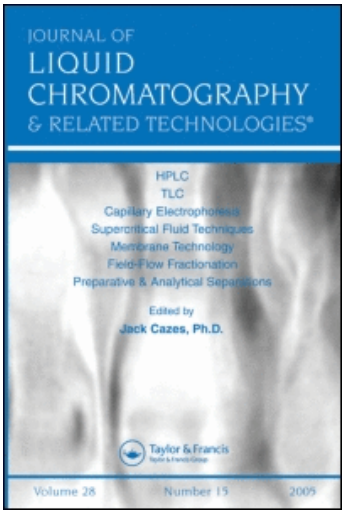

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Densitometric Comparison of the Performance of Stahl-Type and Sandwich-Type Planar Chromatographic Chambers

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ABSTRACT

Over the years, conventional Stahl-type thin-layer chromatography (TLC) chambers (i.e., N-chambers) have attracted criticism for more than one reason. For example, practitioners have questioned the value of the time-consuming procedure of saturating these chambers with mobile-phase vapor, and those who focus on the theory of planar chromatography, have complained that the well-known stepwise diminishing of the rate of capillary flow of the mobile phase in the ascending mode makes computational optimization of solute retention more difficult and less accurate than it could be at constant flow. Everybody also readily agrees that mobile phase consumption is much higher in N-chambers than in sandwich

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chambers (S-chambers). The economic superiority of S-chambers cannot, however, be regarded as a decisive factor when it comes to making a choice of the TLC equipment, because physicochemical aspects of the chromatographic process are more important. In the work discussed in this paper, we compared the N-type and S-type chambers in terms of mobile phase flow rate, the R_F values obtained, and the densitometrically established concentration profiles of the test analytes. For this comparison, we selected several phenyl-substituted alcohols and acids, low-activity adsorbents (cellulose powder and chromatographic paper), and low-polarity solvents (*n*-octane and decalin) as the components of the chromatographic systems investigated. The overall conclusion from the results obtained is that the physicochemical performance of the S-chamber is comparable with that of the N-chamber, and the only indisputable superiority of the S-chamber is the more economical use of the mobile phases.

Key Words: Planar chromatography; Densitometry; Concentration profiles of bands; Microcrystalline cellulose; Chromatographic paper; Stahl-type chamber (i.e., N-chamber); Sandwich-type chamber (i.e., S-chamber).

INTRODUCTION

Planar chromatography performed in conventional Stahl-type chambers (N-chambers) is regarded as inferior to column chromatography, because the stationary phase (either chromatographic paper or thin layer) is used in the form of an "open bed." There is awareness that the "closed bed" of stationary phase contained in chromatographic columns eliminates a number of physicochemical shortcomings inherent in the planar mode.

For this reason, the development of planar chromatography has sometimes consisted of imitating working conditions typical of column chromatography, in the first instance by converting the open bed of stationary phase to a less open bed. The simplest way of achieving this goal was to introduce Sandwich-type chambers (S-chambers), in which the void volume surrounding the plate (or the chromatographic paper) is much smaller than in the N-chambers.

Thorough comparison of the N-type and the S-type chambers in terms of the quality of the respective chromatographic bands is still pending. One reason is the difficult choice of reliable analytical tools and of adequate physicochemical criteria for such a comparison. It seems, however, that with the increasing popularity of densitometry, the lack of such a thorough comparison can no longer be excused.

The aim of the work discussed in this paper, was to compare retention in N- and S-type chromatographic chambers. As criteria for our comparison, we

selected the mobile phase flow rate, R_F values of the test analytes used, and the densitometrically established concentration profiles. We selected analytes (i.e., the alcohols and acids) able to interact effectively by hydrogen bonding and low-activity/low-polarity chromatographic systems, to minimize interference of the stationary and mobile phases with the self-association of the test analytes. The practical usefulness of similar chromatographic systems has been successfully demonstrated in earlier work.[1–3] The results obtained and the conclusions drawn, are presented in the forthcoming sections of this paper.

EXPERIMENTAL

Test Solutes

Selected Alcohols

In this study, we used solutions of 4-phenyl-1-butanol (0.5, 1.0, 1.5, and 2.0 mol L^{-1}) and 5-phenyl-1-pentanol (0.25, 0.5, 0.75, 1.0, 1.5, and 2.0 mol L^{-1}) in carbon tetrachloride. Both alcohols were analytical-grade products manufactured by Merck KGaA (Darmstadt, Germany).

Selected Acids

We used solutions of 3-phenylpropionic acid (0.2, 0.3, 0.4, and 0.5 mol L^{-1}), 2-phenylbutyric acid (0.2, 0.3, 0.4, and 0.5 mol L^{-1}), and 4-phenylbutyric acid (0.2, 0.3., 0.4, 0.5, 0.75, and 1.0 mol L^{-1}) in 2-propanol. All these acids were analytical-grade products manufactured by Merck.

Thin-Layer Chromatography

Thin-layer chromatography (TLC) was performed on glass-backed plates precoated with microcrystalline cellulose (10 cm × 20 cm; Merck, cat. #1.05730). After spotting the solutions of the alcohols and acids (3 μL), the layers were developed with *n*-octane (alcohols) or decalin (acids) to a distance of 15 cm, at ambient temperature, in the two types of chromatographic chambers: (i) ascending mode in a conventional N-chamber (Camag, Muttenz, Switzerland) previously saturated with mobile phase vapor for 20 min; and (ii) horizontal mode in a DS-II S-chamber (Chromdes, Lublin, Poland) saturated with mobile phase vapors in the same way. The volume of mobile phase used in the N-chamber was 20 mL; in the S-chamber it was 6 mL. After development, the chromatograms were carefully dried with a hairdryer.

Paper Chromatography

Paper chromatography was performed on Whatman-1 and Whatman-3 chromatographic paper (Whatman, Maidstone, UK) cut into 10 cm × 20 cm rectangles. After spotting the solutions of the alcohols and acids (5 μL), the paper sheets were developed with *n*-octane (alcohols) or decalin (acids) to a distance of 15 cm, at ambient temperature, in the two types of chromatographic chamber: (i) ascending mode in a conventional N-chamber previously saturated for 20 min; and (ii) horizontal mode in a DS-II S-chamber, saturated in the same way. Again, the volumes of mobile phase used in the N-type and S-type chambers were 20 mL and 6 mL, respectively. After development, the chromatograms were carefully dried with a hairdryer.

Densitometric Detection

Densitometric detection of developed zones was performed with the Desaga (Heidelberg, Germany) model CD 60 densitometer equipped with the Windows-compatible ProQuant program package. Concentration profiles were recorded in ultraviolet (UV) light (in the reflectance mode) at a wavelength of 260 nm; the dimensions of the rectangular light beam were 0.4 mm width and 0.02 mm height.

RESULTS AND DISCUSSION

The results obtained are shown in Tables 1–7 and the selected densitograms are given in Figs. 1–4. Because of the importance of the comparison discussed, we decided to present our data in numerical form (Tables 1–7), thus enabling detailed inspection of the respective R_F values and the concentration profile baseline widths, *w*. The numerical values given in the tables are mean values calculated from results of replicate analyses (the number of replicates, *n*, was usually between 3 and 10). The reproducibility of the results (i.e., R_F and *w* values) obtained by use of the N- and S-chambers, was of exactly the same order (despite the moderate reproducibility of results from S-chambers articulated elsewhere[4]). It was an ultimate goal of this study to compare the simplest physicochemical characteristics of solute retention in planar chromatography (the two parameters R_F and *w*, and the respective concentration profiles) for the N-type and S-type chromatographic chambers. The results obtained revealed several general trends, although with occasional exceptions.

In general, the higher the velocity of development of the chromatogram, the lower the *w* value of the chromatographic band. This regularity is

Table 1. Numerical values of the R_F coefficients and concentration profile widths (w) of chromatographic bands of 4-phenyl-1-butanol developed with *n*-octane on whatman-3 chromatographic paper.

Concentration ($mol\,L^{-1}$)	N-chamber (mean development velocity: 5.16 $mm\,min^{-1}$)		S-chamber (mean development velocity: 6.23 $mm\,min^{-1}$)	
	R_F	w (mm)	R_F	w (mm)
0.5	0.95	14.0	0.95	13.5
1.0	0.92	24.0	0.89	20.2
1.5	0.88	31.5	0.82	27.9
2.0	0.83	38.6	0.78	38.5

readily understandable if we reflect on the relationship between chromatogram development time and the impact of diffusion (the shorter the development time, the less pronounced is diffusion and the lower the peak width).

With all of the test solutes considered, the smaller the amount of sample developed (i.e., the lower the concentration of the spotted solution), the closer to each another become the pairs of the R_F values, irrespective of the chromatographic chamber used (occasionally the values are even identical). This is because the smaller the amount of sample developed, the greater is the probability of partitioning of the analyte between the stationary and mobile phases in the linear range of the adsorption isotherm. Evidently, differences between the construction and use of the two types of chambers differentiates

Table 2. Numerical values of the R_F coefficients and concentration profile widths (w) of chromatographic bands of 5-phenyl-1-pentanol developed with *n*-octane on whatman-1 chromatographic paper.

Concentration ($mol\,L^{-1}$)	N-chamber (mean development velocity: 4.71 $mm\,min^{-1}$)		S-chamber (mean development velocity: 5.95 $mm\,min^{-1}$)	
	R_F	w (mm)	R_F	w (mm)
0.25	0.97	14.2	0.95	15.4
0.50	0.89	20.8	0.92	20.4
0.75	0.82	28.4	0.89	24.3
1.00	0.79	35.6	0.82	31.1

Table 3. Numerical values of the R_F coefficients and concentration profile widths (w) of chromatographic bands of 5-phenyl-1-pentanol developed with *n*-octane on whatman-3 chromatographic paper.

Concentration ($mol\,L^{-1}$)	N-chamber (mean development velocity: $5.61\,mm\,min^{-1}$)		S-chamber (mean development velocity: $6.23\,mm\,min^{-1}$)	
	R_F	w (mm)	R_F	w (mm)
0.25	—	—	0.96	14.1
0.50	0.90	19.8	0.95	18.0
0.75	—	—	0.92	25.3
1.00	0.83	30.8	0.89	30.2
1.50	0.77	41.9	0.82	37.0
2.00	0.75	45.4	0.80	39.3

the process of retention more effectively in the non-linear range of the isotherm, rather than the linear range.

The velocity of development of paper chromatograms in the S-chamber is visibly higher than in the N-chamber. It can be rightly speculated that, because of the relatively large pores in chromatographic paper, capillary forces in the ascending (i.e., vertical) development mode are substantially opposed by gravitational forces and, hence, the development process in a N-chamber undergoes gradually increasing retardation. Development of chromatograms in the horizontal mode cannot be hindered in this way and, therefore, the development process in the S-chamber proceeds unretarded by gravity.

The opposite trend is observed when developing thin-layer chromatograms. The velocity of development of chromatograms in the N-chamber is

Table 4. Numerical values of the R_F coefficients and concentration profile widths (w) of chromatographic bands of 5-phenyl-1-pentanol developed with *n*-octane on microcrystalline cellulose-coated glass plates.

Concentration ($mol\,L^{-1}$)	N-chamber (mean development velocity: $2.06\,mm\,min^{-1}$)		S-chamber (mean development velocity: $1.67\,mm\,min^{-1}$)	
	R_F	w (mm)	R_F	w (mm)
0.25	0.94	12.5	0.86	14.4
0.50	0.82	15.9	0.81	22.4
0.75	—	—	0.75	32.7
1.00	0.76	20.4	0.71	36.2

Table 5. Numerical values of the R_F coefficients and concentration profile widths (w) of chromatographic bands of 3-phenylpropionic acid developed with decalin on microcrystalline cellulose-coated glass plates.

Concentration ($mol\,L^{-1}$)	N-chamber (mean development velocity: $0.77\,mm\,min^{-1}$)		S-chamber (mean development velocity: $0.73\,mm\,min^{-1}$)	
	R_F	w (mm)	R_F	w (mm)
0.2	0.93	8.3	0.94	11.4
0.3	0.91	9.4	0.90	17.8
0.4	0.89	9.7	0.86	20.8
0.5	0.79	17.7	0.78	26.0

Table 6. Numerical values of the R_F coefficients and concentration profile widths (w) of chromatographic bands of 2-phenylbutyric acid developed with decalin on microcrystalline cellulose-coated glass plates.

Concentration ($mol\,L^{-1}$)	N-chamber (mean development velocity: $0.75\,mm\,min^{-1}$)		S-chamber (mean development velocity: $0.73\,mm\,min^{-1}$)	
	R_F	w (mm)	R_F	w (mm)
0.2	0.91	7.9	0.93	10.4
0.3	0.86	12.8	0.90	13.5
0.4	—	—	0.86	14.6
0.5	0.80	15.8	0.84	13.7

Table 7. Numerical values of the R_F coefficients and concentration profile widths (w) of chromatographic bands of 4-phenylbutyric acid developed with decalin on microcrystalline cellulose-coated glass plates.

Concentration ($mol\,L^{-1}$)	N-chamber (mean development velocity: $0.75\,mm\,min^{-1}$)		S-chamber (mean development velocity: $0.73\,mm\,min^{-1}$)	
	R_F	w (mm)	R_F	w (mm)
0.2	0.90	12.5	0.90	13.5
0.3	0.87	14.9	0.86	19.8
0.4	0.85	19.0	0.80	40.6
0.5	0.82	25.0	0.79	43.8
1.0	0.68	54.7	0.64	67.7

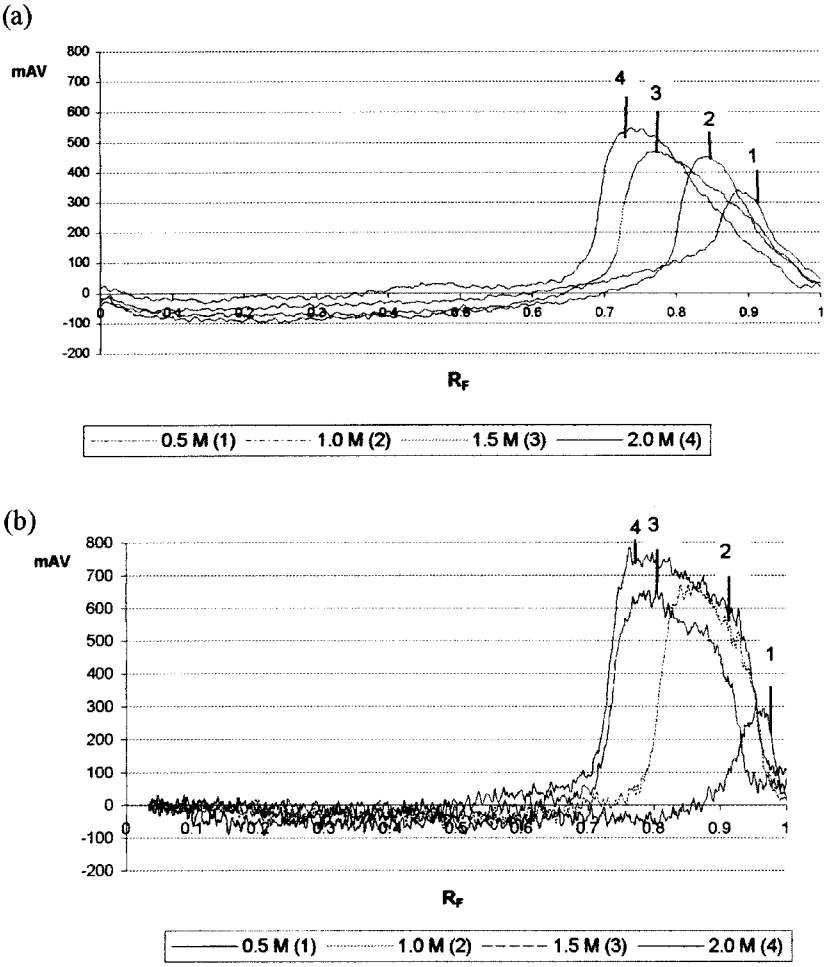

Figure 1. Comparison of the concentration profiles of 5-phenyl-1-pentanol developed with *n*-octane on whatman-3 chromatographic paper in (a) N-chamber and (b) S-chamber (showing the dependence on the concentrations of the test analyte solutions).

somewhat (sometimes insignificantly) higher than in the S-chamber, which obviously cannot be attributed to the impact of gravity. Because the diameters of the pores in microcrystalline cellulose layers are usually much smaller than in chromatographic papers, capillary forces are much more pronounced and far less susceptible to gravity. Feeding of horizontally developing chromatograms with mobile phase is, however, substantially different from feeding

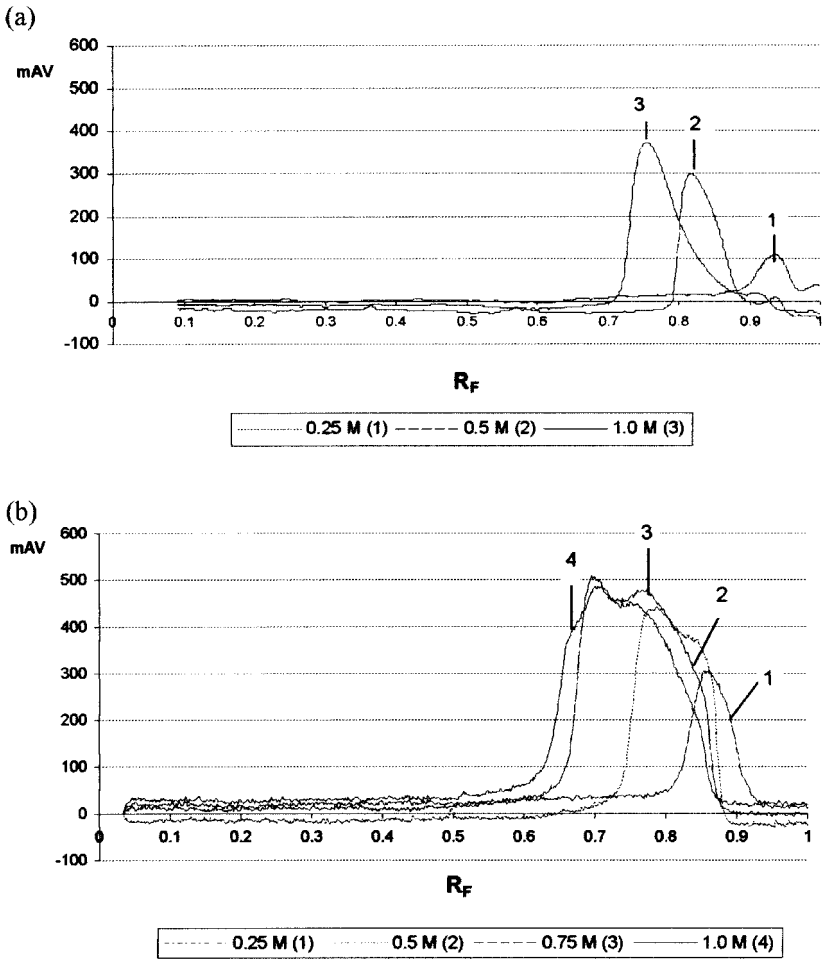

Figure 2. Comparison of the concentration profiles of 5-phenyl-1-pentanol developed with *n*-octane on microcrystalline cellulose in (a) N-chamber and (b) S-chamber (showing the dependence on the concentrations of the test analyte solutions).

them in the vertical position. In the N-chamber, the lower edge of the vertically placed chromatogram (either thin-layer or paper) is dipped in the mobile phase, whereas in the S-chamber, the edge of the horizontally placed chromatogram is fed with mobile phase from a trough through a slit, by capillary action. It seems, that the evident hydrodynamic difference in the mode of

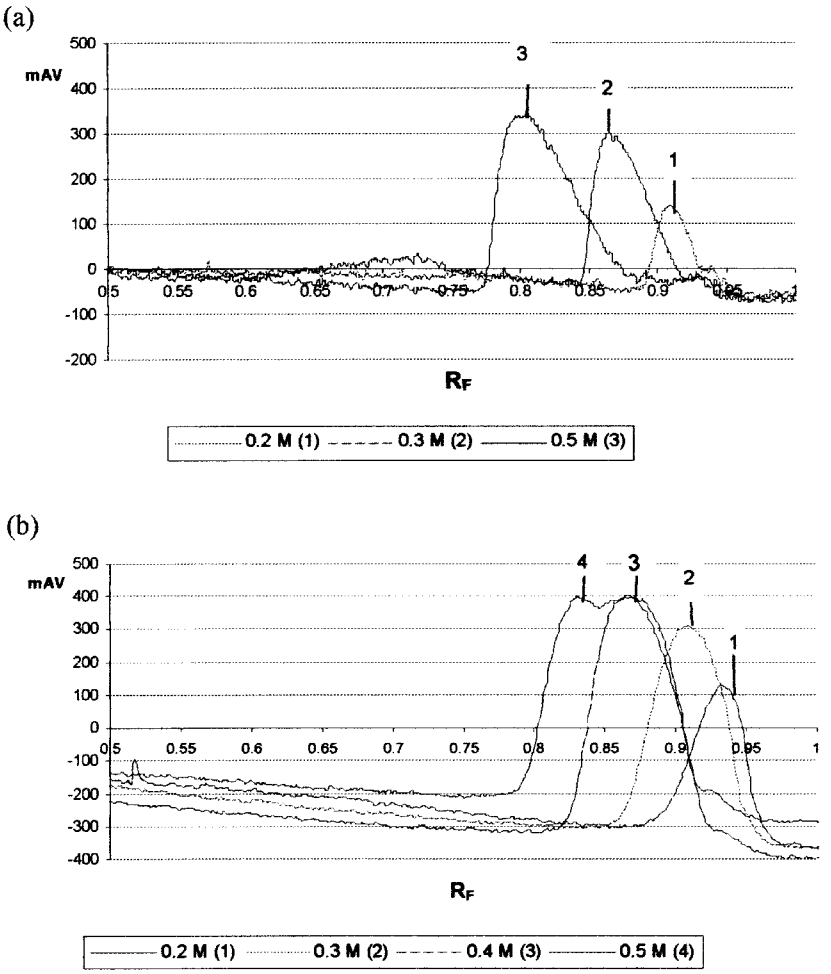

Figure 3. Comparison of the concentration profiles of 2-phenylbutyric acid developed with decalin on microcrystalline cellulose-coated glass plates in (a) N-chamber and (b) S-chamber (showing the dependence on the concentrations of the test analyte solutions).

feeding negatively affects development of thin-layer chromatographic plates (by prolonging the development time), whereas it has no perceptible effect on the development of paper chromatograms.

Selected densitograms obtained from chromatograms developed in N-type and S-type chambers are compared in Figs. 1–4. For all the analytes

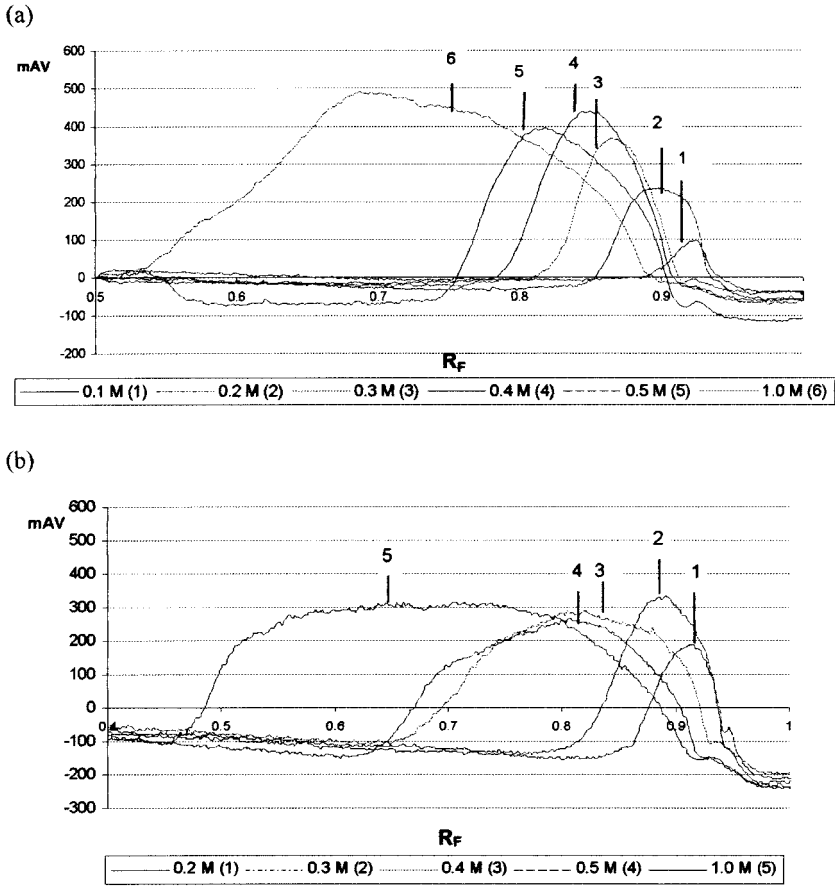

Figure 4. Comparison of the concentration profiles of 4-phenylbutyric acid developed with decalin on microcrystalline cellulose-coated glass plates in (a) N-chamber and (b) S- chamber (showing the dependence on the concentrations of the test analyte solutions).

and the two chromatographic chambers investigated, the lengthwise concentration profiles of the chromatographic bands resemble anti-Langmuir-type peak profiles (the best examples of anti-Langmuir profiles can be seen in Fig. 2). These results persuasively suggest that lateral interactions play the predominant role in the formation of these concentration profiles.

Other important information contained in these results is that the deterioration of the lengthwise concentration profiles of chromatograms developed in

the S-chamber (particularly for the largest amounts of the analytes) is worse than for their counterparts obtained in the N-chamber. In general, the diffusive parts of these peaks are less emphasized and their triangular anti-Langmuir shape is distorted, resulting in a trapezoidal-like shape (see, for example, Fig. 4). Occasionally (e.g., Fig. 3), the multi-peak profiles for the highest concentrations of the analytes developed in the S-chamber give evidence of a limited hydrodynamic stability of the eluent flow in this chamber. The reason for these multi-peak profiles is most probably the hydrodynamic instability of the eluent flow in the S-chamber.

From these results, the overall conclusions are that the physicochemical performance of the S-chamber is comparable with (and occasionally worse than) that of the N-chamber, and that the only indisputable superiority of the S-chamber over the N-chamber consists in more economical use of the mobile phase.

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