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Parallel-current open-tubular liquid chromatography with fluorimetric detection

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ABSTRACT

Some characteristics of the flowing retentive film in parallel-current open-tubular liquid chromatography with a cyclohexanol–water mobile phase and fluorimetric detection are considered. The internal surface of a fused-silica capillary was modified with octamethylcyclotetrasiloxane (D_4 reagent).

INTRODUCITON

Recently, a new mode of open-tubular liquid–liquid chromatography designed as parallel-current open-tubular liquid chromatography (PC-OTLC) was suggested [1–3]. In PC-OTLC, both the mobile phase and the retentive layer move in the same direction, the retentive layer at a lower velocity than the mobile phase. The internal surface of the capillary is modified so that it is wettable by the retentive phase and it is covered with a retentive film. A mathematical description of the retention of an analyte in an open-tubular column of circular cross-section has been derived [1].

In previous paper [2], we suggested criteria for solvent pair optimization on a glass capillary under the conditions of RP-PC-OTLC with electrochemical detection. When the surface layer is not spread uniformly around the inner capillary circumference, the average linear velocity of the film increases and the film cross-section decreases [2]. Consequently, both the solute retention and the phase ratio are lower then those calculated for a uniform film thickness. For maximizing the phase ratio (ϕ), the liquids with a maximum ratio of their viscosity to the interfacial tension (γ/η) between the two flowing liquids is to be preferred. Cyclohexanol represents the optimum choice amongst the liquids examined for RP-PC-OTLC [2].

For reversed-phase systems, the internal surface of the separation capillary should be hydrophobic. Therefore, the siliceous surface of the capillary should be suitably modified. The techniques for surface modification of capillaries for OTLC are based on methods known in gas chromatography [2–5]. Of the methods of glass modification examined, persilylation with octamethylcyclotetrasiloxane (D_4 reagent) is the optimum procedure giving a retention close to the theory [1]. Previously, we used glass capillaries for PC-OTLC [1–3]. Fused silica offers some advantages over with glass. However, the properties of the interface of these two materials with liquids are different [6,7]. On the basis of experience with the glass capillaries, we used persilylation with D_4 reagent in this study with fused-silica capillaries. Also, reversed-phase chromatography with the system water–cyclohexanol was adopted for model separations. In this work, we studied the basic characteristics of the production, rinsing and destruction of the retentive film under different PC-OTLC conditions.

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In previous studies [1–3] we employed an electrochemical detector with a platinum microelectrode. Fluorescence detection offers the highest level of sensitivity obtainable in HPLC [8]. On-column fluorescence detection [9,10], in which the capillary column itself is used as a flow cell, is superior to postcolumn detection because of the negligible extra-column band broadening [11]. Also, on-column detection provides increased sensitivity compared with postcolumn detection [12] owing to the concentration of the solute in the retentive phase. The applicability of fluorimetric detection in PC-OTLC is demonstrated in this study.

EXPERIMENTAL

Capillary preparation

Fused-silica capillaries of 0.2 mm O.D. and 0.035 mm I.D. were purchased from the Institute of the Chemistry of Glass and Ceramic Materials, ČSAV (Prague, Czech Republic). The internal surface of the capillaries was modified with D_4 reagent [2]. The procedure was carried out once or twice. The length of the capillaries used in PC-OTLC experiments was 5–6 m. Measurements were carried out in a linear velocity range from 1.3 to 85 mm s^{-1}.

Chromatograph

The chromatographic system used has been described previously [13]. The pressure source was a VCM 300 micropump (Development Works, Czechoslovak Academy of Sciences, Prague, Czech Republic). The sample was injected using a six-port laboratory made valve with a 20-μl loop and a flow splitter. The splitting ratio was 1:500–7500. The capillary column was immersed in a water-bath connected with a U8 thermostat (MLW Prüfgeräte-Werk, Medingen/Sitz Freital, Germany). The column was maintained at 20.0–90.0°C; the mobile phase temperature was 20.0°C. For on-column fluorimetric detection we adapted [14] a Kratos FS 950 fluorimeter (Schoeffel Instruments) (see Fig. 1). To obtain a detection window, the polyimide coating was removed by burning from an 8-mm long section of the capillary. The radiation obtained from a low-pressure mercury lamp passed through a 254-nm interference excitation filter and fell on the cell. The emitted light passed through a 320-nm cut-off filter to a photomultiplier tube. The detector signal was monitored with a TZ 4100 line recorder (Laboratory Instruments, Prague, Czech Republic).

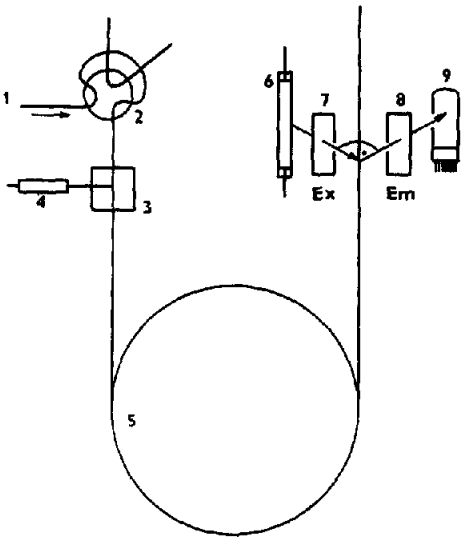

Fig. 1. Experimental arrangement of the chromatograph with fluorimetric detection. 1 = Mobile phase supply; 2 = six-port valve; 3 = splitter; 4 = restrictor; 5 = fused-silica capillary column (35 μm I.D.) immersed in a water-bath; 6 = mercury discharge lamp; 7 = 254-nm interference filter; 8 = 320-nm cut-off filter; 9 = photomultiplier tube.

Mobile phase

The pumped liquid was water saturated with cyclohexanol at 20.0°C. The differences in the solubilities of cyclohexanol in water between the pumped liquid temperature and the column temperature (20–90°C) used for the calculations are based on the interpolation of the reported data [15] and they were recalculated to volumetric units.

Chemicals and test solutes

The model analytes were salicylic acid (marker of the dead time, t_{ro}), DL-tryptophan, hydroquinone, catechol, resorcinol, indolylacetic acid, sulphanilamide, aniline sulphate, 2-methylaniline, 2,6-dimethylaniline and phenol. The test solutes and other chemicals used were purchased from Lachema (Brno, Czech Republic). D_4

reagent was obtained from VCHZ Synthezia (Kolín, Czech Republic).

Viscosity measurement

The viscosities of co-existing liquids in the cyclohexanol–water system at 20, 50 and 70°C were measured using a thermostated Ubbelohde viscosimeter. The liquid densities needed were determined pycnometrically. Using reference values for water [16], the densities of the aqueous and organic phases were determined. The date obtained are summarized in Table I.

Determination of the distribution constant, K_i

The solute K_i was determined by the method described previously [17]. A known amount of the analyte was dissolved in the aqueous phase and the absorbance at 278 nm for phenol was determined. The solution was then equilibrated for 2 days at 20 and 70°C with a known amount of cyclohexanol saturated with water and the absorbance of the aqueous phase was measured again. The K_i values for phenol at 50°C were taken from ref. 1. The data obtained are summarized in Table I.

Calculations

The experimental values of the reduced retention, k_i^*, were determined from the observed t_{ri} and t_{ro} values; the calculated – theoretical k_i^* value is related to the phase ratio ϕ, the flow ratio of the retentive and mobile phases q ($q = F_r/F_m$) and the liquid–liquid distribution coefficients of phenol, K_i, through the equation [1]

$$k_i^* = (\phi - q)/(1/K_i + q) \quad (1)$$

The theoretical value of ϕ was calculated from solubility and viscosity data using the equations [1]

$$q = \Delta s/(1 - \Delta s) \quad (2)$$

$$\phi = (1 - t + 1/\Delta s)/\{1/\Delta s - [1 + t(1/\Delta s - 1)]^{1/2}\} - 1 \quad (3)$$

where t is the viscosity ratio of the retentive and the mobile phases, $t = \eta_r/\eta_m$. In the suggested method, q is determined by the solubility difference of the retentive phase in the mobile phase at temperatures T_1 and T_2, $\Delta s = (s_{T_1} - s_{T_2})$. The linear velocity of the mobile phase was determined from the retention time of salicyclic acid (t_{ro}) and the capillary length.

RESULTS AND DISCUSSION

Long-term retention stability

This was tested by flushing the capillary with up to 1000 dead volumes of the mobile phase at 50°C (see Fig. 2). The linear flow velocity determined from the elution time of the unretained compound was 34.4 mm s^{-1}. The average k_i^* for phenol was 4.5 with fluctuations of up to 7.8%.

Time of retentive phase generation

This was tested by the measurement of the phenol k_i^* during column equilibration (see Fig. 3). The surface-modified capillary tempered at 20°C was flushed with distilled water until the retention of phenol vanished. The flow was then switched to mobile phase and the capillary temperature was increased to 50°C or 70°C (see

TABLE I

SOME EXPERIMENTAL AND CALCULATED CHARACTERISTICS OF THE PC-OTLC OF PHENOL IN THE CYCLOHEXANOL–WATER SYSTEM

T_2 (°C)	Δs (wt.%) [15]	q $\times 10^3$	η_{RP} (mN s m^{-1})	η_{MP} (mN s m^{-1})	t	ϕ	K_i	k_i^*	
								Exp.	Calc.
20	–	–	23.37	1.14	20.50	–	18.50	–	–
50	0.93	9.39	3.97	0.57	6.96	0.265	16.47	4.50	3.65
70	0.97	9.79	1.88	0.43	4.37	0.217	13.97	2.54	2.55

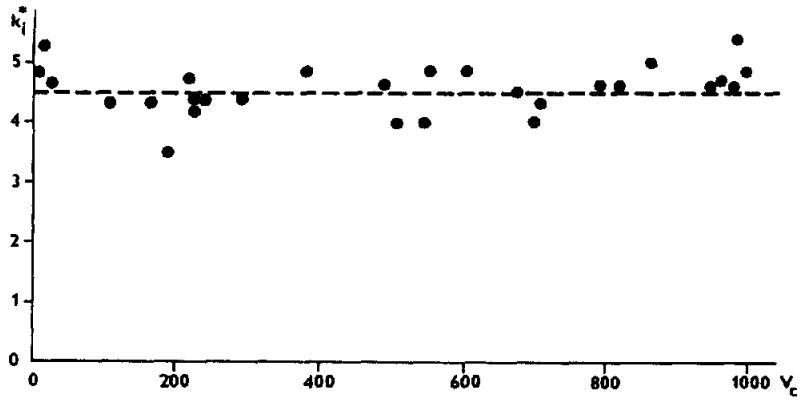

Fig. 2. Effect of the total volume of the mobile phase passed through the column on the reduced retention of phenol (k_i^*) under steady-state conditions. Column, fused-silica capillary (5 m × 35 μm I.D.) twice modified with D_4 reagent; column temperature, 50°C; mobile phase, water saturated with cyclohexanol at 20°C; solutes, salicylic acid (dead time marker) and phenol; splitting ratio, 1:7500; linear flow velocity, 34.4 mm s^{-1}; fluorimetric detection. Broken line = average k_i^* value of phenol; V_c = column volumes.

curves 1 and 2, respectively). Following curve 1 for 50°C, a nearly steady state was obtained after flushing with 17 V_c (column volumes); k_i^* for phenol was 3.7. Subsequently, the increase in k_i^* was slower until the steady state with $k_i^* = 4.4$ for phenol was obtained after 35 V_c. Similarly, following curve 2 for 70°C, a steady-state k_i^* for phenol of 2.35 was obtained after flushing with about 31 V_c. Curve 3 corresponds to the following experiment: the capillary was equilibrated with a flow of the mobile phase at 50°C, then it was left at 20°C without any flow for 16 h. The segmentation of the retentive film is expected within this time period [2,4]. The capillary was then tempered at 50°C and the flow of the mobile phase was renewed. The flushed volume needed for the regeneration of the retentive film was about four dead volumes, as follows from the retentions of the model analyte.

Time for retentive film elution

This was tested, similarly to the above, by the measurement of phenol retention (see Fig. 4). The capillary column was equilibrated at 50 and

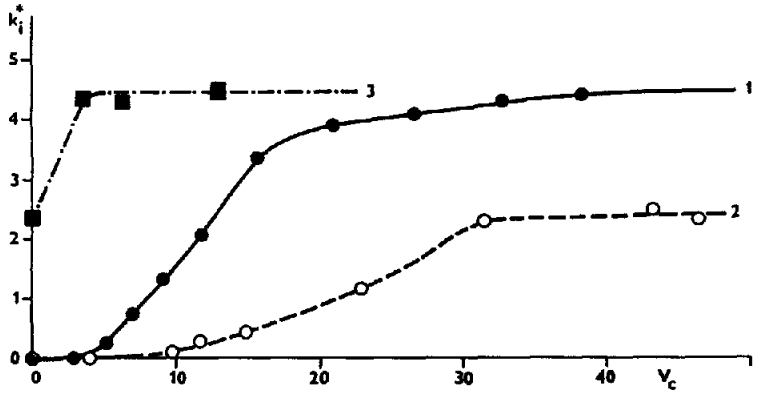

Fig. 3. Eluted volume necessary for generation of retentive layer by temperature rise. 1 = Temperature rise 20 to 50°C; 2 = temperature rise 20 to 70°C; 3 = column temperature 50°C, segmented film of retentive layer spread by flow only. V_c = column volumes; k_i^* = reduced retention of phenol. For other conditions, see Fig. 2.

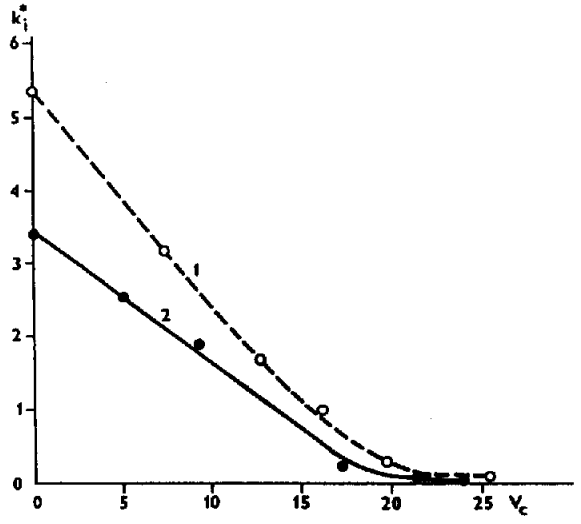

Fig. 4. Eluted volume necessary for removal of retentive layer by temperature drop. 1 = Temperature drop 50 to 20°C; 2 = temperature drop 70 to 20°C; V_c = column volumes; k_i^* = reduced retention of phenol. For other conditions, see Fig. 2.

70°C (see curves 1 and 2, respectively). After cooling the capillary to 20°C, the phenol retention continuously decreased and finally vanished when the capillary was flushed with about 25 V_c.

Dependence of retention on mobile phase velocity

As follows from the previous considerations [2], a decrease in u to below some critical value, u_c causes instability of the retentive film and the formation of segmented flow. Such conditions lead to decreases in the solute retention. In addition to this phenomenon, the presence of the segmented flow can also be reproducibly followed with the fluorimetric detector used, which displays increased noise under such conditions. Fig. 5, curves 1 and 2, represent the k_i^* *versus* u dependences in the capillary tempered at 50 and 70°C, respectively. In both instances, the pumped liquid was water saturated with cyclohexanol at 20°C. The calculated phase ratio in the capillary, ϕ, was 0.267 and 0.217, respectively. Curve 1a corresponds to the k_i^* *versus* u dependence at 50°C, when the capillary was flushed with water which contained cyclohexanol only in an amount corresponding to 97% saturation at 20°C. Then, the calculated ϕ was 0.244 and k_i^* for phenol was 3.0. With a decrease in u at 50°C, a marked increase in the phenol retention can be observed at around u_c. The probable explanation could be that ϕ increases under such conditions. As follows from Fig. 5, the critical velocity decreases with decrease in ϕ, as expected.

Temperature dependence of the solute retention

As supposed previously [1], the solute retention could be controlled by the temperature of

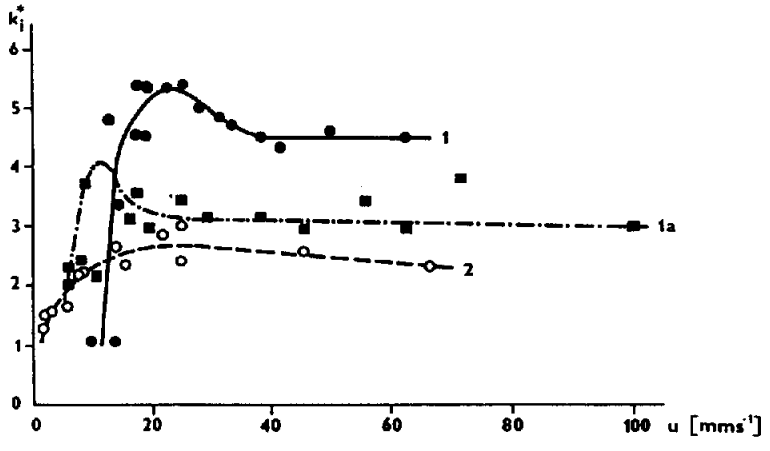

Fig. 5. Effect of flow velocity on reduced retention. 1 = Column temperature 50°C; 1a = column temperature 50°C, pumped liquid diluted with water (ϕ = 0.244); 2 = column temperature 70°C. u = Linear flow velocity (mm s^{-1}); k_i^* = reduced retention of phenol. For other conditions see Figs. 2 and 3.

the capillary column used in PC-OTLC. It follows from the nature of the method that k_i^* *versus* T_c dependence is a complex function of the solubilities and viscosities and their temperature dependences. To obtain an idea of the k_i^* *versus* T_c function, the amount of the data should be known. Nevertheless, some conclusions can be drawn from the above-mentioned measurements at 20, 50 and 70°C. In Fig. 6, the probable course of the k_i^* *versus* T_c function is shown as the broken curve. It was obtained from eqn. 1, where the viscosity and the solubility data needed were obtained by interpolation between the measured values (see Table I). The open points in Fig. 6 are the values calculated from the values in Table I and the closed points are the measured retentions of phenol at the respective temperatures. Although the calculated curve is only a rough approximation, it is in qualitative agreement with the experimental values. Further, the curve indicates that around ambient temperature, a steep dependence of retention on temperature occurs in the system used and, therefore, the strong demands are imposed on the instrument thermostating. In this way, the fluctuations of the analyte retentions can be explained.

Influence of acetonitrile concentration in the sample

To inject less water-soluble analytes, they should be dissolved in an organic or aqueous–organic solution. However, the injection of an organic solvent into the PC-OTLC column may disturb the steady-state conditions within the capillary. In order to obtain an idea about the possible disturbance, a model analyte (phenol) was injected in water–acetonitrile mixtures. A 24-nl volume of 5 m*M* solution was injected using a splitting ratio of 1:500. As follows from Fig. 7, concentrations of acetonitrile from 0 to 60% (v/v) have no influence on the solute retention. At higher concentrations, the phenol k_i^* value decreases from 4.4 to 3.4, which corresponds to a solution of phenol in pure acetonitrile. The decrease in retention can be caused by

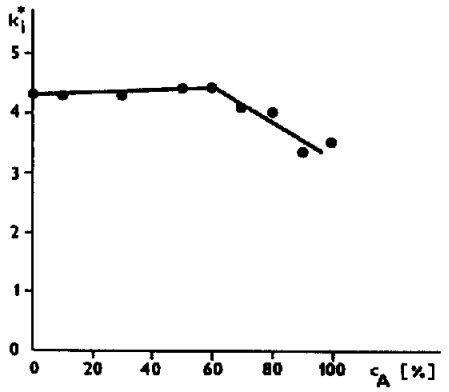

Fig. 7. Effect of sample acetonitrile concentration on reduced retention. c_A = Sample acetonitrile concentration (%, v/v); k_i^* = reduced retention of phenol; splitting ratio, 1:500. For other conditions, see Fig. 2.

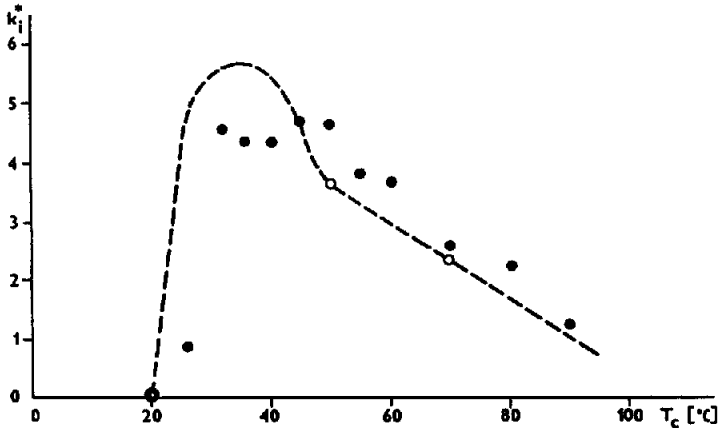

Fig. 6. Effect of column temperature on reduced retention. ● = Experimental points; ○ = calculated points; broken line = theoretical curve. T_c = column temperature (°C); k_i^* = reduced retention of phenol. For other conditions, see Fig. 2.

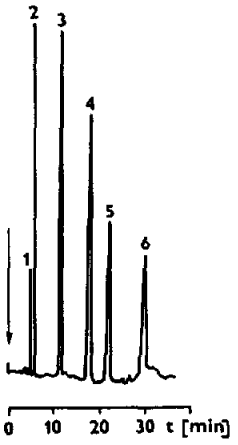

Fig. 8. PC-OTLC separation of a test mixture dissolved in acetonitrile–water (80:20%). Linear flow velocity, 17.9 mm s^{-1}. Peaks: 1 = salicylic acid (dead time marker); 2 = sulphanilamide; 3 = aniline sulphate; 4 = 2-methylaniline; 5 = phenol; 6 = 2,6-dimethylaniline. For other conditions, see Fig. 2.

a transient decrease in the film thickness and/or interfacial tension. An example of a chromatogram of the compounds dissolved in water–acetonitrile mixture (20:80, v/v) is shown in Fig. 8.

Influence of surface modification

In all the above experiments, a fused-silica capillary modified twice with D_4 reagent was used. As has been found previously, modification of the glass surface plays an important role in PC-OTLC [2]. To test the influence of modification of the fused silica on the performance of the capillary, the separation on a fused-silica capillary modified with only one persilylation step with D_4 reagent was carried out (see Fig. 9). It was found that the retentions are lower than those with the twice-modified capillary. Under otherwise identical conditions, a phenol k_i^* value of 3.0 was found at $u = 7.15$ mm s^{-1} and $T_c = 50°C$.

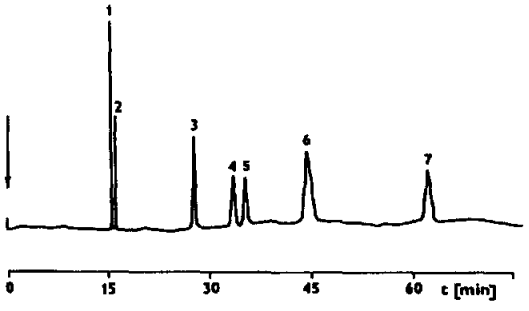

Fig. 9. PC-OTLC separation of a test mixture. Column, fused-silica capillary (6 m × 35 μm I.D.) modified once with D_4 reagent; linear flow velocity, 7.15 mm s^{-1}. Peaks: 1 = salicylic acid (dead time marker); 2 = DL-tryptophan; 3 = hydroquinone; 4 = catechol; 5 = resorcinol; 6 = indolylacetic acid; 7 = phenol. For other conditions, see Fig. 2.

CONCLUSION

The applicability of fluorimetric detection in RP-PC-OTLC was demonstrated. In comparison with electrochemical detection, it is less sensitive to the presence of the two phases in the detection cell, but the segmented flow causes the elevated noise. Simultaneously, the different selectivity also enables the number of detectable analytes to be increased.

Further, the applicability of the fused-silica capillaries in PC-OTLC was demonstrated. Unlike glass capillaries, the retentions found may differ considerably from the predicted values, either negatively or positively. The dynamic properties of the method seem to be advantageous for convenient changes of the separation system. Proper instrument thermostating and/or the use of a not fully saturated pumped liquid may improve the reproducibility of retention. Further studies are needed in order to elucidate all the observed phenomena and to make the method more generally applicable.

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