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# Study of the characteristics and analytical application of a capillary chromatograph with an electrochemical detector

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## ABSTRACT

The characteristics of a capillary liquid chromatograph consisting of a syringe pump, microinjector, fused-silica capillary columns of I.D. 0.2–0.4 mm packed with different sorbents and an electrochemical detector of the amperometric type were investigated. The relative standard deviation of the retention times was $<0.3\%$ and that of the height of the chromatographic peak was $<1\%$. The detection limit for catecholamines was 0.1 pg. Low extra-column spreading makes it possible to attain a separation efficiency with a minimum reduced plate height of 2.1–2.9. Examples are given of the application of the capillary liquid chromatograph to the separation of phenol compounds and amino acids in model and real samples.

## INTRODUCTION

In recent years, miniaturization in high-performance liquid chromatography has been related to the application of packed columns manufactured from flexible fused-silica capillaries less than 0.5 mm in diameter [1–4]. The advantages of capillary columns are a higher efficiency and more extensive possibilities of applying high-speed analysis than in columns of the usual diameter [5,6]. An important advantage of capillary columns is a considerable decrease in sample volume.

However, the decrease in column diameter leads to the necessity for a corresponding decrease in spreading in various assemblies of the chromatographic system: injector, detector and connections. Moreover, the optimum volume flow-rates in capillary liquid chromatography are a few microlitres per minute, which imposes stringent requirements on the pump system.

The application to capillary liquid chromatography using various detector types with cells exhibiting low spreading usually leads to a decrease in concentration sensitivity. The mass sensitivity in capillary column chromatography is much higher than that with columns of the usual diameter [7]. The use of detectors based on laser [8–11] and electrochemical [12–15] principles makes it possible to increase the sensitivity of determination. In the former instance this increase is due to the ability of laser radiation to focus in extremely small volumes and in the latter it results from the application of microelectrodes.

In this paper a capillary liquid chromatograph based on packed fused-silica capillary columns is described. The main characteristics of the column, micropump

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and electrochemical detector of the amperometric type used with the aim of application to highly sensitive chromatographic analysis are reported.

## EXPERIMENTAL

The chromatograph consisted of a Milichrom syringe micropump (Scientific and Industrial, Nauchpribor, U.S.S.R.) with a volume of 2.5 ml or a pump of a KhZh-1311 microcolumn chromatograph (Special Design Technological Office, Scientific and Technological Section of the Academy of Sciences of the U.S.S.R.) with a 1.25-ml volume and flow-rates of the eluent from 0.1 to 100 $\mu$l/min. The injector was a 30-nl microvalve (Special Design Technological Office, Scientific and Technological Section of the Academy of Sciences of the U.S.S.R.). The injector was connected with the fused-silica column without intermediate capillaries. The column was packed with reversed-phase sorbents: Separon Si-$C_{18}$, 10 $\mu$m (Lachema, Brno, Czechoslovakia), Separon Si-$C_{18}$, 7.5 $\mu$m, LiChrosorb RP-18, 5 $\mu$m (Merck, Darmstadt, F.R.G.) and Nucleosil $C_{18}$, 3 $\mu$m (Macherey-Nagel, Düren, F.R.G.). Columns of I.D. 0.2–0.4 mm and length 50–500 mm were packed by a slurry method [15]. The outlet of the column was connected with the cell of an electrochemical detector designed and manufactured in our laboratory [16]. The measuring cell had dual electrodes and was designed in the thin-layer variant [15] with working electrode made of polished glassy carbon or of the "rod-in-flow" construction [17] with a working electrode made of gold, platinum or copper wire 0.1–0.5 mm in diameter. A stainless-steel capillary was used as reference electrode. The effective volume of the detector cell was 5–50 nl, depending on the column diameter and the spacer thickness. The time constant for the detector was 0.1 s. The detector signal was recorded with an LKS4-003 recording potentiometer (Scientific and Industrial, Nauchpribor, U.S.S.R.).

Eluents were prepared with distilled water, chromatographically pure acetonitrile (Merck), sodium perchlorate (chemically pure), EDTA (pure for analysis), orthophosphoric acid (chemically pure), sodium dihydrogenphosphate (pure for analysis) and potassium dihydrogenphosphate (pure for analysis). Model solutions were prepared using a set of amino acids (Merck) and catecholamines (Serva, Heidelberg, F.R.G.) pure for biochemical purposes.

## RESULTS AND DISCUSSION

The analytical properties of a liquid chromatograph depend mainly on the separating ability of the column. As has been shown previously [1–5,15,18], the efficiency of columns made from fused-silica capillaries of I.D. $< 0.4$ mm packed with sorbents with mean particle diameters of 3.5 and 10 $\mu$m exceeds that of columns with common diameters. Table I gives some characteristics of fused-silica columns packed by the procedure described in ref. 15.

Here the diameter of the sorbent particles, $d_p$, is taken as the value given by the manufacturer. The plate number, $N$, was found from the phenol peak in a chromatogram obtained using acetonitrile–water (3:7) + 0.1 $M$ $NaClO_4$ as eluent. The column resistance parameter, $\varphi$ [19], was calculated from the equation

$$\varphi = \Delta p d_p^2 / ULη \qquad (1)$$

TABLE I

CHARACTERISTICS OF FUSED-SILICA CAPILLARY COLUMNS PACKED WITH REVERSED-PHASE SORBENTS

Average values of the parameters for three different columns according to the data from three chromatograms.

| Column dimensions (mm) | Sorbent | Mean particle diameter, $d_p$ ($\mu$m) | Minimum reduced plate height, $h_{min}$ | Column resistance parameter, $\varphi$ | Separation impedance, $E$ |
|---|---|---|---|---|---|
| 430 × 0.32 | Separon Si-$C_{18}$ | 10 | 2.1 | 603 | 2650 |
| 220 × 0.32 | Separon Si-$C_{18}$ | 7.5 | 2.6 | 736 | 4950 |
| 250 × 0.32 | LiChrosorb RP-18 | 5 | 2.3 | 847 | 4480 |
| 100 × 0.34 | Nucleosil $C_{18}$ | 3 | 2.9 | 471 | 3960 |
| 1000 × 0.20[a] | Spherisorb ODS | 5 | 2.52 | 992 | 5393 |

[a] According to the data in ref. 2.

where $d_p$ is the mean particle diameter, $\Delta p$ is the pressure drop in the column, $\eta$ is the viscosity of the mobile phase [20], $L$ is the column length and $U$ is the linear velocity of the mobile phase.

The separation impedance, $E$ [21], was determined from the equation

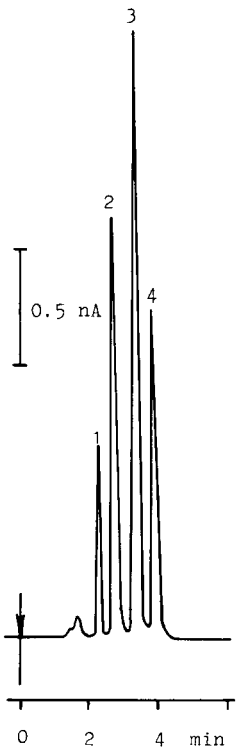
$$E = h^2\varphi \tag{2}$$

where the minimum reduced plate height, $h$, is 2.1–2.9 for various columns packed with different sorbents.

An important aspect of columns made of flexible fused-silica capillaries is the possibility of packing relatively long high-performance columns.

The efficiency of a separation system with capillary columns is much higher than that of high-performance liquid chromatography (HPLC) on the usual scale or that in which sorbents with smaller particle diameters are used. This is equivalent to an increase in chromatographic efficiency at the same operating pressure. In capillary HPLC, it is possible to increase the column length, *i.e.*, to increase the separation efficiency at moderate pressures.

The efficiency of a separation system with capillary columns is greatly affected by the extra-column spreading in the injector, the detector cell and connections. In a high-speed analysis, the speed of response of the detection assembly is also important [6]. In accordance with ref. 22, dispersion in an injector 30 nl in volume is $2.6 \cdot 10^{-4}$ $\mu l^2$ and that in a 2 mm × 0.2 mm I.D. capillary which connects the injector with the column, $\sigma^2_{cap}$, is $1.32 \cdot 10^{-3}$ $\mu l^2$ for phenol at a flow-rate of 3 $\mu$l/min. The effect of the time constant of the detector, $\sigma^2_\tau$, is $1.70 \cdot 10^{-4}$ $\mu l^2$. The experimental value of the total extra-column spreading is 0.004 $\mu l^2$. Hence the contribution to extra-column



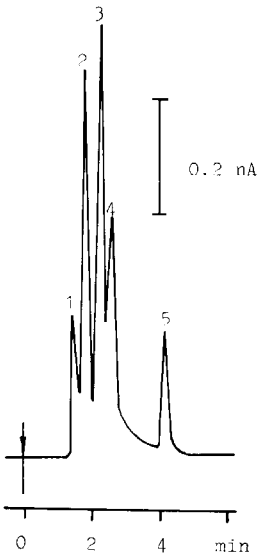




Fig. 1. Chromatogram of catecholamines. Peaks: 1 = Noradrenaline (18 pg); 2 = adrenaline (32 pg); 3 = DOPA (108 pg); 4 = dopamine (71 pg). Column, 52 × 0.34 mm I.D., LiChrosorb RP-18 (5 μm); eluent, 0.1 *M* $NaClO_4$ + $H_3PO_3$ (pH 2.3) + 1 m*M* EDTA in distilled water–acetonitrile (95:5); flow-rate, 4.9 μl/min; sample volume, 30 nl; electrochemical detector, $E$ = +1.4 V (Au).

Fig. 2. Chromatogram of a mixture of amino acids. Peaks: 1 = D,L-Serine (0.43 ng); 2 = D,L-3,4-dihydroxyphenylalanine (3.22 ng); 3 = L-leucine (2.94 ng); 4 = D,L-valine (4.20 ng); 5 = D,L-phenylalanine (3.87 ng). Column, 100 × 0.24 mm I.D., LiChrosorb RP-18 (5 μm); eluent, 0.1 *M* phosphate buffer (pH 7.1); flow-rate, 2.0 μl/min; sample volume, 30 nl; electrochemical detector, $E$ = 0.0 V (Cu).

spreading of the detection system, including the connection of the amperometric detector with the outlet of the column and the effective volume of the measuring cell, is $1.25 \cdot 10^{-3}$ $\mu l^2$. The spreading in the detector found here is much lower than that reported [14,23] for microdetectors of the amperometric type and determined by Chervet *et al.* [24] for a cell of a spectrophotometric detector operating in a system with packed fused-silica columns.

The use of syringe pumps makes it possible to separate substances on fused-silica columns of I.D. 0.2–0.4 mm with a good reproducibility. Thus, at flow-rates of more than 1 μl/min, the relative standard deviation of the retention volumes of substances with $V_R > 10$ μl was $< 0.3\%$ ($n = 3$). The high stability of pump metering ensures that the noise of the amperometric detector is at the level of 2–3 pA. This low noise level makes it possible to determine substances that exhibit electrochemical activity with high sensitivity (Fig. 1). The adrenaline signal ($I$, pA) of this detector is linear in the concentration range 20 μg/l–100 mg/l ($r = 0.98$).

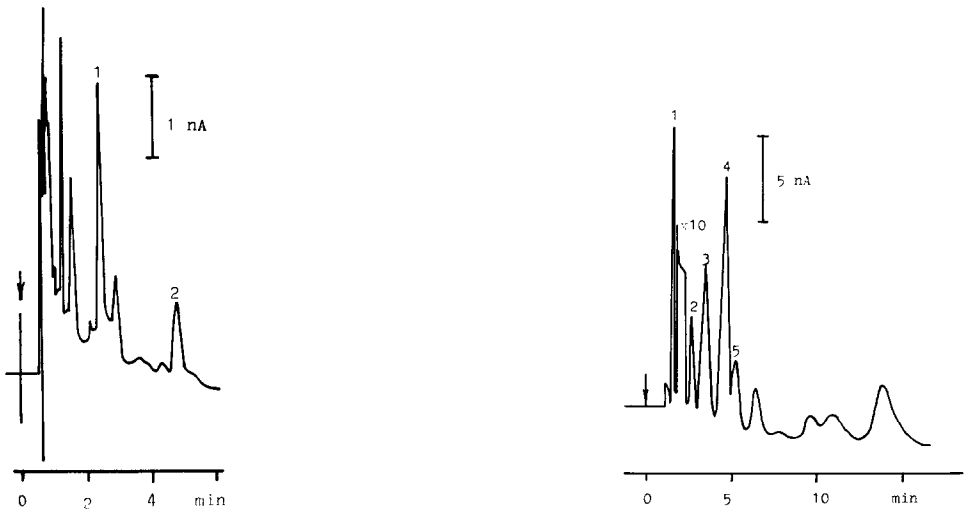



Fig. 3. Chromatogram of a product of wood cooking. Column, 60 × 0.34 mm I.D., LiChrosorb RP-18 (5 μm); eluent, 0.05 *M* $NaClO_4$ + $H_3PO_4$ (pH 2.0) + 2 m*M* EDTA in water–acetonitrile (70:30); flow-rate, 2.0 μl/min; sample volume, 30 nl; electrochemical detector, $E = +1.2$ V (Au). Identified substances: 1 = phenol (8.7 mg/l); 2 = *o*-cresol (6.1 mg/l).

Fig. 4. Chromatogram of a reaction mixture of a dopamine homopolymer. Peak: 1 = Dopamine; 2 and 3 = isomeric dopamine dimers; 4 and 5 = isomeric dopamine trimers; other peaks were not identified. Column, 60 × 0.34 mm I.D., Nucleosil $C_{18}$ (3 μm); eluent, 0.1 *M* $NaClO_4$ + $H_3PO_4$ (pH 2.5) + 1 m*M* EDTA in distilled water–methanol (60:40); flow-rate, 3.4 μl/min; sample volume, 30 nl; electrochemical detector, $E = +1.2$ V (Au).

The relative standard deviation of the height of the chromatographic peak for the concentration of adrenaline of 1 mg/l is 0.8% ($n = 10$).

The chromatographic analysis of amino acids (Fig. 2) is characterised by a considerably lower sensitivity. As has been shown elsewhere [25], a copper electrode is dissolved in the presence of amino acids with the formation of complex compounds. As our investigations have shown, the dissolution of the oxide layer is the limiting process, leading to low electrochemical efficiency. For instance, the electrochemical efficiency for serine at an elution rate of 2 μl/min is 0.11% and that for leucine is about 0.02%. Under these conditions, an electrochemical detector is unsuitable.

The use of a capillary liquid chromatograph for the analysis of real samples makes it possible to decrease considerably the volume of the eluent, the amount of the sorbent and the mass (volume) of the sample. Thus, in waste water from the pulp and paper industry, it is possible to determine individual phenol compounds with high selectivity and sensitivity (Fig. 3).

A sample of a wood cooking liquor was introduced into the chromatograph after precipitation and separation of lignin residue by centrifugation. Satisfactory reproducibility of the results of the determination of electroactive substances in real mixtures over several hours of continuous work was established. When the counter electrode becomes contaminated, which is manifested by a decrease in sensitivity, electrochemical purification at a constant voltage of 2 V and variable polarity is carried

TABLE II

MINIMUM DETECTABLE AMOUNTS OF VARIOUS SUBSTANCES DETERMINED WITH THE AID OF AN AMPERIOMETRIC DETECTOR AFTER THEIR SEPARATION ON A FUSED-SILICA CAPILLARY COLUMN

| Substance | Concentration (μg/l) | Mass (pg) | Substance | Concentration (μg/l) | Mass (pg) |
|---|---|---|---|---|---|
| *Catecholamines* | | | *Phenols* | | |
| Adrenaline | 3.5 | 0.14 | Phenol | 42 | 1.7 |
| Noradrenaline | 3.0 | 0.12 | *o*-Methoxyphenol | 15 | 0.6 |
| DOPA | 6.0 | 0.24 | *p*-Methylphenol | 10 | 0.4 |
| Dopamine | 3.3 | 0.33 | *o*-Methylphenol | 14 | 0.6 |
| | | | 3,4-Dimethylphenol | 15 | 0.6 |
| *Amino acids* | | | 3,5-Dimethylphenol | 21 | 0.8 |
| Serine | 250 | 10 | | | |
| Valine | 2250 | 89 | | | |
| 3,4-Dihydroxyphenylalanine | 825 | 33 | | | |
| Leucine | 650 | 26 | | | |
| Phenylalanine | 2750 | 110 | | | |

out. Under laboratory conditions, the use of capillary chromatography is very advantageous because valuable information about the relationships involved in syntheses may be obtained. High-speed analysis on short capillary columns (Fig. 4) is promising in investigations of the kinetics of reactions.

The detection limits for various substances listed in Table II show that capillary HPLC with an amperometric detector exhibits a high concentration sensitivity. The minimum recorded catecholamine concentrations at a signal-to-noise ratio of 2 are 3 μg/l and those of phenol and its derivatives are 10 μg/l. The mass sensitivity in capillary HPLC is at the femtomole level. This sensitivity allows the application of this method to trace determinations, especially when only small sample volumes are available.

## CONCLUSIONS

The construction of a capillary liquid chromatograph with a highly sensitive amperometric detector has been proposed. The main characteristics of capillary columns packed with reversed-phase sorbents with mean particle diameters of 3, 5, 7.5 and 10 μm were studied and extra-column volumes of the chromatographic system were optimized. It was shown that capillary HPLC exhibits high efficiency and sensitivity and is very economic.

The low noise level of the amperometric detector and the good reproducibility of the retention volumes of the analytes show that capillary HPLC with an electrochemical detector is very promising for the determination of trace amounts of individual catecholamines, amino acids and phenols in model and real samples.

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