Reducing the duration of separation by two-dimensional TLC on the plates of small size using the S_{min}-chamber

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ABSTRACT

The new rapid TLC analysis is proposed based on simultaneous using two-dimensional chromatography on the plates of the small size and the S_{min}-chamber with the minimal gas volume. It was shown that the proposed method on the plates of the size 5x5 cm is characterized by substantially less expenditure of time (by 25-30%) to compare with separation on the plates of the size of 10x10 cm. The implementation of the proposed method is not complicated and it can be recommended for use in analytical practice.

Skrócenie czasu rozdzielania za pomocą dwuwymiarowej TLC na płytkach o małych wymiarach przy użyciu komory S_{min}.

STRESZCZENIE

Zaproponowano nowy sposób szybkiej analizy TLC polegający na zastosowaniu dwuwymiarowej chromatografii na małych płytkach w komorach S_{min} o małej pojemności. Wykazano, że zaproponowany sposób, przy zastosowaniu płytek o wymiarach 5x5 cm, charakteryzuje się znacznie krótszym czasem rozwijania chromatogramów (o 25-30 %) w porównaniu z rozwijaniem na płytkach o wymiarach 10x10 cm. Wprowadzenie zaproponowanej metody nie jest skomplikowane i może być zalecane do stosowania w praktyce analitycznej.

1. INTRODUCTION

TLC is simple, economical and sufficiently efficient method of liquid chromatography characterized by the wide sphere of application [1]. The significant feature of TLC is the possibility of rapid change of separation conditions and not high requirements for the preparation of a sample for chromatographic separation. In association with two-dimensional chromatography, allowing more complete use of separating surface of plate, find more and more practical application [2-4].

The significant characteristic of any analytical method is rapidness, which often determines its practical application. The rapidness in TLC is determined by the movement of mobile phase front on a chromatographic plate. The substantial drawback of TLC is the gradual decrease of separation velocity when increasing the distance traversed by a mobile phase. That is why the reduction of separation duration is very important.

2. EXPERIMENTAL

Chromatographic separation was carried out on the Silica gel plates (Merck, Darmstadt, Germany) on the aluminum or glass support of the size of 10x10 and of 5x5 cm (the thickness of the adsorption layer is 0,25 mm).

In this work the one-dimensional development of chromatograms (ID) was performed using 10x10 cm plates and two-dimensional development (2D) was performed using 5x5 cm plates. The two-dimensional development was performed in two variants: 1) two-dimensional development in perpendicular directions using the same mobile phase for the two dimensions and 2) traditional two-dimensional development in perpendicular directions using two different mobile phases.

One-dimensional as well as two-dimensional development of chromatograms was carried out in the ascending mode of elution both in the S_{min}-chamber (Fig. 1) with the distance *d* between the sorption layer and the chamber wall *d*=0,1 mm [5] and in the traditional N-chamber for the plates of the size of 10x10 cm without previous saturation. The important difference of the chambers used is the volume of gas space above the adsorbent of chromatographic plate. In the N-chamber for the plate of 10x10 cm the volume of gas space is ~1200 cm^3 and in the S_{min} chamber it is ~1 cm^3 and for the plate of 5x5 cm plates it is ~0,25 cm^3. This difference influenced very much on the time of development and not so much on the effectiveness of separation.

The research was conducted using the following mobile phases with different viscosity: toluene ($\mu=0{,}5\cdot10^3$ Pa·s), ethanol ($\mu=1{,}0\cdot10^3$ Pa·s), acetone ($0{,}3\cdot10^3$ Pa·s), ethyl acetate ($\mu=0{,}4\cdot10^3$ Pa·s) pro-

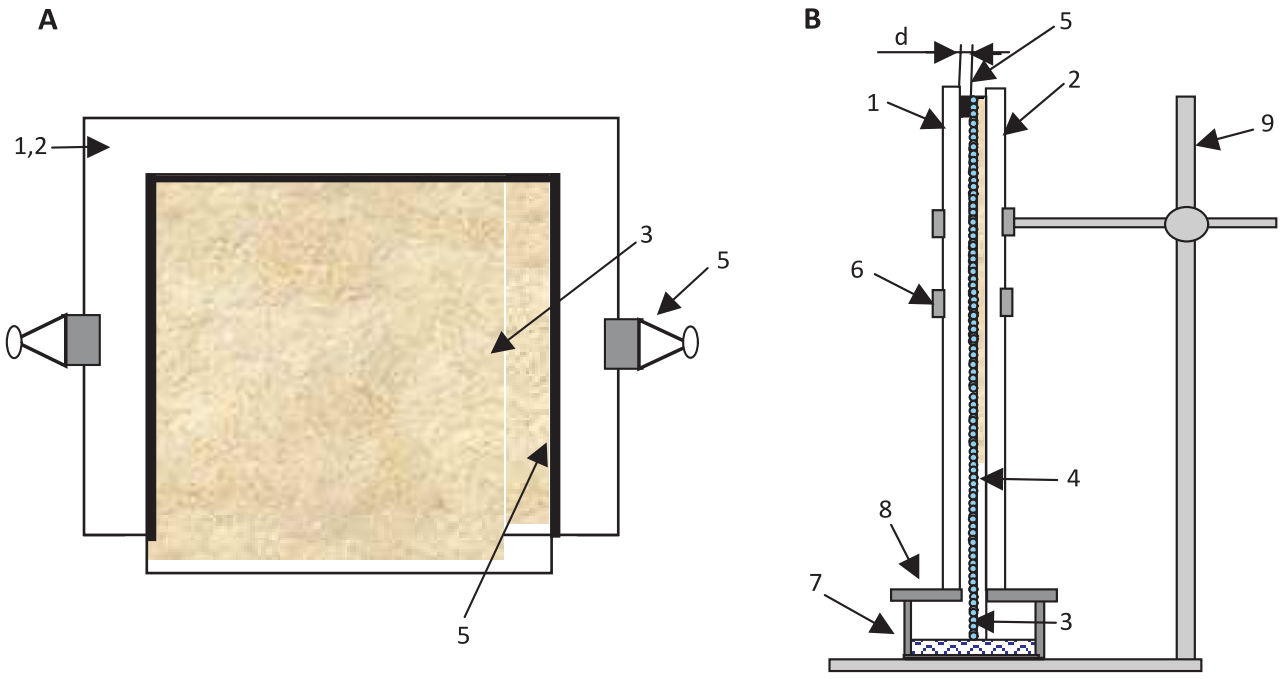

Figure 1. A scheme of small volume S-chamber for TLC.
(A) a general chamber scheme (the device), (B) general view of the chamber and its practical use.
1 – upper cover glass plate, 2 – lower cover glass plate, 3 – adsorption layer of chromatographic plate, 4 – base of TLC plate, 5 – limiter recording the distance between the adsorption layer of TLC plate and upper cover glass plate, 6 – spring clamps, used to build the whole structure 7 – container with mobile phase, 8 – sealing cap for container with a mobile phase, 9 – mounting tripod

duced by "Khimmed", Moscow, Russia; the viscosity values are given for the temperature of 20°C. The separated mixtures were composed of the following dyes: Siba-F II, Indophenol, Ariabel Red, Sudan Blue, Sudan II, Dimethylaminoazobenzene, Brilliant Green, Rhodamine C, Erythrosine, Neutral Red, Methyl Red, Orange G.

Chromatographic separation was carried out at the temperature of 20±1°C.

3. THEORY

The search of new ways to increase the rapidness of analytical separation in TLC is the main direction of its development and main task of this work.

In the traditional version of TLC migration of a mobile phase on the plate adsorption layer can be described by the following equation (see, for example, [6, 7]):

$$Z^2 = \chi \cdot t \qquad (1)$$

where Z is the distance from the line of the contact of the mobile phase with the plate adsorption layer to the line of the front of the mobile phase on the plate, t is the time (the duration) of elution, χ is the constant of the flow. The constant of the flow [6], is determined by the following equation based on the works of Giddings et al. [8] and Guiochon et al. [9]:

$$\chi = 2k_0 \cdot d_p \cdot \gamma / \eta \qquad (2)$$

where k_0 is the parameter of permeability depending on the inner structure of the adsorption layer, d_p is the diameter of the particles of the sorbent of the sorption layer, γ is the surface tension of the mobile phase, η is the viscosity of the mobile phase.

The equations (1) and (2) can be represented in the following form:

$$t = (1/\chi) \cdot Z^2 = (\eta / 2k_0 \cdot d_p \cdot \gamma) \cdot Z^2 \qquad (3)$$

The main feature of traditional TLC is the substantial increase in the duration of the experiment with the increase in the distance for which the mobile phase front traversed on the plate. The equations (1) and (3) reflect the fact that the migration of the mobile phase front on the plate takes place unevenly and with the slowdown.

The dependence of linear velocity of mobile phase front on the distance covered by the front for 5 relatively often used mobile phases is shown in Figure 2. In this figure two regions of the $\upsilon = f(Z)$ dependence can be isolated: A for relatively high velocity of mobile phase and B for low velocity of mobile phase. The average velocities of mobile phase front are given in Table 1.

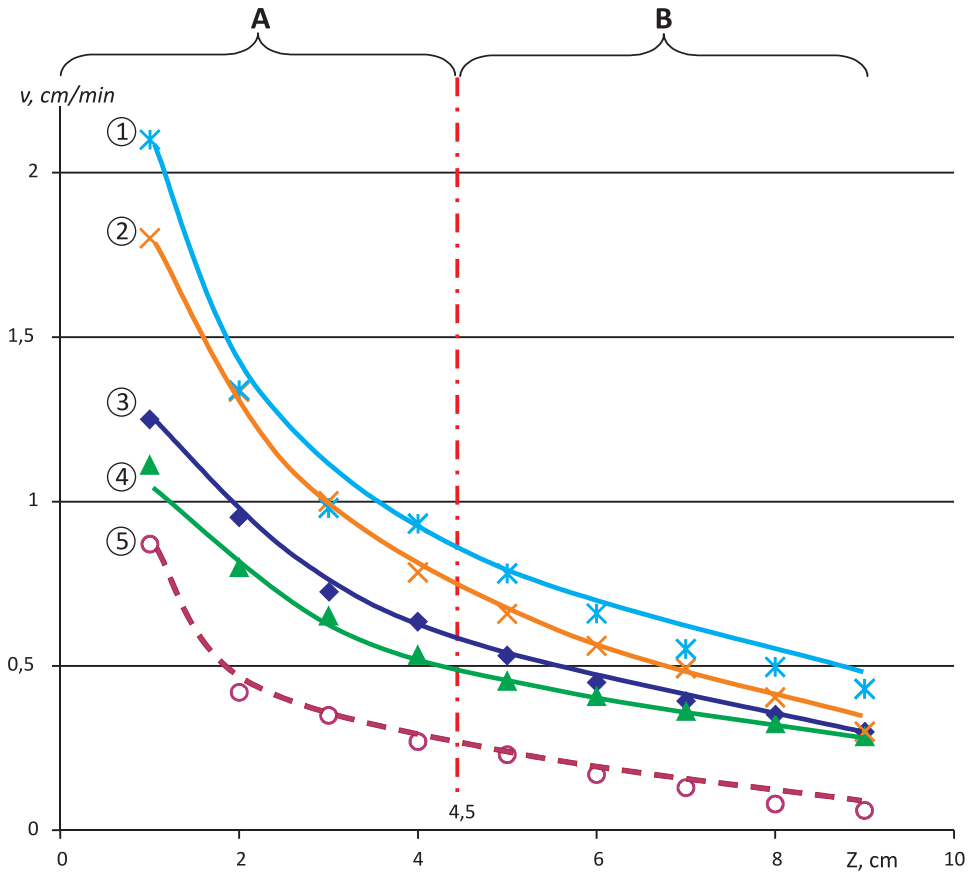

Figure 2. The dependence of mobile phase velocity on the distance of chromatogram development. Mobile phase: 1) acetone, 2) ethyl acetate, 3) light petroleum, 4) chloroform, 5) toluene

Consequently, the decrease in the sizes of the TLC plate will permit to decrease the time necessary for wetting the plate smaller in size, thus making the velocity of the mobile phase migration more even. Thus, if the sizes of the plate are n-times decreased, the time of the plate wetting, that is the duration of the analysis, will be n^2 times increased.

$$n = Z_{F2}(t_2) / Z_{F1}(t_1) \qquad (4)$$

$$Z_{F2} / Z_{F1} = (t_2 / t_1)^{1/2} = n \qquad (5)$$

$$t_2 / t_{1=} n^2 \qquad (6)$$

$$t_2 = (Z^2_{F2}(t_2) / Z^2_{F1}(t_1)) \cdot t_1 \qquad (7)$$

It is necessary to note, that in chromatography when decreasing the length of the sorption layer on which separation takes place the value of the coefficient of the peak resolution, R_S, of separated solutes is also decreased in

Table 1. The mean velocity of mobile phase front in the range A and B (Fig. 2)

Mobile phase	Mean velocity, cm/min		v_A/v_B
	Range A (v_A)	Range B (v_B)	
acetone	1.34	0.58	2.31
ethyl acetate	1.23	0.48	2.56
light petroleum	0.77	0.37	2.08
chloroform	0.89	0.40	2.25
toluene	0.53	0.20	2.65

Table 2. The duration of wetting of the TLC plates Silica gel (Merck, Darmstadt, Germany) of the size of 5x5 cm and of 10x10 cm by some mobile phases in the S_{min}-chamber [9]

Mobile phase	The relation $(\gamma^2/\eta)\cdot 10^3$ [2]	Duration of the plate wetting by the mobile phase, min		
		Size of the plate		$t_{10x10cm}/2\cdot t_{5x5\ cm}$
		5x5 cm	10x10 cm	
acetone	7.4	6.4	16.1	1.25
ethyl acetate	5.3	7.6	19.8	1.30
chloroform	4.7	9.4	23.0	1.22

proportion to the square root of the length of the sorption layer (~$\sqrt{L}$, L is the length of the "working" sorption layer). That is why to save peak resolution approximately equivalent to separation on the plate of big sizes (for example, on the plate of 10x10 cm) it seemed expedient when using the plate decreased in size (for example, 5x5 cm) to carry out the additional and the second separation on this plate in another direction (for example, perpendicular one), that is to use two-dimensional planar chromatography with the application of one mobile phase for separation. In this case the total length of the distance of separation on the decreased plate (5x5 cm) will be equivalent to the distance of single separation on the plate of 10x10 cm. It is possible, however, when conducting the second separation to use another (advisably less viscous) mobile phase.

Based on the approximate estimate given above it might be supposed that two-times decrease in the plate size will permit to up to ~4 times reduce the duration of chromatographic separation. It is really possible that the gain in time will be sufficiently big, but less than the limiting value mentioned above. In the Table 2 the data on the duration of the plate wetting by some mobile phases on the plates of the different size are given.

As the given data show, the using of the plates of the size of 5x5 cm permits to reduce the duration of the analysis to by 30% in comparison with the plates of the size of 10x10 cm when traversing the same distance by the mobile phase.

The idea of the increase in the rapidness of TLC when decreasing the sizes of the plate was considered by us earlier [10], however, in the given work we used the possibility of the additional decrease of the experiment duration when conducting chromatographic separation in the S_{min}-chamber [5], but not in the N-chamber [10]. Earlier in our work [5] it was shown that the using of the S_{min}-chamber permits to decrease the separation duration and to increase the efficiency of separation.

4. RESULTS AND DISCUSSION

4.1. The rapid separation of the analyzed mixtures components by two-dimensional TLC using one mobile phase in two perpendicular directions

The duration of the plate wetting by the mobile phase was determined for one-dimensional planar chromatography (the plates of the size of 10x10 cm) and two-dimensional TLC using one mobile phase (the plates of the size of 5x5 cm). Each variant of the chromatographic separation was performed in the N-chamber, and in the S_{min}-chamber. The results obtained are given in the Table 3.

Performing the two-dimensional development using the 5x5 cm plates it is necessary to consider the time for drying a plate before the second development. The drying is performed at 60°C using a fan during ~1 min.

Table 3. The total duration of the mobile phase front migration on the plate when wetting it by the mobile phase of 8 cm of the adsorption layer when implementing one-dimensional and two-dimensional separation and using the same mobile phase

Type of the chamber	The plates of the size of 5x5 cm	The plates of the size of 10x10 cm	The plates of the size of 5x5 cm	The plates of the size of 10x10 cm
	toluene mobile phase (μ=0,5·10^3 Pa·s)		*ethanol mobile phase* (μ=1,0·10^3 Pa·s)	
S_{min}-chamber	8.4 min *(+1 min)*	28 min	33 min *(+1 min)*	49 min
N-chamber	10 min *(+1 min)*	35 min	42 min *(+ 1min)*	70 min

The results given in the Table 3 agree with the conclusions drawn earlier about that when traversing the same distance by the mobile phase front the process takes place more rapidly as the result of two processes of separation on the plates of the small size [7]. It should be noted that when carrying out separation in the S_{min}-chamber [6] the process is performed more rapidly than in the N-chamber while the difference is observed not only when passing by the mobile phase front the considerable distance (more than 5 cm). The mobile phase velocity on the distance of first 3 cm is much higher than after passing the distance of 5 cm and it is the same phenomenon at S_{min} chamber and N-chamber. Let us note that in the case of using the S_{min}-chamber and carrying out chromatographic separation on the plates of 5x5 cm separation when using toluene as the mobile phase takes place 16% more rapidly in comparison with the N-chamber, and on the plates of 10x10 cm it does 20% more rapidly. This result can be explained by the sharp decrease of the velocity of mobile phase migration with the decrease of the plate length i.e. the difference between speed of mobile phase at the beginning of a plate and its end is higher on longer distance.

When using ethanol as the eluent the separation takes place 22% and 30% more rapidly, respectively. The more the mobile phase viscosity is, the more noticeable the benefits of the using of the S_{min}-chamber are (in comparison with the N-chamber). The value of the observed effect can make up more than 30%.

Thereby, the data obtained as the result of the research conducted testify that the application of the S_{min}-chamber for separation on the plates of the small size permits to achieve more rapid separation what makes the TLC method more attractive for the analysts.

The significant characteristic in TLC used for identification of compounds is the retention values. In the Table 4 the values R_f of the analytes when implementing one-dimensional and two-dimensional separation using the same mobile phase on one plate in two directions are given.

The retention values given in the Table 3 permit to draw the conclusion that the distance traversed by the analyzed compounds on the plates of 5x5 cm is substantially less than the distance traversed by the same compounds on the plates of 10x10 cm. Nevertheless, as we can notice, the substances separate from each other on the tangible distance what does not impede their identification. However, it should be noted that when performing separation in the S_{min}-chamber longer migration distance of the chromatographed compounds is observed.

One of most significant chromatographic characteristics of the process of separation in TLC is the separation factor Rs. The values of the separation factor are given in the Table 5. When calculating peak resolution the distance between neighboring zones was taken into account; when carrying out one-dimensional separation the distance between zones positioned on the same straight line was taken into account, when carrying out two-dimensional

Table 4. The retardation factors (R_F) of the analyzed compounds when using two-dimensional and one-dimensional separation (the R_F values for two-dimensional separations are given for each direction)

The analyzed compounds	S_{min}-chamber		N-chamber		S_{min}-chamber	N-chamber
	The plates of the size of 5x5 cm				The plates of the size of 10x10 cm	
	Toluene mobile phase					
	1D	2D	1D	2D	1D	1D
Siba-FII	0.03	0.03	0.02	0.02	0.04	0.02
Indophenol	0.09	0.09	0.07	0.07	0.15	0.10
Ariabel Red	0.17	0.17	0.16	0.16	0.25	0.21
Sudan Blue	0.26	0.26	0.25	0.25	0.36	0.32
Sudan II	0.38	0.38	0.37	0.37	0.41	0.38
Dimethylaminoazobenzene	0.57	0.57	0.55	0.55	0.66	0.58
	Ethanol mobile phase					
	1D	2D	1D	2D	1D	1D
Brilliant Green	0.12	0.12	0.09	0.09	0.15	0.11
Rhodamine C	0.30	0.30	0.27	0.27	0.36	0.31
Erythrosine	0.80	0.80	0.68	0.68	0.83	0.75

separation the distance between the nearest zones positioned on the plate after the second separation was taken into account.

The peak resolution when using two-dimensional separation with the same mobile phase in two directions on the plates of the size of 5x5 cm in all the cases is on average 10-15% more than on the plates of 10x10 cm when single traversing by the mobile phase front of the same distance (8 cm). At the same time the meanings of the separation factor obtained when implementing the chromatographic process in the S_{min}-chamber both on the plates of the size of 5x5 cm and on the plates of the size of 10x10 cm are ~10-15% higher than the meanings obtained in the N-chamber.

Thereby, in spite of that the substances on the plates of the decreased size have less meanings of retardation the separation factor between them is not less than on the plates of the size of 10x10 cm.

4.2. Two-dimensional TLC using the different mobile phases in two directions

To obtain better resolution, in some cases instead of using the same mobile phase in two-directions of chromatogram development the using of two different mobile phases is advisable.

The using of the method of 2D TLC also permits to apply the different mobile phases in the mode of 2D with applying the second mobile phase different than the first one. It permits the obtaining of mixture components separation which were not resolved during the first development. When performing the traditional version of two-dimensional TLC two dyes mixtures were used with the application of the different combination of the mobile phases. In the Table 6 the data on the separation duration of the two mixtures are given.

The data on duration of two-dimensional separation given in the Table 5 permit to draw the conclusion that application of the S_{min}-chamber to both 5 cm x 5 cm and 10 cm x 10 cm plates permits to obtain

Table 5. The Rs values obtained at the different separation conditions

The couples of the separated compounds	S_{min}-chamber	N-chamber	S_{min}-chamber	N-chamber
	The plates of the size of 5x5 cm		The plates of the size of 10x10 cm	
	Toluene mobile phase			
Siba-FII-Indophenol	2.0	1.7	1.8	1.5
Indophenol-Ariabel Red	3.3	3.0	3.1	2.8
Ariabel Red-Sudan Blue	2.3	2.2	2.2	2.0
Sudan Blue-Sudan II	5.5	5.4	5.4	5.3
Sudan II - Dimethylaminoazobenzene	3.8	3.7	3.7	3.6
	Ethanol mobile phase			
Brilliant Green-Rhodamine C	3.7	3.5	3.2	3.0
Rhodamine C-Erythrosine	8.7	8.0	8.5	7.6

Table 6. The characteristics of the mobile phase front migration on the plate when performing two-dimensional TLC

Type of the chamber	The development duration *t*, min					
	The plates of the size of 5x5 cm			The plates of the size of 10x10 cm		
	Separation of dyes mixture: Brilliant Green, Rhodamine C, Erythrosine, Neutral Red, Methyl Red, Orange G.					
	1D, acetone mobile phase	2D, ethyl acetate mobile phase	**Total duration**	1D, acetone mobile phase	2D, ethyl acetate mobile phase	**Total duration**
S_{min}-chamber	4.2	5.1	9.3	15.4	19.8	35.2
N-chamber	5.3	5.4	10.7	18.0	24.3	42.3
	Separation of dyes mixture: Siba-FII, Indophenol, Ariabel Red, Sudan Blue, Sudan II, Dimethylaminoazobenzene, Brilliant Green, Rhodamine C, Erythrosine					
	1D, toluene mobile phase	2D, ethanol mobile phase	**Total duration**	1D, toluene mobile phase	2D, ethanol mobile phase	**Total duration**
S_{min}-chamber	4.2	16.5	20.7	28.0	49.0	77.0
N-chamber	5.0	21	26.0	35.0	70.0	105

Table 7. The retardation factor values of the chromatographed compounds

Compounds	Separation in the S_{min}-chamber			Separation in the N-chamber without previous saturation		
	The plate of 5x5 cm	The plate of 10x10 cm	R_{F5}/R_{F10}	The plate of 5x5 cm	The plate of 10x10 cm	R_{E5}/R_{E10}
	1D (acetone mobile phase)					
Brilliant Green	0.08	0.06	***1.33***	0.08	0.05	***1.60***
Rhodamine C	0.35	0.31	***1.12***	0.33	0.29	***1.13***
Erythrosine	0.55	0.53	***1.04***	0.54	0.52	***1.04***
Neutral Red, Methyl Red, Orange G	0.95	0.91	***1.04***	0.98	0.93	***1.05***
	2D (ethyl acetate mobile phase)					
Brilliant Green	0	0	***0***	0	0	***0***
Rhodamine C	0.10	0.11	***0.91***	0.10	0.08	***1.25***
Erythrosine	0.38	0.39	***0.97***	0.38	0.36	***1.06***
Neutral Red	0.40	0.45	***0.89***	0.40	0.40	***1.00***
Methyl Red	0.88	0.90	***0.98***	0.85	0.85	***1.00***
Orange G	0.95	0.97	***0.98***	0.90	0.95	***0.94***

Table 8. The values of peak resolution of separation of the dyes mixture by the method of two-dimensional TLC

The compound	Rs values in the S_{min}-chamber			Rs values in the N-chamber		
	The plate of 5x5 cm	The plate of 10x10 cm	R_{S5}/R_{S10}	The plate of 5x5 cm	The plate of 10x10 cm	R_{S5}/R_{S10}
Separation of dyes mixture: Brilliant Green, Rhodamine C, Erythrosine, Neutral Red, Methyl Red, Orange G.						
Result of two-dimensional separation (1D acetone mobile phase, 2D ethyl acetate mobile phase)						
Brilliant Green - Rhodamine C	2.1	2.3	***0.91***	1.7	2.0	***0.85***
Rhodamine C - Erythrosine	3.5	4.1	***0.85***	3.2	3.7	***0.87***
Erythrosine - Neutral Red	2.0	2.3	***0.87***	1.4	1.8	***0.78***
Neutral Red - Methyl Red	5.3	5.5	***0.96***	5.2	5.2	***1.00***
Methyl Red - Orange G	9.1	10	***0.91***	9.0	10	***0.90***
The average meaning			***0.90***			***0.88***
Separation of dyes mixture: Siba-FII, Indophenol, Ariabel Red, Sudan Blue, Sudan II, Dimethylaminoazobenzene, Brilliant Green, Rhodamine C, Erythrosine						
Result of two-dimensional separation (1D toluene mobile phase, 2D ethanol mobile phase)						
Siba-FII - Indophenol	1.7	1.7	***1.00***	1.5	1.5	***1.00***
Indophenol - Ariabel Red	2.9	3.0	***0.98***	2.7	2.8	***0.96***
Ariabel Red - Sudan Blue	2.2	2.2	***1.00***	2.0	2.0	***1.00***
Sudan Blue - Sudan II	5.4	5.4	***1.00***	5.3	5.3	***1.00***
Sudan II - Dimethyl- aminoazobenzene	3.6	3.7	***0.97***	3.5	3.6	***0.97***
Dimethyl- aminoazobenzene - Brilliant Green	1.6	1.5	***1.07***	1.0	1.0	***1.00***
Brilliant Green - Rhodamine C	3.3	3.2	***1.03***	3.1	3.0	***1.03***
Rhodamine C - Erythrosine	8.5	8.5	***1.00***	7.6	7.6	***1.00***
The average meaning			***1.01***			***0.99***

15-25% reduction of time of separation relative to N-chamber depending on the mobile phase used.
In the Table 7 the values of retardation factor of the chromatographed compounds when performing two-dimensional separation on the plates of the different size are given.
In the Table 7 the data for compounds separated in first direction (part 1D) and for compounds separated in second direction (part 2D) using a new mobile phase are given. In the part 2D there are given data for compounds not separated in first direction of development.
The data on the retardation factor values of the compounds when separating the dyes mixture of Siba-FII, Indophenol, Ariabel Red, Sudan Blue, Sudan II, Dimethylaminoazobenzene, Brilliant Green, Rhodamine C, Erythrosine are not given since they are similar to the data obtained when carrying out one-dimensional separation (see Table 4).
The data obtained don't permit to draw the conclusion on that when performing the process on the plates of the size of 5x5 cm the substances are characterized by more meanings of the retardation factor values or, on the contrary, this is especially shown when implementing the process on the plates of the size of 10x10 cm. However, it should be noted, that the maximal movement of the compounds is observed in the S_{min}-chamber.

In Table 8 the data on the separation factor using 2D TLC method are given. Data listed in the Table 8 show that the average Rs in implementing a two-dimensional separation on plates of different sizes (5x5 and 10x10 cm) slightly differ (the maximum difference of 12%). This confirms the appropriateness of the use of plates of small size (5x5 cm) for the chromatographic separation, since in this case, the duration of the analysis is reduced to 40% compared with the duration of the separation process on the plates 10x10 cm, but efficiency of the separation is almost not reduced (5-10%). It is important to note that the Rs values, obtained in different chambers practically do not differ from each other, the maximum discrepancy is 2%.

5. CONCLUSIONS

A systematic study of the chromatographic characteristics of separating a mixture of dyes using two-dimensional chromatography on the plates of size 5x5 and 10x10 cm, using Smin-chamber and traditional N-chamber was carried out. It was shown that the noticeable increase in the rapidness of component mixtures separation in comparison with one-dimensional separation on the plates of 10x10 cm can be obtained using, first, two-dimensional separation on the plates of 5x5 cm and, second, carrying out separation in the S_{min}-chamber.

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