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The use of an S_{min} chamber in two- and multidimensional thin-layer chromatography

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The applications of an S_{min} chamber, which was proposed recently, and a traditional N chamber (without pre-saturation) for the implementation of multidimensional separations have been compared; the S_{min} chamber has been found preferable in terms of the main chromatographic parameters (analysis time, efficiency and separating ability).

Current analytical chemistry requires methods that provide a maximum amount of data as rapidly as possible.[1] Multidimensional planar chromatography is such a method. Multidimensional thin-layer chromatography (TLC) has been extensively used: ~10% publications on TLC deal with *n*-dimensional planar chromatography. To improve the speed and efficiency of multidimensional TLC, we proposed a new S_{min} chamber[2] characterized by a minimum distance between the plate absorption layer and the chamber wall (d = 0.1–0.2 mm), and hence a small volume of the chamber gas space (for a 10×10 cm plate, the chamber volume is 1–2 cm^3). Chambers with minimized gas volumes exhibit better chromatographic characteristics in comparison with the most popular N chambers, the gas space volume in which exceeds 1000 cm^3. The N chambers are most widely used for separation (in more than 60% published works). However, chamber presaturation, which improves the reproducibility of results, is performed only in rare cases. Therefore, it seemed expedient to compare the most popular TLC chambers with the chambers we believe to be optimal. The new S_{min} chamber version was previously employed for only one-dimensional separations, but it seemed reasonable to use it for multidimensional separations.

Previously, Geiss and Schlitt[3] used an S chamber to implement two-dimensional TLC where the distance (d) between the adsorption layer and the chamber wall was 1 mm. Fehriger and Westfall[4] also separated dichlorodiphenyltrichloromethylmethane (DDT) derivatives in an S chamber, but the distance d was 1.5 mm. Since the duration and performance parameters were virtually the same at d = 1.0–4.0 mm,[2] no principal differences other than reproducibility were observed between S and N chambers.

The use of an S_{min} chamber[2] with improved visualization opens up new prospects in the practical application of *n*-dimensional TLC.

In our experiments,[†] to estimate the separation quality[7,8] of the test mixtures, the following chromatographic parameters were determined (ascending elution mode; 24 °C): the separation efficiency (H/μm),[9] the migration of separated compounds (R_f), the resolution factor (R_s), the error of measurement and the total duration of chromatographic *n*-dimensional separation processes in various chambers.

In order to assess the advantages and disadvantages of an S_{min} chamber in two-dimensional separation, the process was also performed in an N chamber. Note that the separation of dyes was accompanied by adding 4 cm^3 of a mobile phase to both the N chamber and the feeding source of the S chamber. Table 1 summarizes the results.

Table 1 Characteristics of the mobile phase front motion along the plate in two-dimensional TLC (silica gel plates, Merck).

Distance	Duration t/min			
	Separation in an N chamber		Separation in an S_{min} chamber (d = 0.1 mm)	
	Direction 1, acetone as the mobile phase	Direction 2, ethyl acetate as the mobile phase	Direction 1, acetone as the mobile phase	Direction 2, ethyl acetate as the mobile phase
1 cm	0.5	0.5	0.4	0.5
2 cm	1.9	2.4	1.3	1.5
3 cm	3.4	3.8	2.5	3.0
4 cm	5.0	5.9	4.2	5.1
5 cm	8.3	9.8	6.4	7.6
6 cm	11.7	12.8	9.1	10.7
7 cm	15.1	16.3	12.7	14.2
8 cm	18.9	24.3	16.1	19.8
	Overall analysis duration, min			
	43.3±0.5		35.9±0.5	

Separation on 10×10 cm plates was accompanied by a gradual decrease in the velocity as the plate was wetted by the mobile phase; furthermore, the velocity and wetting duration were considerably affected by the type of the chamber. As shown recently,[2] the fastest separation was achieved in the S_{min} chamber. Thus, two-dimensional chromatography in an S_{min} chamber allowed us to shorten the chromatographic separation time by 17%.

Table 2 shows experimental data on the mobility (R_f) of the chromatographed compounds depending on the separation conditions.

[†] TLC plates: silica gel (Merck, Germany) on a glass support (10×10 cm; adsorption layer thickness, 0.25 mm) and PTLC-AF-V-UF (IMID, Russia) with a silica gel adsorption layer (0.19–0.20 mm thick) on an aluminum support. The mobile phases were acetone, ethyl acetate, toluene–ethanol (6.5:3.5, v/v) and ethanol–acetic acid (9:1, v/v). The mixtures of Brilliant green, rhodamine C, erythrosin, neutral red, methyl red and orange G were test materials for two-dimensional TLC. The mixtures for four-dimensional TLC[5] contained Ciba-F II, Indophenol, Ariabel red, Sudan blue, Sudan IV, dimethylaminoazobenzene, Methyl orange, Bromophenol red, Acid red, Metanil yellow, Bromothymol dark blue, Xylene cyanol FF, Neutral blue, Crystal violet, Acridine orange, Rosanilin II, Methyl red, Fluoresceine, Thymol blue, Bromophenolblau and Methyl blue. The S_{min} chamber and a most commonly used[6] N chamber (IMID, Russia) for 10×10 cm plates without pre-saturation were compared.

Table 2 R_f values of the separated compounds (silica gel plates, Merck).

Compound	Separation in an N chamber	Separation in an S_{min} chamber (d = 0.1 mm)	$R_f(N)/R_f(S_{min})$
Direction 1, acetone as the mobile phase			
Brilliant green	0.05	0.06	0.83
Rhodamine C	0.29	0.31	0.94
Erythrosin	0.56	0.58	0.97
Non-separated zone	0.91	0.93	0.98
Error (P = 0.95)	6–8%	3–5%	
Direction 2, ethyl acetate as the mobile phase			
Rhodamine C	0.08	0.11	0.73
Erythrosin	0.36	0.39	0.92
Neutral red	0.40	0.45	0.89
Methyl red	0.85	0.90	0.94
Orange G	0.95	0.97	0.98
Error (P = 0.95)	7–9%	3–5%	

Note that, although the values of R_f for the separated compounds measured in N and S_{min} chambers are close to each other, the retardation factors for all of the compounds measured in the N chamber were somewhat smaller than those measured in the S_{min} chamber regardless of the mobile phase used, *i.e.*, $R_f(N)/R_f(S_{min}) < 1.0$.

The separation of a dye mixture along the first direction resulted in the separation of three compounds, whereas four compounds formed one combined zone. However, as the separation was then continued in the second direction, the two zones corresponding to Rhodamine C and Erythrosin, which were separated along the first direction, continued to move, while the unseparated zone became resolved into three compounds (see Table 2).

Table 3 Separation efficiency (H/μm) of a dye mixture in two-dimensional TLC (silica gel plates, Merck) performed in N and S_{min} chambers.

Compound	Separation in an N chamber	Separation in an S_{min} chamber (d = 0.1 mm)	$H(S_{min})/H(N)$
Direction 1, acetone as the mobile phase			
Brilliant green	75	70	0.93
Rhodamine C	34	30	0.88
Erythrosin	22	21	0.95
Non-separated zone	22	21	0.95
Error (P = 0.95)	6–9%	3–4%	
Direction 2, ethyl acetate as the mobile phase			
Rhodamine C	63	61	0.97
Erythrosin	48	47	0.98
Neutral red	45	44	0.98
Methyl red	13	11	0.85
Orange G	10	9	0.90
Error (P = 0.95)	6–8%	4–5%	

Table 4 Resolution factors (R_s) on silica gel plates (Merck).

Compound	Separation in an N chamber	Separation in an S_{min} chamber (d = 0.1 mm)	$R_s(N)/R_s(S_{min})$
Direction 1, acetone as the mobile phase			
Brilliant green–Rhodamine C	3.0	3.2	0.94
Rhodamine C–Erythrosin	7.6	8.5	0.89
Erythrosin–non-separated zone	3.6	3.7	0.97
Direction 2, ethyl acetate as the mobile phase			
Neutral red–Methyl red	7.2	7.5	0.96
Methyl red–Orange G	9.7	10	0.97

Table 5 Chromatographic parameters in four-dimensional TLC separation of a dye mixture in N and S_{min} chambers.

Mobile phase	Separated components	$R_f(N)/R_f(S_{min})$	$H(S_{min})/H(N)$	$t(N)/t(S_{min})$
Toluene–ethanol (6.5:3.5)	Methyl red	0.64	0.67	1.17
	Siba-F II	0.67	0.67	
	Fluorescein	0.66	0.82	
Ethanol–acetic acid (9:1)	Thymol blue	0.67	0.92	1.31
	Crystal violet	0.64	0.89	
	Bromophenolblau	0.67	0.67	
	Neutral blue	0.67	0.79	
	Methyl blue	0.68	0.86	
	Xylene xanoll FF	0.66	0.68	
Acetone	Acridine orange	0.87	0.92	1.15
	Metanil yellow	0.90	0.95	
	Bromothymol dark blue	0.81	0.83	
	Bromophenol red	0.94	0.87	
	Acid red	0.88	0.95	
	Methyl orange	0.90	0.92	
	Methyl red	0.97	0.95	
Toluene	Indophenol	0.63	0.68	1.14
	Ariabel red	0.64	0.61	
	Sudan blue II	0.67	0.85	
	Sudan IV	0.69	0.71	
	Dimethylaminoazobenzene	0.70	0.81	
	Rosanilin II	0.65	0.85	

Table 3 indicates that separation in an S_{min} chamber was somewhat more efficient than that in an N chamber.

Analysis of R_s values for various pairs of chromatographed compounds (Table 4) shows that separation was improved with the use of an S_{min} chamber.

In this study, an S_{min} chamber was applied to a four-dimensional TLC technique.[6] A dye mixture containing 21 components was separated in an S_{min} chamber with d = 0.2 mm on PTLC-AF-V-UF plates. According to the data in Table 5, separation in an S_{min} chamber is more efficient and takes a shorter time and the advantages of an S_{min} chamber in separation depend on the mobile phase used.

Thus, the use of an S_{min} chamber in multidimensional TLC is more expedient, as compared with that of an N chamber. Note that errors in the chromatographic characteristics determined in the S_{min} and N chambers were no greater than 5 and 9%, respectively.

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