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New thin-layer chromatography plate with a closed sorbent layer and details of its application

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Abstract

New variants of traditional-type TLC plates with a sorbent layer were closed by a film transparent to UV and their characteristics were studied. Retention values and movement of the mobile phase front were investigated. Advantages of TLC variants are discussed. To shorten the chromatographic process, a TLC variant was suggested where one end of the plate was connected to a vacuum source and the other end was connected to a mobile-phase source. Some applications for the method developed are briefly discussed.

Keywords: Stationary phases, TLC; Thin-layer plates; Closed sorbent layer; Forced-flow thin-layer chromatography

1. Introduction

TLC is a practical, important, widely used and well-developed analytical technique [1–9]. The following advantages of the method have influenced its widespread use: (1) ease of use of the equipment and the experimental technique, (2) the possibility of varying a large number of experimental parameters, (3) its economic feasibility, and (4) its high productivity (it is possible to separate simultaneously 20 or more samples on one plate). However, when TLC is compared to HPLC it has a number of disadvantages: (1) lower reproducibility of retention values and other chromatographic parameters, (2) longer separation process, and (3) greater difficulty and complexity of the process automatization.

Some years ago Geiss [8] identified a number of the most significant disadvantages of TLC:

1. It has a limited resolving power due to the short (3–10 cm) separation length (exception: over-pressured TLC).
2. The advantage of RP layers cannot be completely used at present (problems of wetting, demixing); the difficulties may diminish with technical progress in the future.
3. As an 'open' system, TLC depends on environmental factors, e.g. influence of relative humidity on hydrophilic layers (control necessary); open layers act as scrubbers for impurities in the air which may impair quantitative results, especially in HPTLC. Problems arise with volatile, oxygen- and light-sensitive samples.

The purpose of this work was to examine some new variants of TLC on plates with a sorbent layer closed by a polymer film, to study the possibility of using TLC variants with controlled flow of the mobile phase on plates of this type and to study the

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characteristics of the chromatographic process in the variants. The TLC variant suggested allows the elimination of some of the above disadvantages.

The TLC variant on plates with an unfixed sorbent layer closed with a cover glass was originally described in 1947 [10]. When carrying out the experiment, the sample to be analysed and the mobile phase required for the separation by circular chromatography were sequentially supplied to the sorbent layer through a hole in the center of the cover glass. Williams [10] noted that use of a closed sorbent layer prevented evaporation of the mobile phase and adsorption of environmental foreign matter. It should be noted that separated zones obtained on the plane chromatograms appear as distorted circles. It seems likely that this phenomenon is related to irregularities of the unfixed sorbent layer. Later on these plates were not of practical use. This is explained by the poor separation results and the complexity of their practical realization. The proposed new planar chromatographic systems (i.e. standard chromatographic systems (plates) with closed sorbent layer) have a nonseparable construction. In our opinion they are characterized by the following advantages: (1) the adsorption properties of the plates are more standard since they are covered with a polymer layer and are protected against environmental action (for example, moisture), (2) application of the new plates allows simplification of the equipment and the technology of their practical use; in the standard technique there is no need to use a special chamber and forced-flow planar liquid chromatographic techniques may be realized with simple equipment.

Later these systems were used in overpressured thin layer chromatography (OPTLC) (see, for example Refs. [11–17]) and rotation planar chromatography [18]. However, the systems consisted of separate components (in the known publications) which were assembled/prepared by the analyst just before their use. The systems proposed here are planar liquid columns and are quasi-nonseparable. They are manufactured in advance by connection of the possible polymer planar material with an upper sorbent layer of the TLC standard plate. Only one of the many possible constructions of the planar column and materials used is described here.

In the present work the following variants of TLC with the sorbent layer closed by a transparent

polymer layer were considered: (1) the classic variant of TLC where the mobile phase moves along the plate by the action of capillary forces but without using any chamber; (2) forced-flow TLC where the top of the plate is connected to a vacuum source. This allowed speeding up of the mobile-phase movement. We also paid great attention to the chromatographic process characteristics in the above variants. In our opinion, the results obtained are of particular interest for understanding some of the characteristics of the process in classic TLC.

In order to demonstrate the novelty of the proposed plates with the closed sorbent layer we compared them with closed chromatographic plates in OPTLC (Table 1). As can be seen, the proposed plate and the possible fields of application are essentially different from the known ones.

2. Experimental

The preparation process for the TLC plate with the adsorption layer closed with polymer film is described in Refs. [19,20].

Initially, commercially available plates are coated with a sorbent layer (for example, silica gel) and heated at 120°C for 0.5–1.0 h, just before coating with a polymer film. This means that the plates have an activated sorbent layer. Then degreased polyethylene film, polyfluoroethylene film (0.1 mm thickness), thermostable rubber and a metal plate are placed on the activated layer of the TLC plate. The multilayer system was uniformly pressed, placed into an air oven, heated to 100°C and kept at that temperature for 1 min. Then, the multilayer system was cooled and disassembled. The plates with polymer film could be of different type depending on their further application (Fig. 1 and Fig. 2).

As initial plates we used plates with silica gel produced by Merck (Germany, Kieselgel 60 F 254), Macherey-Nagel (Germany, Sil G-50), Kavalier (CSSR, Silufol UF 254). We also used plates manufactured by Lyane Kalur (Estonia, Silicagel KSKG-ATSKh), EROKHEM (Armenia, Armsorb TSKh-S). Standard plates for routine analysis with polyethylene film on the cellulose layer (supplied by Merck and Kavalier) and with sorbent layer for reserved-phase chromatography (supplied by Merck) were obtained.

Table 1
Comparison of traditional TLC plates (closed) in an OPTLC device with proposed plates (closed) with transparent polymer film firmly fastened to the surface of the sorbent layer

Plate characteristics	Closed plate in traditional OPTLC	Proposed plate with closed adsorption layer
Construction		
(1) type	Separable	Nonseparable
(2) weight	Some kilos	Some grams
Place of closed system realization	OPTLC device	Anywhere, independent of device
Lifetime	Usually corresponds to the duration of the chromatographic process in OPTLC	Without limit
Environmental influence	Great (for example, moisture etc.)	Minor
Application fields	OPTLC	(1) Traditional TLC (practically without special chamber) (2) Forced-flow TLC using more simple devices
Cost	Expensive	Not expensive

Needless to say that other methods to coat the sorbent layer with a film (preferably transparent) are possible.

The main difference between the classical method of separation on the plates with the a closed sorbent layer and traditional TLC is the absence of the chromatographic chamber, because the plate with the

fixed sorbent layer is the new variant of the plate–unsaturated chamber system. It should be pointed out that in the given method the chamber was realized in the most simple form and in the most ‘clear’ variant. The main variants of TLC plates with closed sorbent layer are shown in Figs. 1 and 2. The analyte samples were applied onto the open sorbent layer

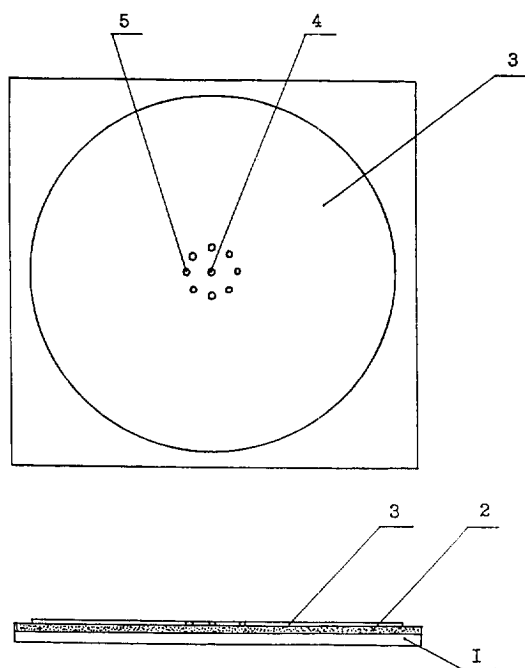


Fig. 1. TLC plate variant with polymer cover on the sorption layer for circular chromatography: 1, support; 2, sorption layer; 3, transparent polymer cover; 4, hole for the mobile-phase feeding; and 5, hole for the samples being analyzed.

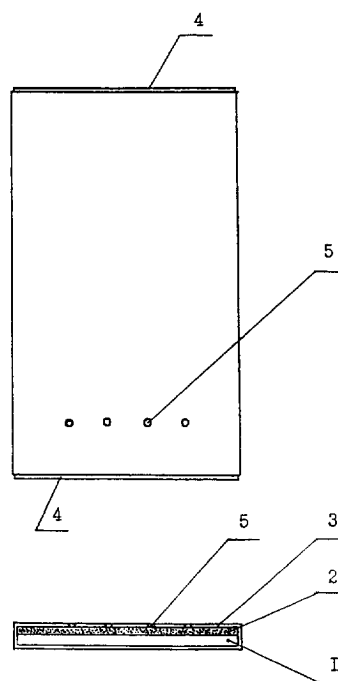


Fig. 2. TLC plate variant with polymer cover of the sorption layer for linear chromatography: 1, support; 2, sorption layer; 3, transparent polymer cover; 4, plate edges clean of polymer cover; and 5, holes for the samples to be separated.

and the elution was started. When using highly volatile mobile phases the sample holes in the polymer film were closed (using for example, a spring-controlled clamp).

To carry out the separation the sorbent layer was connected to the eluent: for a rectangular plate its bottom was placed in a vessel containing the eluent. After separation the polymer film was removed from the sorbent layer. We assume that removal of the polymer film is not always obligatory, especially when the film is transparent to UV.

Significant changes in the macrosurface of the sorbent layer were not observed after removing the polymer film. This was confirmed by the results of scanning the sorbent layer surface with a spectrophotometric densitometer TLC Scan III (Camag, Switzerland). It may be possible to use plates of this type for transparency scanning if the material used as the support is transparent to UV (e.g., polyethylene). To realize forced flow of the mobile phase through the TLC plate, a closed sorbent layer vacuum was used as an additional moving force. The vacuum was applied at the top of the plate, while the bottom of the plate was put into the mobile phase.

The scheme of the device is shown in Fig. 3 [21]. The discharge in the given device could be realized by applying an oil pump or even a water-jet pump.

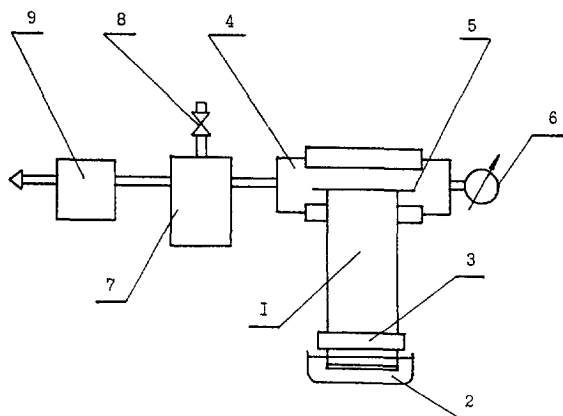


Fig. 3. Scheme of the device for realization of forced-flow TLC when using vacuum: 1, TLC plate with closed sorbent layer (linear chromatography variant); 2, mobile phase source; 3, sealing clip closing the holes for the samples; 4, chamber with impermeable slit-like slot; 5, used for connecting the plate to the vacuum source; 6, vacuum gauge; 7, buffer vessel; 8, valve; and 9, vacuum pump.

3. Results and discussion

3.1. Investigation of the characteristics of the chromatographic process (traditional variant) for TLC plates with a closed sorbent layer and no chamber

The following characteristics of the chromatographic process were studied: retention of analytes and the movement of the mobile-phase front.

As known [1–9], the R_F value is the main retention characteristic in TLC. It is the distance from the origin to the center of the separated zone divided by the distance from the origin to the solvent front (z_F). Fig. 4 shows the relationship of R_F vs. z_F for the pigment methyl red. It was obtained for TLC variants that differed in the saturation of the sorbent layer with solvent (eluent). As can be seen, a constant R_F value (i.e. independence of the retention characteristics with respect to z_F) is observed for only two TLC variants: (1) TLC with a closed sorbent layer, and (2) TLC with a saturated chamber. Comparison of the above variants demonstrates that the former variant is preferable for a number of reasons. Firstly, the duration of the additional stage (saturation) for TLC with a sorbent layer saturated

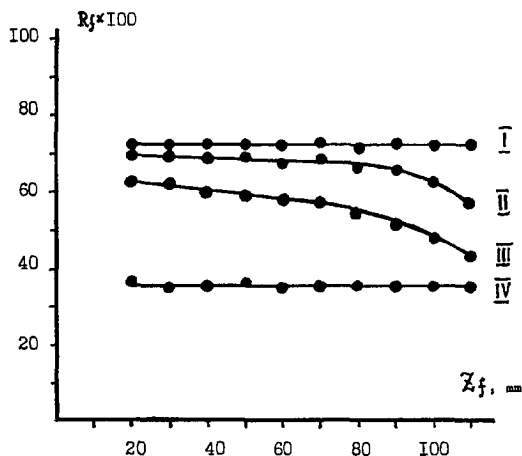


Fig. 4. Dependence of R_F on the distance z_f for methyl red for the different TLC variants on the Kieselgel 60F254 (Merck) plate when using propanol-2 as the mobile phase: I, plate with closed sorption layer (variant of unsaturated chamber); II, unsaturated chamber; III, saturated chamber (without preliminary saturation); and IV, saturated chamber (with preliminary saturation of more than 50 min).

with the eluent vapours is rather long. In our experiments it was no less than 50 min. Secondly, the R_F value for (1) is essentially greater than that for (2). This can be easily explained when taking into consideration the fact that the mobile-phase volume needed for transfer over the same distance is essentially lower for (2). Thirdly, the better reproducibility of the R_F value is an important characteristic; (1) provides better reproducibility (see Table 2).

In addition it should be noted that the former variant is better from the ecological point of view (mobile phase vapours do not evaporate from the sorbent surface as components of the analysing mixture).

The second important characteristic of the chromatographic process in TLC is the movement of the mobile phase front along the sorbent layer.

Many original and important works were devoted to the study of this problem [2,4,6–9,22–27]. However, the authors studied processes that took place on TLC plates with open layers in special chambers or under conditions where some additional force (e.g., pressure) was applied on the mobile-phase movement along the sorption layer.

As known, in linear chromatography the following equation is used:

$$z_f^2 = Kt \quad (1)$$

where z is the migration distance of the mobile-phase front in a time t and K is the kinetic rate factor depending on the experimental conditions.

The movement of the mobile-phase front on plates with open and closed sorbent layers was studied with

silica gel plates manufactured by different firms using various mobile phases. For all mobile phases studied (methanol, ethanol, propanol-1, propanol-2, butanol-1, toluene, acetone and butanone-2) and plates (Kieselgel 60F254, Merck; Sil G-25, Macherey–Nagel; Silufol UF 254, Kavalier; Armsorb TSKh-S, FROKHEM) the same dependencies were obtained. As an example, the dependencies of the kinetic rate factor versus time (see Eq. (1)) are shown in Fig. 5 when studying the movement of the propanol-2 front on Kieselgel 60F254 plates for different variants of TLC. As can be seen, the K value is stable with respect to the migration distance of the mobile phase: (1) on the plates with closed sorbent layer, and (2) in the chamber with the sorbent layer previously saturated with propanol-1 vapours (the duration of the saturation must be no less than 50 min).

The above characteristic of the chromatographic process for a plate with a closed sorbent layer allows us to suggest the equation for the dependence of the zone migration on the time, taking into account the known Eq. (1) and the following equations:

$$l = R_f \sqrt{k} \sqrt{t} \quad (2)$$

$$R_f = 1/z_f \quad (3)$$

where l is the analysing zone migration distance in a time t and R_f is the retention value used in TLC.

On the basis of Refs. [8,22,23] the following equation was suggested [24] for the rate constant for the movement of the solvent front in the sorbent layer saturated with mobile phase:

Table 2
Separation of fat-soluble pigments on the Kieselgel 60F254 (Merck) plates with open and closed sorbent layers at different elution variants

Separated compounds	Elution variants							
	I		II		III		IV	
	R_F	S_f (%)	R_F	S_f (%)	R_F	S_f (%)	R_F	S_f (%)
Azobenzene	0.83	6.9	0.58	3.5	0.44	1.9	0.86	2.1
Butyrous yellow	0.34	5.9	0.23	3.3	0.19	2.2	0.35	1.6
Sudan III	0.16	5.6	0.09	3.6	0.08	2.5	0.15	1.9
Victoria blue	0.13	6.1	0.08	3.5	0.06	3.1	0.12	2.3
Sudan red	0.09	7.0	0.06	4.0	0.04	3.0	0.08	2.4

I, in unsaturated chamber (just after mobile phase feeding); II, in saturated chamber without preliminary saturation; III, in saturated chamber with preliminary saturation for 60 min; IV, plate with closed sorbent layer, unsaturated chamber variant. The mobile phase was toluene.

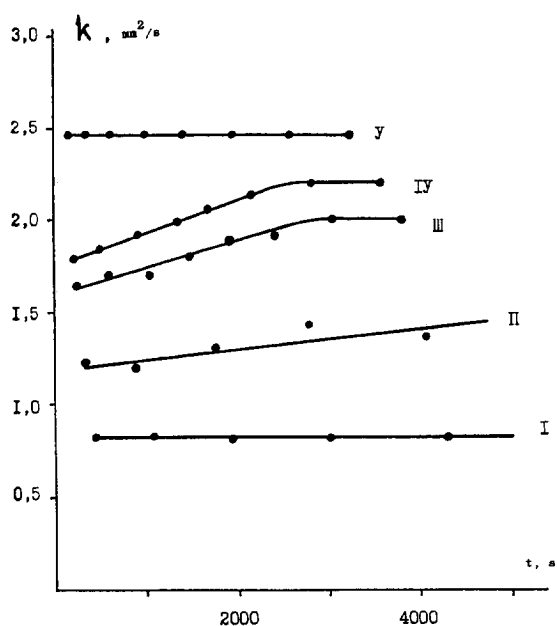


Fig. 5. Dependence of the kinetic rate coefficient on the mobile phase (propanol-2) movement on the TLC Kieselgel 60F254 plate for different variants of the chromatographic process: I, on the closed sorption layer (variant of unsaturated chamber); II, in the unsaturated chamber; III, in the saturated chamber without preliminary saturation of the sorption layer; IV, in the saturated chamber with preliminary saturation for 10 min; and V, in the saturated chamber with preliminary saturation for more than 50 min.

$$\begin{aligned}
 K_{f, \text{sat}} &= 2K_0 d_p (\gamma/\eta) [V_0 / (V_0 - V_v)] \cos\theta \\
 &= 2K_0 d_p (\gamma/\eta) g
 \end{aligned}
 \quad (4)$$

where d_p is the diameter of the sorbent particles, γ is the surface tension, η is the viscosity of the mobile phase, V_0 is the total porosity of the sorbent layer, V_v is the sorbent layer volume occupied by adsorbed vapours of the mobile phase, θ is the wetting angle and $g = V_0 / [V_0 - V_v]$.

For TLC with a closed sorbent layer $V_v = 0$, $g = 1$. Hence:

$$K_{f, \text{clos}} = 2K_0 d_p (\gamma/\eta) \cos\theta \quad (5)$$

Dividing Eq. (4) by Eq. (5) gives:

$$K_{f, \text{sat}} / K_{f, \text{clos}} = g \quad (6)$$

When deducing the above correlation we assumed

that the wetting angle was the same for both TLC variants.

Thus, the g value could be determined from the above equation. It could also be determined from the direct independent experiments. The total porosity V_0 was determined from the data obtained by weighing the plates with the sorbent layer completely wetted with mobile phase after the activation and just after elution. V_v was determined from the data obtained when the plates were in the overweight condition in the saturated chamber under the surface of mobile phase up to the equilibrium saturation of the sorbent layer with the vapours of the mobile phase. The data for the g values were obtained: (1) using Eq. (6), and (2) using the above independent weight method (Table 3). As can be seen, the values obtained with the former method are 1.46 times greater than those obtained by the latter one. The result is independent of the type of TLC plate. This deviation could possibly be explained by the fact that the weighing angles depend on the silica gel saturation with mobile phase vapours. The temperature factor used in traditional TLC is very limited (see, for example, Ref. [1]).

The application of TLC with a closed sorbent layer allows renewed consideration and expediency of the temperature factor used in the separation process. For TLC with a closed layer the temperature influence could appear in: (1) an acceleration of the movement of the mobile phase front (acceleration of the chromatographic process), (2) variation of the R_F value because the equilibrium constant of the compounds chromatographed depends on temperature.

The dependence of the rate constant of the mobile phase movement on the temperature for the Kieselgel 60F254 plate with a closed sorbent layer for different mobile phases is shown in Fig. 6. In the studied range of temperatures, 20–60°C, the K values show a linear relationship with the temperature.

Since, from Eq. (5), a possible linear dependence $K = f(\gamma/\eta)$ for one type of plate exists, this assumption was checked for different mobile phases and temperatures (see Fig. 7). The above dependence is linear. Hence, Eq. (5) is true for TLC with a closed layer; the dependence could be used for the determination of K values for other mobile phases (if tabulated data γ and η are known) and temperatures.

In TLC with a closed sorbent layer R_F increases

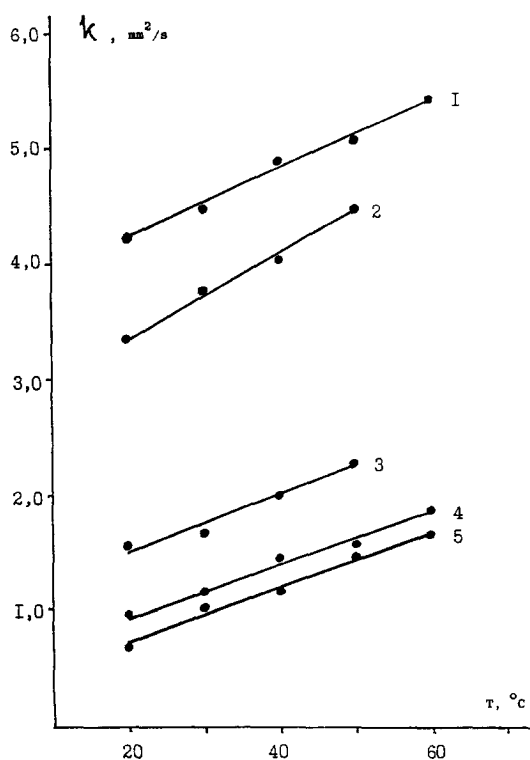


Fig. 6. Dependence of the kinetic rate coefficient of the mobile phase movement on the temperature. Experimental conditions: Kieselgel 60F254 plate with closed sorbent layer; mobile phases: 1, toluene; 2, methanol; 3, ethanol; 4, propanol-1; and 5, butanol-1.

when the temperature increases, while the temperature increase does not essentially degrade the reproducibility of the separation. The change in R_F in the studied range of temperatures, 20–60°C, could be considered to be linear (see Fig. 8).

It should be noted that for definite pairs of compounds (for example, Sudan III, Victoria blue, methyl red and natural red) an improvement of the separation is observed. Hence, the temperature factor could be used for optimization (improvement) of separation conditions on plates with a closed sorbent layer. It is likely that it is also expedient to use temperature programming of the plate and temperature gradient under separation. A GC air oven could be used for the realization of the given and other temperature variants. It follows from the above-mentioned application of the new plates with a closed sorbent layer that there are a number of

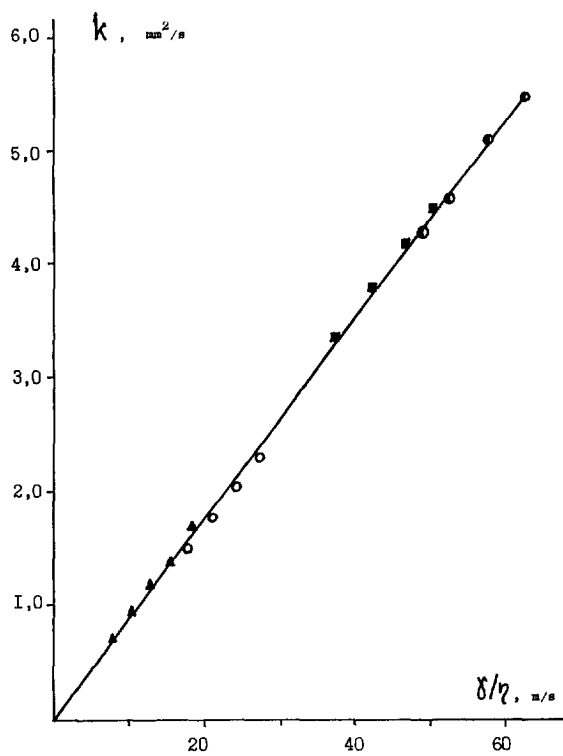


Fig. 7. Dependence of the K value on the penetration coefficient (γ/η), obtained at 20–60°C. Experimental conditions: Kieselgel 60F254 plate with closed sorbent layer; mobile phases: (○) toluene; (■) methanol; (○) ethanol; (▲) butanol-1.

advantages compared to plates with and open layer used traditionally.

3.2. Investigation of the dependence of the chromatographic process in forced-flow TLC on plates with a closed sorbent layer

In traditional TLC the separation of the analytes is carried out under conditions where there is a practically uncontrolled (and far from optimal) flow-rate of the mobile phase. For the optimization of the process it is expedient to use different TLC variants with a forced flow of the mobile phase [2,9,11–16]. Results of vacuum application used to accelerate the mobile-phase movement in TLC were first published by Delvordre et al. in 1992 [25]. However, a vacuum variant of forced-flow TLC was first suggested and realized in 1987 [21]. Both research groups worked independently and the suggested methods are sig-

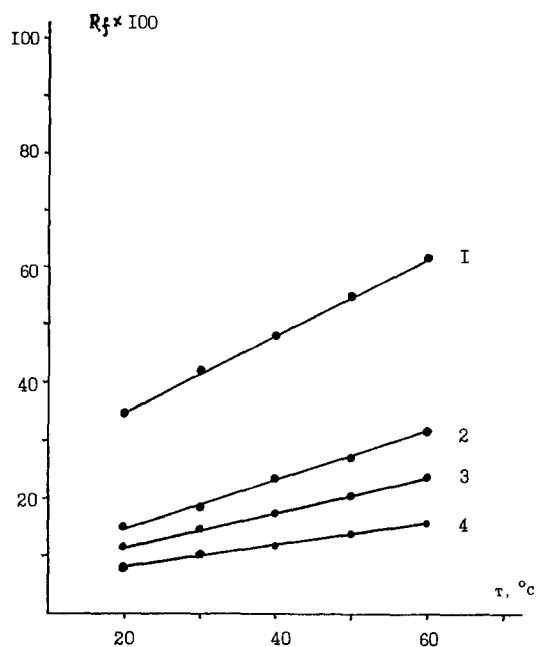


Fig. 8. Effect of temperature on the retention values in TLC with a closed sorbent layer. Experimental conditions: Kieselgel 60F254 plate with a closed sorbent layer; the mobile phase used was toluene. Separated compounds: 1, butyrous yellow; 2, Sudan III; 3, Victoria blue; and 4, Sudan red.

nificantly different. In our opinion the method we suggested in 1987 is more simple. We first studied the characteristics of the mobile-phase movement in the vacuum variant. One of the new simplified TLC variants with controlled flow of the mobile phase could be realized on the plate with a closed sorbent layer when using a vacuum source (a water jet or an oil vacuum pump).

Use of a vacuum as an additional force for the mobile phase allows essentially the acceleration of its movement. Thus, the 150 mm migration of the butanol-1 front on the Kieselgel 60F254 plate with the closed sorbent layer (without additional force $\Delta p = 0$) requires 506.8 min. When using a vacuum in the described system ($\Delta p = 0.5 \text{ kg/cm}^2$) the duration of the elution was shortened by a factor of 4.3 (118.9 min); with an increased vacuum the duration could be shortened to 67.3 min. The dependence of the migration of the mobile phase (z_F) on the time (t) expressed by Eq. (1) follows from Fig. 9.

The rate constants of the mobile phase movement

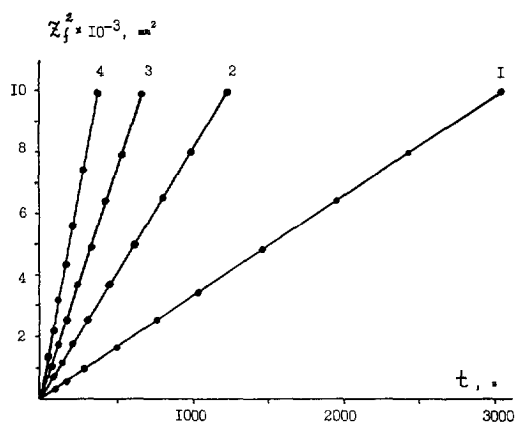


Fig. 9. Dependence of z_F^2 on t in TLC under vacuum at different pressures. Experimental conditions: Kieselgel 60F254 plate with a closed sorbent layer; the mobile phase used was methanol; vacuum values (Δp , kg/cm^2): 1, 0; 2, 0.2; 3, 0.5; and 4, 1.0.

increase with an increasing vacuum (Δp) in accordance with:

$$K_p = A + B\Delta p \quad (7)$$

where A and B are constants related to the given mobile and stationary phases and also the experimental conditions. At $\Delta p = 0$, $K_p = K = A$; hence:

$$K_p = K + B\Delta p \quad (8)$$

As an example, the dependence $K = f(\Delta p)$ is shown in Fig. 10. As can be seen, Eq. (8) fits the experimental data well (correlation coefficient > 0.99). It should be noted that it is necessary to linearly program the discharge Δp – because the resistance to the flow increases with increases in the length of the mobile-phase front – in order to perform the separation under conditions close to the optimal ones.

The use of the additional force for the acceleration of the transfer of the mobile-phase flow in the closed sorbent layer of the TLC plate at a range where the path length of the mobile-phase front has a noticeable value (i.e., more than 10–20 mm) allows an improvement (see Table 4) in the separation efficiency (as well as in the other TLC variants with forced flow).

As can be seen from Table 4, when separating fat-soluble pigments on traditional plates in an unsaturated chamber, the HETP was better by up to a factor of 2, but in the saturated case it was up to 1.5

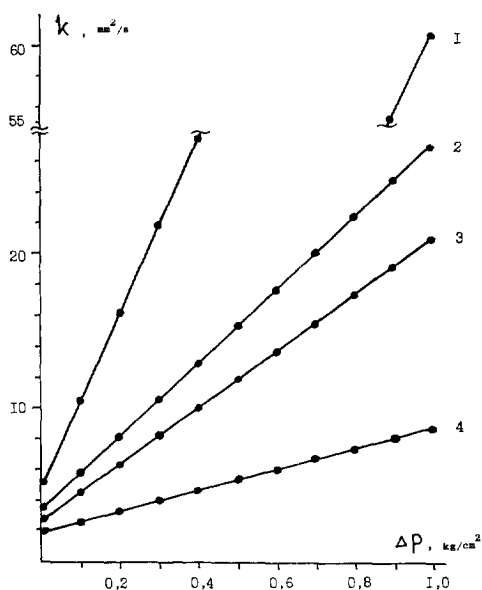


Fig. 10. Dependence of K_p on Δp for different plates with silica gel (closed layer) in TLC under vacuum. Experimental conditions: Kieselgel 60F254 plate with a closed sorbent layer; the mobile phase was methanol. Initial plates: 1, Silufol UF 254; 2, Kieselgel 60F254; 3, Armsorb TSKh-S; and 4, HP TLC SIL G-25.

times worse than when separating in TLC under vacuum on plates with a closed layer at the optimal pressure drop. The analysis times (at a path length of the mobile phase of 80 mm) were 865 s for the unsaturated chamber and 656 s for the saturated one, but on the plate with a closed sorbent layer (at $\Delta p = 0.35 \text{ kg/cm}^2$) it was 459 s. The listed data testify to a number of significant advantages of the described variant of TLC compared with the traditional methods. This variant is more simple than the methods with controlled flow. It provides better ecological safety for the chromatographer. In our opinion, further development of the variants suggested is required. In particular, the realization of the

Table 3
Values of $g = V_0 / (V_0 - V_g)$ obtained by gravimetric determination (g_1) and by calculations using Eq. (6) (g_2) for the studied TLC plates with silica gel and different mobile phases

TLC plate	V_0	V_g	g_1	g_2	g_1/g_2
Kieselgel 60F254	0.81 ± 0.01	0.41 ± 0.01	2.03	2.90	1.43
Sil G-25	0.79 ± 0.01	0.43 ± 0.01	2.19	3.21	1.46
Silufol UF 254	0.83 ± 0.02	0.48 ± 0.02	2.36	3.70	1.56
Armsorb TSKH-S	0.80 ± 0.01	0.40 ± 0.01	2.00	2.84	1.42
ATSKh	0.81 ± 0.01	0.40 ± 0.01	1.98	2.86	1.44

Table 4

HETP for fat-soluble pigments obtained in (I) unsaturated chambers, (II) saturated chambers, and (III) on the plates with a closed sorbent layer in TLC under vacuum

Compound chromatographed	HETP (μm)		
	I	II	III
Azobenzene	50	38	24
Butyrous yellow	62	49	38
Sudan III	57	43	27
Victoria blue	42	34	20
Sudan red	55	49	31

The results were obtained in optimal conditions: plate, Kieselgel 60F254; mobile phase, toluene; path length of mobile phase, 80 mm.

method is of interest for paper chromatography and for electrophoresis. That is why it is expedient to realize the accelerated movement of the mobile phase, applying it on the sorbent layer under pressure.

4. Conclusion

The study gave an opportunity to investigate the regularities of the chromatographic process on plates with an unsaturated layer in the most 'clear' variant.

The results obtained testify to the expediency of the practical application of the new TLC variants and their further development.

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