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TRACE ANALYSIS BY PEAK COMPRESSION SAMPLING OF A LARGE SAMPLE VOLUME ON MICROBORE COLUMNS IN LIQUID CHROMATOGRAPHY

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SUMMARY

Theoretical analyses and practical examples have shown the advantages of microbore columns when injecting solutes dissolved in a non-eluting solvent. In the determination of polynuclear aromatic hydrocarbons and chlorinated phenols, reversed-phase chromatography with amperometric detection yielded detection limits from 20 to 280 ng l^{-1} in the sample. The method works with a small amount of sample (1 ml), with simple injection and without a pump for flushing the injection loop.

INTRODUCTION

The present state of the art of microbore columns requires small detection cells, connections and injection volumes[1–3]. Such requirements are mainly due to the need to maintain the resolution obtained on microbore columns. Generally, the reduction of injection volumes leads to a decrease in sensitivity of chromatographic analyses. The adverse effect of injecting larger sample volumes on the efficiency of separation can be overcome by dissolving the solute in a non-eluting solvent (peak compression sampling), as has been shown[4,5] for 150 × 4.6 mm I.D. columns. This method of injection also allows the sensitivity of the analysis to be increased in proportion to the increase in the sample volume injected.

However, the sample volume injected by this technique cannot be increased infinitely. In the ideal case, the upper limit is determined by the ratio of the eluting forces of the mobile phase and those of the non-eluting solvent for the sample. However, this limit is actually reduced owing to the mixing of the mobile phase with the solute solution in the injection loop during the flushing of the sample from the injection loop on to the column.

An alternative approach to injecting large sample volumes into the column consists in using an enriching pre-column, but this technique necessarily requires a pump for flushing the pre-column. This approach has been used with advantage when a large enough sample is available as, for instance, in the determination of polynuclear aromatic hydrocarbons (PAHs) in water[6,7]. The sample volume can be significantly reduced by using microbore columns together with microbore pre-columns[8–12]. With UV detection, the detection limits vary from units to tens of μg l^{-1} in water samples, with the sample sizes range from 0.1 ml to 10 ml.

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In this paper, the analysis of theoretical relationships demonstrates the advantages of injecting samples in non-eluting solvents into microbore columns, compared with common columns. These advantages are lower sample consumption, lower pressure for filling the empty injection loop and a lower dispersion of the sample zone in the injection loop. Examples of these advantages are given in the analyses of trace concentrations of PAHs and chlorinated phenols in water by reversed-phase liquid chromatography with electrochemical detection. On the basis of electrochemical experiments[13] and quantum chemical calculations[14], an amperometric detector with a platinum anode was used, giving detection limits for PAHs and chlorinated phenols ranging from 20 to 280 ng l^{-1} (20–280 ppt).

THEORETICAL

If $\sigma^2_{V_c}$ is the volumetric variance of the chromatographic process on the column and $\sigma^2_{V_0}$ is the volumetric variance of the injection then, for a given highest permitted distortion of the chromatographic zone at the column outlet, the following relation should be maintained:

$$\sigma^2_{V_c} > \frac{\sigma^2_{V_0}}{k_1} \tag{1}$$

where k_1 is a constant depending on the magnitude of the permitted distortion and proportional to the ratio of the eluting forces of the mobile phase and those of the sample solvent. For injection by means of a six-port valve, with the injection loop consisting of a long capillary of inner diameter d_l, the right-hand side of eqn. 1 can be expressed as

$$\frac{\sigma^2_{V_0}}{k_1} = \frac{V^2_{inj}}{k_2 \cdot 12} + \frac{d_l^2 \, V_{inj} F}{k_3 \cdot 96 \cdot D} \tag{2}$$

Where V_{inj} is the volume of the injection loop, F is the volumetric flow-rate of the mobile phase and D is the diffusion coefficient of the solute in the sample injected. The first term on the right hand side of eqn. 2 represents the contribution to the zone variance due to the sample volume. The constant $k_2 = (k_0 + 1)^2/(k + 1)^2$ represents the magnitude of the injection effect of the zone variance due to the ratio of the elution strength of the mobile phase and that of the sample solvent. Here, k_0 is the capacity ratio of the solute during elution by the sample solvent, and k is the capacity ratio of the solute during elution by the mobile phase. The second term on the right-hand side of eqn. 2 expresses the contribution due to the mixing of the mobile phase with the sample solution in the injection loop, assuming that both liquids are completely mixible and that the diffusion coefficient of the solute in the mobile phase, D_m, is identical with that in the sample solvent, D. As the elution strength of the mobile phase is higher than that of the sample solvent, the relation $k_3 < k_2$ is valid. A more detailed expression of the magnitude of k_3 requires complex calculations. When the sample is injected with the mobile phase as a sample solvent, the relation $k_1 = k_2 = k_3 = 1$ is valid.

For the purpose of optimizing the dimensions of the injection loop of a certain

volume, V_{inj}, it follows that the second term on the right-hand side of eqn. 2 must be smaller than the first term, *i.e.*,

$$\frac{V_{inj}^2}{k_2 \cdot 12} > \frac{d_l^2 \, V_{inj} \, F}{k_3 \cdot 96 \cdot D} \tag{3}$$

assuming that the linear velocity of the mobile phase is higher than that corresponding to the minimum dependence on u of the height equivalent to a theoretical plate, H, on the column.

Relation 3 can be rearranged by using the dependence $\sigma_{V_c}^2$ on the flow-rate of the mobile phase, F, and on the particle diameter, d_p, of the column packing. We can then write

$$\sigma_{V_c}^2 = \frac{V_0 \, d_p^2 \, F}{D_m} \cdot f(k) \tag{4}$$

where V_0 is the dead volume of the chromatographic column and $f(k)$ is a coefficient expressing the numerical constants and the dependence $\sigma_{V_c}^2$ on the capacity ratio, k. By combining eqns. 1, 2 and 4, we obtain

$$\frac{V_0 d_p^2 \, F}{D_m} \cdot f(k) > \frac{V_{inj}^2}{k_2 \cdot 12} + \frac{d_l^2 \, V_{inj} \, F}{k_3 \cdot 96 \cdot D} \tag{5}$$

If relation 3 is valid, addition of inequalities 3 and 5, cancelling out the term with k_2, and algebraic rearrangement under the assumption that $D_m = D$, gives the following relation for the optimum capillary diameter of the injection loop, d_l:

$$d_l^2 < \frac{V_0}{V_{inj}} \cdot d_p^2 \, g(k) \tag{6}$$

where $g(k)$ is given by $g(k) = f(k) \cdot 48 \cdot k_3$.

It follows from relation 6 that the smaller the particles of the column packing used and the larger the sample volume to be injected on a certain column, the smaller the capillary diameter of the injection loop should be in order not to exceed a certain distortion of the chromatographic zone due to injection. For a certain ratio of the sample volume and the column packing, however, it follows from relation 6 that the same diameter of the injection loop should be maintained. In this instance, the length of the injection loop increases with increasing volume of the column. To maintain a constant V_0/V_{inj} ratio, not only the volume of the sample used increases with increasing column diameter, but, to maintain the separation efficiency, the pressure required to flush the injection loop will also increase.

EXPERIMENTAL

A Model HPP 4001 linear pump (Laboratorní přístroje, Prague, Czechoslovakia) was used to supply the mobile phase. A laboratory-made six-port injection valve was modified to minimize connections to the column and injection loop by

means of a method previously described[15]. The injection loops consisted of stainless-steel capillaries. The 0.1-ml loop was 2 m long with I.D. 0.25 mm and the 1 ml-volume loop was 5 m long with I.D. 0.5 mm. The injection loops were loaded by all-glass injection syringes of capacity 1 and 20 ml, respectively. The solute solutions were prepared by dissolving weighed portions in pure acetonitrile give a concentration of *ca.* 10 ppm and by further dilution 1000- and 10,000-fold with distilled water to the required concentration.

In order to separate the injected sample microbore columns were used that were operated as compact glass columns (CGC) (Laboratorní přístroje) adapted to microbore columns (0.7 mm I.D., 7 mm O.D., 150 mm long). The columns were packed with LiChrosorb RP-18 (Merck, Darmstadt, F.R.G.) of particle diameter 7 μm. The previously described viscosity techniques[15,16] were applied to pack the microbore columns. The components of the effluent leaving the column were detected with a Model EMD 10 electrochemical detector (Laboratorní přístroje) with a detection cell similar to that previously described[15,17]. A voltage of 1.2 V was applied to the electrochemical cell. The mobile phase was prepared from distilled water, acetone and analytical-reagent grade sodium perchlorate; the acetonitrile (Avondale Laboratories, U.K.) had to be purified[18]. A TZ 4100 line millivoltmeter (Laboratorní přístroje) was used to record the chromatograms.

RESULTS AND DISCUSSION

The practical consequences of relation 6 are demonstrated in Table I, which compares two columns of identical separation efficiency, operated under the same conditions, *i.e.*, with the same number of theoretical plates, N, retention times, t_R, particle diameters, d_p, length, L, and linear velocity, u, with the same V_{inj} to V_0 ratio (sample volume to column volume). The sample volume was 1 ml for the microbore column and 32.4 ml for the ordinary column. With the microbore column, the injection loop can be flushed within a reasonable time (see Table I, first line) at 0.1 MPa pressure, which can easily be produced by means of a common injection syringe. This cannot be accomplished with the ordinary columns without employing a pump to flush the injection loops (Table I, second line). This leads to even higher sample

TABLE I

COMPARISON OF PEAK COMPRESSION SAMPLING WITH COLUMNS OF 0.7 AND 4 mm I.D.

Column	$\frac{V_{inj}}{V_0}$	V_{inj} *(ml)*	d_i *(mm)*	t_w* *(sec)*	$\frac{d_i^2\ V_{inj}}{V_0\ d_p^2\ g(k)}$ *(arbitrary units)*
150 × 0.7 mm I.D., V_0 = 43 μl	23	1.0	0.5	33	1
150 × 4 mm I.D., V_0 = 1410 μl	23	32.4	0.5	34,600	1
	23	32.4	1.6	33	10
	4.8	6.8	1.1	33	1

* t_w = Time required to flush the injection loop with one volume, V_{inj}, at a pressure drop of 0.1 MPa and a viscosity coefficient η = 0.001 Pa sec (Water).

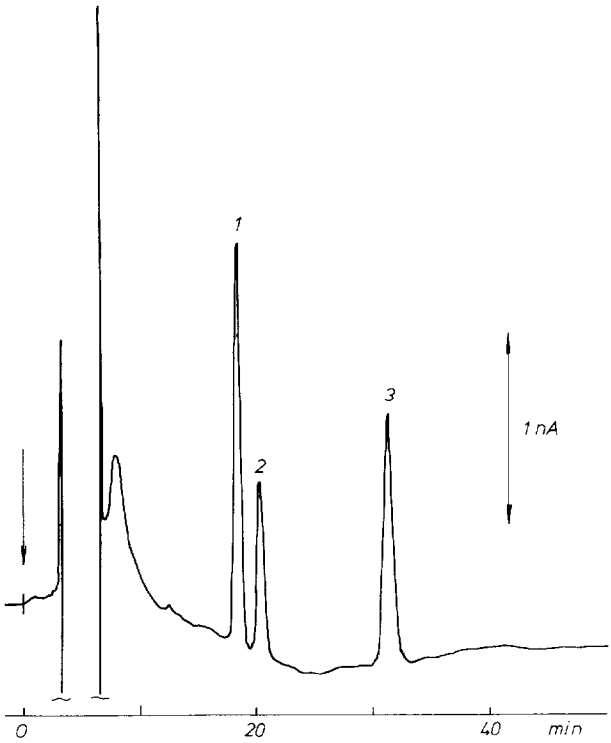

Fig. 1. Chromatogram of a mixture of PAHs in water. Glass column, 150 × 0.7 mm I.D.; packing, LiChrosorb RP-18 (7 μm). Mobile phase: acetone water (75:25, v/v) + 0.1 *M* $NaClO_4$; u = 0.95 mm sec^{-1}. Detector: EMD 10, Pt electrode, potential +1.2 V. Sample volume: 0.1 ml. Solutes: 1 = perylene, 12 μg l^{-1}; 2 = 1,2-benzopyrene, 9 μg l^{-1}; 3 = 20-methylcholanthrene, 54 μg l^{-1}.

consumption. On the other hand, an easily rensed injection loop brings about a substantial increase in the dispersion of the injection loop (Table I, line 3), and this can significantly impair the resulting separation efficiency of the analysis. Keeping the same dispersion and the same conditions for flushing the loop inevitably leads to a reduction in the attainable V_{inj}/V_0 ratio (Table I, line 4).

The potential injection of large sample volumes into packed microbore columns in a non-eluting solvent and the assumptions derived in the theoretical section were tested by analysing aqueous solutions of PAHs and chlorinated phenols. Loops of volume 0.1 and 1 ml were used in order to inject aromatic hydrocarbons (perylene, 1,2-benzopyrene and 20-methylcholanthrene). The chromatograms are shown in Figs. 1 and 2. For the 0.1-ml injection loop, the values of the height equivalent to a theoretical plate, H, were 27, 25 and 28 μm for the individual solutes at a linear velocity of the mobile phase of u = 0.95 mm sec^{-1}; for the 1-ml loop the corresponding values of H were 29, 31 and 28 μm at u = 0.9 mm sec^{-1}. The efficiency of a chromatographic system with both loop volumes is comparable to that measured on packed microbore columns with sample injections of 0.2 μl[19].

On injection of 0.1 ml of an aqueous solution of aromatic hydrocarbons, the last solute (20-methylcholanthrene) with a capacity ratio of k = 8.5 was eluted from

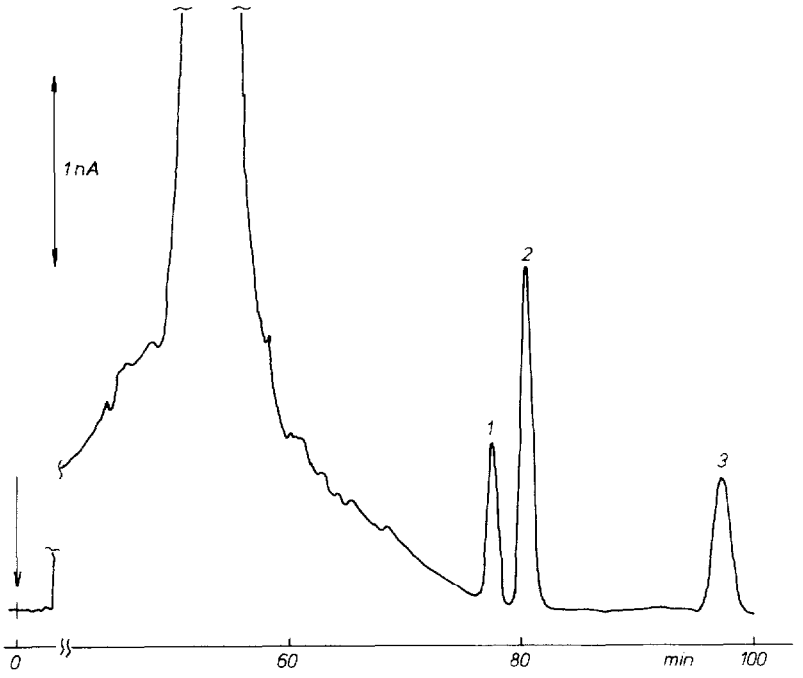

Fig. 2. Chromatogram of a mixture of PAHs in water. Sample volume: 1 ml. Solutes: 1 = perylene, 1 $\mu g\ l^{-1}$; 2 = 1,2-benzopyrene, 2.7 $\mu g\ l^{-1}$; 3 = 20-methylcholanthrene, 5.2 $\mu g\ l^{-1}$. Other data as in Fig. 1.

TABLE II

DETECTION LIMITS OF POLYNUCLEAR AROMATIC HYDROCARBONS AND CHLORINATED PHENOLS IN WATER

Column: 150 × 0.7 mm I.D., LiChrosorb RP-18 (7 μm). Mobile phase: A, acetone–water (75:25, v/v) + 0.1 *M* $NaClO_4$; B, acetonitrile–water (60:40, v/v) + 0.1 *M* $NaClO_4$ + 10^{-3} *M* $HClO_4$.

Loop volume (ml)	*Mobile phase*	*Solute*	*Detection limit (ng l^{-1})*
0.1	A	Perylene	170
		1,2-Benzopyrene	260
		20-Methylcholanthrene	1250
1.0	A	Perylene	40
		1,2-Benzopyrene	60
		20-Methylcholanthrene	260
1.0	B	44-Chlorophenol	20
		2,4-Dichlorophenol	50
		2,4,6-Trichlorophenol	80
		Pentachlorophenol	280

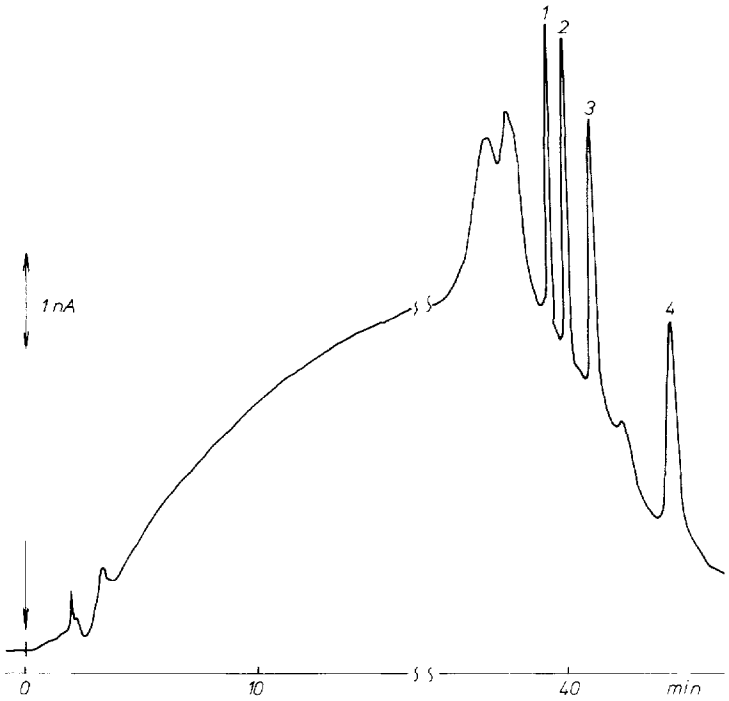

Fig. 3. Chromatogram of a mixture of chlorinated phenols in water. Glass column, 150 × 0.7 mm I.D.; packing, LiChrosorb RP-18 (7 μm). Mobile phase: acetonitrile–water (60:40, v/v) + 0.1 *M* $NaClO_4$ + 10^{-3} *M* $HClO_4$; u = 1.4 mm sec^{-1}. Detector: EMD 10, Pt electrode, potential +1.2 V. Sample volume: 1 ml. Solutes: 1 = 4-chlorophenol, 1.6 μg l^{-1}; 2 = 2,4-dichlorophenol, 4 μm l^{-1}; 3 = 2,4,6-trichlorophenol, 6 μm l^{-1}; 4 = pentachlorophenol, 14.7 μg l^{-1}.

the column at a linear velocity of the mobile phase of 0.95 mm sec^{-1} within 30 min, *i.e.*, only 6 min later that it would have been eluted on injecting an adequately small sample volume in an eluting solvent. The time of analysis was about three times longer when 1 ml of an aqueous solution of the sample was injected.

A number of PAHs can be oxidized in mixed water–organic solvents on a platinum electrode[13,14]. This fact forms the basis for the amperometric detection of these hydrocarbons, *i.e.*, compounds with high biological activity, such as 1,2-benzopyrene, 1,2,5,6-dibenzanthracene, 20-methylcholanthrene, 1,2-benzoperylene, 2,3-benzofluoranthene as well as pyrene and anthracene. On the other hand, several biologically inactive PAHs are oxidized in water–organic media at higher potentials, and this fact can be used for the selective detection of the major part of priority PAH pollutants (EPA)[20] in complex mixtures. Our experiments support the feasibility of amperometric detection of PAHs with the aid of platinum electrodes. The detection limits of PAHs in the injected aqueous solutions for the two injection loop volumes are listed in Table II.

Another example of peak compression sampling for microbore columns is illustrated by the analysis of an aqueous solution of chlorinated phenols. The chromatogram of this compound is shown in Fig. 3; the detection limits are listed in Table II.

CONCLUSIONS

When the technique of peak compression sampling is applied to packed microbore columns, detection limits of the order of tens to hundreds of ng l^{-1} of PAHs and chlorinated phenols can be obtained by injecting a sample of 1 ml. No enriching pre-columns or other methods of sample concentration are needed for this approach.

The efficiency of the chromatographic separation is the same as that by the common injection method, *i.e.*, by injecting a small volume of sample dissolved in the mobile phase.

For injections of 0.1-ml samples, the analysis time is comparable to that of the usual injection method and it is about three times longer when a 1-ml sample is injected, but there is no need for further handling of the sample.

It has been shown by theoretical analysis and in practice that peak compression sampling increases the sensitivity of the analysis on microbore columns more distinctly than is the case for 4-mm I.D. columns.

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