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Variants of Thin-layer Chromatography with Controlled Flow of the Mobile Phase

V.G.Berezkin and V.V.Buzaev

The review is devoted to the methods for controlling the flow of the mobile phase which have been developing successfully in thin-layer chromatography (TLC). Their use makes it possible to eliminate some of the principal disadvantages of the traditional TLC with retention of the majority of its advantages. The continuous variant, the continuous variant with flow type detection, the centrifugal variant of TLC, and also the variant of TLC under pressure are examined. It is shown that control of the flow of the mobile phase makes it possible not only to employ TLC successfully for the selection of separation conditions in column liquid chromatography (CLC) but also to approach CLC as regards performance and speed. At the same time it gives rise to new possibilities for the automation of TLC.
The bibliography includes 143 references.

CONTENTS

I.	Introduction	382
II.	Classification of TLC methods with controlled flow of the mobile phase	383
III.	Continuous combined (two-stage) TLC	384
IV.	Continuous single-stage TLC with flow detection	385
V.	Continuous TLC with a moving attached layer of sorbent	388
VI.	Centrifugal TLC	389
VII.	Thin-layer chromatography under pressure	390

I. INTRODUCTION

The method of thin-layer chromatography, proposed in 1938 by the Soviet scientists Izmailov and Shraiber,[1] is at present one of the principal procedures in liquid chromatography. It is used in the study of organic[2] and inorganic[3] substances and has found applications in biochemistry and pharmacology,[4,5] in medicine,[6] and in the analysis of the environment,[7] polymers,[8] etc. Such wide-scale application of TLC can be accounted for by the following advantages of this method: (1) the simplicity of the equipment and of the experimental methods, (2) the possibility of altering purposefully and relatively simply a large number of parameters optimising thereby the separation process, (3) economy, (4) the possibility of separating simultaneously and independently a large number of samples in the course of a single determination (up to 20 samples). Important features of TLC such as the possibility of the rapid and simple replacement of the mobile and stationary phases, which determine the selectivity of the method,[4,9-15] and of using the two-dimensional separation technique,[4,9,10,16] which significantly increases the real resolving power of the method must also be specially emphasised. The advantages of TLC include also the fact that on a plate it is possible to analyse all the mixture components and not only those which have reached the detector, as happens in column chromatography.

The commonest variant of liquid chromatography is at the present time column liquid chromatography (CLC) and especially its modern version—high performance liquid chromatography (HPLC). However, the features of TLC mentioned above ensure that it has many advantages over CLC, which have also been responsible for the fairly wide scale employment of TLC in analytical practice. Furthermore, after comparing CLC and TLC, certain investigators give preference to the latter.[17-19] It has been suggested in a review[20] that, although HPLC is superior to high performance TLC (HPTLC) as regards efficiency and the number of components separated, nevertheless the difference between them is not fundamental.

However, compared with HPLC, traditional TLC suffers also from certain disadvantages; (1) a lower reproducibility of the retention parameters, (2) usually a longer separation time, and (3) the existence of certain complicating factors which may have a negative influence on the reproducibility of the chromatographic process (for example, the separation into layers of the solution of the multicomponent mobile phases as they move through the sorption layer,[21] which has been observed in many instances, and the evaporation and adsorption of the vapour of the mobile phase), and (4) a more laborious and complex automation of the entire process—from the introduction of the sample and the preparation of the chromatographic plate up to the quantitative assessment of the results obtained.

The majority of the disadvantages of traditional TLC are associated with the use of a non-stationary virtually uncontrolled flow of the mobile phase (see, for example, Refs. 4, 9, 10, and 22-24). The flow rate of the mobile phase in the layer signigicantly during elution and the distance traversed by the front of the mobile phase Z_f is quadratically related to the elution time t:[9,22-25]

$$Z_f^2 = kt \ , \qquad (1)$$

where k is the rate constant, which depends on the nature of the mobile and stationary phases.

It follows from Eqn.(1) that the rate of movement of the mobile phase front diminishes appreciably as one moves away from the site where the eluent is supplied. It is also essential to note that the pressure drop caused by capillary forces precludes the achievment of optimum rates of movement of the mobile phase throughout the elution zone. For this reason, elution on most of the plate takes place in traditional TLC at rates below the optimum values.

The non-stationary nature of the flow of the mobile phase, the formation of an unsaturated zone in the region of the eluent near the front, in which the content of the solvent relative to the sorbent differs significantly from the equilibrium value,[9,25-27] and the processes involving the evaporation and adsorption of the components of the mobile phase[28] have

an appreciable negative influence on the effectiveness of the separation, the reproducibility of the retention parameters, and the chromatographic process time. A number of studies[29–33] have been devoted to the optimisation of the chromatographic process in TLC, mainly by means of the concerted selection of the sorbent, the mobile phase, and the length of the chromatographic plate. In our view the creation of, firstly, a stationary flow, and, secondly, the optimum flow of the mobile phase, or one approaching the optimum, also plays an important role in the optimisation of the separation process in TLC. The use and development of elution methods which make it possible to obtain a stable flow of the mobile phase (which it is desirable to contol) is one of the promising trends in TLC, because, in combination with the known methods for the optimisation of separation conditions,[29–33] it helps to overcome the principal disadvantages of the traditional TLC, among those enumerated above, retaining at the same time most of its advantages. This is why the present review is devoted to the consideration of methods of TLC with a controlled flow of the mobile phase (TLC-CF).

II. CLASSIFICATION OF TLC METHODS WITH CONTROLLED FLOW OF THE MOBILE PHASE

Variants of TLC in which a stationary flow of the mobile phase, controlled only by employing special methods involving elution and action on the chromatographic system by various physical forces can also be usefully included among TLC-CF methods. Among the variants of TLC-CF known at the present time, it is possible to distinguish two groups: continuous TLC methods and TLC methods with forced flow of the mobile phase. Such subdivision is caused primarily by the difference between the forces responsible for the movement of the mobile phase. In the first group of methods, these are capillary forces. This group includes the continuous combined (two stage), the continuous single-stage (flow), and the continuous TLC methods with a moving attached layer of the sorbent.

In continuous two-stage TLC the sample is deposited on the dry sorbent after which the mobile phase is eluted up to the point where it is removed from the bed under the conditions of the usual TLC with a variable rate of elution (first stage); next, until the end of separation, the rate of elution remains constant (second stage). The rate of elution in the second stage depends on the length of the elution zone, i.e. on the distance between the site where the eluent is supplied and the site where it is removed.

In continuous single-stage TLC the sample is injected directly into the steady flow of the mobile phase. The rate of elution from the instant of the introduction of the sample up to the end of its separation is constant.

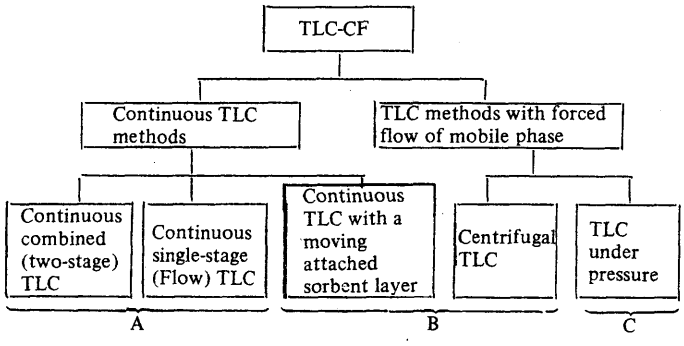
In the third method of this group, the sorbent, attached to a flexible support, moves in the direction opposite to that of the component to be separated. The sample can be deposited on the dry sorbent or can be injected into the flow of the mobile phase.

The term "forced flow TLC" was introduced by Kalász[34] quite recently. It has been used directly to designate the method of TLC under pressure (TLC-P),[35] in which the movement of the mobile phase in the sorbent layer, as in HPLC, is achieved as a result of the excess pressure at the entry of the eluent into the chromatographic system. However, we think that it is more useful to include in the concept of "forced flow TLC" (TLC-FF) all the variants of TLC in which any external factor, for example, the centrifugal force or a magnetic field, acting on the given chromatographic system, determines the character of the movement of the mobile phase. Accordingly, centrifugal TLC, where the eluent moves mainly under the influence of centrifugal forces, has been assigned by the present authors to TLC methods with forced flow of the mobile phase (TLC-FF), like TLC-P.

We may note that descending TLC,[36] in which the force of gravity increases the rate of movement of the mobile phase compared with the ascending and horizontal variants of elution, could also be included in TLC-FF. However, because of the week influence of the gravitational component on elution,[37] this method is regarded only as a variant of continuous TLC.

A classification of the methods of TLC-CF is presented below. It demonstrates the possibilities of different variants of TLC-CF as regards the control of the flow of the mobile phase. In the TLC-P method (group C) the range of flow rates generated is virtually the same as in HPLC. The use of group B methods shortens the time of analysis by a factor of 2–5 and that of group A methods by a factor of 1.5–3 compared with traditional TLC.

Scheme



It is noteworthy that this proposed subdivision of TLC-CF methods into two groups is fairly arbitrary. For example, continuous elution conditions are realised in both centrifugal TLC and in TLC-P.

Table 1. Principal variants of liquid chromatography.

Characteristic	Traditional TLC	TLC with a controlled flow of mobile phase	CLC
Type of sorption system used	open	open or closed	closed
Cause of movement of mobile phase in sorption layer	capillary forces	capillary forces or pressure drop across layer	pressure drop
Flow rate of mobile phase lengthwise along the layer	variable	constant (controlled)	constant (controlled)
Possibility of controlling the flow of mobile phase	does not exist	exists	exists
Method of detecting components	on sorbent layer (static)	static and dynamic	in stream of mobile phase (dynamic)
Detection sensitivity*	high	high	high
Reproducibility	moderate	high	high
Number of samples analysed simultaneously	large	large	limited (usually one)
Use of sorption layer	single**	single**	repeated
Consumption of sorbent in series of analyses	large	minimal	minimal

*CLC has the following advantages over traditional TLC: 1) absence of sorbent in the detection zone and 2) long light path in the measuring cell.

**Certain types of chromatographic plates in TLC can be used repeatedly after regeneration.

The principal variants of liquid chromatography are compared in Table 1 in terms of the sorption system employed, the character of the forces responsible for the movement of the mobile phase, etc.

III. CONTINUOUS COMBINED (TWO-STAGE) TLC

The term "continuous chromatography" is used in TLC in two senses. Firstly, in order to designate the process involving the separation of the sample which is continuously supplied into the chromatographic system. In continuous separation the mobile phase, on the one hand, and the sample and the sorbent layer, on the other, move in different directions. This method was first proposed by Martin[38] in planar (paper) chromatography and was realised by Guiochon et al.[29] Secondly the term is used to designate the variant of TLC in which only the flow of the mobile phase is continuous and the volume of this phase is greater than that necessary to wet the sorbent throughout the length of the chromatographic plate. Here and henceforth we shall only use the second meaning of the term "continuous chromatography".

The principal aim of continuous TLC is the improvement of the separation of the mixture analysed by increaseing the distance over which the components to be separated migrate.

The initial variant of continuous TLC was the combined (two-stage) method. It was first used[40] in ascending chromatography on a free layer of alumina. The component to be separated was deposited on a dry layer of sorbent and elution was then carried out. The mobile phase was evaporated from one end of the chromatographic plate. In this case the separation process proceeds in exactly the same way as in the usual TLC after the attainment of the edge of the plate. The eluent reaches the edge of the plate, as stationary flow of the mobile phase is established along the sorbent layer. The flow rate is determined by the rate of migration of the front of the mobile phase at the end of the elution zone. Changes in the rate of movement of the solvent for the given combination of the mobile and stationary phases can be achieved by altering the length of the elution zone. [In a recently published study† an attempt was made to optimise the separation conditions in continuous TLC as a function of the rate constant of the movement of the eluent, the degree of separation, and the R_f for the components separated.]

Somewhat later Stanley and Vannier[41] and Mistryukov[42] developed novel and elegant methods for descending two-stage continuous TLC. Interesting procedures for the application of this variant of TLC have also been described in other communications.[43,44] However, the method involving constant flow of the mobile phase, proposed by Truter and Van Den Eijnden[45–47] and also the method of the gradient flow of the mobile phase or the evaporation method[48,49] are most frequently used. The differences between them are illustrated fairly clearly in Fig.1.

In order to carry out continuous TLC under identical conditions, Truter[45,47] suggested that a special lid be employed for the chromatographic chamber with a slit in which the plate is inserted. The mobile phase rapidly evaporates outside the limits of the chamber. Later a similar design was proposed independently by Van Den Eijnden[46] and was successfully applied in subsequent investigations.[50]

We may note certain useful modifications of Truter's method. In one investigation[51] a layer of charcoal was proposed to accelerate the process involving the "removal" of the mobile phase from the end of the chromatographic plate. The use of a paper wick for the removal of the eluent from the plate and for its supply through a slit to the outside of the chamber for the purpose of subsequent evaporation has been described.[52,53] The sorbent itself can be used to collect the mobile phase by placing a thick layer of the former ("roller") on the edge of the chromatographic plate.[54–56]

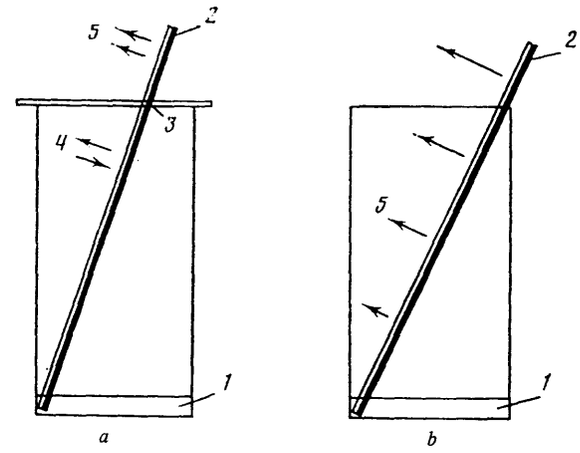


Figure 1. Continuous TLC methods:[20] *a*) the method of the constant flow rate of the mobile phase;[45–47] *b*) the method of the flow rate gradient of the mobile phase along the layer:[48,49] 1) mobile phase; 2) thin-layer plate on which separation takes place; 3) slit in the lid of the chamber through which the upper end of the plate passes; 4) equilibrium in the sorption and desorption of the mobile phase vapour (the gas phase in the chamber is saturated by the mobile phase vapour); 5) evaporation of the mobile phase from the plate (a flow rate gradient of the mobile phase arises as a result of evaporation along the plate).

Thus a chamber saturated by the vapour of the mobile phase is used in Truter's method (Fig.1*a*) and its modifications and the solvent is evaporated outside its limits. For this reason, the rate of movement of the mobile phase along the plate is constant within the chamber.

Subsequently, chambers with a small volume of the gas phase came to be widely used. The first chamber of this type was proposed by Brenner and Niederwieser[57] specially for continuous TLC.[57] It was subsequently significantly improved by a number of investigators.[58–62] In our view, a novel chamber distinguished by simplicity and convenience in use, described by Soczewiński and co-workers,[61,62] merits special attention.

In the second known variant of continuous TLC (Fig.1b), the mobile phase is evaporated from the chromatographic plate already in the separation zone and during separation.[48,49] As a result of the partial evaporation of the eluent, the rate of movement of the mobile phase diminishes from the beginning to the end of the plate. This negative flow rate gradient results in a decrease of the width of the band (the band is compressed) and the separation is improved, which is especially important for the analysis of impurities. However, the main disadvantage of this interesting variant of continuous chromatography is inadequate reproducibility of the experimental conditions, which is naturally reflected also in the

†E.K.Johnson, M.J.Wenning, R.E.J.Tecklenburg, and D.Nurok, J.High Resolution Chromatography and Chromatography Comm., 1986, **9**, 285.

reproducibility of the retention parameters, the effectiveness, and the separation factors for the compound analysed.

Subsequently a new method, based on the intensification of the evaporation process—the so called "hot plate" method—was proposed and developed.[63,64] In this method a zone of increased temperature is created on the plate where accelerated evaporation results in an appreciable gradient of the flow rate of the mobile phase, leading to the compression of the chromatographic bands.

The variants of continuous TLC described (the evaporation and hot plate method) can be usefully employed, in our view, mainly for the concentration of impurities in analysis.

In the practical application of continuous chromatography, the isothermal variant is most often used.

All the variants examined presuppose a two-stage continuous TLC scheme. The presence of the first chromatographic stage and the deposition of the specimen to be separated on the dry sorbent greatly reduce the reproducibility and effectiveness of the continuous TLC method. Apart from this, the separation conditions selected with the aid of this method are fairly difficult to use in HPLC. This in fact stimulated the development of the single-stage continuous TLC method in which the sample is injected directly into the flow of mobile phase.[65] An important feature of this method is the detection of the components to be separated in the course of elution as in HPLC. For this reason, the single-stage continuous TLC will be considered in greater detail below in the Section devoted to the single-stage TLC with flow detection.

The continuous TLC method with sample injection into the stream of eluent is frequently referred to as flow TLC,[66] and the two-stage variant as continuous TLC. The concepts of "two- and single-stage continuous TLC" introduced in this review reflect the significant differences between these variants of TLC. This is necessary also because in the literature the flow variant is frequently referred to as continuous and conversely, although, as shown above, they differ fundamentally.

IV. CONTINUOUS SINGLE-STAGE TLC WITH FLOW DETECTION

An important independent stage in any chromatographic method is the assessment of the results of separation or the detection stage. In continuous TLC the following procedures can be used to determine the qualitative and quantitative compositions of the mixture analysed: (1) scanning (optical) of the plate with the separated chromatographic bands (see, for example, Refs.23 and 24), (2) continuous measurement of the concentrations of the compounds analysed in the moving eluent on the chromatographic plate or beyond it, and (3) a combination of both procedures: a method of detection in the flow of mobile phase for "light" components and a scanning method for "heavy" components or in general for components which are not eluted under the given conditions.

The first and third procedures appear to us more laborious compared with the second but they are optimal in the solution of many analytical problems. At the present time scanning is the commonest method for the automatic assessment of the results of separation in TLC.

The application of the second method actually implies the creation of a liquid-type thin-layer flow chromatograph. The role of the column in the latter is fulfilled by the thin-layer plate whose absorption layer is open from at least one side. Since in our view unjustifiably little attention has been devoted in the literature to the second variant of continuous TLC, although it makes it possible to reduce significantly the amount of labour involved in TLC whilst retaining its traditional advantages, it appears useful to discuss this method in greater detail. Previously it was considered only in a monograph[67] and in a review.[68]

The consideration of the method of continuous TLC with flow detection in a review devoted to different ways of controlling the mobile phase can be justified by the fact that the single-stage continuous (flow) TLC method was first devised precisely as a variant of continuous TLC with flow detection. The application of a flow detector assisted in a more successful development of continuous TLC.

The method of continuous TLC was initially developed for the rapid selection of experimental conditions in column liquid chromatography.[65]

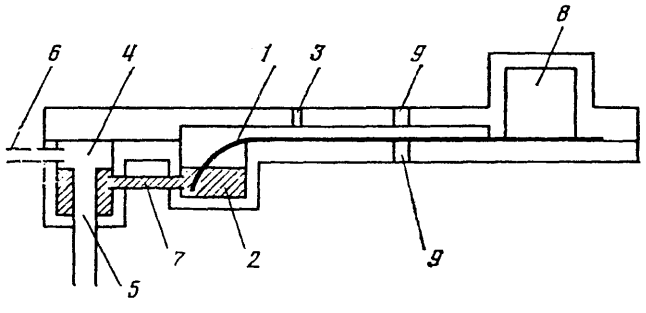


Figure 2. Schematic illustration of the first thin-layer chromatograph:[65] 1) TLC plate on a flexible transparent support; 2) reservoir for the mobile liquid phase; 3) aperture in the cover plate for the introduction of the sample onto the chromatographic plate; 4) preliminary chamber for the mobile phase with the drain tube *5*; 6) tube for the supply of the mobile phase; 7) connecting tube; 8) reduced pressure chamber for the evaporation of the mobile phase; 9) apertures for detection.

The thin-layer chromatograph employed by Schmid et al.[65] is shown in Fig.2. The constancy of the supply of the mobile phase is ensured by maintaining a virtually constant level of liquid in the working reservoir *2*, which communicates with the chamber *4*. The sample is injected from a micropipette via the aperture *3* in the upper lid. We may note that, in order to compensate for the possible errors in the measurement, a double chromatographic system is used. The two parts of a single flexible thin-layer plate work independently: in the chromatograph there are two apertures in the upper lid for the injection of the samples analysed and two apertures for the light guides with the aid of which the results of separation are recorded. The light guides, connected to a light source, are fitted with a green filter (550 nm). A photoresistor is used as the radiation detector. The liquid mobile phase is removed from the plate in chamber *8* under vacuum. The distance between the site of sample injection and the detection region is about 4 cm.

Fig.3 presents as an example the chromatogram of a mixture of dyes obtained on a thin-layer chromatograph of the first type. The parameters of the chromatographic bands of the dyes separated are listed in Table 2.[65] Taking into account the fact that the data presented in Table 2 refer to a short length of the layer (4 cm), they must be regarded as entirely satisfactory. For comparison, we may note that the columns used in the initial period of the development of gas–liquid chromatography were characterised by an efficiency close to that given in Table 2.

The method of continuous TLC with flow detection has been subjected to a general assessment.[69] It was shown that the continous-eluent planar chromatography can be used to separate both organic and inorganic compounds and that not only inorganic but also organic adsorbents, including ion-exchange adsorbents, can be employed as the stationary planar phase. In many instances it is useful to employ chromatographic paper as the sorbent in planar chromatography. Different gradient methods for the improvement of separation and, in particular, methods based on the use of sorption layers of different shape are of interest.[70,71]

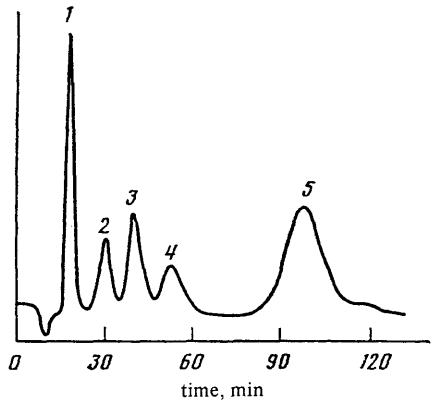


Figure 3. Chromatogram of a mixture of azo-dyes obtained on the first thin-layer chromatograph;[65] separation conditions: stationary phase—"Polygram Sil C" TLC plate, thickness of silica gel layer 0.25 mm, mobile phase—toluene mixed with 2% of ethanol; the designation of the peaks correspond to the numbers of the dyes in Table 2.

Table 2. Characteristics of the separation of azo-dyes under the conditions of continuous TLC with flow detection (mobile phase—toluene).[65]

Dye	*t*, min	*c*, mm	*N*, t.p.
N-Ethyl-1-[(*p*-phenylazophenyl)azo]-2-naphthylamine	17.5	4.5	648
1-[(*p*-n-Butylphenyl)azo]-2-naphthol	39	10	654
1-[(*p*-Methoxyphenyl)azo]-2-naphthol	61	17	501
1-[(*m*-Methoxyphenyl)azo]-2-naphthol	90	25	506
1-[(*o*-Methoxyphenyl)azo]-2-naphthol	162	41	683

Notation: *t* is the retention time, *c* the width of the separated component band, and *N* the number of theoretical plates (t.p.).

Continuous planar chromatography with flow detection makes it possible to employ the advantages of two main methods of liquid chromatography: the planar method characterised by flexibility and ease of replacement of the column-plate, economy, etc. and the column method which ensures, a high reproducibility of the retention parameters, a high degree of automation of the process, etc.

The method of continuous-eluent chromatography with flow detection can be applied in two versions: version I—the specimen analysed is deposited on the dry sorption layer and elution is then carried out by means of a flow of the mobile phase (the traditional method of sample deposition in TLC) and the components separated are detected by means of a flow detector; version II—the specimen analysed is deposited on the sorption layer wetted by the moving mobile phase and the components separated are detected by the flow detector (the method used in continuous TLC[61]).

In order to assess these versions of sample injection in continuous TLC, two series of measurements, comprising seven repeated separations on the same plate, were carried out.[69] The average rates of movement of azo-dyes on the TLC plate obtained using both versions as well as the reproducibility of these quantities are presented in Table 3.[69] The maximum deviation of the rate of movement of the chromatographic band from its average value (ψ_{max}, %) was used as the characteristic of the reproducibility. The measurements were performed using two mobile phases: benzene and toluene. Table 3 shows that the linear rate of migration of the chromatographic band (or the retention parameter) is reproducible, under conditions where the sample is injected into the stream of eluent (version II), with a random error 6–10 times smaller than in the deposition of the sample on the dry sorbent layer (version I). The time of analysis, determined from the retention time of the last extra component, was the same for both versions, but the retention times of light components are shorter for version I, apparently because of the higher flow rate in the initial period of the spreading of the mobile phase through the dry sorbent and the presence of a region of unsaturation near the solvent front.

Table 3. The average rates of migration of azo-dyes U_a on the chromatographic plate and the characteristic of their reproducibility $\psi_{max} = (\Delta U_{max}/\bar{U}_a) \times 100\%$ under the conditions of continuous TLC with the substance analysed introduced onto the dry layer of sorbent (variant I) and on the sorption layer wetted by the moving eluent (variant II).[69]

Sorbent (stationary phase)	Azo-dye	$\bar{U}_a$, mm min^{-1}				ψ_{max}, %	
		benzene (I)	toluene (I)	benzene (II)	toluene (II)	benzene (I)	toluene (I)
KSK silica gel + 5% of gypsum	*1*	1.82	1.70	1.27	0.99	22	2
	2	0.95	0.37	0.69	0.66	20	0
	3	0.57	0.46	0.54	0.38	7	2
	4	0.42	0.32	0.41	0.28	12	2
	5	0.30	0.21	0.30	0.20	10	0
Kieselgel 60F 254	*1*	2.40	2.18	2.00	0.92	16	1
	2	1.72	0.71	1.33	0.42	21	1
	3	0.90	0.33	0.67	0.24	13	0
	4	0.50	0.22	0.45	0.18	8	2
	5	0.30	0.10	0.31	0.11	4	2
Silpearl + 5% of gypsum	*1*	3.87	2.50	2.29	2.00	13	1
	2	1.56	0.92	1.21	0.88	16	1
	3	1.04	0.58	0.89	0.51	11	0
	4	0.65	0.33	0.60	0.30	8	2
	5	0.48	0.22	0.48	0.22	6	0

Notation: ΔU_{max} is the maximum deviation from the average rate of migration of the azo-dye in a series of experiments; 1) azobenzene; 2) Dimethyl Yellow; 3) *p*-methoxyazobenzene; 4) *p*-aminoazobenzene; 5) Sudan-III.

Analogous results have been obtained in the separation of aminoacids. Chromatograms of mixtures of dyes and aminoacids are presented in Fig. 4. The variation of the base line in the region of light (weakly retained) components on chromatograms *a* and *c* can be accounted for by the fact that, when the sample analysed is deposited on the dry sorbent,

the arrival at the detector of the front of the mobile phase is accompoanied by a gradual alteration of the signal as a result of the movement through the detector of the unsaturation zone with a variable content of the eluent. When the sample is deposited on the sorbent layer and the stream of eluent is moving at a constant rate, this phenomenon is naturally not observed (chromatograms *b* and *d*).

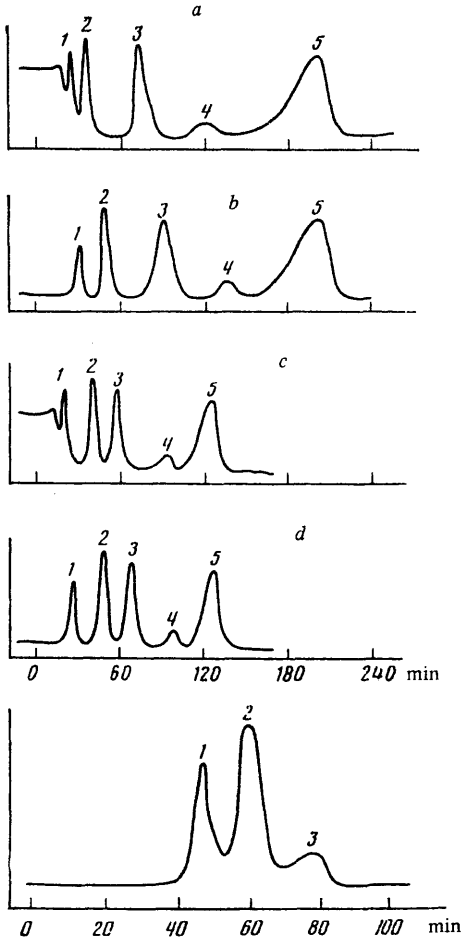


Figure 4. Chromatograms of mixtures of azo-dyes and amino-acids obtained on a thin-layer chromatograph:[69] *a*)–*d*) separation of a mixture of five azo-dyes (the designations are indicated in Table 3) on plates with "Kieselgel 60F 254" silica gel from the Merck Company (*a* and *b*) and "Silpearl" silica gel from the Cavalier Company (*c* and *d*); mobile phase—benzene, 600 nl sample introduced onto the dry layer of sorbent (*a* and *c*) or onto a layer of sorbent with a moving stream of eluent (*b* and *d*); *e*) separation of a mixture of 4-amino-salicylic acid (1), tryptophan (2), and histidine (3); stationary phase—"Silpearl" silica gel + 5% of gypsum, mobile phase—70:30 ethanol–water mixture; sample introduced onto the layer of sorbent with a flowing eluent; distance from the point of sample injection to detection zone 40 mm.

Since in the case where the sample is injected on the sorbent layer with the eluent in continuous TLC with flow detection (CTLC-FD) the separation process does not differ in essence from those occurring in column chromatography, this version of TLC is apparently one of the most rational procedures for the selection of optimum separation conditions in liquid column chromatography, to which attention was drawn by Schmid and Cramer.[65] The possibilities of CTLC-FD as regards the optimisation of separation conditions have been demonstrated in relation to the analysis of a mixture of dyes.[72] However, in our view, this method is undoubtedly also of independent importance. For this reason, we may note some of its positive features. Firstly, CTLC-FD makes it possible to replace the mobile and stationary phases easily and simply. Secondly, the method permits the analysis of specimens containing components which are not in fact eluted, since the periodic replacement of the thin-layer plate presents no difficulties. Thirdly, the method approaches column chromatography as regards the level of automation.

It is important to note that the injection of the sample into the stream of mobile phase beyond the line of the solvent front makes it possible to obtain more symmetrical peaks.[62] Thus this method of sample injection is of interest also for the usual variants of TLC. CTLC-FD occupies an intermediate position between the two- and single-stage variants of continuous TLC, since the eluent flow has not yet reached a steady state despite the specimen being injected into it.

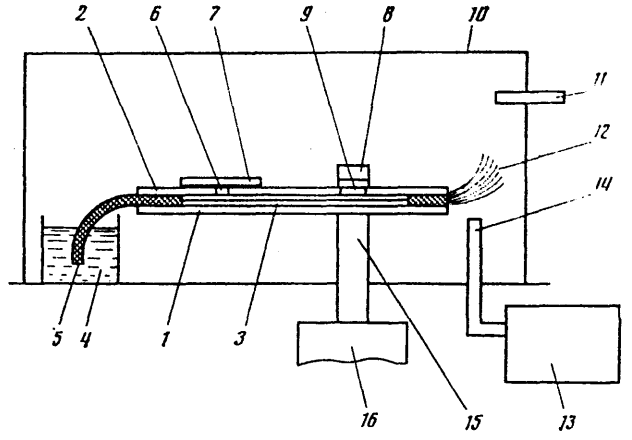


Figure 5. Schematic illustration of a thin-layer chromatograph:[69] 1) base (lower lid) of the chamber for the thin-layer plate; 2) upper lid of the chamber; 3) plate with sorption layer; 4) compartment for the mobile phase; 5) paper wick; 6) window for the introduction of the sample to be analysed; 7) lid; 8) radioluminescent light source; 9) slit diaphragm; 10) opaque jacket; 11) tube for the removal of air with the mobile phase vapour; 12) wick for the evaporation of the mobile phase; 13) air microcompressor; 14) pipeline for the supply of air; 15) light guide; 16) photoelectric multiplier.

The design of the apparatus is important in any experimental method. A thin-layer chromatograph, differing somewhat from the first instrument,[65] has been described by Berezkin et al.[69] This liquid-type chromatograph is illustrated schematically in Fig.5. A wick exposed to a strem of air and not an evacuated chamber, as in the study of Schmid et al.,[65] is used here to remove the solvent from the chromatographic plate. The employment of the wick for the supply of solvent to the plate permitted a greater variety of types of plates

which can be used in the thin-layer chromatograph. In particular, in this instrument one can employ plates on a solid support. The use of a radioluminescent light source[73] permitted a simplification of the design and the employment of sources with different maxima in their emission spectra permitted the expansion of the region of applicability of the spectral detector. Not only glass but quartz plates are used as the material of the support for the adsorption layer, which creates the possibility of measurements in the ultraviolet part of the spectrum.[69] Other solutions have also been described.[74] They are based on the use of a small aperture in the sorption layer on the plate, which is usually opaque, as the optical microcuvette of the detector, where the concentrations of the compounds separated in the stream of mobile phase are measured. The filling of this aperture by the mobile phase does not entail an appreciable distortion of the chromatographic bands of the components separated. Fig.6 illustrates the possible types of microcuvettes in a thin-layer chromatographic plate.[74] The application of microcuvettes increases the sensitivity of the determination by a factor of 1.5–10 compared with variants in which there is a thin layer of sorbent in the path of the measured emission.[75] The diameter of the microcuvette does not exceed 1–3 mm. The possibility of using microcuvettes for the electrochemical detection of compounds separated has also been noted.[75]

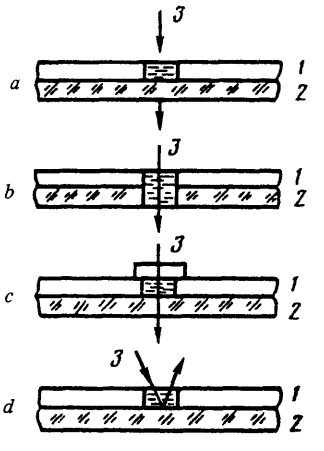


Figure 6. Types of optical flow microcuvettes in the TLC plate:[74] *a*) microcuvette in the sorption layer; *b*) microcuvette in the sorption layer and the support; *c*) closed microcuvette in the sorption layer; *d*) microcuvette in the sorption layer deposited on a support with a reflecting surface; 1) sorption layer; 2) support; 3) direction of light flux.

The efficiency of the separation when plates prepared in the laboratory with a thickness of the layer of 0.4 mm and a working length of the layer of 100 mm are used was $N = 700$ theoretical plates for the Sudan dye. The use of a plate from the Merck Company for high performance TLC made it possible to increase the efficiency to 3450 theoretical plates (for a working length of the layer of 60 mm).[69] This is significantly greater than the value obtained by Schmid et al.[65]

An interesting and promising trend in the development of flow TLC is its use for the separation of various compounds on various mobile phases consisting of two and more components. In chromatography under the conditions of single-stage continuous TLC, the "separation into layers" of the components of the mobile phase is unlikely, in contrast to traditional TLC.[21] This makes it possible to employ complex mobile phases for the separation of various sytems[72,76,77] and also to carry out the separation under the conditions of gradient elution.[78] The method and theory of continuous TLC separation using multicomponent mobile phases have been described in a recent study#. In the same investigation it was also shown that the data on separation obtained by continuous TLC agree satisfactorily with the results of separation by the HPLC method and can therefore be usefully employed for the optimisation of the separation conditions in HPLC.

The CTLC-FD method can be used successfully to estimate the separating properties of solvent powders,[79] which leads to yet another possibility, in principle, of employing TLC for physicochemical measurements.

We believe that, in analysing the prospects for the development of continuous TLC with flow detection, one should turn ones attention to the possibility of using slightly modified standard equipment for CTLC-FD, for example, a chromatograph with a U-chamber[80] as well as detectors based on the employment of laser-excited fluorescence,[81] photoionisation,[82] photoacoustic spectroscopy,[83] etc.

V. CONTINUOUS TLC WITH A MOVING ATTACHED LAYER OF SORBENT

A way[29,32,84] of increasing the efficiency of the separation per unit time is to increase the rate of elution and the distance over which the separation takes place. High flow rates of the mobile phase and high rates of continuous TLC can be achieved by shortening the elution zone,[37] which decreases in its turn the total number of effective theoretical plates. Separation in continuous TLC using a long elution zone entails a considerable fall in the flow rate of the eluent and hence a significant decrease of the specific efficiency.

Saunders and Snyder[85] have proposed an interesting solution of the problem of increasing the specific and general efficiency by increasing the rate of elution with a simultaneous increase of the distance over which the components separated migrate in the course of analysis under the conditions of continuous TLC. They developed a method in which an infinite strip on which the sorbent has been deposited moves in the direction opposite to that of the movement of the eluting substances. This method has been called drum TLC.[85] It is based on the fact that in the course of elution the distance between the front of the mobile phase and the site where it is supplied remains unchanged. By maintaining this distance as small as possible, one achieves high rates of elution. The separation in drum TLC takes place in the following manner. The plate on a flexible base is attached to a cylinder, the specimen to be separated is deposited, and then the cylinder is lowered into the eluent in such a way that the specimen remains above the surface of the liquid eluent. After the front of the mobile phase has reached the evaporation line, the drum is made to rotate against the direction of the migration of the eluent at a rate equal to that of the frontal migration but having the opposite sign. Thus the separating layer migrates relative to the spot and the immersion and

#E.Soczewiński, in "Planar Chromatography", Vol.1 (Edited by R.E.Kaiser), Huethig, Heidelberg, 1986, p.79.

evaporation (of the front) lines at the same rate. Consequently the components separated can be eluted through a sorption layer of any length at a fairly high rate. The authors[85] showed that the method of drum TLC is a more effective and shorter analytical procedure than the traditional and usual continuous TLC and its efficiency is also superior to that of centrifugal TLC.

However, drum TLC, which gives rise to highly effective and rapid separation, suffers from a series of limitations. Firstly, this method makes it possible to separate only substances which differ only slightly in their R_f. Secondly, it is fairly difficult to maintain the necessary rate of rotation of the drum in the separation of colourless substances. Nevertheless, this method, which is relatively unsuitable for the separation of multicomponent mixtures consisting of substances with markedly different R_f, can prove to be extremely effective for the separation and analysis of substances having similar properties, for example, optical isomers.

A principle analogous to that employed in drum TLC has been used in the method of three-chamber TLC.[86] In this case the chromatography is achieved on a long looped foil strip coated by a thin layer of sorbent. During its movement, the foil enters the elution device consisting of three glass chambers (Fig.7).

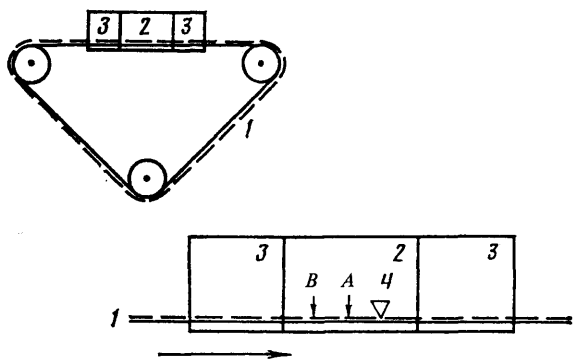


Figure 7. Schematic illustration of three-chamber TLC:[86] 1) foil with chromatographic layer; 2) main chamber; 3) lateral chambers; 4) channel for the supply of the solvent; A) and B) substances to be separated.

The separation takes place in the middle chamber. The other two serve for the evaporation of the solvent from the layer of sorbent. The rate of movement of the foil with the adsorbent is regulated by means of an electric motor connected to rollers. The mixture separated is deposited on the sorbent and placed in the middle chamber through which eluent is then supplied. During chromatography, the rate of movement of the strip should be such that the component to be determined is at the same distance from the eluent front throughout. At the end of the chromatographic process, the speed of the chromatogram is increased and all the components separated are moved in succession to the side opposite to that where the eluent is supplied. After drying each chromatographic band, a clear cut separation of the specimen over a comparatively short distance is achieved.

A method similar to drum TLC has been described by Halpaap and Bausch.[87] In this method the film with the sorbent layer is rewound from one coil to another coil underneath. As in drum TLC, the latter is immersed in the reservoir with the eluent. In this case the separation section is much longer than the circumference of the cylinder in drum TLC. The authors[87] carried out the separation process on sections of the sorption layer of different lengths.

It is noteworthy that the continuous TLC methods considered here are variants of eluent chromatography with the layer of sorbent moving relative to the point of supply of the eluent.[88,89]

VI. CENTRIFUGAL TLC

Centrifugal chromatography, first proposed[90] as a variant of paper chromatography, was subsequently used in TLC.[91,92] This method is applied in two versions: the linear and radial versions.[85] In the radial or circular version of the centrifugal TLC, the system chromatographed rotates about its axis so that the movement of the mobile phase is accelerated by centrifugal forces, as in the linear version. In the radial centrifugal TLC, the mobile phase is supplied to the centre of a circular sorption system. The flow rate of the mobile phase can be regulated by means of a micropump. According to the authors,[85] centrifugal forces play the same role as pressure in HPLC.

In linear centrifugal TLC, the quantity equivalent to pressure in HPLC can be expressed by the relation[85]

$$P = \rho\omega^2 Z_f^2/2 , \qquad (2)$$

where ρ is the density of the mobile phase, ω the angular velocity of the plate, and Z_f the distance from the point of supply to the front of the mobile phase.

On the basis of the foregoing, centrifugal TLC can be classified as a TLC method with a controlled forced flow. The flow is stabilised by employing continuous TLC conditions and the rate of elution is regulated by the rate of rotation of the support. We may note that the rate of elution is directly proportional to the square of the angular velocity.[85]

A detailed critical review on centrifugal chromatography,[93] written in 1964, has not lost its importance today. The authors[4] believe that the economy of time in the elution of thin-layer chromatograms by centrifugal TLC is not very great. It is impossible to agree fully with this view. For very long separations, this method is undoubtedly economically justified and it has continued to be used in analytical practice in recent years: the reversed phase centrifugal TLC has been used successfully for the separation of polar compounds of plant origin§ and the ion-exchange version of centrifugal TLC has been employed for the determination of Fe, Co, Ni, Cu, Hg, and other metals¶. Different aspects of the application of centrifugal TLC have been discussed in a number of studies.[94-96] The possibilities of centrifugal TLC for preparative separation have been demonstrated by Stahl and Müller,[97] who showed that the specific sample charge in this method can amount to between 10 and 1000 mg per millimetre of the thickness of the absorption layer for one compound. The results of a detailed study[97] are of interest not only for preparative but also for analytical chromatography. Fig.8 [97] illustrates the dependence of the separation time on the overall flow rate of the mobile phase. It follows from the data presented that an increase in the flow rate of the mobile phase leads to a significant shortening of the chromatographic process, which is particularly pronounced in the region of low rates. Fig.9 [97] presents the dependence of the separation

§ F.Orsini and L.Verotta, J.Chromatog., 1985, **349**, 69.

¶ Song Yirong and Yin Bohai, Xibei Daxue Xuebao, Ziran Kexueban, 1985, **15**(2), 56; Chem.Abs., 1986, **104**, 179, 193.

factors for hydrazones (dyes) on the volume rate of supply of the mobile phase and the rate of rotation of the chromatographic system. The maximum resolution for the average flow rate of the mobile phase (5.2–3.5 ml min^{-1}) was obtained in the region of relatively low rates of rotation of the chromatographic system (~750 rev min^{-1}).

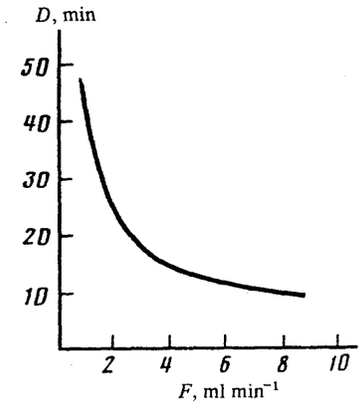


Figure 8. Dependence of the separation time D on the volume flow rate of the mobile phase F; experimental conditions: stationary phase—silica gel 60 with 1.5% of acronal, mobile phase—80:20 hexane–ethyl acetate mixture, rate of rotation 750 rev min^{-1}.[97]

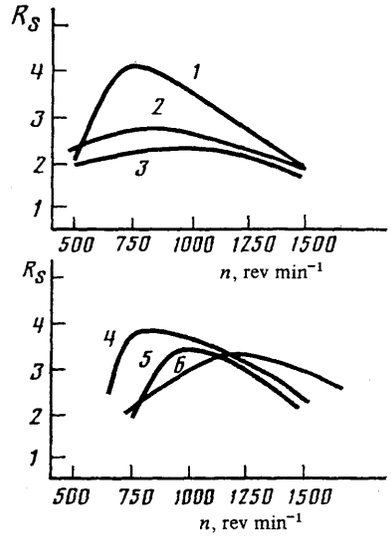


Figure 9. Dependence of the separation factor R_S for hydrazone dyes on the rate of rotation of the chromatographic system n;[97] experimental conditions: stationary phase—silica gel 60 with 1.5% of acronal, mobile phase—80:20 hexane–ethyl acetate mixture, volume rate of supply of mobile phase to the chromatographic system (ml min^{-1}): 1) 3.5; 2) 2.1; 3) 1.1; 4) 5.2; 5) 7.1; 6) 8.8.

In comparing preparative centrifugal TLC with traditional preparative TLC and column chromatography, the authors[97] arrived at the justified conclusion that this method has definite advantages.

Attention should be drawn to the recent appearance of several new methods and variants of apparatus involving centrifugal TLC.[98–100] A new method of planar preparative chromatography—sequential centrifugal layer chromatography (SCLS)—has been described.[98] According to the authors of the method, it combines the advantages of preparative and analytical centrifugal, antiradial, and sequential TLC. The apparatus for SCLC makes it possible to carry out circular chromatography with centrifugation at different rates of rotation. The supply of the eluent to any point on the plate makes it possible to separate additionally the individual components using individual mobile phases. Furthermore, there is a possibility of carrying out antiradial chromatography under the influence of capillary forces. Thus the SCLC method is in fact a hybrid procedure which expands the possibilities of centrifugal TLC.

A new centrifugal countercurrent chromatograph for partition chromatography has been proposed by Japanese investigators.[99] A rapid preparative separation of a series of mixtures of aminoacids, sugars, and saturated aliphatic acids has been achieved with its aid.

The possibilities of centrifugal TLC for a significant increase of the flow rate of the mobile phase in HPTLC have been demonstrated.[101] It has been shown that the results obtained in three-minute separation with centrifugation are equivalent to 12-minute separation with the aid of the usual circular TLC.

The method of centrifugal paper chromatography, its possibilities, and the prospects for its development have been considered in a review.[102]

The possibility of obtaining high flow rates of the mobile phase in centrifugal chromatography are limited. It is believed[85] that the use of rates of rotation at which pressures above 1 atm are created [see Eqn.(2)] would cause the rupture of the column of solvent and the mixing of air with the solvent at the point of its introduction. Yet another limitation is associated with the application of an open layer of sorbent in centrifugal chromatography: the increase in the resistance of the layer of sorbent with increase in the rate of elution can lead to the "spilling" of the eluent through the sorbent. This is in fact why centrifugal chromatography does not permit the attainment of the same high flow rates of the mobile phase as in HPLC.

VII. THIN-LAYER CHROMATOGRAPHY UNDER PRESSURE

The simplest, effective, and traditional method of attaining a high flow rate of the mobile phase or the layer of sorbent involves the creation of an excess pressure at the start of the sorption layer. However, an enclosed layer must be used to apply this method. This solution was proposed for TLC in 1977 by Hungarian investigators.[35] In fact, this is a variant of thin-layer column liquid chromatography, because the column contains a thin layer of sorbent. Thin-layer plates with an enclosed layer were known earlier in planar chromatography (see, for example, Refs.103–105), but they were not used in liquid chromatography.

A schematic illustration of a simple and novel apparatus[35,106,107] for TLC under pressure is presented in Fig.10. It is especially noteworthy that standard plates of any type can be used in this apparatus. The support of the chromatographic plate serves as the lower wall of the planar column, while the upper wall consists of the polymeric film-membrane *8*, which is clamped to the adsorption layer *6* of the plate *7* under the pressure of gas or liquid. The pressure at the inlet to a planar chromatographic column should naturally be lower than the external pressure used to clamp the polymer film to the

adsorption layer in order to form a closed chromatographic system—"column". Owing to edge effects, which significantly disturb the front of the mobile phase and disorganise the solvent stream under the conditions of increased pressure, in applying the linear TLC-P method it is essential to use either special plates with the sorption layer on a silica gel, alumina, or talc base[108] alternatively a material inert towards the given mobile phase must be deposited on the edges of standard plates. Paraffin or various plastics are used as materials of this kind. Fig.11 illustrates plates for TLC-P. In order to form the linear front of the mobile phase over the adsorption layer of the TLC plate, an insert with a channel connected to the aperture through which the solvent is supplied to the planar chromatographic system is introduced. The channel can be made also directly in the adsorption layer having removed part of the sorbent.[109] Under the conditions of increased pressure, the solvent very rapidly fills the capillary channel, which thus becomes a linear source of the solvent and actually fulfills in TLC-P the role of a small bath with a mobile phase used in the usual TLC. Fig.11 illustrates two versions of linear TLC on standard plates: *a*—elution in one direction and *b*—elution in two opposite directions. Two dimensional separation,[110] which is known to be characterised by the maximum peak capacity compared with other TLC methods, can be achieved by means of TLC-P.

Other variants of traditional TLC, differing in the form of the sorption layer, can also be used successfully in the TLC-P method: the triangular,[71] anti-circular,[111] and circular[23] variants. The use of circular chromatography in this variant of TLC has been described, for example, in Refs.107, 109, and 112. It is apparently simplest to apply this variant of TLC-P in practice, since there is virtually no need to cover the edge of the plate by any material. The samples analysed are usually deposited on the plate in a preliminary step before the plate is placed in the TLC-P apparatus. A number of studies[113–116] have been devoted to questions of the optimisation of the operating conditions in TLC-P. Kalász and Nagy[116] believe that the optimum linear flow rate of the mobile phase depends on the size of the particles of the sorption layer and is in the range 0.10–0.28 mm s^{-1}. The TLC-P method was subsequently developed in other investigations.[117–120]

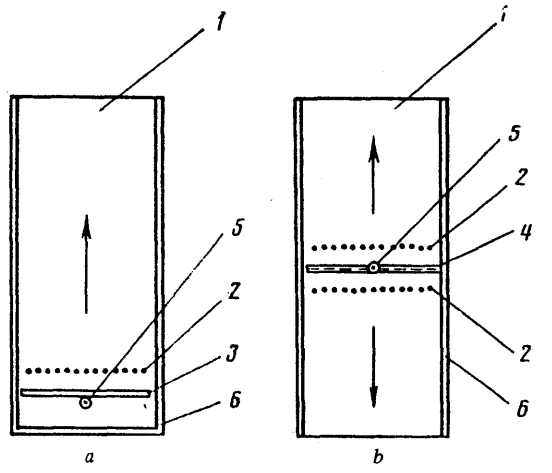


Figure 11. Linear elution in one direction (a) and in two opposite directions (*b*) in TLC-P:[109] 1) chromatographic plate; 2) region of deposition of samples analysed on the sorbent layer; 3) capillary channel in the adsorption layer for the organisation of the linear mobile phase front; 4) insert with a capillary channel for the mobile phase; 5) inlet for eluent; 6) edge impregnated with inert material.

The characteristics of the movement of the mobile phase front in TLC-P differ from those familiar in traditional TLC, for which a quadratic law holds[9,22–25] [see Eqn.(1)], while in the vicinity of the front there is an unsaturation zone.[7,27] It has been established (see, for example, Lengyel et al.[109]) that the following equation is valid for linear TLC-P:

$$Z_f = k_{\text{TLC-P}}t, \qquad (3)$$

where Z_f is the distance of the solvent front from the line at which the mobile phase is introduced, t the time, and $k_{\text{TLC-P}}$ a constant. This relation holds under the following conditions: (1) the volume rate of supply of the mobile phase in

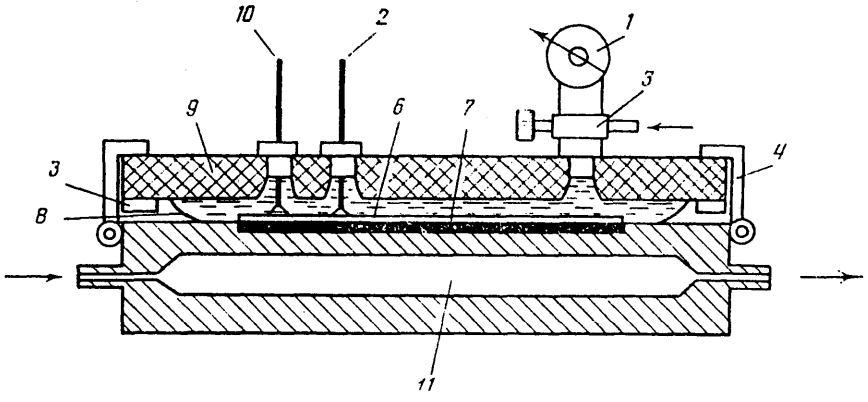


Figure 10. Schematic illustration of the apparatus for TLC under pressure:[106] 1) manometer; 2) device for the introduction of the specimen to be analysed under pressure; 3) inlet for gas or liquid; 4) clamps; 5) sealing washer; 6) sorbent layer; 7) glass or plastic support; 8) polymer membrane; 9) transparent lik; 10) device for the introduction of the mobile phase; 11) thermostatic device.

the chromatographic system F is constant and (2) the layer of sorbent is regularly packed so that its width is b, its thickness d, and the free volume fraction is β. The time t during which the mobile phase front migrates over a distance Z_f is defined by the relation

$$t = (Z_f bd\beta)/F ,$$

whence

$$Z_f = (F/bd\beta)t . \qquad (4)$$

It follows from Eqns.(3) and (4) that

$$U_f = k_{\text{TCL-P}} = F/bd\beta,$$

where U_f is the linear rate of migration of the front.

Knowing the parameters b, d, and β of the sorption layer and also the volume rate of supply of the mobile phase to the system, it is possible to determine the linear rate of supply of the mobile phase.

On the basis of material balance equations, the following relations can be formulated for circular TLC-P:

$$t = \pi Z_{f\text{-}r}^2 d\beta/F ,$$

where Z_{f-r} is the radius of the mobile phase front on the chromatogram and

$$Z_{f\text{-}r} = \left(\frac{Ft}{\pi d\beta}\right)^{1/2} , \qquad (5)$$

$$U_{f\text{-}r} = \frac{dZ_{f\text{-}r}}{dt} = \frac{1}{2}\left(\frac{F}{\pi d\beta t}\right)^{1/2} ,$$

or

$$U_{f\text{-}r} = \frac{1}{2}\,\frac{F}{\pi d\beta Z_{f\text{-}r}} .$$

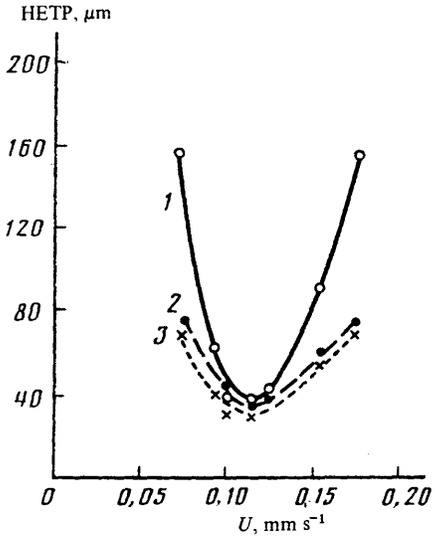


Figure 12. Dependence of the HETP on the linear flow rate of the mobile phase U;[131] stationary phase—200 × 200 mm plate (Fixion 50 × 8), mobile phase—sodium citrate buffer at pH 4.2; 1) arginine; 2) lysine; 3) histidine.

Thus the linear flow rate of the liquid mobile phase under the conditions of circular TLC-P is inversely proportional to Z_{f-r}. Consequently, under these conditions, the linear rate of migration of the eluent is not constant, decreasing as the point of the measurement moves away from the site at which the mobile phase is introduced. However, it is known that the efficiency of a chromatographic system, characterised by the height equivalent to the theoretical plate (HETP), depends significantly on the linear rate of migration of the mobile phase and this dependence has a sharp optimum. As an example, we may quote the dependence of the HETP on the linear flow rate of the mobile phase (see Fig.12). It follows from Fig.12 that the HETP depends fairly markedly on the linear rate of migration of the mobile phase. It therefore appears useful to carry out the chromatographic separation at a constant linear rate also in circular chromatography. This condition can be achieved by programming the flow rate of the eluent.

Since the flow rate of the mobile phase decreases as the front moves in the known variants of circular TLC-P, the HETP increases. As an example, Fig.13 presents data for the variation of the HETP as a function of the distance traversed by the mobile phase front. Evidently in linear TLC-P the efficiency hardly changes but it diminishes sharply when the elution methods traditional for TLC are used. It is noteworthy that the maximum efficiency is also attained in the case of linear TLC-P.

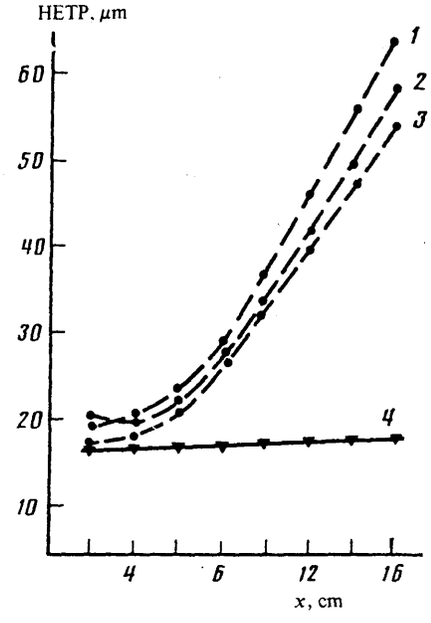


Figure 13. Dependence of the HETP on the distance traversed by the mobile phase front x [126] under the conditions of traditional TLC (curves *1–3*) and TLC-P (curve *4*): 1) unsaturated chamber; 2) ultramicrochamber; 3) saturated chamber; experimental conditions: stationary phase—silica gel 60 with 5 μm grain size, mobile phase—methylene chloride, sorbate—Butter Yellow dye.

Fig.14 presents two densitograms (chromatograms) for a mixture of compounds used as doping agents.[121] All the compounds are separated satisfactorily by the TLC-P method (Fig.14*a*) after 25 min; when traditional TLC is used (Fig. 14*b*), certain compounds cannot be separated at all (for example, compounds 2 and 3 and 5 and 6), while others are separated unsatisfactorily (for example, compounds 3 and 4 and 7 and 6), the separation time amounting to 95 min (almost four times more than in TLC-P).

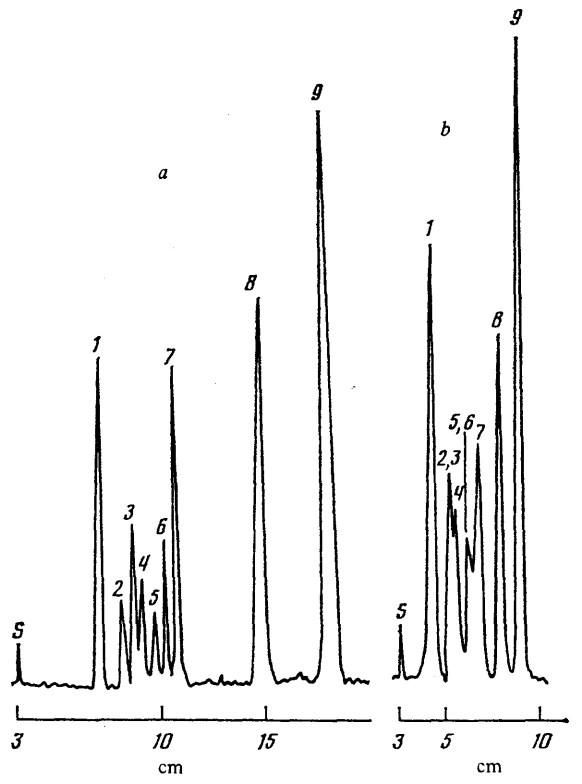


Figure 14. Separation of doping agents by TLC-P (*a*) and traditional TLC (*b*) methods:[121] 1) strychnine; 2) ephedrine; 3) methamphetamine; 4) phenmetrazine; 5) methylphenidate; 6) amphetamine; 7) desopimon; 8) coramin; 9) caffeine; *S*–start; stationary phase–silica gel 60 from the Merck Company, mobile phase–25 : 17 : 8 : 4 : 6 1-butanol–chloroform–ethyl ethyl ketone–water–acetic acid mixture; flow rate (for TLC-0.85 cm min^{-1}.

Table 4. Some examples of the practical use of thin-layer chromatography under pressure.[108]

Compound analysed	Mobile phase
Essential oil	methylene chloride or benzene
Lipophilic dye	methylene chloride
Corticosteroids	nitromethane
Bile acids and their derivatives	ethyl acetate
Ergot alkaloids	acetonitrile
Dinitrophenylhydrazones of aliphatic aldehydes	diisopropyl ether
Essential oil	benzene–ethyl acetate (98 : 2)
Adducts of dimedone with aliphatic aldehydes	chloroform–ethyl acetate (95 : 5)
Aminoacids derived from nucleic acid bases, nucleosides	n-butyl alcohol–glacial acetic acid–water (4 : 1 : 1)
Poppy alkaloids	acetonitrile–water mixtures
Digitalis alkaloids	acetonitrile–water mixtures
Dinitrophenylhydrazones of aliphatic aldehydes	methanol–water and acetonitrile–water mixtures

The TLC-P method suffers from no limitations as regards the type of compounds to be analysed and is used to separate compounds of different classes: aminoacids and their derivatives,[122–124] the aflatoxins B_1, B_2, C_1, C_2, and M_1,[125] dyes,[126] and organic acids and their derivatives.[127] This method is used in reversed phase[128–130] and ion exchange TLC[131] and in phytochemistry.[132] Certain classes of compounds separated and the systems of mobile phases used for their separation are presented in Table 4. The possibilities of the TLC-P method have also been discussed in other studies.[133–142]

A variant of TLC-P different from that employed by Hungarian investigators[106,107] has been recently proposed by Kaiser and Rieder.[143] In this case the plate is positioned on a thermostatted base with the sorption layer downwards and is clamped to the latter with the successive deposition from above onto the support of the plate of a layer of silicon rubber and a thermostatted coating using a press developing a force up to 20 tonnes. The eluent is supplied from below onto the sorption layer at the centre of the plate or along its periphery. In the former case the circular version of TLC-P is achieved and in the second the antiradial version obtains. The eluent passes from a vessel, in which an excess pressure is established by supplying an inert gas at a high pressure. The main advantage of this variant of the device for TLC-P is the possibility of the relatively simple application of antiradial TLC-P.

The extensive experience gained in the development and study of the possibilities of the TLC-P method makes it possible to recommend it for a wide range of applications. At the present time Hungarian specialists have initiated the manufacture of commercial apparatus for TLC-P.[108,114,136,140]

In considering the prospects for the TLC-P method, we should like to draw attention to the usefulness of its variant with flow detection. When this method is employed for preparative separation, it is desirable to use one detector placed behind the sorption layer. We may note that we are unaware of studies in which the possibilities of the TLC-P method have been assessed as regards preparative separation, although this aspect of its practical application appears to be of great interest.

The variant of continuous TLC-P with a series of independent detectors, in which the chromatographic plate functions as a set of individual columns arranged in parallel, for the simultaneous and independent separation of a series of samples is promising for analytical separation.

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Institute of Petrochemical
Synthesis,
USSR Academy of Sciences,
Moscow