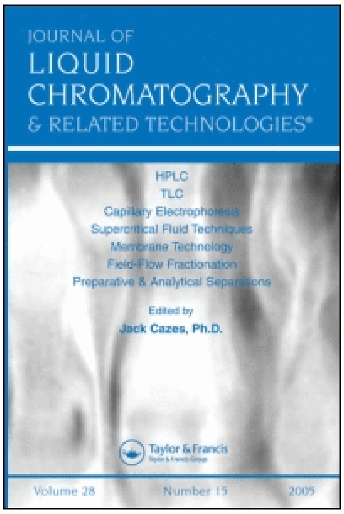

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The Impact of Mobile Phase Pressure and Velocity on the Development of Chromatograms in TLC and OPLC—a Comparison

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The Impact of Mobile Phase Pressure and Velocity on the Development of Chromatograms in TLC and OPLC—a Comparison

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Abstract: Overpressured-layer chromatography (OPLC) has been widely recommended in many scientific publications as a special thin-layer chromatographic technique with forced flow of mobile phase that largely out-performs classical thin-layer chromatography (TLC) with its spontaneous low-pressure capillary flow. Some authors even claim that OPLC can be regarded as a successful bridge between TLC and high-performance liquid chromatography (HPLC). The essential difference between OPLC and TLC is the nature of the force that, in each of these two modes of planar chromatography, pushes the mobile phase through the pores of the stationary phase. It was the objective of this study to compare the impact of capillary and forced flow on analyte retention and separation quality. As test analytes, we selected three different hydrocarbons, tetralin, phenanthrene, and anthracene; these are, respectively, mono-, bi-, and tricyclic aromatic compounds with no functionality and, hence, are unable to participate in lateral interactions that might eclipse the basic effects of retention. In the most advanced OPLC systems, development of the thin-layer chromatograms resembles that in HPLC as closely as possible (e.g., the stationary phase bed is preconditioned with the mobile phase and the samples are applied on-line to the sorbent layer, without interrupting the flow of mobile phase and without drying the initial

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spots). With the Cobrabid OPLC apparatus used in this study, however, the only possibility was to develop dry layers with the dried spots of samples applied off-line. Therefore, the only difference between development of TLC and OPLC chromatograms was, in fact, the pressure (and consequently the flow rate) of the mobile phase. Surprisingly enough, values of the retardation factor (R_F) obtained for our test analytes by OPLC were always substantially lower than those obtained by TLC, which, under the conditions of our experiment, was proof of the poorer selectivity of OPLC compared with TLC. Two physical explanations (either alternative, or complementary) are offered to explain how the elevated pressure of the mobile phase in OPLC results in much lower numerical values of R_F than in TLC.

Keywords: TLC, OPLC, Densitometry, Hydrocarbons

INTRODUCTION

Overpressured-layer chromatography (OPLC) was introduced for the first time 25 years ago[1] and became known as a technique intermediate between thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC), supposedly combining the advantages of both.[2–4]

The main difference between planar and column chromatography is the force pushing the mobile phase through the stationary phase bed. In TLC, the mobile-phase flows through the stationary phase by capillary force. Hence, the velocity of the eluent is not linear, in contrast with OPLC, which is a forced-flow technique using an external pressure (by application of a pump).[5,6] In OPLC, the velocity of the mobile phase is linear and constant over the entire separation distance. In this system, the vapor phase above the sorbent layer is eliminated, hence, OPLC in a sense resembles HPLC on a column, although having a very thin but wide cross section.[1,7–9]

The inventors and practitioners of OPLC claim that, owing to its high flexibility, the technique can be used for both analytical and preparative purposes,[10] and OPLC has often been used for separation, identification, and quantitative determination of different classes of compounds.

In this study, we used OPLC and classical TLC to study and compare the impact of mobile-phase velocity (and indirectly pressure) on the separation behavior of pairs of test analytes. As test analytes, we purposely chose three aromatic hydrocarbons, i.e., species lacking any functionality and, therefore, unable to participate in lateral interactions and interacting only weakly with the other components of the chromatographic system (i.e., the stationary and mobile phases). It was our intention to select test analytes that were unable to obscure the effect with intermolecular interactions of any kind.

In our experiments, we used an OPLC chromatograph manufactured by the firm Cobrabid (Warsaw, Poland); further technical details are given in the Experimental section. The classical variant of TLC was performed in a Stahl-type open-space chromatographic chamber in which migration of the mobile phase and the analytes was a consequence of the action of capillary forces only. The results obtained are presented and discussed in forthcoming sections of this paper.

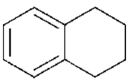
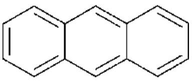
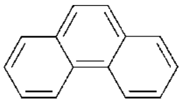
EXPERIMENTAL

TLC and OPLC

Chromatography was performed with the three test analytes listed in Table 1, with (i) separate solutions of anthracene (concentrations 0.050 and 0.075 mol L^{-1}), phenanthrene (concentrations 0.10 and 0.20 mol L^{-1}), and tetralin (concentration 2.0 mol L^{-1}), and with (ii) binary mixtures of anthracene and tetralin (concentrations of anthracene 0.050 and 0.075 mol L^{-1} and concentration of tetralin 2.00 mol L^{-1}), and binary mixtures of phenanthrene and tetralin (concentrations of phenanthrene 0.10 and 0.20 mol L^{-1} and concentration of tetralin 2.00 mol L^{-1}). The analytes were dissolved in toluene. The stationary phase employed was silica gel 60 F_{254} (precoated aluminum-backed TLC plates manufactured by Merck, Darmstadt, Germany; #1.05554.0001), with *n*-hexane as the mobile phase. The volume of the test samples applied to the plates (irrespective of their concentration) was 1 μL.

TLC development was performed in the ascending mode in a Stahl-type open-space chromatographic chamber previously saturated with mobile phase vapor for 20 min. Migration of the mobile phase and the analytes was a result of the action of capillary forces only. OPLC was performed in a Cobrabid (Warsaw, Poland) KB 5121 OPLC chromatograph (designed for

Table 1. The molecular structures of the investigated analytes

Analyte	Molecular structure
Tetralin	
Anthracene	
Phenanthrene	

100 mm × 200 mm chromatographic plates) equipped with the KB-5533 syringe pump. The conditions used for development of the chromatograms were:

(i) the external pressure applied in the direction perpendicular to the Teflon plate covering the adsorbent surface was 7 atm; the mobile phase was pumped through the adsorbent layer between the aluminum backing plate and the Teflon covering plate; and
(ii) the typical mobile-phase flow rate was 4.0 mm min^{-1}.

In OPLC, the mobile-phase flow was a consequence of mechanical force, although the pumping pressure was unknown (because the apparatus was not equipped with a pressure gauge).

Another experiment was also performed with phenanthrene (concentration 0.10 mol L^{-1}) and a phenanthrene–tetralin mixture (concentrations 0.10 and 2.0 mol L^{-1}, respectively) developed at three different flow rates, 1.2, 4.0, and 6.2 mm min^{-1}.

In both TLC and OPLC, the migration distance of the mobile-phase front was 14 cm. Each experiment was repeated from three to six times ($n = 3-6$), and each numerical R_F value was the mean with $\pm$ 0.02 R_F units deviation.

Densitometry

All of the thin-layer chromatograms obtained in this study were evaluated by means of densitometry. Densitograms were acquired with the Desaga (Heidelberg, Germany) model CD 60 densitometer, equipped with Windows-compatible ProQuant software. Concentration profiles were recorded in ultraviolet (UV) light (in the reflectance mode) at 254 nm. The dimensions of the rectangular light beam were 0.02 mm × 0.4 mm. The densitograms obtained were relatively smooth and, therefore, needed no extra smoothing.

RESULTS AND DISCUSSION

The results given in Tables 2 and 3 and Figures 1–5 reflect the impact of the mobile-phase flow rate, arising from capillary forces in TLC and from forced flow in OPLC, on the chromatographic behavior of the separated pairs of analytes and, in the first instance, on their R_F values. From these results, one can draw general conclusions and compare the two planar chromatographic techniques. The numerical values of R_F originating from classical TLC are substantially larger than those originating from OPLC, both for the three test analytes chromatographed as single species and for the binary mixtures. Moreover, the differences (ΔR_F) between the retardation

Table 2. Comparison of R_F values obtained by TLC and OPLC for different amounts of anthracene, tetralin, and their binary mixture

		Retardation factor, R_F	
Analyte	Concentration (C_m) (mol L^{-1})	TLC development velocity 1.81 mm min^{-1}	OPLC development velocity 4.00 mm min^{-1}
Anthracene	0.050	0.47	0.14
	0.075	0.47	0.14
Tetralin	2.0	0.78	0.43
Binary mixture	0.050:2.0	0.49	0.15
		0.79	0.37
	0.075:2.0	0.49	0.18
		0.78	0.37

coefficients for anthracene and tetralin obtained by TLC are significantly larger than those obtained by OPLC ($\Delta R_F \approx 0.29-0.30$ for TLC compared with $\Delta R_F \approx 0.19-0.22$ for OPLC). In general terms, similar results were obtained for binary mixtures of phenanthrene and tetralin ($\Delta R_F \approx 0.30-0.31$ for TLC compared with $\Delta R_F \approx 0.24$ for OPLC). It is readily apparent that the thin-layer chromatography separation of anthracene and tetralin is complete, whereas with OPLC it is only partial.

The working conditions employed in OPLC, i.e., a mobile-phase flow rate that resulted in shortening of the development time by a factor of two or three, did not result in improvement of the separation. In view of this, it must be

Table 3. Comparison of R_F values obtained by TLC and OPLC for different amounts of phenanthrene, tetralin, and their binary mixture

		Retardation factor, R_F	
Analyte	Concentration (C_m) (mol L^{-1})	TLC development velocity 1.81 mm min^{-1}	OPLC development velocity 4.00 mm min^{-1}
Phenanthrene	0.10	0.44	0.23
	0.20	0.45	0.22
Tetralin	2.0	0.78	0.43
Binary mixture	0.10:2.0	0.45	0.22
		0.76	0.46
	0.20:2.0	0.46	0.22
		0.76	0.46

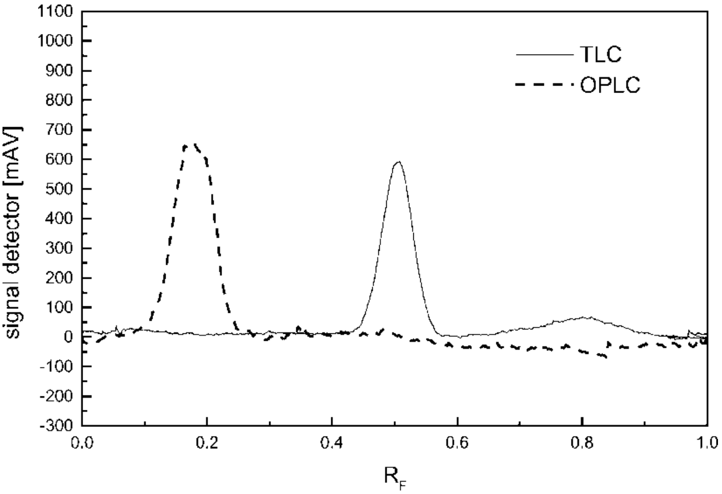


Figure 1. Comparison of the TLC and OPLC densitograms obtained from anthracene ($0.05\,\mathrm{mol\,L^{-1}}$). Silica gel 60 F_{254} was used as stationary phase and *n*-hexane as mobile phase. The volume of sample applied to the plate was always 1 μL. The mean velocities of *n*-hexane migration were, respectively, $1.81\,\mathrm{mm\,min^{-1}}$ for TLC and $4.00\,\mathrm{mm\,min^{-1}}$ for OPLC.

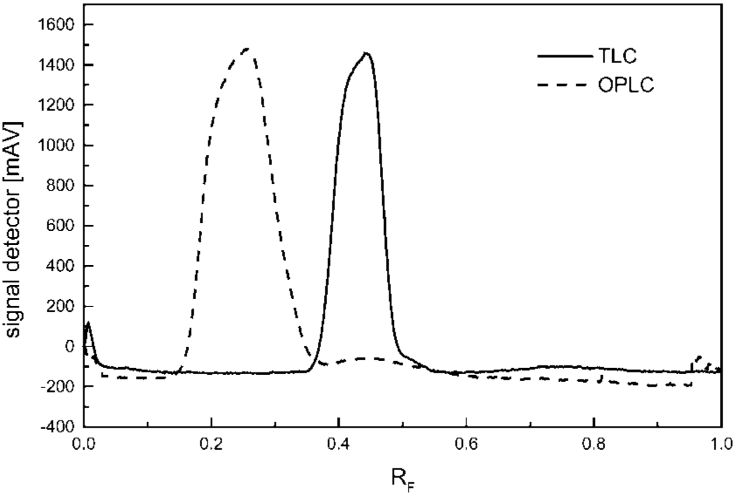


Figure 2. Comparison of the TLC and OPLC densitograms obtained from phenanthrene ($0.20\,\mathrm{mol\,L^{-1}}$). See Figure 1 for conditions.

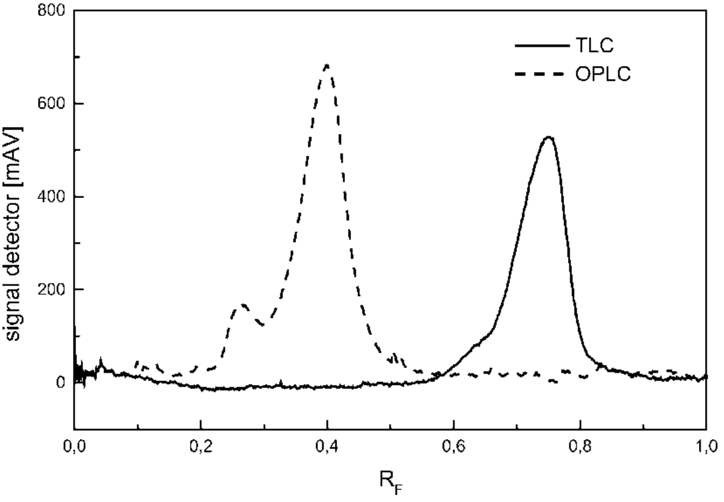


Figure 3. Comparison of the TLC and OPLC densitograms obtained from tetralin (2.0 mol L^{-1}). See Figure 1 for conditions.

stated that in our experiments the separation performance of TLC proved to be substantially better than that of OPLC. This phenomenon can be interpreted in one way or another. It seems that the enhanced flow rate characteristic of OPLC made the time needed for dissolution of the test analytes at the origin of the chromatographic plate even less compatible with the rate of eluent migration along the sorbent layer than it was in conventional TLC.

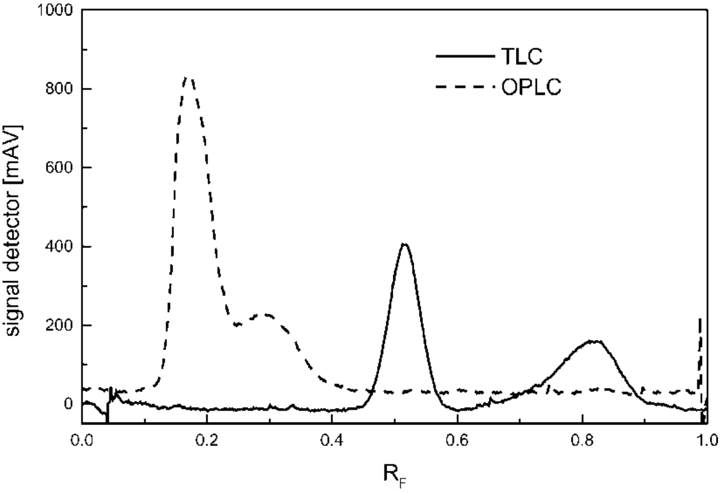


Figure 4. Comparison of TLC and OPLC separations of the binary mixture of anthracene (0.05 mol L^{-1}) and tetralin (2.0 mol L^{-1}). See Figure 1 for conditions.

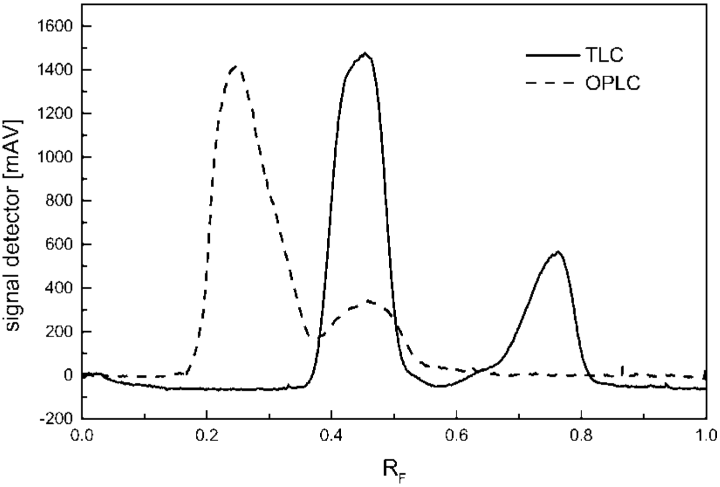


Figure 5. Comparison of TLC and OPLC separations of the binary mixture of phenanthrene (0.20 mol L^{-1}) and tetralin (2.0 mol L^{-1}). See Figure 1 for conditions.

Moreover, the enhanced pressure of the mobile phase in OPLC undoubtedly increased its penetration inside the silica pores, thus increasing the specific surface area of the sorbent, which resulted in more pronounced adsorption of the test analytes on the sorbent surface and, consequently, in the lower numerical values of the retardation factor, R_F, compared with TLC. From analysis of the data in Table 4, it seems that the second phenomenon prevails over a hypothetical decrease of the dissolution process. In OPLC, the mobile-phase velocity (1.2 mm min^{-1}) is a factor of 0.66 smaller than that in TLC (1.81 mm min^{-1}), and the R_F values obtained (e.g., 0.25 and 0.26 for phenanthrene) are approximately a factor of 0.6 smaller than those obtained by TLC (between 0.44 and 0.46 for the same analyte).

Table 4. Comparison of R_F values obtained by OPLC, at three different development velocities, for phenanthrene and for the constituents of the phenanthrene–tetralin binary mixture

		Retardation factor, R_F		
Analyte	Concentration (C_m) (mol L^{-1})	1.2 mm min^{-1}	4.0 mm min^{-1}	6.2 mm min^{-1}
Phenanthrene	0.10	0.25	0.23	0.19
Binary mixture	0.10:2.0	0.26	0.22	0.20
		0.50	0.46	0.36

Finally, it must be mentioned that the concentrations of the test analytes and the amounts applied were relatively high, and from their densitometrically recorded concentration profiles (particularly the back-tailing of the tetralin profile) it is apparent that chromatography was performed within the nonlinear range of the adsorption isotherm. Performing the experiments discussed in this paper, under this preparative layer chromatographic condition, certainly had an additional (although by no means crucial) impact on the results presented, more specifically on the numerical values of the retardation coefficient, R_F, and on the efficiency of separation of the test mixtures in both modes of planar chromatography.

CONCLUSIONS

It seems that the results presented in this paper can be explained in the following two, most probably complementary, ways:

The R_F values for different amounts of anthracene, phenanthrene, and tetralin chromatographed separately and as binary anthracene–tetralin and phenanthrene–tetralin mixtures are substantially lower in OPLC than in TLC. This difference is most probably because the higher the pressure of mobile phase the better is its penetration of the adsorbent's pores and, hence, the stronger is the adsorption.

The mobile phase flow rate in OPLC is evidently much (i.e., more than two to three times) higher than in TLC. This increased mobile phase flow rate in OPLC is less compatible with the rate of dissolution of the analytes at the origin than it is for the much slower capillary flow. Hence in OPLC (with much more rapid mobile-phase flow), the relative retardation of the analytes because of their slow dissolution is evidently greater than in ascending TLC, because of the action of capillary forces (and much slower mobile-phase flow).

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