Performance of reversed-phase parallel-current open-tubular liquid chromatography columns and comparison with theory

Marie Horká*, Vladislav Kahle, Miloš Krejčí, Karel Šlais

Institute of Analytical Chemistry, Academy of Sciences of the Czech Republic, Veveří 97, 611 42 Brno, Czech Republic

Abstract

The capillary column performance in reversed-phase parallel-current open-tubular liquid chromatography (RP-PC-OTLC) was studied with the use of cyclohexanol–water. The reduced retention and the height equivalent to a theoretical plate were studied as a function of the liquid velocity and the content of cyclohexanol in the capillary at constant column temperature. The effect of microemulsion formation on band broadening and retention is discussed. The plate heights obtained were compared with the Golay equation.

1. Introduction

In conventional capillary columns used widely in GC, LC or SFC, the inner surface of open capillaries of circular cross-section is coated with a uniform film of stationary phase. There is only one flow conduit in the tube. For an idealized model, the separation efficiency can be expressed with the help of the Golay equation [1]. For real capillaries, the stationary phase film tends to distribute non-uniformly on the wall, which causes some deviation from the simple theory [2].

In parallel-current open-tubular liquid chromatography (PC-OTLC) [3–6], both the mobile phase and the surface layer move in the same direction, but with different velocities. According to the idealized model [3], the ratios of the flow-rates and viscosities of the two phases determine the thickness and the velocity of the retentive layer. Further, it was found [4] that some minimum average velocity of the mobile phase is necessary for the maintenance of annu-

lar flow of the retentive liquid. The magnitude of this minimum velocity, termed here the critical velocity, is dependent on the interfacial tension between the two liquids. In PC-OTLC experiments [3–6], a two-phase flow was generated by a decrease in the solubility of the retentive liquid at the capillary inlet. This can be conveniently achieved by having a difference in the temperature of the liquid entering the capillary and the capillary temperature.

As the reversed-phase (RP) mode of LC is frequently used, we have examined [4] the relevant properties of some partially water–soluble organic liquids potentially suitable as retentive phases in RP-PC-OTLC. Of the compounds examined, cyclohexanol appeared to be the most promising retentive liquid.

Finally, the properties of the capillary inner surface also influence the thickness and distribution of the retentive layer on the inner surface and, consequently, the separation efficiency [4]. According to the model used [3], varying the concentration of retentive liquid and/or the solution temperature should be useful for controlling the phase ratio in the capillary. How-

---

* Corresponding author.

ever, temperature variations in the solution containing partially water-soluble organic liquids can generate dispersion systems, including microemulsions [7,8]. Further, one of the most commonly encountered complications affecting the homogeneity of capillary flow is that arising from variations in the solid–liquid interfacial tension in the system [8]. Liquid flow at a surface arising from surface tension gradients is commonly refered as Marangoni flow [7–9]. In multicomponent systems, surface tension gradients usually arise as a result of adsorption-related phenomena [8].

In order to evaluate the possibilities of controlling the method performance, we studied here the solute retention and dispersion characteristics in RP-PC-OTLC as a function of the mobile phase velocity and concentration of cyclohexanol in the liquid entering the capillary. A method for calculation of the axial zone dispersion in a cylindrical capillary with two flowing concentric annular layers was suggested by Gill [10]. However, this approach leads to equations that are too complicated for practical use. As, under the conditions examined here, the retentive layer is thin and its average velocity is only a small fraction of the mobile phase velocity, the contribution to the dispersion in the retentive phase caused by its flow was neglected for the first approximation. Therefore, the plate height found was compared with the Golay equation, in which actual solute retention characteristics and layer thickness were incorporated.

2. Calculations

In open-tubular chromatography with an immobile, uniformly distributed retentive layer, the capillary performance can be described with help of the Golay equation [1]:

$$
\begin{aligned}
H = {} & 2D_M/u_M \\
& + [(1 + 6k + 11k^2)R^2/24(1 + k)^2 D_M \\
& + 2kd_f^2/3(1 + k)^2 D_R]u_M
\end{aligned}
\tag{1}
$$

where H is the height equivalent to a theoretical plate, R is the inner radius of the capillary column, u_M is the linear velocity of the mobile phase, D_M and D_R are the diffusion coefficients of solutes in the mobile and retentive phase, respectively, d_f is the thickness of the retentive phase film and k is the capacity ratio of the solute for the immobile retentive layer. The plate height is related to the width of the Gaussian zone by the equation $H = L/(t_{ri}/\sigma_i)^2$, which can be used for the calculation of H from the chromatogram. The capacity ratio can be related to the retention time of the analyte, i, in a capillary with an immobile retentive phase, t_i, and the elution time of the unretained compound, t_0, as $k = t_i/t_0 - 1$. On the other hand, k is related to the analyte distribution constant, K_i, and phase ratio, $\phi = S_R/S_M$, as $k = \phi K_i$, where S_R and S_M are the cross-sections of the retentive and mobile phase, respectively.

As the retentive phase flow influences the analyte retention, the term reduced retention, k_i^*, was introduced for the description of retention in PC-OTLC [3]. For practical reasons, it can be related, similarly to k, to the retention time of the solute in the capillary with a mobile retentive phase, t_{ri}, and t_0 as

$$
k_i^* = t_{ri}/t_0 - 1
\tag{2}
$$

The relation of k_i^* to the phase cross-section ratio, ϕ, and to the liquid–liquid distribution constant of the solute, K_i, was found as [3]

$$
k_i^* = (\phi - q)/(1/K_i + q)
\tag{3}
$$

where q is the flow ratio of the retentive and mobile phases, $q = F_R/F_M$. As long as the temperature-induced changes in the liquid solubility are used for the generation of the two-phase flow, q may be related to the difference between the mass fraction of the retentive phase dissolved in the liquid entering the capillary, w, and the mass fraction of the dissolved retentive liquid in the mobile phase saturated by this liquid at the capillary temperature, w_T. Provided that F_R is less than 1% of F_M, q can be written simply as

$$
q = F_R/F_M = \Delta w/(1 - \Delta w)
\tag{4}
$$

where

$$\Delta w = w - w_T \qquad (5)$$

It can be seen from Eqs. 4 and 5 that both w determined by the composition of the inlet liquid and w_T determined by the column temperature can be used for the control of q. As the capillary temperature also influences other important parameters, including the viscosity and the surface tension [4,6], the variation in w seems to have the simplest relation to the capillary performance.

Based on the solubility and viscosity values, the phase ratio can be calculated as [3]

$$\phi = S_R / S_M$$
$$= (1 - t + 1/\Delta w)/\{1/\Delta w -$$
$$[1 + t(1/\Delta w - 1)]^{1/2}\} - 1 \qquad (6)$$

where t is the viscosity ratio of the retentive and the mobile phases, respectively, $t = \eta_R / \eta_M$. Alternatively, the phase ratio can be calculated from Eq. 3 when k_i^*, K_i and q are known. The amount of the retentive phase in the capillary can also be visualized as the thickness of the retentive layer, d_f, which can be expressed with the help of Eq. 3 as

$$d_f = R\{1 - 1/[1 + k_i^*/K_i + q(k_i^* + 1)]^{1/2}\} \qquad (7)$$

Alternatively to the description of the retention in terms of k_i^*, the capacity factor, k_i', defined similarly as in micellar electrokinetic capillary chromatography (MECC), was suggested [11]. Thus, $k_i' = \phi K_i$, which is simpler than Eq. 3. However, when calculating k_i' from the chromatogram, the following equation should be used [11]:

$$k_i' = (t_{ri} - t_0)/t_0(1 - t_{ri}/t_r) \qquad (8)$$

which indicates the need to know the retention time of the compound completely dissolved in the retentive phase, t_r. The relationship of the last variable to other characteristics may be expressed as [11,12]

$$u_R / u_M = q/\phi = t_0 / t_r \qquad (9)$$

where u_R and u_M are the average linear velocities of the retentive and the mobile phase,

respectively. Thus, the smaller q/ϕ is, the larger is the t_r value obtained and k_i' is closer to both k and k_i^*. On the other hand, reading t_r from the chromatogram becomes more difficult owing to the dispersion of the zone of the compound used as the tracer of u_R.

3. Experimental

3.1. Capillary preparation

Fused-silica capillaries of 0.2 mm O.D. and 35 μm I.D. were purchased from Laboratory of Chemistry of Glass and Ceramic Materials (Academy of Sciences of the Czech Republic, Prague, Czech Republic). A Simax-type glass tube (Kavalier, Sázava, Czech Republic) of 7 mm O.D. and 0.6 mm I.D. was drawn using a glass-drawing machine to obtain a glass capillary of 0.7 mm O.D. and 17 μm I.D. The internal surface of capillaries were twice modified with D_4 reagent [4]. The length of the capillaries used in PC-OT-LC experiments was 4–5 m.

3.2. Chromatograph

The chromatographic system used has been described previously [13]. A VCM 300 micropump (Development Works, Czechoslovak Academy of Sciences, Prague, Czech Republic) was used. The sample was injected using a laboratory-made six-port valve with a 20-μl loop and a flow splitter. The splitting ratio was 1:3500 or 1:7500. 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 50.0°C. For on-column fluorimetric detection we adapted [6] a Kratos FS 950 fluorimeter (Schoeffel Instruments). The excitation wavelength was 254 nm and the emission wavelength was 320 nm. An EMD 10 electrochemical detector (Laboratory Instruments, Prague, Czech Republic) was equipped with a thin-layer microcell similar to that described previously [14]. The detector signal was monitored with a TZ 4100 line recorder (Laboratory Instruments).

3.3. Mobile phase

The pumped liquid was water with a known concentration of cyclohexanol. The solubility difference between the concentration of the retentive phase in the mobile phase in the pumped liquid and the concentration of the retentive phase in the mobile phase at the column temperature, Δw, was in the range 0.01–0.96 wt.%.

3.4. Chemicals and test solutes

The model analytes were salicylic acid (marker of the dead time, t_o), hydroquinone, phenol and carbazole. 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). The viscosity ratio of coexisting liquids in the cyclohexanol–water system at 50°C, $t = 7.0$, was taken from Ref. [3]. The solute distribution constant K_i between coexisting cyclohexanol and water phases was taken from Ref. [3]. The K_i values for phenol and hydroquinone at 50°C were 16.7 and 4.9, respectively. The logarithm of the octanol–water distribution constant of carbazole is reported to be 3.29 [15]; a similar value is expected for the cyclohexanol–water system. The diffusion coefficients of solutes in the retentive and mobile phases, D_R and D_M, respectively, for phenol are $D_M = 4.5 \cdot 10^{-3}$ mm^2 s^{-1} and $D_R = 4.4 \cdot 10^{-4}$ mm^2 s^{-1} [4].

3.5. Measurements

The measurements were carried out in a linear velocity range from 1.0 up to 37 mm s^{-1}. The linear velocity of the mobile phase was determined from the retention time of salicylic acid (t_o) and the capillary length. The test solutes for the determination k_i^* and H were hydroquinone and phenol. The k_i^* values used in place of k in the Golay equation were determined from Eq. 2. The value of d_f was calculated from K_i, k_i^* and q with the use of Eq. 7. The values of u_c and d_i used are the averages from the values obtained with both test solutes [4]. Care was taken to use

an arrangement of the instrumentation that does not affect the measurements owing to the extra-column band broadening [13,16]. Thus, the observed peak widths and calculated plate heights can be considered as the result of the processes within the capillary.

4. Results and discussion

4.1. Retention phase velocity

The average linear velocity of the retentive layer was determined both from the dead time, viscosity and solubility data and from the retention of carbazole. Carbazole was chosen owing to its suitable detection characteristics and high hydrophobicity. Therefore, Eq. 9 can be used for the estimation of u_R with sufficient precision. For the fused-silica capillary and the pumped liquid with $\Delta w = 0.81$ wt.% and $u_M = 8.33$ mm s^{-1}, $u_R = 0.31$ mm s^{-1} was found from the evaluation of t_o and t_r. Similarly, $u_R = 0.35$ mm s^{-1} was found for $\Delta w = 0.51$ wt.% and $u_M = 12.5$ mm s^{-1}. These values of u_R are comparable with the u_R values 0.28 and 0.33 mm s^{-1}, respectively, which were obtained from q and ϕ values calculated from the solubility and viscosity data using Eqs. 4–6. Together with the previously reported values of ϕ and q [3], the values presented here indicate that, in the system studied, the contribution of the retentive layer to the total flow in the capillary is less than 1%. Thus, when using the Golay equation for the calculation of the theoretical plate height, the error due to neglecting the retentive layer flow can be expected to be within a few per cent of the calculated value. Further, the similar values obtained in different ways support the assumption of the existence of a flowing annular retentive layer under the conditions specified.

4.2. Retention and the plate height in RP-PC-OTLC

The performance of the system studied was evaluated from the k_i^* vs. u_M and H vs. u_M dependences for several values of Δw and for

phenol as solute (Figs. 1–5). In the graphs shown, line 1 (○) represents plate height, H_1, values calculated from the observed retention time of the peak, the peak variance and the capillary length, line 2 (●) represents H_2 values calculated from Golay equation for immobile films with the use of actual k_i^* and d_f values and line 3 (▽) corresponds to k_i^* calculated from the retention times. In all of the figures, a marked decrease in k_i^* can be observed at certain u_M values. This phenomenon was also observed previously [4,6] and was ascribed to the disturbance of the flow of the retentive layer due to the predominance of the surface tension forces over the viscous flow. The velocity at which the decrease in k_i^* occurs was termed the critical velocity, u_c [4]. The observed u_c values and k_i^* corresponding to u_c, k_{ic}^*, are summarized in Table 1. It can be seen that the solute retention

Fig. 2. Dependence of H_1, H_2 and k_i^* for phenol on u_M in RP-PC-OTLC in a fused-silica capillary column. Conditions: 5 m × 0.035 mm I.D. fused-silica capillary modified with D_4 reagent; column temperature, 50°C; pumped liquid, water saturated with cyclohexanol at 20°C, corresponding to $\Delta w = 0.96$ wt.%; solutes, salicylic acid (dead time marker), hydroquinone and phenol; splitting ratio, 1:3500; linear velocity of mobile phase flow, $u_M = 1–38$ mm s^{-1}; fluorimetric detection. Line 3a (▼) = reduced retention k_i^* for hydroquinone calculated from retention time. Other symbols as in Fig. 1.
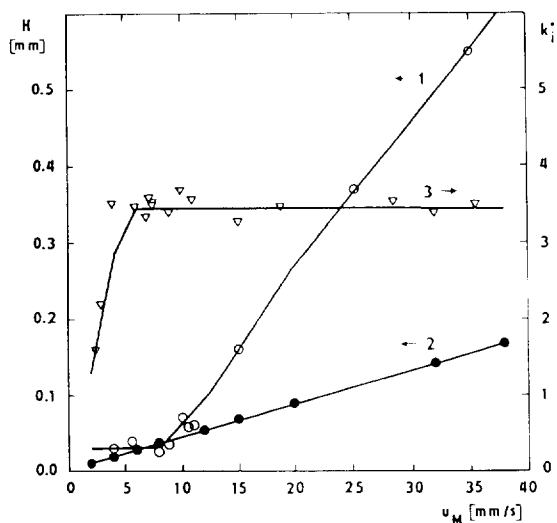

Fig. 1. Dependence of H_1, H_2 and k_i^* on u_M for phenol in RP-PC-OTLC in a glass capillary column. Conditions: 4 m × 0.017 mm I.D. glass capillary modified using D_4 reagent; column temperature, 50°C; pumped liquid, 100 mmol l^{-1} NaClO$_4$ and 1 mmol l^{-1} acetic acid in water saturated at 20°C with cyclohexanol ($\Delta w = 0.96$ wt.%); solutes, salicylic acid (dead time marker) and phenol; splitting ratio, 1:7500; mobile phase velocity, $u_M = 2–38$ mm s^{-1}; electrochemical detection. Line 1 (○) = H_1 values calculated from observed retention time of the peak, variance of the peak and capillary length; line 2 (●) = H_2 values calculated from the Golay equation for immobile films from k_i^* and d_f; line 3 (▽) = reduced retention k_i^* for phenol calculated from retention time.
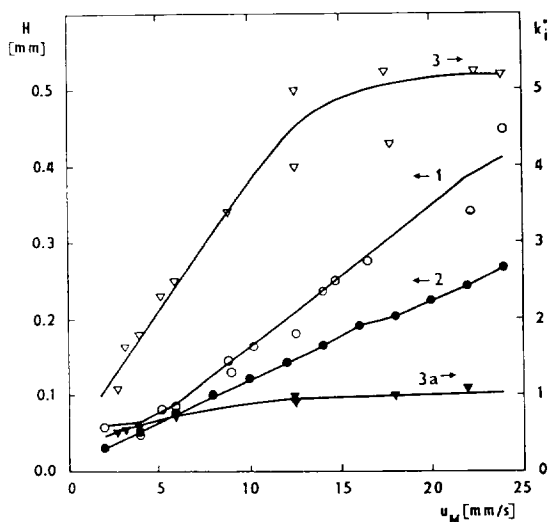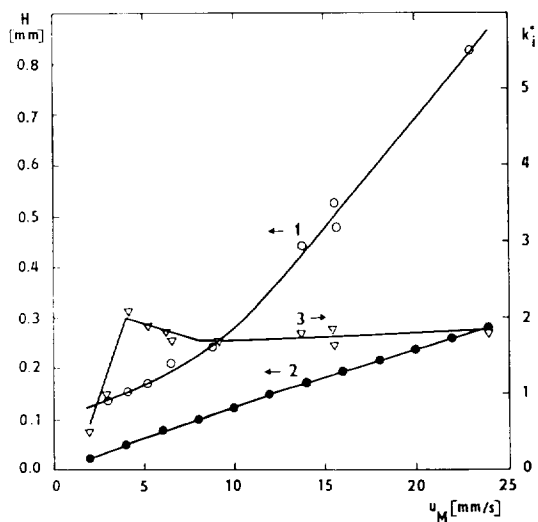

Fig. 3. Dependence of H_1, H_2 and k_i^* for phenol on u_M with $\Delta w = 0.31$ wt.%. Other conditions and symbols as in Fig. 2.
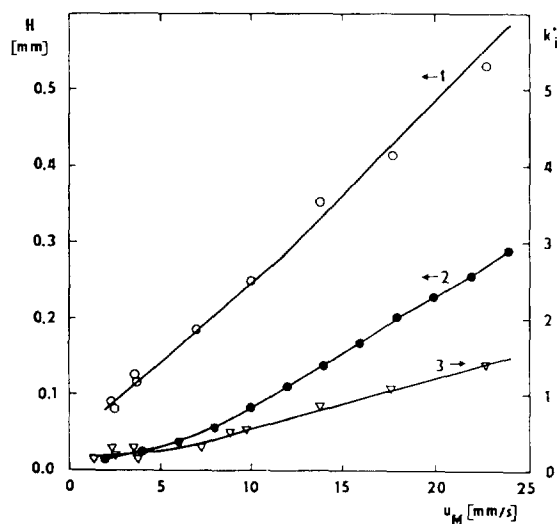
Fig. 4. Dependence of H_1, H_2 and k_i^* for phenol on u_M with $\Delta w = 0.11$ wt.%. Other conditions and symbols as in Fig. 2.

can be efficiently controlled by the amount of the retentive phase introduced at the capillary inlet.

Based on the comparison of u_c, k_{ic}^* and d_f values in Table 1, and also from the dependence of k_i^* and H on u_M, it can be concluded that the flow pattern depends strongly on Δw. As indicated in the Introduction, cyclohexanol is partially soluble in water [3,17] and may form a
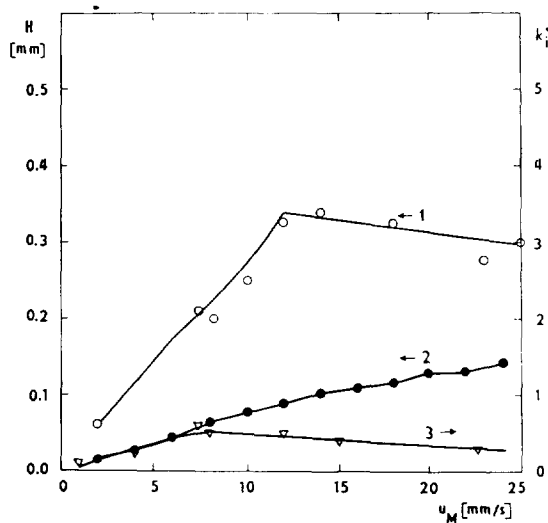
Table 1
Dependence of u_c and k_{ic}^* for phenol, ϕ and d_f for glass and fused-silica capillaries on the concentration of cyclohexanol in the pumped liquid

Capillary column	Δw (wt.%) (Eq. 5)	u_c (mm s^{-1})	k_i^* (Eq. 2)	ϕ (Eq. 6)	d_f (μm) (Eq. 7)
Glass	0.96	4.8	3.40	0.235	0.8
Fused silica	0.96	12.2	4.40	0.315	2.2
	0.51	7.5	3.10	0.206	1.6
	0.31	3.0	1.30	0.084	0.7
	0.11	5.7	0.30	0.019	0.2
	0.01	7.9	0.52	0.031	0.2

dispersion system with water [7,8]. Therefore, we examined the properties of the dispersion of cyclohexanol–water in macroscopic volume (cylinder 100 ml) at the temperature of the capillary (Fig. 6). The time dependence of the amount of the fraction of cyclohexanol in the emulsion droplets, w_e, is shown. This quantity was evaluated from w_T, the amount of the sedimented cyclohexanol-rich layer, and several values w in the range 0.01–0.96 wt.%. An increase in temperature from 20 to 50°C leads to
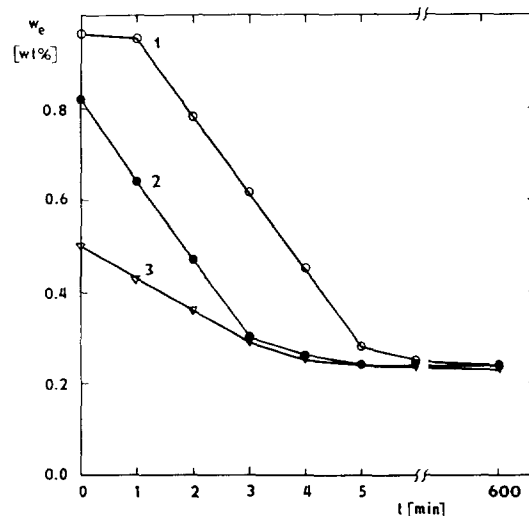

Fig. 5. Dependence of H_1, H_2 and k_i^* for phenol on u_M with $\Delta w = 0.01$ wt.%. Other conditions and symbols as in Fig. 2.

Fig. 6. Characterization of cyclohexanol–water solutions at a column temperature of 50°C. w_c (wt.%) = Concentration of cyclohexanol in the microemulsion at 50°C (macrovolume experiment); t = time (min). Δw = (1) 0.96, (2) 0.81 and (3) 0.51 wt.%. For explanation, see text.

a decrease in the solubility of cyclohexanol [17] and an emulsion appears. The amount corresponds to Δw given by Eq. 5. Then, the emulsion content decreases, which can be followed from the amount of sedimented layer. It follows from Fig. 6 that a microemulsion can exist for up to 10 h. After that time, the amount of cyclohexanol in the form of microemulsion droplets is $w_e = 0.24$ wt.% (see Fig. 6). Similar behaviour can also be expected in the capillary. However, the time for the sedimentation of the cyclohexanol liquid layer should be expected to decrease according to the characteristic dimension, which is about 1000 times smaller in the capillary. For $\Delta w = 0.11$ and 0.01 wt.%, the amount of cyclohexanol in the solution appears to be insufficient for the formation of a long-term stable microemulsion.

In order to visualize the departure of the observed H_1 from the calculated H_2 as a function of u_M around u_c, the relative values of the height equivalent to a theoretical plate, $h_r (= H_1/H_2)$. on the relative velocity of the mobile phase, $v_r (= u_M/u_c)$, were plotted in Fig. 7. At $v_r > 2.5$, $h_r = 2$–3 for all Δw values studied. Hence the capillary performance is not very different from the proposed model. At $v_r < 2.5$, h_r is in the range 1–2 for $\Delta w = 0.96$ wt.% (open circles) and
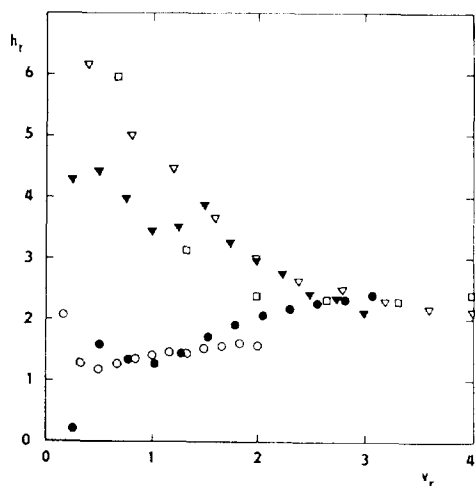

Fig. 7. Dependence of the relative height equivalent to a theoretical plate, h_r. on relative mobile phase velocity, v_r. $\Delta w = (\bullet)$ 0.96, ($\bigcirc$) 0.51, ($\square$) 0.31, ($\triangledown$) 0.11 and ($\blacktriangledown$) 0.01 wt.%.

$\Delta w = 0.51$ wt.% (closed circles). Similarly to the above conditions, a regular annular layer should be present. For $\Delta w = 0.31$, 0.11 and 0.01% (squares and open and closed triangles in Fig. 7, respectively) and $v_r < 2.5$, h_r increases markedly with decrease in v_r. Hence, the dispersion is far greater than predicted by Golay equation and a regular surface layer can no longer be expected. Additionally, k_{ic}^* decreases with decrease in Δw, but for the lowest Δw examined it is higher than for $\Delta w = 0.11$ wt.% (see Table 1).

The above observations can probably be explained as follows. Above u_c, the mobile phase velocity is sufficient to maintain the regular annular shape of the retentive layer with cross-section determined by Eqs. 4–6. For u_M close to u_c and lower, the retentive layer becomes unstable so that the amount of retentive liquid in the layer decreases [3]. Depending on Δw, two limiting cases may occur:

(1) When Δw is large enough for the existence of the microemulsion (i.e., more then about 0.24 wt.% here), the part of the retentive liquid that is above the amount remaining in the layer can move as microemulsion droplets. Both the microemulsion and a decrease in the annular cross-section lead to a considerable decrease in k_i^*. On the other hand, the contact of the microemulsion droplets with the surface continuously smooths the irregularities in the surface layer. Similarly to the transport of the solute in the micellar systems [18], the existence of microemulsion droplets does not seriously impair the diffusion transport of the analyte. Thus, with decrease in v_M, h_r remains constant or decreases to approach unity.

(2) When Δw is small so that an appreciable amount of microemulsion cannot appear, the part of the retentive liquid that is not retained on the surface moves in the form of large droplets or, at very low velocities, in the form of segments. As mentioned previously [3], such conditions approach close to segmented liquid–liquid flow analysis. Simultaneously, owing to the capillary surface irregularities, the retentive liquid remaining on the surface is no longer in the form of a regular annular layer. Naturally, the zone dispersion increases markedly owing to both phenomena. The absence of a microemul-

sion also decreases the transport of the solute by the mobile phase so that the decrease in k_i^* is smaller. Additionally, as the surface coverage is low, the properties of the inner capillary surface strongly influence the regularity of the surface film of the retentive liquid.

As the liquid system within the capillary is under continuous movement, the microemulsion content probably differs from that observed under the stationary conditions. Nevertheless, the suggested model should be useful for the qualitative explanation of the discussed phenomena.

5. Conclusions

Above the critical velocity of the mobile phase, the performance of RP-PC-OTLC can be explained in terms of the model comprising the regular flowing surface layer of the retentive liquid. While the plate height is comparable to the values given by the Golay equation, the solute retention can be controlled by the amount of the retentive phase introduced at the top of the capillary column.

The behaviour of PC-OTLC with the mobile phase velocity below its critical value can be explained in terms of the suggested model involving the formation of a microemulsion. Owing to the high retention and efficiency, the system examined with a larger amount of the retentive liquid promises a better capillary performance.

References

[1] M.J. Golay, in D.H. Desty (Editor), *Gas Chromatography*, (Butterworths, London, 1958), p. 36.
[2] J.C. Giddings, *Unified Separation Science*, Wiley, New York, 1991.
[3] K. Šlais, M. Horká and K. Klepárník, *J. Chromatogr.*, 605 (1992) 167.
[4] K. Šlais and M. Horká, *J. Microcol. Sep.*, 5 (1993) 63.
[5] K. Šlais and M. Horká, *J. High Resolut. Chromatogr.*, 16 (1993) 194.
[6] M. Horká, V. Kahle and K. Šlais, *J. Chromatogr. A*, 660 (1994) 187.
[7] L.M. Prince, *Microemulsions Theory and Practice*, Academic Press, New York, 1977.
[8] D. Myers, *Surfaces, Interfaces, and Colloids, Principles and Applications*, VCH, New York, 1991.
[9] M. Krejčí, *Trace Analysis with Microcolumn Liquid Chromatography (Chromatographic Science Series*, Vol. 59), Marcel Dekker, New York, 1992.
[10] W.N. Gill, *AIChE J.*, 15 (1969) 745.
[11] P.G.H.M. Muijselaar and C.A. Cramers, *J. Microcol. Sep.*, 5 (1993) 187.
[12] K. Šlais, M. Horká and K. Klepárník, *J. Microcol. Sep.*, 5 (1993) 189.
[13] K. Janák, M. Horká and M. Krejčí, *J. Microcol. Sep.*, 3 (1991) 203.
[14] K. Šlais and M. Krejčí, *J. Chromatogr.*, 235 (1982) 21.
[15] A. Leo, C. Hansch and D. Elkins, *Chem. Rev.*, 71 (1971) 525.
[16] K. Janák, M. Horká and M. Krejčí, *J. Microcol. Sep.*, 3 (1991) 115.
[17] R. Stephenson and J. Stuart, *J. Chem. Eng. Data*, 31 (1986) 56.
[18] S. Terabe, K. Otsuka and T. Ando, *Anal. Chem.*, 61 (1989) 251.