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EFFECT OF LAPLACE–MARANGONI FLOW IN THE STATIONARY PHASE ON THE CHROMATOGRAPHIC PROPERTIES OF GLASS CAPILLARY COLUMNS

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SUMMARY

Variations in the properties of the column (efficiency, capacity ratio) can be attributed to the Laplace–Marangoni flow in the stationary phase. The presence of surfactants (solutes or stationary or mobile phase) is responsible for the flow in the stationary phase. This flow causes the differences in film thickness to increase so that droplets are generated that can be washed out of the column by the liquid mobile phase. The droplets formed hinder or interrupt the mobile phase flow in small-bore columns. A scanning electron microscope photograph of the droplets formed in the column is presented.

The effects were tested in columns of I.D. 15–30 μm. DC-550, OV-101 and Apiezon K were used as stationary phases.

INTRODUCTION

Capillary liquid chromatography makes it possible in some instances to investigate and to interpret phenomena that could not be explained when columns with larger diameters or adsorption and permeation systems with pore diameters close to those of the capillary columns used are used.

Small values of diffusion coefficients in liquid systems lead to the use of capillary columns with small inner diameters. Small values of the ratio of stationary to mobile phase volume are responsible for a preference for liquid–liquid systems. Attainment of acceptable capacity ratios assumes that such large distribution constants are applied that they can hardly have a linear isotherm within a sufficient concentration range in adsorption or ion-exchange systems.

The preparation and application of capillary columns in liquid–liquid systems raises several points of interest that were observed earlier, but have not been explained up to now:

(1) The capacity ratios in columns decrease with time[1], which is obviously associated with washing out of the liquid stationary phase from the column.

(2) Simultaneously, the column efficiency decreases and the height equivalent to a theoretical plate increases, although it should not do so according to theory. Fig. 1 shows an example illustrating this fact.

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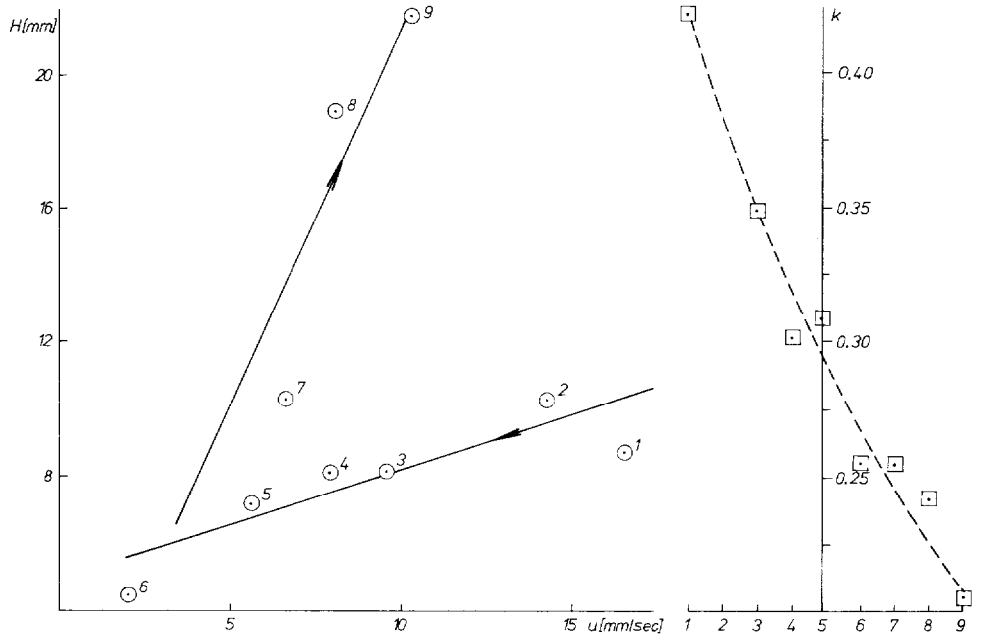

Fig. 1. Change in the height equivalent to a theoretical plate, H, and the capacity ratio, k, depending on the sequence of experiments nos. 1–9. Slope of the dependence of H *vs.* u for experiments 1–6, m_{1-6} = 0.308 sec; for experiments 6–9, m_{6-9} = 2.231 sec.

(3) The permeability of the capillary column changes and in some instances[1,2] reversible blocking of the column occurs, which can be suppressed by means of increased pressure or sometimes also by changing the temperature[2]. At other times, the flow of liquid cannot be re-established, even with a pressure of several tens of MPa.

(4) In some instances, changes occur in both the permeability and efficiency of the column, even when the mobile phase does not flow through the column for some time. This phenomenon has also been produced in capillary columns prepared for and applied in gas chromatography. This effect is occasionally encountered in practical gas chromatography, most frequently in the analysis of surfactants.

Secondary flow arising as a result of concentration gradients of the surfactant at a liquid–liquid or liquid–gas interface, called the Marangoni effect[3,4] and also the Laplace–Marangoni effect, serves as the background for the explanation of the above phenomena. This paper describes the dynamics of changes in the chromatographic properties of glass capillary columns associated with possible secondary flow in the stationary liquid phase. Glass columns of I.D. 15–31 μm, coated with liquid stationary phases, were used for these experiments.

EXPERIMENTAL

We used glass capillary columns in liquid chromatography in the apparatus described earlier[2]. Samples were applied with the aid of a splitter[5]. A three-electrode electrochemical detector[5] with a cell volume of less than 1 nl, a flame-ionization

TABLE I

PARAMETERS OF THE CAPILLARY COLUMNS USED

Mobile phase: 10^{-3} *M* $HClO_4$ in distilled water. TMCS = trimethylchlorosilane.

Column No.	*Length (m)*	*Inner diameter (μm)*	*Surface modification*	*Stationary phase*
1	7.5	31	TMCS	Apiezon K
2	5.5	18	TMCS	Apiezon K
3	6.0	16	TMCS	Apiezon K
4*	6.0	16	TMCS	Apiezon K
5	5.6	15	None	Apiezon K
6**	5.1	15	None	DC-550
7	20.0	100	TMCS	OV-101
8	20.0	100	TMCS	OV-101

* Saturated with stationary phase.

** Etched with 20% HCl, washed with 1% HCl, dried in H_2 at 250°C, hexamethyldisilazane (HMDS).

detector[2], an alkali flame-ionization detector[6] and an adapted two-electrode electrochemical detector[7] with a cell volume of *ca.* 4 nl were used for detection.

The glass capillary columns were prepared as described elsewhere[2]. Table I lists the parameters of the capillary columns used. The dynamic method was used for column coating. In some instances, the internal surface of the capillaries was roughened with 1,1,2-trifluoroethyl methyl ether, silanized and, finally, coated dynamically. DC-550, OV-101 and Apiezon K (Applied Science Labs., State College, PA, U.S.A.) were used as stationary phases. In several instances, SE-54 stationary phase was immobilized by the action of benzoyl peroxide[8].

A Fractovap 2101 AC chromatograph (Carlo Erba, Milan, Italy) was used for gas chromatography with hydrogen as the carrier gas. Glass tubes made of Sial glass were used.

Background of flow in the stationary phase

A surface tension gradient at the interface[4] gives rise to the Laplace–Marangoni effect, resulting in flow of the liquid at the interface. The liquid with a lower surface tension spreads on the surface posessing a higher surface tension. If the surface of the capillary is curved, then convex curvature gives rise to capillary forces that draw the liquid off the flatter parts of the film into the more curved parts[4] (Laplace effect). As a result of the flow produced in the liquid, tension occurs at the interface that evokes a counter flow (Marangoni effect). This flow has been described mathematically several times (*e.g.*, ref. 9). However, the application of the mathematical treatment to real situations is very difficult, if possible at all.

The Laplace Marangoni effect can result[10] from concentration, thermal or electrical charge density gradients, as a consequence of which surface tension at the interface arises. All of the above effects may occur in chromatography. The last one was described in connection with electrokinetic effects in liquid chromatography[11]. The Laplace–Marangoni effect can cause changes in the interfacial surface area[12] and, as a result, can also change the thickness of the liquid film[13–15].

Decrease in capacity ratios

Non-homogeneity of the surface modification or non-homogeneity of surface properties of the internal surface of glass capillary columns can be the reason for non-homogeneity of the thickness of the stationary phase film. The presence of surface-active substances in a stationary phase–mobile phase–solute chromatographic system leads to Laplace–Marangoni flow, which increases the difference in the film thickness. Under these circumstances, droplets of stationary phase may be generated, which are connected with the surface of the column by adsorption forces and increase in volume[14]. As a consequence of the increase in droplet volume with a constant basal area, the wetting angle increases to the critical value, at which point the droplet or part of it is released and produces a sphere with of diameter d_b. Non-homogeneity in the stationary phase thickness and the tendency to produce droplets were observed in glass capillary columns for gas chromatography[16], and as an example a scanning electron microscope (SEM) photograph is presented in Fig. 2.

In order to investigate further the development of the hydrodynamic system of the capillary column, it must be assumed that, depending on the surface properties of both the stationary and the mobile phases, the droplet diameter may be in the range $d_f \leqslant d_b \leqslant d_c$, where d_f is the film thickness and d_c is the column diameter. If $d_b \ll d_c$, the spheres of stationary phase can be very easily carried along by the mobile

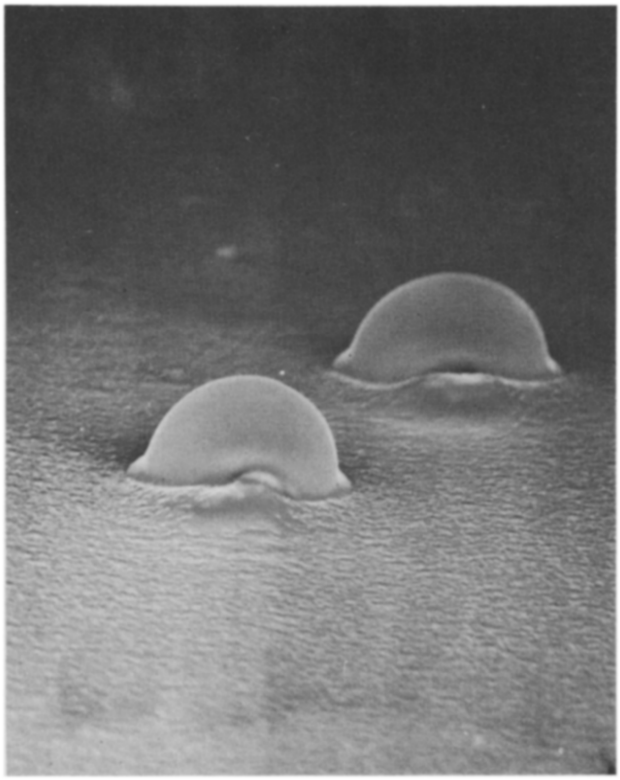

Fig. 2. SEM photograph of the liquid stationary phase (UCON LB-550X). Glass WCOT column, d_c = 250 μm. Magnification 3000 times.

phase flow. They can be trapped on other droplets of stationary phase present in the column, increase the volume of these droplets and, ultimately, be again released as spheres into the stream of mobile phase. Hence a certain ficticious retention will be observed. The rate at which the stationary phase is lost does not correspond to the mobile phase flow-rate. In some instances, the stationary phase is stabilized to such an extent that the droplets cannot grow any longer in this way, and thus they are forced to stay where they are adsorbed, thereby retarding the loss of stationary phase from the column. Fig. 3 shows the course of the loss of stationary phase from the column (characterized as a decrease in the relative capacity ratio, k_{rel}). If the mobile phase is saturated with the stationary phase prior to entering the column, a substantially smaller decrease in the stationary phase volume occurs, but nevertheless, the biggest droplets of stationary phase are eluted from the column under these circumstances. The surface created in this way is less inhomogeneous and, consequently, the column efficiency increases to a constant value which does not vary noticeably with time. Experimental verification of this phenomenon is obvious from Table II.

If $d_b < d_c$, the droplet created on the surface of the stationary phase film can make the column cross-section narrower. Under these conditions, the permeability of the column varies and at lower pressures the flow may be interrupted at the mobile phase inlet, as was demonstrated in Fig. 4 in ref. 17. By increasing the inlet pressure, flow with the initial dependence of the flow-rate on pressure can be re-established. Apparent restriction of the cross-section was illustrated in Fig. 5 and Table IV in ref. 2.

If $d_b \approx d_c$, the droplet on the stationary phase film is not released in the shape of a travelling sphere, but produces a column of liquid. Under certain circumstances, the ratio between the adsorption forces and the surface tension then permits penetration of the mobile phase or the mobile phase with the solute through this column of liquid. The penetration proceeds in the way often described in association with the Marangoni effect. The moving heterogeneous liquid (or gas) penetrates the col-

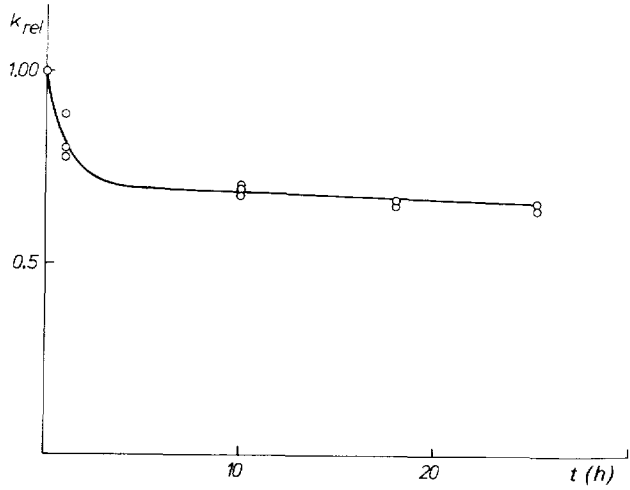

Fig. 3. Dependence of the decrease in the relative capacity ratio, k_{rel}, on time. $-k_1 = 0.05$; $-k_2 = 0.19$; $-k_3 = 0.61$.

TABLE II

CHANGE IN THE EFFICIENCY OF THE COLUMN SATURATED WITH THE MOBILE PHASE

Sequence of the experiment	k_{rel}	h/v
1	1.00	0.47
2	1.00	0.34
3	0.78	0.07
4	0.78	0.09
5	0.78	0.17
6	0.79	0.16

umn of stagnant liquid, which does not change its position in the capillary during the penetration. In this, it differs from bubble flow. We have observed this phenomenon visually during the penetration of nitrogen through a water column at the end of a glass capillary column of I.D. 100 μm. When working with a flame-ionization detector and capillary columns, the above effect could be observed through the detector response. This detector, like the mass detector, reacts rapidly enough to the change in the flow-rate. It is obvious from Fig. 4 that the solute flow at the column outlet is interrupted at relatively regular intervals. DC-550 stationary phase is transferred towards the column outlet. Blocking of the column occurs here, and the solute penetrates through the column of stationary phase in discrete quanta. Another solute

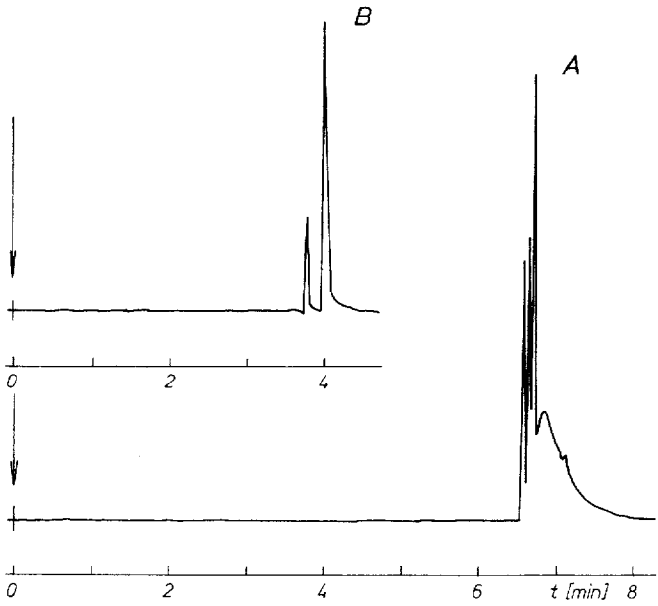

Fig. 4. Example of chromatograms. (A) Penetration of solute, 1,2-dibromo-2,2-dichloroethyldimethylphosphate (Naled), through the column of the stationary phase. (B) Normal elution of the solute (trimethyl ester of phosphoric acid) from the same column under the same conditions. Detector, flame ionization; column, 3.5 m × 16 μm I.D.; stationary phase, DC-550; mobile phase, water–methanol (1:1) + 50 ppm of nitrobenzene.

is eluted from the column without the above effect, because during the analysis time no column of liquid exists at the end of the capillary. In some instances, the flow was interrupted and could not be re-established, even with a pressure of 40 MPa. It was sufficient to break several millimeters off the end of the column to re-establish the flow to the initial extent. We assume that in the case described above the column of stationary phase was longer and impeded the penetration of the mobile phase. In other instances, the interrupted flow could be re-established by increasing the column temperature. Sometimes, the flow of the mobile phase through the column was interrupted irreversibly. From the experiments described above it is obvious that the interruption of the mobile-phase flow is not caused by mechanical blocking of the column. The reproducibility of the experiments is relatively low, as the changes in the shape of the stationary phase film can neither be forecast nor significantly controlled.

Decrease in column efficiency depending on the decrease in capacity ratio

Deficiency or non-homogeneity in the thickness of the stationary phase film gives rise to decreased column efficiency. The change in column efficiency is obvious from Fig. 1. The numbers of the experimental points designate the sequence of the experiments. A conclusive change in slope, m, can be observed for the series of experiments 1–6 and 6–9. The decrease in capacity ratios was monotonous. The change in the film shape associated with the flow and, consequently, also with the loss of the stationary phase leads to a decrease in column efficiency, depending on the decrease in capacity ratios in the column. In order to evaluate this effect, we started from the known equation[18] for the reduced height equivalent to a theoretical plate, h_t:

$$h_t = \frac{2}{v} + \left[\frac{1 + 6k + 11k^2}{96(1 + k)^2} + \frac{k}{(1 + k)^2 d_c^2} \cdot \frac{D_m}{D_s} \cdot q d_f^2\right] v \tag{1}$$

which can be expressed in a simplified form with the aid of coefficients of mass transfer in the mobile and stationary phases, C_m and C_s, respectively, and the coefficient of longitudinal diffusion, B:

$$h_t = \frac{B}{v} + (C_m + C_s)\, v \tag{2}$$

In eqn. 1, values of v (the reduced flow-rate of the mobile phase), k (capacity ratio) and d_c (inner diameter of the column) can be determined experimentally, and those of D_m and D_s (diffusion coefficients in the mobile and stationary phases, respectively) can be found in tables. The product qd_f^2 (configuration factor, q, and thickness of the stationary phase, d_f) is difficult to determine experimentally and always involves considerable uncertainty with respect to the instantaneous state of the stationary phase film, which is difficult to define. It was simultaneous assumed that product qd_f^2 is a quantity involving not only a change in the amount of stationary phase in the column, but also a change in the shape of the film. We therefore started from a comparison of the value of h_t, calculated theoretically, with the value h_e, determined experimentally, of the reduced height equivalent to a theoretical plate. The value of

h_e is the sum of the column contributions to the height equivalent to a theoretical plate, h_c, and the extra column contribution to the peak broadening, h_{ec}. Hence

$$\Delta h = h_e - h_t = h_c + h_{ec} - h_t \tag{3}$$

In view of the fact that eqns. 1 and 2 describe peak broadening in the column, they are valid for $k = 0$, $C_s = 0$ and, thus, $h_c - h_t = 0$ and

$$\Delta h = h_{ec} \tag{4}$$

For $k \neq 0$

$$\Delta h = h_e - h_t = (C_{se} - C_{st})v$$

$$= [(qd_f^2)_e - (qd_f^2)_t]v \cdot \frac{k}{(1 + k)^2} \cdot \frac{D_m}{D_s d_c^2} \tag{5}$$

where the subscripts e and t designate experimental and theoretical values, respectively.

Because in all instances $(qd_f^2)_e \gg (qd_f^2)_t$ and $(qd_f^2)_t$ in eqn. 5 did not contribute more than *ca.* 0.5%, it can be neglected and hence

$$d_f \sqrt{q} = \sqrt{h \cdot \frac{(1 + k)^2}{vk} \cdot \frac{D_s d_c^2}{D_m}} \tag{6}$$

Glass capillary columns 1–5, the parameters and the preparation of which are given in Table I, were used for the experiments. The columns in which the mobile phase was not saturated with the stationary phase showed a decrease in the relative capacity ratio, k_{rel}, in the interval from 1 to 0.23 over the course of several days. When the decrease was not significant, the mobile phase flow was mostly blocked prior to the end of the experiment. The apparatus was not dismantled in the course of work on one column. Extra-column contributions to peak broadening can therefore be considered constant in one particular experiment, and all changes in the efficiency of the system can be ascribed only to the change in the shape and amount of stationary phase in the column. Fig. 5 shows an example of the experiment evaluated according to eqn. 6.

It is obvious that with a decreasing capacity ratio, k_{rel}, the volume of the stationary phase, V_s, also decreases at an assumed constant value of the distribution constant, K_D, and, consequently, so does the total amount of stationary phase on the surface of the column and thus also the thickness of the stationary phase, d_f, corresponding to the homogeneous distribution:

$$V_{s\,rel} = k_{rel} V_m / K_D = \pi d_c d_{f\,rel} L \tag{7}$$

The value of qd_f^2, determined experimentally, increases. In accord with the assump-

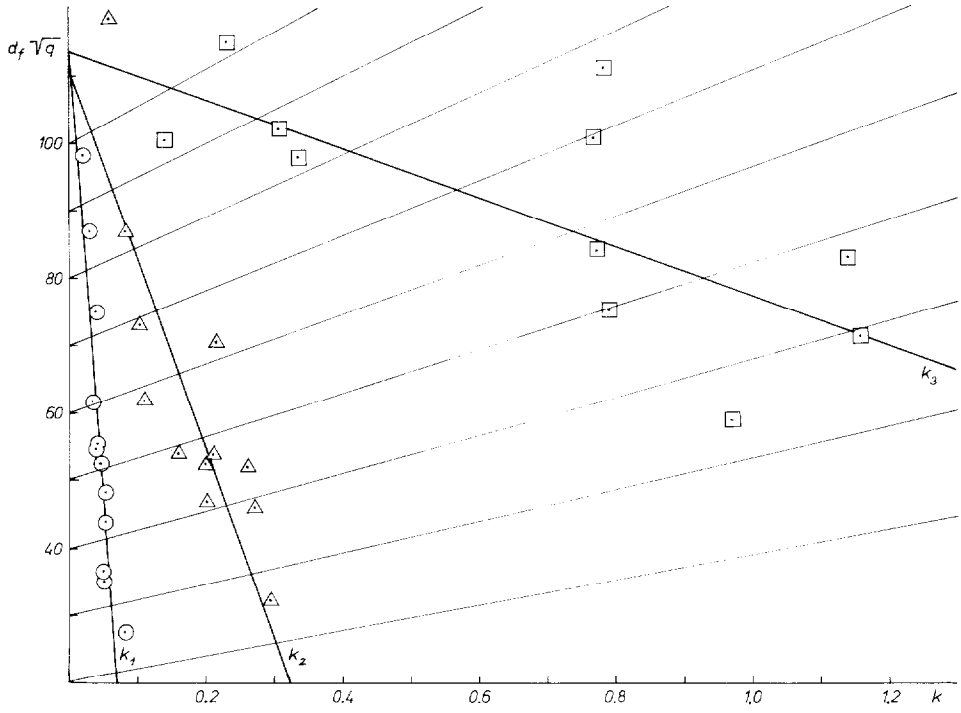

Fig. 5. $d_f\sqrt{q}$ (eqn. 6) as a function of the change in capacity ratio, k.

tion in eqn. 4 for $k = 0$, $d_f\sqrt{q}$ is a constant, regardless of the initial capacity ratio of the solute. The experimental variance of the values is ± 1.9% in this case. The average of the values of $d_f\sqrt{q}/d_c$ is 3.16 for columns of I.D. 15–31 μm (nos. 1–5), which suggests that extra-column contributions to the peak broadening did not differ significantly in these instances.

It follows further from the experiments that the functional dependence of the reduced height equivalent to a theoretical plate on the capacity ratio is different in the present case from that assumed in eqn. 1 from which dependence (6) is also derived. It is obvious from Fig. 5 that the slope of the straight lines connecting the values for k_1, k_2 and k_3 increases with decreasing relative capacity ratio. The slopes of these straight lines, with correlation coefficients depending on the relative capacity ratio, are presented in Table III. It follows from the experiment evaluated in this way that the capacity ratios of solutes decrease with time, while the inhomogeneity of the film of the stationary phase intensifies. This inhomogeneity is more significant for higher capacity ratios in the range of the measured values.

Decrease in column efficiency depending on time without application of the liquid mobile phase

In view of the fact that the Laplace–Marangoni flow arises at the interface, it must occur regardless of whether phase heterogeneity of the system is produced by contact of two immiscible liquids (LLC) or by contact of liquid and gas (GLC).

TABLE III

SHAPE OF THE DEPENDENCE OF $d_f\sqrt{q} = f(k)$ ACCORDING TO EQN. 6

k_{rel}	*Slope (m)*	*Correlation coefficient (R)*
1.00	40.16	1.00
0.98	42.42	1.00
0.93	85.52	0.93
0.78	56.95	0.99
0.77	75.20	0.97
0.42	129.78	0.97
0.42	107.89	0.96
0.34	156.00	0.97

Columns with parameters acceptable for operation in both a gas–liquid system and a liquid–liquid system were selected for the experiment. These were columns 7 and 8 (Table I). Column 8 served as a reference and efficiencies were measured in it at a constant flow-rate of the carrier gas. *n*-Decane was used as a solute. The column chosen for the experiments (7) was tested in a Fractovap 2101 AC gas chromatograph and 4000 theoretical plates per metre were obtained. The reference column provided an efficiency of 2500 plates per metre at the same time and under the same conditions. These values are shown in Fig. 6 as $n_{rel} = 1$. Having been tested in gas chromatography, column 7 was used for liquid chromatography. A decrease in the efficiency, also expressed in Fig. 6, was observed with this column. When the experiments were finished, the column was dried with nitrogen and again tested in the gas chromatograph under identical conditions. A decrease in column efficiency obviously occurred after the film of the stationary phase had been disturbed by the liquid mobile phase. After repeated column conditioning in gas chromatography, the column was used to analyse ethoxyphenols (Wool Research Institute, Brno, Czecho-

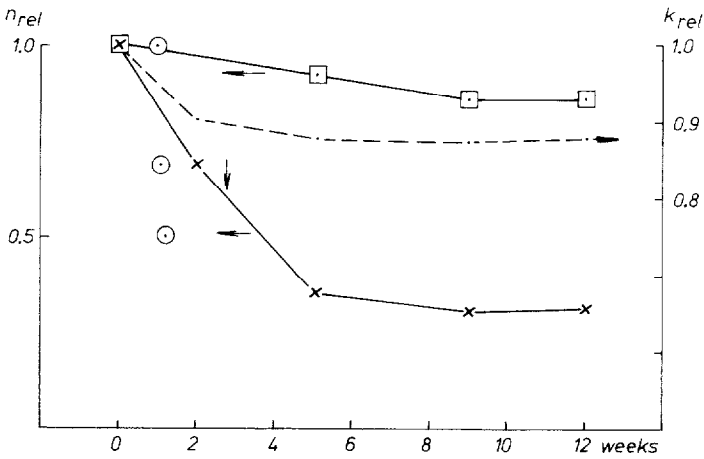

Fig. 6. Decrease in the efficiency of the capillary column in gas chromatography. ⊡, Reference column; +, measured column; ⊙, experiments in liquid chromatography. Test mixture, ethoxyphenols.

slovakia) as surface-active agents. After the injection of these substances, the column was closed and stored at room temperature for about 3 weeks. After this time, an additional decrease in the efficiency was observed, in spite of the fact that the amount of stationary phase in the column had changed insignificantly. This is in accord with the assumption that the gaseous mobile phase is not capable of mechanically eluting the stationary phase. However, the concentration gradient of a surface-active agent causes flow and destruction of the stationary phase film structure. The difference in the decrease in column efficiency in the reference and the tested columns is obvious from Fig. 6.

CONCLUSION

It has been demonstrated that the distribution, form and amount of liquid stationary phase in glass capillary columns in LC changes with time in the presence of surface-active agents. Laplace–Marangoni flow gives rise to inhomogeneities in the film thickness, in some instances to droplets of the stationary phase, causing a decrease in column efficiency and a decrease in the amount of stationary phase in the column. In accord with this, the inhomogeneity of the stationary phase distribution appears to be greater the higher is the solute capacity ratio in the column.

The application of a surface-active solute to the column in gas chromatography leads also to a decrease in the efficiency of a capillary column in gas chromatography. The process proceeds even without a flow of the carrier gas.

In order to improve the ratio of the stationary and mobile phases, the inner diameter of the column must be reduced. In a liquid–liquid system, a column I.D. of $d_c = 5$ μm is probably the limit. Disturbances in mobile phase flow and a rapid decrease in the efficiency occur in columns with smaller diameters, owing to the phenomena described above. It can be expected that even when columns with non-circular cross-sections[19–21] are used, smaller dimensions of the column will be unsuitable for liquid–liquid systems due to the effects described above. In the application of adsorption systems, it will be necessary to prevent even the temporary formation of two heterogeneous phases in the column.

Saturation of the mobile phase with the stationary phase can nevertheless stabilize capacity ratios in the column, but even under these circumstances a decrease in column efficiency occurs.

The pore dimensions of supports of gas chromatographic stationary phases, similar to the interstitial capillaries in liquid chromatography, are close to the dimensions of the columns we have studied, and effects of Laplace–Marangoni flow in the stationary or stagnant phase on the stability of packed chromatographic columns therefore cannot be excluded.

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