voltage is growing more positive at the same time that the anode voltage is of positive direction.

The chart recorder connected to the conductivity bridge is a Kent multelec single point potentiometer, 0—10 mV. As already mentioned, the galvanometer and the slidewire in the recorder are the parts used. All the other electrical components and leads are disconnected. Certain precautions have been taken in order to avoid 50-cycle interferences from the motor and its cables. Therefore, all leads between the recorder and the brigde device have

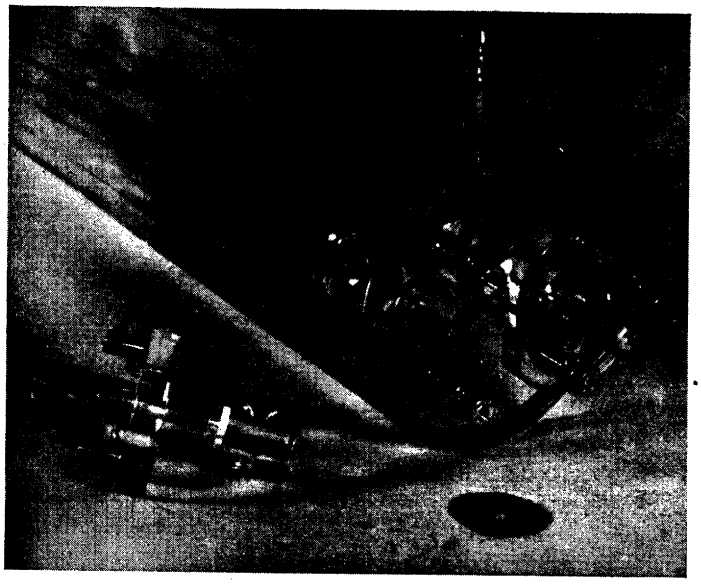

Fig. 3. Photograph of the conductivity cells.

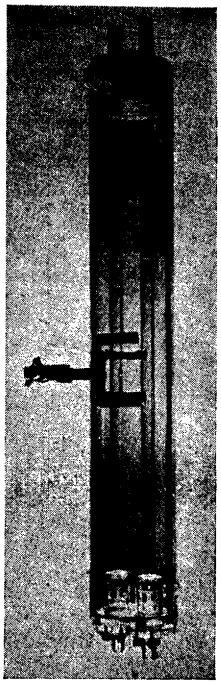

Fig. 4. Photograph of the columns and the jacket.

been carefully screened. This has also been done with the leads to the conductivity cells. A paper speed of 1 inch per hour is usually convenient.

Conductivity cell. In order to obtain a capacitive balance of the bridge two equal cells have been used. They are placed in the bottom of the columns and are filled with small glass beads (diameter 0.1 mm). Different constructions have been tried, and that found most suitable is shown in Fig. 3 and 4. The columns are made of "perspex" and the cells are easily removable. They are fastened to the columns by screws and rubber packing. The beads are held by a glass filter in the bottom of the cell. The electrodes are made of platinum and inserted in a niche in the wall. The surface facing the lumen is platinized and the wires from the electrodes are led through the walls down to the under-surface where terminal screws are placed. The columns mostly used have the dimensions 10 × 560 mm.

In these the conductivity cell constant is 0.20 cm^{-1}. A larger column with a diameter of 32.5 mm has been built for preparative work. To prevent temperature variations the columns are fitted with a jacket through which thermostated transformer oil is pumped. The temperature of the latter is kept constant to within $\pm$ 0.1° C. Greater temperature variations are also prevented in the room where the apparatus is located. The conductivity cell is placed in the bottom of the column largely in order to reduce the risk of mixing the fluid leaving the column. This might occur if the effluent were made to go through a capillary tube with an extension for the cell.

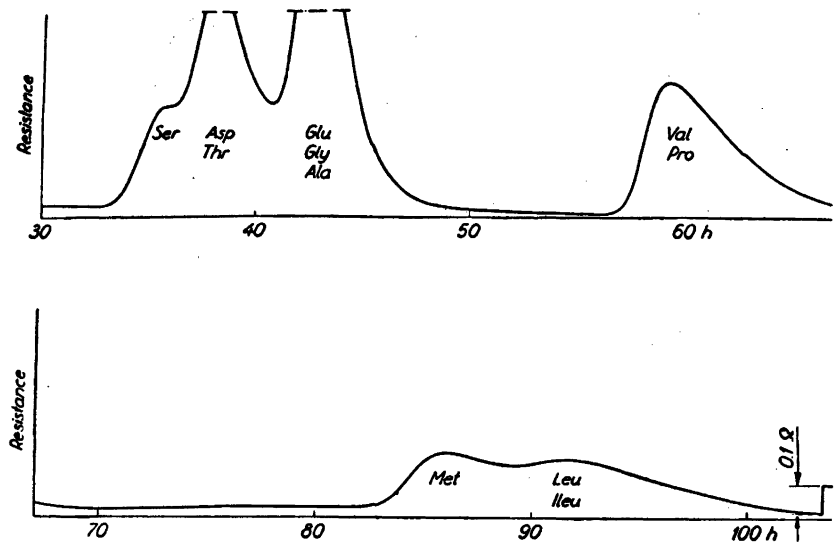

Fig. 5. Elution of amino acids from 1.0 ml of a casein hydrolysate (total nitrogen 44.8 mg) with 1.0 N HCl from a Dowex 50 column (2 % DVB).

Column dimensions: 10.3 × 387 mm. Flow rate: 1.36 ml/hr. Chart speed: 1 inch/hr. Abbreviations of amino acids according to Brand and Edsall [9].

EXPERIMENTS

The apparatus has been used for experiments with amino acids and phosphorus-containing amino acids and peptides.

Amino acids. The best procedures for chromatographic separations are those described by Stein and Moore [4,5]. The principle is elution chromatography on the strong cation exchanger, Dowex 50 (8 % cross-linking), by hydrochloric acid or various buffer mixtures. The last procedure gives the best resolution but works at a pH near the isoelectric point, making unsuitable the conductivity recording of the amino acids. Drake [3] has also used 0.1 *N* HCl for his separation of amino acids. He found that it was possible to elute serine, threonine, glycine, alanine and valine with that strength of hydrochloric acid although there was some overlapping and valine gave a low and broad peak. To reduce the adsorption to the resin, Dowex 50 with lower cross-linking (2 % DVB) and higher hydrochloric acid concentrations (1.0 *N*) have been tried. Fig. 5 shows such an experiment, but there is still some overlapping. For quantitative determinations a preliminary separation into groups is thus necessary. Using 1 *N* hydrochloric acid the cell resistance is 19.6 ohms. With 100 ohms in each of the arms containing the slidewire the apparatus easily detects 0.025 ohms corresponding to a sensitivity of 0.125 % of the total resistance.

Phosphorus-containing amino acids and peptides. The usefulness of the separation of these substances by elution with 0.01 *N* hydrochloric acid from a Dowex 50 column has been shown [6,7]. As a consequence of the low acid concentration employed, the compounds may be indicated with high sensitivity by measuring the conductivity. Investigations [8] in this laboratory indicate that elution with 0.5 *N* formic acid from a bed of a strong anion exchanger (Dowex 2, formate form, 10 % DVB) is also an excellent chromatographic system for this type of substances. Fig. 6 gives a photograph of a curve indicating phosphoserine and phosphothreonine in such an experiment. Because of the dissociation of the phosphoric acid group the peaks given correspond to a rise of the con-

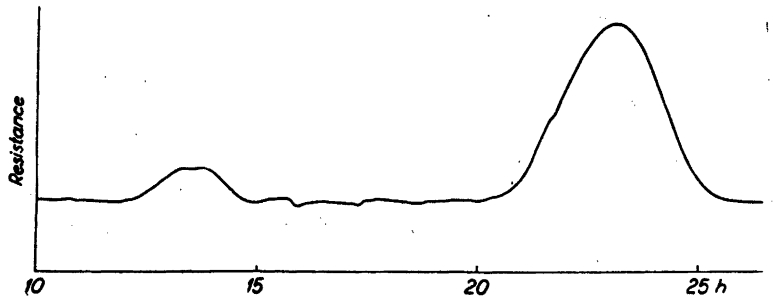

Fig. 6. Elution of phosphoserine (12.5 mg) and phosphothreonine (2.9 mg) with 0.5 N HCOOH from a Dowex 2 column (10 % DVB).
Column dimensions: 10.3 × 390 mm.
Flow rate: 12.1 ml/hr. Chart speed: 1 inch/hr.

ductivity, in contrast to the lowering of conductivity caused by the ordinary amino acids. The resistance of the cell with 0.01 *N* HCl and 0.5 *N* HCOOH is 1 270 and 1 540 ohms respectively. The sensitivity is about 0.05 % (100 ohms in each of the bridge arms containing the slidewire).

QUANTITATIVE DETERMINATIONS

Formulas for the estimation of amino acids by conductivity measurement are given by Drake [3].

To check the possibility of using the apparatus for quantitative determinations several curves were collected from elution of phosphoserine from Dowex 50 with 0.01 *N* hydrochloric acid. Different amounts and different flow rates were used.

The general formula for the determination of a substance in a flowing solution:

$$N_B = \int_{V_1}^{V_2} c_B \mathrm{d}V$$

(where N_B is the amount of a special substance B, c_B the concentration of the substance and V the passing volume) can be transformed to a form more suitable for calculations. Putting

$$c_B = k_1 R = k_1 k_2 b \text{ and}$$
$$V = k_3 t = k_4 a$$

(here R is the change in resistance in the conductivity cell due to the substance to be recorded, t is the time and a and b are the abscissa and ordinate respectively, expressed in cm) the formula can be transformed to

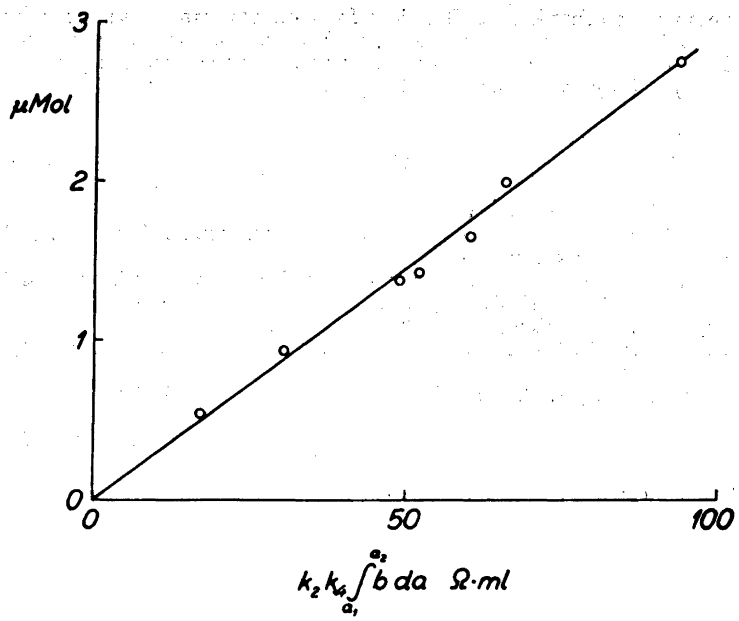

Fig. 7. Abscissa: $k_2k_4 \int_{a_1}^{a_2} b\ \mathrm{d}a$. *Ordinate: amount of phosphoserine (micromoles).*

$$N_B = k_1k_2k_4 \int_{a_1}^{a_2} b\ \mathrm{d}a$$

The area $\int_{a_1}^{a_2} b\ \mathrm{d}a$ can be determined with a planimeter, and k_2 and k_4 can be determined for each run. k_1 has to be determined for every system and measuring cell. Fig. 7 shows a curve, where N_B is plotted against $k_2k_4 \int_{a_1}^{a_2} b\ \mathrm{d}a$ indicating good linearity. k_1 for this system is 0.029 compared with $k_1 = 0.086$, when phosphoserine is eluted with 0.5 N formic acid through the same conductivity cell.

SUMMARY

A short discussion is given of earlier apparatus for automatic conductivity measurement of the effluent from chromatographic columns. A modification of an earlier design by James *et al.* is described. The conductivity bridge contains identical measurement and comparison cells. The modified apparatus has been of great value in the separation of ordinary amino acids and phosphorus-containing amino acids and peptides on ion exchange columns.

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X-ray Crystallographic Data on Selenotrithionates

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Selenotrithionic acid, $Se(SO_3H)_2$, was discovered by Rathke [1] in 1865, two and a half decades after the preparation of the first salts of trithionic acid, $S(SO_3H)_2$, by Langlois [2].

Whereas crystal data on potassium, rubidium and cesium trithionate were reported by Mackenzie and Marshall [3] in 1908, and Zachariasen [4] in 1934 worked out the crystal structure of potassium trithionate by X-ray methods, the only crystallographic data available on selenotrithionates are from a morphological study of potassium selenotrithionate by Rathke [5,6] in 1870.

The chemical evidence concerning the constitution of selenotrithionic acid and selenotrithionates, *e.g.*, the formation reaction from selenium acetylacetonate and hydrogen sulphite [7]:

$$(C_5H_8O_2Se)_2 + 4\ HSO_3^- = 2\ Se(SO_3)_2^{--} + 2\ C_5H_8O_2 \qquad (1)$$

and the nucleophilic displacement equilibrium [8] involving selenotrithionate/selenopentathionate and thiosulphate/sulphite ions:

$$Se(SO_3)_2^{--} + 2\ S_2O_3^{--} \rightleftharpoons Se(S_2O_3)_2^{--} + 2\ SO_3^{--} \qquad (2)$$

leave little doubt that the selenotrithionate ion is built up of a divalent selenium atom to which are attached two sulphonate groups. Thus, selenotrithionate is derived from trithionate by substitution of selenium for the middle sulphur atom. Two organic analogues, *viz.*, sulphur and selenium dibenzenesulphinate, arise from the acids by substitution of phenyl for the hydroxyl groups. The benzenesulphinates form isomorphous crystals [9], and the detailed structures have been determined recently by Mathieson and Robertson [10] and Furberg and Öyum [11].

In the present study, crystallographic measurements have been made on ammonium, potassium, rubidium, cesium and barium selenotrithionate, with the purpose of establishing whether isomorphism exists between these crystals and those of the corresponding trithionates. No such isomorphism has been found. In view of the undoubtedly similar constitution of the

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