

CHROM. 10,472

A CONDUCTIMETRIC DETECTOR WITH A WIDE DYNAMIC RANGE FOR LIQUID CHROMATOGRAPHY

VRATISLAV SVOBODA and JAROSLAV MARŠÁL

Institute for Research, Production and Application of Radioisotopes, 17004 Prague 7 (Czechoslovakia)

SUMMARY

A conductimeter with an alternating symmetrical square wave measuring voltage (1 V, 2.5 kHz) and logarithmization circuit has been constructed and its application studied using a symmetrical cell of 3 μ l internal volume. Its dynamic range is about six orders of magnitude. No manual switching of ranges is necessary and therefore unattended operation as an on-line chromatographic detector is possible. Examples of inorganic salts chromatographed on a Sephadex G-10 column are presented.

INTRODUCTION

The concentration of inorganic compounds in effluents from liquid chromatography columns may change very rapidly, sometimes by even more than four orders of magnitude within several seconds. This effect is very pronounced in gel chromatography, when peaks of salts are highly asymmetric with steep shoulders or fronts, probably owing to ion exclusion effects.

When using a conductimeter with a linear scale, *i.e.*, with the signal proportional to the conductivity of the solution, then the dynamic range is limited at the lower end by the noise and drift of the recording chain and frequent switching of ranges is therefore necessary. This is usually performed manually and is not only time consuming but also susceptible to human errors. If it is done automatically, then the reading of the graphical record may become complicated in regions of rapid ascent or descent of the effluent conductivity. We have therefore tried to construct an instrument in which the signal is approximately proportional to the logarithm of the measured conductivity.

Flow-through conductimetric detectors are frequently used in liquid chromatography, but optimization of their design has never been thoroughly studied¹. Most conductimetric detectors described recently^{2–5} were built so as to minimize the cell volume. This is most pronounced in detectors for isotachopheresis, where sharp boundaries of zones have to be measured quickly and with minimal distortion. In order to offset the influence of the fluctuation of the regulated high-voltage source, a conductimeter operating at high frequency (above 100 kHz) was developed². However, at these frequencies the ohmic conductivity of electrolytes is lower than the

d.c. conductivity. Obviously, this detector could also be used in high-performance liquid chromatography (HPLC), its advantage being the higher immunity to electrical noise.

Conductimetric cells constructed specifically for use in HPLC were described by Pecsok and Saunders³ and later by Tesařík and Kaláb⁴. Their designs were very similar in both basic characteristic and performance. Cells formed an integral part of columns; their dead volume was low (5 and 2.5 μl , respectively) and their cell constants were near to unity (0.55 and 0.8 cm^{-1} , respectively). The cells had to be isolated from metallic columns, or were adapted for use with glass columns only. For measurement of resistivity commercial instruments with several manually switchable linear ranges were used in both instances (Radiometer CDM2e and Radelkis OK-102/1). For testing a short column packed with glass beads was used and deionized water was used as the solvent. The minimal detectable concentrations of potassium chloride (signal equal to twice the peak-to-peak noise amplitude) were $5 \cdot 10^{-8}$ and $1.5 \cdot 10^{-7}$ $\text{mole} \cdot \text{l}^{-1}$, respectively.

Jackson⁵ studied the use of a high-frequency conductimetric detector with external electrodes; however, the dead volume was very high (ca. 2 ml). The minimal detectable concentration with this instrument was probably similar to those mentioned above.

EXPERIMENTAL

The conductivity, G , measured by the electronic circuit is determined by the specific electrical conductivity of the measured solution and by the cell constant of the measured solution and by the cell constant of the measuring cell. Hence the cell design may affect to some extent the range of specific conductivities measurable by a particular instrument.

In our instrument, the range of measurable conductivities extends from about 10^{-8} S to more than 10^{-2} S. For measurement a rectangular alternating voltage with amplitude ± 1 V and frequency 2.5 kHz is used. The output voltage in this range is proportional to a function approximating to the logarithm of the conductivity.

The electronic circuitry is illustrated in Fig. 1. The instrument consists of three principal parts: a generator of alternating voltage (OA1), a logarithmic alternating current-voltage converter (OA2) and a d.c. amplifier (OA3).

For generation of the logarithmic function use is made of the fact that for most transistors the relationship between the base-emitter current and voltage is nearly logarithmic. Hence the alternating measuring voltage of ± 1 V from the generator is carried to the positive input of the operational amplifier OA2. Owing to the back-loop, virtually the same voltage is also on the other input of OA2. The resistance of the condenser C_V can, in the range of conductivities applying here, be neglected and therefore for the current flowing through the cell, I_x , and the generator voltage, U_A , we have

$$I_x \approx U_A \cdot G_x \quad (1)$$

At the output from OA2 we then have an alternating voltage with amplitude given by

$$U_b = \pm (U_A + k \log I_x + k) \quad (2)$$

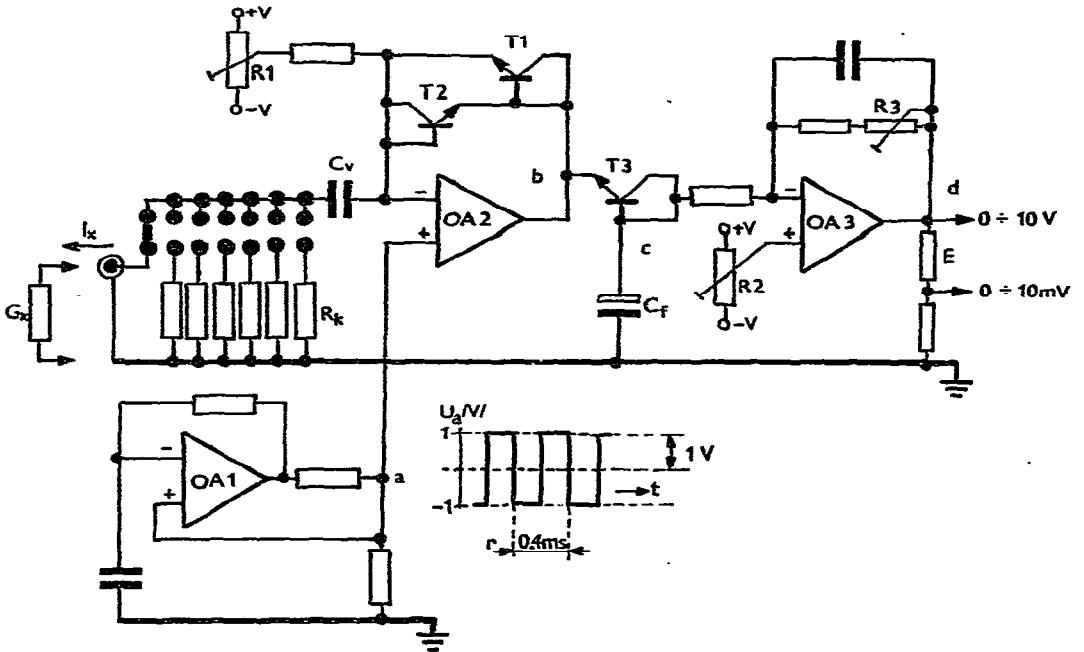


Fig. 1. Wiring diagram of conductimeter. OA1, OA2, OA3 are operational amplifiers, T1, T2, T3 transistors, and R1, R2, R3 potentiometers.

and after rectification we obtain

$$U_c = -(U_A + k \log I_x + k - U_{T_2}) \tag{3}$$

After amplification of this signal by OA3 we finally obtain the output voltage:

$$U_a = A \log I_x + B \tag{4}$$

The constants *A* and *B* are set by potentiometers *R*₂ and *R*₃ so that the output voltage ranges from 0 to +10 V (see Fig. 2). The temperature drift of the base-emitter voltage

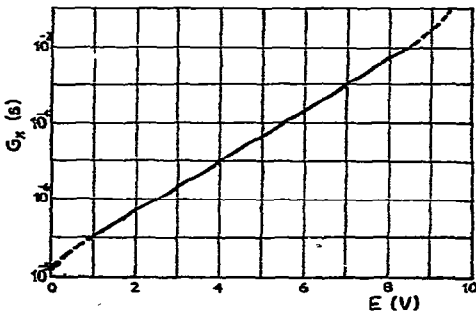


Fig. 2. Calibration curve of conductimeter. *G*_x = measured conductivity (S), *E* = output voltage (V).

of transistors T_1 and T_2 is compensated for by temperature drift of the voltage on transistor T_3 .

The correct functioning of the instrument can be checked by a set of fixed resistors, R_k , which can be selected by a switch.

A d.c. power supply of ± 12 V is used. The whole electronic unit is built on a single printed circuit board of dimensions 100×160 mm. On the front panel of the instrument are located only two switches: an on-off switch and an external-internal calibration switch. More detailed information on the construction of the circuit is available from the authors on request.

Because of the extreme simplicity of the design, the instrument is very dependable. Two instruments have been in use in our laboratory for more than 2 years, during which period only one repair of a calibration switch was needed.

The conductimetric cell (Fig. 3) is symmetric, both outer electrodes being earthed and the internal electrode being attached to the voltage source. The electrodes are made of stainless steel and isolators of polytetrafluoroethylene. The total volume of this detector is about $3 \mu\text{l}$ and this can easily be reduced further if necessary.

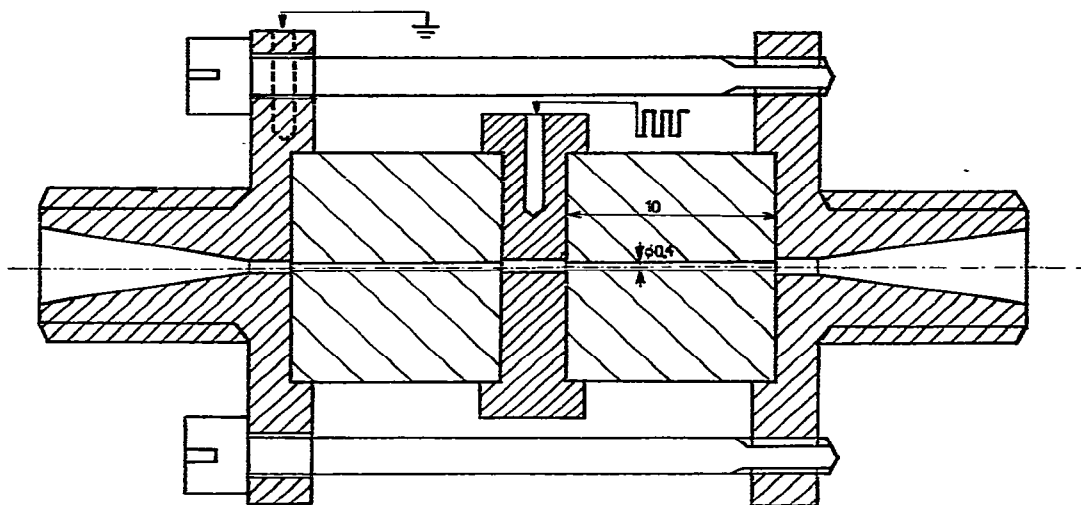


Fig. 3. Diagram of conductimetric cell. Materials used: stainless steel and PTFE. Dimensions in mm.

RESULTS AND DISCUSSION

A typical chromatogram of sodium perchlorate on Sephadex G-10 is illustrated in Fig. 4. This is a characteristic example of a peak with a steep front edge; the total change in conductivity ranges from about $4 \cdot 10^{-8}$ to $1.5 \cdot 10^{-2}$ S, *i.e.*, just about the whole range of our instrument. Fig. 5 illustrates a set of chromatograms recorded with various amounts of potassium chloride injected on a Sephadex G-10 column, and is an example of a peak with a steep shoulder. In curve 6 the full range of the instrument is used for recording. Thus with the same instrument setting two peaks representing amounts of sample differing by a factor 10^4 can be recorded and quanti-

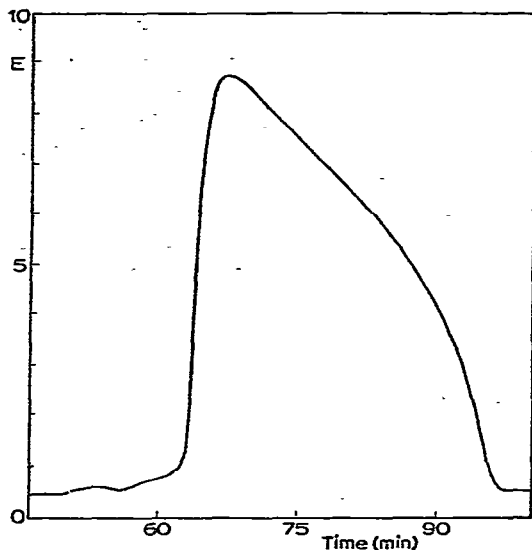


Fig. 4. Shape of a peak with steep front edge. Column dimensions, 1000×4 mm, packed with Sephadex G-10; eluent, $0.01 M$ ammonia solution in water; pumping speed 10 ml/h; sample, 61 mg sodium perchlorate.

tatively evaluated. This is a major advantage over the previously described conductimeters²⁻⁵, with which unattended recording over a wide dynamic range was virtually impossible. The relationship between amount of sample and peak area is non-linear, even when the pen deflection is strictly proportional to the measured conductivity^{3,4}. Therefore, even in these instances a calibration graph constructed over the whole range of concentration s_V is necessary.

The symmetrical construction of the conductimetric cell led to a suppression of ground-loop currents and high-frequency pick-up, both of which increase the drift and noise. Therefore, metallic columns can be used and all connections can be made

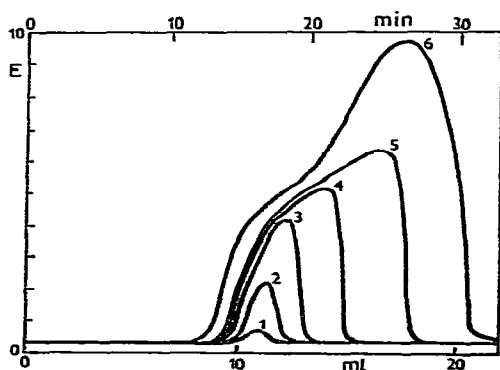


Fig. 5. Influence of sample amount on the peak shape. Column dimensions, 1260×4.8 mm, packed with Sephadex G-10, eluent $1 \cdot 10^{-5} M$ Tris in water, pumping speed 0.67 ml/min. Various amounts of KCl: 1: $10 \mu\text{g}$, 2: $50 \mu\text{g}$, 3: $250 \mu\text{g}$, 4: 1 mg, 5: 5 mg, 6: 100 mg. Vertical axis: $0-10$ V (see Fig. 2).

by using common stainless-steel capillaries with ferrules. In contrast to previous designs we could therefore increase the cell constant to $ca. 4 \cdot 10^2 \text{ cm}^{-1}$. This further permits an increase in the precision of measurements at high concentrations, which are important with many samples of biological interest. The upper measuring limit of our instruments probably extends to about two orders of magnitude higher than those described previously^{3,4}. On the other hand, we do not believe that in most experiments in which conductimetric detectors are used deionized water is the solvent of choice. Usually, even in gel filtration or ion-exclusion chromatography, the pH of the solvent has to be maintained either above or below 7. Therefore, the conductivity of the solvent in most instances is higher than that of conductimetric water and the published ultimate minimal detectable concentrations are mostly only of academic interest. Even so, the maximum concentration of potassium chloride in peak 1 (Fig. 5) is only $1.2 \cdot 10^{-7} \text{ mole} \cdot \text{ml}^{-1}$ (the measured amount of potassium chloride within the detector is about 27 ng) and the minimal detectable concentration, owing to the very low noise of the whole measuring chain, is about $6 \cdot 10^{-6} \text{ mole} \cdot \text{l}^{-1}$. The total dynamic range is about six orders of magnitude, which is about two orders of magnitude greater than that reported earlier⁴.

There is no point in comparing the absolute amplitude of the noise and the output from the amplifier, as was done in earlier papers^{3,4}, because of the different (and unpublished) electronic gains.

The advantages of the detector can be used fully only in eluents with relatively low concentrations of ionizable components (*e.g.*, 0.001 *M* ammonia, formic acid in water) and therefore it can be expected to be applied mainly in gel filtration and ion-exclusion chromatography.

REFERENCES

- 1 M. Krejčí, Z. Pechan and Z. Deyl, in Z. Deyl, K. Macek and J. Janák (Editors), *Liquid Column Chromatography*, Elsevier, Amsterdam, 1975, p. 148.
- 2 S. Stankoviansky, P. Čičmanec and D. Kaniansky, *J. Chromatogr.*, 106 (1975) 131.
- 3 R. L. Pecsok and D. L. Saunders, *Anal. Chem.*, 40 (1968) 1765.
- 4 K. Tesařík and P. Kaláb, *J. Chromatogr.*, 78 (1973) 357.
- 5 A. Jackson, *J. Chem. Educ.*, 42 (1965) 447.