A CONDUCTIVITY DETECTOR FOR LIQUID CHROMATOGRAPHY WITH A CELL VOLUME OF 0.1 µl

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A conductivity detector with a cell volume of 0·1 µl has been devised to fit glass micropacked columns with an inner diameter of 0·5 mm. D.c. current was used in the measurements, the voltage applied to the electrodes was 30 V. Organic acids separated in a reverse-phase system were detected; the minimum detectable concentrations were $6 \cdot 10^{-7}$—$1 \cdot 10^{-5}$ mol l^{-1} according to the acid concerned and the composition of the mobile phase. The linear range of the detector is 400.

Small-diameter (~1 mm) packed columns for liquid chromatography have recently attracted attention. Unless they are several metres long, the use of such columns necessitates miniaturization of the equipment, particularly the injection system, the detecting cells, and their connection to the column.

The contribution of the detecting cell to the peak spreading is negligible if the cell volume V_D is less than 1/10 of the peak volume V_p (ref.[1]). Assuming that the peak has a Gaussian shape, the peak volume can be represented as[2]

$$V_p = 4\sigma = \pi d^2 L \varepsilon_T (1 + k)/\sqrt{N} ,$$

where d and L are the diameter and length of the column, respectively, ε_T is its total porosity, k is the capacity ratio of solute, and N is the number of theoretical plates. In order to achieve an efficiency of 25 000 theoretical plates, for a nonsorbed component ($k = 0$) on a column 50 cm long with an inner diameter of 0·5 mm, the volume of the detecting cell must be reduced to less than 0·19 µl.

We chose a conductivity detector for the detection of components separated on small-diameter packed columns, because its detecting cell can be easily miniaturized. Conductivity detectors are suited to the detection of substances that are ionized in the mobile phase, and so they find application particularly in ion exchange chromatography[3–8] where water serves as the mobile phase. A commercial conductivity detector has been described[9] for use with reverse-phase systems (methanol, water); for organic acids, the minimum detectable concentration achieved in optimum conditions was 10^{-5} mol l^{-1} using a cell of a volume of 2·5 µl.

D.c. or a.c. current can be used for conductivity measurements of solutions, the latter alternative being more commonly used in practice because problems associated with the electrode polarization are thus avoided. On the other hand, problems arise from the capacity of the electric circuit including the capacity of the measuring cell, the capacity of the electrode double layer, and also the capacity of the connection with the measuring instrument; in miniature apparatus the last-mentioned capacity plays the major role.

Conductivity measurements using d.c. current are used, *e.g.*, for the zone detection in isotachophoresis experiments[10]. If, by design, a high resistance constant of the cell is achieved (small cross-section, large electrode spacing), a sufficiently high voltage can be applied to the electrodes so that the voltage loss arising from polarization does not play a part.

In the present work, two geometries of a miniature flow-through cell have been tested in conjunction with packed microcolumns. By applying a d.c. voltage a higher sensitivity was attained as compared with an a.c. polarizing voltage. In the arrangement used, the optimum sensitivity is not deteriorated by the presence of small quantities of ionizable substances in the mobile phase, necessary for obtaining symmetric peaks for the organic acids analyzed.

EXPERIMENTAL

The measurements were carried out on a home-made apparatus. The chromatographic glass columns used were 50—100 cm long, inner diameter 0·4—0·5 mm, outer diameter 0·9 mm, coil diameter 10 cm. The solutes were delivered by means of a six-way valve with a 8 µl loop, after which a flow splitter was inserted. The glass column was attached to the injecting equipment so that it reached, over the splitter, as far as the immobile section of the valve. The splitting ratio was varied within the range of 1 : 20 to 1 : 50.

The columns were packed with Silpearl C_{18}, particle diameter 30—40 µm, prepared at our Institute from Silpearl silica gel of Kavalier (Votice), and with Separon Si C_{18} with a mean particle diameter $d_p = 10$ µm (Laboratorní přístroje, Prague). Suspensions were used for packing the columns.

The mobile phase was 40—60% (V/V) aqueous methanol containing 0·01—0·1% (m/m) lauric acid. Acetic, propionic, butyric, valeric, caprylic, oxalic, malonic, succinic, glutaric, adipic, maleic, citric, and benzoic acids served as the testing solutes.

The layout of the detecting cell and the conductivity detector circuit are shown in Fig. 1. Two detecting cells were designed, constituted by capillaries into which electrodes are inserted. In cell A, platinum wires 0·1 mm in diameter served as the electrodes, the inner diameter of the capillary was 0·15 mm, the electrode spacing was 5 mm. The volume of cell A was 0·1 µl. In cell B, the electrodes were Ni–Cr wires 0·35 mm in diameter, the capillary had an inner diameter of 0·4 mm, and the electrode spacing was 1 mm; the cell volume was 0·12 µl.

The cell was inserted directly into the end of the chromatographic column and sealed to it with epoxy resin. To the other end of the cell was attached, also by means of epoxy resin, an outlet capillary (i.d. 0·5 mm). The whole assembly was protected by a glass capillary fixed to the column and to the outlet capillary with epoxy resin.

The electrodes were connected to a d.c. power supply, the voltage being variable over a range of 0—30 V. The current was directly recorded on a Kompensograph III recorder (Siemens, FRG).

RESULTS AND DISCUSSION

For an assessment of the effect of the electrode polarization, the dependence of the base current (for the pure mobile phase) on the voltage applied was measured on the two cells. The mobile phase contained 40% (V/V) methanol and 0·01% (m/m) lauric acid in water. The results are presented in Fig. 2. For detecting cell A the dependence was linear over the entire region applied (1—30 V), hence, the electrode polarization clearly did not affect the quantity measured. A supply voltage of 30 V was then chosen for subsequent measurements. For detecting cell B the dependence of the base current on voltage was nonlinear over the 1—7 V range and linear at higher voltage. A working voltage of 20 V was then chosen with regard to the signal/noise ratio. The base current for cell B was about 100 times higher than for cell A. The differences in the base current levels correspond with the differences in the dimensions size and, consequently, in the resistance constant values.

The detector was tested by using organic acids as the solutes. Their minimum detectable concentrations determined on both types of detecting cell are given in Table I., The mobile phase consisted of 40% (V/V) methanol and 0·01% (m/m) lauric acid in water. Owing to the more favourable signal/noise ratio, the minimum detectable concentrations (*i.e.* those inducing a peak height equal to double noise

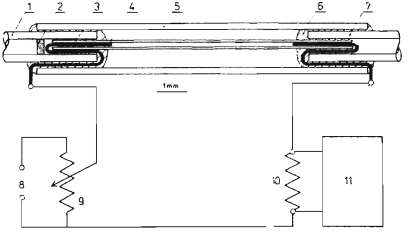

FIG. 1

Schematic diagram of the design and connection of the conductivity detector. **1** Packed glass microcolumn, **2** wool plug, **3** platinum wire 0·1 mm i.d., **4** glass capillary 0·15 mm i.d., **5** glass capillary 1·3 mm i.d., **6** epoxy resin, **7** outlet glass capillary, **8** battery 30 V, **9** potentiometer 10 kΩ, **10** resistor 100 kΩ, **11** recording millivoltmeter

level) were about ten times lower for cell A, where the peak height corresponded to a change in the specific conductivity of eluate of $2 . 10^{-8}$ S cm^{-1} (*i.e.* 0·3% base current).

TABLE I

The minimum detectable concentrations of organic acids

Acid	Minimum detectable concentration μmol l^{-1}	
	cell A	cell B
Acetic	0·67	5·0
Propionic	0·54	5·4
Caprylic	0·89	16·0
Benzoic	0·31	4·4
Oxalic	1·5	17·0
Citric	2·0	14·0

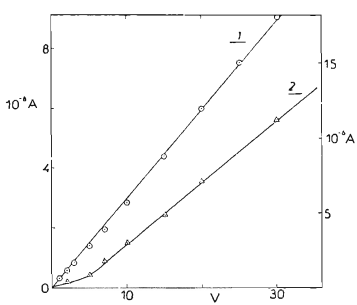

FIG. 2

Dependence of the base current on the voltage applied. *1* Cell A, *2* cell B

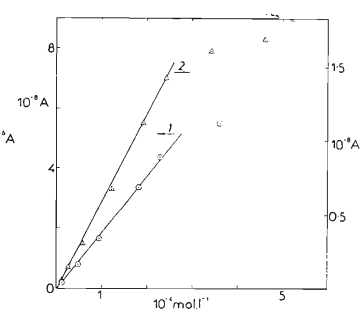

FIG. 3

Linear range of the detector. Solute: propionic acid, mobile phase: 40% (V/V) methanol with 0·01% (m/m) lauric acid in water. *1* Cell A, *2* cell B

The linearity ranges were established for the both cells using propionic acid as solute and 40% (V/V) methanol with 0·01% (m/m) lauric acid in water as the mobile phase. The results are depicted in Fig. 3. The response was linear, in both cases, up to a concentration of approximately $2{\cdot}4 \cdot 10^{-4}$ mol l^{-1}; so, taking into account the minimum detectable concentrations, the linearity for cell A is about 400, for cell B, about 40.

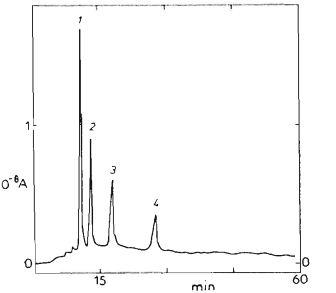

FIG. 4

Chromatogram of a mixture of monocarboxylic acids. Column: $L = 75$ cm, $d_i = 0{\cdot}5$ mm; stationary phase: Separon Si C_{18}; mobile phase: 40% (V/V) methanol with 0·01% (m/m) lauric acid in water; $F_m = 9$ µl min^{-1}. Acids: *1* acetic, *2* propionic, *3* butyric, *4* valeric

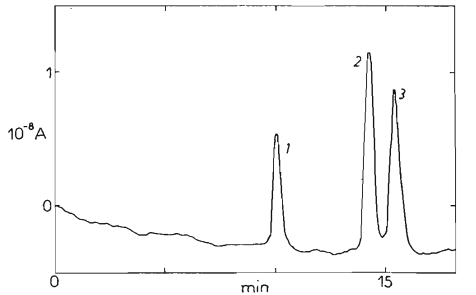

FIG. 5

Chromatogram of a mixture of dicarboxylic acids. Column: $L = 75$ cm, $d_i = 0{\cdot}5$ mm; stationary phase: Separon SiC_{18}; mobile phase: 60% (V/V) methanol with 0·1% (m/m) lauric acid in water; $F_m = 6$ µl min^{-1}. Acids: *1* oxalic, *2* succinic, *3* adipic

TABLE II

Dependence of the minimum detectable concentrations of organic acids (μmol l^{-1}) on the mobile phase composition

Acid	K_a (20°C)	60% (V/V) methanol with			50% (V/V) methanol with	40% (V/V) methanol with
		0·01% (m/m) lauric acid	0·05% (m/m) lauric acid	0·1% (m/m) lauric acid	0·01% (m/m) lauric acid	0·01% (m/m) lauric acid
Acetic	$1{\cdot}75 \cdot 10^{-5}$	9·4	12	13	1·5	0·67
Propionic	$1{\cdot}34 \cdot 10^{-5}$	5·3	5·4	7·9	0·23	0·54
Butyric	$1{\cdot}54 \cdot 10^{-5}$	5·8	6·0	8·6	0·35	0·65
Valeric	$1{\cdot}46 \cdot 10^{-5}$	6·4	6·9	9·3	0·42	0·67
Caprylic	$1{\cdot}27 \cdot 10^{-5}$ [a]	9·1	12	—	0·59	0·89
Benzoic	$6{\cdot}24 \cdot 10^{-5}$	2·0	1·6	2·4	7·5	0·31
Oxalic	$6{\cdot}5 \cdot 10^{-2}$ [a] $6{\cdot}1 \cdot 10^{-5}$	3·7	3·3	2·5	3·3	1·5
Malonic	$1{\cdot}58 \cdot 10^{-3}$ [b] $2{\cdot}16 \cdot 10^{-6}$	—	5·9	—	—	1·0
Succinic	$6{\cdot}07 \cdot 10^{-5}$ $2{\cdot}30 \cdot 10^{-6}$	9·2	9·1	19	2·8	1·6
Glutaric	$4{\cdot}58 \cdot 10^{-5}$ [a] $3{\cdot}89 \cdot 10^{-6}$	9·3	8·1	21	2·5	—
Adipic	$3{\cdot}7 \cdot 10^{-5}$ $3{\cdot}22 \cdot 10^{-6}$	7·6	15	29	3·8	—
Maleic	$1{\cdot}0 \cdot 10^{-2}$ [a] $5{\cdot}5 \cdot 10^{-7}$	2·6	2·2	3·2	2·1	—
Citric	$6{\cdot}7 \cdot 10^{-4}$ $1{\cdot}6 \cdot 10^{-5}$ $4{\cdot}1 \cdot 10^{-6}$	7·0	9·2	—	2·3	2·0

[a] At 25°C; [b] at 18°C.

The minimum detectable concentrations were also investigated in dependence on the composition of the mobile phase for cell A. The results, along with the dissociation constant values for the acids in water[11], are given in Table II. The minimum detectable concentrations of monocarboxylic acids are clearly lower (the detector response is higher) for lower methanol contents. The dissociation constant of acetic acid, which is $4{\cdot}7 \cdot 10^{-6}$ in 40% (V/V) methanol, decreases down to $2{\cdot}0 \cdot 10^{-6}$ on passing to 60% (V/V) methanol[12]. The concentration of lauric acid in the mobile phase of 60% (V/V) methanol affects the minimum detectable concentrations of monocarboxylic acids only slightly. The response to the various monocarboxylic acids decreases with increasing number of carbon atoms in the molecules (acetic acid, inducing a lower response than propionic acid, is an exception). As the number of carbon atoms increases, the dissociation constants of the acids in water decrease; in the water–methanol–lauric acid system the dissociation constants can be expected to follow the same trend. The mobility of the acid anion decreases somewhat in this series too.

The responses to dicarboxylic and tricarboxylic acids in mobile phases of 60% (V/V) methanol are comparable with those to monocarboxylic acids (except for oxalic acid inducing a higher response), and practically do not vary with changing contents of methanol and/or acid. In the mobile phase of 40% (V/V) methanol the responses to dicarboxylic acids are lower than to monocarboxylic acids. Thurman, who has observed this effect as well, ascribes this fact to the different solvation of the acid anion[9].

Examples of detection of monocarboxylic acids in a mixture and of dicarboxylic acids in a mixture are shown, for cell A, in Figs 4 and 5, respectively. In the former case, 0·4 μl was injected of a mixture containing $1 \cdot 10^{-3}$% (m/m) acetic acid and $5 \cdot 10^{-4}$% (m/m) propionic, butyric, and valeric acids in the mobile phase. In the latter case, the sample injected (0·25 μl) contained $2 \cdot 10^{-3}$% (m/m) oxalic acid and $1 \cdot 10^{-2}$% (m/m) succinic and adipic acids in the mobile phase.

The results obtained demonstrate that the conductivity detector devised is suitable for detection in conjunction with packed glass microcolumns. Of the two cell types, the longer one with the smaller diameter (cell A) suits better owing to the lower base current and the more favourable signal/noise ratio. With the d.c. voltage applied, no effect of the electrode polarization has been observed. The minimum detectable concentration of acetic acid is $7 \cdot 10^{-7}$ to $1{\cdot}3 \cdot 10^{-5}$ mol l^{-1}, according to the composition of the mobile phase.

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