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Comparison of a DS-horizontal chamber and a new S_{min} chamber in TLC separation

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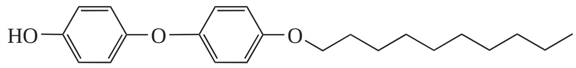
The chromatographic characteristics of a popular DS-horizontal chamber (Chromdes, Poland) and an S_{min} chamber with a minimum gas volume (Institute of Petrochemical Synthesis of the Russian Academy of Sciences) have been experimentally evaluated.

Since traditional TLC employs volatile compounds as mobile phases, separation in planar TLC is carried out in special closed chambers; the chambers most popular in practical chromatography include N chambers (internal gas volume of ~1000 cm^3) and S chambers with smaller gas volumes.[1–5]

Recently, the Institute of Petrochemical Synthesis of the Russian Academy of Sciences introduced a new S_{min} chamber whose distinctive features include a very small distance d between the adsorption layer of the chamber plate and the chamber cover (d = 0.1–0.2 mm) and hence a much smaller gas volume. The S_{min} chamber is characterised by fast and efficient separation.[6–9]

To access the practical value of the S_{min} chamber, it appeared necessary to compare it with commercially available S chamber. We chose a DS-horizontal chamber (Poland), which was successfully used for many years as the reference one.[†] Note that the first data obtained with a DS-horizontal chamber were published in 1990.[10]

We compared the chromatographic chambers (DS and S_{min}) based on the separation results of ethers, vitamins and dyes. The experimental studies of the chromatographic chambers were performed on the same day using both an S_{min} chamber with vertical and horizontal arrangement of the plate and a horizontal



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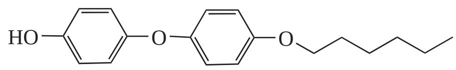

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DS chamber (Chromdes, Poland). Note that the latter one was successfully used to separate compounds of various classes.[†]

The separation of various mixtures by the same chromatographic procedure was carried out in parallel using, first, a DS-horizontal chamber[†] presaturated for 10–20 min with mobile phase vapours until equilibration in the chamber (the reservoir was filled with ~8 cm^3 of the mobile phase) and second, an S_{min} chamber[6,7] whose reservoir contained 3 cm^3 of the mobile phase for feeding the plate. The main difference of the chambers used was that they had different characteristic distances d between the sorption layer of the TLC plate and the cover glass (chamber lid): 2 mm in the DS chamber (Chromdes) and 0.1 mm in the S_{min} chamber.[5]

Table 1 Chromatographic parameters of components of compounds **1** and **2** being separated in DS and S_{min} chambers.

Compounds being separated	Mobile phase	No. of component	R_f[a]		Rs		Duration		
			DS chamber	S_{min} chamber	DS chamber	S_{min} chamber	DS chamber, t_1/min	S_{min} chamber, t_2/min	$\rho_t = (t_2 - t_1)/t_2$
Reaction mixture formed in the preparation of ether **1**	THF–toluene (1:20)	1	0.06	0.04	$Rs_{1,2}$ = 14.7	$Rs_{1,2}$ = 13.7	25	30	0.17
		2	**0.33**	**0.31**	$Rs_{2,3}$ = 15.3	$Rs_{2,3}$ = 15.1			
		3	0.91	0.97	Rs_{mean} = 15.0	Rs_{mean} = 15.5			
	$CHCl_3$	1	0.07	0.06	$Rs_{1,2}$ = 8.7	$Rs_{1,2}$ = 8.8	18	21	0.14
		2	**0.24**	**0.25**	$Rs_{2,3}$ = 10	$Rs_{2,3}$ = 11			
		3	0.85	0.85	Rs_{mean} = 9.35	Rs_{mean} = 9.9			
Reaction mixture formed in the preparation of ether **2**	THF–toluene (1:20)	1	0.06	0.04	$Rs_{1,2}$ = 14	$Rs_{1,2}$ = 14	25	30	0.17
		2	**0.33**	**0.29**	$Rs_{2,3}$ = 15	$Rs_{2,3}$ = 18			
		3	0.81	0.90	Rs_{mean} = 14.5	Rs_{mean} = 16			
	$CHCl_3$	1	0.07	0.06	$Rs_{1,2}$ = 2.4	$Rs_{1,2}$ = 2.3	18	21	0.14
		2	0.17	0.14	$Rs_{2,3}$ = 3.6	$Rs_{2,3}$ = 6.4			
		3	**0.36**	0.27	$Rs_{3,4}$ = 2.0	$Rs_{3,4}$ = 3.0			
		4	0.43	**0.36**	$Rs_{4,5}$ = 6.9	$Rs_{4,5}$ = 4.8			
		5	0.78	0.78	$Rs_{5,6}$ = 2.7	$Rs_{5,6}$ = 2.7			
		6	0.91	0.91	Rs_{mean} = 3.5	Rs_{mean} = 3.8			

[a] The marked components correspond to pure ethers, whereas other ones are impurities.

[†] See *www.chromdes.com*.

All separations were carried out using Silica gel 60 F_{254} plates (Merck, Germany) on a glass support with an adsorbent layer thickness of 0.25 mm. The mobile phase components were chloroform, ethanol, butan-1-ol, propan-2-ol, tetrahydrofuran, toluene and methanol (Merck, Germany). The following main characteristics were determined: visualization times (plate wetting time) *t*, retention times of test compounds R_f and separation factors Rs. UV detection was performed at λ = 366 and 258 nm.

Ethers **1** and **2** were used as stationary phases to compare separation in S chambers. Table 1 shows the results of tests using (1) two-component THF/toluene (1:20, v/v) and (2) one-component $CHCl_3$ mobile phases.

When analysing the mean values in Table 1, it should be taken into account that, in terms of the mean $\bar{R}_s$, which is one of the main characteristics of the chromatographic method, the S_{min} chamber has a somewhat better resolution, however, the duration of the chromatographic process is longer for this chamber if the plate is arranged vertically. It is reasonable that a longer time is required to wet the plate in the S_{min} chamber (by 5 min if a THF–toluene mixture is used and by 3 min if chloroform is used as the mobile phase), since the separation was carried out using a vertical arrangement of the plate, where gravity decreased the plate wetting time.

The use of various mobile phases for the separation of multi-component vitamin mixtures has been reported.[11–23] However, we achieved the best separation of the maximally possible number of vitamins[‡] in a sample using procedures reported previously[18,22] (Table 2).

Not all of the vitamins in the test mixture could be separated. However, two-dimensional separation using the S_{min} chamber tooks 15% longer. Similar mobility values were obtained for both chambers being compared.

Table 2 Chromatographic parameters R_f of the separated compounds of 'Falvit' product obtained in DS and S_{min} chambers.

Vitamin	Elution 1, mobile phase: $CHCl_3$–EtOH–H_2O (83:16:1, by volume)		Elution 2, mobile phase: BuOH–Pr^iOH–H_2O (5:3.5:5, by volume)	
	DS chamber (19.5 min)	S_{min} chamber (22.5 min)	DS chamber (115 min)	S_{min} chamber (132 min)
C	0.40	0.41	0.73	0.73
PP	0	0	0	0
E	0.98	1.00	0.98	1.00
B_5	0	0	0	0
B_6	0.20	0.22	0.64	0.65
B_2	0.23	0.24	0.67	0.67
B_1	0.01	0.02	0.03	0.04
A	0.98	1.00	0.98	1.00
H	0	0	0	0
D_3	0.23	0.24	0.01	0.02
B_{12}	0.42	0.41	0.67	0.67
B_9	0.24	0.24	0	0

[‡] 'Falvit' multivitamins (Przedsiębiorstwo Farmaceutyczne Jelfa SA) with the following composition were analysed: vitamin C, 60 mg; vitamin PP, 18 mg; vitamin E, 10 mg; vitamin B_5, 6 mg; vitamin B_6, 2 mg; vitamin B_2, 1.6 mg; vitamin B_1, 1.4 mg; vitamin A, 800 μg; vitamin H, 150 μg; vitamin D_3, 5 μg; vitamin B_{12}, 1 μg; vitamin B_9, 200 μg.

The following samples of single vitamins were used to interpret the chromatographic zones: vitamin E [α-tocopherol acetate and α-tocopherol (Sigma-Aldrich, USA)], vitamin A (Sigma-Aldrich, USA), vitamin B_1 (Polfarmex, Poland), vitamin B_2 (Zestov Familijny hecpharma), vitamin PP (Polfarmex, Poland), vitamin B_6 (Polfarmex, Poland), vitamin C (Nature's Sunshine Products, USA), vitamin B_9 (Polfarmex, Poland), vitamin B_{12} (Polfarmex, Poland), vitamin D_3 (Metagenics, USA), vitamin H (Hangzhou Hetd Industry Co., China) and vitamin B_5 (Solgar, USA).

Table 3 Chromatographic characteristics of dye mixture separation in the horizontal DS chamber and vertical S_{min} chamber (mobile phase, $CHCl_3$).[a]

Compounds being separated	R_f		Rs	
	DS chamber (horizontal)	S_{min} chamber (vertical)	DS chamber (horizontal)	S_{min} chamber (vertical)
Sudan black (1)	0.04	0.04	$Rs_{1,2}$ = 1.3	$Rs_{1,2}$ = 1.3
Artisil blue (2)	0.19	0.19		
Sudan yellow (3)	0.31	0.31	$Rs_{2,3}$ = 1.2	$Rs_{2,3}$ = 1.2
Fatty orange (4)	0.50	0.50	$Rs_{3,4}$ = 1.5	$Rs_{3,4}$ = 1.5
Fat red 7B (5)	0.86	0.86	$Rs_{4,5}$ = 2.5	$Rs_{4,5}$ = 2.5

[a] Duration was 16 min for the DS chamber and 19.4 min for the S_{min} chamber.

Table 3 summarizes the results obtained in the separation of Test Dye Mixture II (Camag) with chloroform as a mobile phase. Judging from the R_f values, the separation results are identical for the horizontal DS chamber and the vertical S_{min} chamber, but separation takes 20% longer (3.4 min) for the S_{min} chamber. In view of this, it appeared feasible to test an S_{min} chamber with the horizontal movement of a mobile phase (Figure 1).

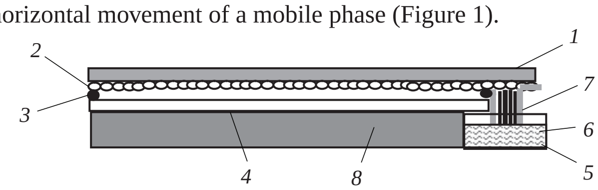

Figure 1 Schematic diagram of an S_{min} chamber with horizontal plate arrangement: (*1*) TLC plate support, (*2*) plate adsorption layer, (*3*) restricting elements (*d* = 0.1 mm), (*4*) cover glass of the chamber, (*5*) mobile phase source, (*6*) mobile phase, (*7*) device for feeding the plate with the mobile phase (filter paper fixed between aluminium sheets) and (*8*) chamber support.

As mentioned above, the characteristic distances were *d* = 2 mm for the DS chamber and *d* = 0.1 mm for the S_{min} chamber; the method for feeding the plate with the mobile phase also differed. Note that the DS chamber was pre-saturated with mobile phase vapour for 15–20 min before the chromatographic process. The chromatographic separation results are shown in Table 4.

The use of an S_{min} chamber favours an increase in the mobility of compounds which are retained most strongly (R_f < 0.1). This is explained by the fact that the implementation of the chromatographic process involved pre-saturation (~10%) of the DS chamber with mobile phase vapour. However, the analysis duration is the same in both cases, which may also be due to the fact that, before the experiment, the gas phase of the DS chamber contained mobile phase vapour adsorbed by the plate layer, thus favouring plate wetting by the mobile phase.

Additional experiments to compare an S_{min} chamber with horizontal and vertical plate arrangements have shown that the difference in analysis duration ranges from 10% (for ethanol) to 16% (for toluene, methanol); hence, the dependence of the duration of plate wetting by the mobile phase on *d* is not so pronounced for a horizontal S chamber as for a vertical one.

Table 4 Chromatographic characteristics of dye mixture separation in the horizontal DS and S_{min} chambers (mobile phase, $CHCl_3$).[a]

Compounds being separated	R_f		Rs	
	DS chamber (horizontal)	S_{min} chamber (horizontal)	DS chamber (horizontal)	S_{min} chamber (horizontal)
Sudan black (1)	0.04	0.10	$Rs_{1,2}$ = 1.3	$Rs_{1,2}$ = 0.9
Artisil blue (2)	0.19	0.19		
Sudan yellow (3)	0.31	0.31	$Rs_{2,3}$ = 1.2	$Rs_{2,3}$ = 1.2
Fatty orange (4)	0.50	0.50	$Rs_{3,4}$ = 1.5	$Rs_{3,4}$ = 1.5
Fat red 7B (5)	0.86	0.86	$Rs_{4,5}$ = 2.5	$Rs_{4,5}$ = 2.5

[a] Duration was 16 min for the DS chamber and 15.4 min for the S_{min} chamber.

Thus, the experimental comparison of a commercial DS chamber (Chromdes, Poland), and the S_{min} chamber developed at the Institute of Petrochemical Synthesis has demonstrated the similarity of the chromatographic characteristics obtained in the separation of the same mixture in both chambers. Meantime, the analysis using the S_{min} chamber in ascending mode took longer (at most by 15%). This difference is due to the fact that, first, the plate in the horizontal DS chamber is pre-saturated by ~10% with mobile phase vapours, resulting in occurrence of 'saturated' TLC, and second, the resisting forces are much smaller in horizontal motion of the mobile phase than in an ascending mobile phase stream.

Taking into account the high separation efficiency, simplicity and low cost of the S_{min} chamber (~500 Euro[†] for a DS chamber against ~25 Euro for an S_{min} chamber), we believe that the S_{min} chamber can be recommended for analytical practice along with the commercially available horizontal DS chamber.

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