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MINIMIZATION OF EXTRA-COLUMN EFFECTS WITH MICROBORE COLUMNS USING ELECTROCHEMICAL DETECTION

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SUMMARY

The characteristics of a simple two-electrode electrochemical detector (cell volume 20 nl) for microbore columns are described. An injection valve is connected to the microbore columns so as to provide low sample dispersion during injection. The applications described show that the equipment fully exploits the advantageous features of short columns down to 50 × 0.5 mm. The minimum detectable quantity when using small columns is 3 pg of pyrocatechol, the linear dynamic response range exceeding three orders of magnitude.

INTRODUCTION

The use of packed microbore columns in liquid chromatography offers a number of advantages such as savings in mobile phase and sorbent, increased mass sensitivity in detection and shortening of the analysis time. However, such columns (< 1 mm) impose heavy demands on the chromatographic equipment[1–3]. As far as detection is concerned, the cell volume should be miniaturized so as to comply with the condition[1,4]

$$V_{det}^2 \leqslant 0.16\ \sigma_{V,col}^{0^2} \tag{1}$$

where $\sigma_{V,col}^{0^2}$ is the variance of the volumetric dispersion of the non-sorbed solute in the column expressed in volume units and V_{det} is the volume of the detector cell. Compliance with this condition ensures an adequately low dispersion of the eluted zone in the cell.

The volume of the sample injected should also be minimized so as to produce a dispersion of the eluted zone not exceeding 10%[1,4]:

$$\sigma_s^2 = V_s^2/12 \leqslant 0.1\ \sigma_{V,col}^{0^2} \tag{2}$$

Here, V_s is the volume of the sample injected and σ_s^2 is the variance of distortion of the eluted zone due to the sample volume, expressed in volume units. Even though the commonly used capillary connections between the column and the injection valve and

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the detector, respectively, are not limiting to the dispersion of zones for columns of about 4 mm I.D.[3,4], primary attention should be given to the connections when using packed microbore columns. The variance of the volumetric zone dispersion in a capillary, $\sigma^2_{V,cap}$, expressed in volumetric units, can be characterized, for instance, by the relation[5–7]

$$\sigma^2_{V,cap} = \pi d^4_{cap} FL/384\ D \tag{3}$$

where d_{cap} is the diameter of a capillary of circular cross-sectioned area, F is the volumetric flow-rate of the mobile phase, L is the length of the capillary and D is the diffusion coefficient of the solute.

Amperometric detectors make it possible to design miniature detection cells[8]. Detectors of cell volume 0.300[9–11], 0.150[12] and 0.035 μl[13] are suitable for operations using microbore packed columns. An electrochemical detector of cell volume < 1 nl[8] has been constructed and used to detect zones eluted from capillary columns in liquid chromatography.

All of the detectors mentioned were designed as three-electrode detectors with their cells constructed from insulating material. Recently, a 20-nl cell volume detector was designed[14] to be used with microbore columns in liquid chromatography, based on a two-electrode combination with a cell constructed from metal.

A disadvantage of the two-electrode arrangement is the uncertainty in the working potential of the working electrode. In liquid chromatography, however, the composition of the mobile phase frequently differs greatly from that of the solutions in which the electrochemical behaviour of the solutes is characterized, if it is characterized at all. In any case, the optimum potential for the detection of solutes should be determined experimentally for the particular mobile phase and electrodes. Besides, the design of some miniature electrochemical flow cells does not permit the optimum functioning of the reference electrode as far as the definition of the working electrode potential is concerned, since the resistivity path of the electrolyte is generally the same for both the reference and the auxiliary electrode.

This paper describes the application of a two-electrode detector together with an injection valve for microbore columns. The dependence of dispersion, linearity and response on mobile phase flow-rate were investigated.

Injection techniques appropriate to microbore packed columns are the "stop-flow" method[15] and the use of 0.2-μl[1] and 0.5-μl[16] Valco injection valves. A specially designed 0.02-μl[17] injection valve has also been reported. Optimization of the connection of the valve injection loop to the column was emphasized for all of these designs. However, as follows from a study dealing with 1000 × 1.0 mm columns[16], dispersion of the zones due to extra-column effects has also been observed with this optimized equipment.

EXPERIMENTAL

Fig. 1 depicts the set-up of the injection valve, the packed microbore columns and the electrochemical detector. A home-made four-port injection valve with loop volumes 0.05 or 0.2 μl was tested for injection. The chromatographic columns were made of 0.5 mm I.D. glass tubes to the ends of which a metal screw was attached with

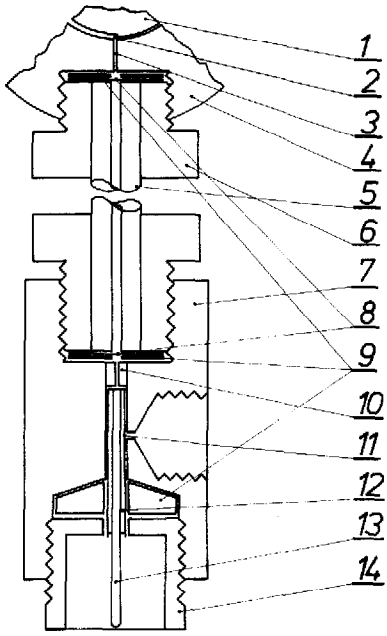

Fig. 1. Connection of the injection valve to the column and of the column to the detector. 1 = Valve rotor; 2 = sample volume; 3 = capillary port (3 × 0.25 mm); 4 = valve body; 5 = glass column; 6 = column screw; 7 = detector body; 8 = filter-paper; 9 = PTFE seal; 10 = glass capillary (0.05 mm I.D.); 11 = stainless-steel capillary; 12 = PTFE insulation; 13 = platinum wire; 14 = connector.

the aid of epoxide resin. The columns were packed by means of viscosity techniques using a 15% adsorbent suspension in a ethanol–glycerol (50:50, v/v) at 200 atm. The method of connecting the detector to the columns is obvious from Fig. 1, which also depicts the cross-section of the detector cell. The detector cell was polarized by a voltage of 1.3 V from a potentiometer connected to a dry battery. In order to measure and record the current of the platinum working electrode, a Kompensograph III (Siemens) compensating millivoltmeter with its 100-kΩ resistor connected to the input was employed. The time constant of the recording device was 0.2 sec.

A modified linear type HPP 4001 injector (Laboratorní Přístroje, Prague, Czechoslovakia) was used to supply the mobile phase, acetonitrile–water (50:50, v/v) + 1% acetic acid + 0.1 *M* $NaClO_4$. Separon SI C 18 (Laboratorní Přístroje) of mean particle diameter 10 and 6 μm was used as sorbent.

RESULTS AND DISCUSSION

Measurements of extra-column dispersion when using the injector and detector described were carried out on the equipment shown in Fig. 1 but to which, instead of the chromatographic column, a glass capillary (25 mm × 50 μm I.D.) was connected. The injection of a hydroquinone solution into the mobile phase at differing flow-rates and using 0.05-μl and 0.2-μl injection loops yielded zones approaching Gaussian-shaped curves. The volumetric variances, $\sigma^2_{v.ex}$, of these zones resulting from disper-

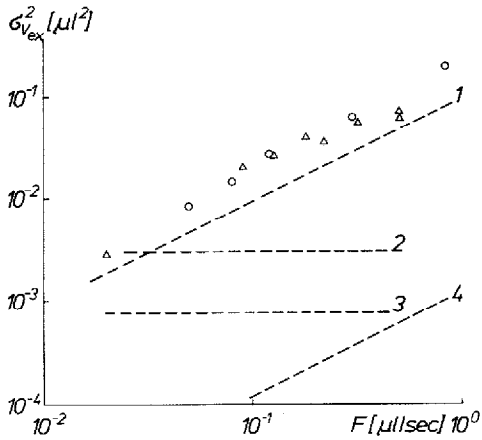

Fig. 2. Extra-column variances of the equipment described. ○ and △, measured variances when using a 0.2-μl or 0.05-μl injection loop. Curves: 1 = variance due to the capillary port (3 × 0.25 mm) (3 in Fig. 1) in the valve, calculated according to eqn. 3; 2 and 3 = variances due to the volume of the sample injected, calculated according to eqn. 2, for 0.2 μl and 0.05 μl volume, respectively; 4 = variance due to the connecting capillary (25 × 0.05 mm) calculated according to eqn. 3.

sion within the connection of the injector to the detector were measured. These variances are plotted in Fig. 2. This figure also depicts variances due to the magnitude of the volume injected and calculated according to eqn. 2, variances caused by the capillary port (3 × 0.25 mm) in the valve body and those caused by the glass capillary (25 × 0.05 mm) connecting the injector to the detector. The last two types of variance were calculated according to eqn. 3 under the assumption that the diffusion coefficient of the solute was equal to $1 \cdot 10^{-3}$ mm^2/sec. As shown in Fig. 2, neither the glass capillary connected instead of the column nor the injection loop volume affect the extra-column dispersion of the zones for the flow-rates investigated. The short capillary port in the valve body is the limiting element for reducing extra-column dispersion in the arrangement described.

The volumetric variance of the detection cell of volume 0.02 μl[14] is insignificant compared to the total extra-column variance for the flow-rates considered. Based on the measurements described above, a valve with an injection loop volume of 0.2 μl was used in subsequent operations. The linear dynamic range of the detector response was determined on equipment arranged as described, in which a column (50 mm × 0.5 mm I.D.) packed with a sorbent having a particle diameter of 10 μm was coupled between the injector and the detector. The pyrocatechol concentrations in the detection cell were calculated from the concentration of the pyrocatechol solution injected and from the heights and widths of the peaks obtained (capacity ratio, $k' = 0.25$). The concentrations are plotted in Fig. 3 against the current measured at the peak maximum. Fig. 3 also shows the current corresponding to the double peak-to-peak noise. The minimum detectable pyrocatechol concentration, $1.5 \cdot 10^{-7}$ mol/l (0.0165 ppm), was determined from the magnitude of this current and from the dependence of the current on the solute concentration in the detector.

With the same arrangement, the dependence of the electrochemical efficiency of the detector cell on the volumetric flow-rate of the mobile phase was determined by

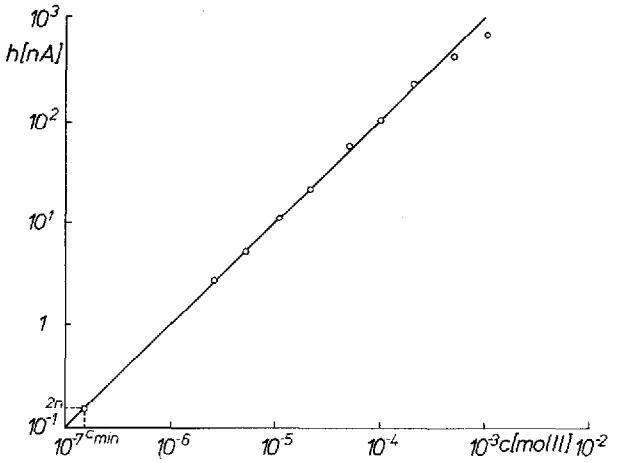

Fig. 3. Linear dynamic range of the detector response. Solute: pyrocatechol. Sample volume: 0.2 μl. Column: 50 × 0.5 mm, packed with Separon SI C 18 (d_p = 10 μm). Mobile phase: water–acetonitrile (50:50, v/v) + 1 % acetic acid + 0.1 *M* $NaClO_4$. Applied voltage: 1.3 V. h = Peak height; c_{min} = minimum detectable concentration in the detector; n = peak-to-peak noise.

sampling 0.2 μl of $5 \cdot 10^{-4}$ mol/l pyrocatechol during the variable flow-rate of the mobile phase, F. The relationship established and that of the product of peak area, Q, (μA sec), and mobile phase flow, F (μl/sec), are plotted in Fig. 4. In spite of the small surface area of the working electrode (0.2 mm^2), the electrochemical efficiency of the detector cell varies in the range of 1–10 % with flow-rates that are commonly employed in packed microcolumns (0.05–0.5 μl/sec). The rather flat dependence of the product QF on the mobile phase flow-rate demonstrates that the detector is actually behaving as a concentration detector in this region.

In the same flow-rate range, the detector background current, adjusted to steady state, was independent of the flow-rate within the range 26–27 nA. This feature limits the noise during possible fluctuations in the mobile phase flow-rate. The drift measured at a steady flow-rate was 4 nA/h while the noise amounted to 75 pA.

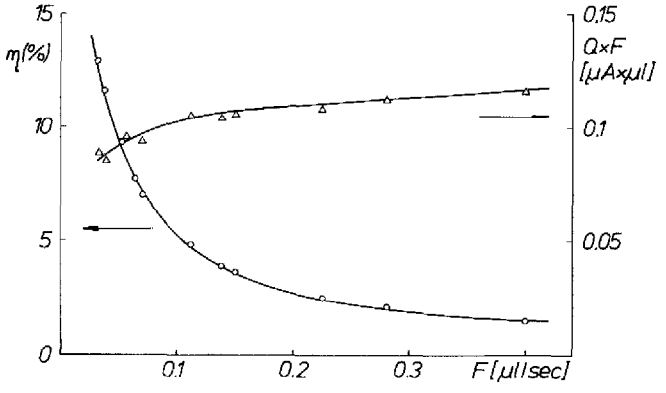

Fig. 4. The electrochemical efficiency of the detector, η (○), and the product of peak area, Q, and mobile phase flow, F (△), as a function of the mobile phase flow. See Fig. 3 for conditions.

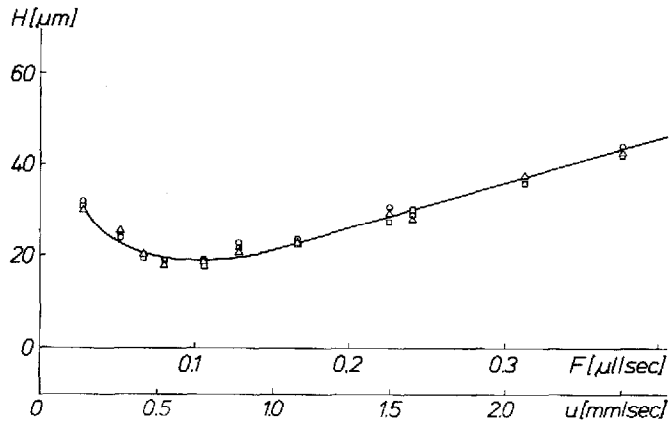

Fig. 5. The height of theoretical plate, H, as a function of linear velocity, u, and volume flow, F, of the mobile phase. Column: 100 × 0.5 mm, packed with Separon SI C 18 (d_p = 6 μm). Solutes: ○ = 1,3-dihydroxynaphthalene, k' = 1.1; △ = 2-naphthol, k' = 2.8; □ = 2-methyl-4-ethylphenol, k' = 4.4. See Fig. 3 for other conditions.

Applications of the above combination of injector and electrochemical detector are illustrated below.

Fig. 5 shows the dependence of the height equivalent to a theoretical plate, H, on the linear velocity of the mobile phase, u, as measured on a column (100 × 0.5 mm I.D.) packed with Separon SI C 18 (Laboratorní Přístroje) having mean particle diameter of 6 μm. As is seen, the efficiency of the small-bore column used is not affected by extra-column zone dispersion. At the same time, a value of H = 19 μm at the minimum in the dependence was obtained for all three solutes.

Fig. 6 illustrates a fast separation of phenols on a column (50 × 0.5 mm) packed with particles of mean diameter 6 μm. For a mobile phase linear velocity, u = 2.0 mm/sec, the peak with capacity ratio k' = 9 is eluted within 250 sec, and the plate height H = 30 μm.

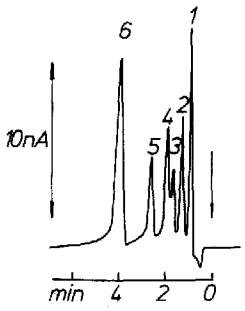

Fig. 6. Separation of alkylated phenols: 1 = phenol; 2 = methylphenol; 3 = 3,5-dimethylphenol; 4 = 2,6-dimethylphenol; 5 = 2-methyl-4-ethylphenol; 6 = thymol. Column: 50 × 0.5 mm, packed with Separon SI C 18 (d_p = 6 μm). Linear velocity: u = 2.0 mm/sec. See Fig. 3 for other conditions.

CONCLUSIONS

Owing to its sensitivity and linear dynamic range, the simple two-electrode amperometric detector described is fully comparable to amperometric detectors of cell volume about 0.3 μl[18]. These characteristics were mainly due to the substantial reduction in the surface of the measuring electrode and to the optimized liquid flow-rate. As has been demonstrated, the reference electrode is not absolutely necessary to obtain optimum sensitivity and linear dynamic range.

It has also been shown that, in order to maintain the high efficiency of small-bore columns, the importance of the connection of the injection valve to the column is at least as great as that of the detector or the injection loop volume.

The equipment described was tested when coupled to short small-bore columns. When using columns (50 $\times$ 0.5 mm I.D.) packed with particles of 10 μm in diameter, the minimum amount of pyrocatechol which can be analyzed is 3.3 pg. The tests performed have shown that such small columns packed with particles of 6 μm in diameter can exhibit the advantages of a small-bore packed column.

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