CHROM. 21 244

LIQUID CHROMATOGRAPHY ON PACKED FUSED-SILICA CAPILLARY COLUMNS WITH ELECTROCHEMICAL DETECTION

V. F. RUBAN*, A. B. BELENKII, A. Ya. GUREVICH and B. G. BELENKII

Institute of Macromolecular Compounds of the U.S.S.R., Academy of Sciences, Bolshoy, 31 Leningrad (U.S.S.R.)

SUMMARY

A chromatographic system for reversed-phase high-performance liquid chromatography on fused-silica capillary columns [height equivalent to a theoretical plate (HETP) = 2–$3d_p$; d_p = mean particle diameter] has been developed. The rate dependences of the column efficiency were investigated and the volumes for sample injection and detection were studied and optimized. An electrochemical detector with a cell volume of 1–100 nl was developed and its main characteristics were investigated. The application of this system to the high-speed ultra-sensitive chromatographic analysis of electrochemically active substances with a detection limit of a few picograms is reported.

INTRODUCTION

High-performance liquid chromatography with the application of fused-silica capillary columns of 0.2–0.4 mm I.D. packed with fine-grained sorbents exhibits advantages due to the high mass sensitivity and low consumption of the sorbent and the eluent. Columns consisting of flexible fused-silica capillaries also make it possible to attain very high efficiency (more than 100 000 plates) and high rates (more than 100 plates/s) of chromatographic separations[1–3]. The application of these columns is particularly promising in chromatographic analyses that require the exclusion of the chemical and adsorbing effects of the column material on the sample components to be separated.

In order to achieve a high efficiency of columns made of flexible fused-silica capillaries, the chromatographic system should include a sample injector and a detector measuring cell of less than 0.1 μl in volume. The most advantageous detectors for liquid chromatography on packed fused-silica columns are laser detectors with induced fluorescence[4–6]. Recently laser-induced photothermal refractometric (absorptiometric)[7,8] and electrochemical (voltamperometric) detectors have been proposed, which allow the minimization of the operating volume without substantial decreases in sensitivity and selectivity[4].

Electrochemical detection in this variant of liquid chromatography is of analytical interest because it is known that amperometric detectors with steel

micro-columns with I.D. 1 mm[9,10] and 1.2 mm[11], glass columns of I.D. 0.7 mm[12], PTFE columns of I.D. 0.5 mm[13], glass capillary columns packed with 30-μm sorbent[14] and open glass[15] and fused-silica capillary columns[16] for the investigation of many classes of substances (phenols, catecholamines, amino acids, drugs and their metabolites, etc.) are used. The use of amperometric detectors with packed silica capillary columns of I.D. 0.35 mm has also been reported[17,18]. The latest investigations used single carbon-fibre microelectrodes of 7 μm diameter, whose size hampers their easy handling and makes the construction of a measuring cell of I.D. 50 μm difficult. The purpose of our work was to construct and investigate a chromatographic system using fused-silica capillary columns packed with fine-grained sorbents and an electrochemical detector with a measuring cell of a simple and compact design. In contrast to similar detectors that have been described[9–11,14], that proposed here permits the direct connection of the separating column and the measuring cell without the use of intermediate capillaries[17,18]. This permits the extra-column spreading to be decreased appreciably, the separation efficiency to be increased and the detection efficiency to be improved.

EXPERIMENTAL

Chromatographic system

To investigate the rate characteristics of the columns and the efficiency of the electrochemical detector and to determine the optimum injection volumes, a system was used in which the eluent was injected by a Shimadzu LC-5A high-pressure pump at a constant rate of 10–100 μl/min or at constant pressure (Fig. 1). After the Rheodyne 7410 injection valve (volume 0.5 μl) the flow is divided; the smaller part enters the fused-silica column and the larger part passes into a side-line. The degree of flow splitting is smoothly controlled with a valve located in the side-line[19]. The degree of splitting controls the eluent flow-rate in the column and the volume of the sample injected into the column (1–500 nl). The outlet of the column is connected with the cell of the electrochemical detector. The adjustment of the potential of the working electrode and the measurements of the polarization current were carried out under d.c. conditions with the aid of an OH-107 recording polarograph (Radelkis) with a time constant of 0.3 s. The eluent flow in the side-line joins that coming from the detector and enters a reservoir containing 20 ml of the initial eluent. Consequently, the
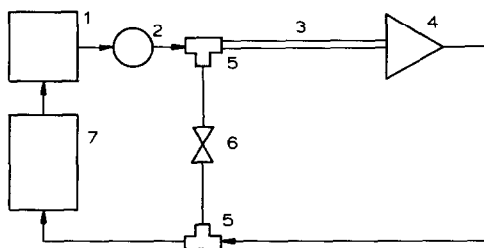

Fig. 1. Hydraulic scheme for chromatographic system: 1 = pump; 2 = injection valve; 3 = fused-silica column; 4 = electrochemical detector; 5 = T-shaped connections; 6 = valve; 7 = vessel containing the eluent.

hydraulic system becomes closed. The eluent circulates along the closed path without losses and can be repeatedly used for carrying out analyses of the same type.

With model mixtures, *i.e.*, when a sample does not contain contaminating components whose concentrations substantially exceed those of the substances under analysis, the circulation of the flow causes a drift of the zero line which is very small in comparison with the noise level. Chromatographic separation was also conducted with the use of a 30-nl injection valve (Special Design Bureau of Analytical Instruments, Academy of Sciences of the U.S.S.R.) in a non-split flow system (see Figs. 5 and 6).

Column packing

The fused-silica capillary columns (5–100 cm × 0.2–0.4 mm I.D.) were packed with Nucleosil 3C$_{18}$ sorbent (Macherey, Nagel & Co.) with a particle size of 3 μm and a Separon Si-C$_{18}$ sorbent (Lachema) with a particle size of 10 μm by a slurry packing procedure similar to that described elsewhere[20,21]. For this purpose, a sorbent suspension in methanol–isopropanol (95:5) at a concentration of 100 mg/ml was prepared, treated ultrasonically (22 kHz) for 3 min and then injected with a syringe into a stainless-steel reservoir. The suspension was displaced from this reservoir with methanol into the fused-silica capillary with the aid of a Shimadzu LC-5A pump. After the column had been packed with the sorbent, methanol was replaced with distilled water and the packed sorbent layer was stabilized for 30 min.

Electrochemical detector

The detector cell (Fig. 2) was manufactured in a variant combining the wall-jet and thin-layer types[22]. It was made of PTFE with polished operating surfaces. The upper part of the cell body (1) had openings for a fused-silica column (2) and silver chloride reference electrode (3). The flow from the column was fed to the polished surface of the glassy carbon working electrode (4). Subsequently, the electrode passed through a rectangular channel 0.1–0.2 mm in width cut in a PTFE sheet (5) 0.01–0.1 mm thick and led to a platinum or stainless-steel capillary (6) acting as the auxiliary electrode.
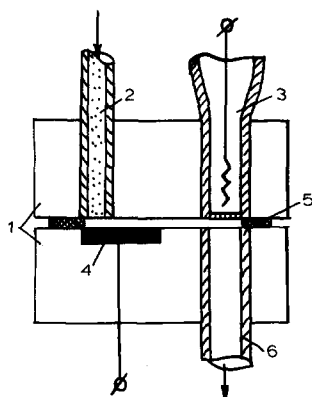

Fig. 2. Design of electrochemical detector cell: 1 = body; 2 = fused-silica column; 3 = reference electrode; 4 = working electrode; 5 = PTFE sheet; 6 = auxiliary electrode.

This construction of the electrochemical detector cell also makes it possible to carry out the recording of electrochemically active substances on two independently polarized glassy carbon (4) and platinum (6) electrodes with the appropriate scheme for the connection of the measuring instrument. The replacement of the lower part of the cell body by a similar part containing a platinum electrode instead of a glassy carbon electrode extends the possibilities of using the detector.

By changing the thickness of the PTFE sheet it is possible to vary the effective cell volume in the range 1–100 nl. In this instance the sensitivity of the electrochemical detector changes; it is considerably affected by the area of the active surface of the working electrode and is controlled by the size of the channel with the PTFE sheet.

Reagents

The eluents were prepared with doubly distilled water, acetonitrile (Chempure), phosphoric acid (specially pure), sodium perchlorate (specially pure) and EDTA (AnalaR).

RESULTS AND DISCUSSION

Efficiency of the chromatographic system

For a successful separation on a chromatographic column, it is necessary to minimize extra-column spreading. According to Kirkland *et al.*[23], when the decrease in column efficiency due to spreading in the injector does not exceed 10%, the volume dispersion of the chromatographic zone is given by

$$\sigma_s^2 = \frac{V_s^2}{12} \leqslant 0.1 \, \sigma_{V_0,\text{col}}^2 \tag{1}$$

where V_s is the sample volume and $\sigma_{V_0,\text{col}}^2$ is the variance of the volumetric dispersion of the non-sorbed solute. For a 430×0.32 mm I.D. column packed with 10-μm Separon Si-C$_{18}$, the experimentally measured volume V_0 is 28.2 μl. The extrapolation of the dependence $N = f(V_s)$ to zero sample volume gives a maximum efficiency of $N = 8200$ plates at an flow-rate of 2.5 μl/min. In this case $\sigma_{V_0,\text{col}}^2 = 0.097 \, \mu l^2$ and, according to eqn. 1, we should have $V_s \leqslant 0.34 \, \mu$l. However, at $V_s \leqslant 0.03 \, \mu$l the decrease in the efficiency of the system does not exceed 10%, as follows from the experimental results. This discrepancy between theory and experiment is due to the existence of the contributions to extra-column spreading which were not taken into account, *e.g.*, the connections of the column with the injection valve and the detector. Spreading in the cell of the electrochemical detector could be neglected because the effective volume of the cell was 1 nl.

The requirement for the miniaturization of the detector volume V_d in column chromatography[23] is expressed by the equation

$$V_d^2 \leqslant 0.16 \, \sigma_{V_0,\text{col}}^2 \tag{2}$$

Comparison of eqns. 1 and 2 leads to the relationship $V_d \approx 0.365 V_s$, *i.e.*, in chromatography on capillary columns the detector volume should be several nanolitres. The decrease in the column diameter and length when sorbents of grain size
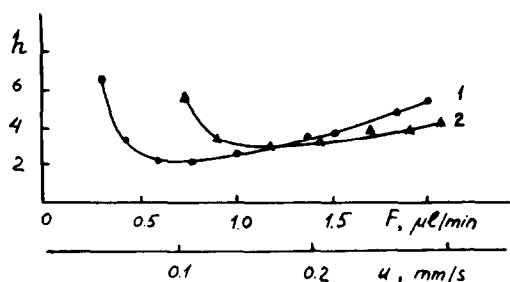
Fig. 3. Rate dependence of reduced HETP: 1, 430 × 0.32 mm I.D. column, Separon Si-C$_{18}$ (10 μm); 2, 100 × 0.34 mm I.D. column, Nucleosil 3C$_{18}$ (3 μm). Eluent, acetonitrile–water (3:7) + 0.1 M NaClO$_4$, 22°C; sample, hydroquinone (k' = 0.72); electrochemical detector, E = +0.5 V (Pt), V_s = 20 nl, V_d = 1 nl.

less than 10 μm are used requires a corresponding decrease in the volume of injection and the detector cell.

The rate dependence for fused-silica capillary columns shown in Fig. 3 indicates that this procedure of column packing makes it possible to attain a high efficiency. Thus, for a 10-μm sorbent at the optimum elution rate, the reduced height equivalent to a theoretical plate (HETP) is 2.1d_p and for a 3-μm sorbent it is 2.9d_p, where d_p is the mean particle diameter.

Detector characterization

Electrochemical detectors used in microcolumn chromatography in which the cell volume is small and the active surface of the working electrode is a few tenths of a square millimetre usually operate under an amperometric regime[12]. The electrochemical efficiency of the detector, η, can be defined as the ratio of the number of electrons absorbed on the working electrode in the oxidation of the substance, Q_A, to that absorbed in the oxidation of the entire mass of the substance being detected, Q_K. The potential of the working electrode corresponds to the conditions of the limiting current. The value of Q_A was calculated from the surface area under the chromatographic peak and Q_K was estimated on the basis of the Faraday law.

It can be seen from Fig. 4 that the electrochemical efficiency of the detector increases with decreasing elution rate. Moreover, the greatest change in η occurs at a rate of less than 2 μl/min, whereas at the maximum rate the column efficiency of the detector is about 25%.

It is possible to determine electrochemically active substances with the aid of an amperometric detector if the linearity of the response has a relatively wide range. Hence, we investigated for phenol the dependence

$$\log I = a \log q + b \tag{3}$$

where I (nA) is the detector signal, q (pg) is the amount of phenol in the sample and a and b are constants. Processing of the experimental data by the least-squares method yields a = 0.99 ± 0.01 and b = −(1.38 ± 0.02) for log q = 2–5. In other words, the detector has linear characteristics over the range under investigation. The noise level in this construction does not exceed 0.12 nA. Hence, according to eqn. 3, the detection level of phenol is 6 pg.
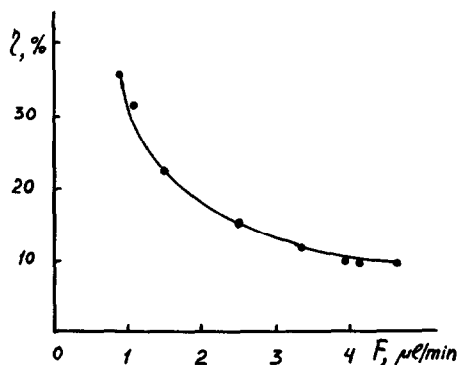
Fig. 4. Electrochemical detector efficiency *vs.* flow-rate. Column, 100 × 0.34 mm I.D., Nucleosil 3C$_{18}$ (3 μm); eluent, acetonitrile–water (3:7) + 0.1 M NaClO$_4$, 22°C; sample, phenol, V_s = 30 nl, mass = 5 ng; electrochemical detector, E = +1.2 V (glassy carbon), V_d = 10 nl.

Analytical application

The chromatographic system was applied to the analysis of a mixture of phenol and its monosubstituted derivatives (Fig. 5). A fused-silica capillary column (100 × 0.34 mm I.D.) packed with a sorbent with a particle size of 3 μm exhibits an efficiency of more than 11 000 plates at the optimum flow-rate. The high elution rate makes it possible to separate these compounds in 6 min with a selectivity sufficient for quantitative analysis.

Fig. 6 shows the chromatographic determination of biogenic amines. The analysis time is less than 1 min. The detection limit for noradrenaline is 7 pg.

CONCLUSIONS

An electrochemical detector with a nanolitre-size cell with platinum and glassy carbon working electrodes, which is a combination of the thin-layer and wall-jet
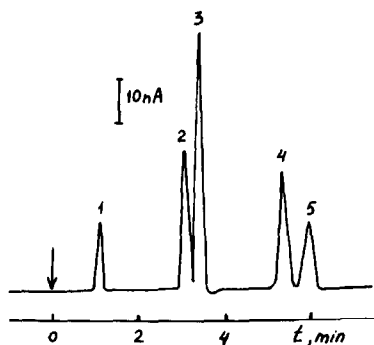

Fig. 5. Chromatography of phenol derivatives. Column, as in Fig. 4; eluent, phosphate buffer solution (pH 2.4)–acetonitrile (7:3), flow-rate 4.6 μl/min, 20°C; electrochemical detector, E = +1.5 V (Pt), V_d = 10 nl; sample volume, 30 nl. Amounts of phenol compounds, 5.75 ng of each: 1 = NaNO$_2$ (k' = 0); 2 = phenol; 3 = guaiacol; 4 = *p*-cresol; 5 = *o*-cresol.
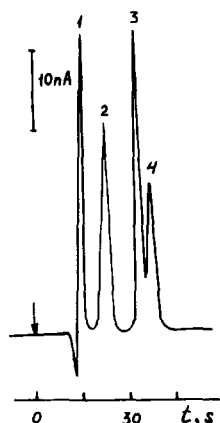
Fig. 6. High-speed analysis of catecholamines: 1 = noradrenaline (1.0 ng); 2 = adrenaline (2.0 ng); 3 = 3,4-dihydroxyphenylalanine (2.0 ng); 4 = 3,4-dihydroxythiramium (1.5 ng). Column, 60 × 0.32 mm I.D., Nucleosil $3C_{18}$ (3 μm); eluent, distilled water + 0.1 M NaClO$_4$ + 1 mM EDTA + H$_3$PO$_4$ (pH 2.0), flow-rate 11 μl/min, 20°C; sample volume, 30 nl; electrochemical detector, E = +1.5 V (Pt), V_d = 10 nl.

versions, allows the maximum use of the efficiency of silica capillary columns with an HETP of 2–3d_p. The suggested chromatographic system makes it possible to perform high-speed analyses of trace amounts of electrically active substances, especially with samples of small volume.

REFERENCES

1 P. Kucera, *J. Chromatogr.*, 198 (1980) 93.
2 C. E. Reese and R. P. W. Scott, *J. Chromatogr. Sci.*, 18 (1980) 479.
3 G. H. Knox, *J. Chromatogr. Sci.*, 18 (1980) 453.
4 R. P. W. Scott (Editor), *Liquid Chromatography Detectors*, Elsevier, Amsterdam, 1986, p. 271.
5 A. Ph. Lobazov, V. A. Mostovnikov, S. V. Nechaev, B. G. Belenkii, J. J. Kever, E. M. Korolyova and V. G. Maltsev, *J. Chromatogr.*, 365 (1986) 321.
6 M. C. Roach and M. D. Harmony, *Anal. Chem.*, 59 (1987) 411.
7 C. N. Kettler and M. J. Sepaniak, *Anal. Chem.*, 59 (1987) 1733.
8 T. O. Nolan and N. J. Dovichi, *Anal. Chem.*, 59 (1987) 2803.
9 A. Carlsson, T. Sharp, T. Zetterström and U. Ungerstedt, *J. Chromatogr.*, 368 (1986) 299.
10 A. Carlsson and K. Lundström, *J. Chromatogr.*, 350 (1985) 169.
11 E. J. Caliguri, P. Capella, L. Botarri and T. M. Mefford, *Anal. Chem.*, 57 (1985) 2423.
12 K. Šlais and O. Kouřilová, *J. Chromatogr.*, 258 (1983) 57.
13 M. Goto, Y. Koyanagi and D. Ishii, *J. Chromatogr.*, 208 (1981) 261.
14 Y. Hirata, P. T. Lin, M. Novotný and R. M. Wightman, *J. Chromatogr.*, 181 (1980) 287.
15 K. Šlais and M. Krejčí, *J. Chromatogr.*, 235 (1982) 21.
16 M. J. N. Hetem, H. A. Glaessens, P. A. Leclercq, C. K. Gramers, V. Pacakova and K. Stulik, in P. Sandra (Editor), *Proceedings of the Eighth International Symposium on Capillary Chromatography*, Vol. II, Hüthig, Heidelberg, 1987, p. 112.
17 M. Goto and K. Shimada, *Chromatographia*, 21 (1986) 631.
18 M. Goto, K. Shimada, T. Takeuchi and D. Ishii, *Anal. Sci.*, 4 (1988) 17.
19 F. J. Yang, *J. Chromatogr.*, 236 (1982) 265.
20 M. Konishi, Y. Mori and T. Amano, *Anal. Chem.*, 57 (1985) 2235.
21 J. C. Gluckman, A. Hirose, V. L. McGuffin and M. Novotny, *Chromatographia*, 17 (1983) 303.
22 W. Kemula and W. Kunter, in E. Pungor, I. Buzás and G. E. Veress (Editors), *Modern Trends in Analytical Chemistry (Analytical Chemistry Symposia Series, Vol. 18)*, Elsevier, Amsterdam, 1984, p. 3.
23 J. J. Kirkland, W. W. Yau, H. J. Stoklosa and C. H. Dilks, *J. Chromatogr. Sci.*, 15 (1977) 303.