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ELECTROCHEMICAL CELL WITH EFFECTIVE VOLUME LESS THAN 1 nl FOR LIQUID CHROMATOGRAPHY

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SUMMARY

The design and the application of an electrochemical detector with volume less than 1 nl is described. The detection limit is less than 0.1 pg. The application of the detector in combination with capillary columns in liquid chromatography yields good separation efficiencies within relatively short analysis times.

INTRODUCTION

Recently it was derived theoretically[1,2] and verified experimentally[3] that the volume of the detection cell or the time constant of the detection restricts the use of capillary columns in liquid chromatography.

The volume of the detector cell, V_{det}, required should be less than half of the standard deviation, σ_V (ref. 1), of the peak of an unsorbed compound expressed in the terms of volumetric units

$$V_{det} \leqslant 1/2\ \sigma_V = \pi d_c^2 L/8\sqrt{N} \tag{1}$$

where d_c is the column inner diameter, L is the column length and N is the number of theoretical plates. For mobile phase velocities higher than the velocity that would correspond to the minimal height equivalent to a theoretical plate, H, N can be expressed as

$$N = \pi\ 24\ D\ L/F_m \tag{2}$$

where D is the diffusion coefficient of the solute and F_m is the volumetric flow-rate of the mobile phase.

The demands imposed on the time constant of the detection, τ_d, are also stringent. In order that the distortion of the signal is less than 5% it is necessary[4] that

$$\tau_d \leqslant 0.32\ \sigma_t = 0.32\ t_R/\sqrt{N} \tag{3}$$

where σ_t is the standard deviation of the peak of an unsorbed compound expressed in

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terms of time units and t_R is the retention time of this peak. Strict technical requirements of the detection follow from eqns. 1–3. The advantages of capillary columns over packed columns can only be realized when the volume of the detector is less than 1 nl and the time constant of the detection is equal to a few tenths of a second.

Attempts at the miniaturization of the detection cell have been made for several years. So far the UV detector has been reduced to 50 nl[5] or to 100 nl[6,7], the fluorometric detector to 53 nl[8] or 100 nl[9] and the electrochemical detector to 150 nl[10]. Very recently a UV detector with an effective volume of 6 nl[11] was reported, making it possible to measure concentrations of 1.5 ng/μl of benzene with a noise level of $1.5 \cdot 10^{-5}$ a.u. These detectors permit operation without substantial distortion of the signal with capillary columns having inside diameters of 30–60 μm[5–9,11]. The use of detectors based on the principle of flame ionization[12] permitted the application of columns with an inside diameter of 10 μm[3].

Liquid chromatography with electrochemical detection has become widespread[13–15]. The advantage of electrochemical detection is its sensitivity; thus, sensitivities higher than those of UV or fluorometric detection[10] can be obtained.

The present paper describes the design[16] and the application of an electrochemical cell with a volume of about 1 nl combined with capillary columns with inside diameters less than 20 μm. The use of this combination permits analysis times comparable with those obtained in existing packed columns. At the same time the detection limit is less than 1 pg.

EXPERIMENTAL

Electrochemical cell

The construction of the electrochemical cell was based on "wall jet"[17–19] electrode, which was miniaturized so that very low flow-rates (*ca.* 1 nl/sec), usually used with capillary columns, could be applied. The design of the cell and its connection to a capillary column is shown in Fig. 1.

Into a stainless-steel capillary (0.8 mm O.D., 0.45 mm I.D.) was inserted a capillary of Simax glass (0.30 mm I.D.) into which a capillary of the same glass (0.25 mm O.D.) was freely inserted. A platinum wire (diameter 0.10 mm) was resin-bonded into the last capillary. The whole assembly was ground at one end perpendicularly to the axis of the capillary to form the auxiliary electrode, comprising a cross-section through the stainless-steel capillary, and the measuring electrode, comprising a cross-section through the platinum wire (Fig. 2). The annular space between the glass capillaries was filled with the mobile phase, thus providing a conducting bridge to a reference electrode. The other end of the capillary assembly was resin-bonded with the vessel for the reference electrode. A silver wire coated with silver chloride and immersed into a saturated solution of potassium chloride in water served as a reference electrode.

All of the measurements were carried out at a measuring electrode voltage of 0.8 V with respect to the reference electrode. The mobile phase enters the vessel with the reference electrode. Through this vessel the platinum wire of the measuring electrode, isolated from the surroundings by a glass capillary, also passed. The assembly was joined to the perpendicularly ground glass capillary column via a plastic tube with a suitable inside diameter such that the bearing surfaces were parallel and in close contact.

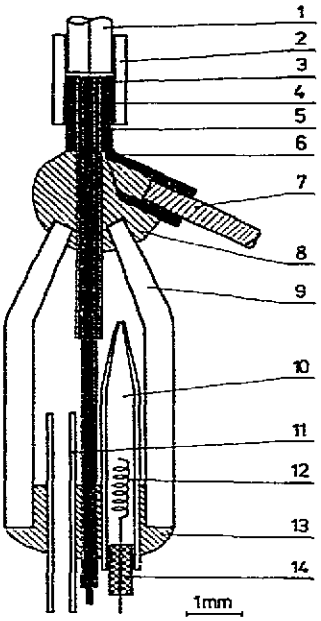

Fig. 1. The measuring electrode and its connection to the capillary column. 1 = Capillary column; 2 = PTFE tube; 3 = platinum wire (diameter 0.1 mm); 4, 5 = glass capillary; 6 = stainless-steel capillary; 7 = inlet wire for the auxiliary electrode; 8, 13 = epoxide resin; 9 = glass vessel for the reference electrode; 10 = reference electrode; 11 = output capillary; 12 = silver wire; 14 = rubber stopper.

As the surface area of the measuring electrode was less than 0.01 mm^2 and the distance between the electrode and the exit of the capillary column was less than 0.1 mm, the volume of the space bounded by the capillary estuary and the reference electrode —"cell"— is less than 1 nl. The surface of the electrode can easily be cleaned by grinding the electrode assembly with a fine sharpening stone.

The electrochemical cell was connected to the electrical circuit as shown in Fig. 3. An alternative arrangement was also tested by omitting the reference electrode and connecting the corresponding amplifier into the circuit as a voltage follower. This variant gave no deterioration of the baseline or of the sensitivity.

Sampling and the column

A combination of a six-port valve and a splitter, Fig. 4, was used for the injection.

Glass capillary columns were drawn from glass tubes of suitable dimensions in the manner described earlier[3,20]. The previous work also describes the determination of the inside diameter of the columns prepared. A column coated with OV-101 silicone oil was used for reversed-phase chromatography. The procedure for stationary phase coating, and the properties of the columns prepared, will be described elsewhere[21].

Distilled water, acidified with $1 \cdot 10^{-3}$ *M* perchloric acid, was used as a mobile phase for all of the measurements. This was pumped without any further treatment into the chromatograph with the aid of a high-pressure pump (Orlita, Giessen, G.F.R.) connected to a device for damping pressure pulses. A Kompensograph III

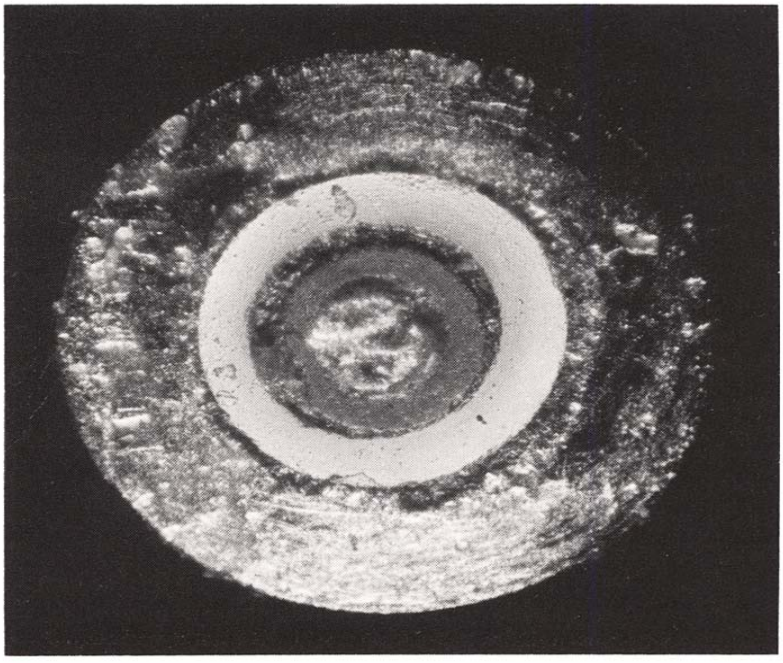

Fig. 2. Photograph of the measuring electrode.

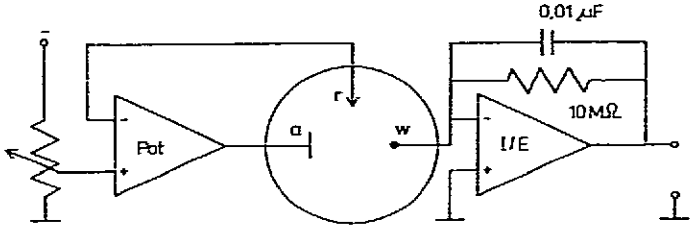

Fig. 3. Diagram of the electrical circuit used. The potentiostat (Pot) and current-to-voltage converter (I, E) are National Semiconductors JFET operational LF 356 N amplifiers. The time constant of the circuit is 0.1 sec. The auxiliary, working and reference electrodes are designated a, w and r, respectively.

recording millivoltmeter (Siemens, Karlsruhe, G.F.R.) with a time constant of 0.2 sec was used to record the chromatograms.

RESULTS AND DISCUSSION

Character of the response

A Sial glass capillary (1.2 m × 14.3 μm I.D.), corresponding to a column volume of 193 nl, was used to measure the character of the response. The solute was a solution of 10 mg/l hydroquinone in the mobile phase. The flow through the splitter was selected so that the volume sampled in the column from the sampling loop (volume 10 μl) was greater than the column volume. The splitting ratio was constant for all the mobile phase flow-rates.

The difference between the background current and the current corresponding

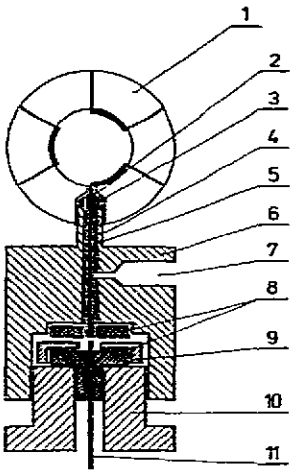

Fig. 4. Diagram of the six-port sampling valve and the splitter. 1 = Six-port valve; 2 = soldered; 3, 8 = PTFE sealing; 4 = stainless-steel capillary (0.2 mm I.D., 1.0 mm O.D.); 5 = stainless-steel tube (1.2 mm I.D., 2 mm O.D.); 6 = splitter body; 7 = splitter output; 9 = sealing holder; 10 = tightening screw; 11 = capillary column.

to the passage of the hydroquinone solution was then measured at various flow-rates of the mobile phase. Table I and Fig. 5 summarize the results obtained. It is obvious that for flow-rates greater than *ca.* 1 nl/sec the detector can be considered as a concentration detector, and is therefore suitable for the evaluation of chromatograms.

TABLE I

DEPENDENCE OF ELECTROCHEMICAL EFFICIENCY OF THE DETECTOR ON THE MOBILE PHASE FLOW-RATE

t_{R^0} *(sec)*	F_m *(nl/sec)*	*Mass flow of hydroquinone (pg/sec)*	*Current for 100% coulometric yield (nA)*	I_d *(nA)*	*Coulometric yield (%)*
103	1.87	18.7	32.81	1.46	4.51
200	0.965	9.65	16.93	1.34	7.86
360	0.536	5.36	9.40	1.25	13.30
580	0.333	3.33	5.85	1.18	20.17
860	0.224	2.24	3.94	1.08	27.42
1300	0.148	1.48	2.59	0.78	30.16

The observed dependence does not correspond with the dependences of the diffusion current on the mobile phase flow-rate derived for wall-jet electrodes[17,18]:

$$I_d = f(F_m^{3/4}) \text{ or } I_d = f(F_m^{1/2})$$

These dependences were, however, derived for flow geometries rather different from that used in the present work.

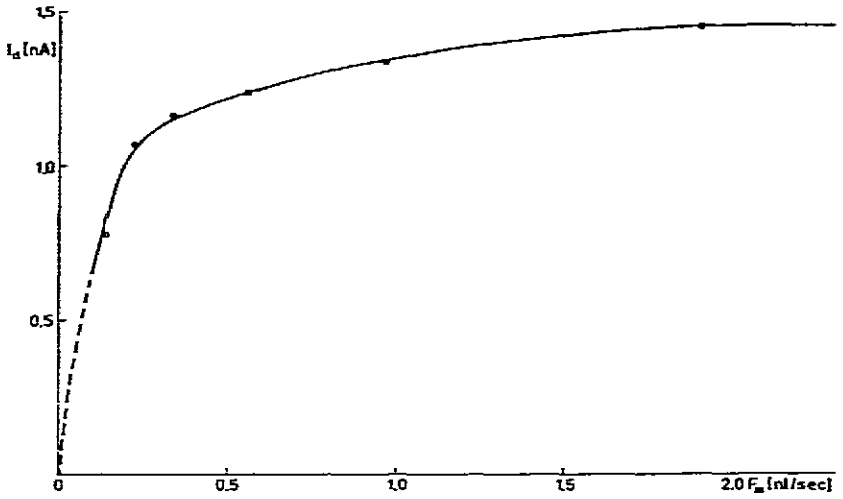

Fig. 5. Dependence of the response value of the amperometric detector, I_d on the mobile phase flow-rate, F_m. Solute: 10 mg hydroquinone per 1 l of the mobile phase.

The observed dependence has the exponential character of the relation derived for thin-layer electrode[22]

$$I_d = nFCF_m [1 - \exp(-\mathrm{Sh} \cdot Dlb/2F_m d)] \quad (4)$$

where I_d is the diffusion current, Sh is Sherwood's number, l, b and d are the length, width and height, respectively, of a thin-layer electrode, n is the number of electrons that participate in the electrode reaction, F is the Faraday constant and C is the solute concentration in the mobile phase. At high flow-rates, when the exponent $(-\mathrm{Sh} \cdot Dlb/2F_m d)$ is small in comparison with unity, the term in the square brackets can be approximated with a positive value of the exponent. Hence the diffusion current ceases to be a function of the flow-rate of the mobile phase and the detector response then has concentration character.

Extra column peak broadening

In order to estimate extra column peak broadening, the dependence of the height equivalent to a theoretical plate, H, on the linear velocity of the mobile phase was measured for the capillary used in the above measurement. The splitting ratio was adjusted to *ca.* 1:6000 and the injection was performed in such a manner that the sampling loop was inserted into the flow for only a fraction of a second. The dependence observed is shown in Fig. 6. It is obvious that for the range of mobile phase flow-rates studied, the value of H observed is approximately four times as high as would be predicted by theory. Hence, it follows that for the capillary used the broadening results chiefly from extra column contributions, *i.e.*, the extra column broadening, $\sigma^2_{ex} \approx 3\sigma^2_{col}$. On the basis of eqns. 1 and 2, it can be calculated that, for the capillary used, the dependence of the broadening on the volumetric flow-rate of the mobile phase is expressed by the relationship

$$\sigma^2_{col} = 0.412 \cdot F_m$$

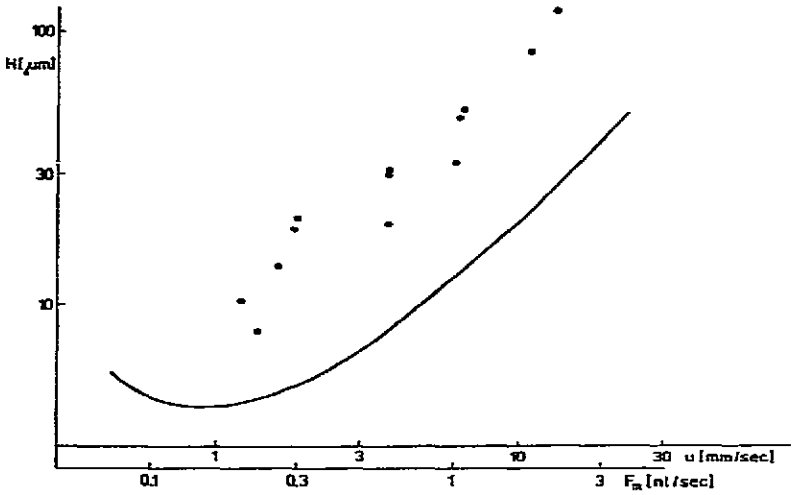

Fig. 6. Dependence of HETP on the linear velocity of the mobile phase. ●, Data measured experimentally with the use of the detector described; ———, calculated dependence for a column of diameter 14 μm and diffusion coefficient $D = 10^{-5}$ cm²/sec.

where F_m is expressed in nl/sec and, as a result, σ^2_{col} is then obtained in nl². Hence the extra column broadening of the chromatographic system for the range of the mobile phase flow-rates used is as follows:

$$\sigma^2_{ex} \approx 1.3 \cdot F_m$$

Detection limits

These were determined in the arrangement used above. An example of the chromatogram observed is shown in Fig. 7. Injection of the pure mobile phase does not provide any noticeable peak. From the measurements of the character of the detector response it can be calculated that the peak observed corresponds to 0.76 pg

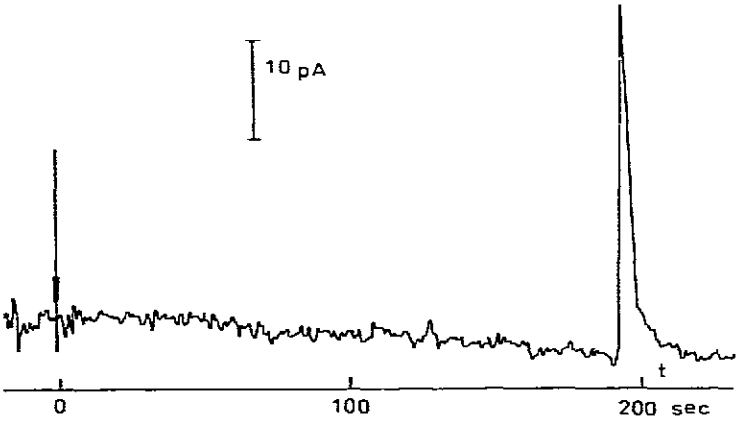

Fig. 7. An example of a chromatogram obtained with the capillary column (1.2 m × 14 μm I.D.). Pressure: 1.0 MPa. The peak obtained corresponds to the injection of 0.76 pg hydroquinone.

of hydroquinone injected into the column. The noise observed is 1.5 pA peak-to-peak. Hence, the signal-to-noise ratio is equal to two and corresponds to the introduction of 0.05 pg hydroquinone into the column or to a concentration of 0.02 ppm at the peak maximum.

An example of the separation of phenols on the capillary column is shown in Fig. 8. The capillary column (2.8 m × 16 μm I.D.) had a volume of 560 nl. On the basis of eqns. 1 and 2 it can be calculated for the elution of an unsorbed compound that the broadening is characterized by the relationship $\sigma^2_{col} = 1.5 \cdot F_m$. Comparison of this result with the relationship characterizing extra column broadening indicates that for the elution of the unsorbed component the extra column broadening will lead to a *ca.* 85% increase in the value of H. At the linear velocity of the mobile phase used —7.2 mm/sec— H for the peak of the unsorbed compound was found to be 45 μm, whereas the theory predicts 20 μm. With respect to the extra column broadening estimated earlier, H should be 37 μm. The discrepancy between this value and the value observed can be ascribed to indeterminacy of the value of the diffusion coefficient (estimated to be $1 \cdot 10^{-5}$ cm²/sec) or to the error in the determination of the diameters of the capillary columns. Nevertheless, for sorbed compounds the extra column broadening is acceptably low for this type of separation, which makes it possible to take advantage of the separation capacity of the capillary column used.

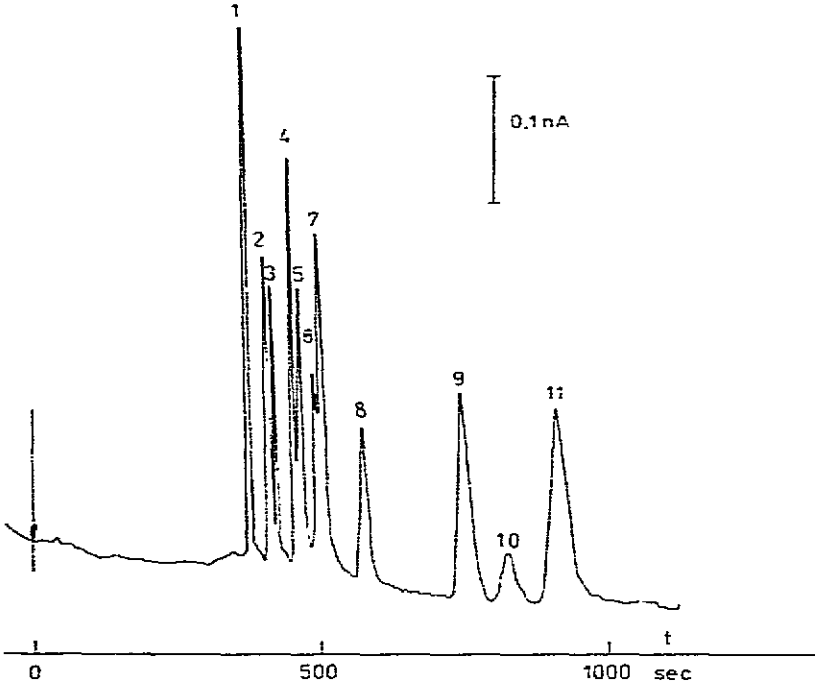

Fig. 8. Separation of phenols by capillary liquid chromatography. Peaks: 1 = hydroquinone (t_R); 2 = 4-methylphenol; 3 = 2-methylphenol; 4 = 3,4-dimethylphenol; 5 = 3,5-dimethylphenol; 6 = 2,3-dimethylphenol; 7 = 2,4-dimethylphenol; 8 = 2,6-dimethylphenol; 9 = 2-methyl-4-ethylphenol; 10 = 2-isopropylphenol; 11 = 2,4,6-trimethylphenol. Mobile phase: 10^{-3} M $HClO_4$ in distilled water; flow-rate 1.7 nl/sec. Pressure: 2.5 MPa. Column: 2.8 m × 16 μm I.D.; stationary phase OV-101. Flow through the splitter: 12 μl/sec.

CONCLUSIONS

A miniature amperometric detector has been designed which enables detection without substantial distortion of the effluent composition from capillary columns with diameters less than 20 μm and with lengths less than 3 m. The electrochemical efficiency is comparable, at the mobile phase flow-rates used, with those of cells employed with packed columns. The small surface area of the measuring electrode results in a low noise (*ca.* < 1.5 pA) with the minimal measuring space (volume less than 1 nl). These characteristics enable the detection of concentrations down to 0.02 ppm at the flow-rates employed which capillary columns.

The described combination of the capillary column with the amperometric detector makes it possible to achieve separations comparable with those obtained in high efficiency packed columns, in respect both of separation efficiency and speed of analysis, was demonstrated for the separation of eleven components within 15 min. The preparation of capillary columns with diameters less than 10 μm will enable full use to be made of their advantages in liquid chromatography.

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