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Application of thermoscanning methods using gas detectors in the quantitative assessment of the results of separation in thin layer chromatography

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ABSTRACT. The review deals with the application of gas detectors in combination with the thermoscanning of adsorption layers in thin layer chromatography for the recording of the results of the separation. Attention is concentrated on a combined method, based on the use of cylindrical thin layer chromatography and a flame-ionisation detector; which fulfills two functions simultaneously: thermoscanning of the adsorption layer with separated organic compounds and detection of the thermoscanning products. The prospects for the development of the method are discussed. The biography includes 179 references.

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I. Introduction

Thin layer chromatography (TLC) is one of the most effective and economical methods in liquid chromatography. At the present time optical scanning methods are most widely used in TLC practice for the quantitative and qualitative assessment of the results of the separation.[1,2] However, the application of these methods is limited, firstly, by the possibility of analysing only the compounds which absorb radiation or fluoresce in parts of the spectrum which are not too inconvenient for measurement and, secondly, by the need for laborious calibration which is usually associated with the high individual selectivity of the method. Therefore, for a series of compounds (lipids, carbohydrates, many polymers of practical importance, etc.), it is necessary to employ in many instances detectors of other types.

Gas detectors, for example the flame-ionisation detector (FID), the katharometer, etc., are used widely and successfully in gas chromatography; they are universal and highly sensitive, especially the FID. Their use for the scanning of TLC plates is therefore undoubtedly a promising application in the development of quantitative TLC methods. However, for analysis with the aid of gas detectors, it is first necessary to achieve transfer of the organic substances in the chromatographic bands from the adsorbed state to the gas phase. This stage is usually based on evaporation, oxidation, or thermal degradation of the substances and can be combined with the detection proper. For example, if the FID is used, then the high temperature of the flame usually ensures the quantitative conversion of the compound analysed into volatile chemical forms.

The characteristic features (and certain fundamental limitations) of this method consist in the fact that the process described above must be performed within a very narrow range to increase the resolving power, especially in the assessment of high performance chromatograms. The evaporation (degradation) band width must apparently be not more than 0.1–0.3 of the width of the chromatographic band. However, firstly, the practical application of the TLC–gas detectors (TLC–GD) method has shown that there exist many problems of practical importance for which a high resolving capacity is not required and, secondly, the possibilities of modern experimental techniques as regards a significant improvement of sensitivity are far from being exhausted.

The problem of detection is one of the central problems in TLC on the successful solution of which many investigators are working. As mentioned in a review devoted to the prospects of TLC,[3] the development of effective and universal detection methods is very important.

II. General characteristics of the thermoscanning method

Fig. 1 presents two main schemes used at present for the quantitative assessment of thin layer chromatograms by the thermoscanning method with subsequent (*a*) and simultaneous (*b*) detection.

In scheme (*a*), a furnace heated to 600–700 °C is moved over the reaction *3* containing the absorption layer with the separated components. In order to increase the temperature gradient, a cooling unit is placed in front of the furnace. As the chromatographic band is heated, the volatile evaporation-degradation products are transferred continuously with the stream of carrier gas (inert or oxidising) to the detector *5* (FID, katharometer, or a detector of another type). According to some investigators, the quantitative characteristics of the detection can be improved if the volatile products are oxidised on copper oxides to CO_2 with subsequent hydrogenation on a nickel catalyst.

A version of the method in which the TLC plate is placed within a rectangular reactor *3* was first proposed by Cotgreave and Lynes,[4] while the method using an adsorption layer deposited on the

inner walls of the reactor tube was developed by a number of investigators.[5-7]

An FID, the size of the flame of which is significantly greater than that normally used in gas chromatography, is employed in scheme (*b*) for the scanning of a cylindrical adsorption layer deposited on the lateral surface of a rod. The FID then fulfills simultaneously the function of a furnace (thermodegrader) and a detector. The method involving the thermoscanning of a cylindrical adsorption layer using an FID was first proposed by Padley.[8,9] A version of this procedure is used most widely nowadays in chromatographic practice.[10]

We shall consider these two groups of methods in greater detail.

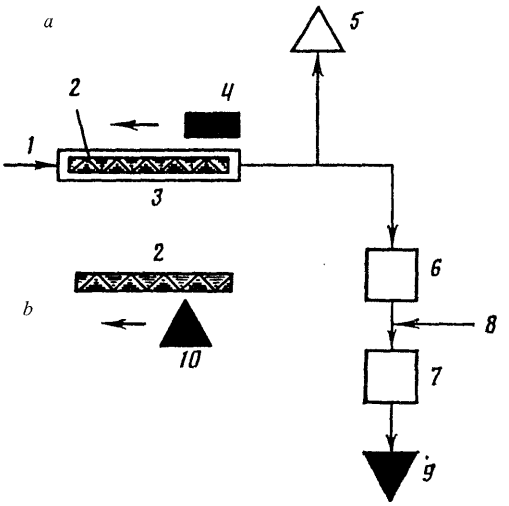


Figure 1. Generalised thermoscanning schemes in TLC: *a*, scheme with separated thermoscanning and detection devices; *b*, scheme for scanning with a thermodetector; *1*, stream of inert gas or oxidant (air); *2*, thin layer chromatogram; *3*, reactor; *4*, scanning furnace; *5*, universal detector (for example, a katharometer or an FID); *6*, reactor with oxidant; *7*, catalytic reactor; *8*, stream of hydrogen; *9*, highly sensitive universal detector; *10*, thermodetector (FID).

III. The thermoscanning method with subsequent detection of the thermoscanning products

The existing versions of this method (Fig. 1*a*) differ in a series of principal characteristics: (1) the position of the adsorption layer relative to the reactor walls; (2) the type of carrier gas used; (3) the function of the gas stream in the reactor; (4) the type of detector used (Table 1). It is noteworthy that Table 1 does not include TLC methods involving the thermoscanning and detection of radioactive compounds,[6,11] in particular, those containing isotopes with soft radiation whose detection in the gas phase is preferable.

The TLC-GD method was first described by Cotgreave and Lynes.[4] The TLC plate (150 × 12 mm, silica gel as the adsorbent on a quartz support) was placed in a heated tube with a rectangular cross-section. The authors[4] justifiably narrowed the limits of applicability of the method developed, indicating only the possibility of analysing compounds evaporated from the adsorption layer and disregarding the possibility of their thermal degradation. However, overall, this study demonstrated the possibility, in principal, of a new aspect of quantitative TLC—thermoscanning of the adsorption layer.

Table 1. Characteristics of the method for the thermoscanning of the adsorption layer with subsequent detection.

Characteristic of method	Characteristics of the application of method	
Form of adsorption component of thin layer	traditional (layer on a rectangular plate) (Ref.4)	tubular (Refs.5-7)
Linkage between adsorption layer and reactor	support not linked to the reactor; quartz support (plate) (Ref.4)	adsorption layer deposited on the inner walls of tubular reactor; reactor plays the role of support (Refs.5-7)
Function of gas stream through reactor	carrier gas transports volatile products to detector (Refs.4-7); the carrier gas is also a reactant, for example, an oxidant, which helps to convert the adsorbed compounds into volatile products	
Type of detector used	gas detector employed, for example, in gas chromatography (thermal conductivity detector, FID) (Refs.4-7)	

It is notable that the development of this method for liquid chromatography with a capillary column has been proposed.[12,13] The mobile phase was used in an amount sufficient for the separation of dyes without their elution from the sorbent (Al_2O_3) layer. The free part of the column, without the sorbent, was filled with an inert friable material and CuO to ensure the completion of the combustion of the dyes. The carrier gas (air) passed through the column to the katharometer. The electric furnace, heated to 650–700 °C at a rate of 1 cm min^{-1}, was moved onto the column in opposition to the stream of air; the CO_2 formed on combustion of the components was recorded by the detector (Fig. 2). This method can be used not only for liquid chromatography, but also, for example, for tubular TLC.

An interesting version of the TLC-GD procedure is the method of tubular TLC in which the inner walls of the tubes serve as the support for the fixed layer of the active sorbent.[5-7] After the separation (and the volatilisation of the mobile phase), the tube is thermoscanned and the products pass through the detector in the stream of carrier gas. Thus the tube fulfills the function of a solid carrier for the sorption layer and of a device for the formation of the gas stream.

In the apparatus for tubular TLC (Fig. 1*a*), one can use a katharometer. For this purpose, the substances separated are oxidised (by the oxygen present in the carrier gas and by the CuO in the adsorbent layer), the resulting water is collected in a trap, and CO_2 is detected.[7] The detector signal is proportional to the carbon content in the chromatographic band. When an FID is used in the TLC-GD method, the sensitivity of the detection of, for example, lipids, is close to the sensitivity of the analysis by column chromatography. It is notable that the separation of, in particular, shark liver oil is the same in both of the above versions, but polar lipids have been detected solely by the TLC-GD method.[7]

A detection system for the thermoscanning of the adsorption layer deposited on the inner walls of the tube has also been described by Mukherjee et al.[14] A quartz or Pyrex tube with a silica gel layer passes through three zones after the separation of, for example,

neutral lipids and bile acids and the removal of the organic eluent—a cooling unit, the pyrolytic furnace, and a thermostat, which prevents the condensation and adsorption of the pyrolysis products.

We may note that, in the method considered, the inner diameter of the reactor tube must be sufficiently large to ensure the rise of the mobile phase solely via the adsorption layer and not via the tube. This creates certain difficulties when an attempt is made to increase the temperature gradient and hence the resolving capacity of the method, which is nowadays used extremely rarely.

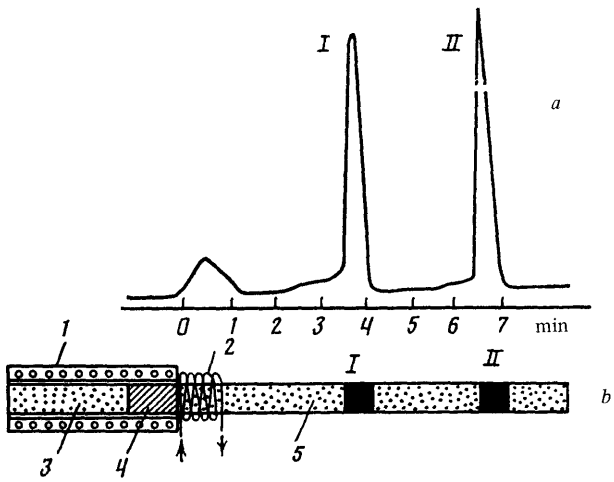


Figure 2. Schematic illustration of the thermoscanning of a capillary column for liquid chromatography (*b*) in the chromatogram (*a*) for azobenzene (I) and sudan red (II) separated on a column with Al_2O_3;[13] *1*, electric furnace; *2*, water-cooled quartz coil; *3*, part of the column filled with solid carrier (Spherochrom); *4*, part of the column filled with CuO; *5*, separating section of the column filled with Al_2O_3.

Fig. 3 illustrates schematically the scanning device of the "Iatroscan" apparatus.[18] The FID signal is known[19,20] to be directly proportional to the rate at which the organic substance enters the flame and, in the case under consideration (for a constant rate of movement of the rod), also to the concentration of the substance in the chromatographic band. In the "Iatroscan" apparatus, ten rods are fixed in a frame which moves in accordance with a definite programme, rod after rod being scanned automatically. The scanning time of a single rod is 30 s and the hydrogen flow rate is 160 ml min^{-1}. The results of the separation on a cylindrical thin layer are recorded by a pen recorder in the form of the usual chromatogram and in the integral form.

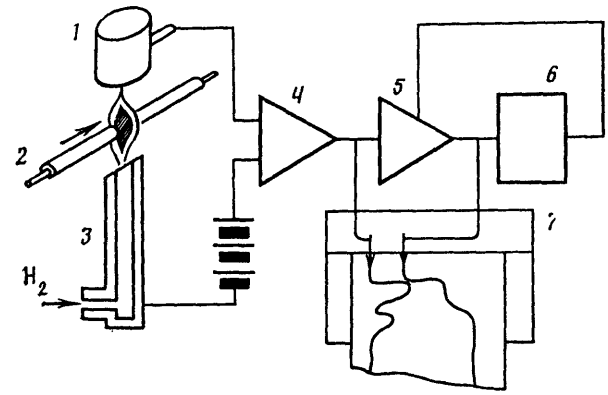


Figure 3. Schematic illustration of a device with an FID for the scanning of a rod with an adsorption layer containing the separated bands:[18] *1*, collector electrode; *2*, chromarod; *3*, burner; *4*, current amplifier; *5*, and *6*, signal integration units; *7*, two-channel pen recorder.

IV. A method for the thermoscanning of a cylindrical adsorption layer with simultaneous detection

The method for scanning a thin adsorption layer with the aid of an FID, described in 1967 [8] (Fig. 1*b*), is simpler than the methods described above in which the thermoscanning and detection stages are separated. An entirely satisfactory separation of glycerides has been achieved using a quartz rod 0.5 mm in diameter and 200 mm long with deposited silica gel. It was shown that the disposition of the rod in the flame and the rate of its movement relative to the flame influence the sensitivity of detection.

A scanning method using an FID was described in 1970.[15] The chromium–nickel alloy strip (215 mm long, 3 mm wide, and 0.1 mm thick) on which a silica gel layer 0.3–0.4 mm thick had been deposited, was used as the chromatographic system. Despite the entirely satisfactory analytical results, this system has not come to be widely used in chromatographic practice.

The development and investigation of repeatedly used rods with an adsorption layer, carried out by Okumura and Kadono[16,17] were very important for the application of Padley's method. This system, which was subsequently modified somewhat, is widely used in practice.

Since a cylindrical adsorption layer is used in this method as the chromatographic system, which is of fundamental importance for this variety of TLC, it is useful to call this version cylindrical TLC (CTLC) and to designate the above combined method as a whole by CTLC-FID.

The first three stages in this method are usual for transitional TLC: the deposition of the sample on the adsorption layer, its separation in a stream of an ascending mobile phase, and the removal of the mobile phase (at an elevated temperature). The preliminary rod activation stage with the aid of FID and the last stage involving the scanning of the rod with the separated bands by the same detector have no analogues in traditional TLC.

At the present time the CTLC-FID method has come to be most widely used in analytical practice compared with other thermoscanning methods. In our view, this can be explained by the following causes: (1) the scanning apparatus and the analytical method are distinguished by simplicity (the FID fulfills simultaneously the thermoscanning and detection functions); (2) the FID is characterised by a wide linear dynamic range, a high sensitivity, and universality also as regards organic compounds (hydrocarbons, lipids, etc.), to which the widely used optical detection methods are almost applicable; (3) the availability of commercial rods of the "Chromarod"

brand and equipment manufactured by the Japanese Company Iatron Laboratories;[18] (4) the high productivity of the method (about 10 analyses in 10 min) by virtue of the high scanning rate.

Since the CTLC-FID method differs from traditional TLC as regards the form of the adsorption layer and the type of scanning and detection, it is necessary to examine the features of this method both in chromatographic separation and in quantitative analysis.

1. Characteristic features of the chromatographic separation by the CTLC method

The widely used cylindrical open chromatographic system (chromarod) consists of a quartz rod on the surface of which a porous layer of silica gel or Al_2O_3 is deposited (Fig. 4). The geometrical parameters of the chromarod are as follows: length 152 mm, diameter 0.9 mm, length of an adsorption layer 120 mm, thickness of adsorption layer 75 μm. After thermoscanning, the rod is automatically activated by the FID, is cleaned, and becomes suitable for re-use. One rod can be used for 100 analyses.

Three types of rods are being manufactured at the present time: chromarod SI, chromarod SII, and chromarod A (since 1987 also chromarod SIII). The first two types of rods are based on silica gel, the particle diameters of which are 10 and 5 μm respectively, chromarod SII being characterised by a higher resolving power. The adsorption layer on chromarod A is obtained from Al_2O_3 particles 10 μm in diameter and is normally employed for the separation of compounds which are unstable on silica gel and also of certain stereoisomers. The adsorption layers on the rods are obtained by sintering the adsorbent particles in the presence of a small amount of readily fusible glass particles added as a binder. The cylindrical shape of the adsorption layer has definite advantages. In this system there are no edge effects disturbing the movement of the eluent front and observed in many cases on the usual rectangular TLC plates. The width of the adsorption layer is small amounting to πd = 2.8 mm (d = 0.9 mm is the diameter of the adsorption layer) and the possible disturbances of the eluent front show a tendency towards "self-healing". For separation the small size of the adsorption system needs only small amounts of the mobile phase, which is frequently extremely expensive.

Further reduction of the rod diameter is justified, which would apparently make it possible (1) to increase the resolving power in the detection of the separated bands by virtue of the increase in the temperature gradient on the rod during scanning, (2) to reduce the uncontrollable losses of the substances analysed during detection, (3) to increase the rate of detection, and (4) to reduce the amount of eluent consumed.

The sample is deposited and separated by the procedure of traditional TLC,[21,22] although, in our view, it would be more justifiable to use special apparatus taking into account the geometry of the rods. The samples (with a volume not exceeding 1 μl) are deposited on the adsorption layer of the rod at a distance of 1–2 cm from its end and the syringe or an applicator of another type must not touch the adsorption layer. The deposited samples on rods fixed in cassettes, which can contain up to 10 rods, are separated in small rectangular chambers for 15–40 min. After the separation, the rods are dried to remove the mobile phase: at a temperature above 100 °C, this takes several minutes. For example, 3 min is sufficient to evaporate a mixture of chloroform, benzene, and formic acid at 130 °C.[23] For labile compounds, the drying can be carried out at low temperatures in a stream of an inert gas or *in vacuo*.

The removal of the mobile phase before scanning is an important stage, since even its traces create an appreciable background and reduce the sensitivity of the detection. Therefore, in our view, it is useful to use as mobile phases substances to which the FID is almost insensitive: water,[19,24] carbon disulphide,[25] ammonia,[26,27] formamide,[25] formic acid,[24] and certain other substances. It is possible and desirable to employ also combinations of these compounds.

It is likewise useful to point out the possibilities of the use of chemically selective inorganic phases in CTLC. Thus organic acids and their methyl esters or triglycerides have been separated on chromarods impregnated with a silver nitrate solution as a function of the degree of unsaturation and isomerisation of the molecules.[28–30] It has been noted[30] that the rods employed are not washed entirely free of silver nitrate even after they have been kept for a long time in aqua regia. The possibility of reusing the rods with silver nitrate has been investigated also in another study.[31]

Another example of the employment of a chemically selective phase is provided by the separation of the glycerides on rods impregnated with a 3% solution of boric acid.[32]

Single separation in CTLC is the simplest but not the only method of chromatographic analysis. The application of repeated elution using several mobile phase systems to improve the separation of samples with a complex composition has been described. For example, a clear separation of the main groups of hydrocarbons present in the residue from the vacuum distillation of petroleum using three consecutive elutions over definite distances has been achieved on a chromarod.[33]

The repeated elution procedure with intermediate scanning of part of the rod is important and methodologically elegant. Thus, in the analysis of blood serum lipids,[34,35] neutral lipids were initially

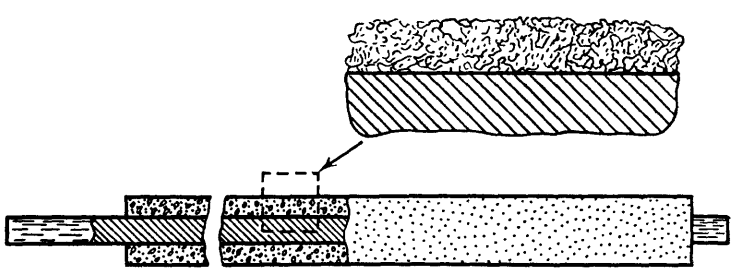


Figure 4. Schematic illustration of the chromarod.[18]

separated and scanned with the aid of an FID and the phospholipids remaining at the start were then separated using a more polar eluent and were also thermoscanned. This version has no direct analogue in traditional TLC and can apparently be regarded as a version of the two-dimensional separation.

It is necessary to differentiate an important application of the CTLC-FID method for the quantitative analysis of components, including impurity components, which cannot be determined by the traditional TLC. The lipid components of the crab have been determined[36] using for the analysis the extract from the part of the TLC plate, which, according to optical scanning data (after the corresponding derivatives have been obtained), did not contain even traces of any compounds. The CTLC-FID method can be used successfully for the analysis of fractions separated by column chromatography and of extracts obtained from sorbent specimens from definite sections of the TLC plate.[10,37]

The combination of the separation on a traditional TLC plate with subsequent separation of individual bands by the CTLC-FID method can also be regarded as one version of the two-dimensional separation.

The moving transporter system proposed earlier[38] is of interest for the analysis of components which cannot be determined by an optical detector especially when packed capillary columns are used in liquid chromatography. A chromarod can apparently be used as such a transporter.

In our view, the CTLC-FID method is promising, since it makes it possible to analyse complex objects rapidly and with a resolving capacity which is sufficient for many practical purposes. It is therefore useful to compare both the versions of chromatography realised in this method as well as those which have not yet been used.

Table 2. Classification of the chromatographic systems used in TLC on the basis of the state of aggregation of the mobile and stationary phases.

Chromatographic system	TLC	
(Mobile phase) [stationary phase]	traditional, on plates	cylindrical, on rods
(Liquid) [solid]		
Traditional, direct phase (non-polar or polar liquid) [polar solid]	is used	is used
Reversed phase (polar liquid) [non-polar solid]	is used	is possible
(Liquid) [liquid-solid]		
Traditional, direct phase (non-polar liquid) [polar liquid-solid]	is used	is possible and desirable
Reversed phase (polar liquid) [non-polar liquid-solid]	is used	is possible

Table 2 presents a classification of various chromatographic systems in traditional and cylindrical TLC. In fact, only one chromatographic system is used in CTLC: (liquid) [polar solid]. The application of other types of chromatography, especially the reversed phase chromatography, is likely to be extremely promising in the future. Here there is apparently a possibility of two approaches: firstly, to obtain the grafted phase each time on the adsorption layer (by standard grafting methods in the gas or liquid phases) and, secondly, to obtain rods with a coating of a carbon adsorbent which will probably also have to be renewed after each use of the rod.

Comparison of the traditional TLC and CTLC in terms of the types of chromatographic processes suggests that at the present time only adsorption is used in CTLC. The employment of partition chromatography will permit, in our view, a significant expansion of the number of parameters for the selection of the optimum solution. The only additional requirement is the use of a volatile compound or substance to which the FID is almost insensitive to the stationary phase.

By using an adsorbent with a known and controllable porosity, it is undoubtedly possible to achieve exclusion CTLC.

Fundamentally new possibilities arise when electromigration methods on cylindrical rods are resorted to. The application of ion exchange to the separation of inorganic and organic ions is also extremely promising. In this version of CTLC, it is desirable to employ optical detectors, for example, the flame-photometric detector, together with the FID.

Table 3 compares the known and possible methods of elution in traditional and cylindrical TLC. In our view, among the elution methods which have not been used in CTLC hitherto, descending and centrifugal elution, whose application would permit an acceleration of the separation process, is of interest.

Table 3. Elution methods in traditional and cylindrical TLC.

Elution method	TLC	
	traditional, on a plate	cylindrical, on a rod
Ascending	is used	is used
Descending	is used	is desirable
Horizontal	is used	is possible
Continuous	is used	is possible
Radial and circular	is used	–
Repeated	is used	is used
Stepwise	is used	is used
Stepwise with intermediate detection of some of the separated compounds and their removal from adsorption layer	is desirable	is used
Two-dimensional	is used	–
Centrifugal	is used	is desirable
Gradient	is used	is desirable

Table 4. Combination of CTLC with other analytical physicochemical methods.

Analytical method	Combination with CTLC
Thin layer chromatography	is used (Refs.10, 36)
Column liquid chromatography	has been proposed (Ref.10)
Gas chromatography	is possible
Mass spectrometry	is used (Ref.10)
Spectroscopic methods	is desirable
Electrochemical methods	is possible

Hybrid methods (see, for example, Zolotov[39]) based on the joint employment of two and more methods, have been increasingly introduced into analytical chemistry and chromatography in recent years. It is therefore of interest to analyse the possibility of combining different methods with CTLC (Table 4).

Thus, it follows from the analysis of Tables 2–4, that the CTLC method has wide prospects for development and the majority of the known separation principles in liquid chromatography can apparently be used successfully in this procedure also.

An important aspect of any analytical method is the estimation of the reliability and reproducibility of the results obtained. We shall now consider the quantitative aspects of the CTLC-FID method.

2. Quantitative aspects of the CTLC-FID method

In the FID method considered, the FID is not used under the normal conditions for which it has been designed.[40,41] Thus the coefficients of sensitivity of this type of detector with respect to lipids differ for the CTLC-FID method from the coefficients for gas chromatography.[42] The main characteristics of the quantitative analysis by the CTLC-FID method are influenced not only by the FID operating conditions (the rod passes through the flame whose dimensions are comparable to those of the rod), but also by the unusual conditions in the detection of organic compounds adsorbed on the porous layer of the rod. The transport of these compounds (or their degradation products) to the hydrogen flame is hindered by the limited mass exchange and also by the high scanning rate (about 4 mm s^{-1}). The adsorbed involatile compounds must be initially subjected to thermal degradation which complicates detection and undoubtedly influences the accuracy of the quantitative determination. It has been noted[43] that, in order to obtain the full signal due to the chromatographic bands of certain substances, repeated scanning of the rod with the summation of the results of all the scans is essential. With increase in the size of the molecule of the substance analysed, the number of scans increases.

In order to avoid repeated scanning, it has been suggested[44] that the rod be rotated through 180°, which improves the contact between the flame and the adsorbed substance.

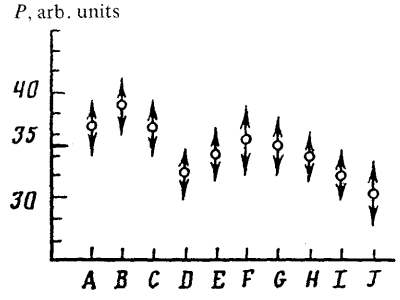


Figure 5. The areas of the peak (P, arbitrary units) of octadecanoic acid for 10 chromarods S (from A to J);[45] sample size 10 μg; the sample deposited on the rod was not eluted and the measurements on each rod were performed ten times.

The reproducibility of the analytical properties of the adsorbent and hence of the results of the quantitative analysis constitutes an extremely important problem in the adsorption chromatogaphy in general and in CTLC in particular. It has been shown[45] that the quantitative results of repeated analysis for chromarods S are different and that this applies both to the average peak area (the points on Fig. 5) and to its standard deviation (arrows). Mares et al.[46] suggest that the reproducibility of the method can be increased in the future by a preliminary selection of the rods (see, for example, Hirayama and Morita[47]).

The large standard deviation is explained by some investigators in terms of the differences in the properties of the rods,[48] while according to others it is due to the inadequate reproducibility of the detection process.[49] Some workers[48,50–53] have concluded that the results of the quantitative determinations by the CTLC-FID method are unfortunately characterised by a low accuracy, although it is sufficient for the solution of many practical problems.

The study[54] in which it has been suggested that the rods with a silica gel layer be impregnated beforehand with a 5% $CuSO_4$ solution appears important in connection with the foregoing. It has been shown that, in the determination of lipids, the base line of the detector is improved and the scatter of the results from rod to rod is diminished. However, it has to be said that the impregnation with copper sulphate shortens the useful life of the rods.[55]

The reproducibility of the results improves with increase in the scanning rates from 3.2 to 4.2 mm s^{-1}.[56] It is of interest that impregnation with copper sulphate alters the relative sensitivities for different substances. For example, the sensitivity with respect to methyl haptanoate increases relative to triglycerides.

The choice of the quantitative determination method is important. Many investigations have been devoted to the analysis of lipids—one of the most important practical applications of the CTLC-FID method.[10,57–61]

It has been shown,[18] in relation to the analysis of glycerides and free acids, that, when the relative and not the absolute peak area is used for the calculations, the "individuality" of the rod is not manifested quite so sharply.

It has been confirmed experimentally that the application of the internal standard method permits, in contrast to the absolute calibration, a significant improvement of the reproducibility of the results of the method. Despite the fact that the use of an internal standard reduces the scatter of the results when one rod is replaced by another,[51,52,62] it is difficult to obtain accurate quantitative analytical results because the relative sensitivity depends on the sample size,[62–64] the ratio of the concentrations of the substance analysed and the internal standard,[65] the scanning rate,[61,66] and whether a biological object or an artificial mixture is analysed.[46]

It has been stated[63] that the CTLC-FID method using an internal standard can yield an approximate result in the study of the lipid composition of marine organisms and deposits as well as sea water, whose determination had been attempted earlier:[67,68] for a <2–3 μg sample, the relative sensitivity coefficients change appreciably and to different extents for lipids of different classes.

The calibration plots for lipids are not linear and cannot always be extrapolated to the zero point.[62,64,69–72] For lipids, aliphatic hydrocarbons, esters, ketones, free aliphatic acids, triglycerides, free alcohols, and free sterols, present in sea water, the calibration plots are described by different equations:[73] for example, $y = 0.07 + 4.19x$ for polar lipids, while $y = -0.72 + 1.39x + 0.09x^2$ for free sterols and, in order to attain an accuracy of the quantitative analysis better than to within 10 rel.%, it is essential to carry out many measurements (see also Tanaka et al.[74]).

The multifactorial experiment on the analysis of triglyceride mixtures of different composition, differing in the acidities of the components, on 10 chromarods S showed[56] that the previous history of the rod, the mixture composition, and the acid composition of the triglyceride are very important for quantitative analysis, the relative sensitivity (with an ester as the standard) gradually diminishing as a function of time. It has also been established that the sensitivity

coefficients for single and mixed triglyceride specimens are not the same[56] and yet the majority of investigators use coefficients obtained by calibration with respect to individual compounds for the analysis of biological objects.[46,52,61,72,74-76]

It is noteworthy that, in the analysis of hydrocarbons, the results obtained by the CTLC-FID method are stable as a function of time. Thus, in the determination of the composition of petroleum on chromarod SII, random oscillations in the absolute values of the signal were observed in the course of 5 days, although they were different for different groups of hydrocarbons.[77]

Thus the nature of the substance influences the error of the measurement. It may be that the scanning of each band or separate groups of bands can be usefully carried out separately under optimum conditions for each band.

Studies designed to improve not only the accuracy of the quantitative analysis but also the sensitivity of the method are of great interest. The size of the sample analysed influences significantly the standard deviation of the signal and it must be greater than 1 μg in order to obtain reproducible results.[46]

It has been shown that the sensitivity of the Iatroscan apparatus in the determination of lipids (combined with a satisfactory accuracy and reproducibility) is a maximum for a hydrogen flow rate >200 ml min^{-1}, a distance between the rod and the detector burner nozzle not greater than 0.8 mm, when the vibration air pump is replaced by a compressor, and when new rods are washed with concentrated nitric acid.[78] The above measures led to an increase in sensitivity by a factor of ~ 2 and to a decrease in background by a factor of ~ 5; the standard deviations of the signal were then 5–15 rel.%. It was possible to determine 0.05 μg of each lipid in a small sample volume (0.2 μl). According to the data in Ref. 18, the sensitivity of the CTLC-FID method is fairly high: the detection limit is about 0.07 μg m^{-1}. Different procedures are used to increase the sensitivity: mounting of the ion collector at a distance of ~ 1.7 mm about the rod,[79] the focussing of the band of the test substance on the rod,[69] and chemical treatment of the rods, for example with iodine vapour, to intensity the FID signal in the analysis of unsaturated lipids.[80]

Comparison with the traditional methods for the solution of the same problem is important for the estimation of the quantitative aspect of the new method. Mares et al.[46] compared the accuracies of the analysis of human blood plasma lipids by the CTLC-FID and gas chromatographic methods. The reproducibility of the former method proved significantly lower, but one must agree with the above authors,[46] who assume that further development of the CTLC-FID method will permit an improvement in this important parameter.

We may note the following important advantages of the CTLC method compared with gas chromatography: (1) the possibility of using involatile and thermally labile compounds for analysis; (2) an unusual selectivity which is frequently difficult to achieve in gas chromatography—for example, group separation of hydrocarbons, phospholipids, etc. is possible; (3) high rate of detection (1 scan in ~ 30 s).

The results of the determination of malthenes and asphaltenes in heavy petroleums by the CTLC-FID method agree well with data obtained by the traditional IP 143 method of the Institute of Petroleum (London).[81] Both methods are characterised by virtually the same reproducibility, but the CTLC-FID method is less laborious: the time required for the analysis of 9 specimens is about 30 min (excluding the time consumed in the weighing and dissolution of the specimens).

In the determination of the group compositions of heavy hydrocarbons, the data obtained by the CTLC-FID method and the standard ASTM D2007 method differ significantly, which is associated with the sharp difference between the fractions actually isolated by the ASTM method and the declared fractions.[33]

When the CTLC-FID method is used to analyse heavy petroleum and coal processing products, a disadvantage of high performance column chromatography, namely the loss of appreciable proportions (from 5 to 15%) of the specimen analysed, is eliminated.[44]

It has been shown[82] that the CTLC-FID method yields reliable quantitative results in the determination of polycyclic aromatic hydrocarbons, in contrast to methods such as liquid–liquid extraction, liquid column chromatography, and gas chromatography.

In conclusion, it must be emphasised that, despite many critical comments concerning the quantitative characteristics of the CTLC-FID method, numerous studies indicate a positive aspect of its practical application for the quantitative analysis of a wide variety of objects—ranging from petroleum to biologically active compounds.

There is no doubt that the further perfection of the apparatus will permit a significant improvement in the quantitative characteristics of this promising method.

3. Practical applications of the CTLC-FID method

The CTLC-FID method is widely used in various branches of science, industry, medicine, protection of the environment, etc. It is of special interest for the study of objects in which the optical detection methods, widely used in liquid chromatography (column chromatography and TLC), are almost inapplicable and the preparation of the corresponding derivatives is complicated or undesirable. We shall quote certain examples of the practical employment of the CTLC-FID method.

(a) Lipids. Tanaka et al.[74] investigated the separation of complex lipid mixtures. The products of the methanolysis of sphingoglycolipid were fully separated on chromarod SII using two consecutive elutions.

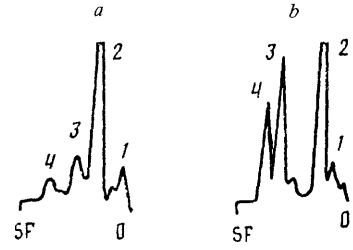


Figure 6. Chromatography of rat brain phospholipids;[83] chromarod S with copper sulphate; eluent: chloroform–methanol–water (68.5:29:2.5) without (*a*) and with (*b*) addition of 0.5% of formic acid; *1*, sphingomyelin; *2*, phosphatidylcholine; *3*, phosphatidylethanolamine; *4*, diphosphatidylglycerol; 0, start; SF, solvent front.

The separation of rat brain phospholipids has been demonstrated[83] on rods impregnated with copper sulphate (Fig. 6*a*). As in the author's previous studies using non-impregnated rods,[75,84] the eluent was a mixture of chloroform with methanol and water. The addition

of formic acid decreased the spreading of the bands of certain compounds (peaks *3* and *4* on Fig.6 *b*) and reduced the peak at the start.

Analysis of lipids is of definite interest also in connection with the diagnosis of certain diseases. For example, a sharp qualitative change in the lipid content of the amniotic fluid of pregnant women occurs in the case of illness.[51,85,86] The determination of lipids by the CTLC-FID method is used in pharamaceutical and clinical studies, in toxicological analysis, and in the analysis of food.[87-89] Data on the quantitative determination of lipids and their derivatives by the CTLC-FID method have been surveyed.[90] Apart from the investigations mentioned here and in the preceding Sections, other studies of lipid objects must be indicated: blood plasma,[91-94] blood serum,[91,92,95] human gums,[96] yeast,[97] micro-organisms,[98] vegetable oils,[99,100,133] as well as glycerides and fatty acids,[95,99,101-117] phospholipids,[118-120] and lipids of other classes.[121-129]

It is notable that the use of computers has expanded in chromatographic practice. Thus the application of computers in the Iatroscan apparatus for the analysis of lipids of biological origin has been described.[130]

In conclusion we may mention that the determination of lipids in various specimens is at present the most thoroughly developed field of the practical application of the CTLC-FID method and its use in this field alone undoubtedly justifies the development of this method.

(b) Petroleum and hydrocarbon fuel. The application of the CTLC-FID method in the determination of the group composition of heavy fuel was first described by Suzuki and Takeuchi.[131,132] The study of Yamamoto and Kawanobe[134] was devoted to a method for the rapid determination of asphaltenes in heavy oils and of the composition of these oils. The method for the determination of malthenes and asphaltenes on rods with a silica gel layer has been successfully tested on 16 specimens of bitumen, heavy petroleums, and synthetic fuels.[81] Components insoluble in benzene were removed before the analysis.

The liquid products of the hydrogenation of coal from Japanese deposits were resolved into the following fractions: saturated, polycyclic aromatic, and heteroaromatic compounds, tars, and asphaltenes.[135]

Other examples of the use of the CTLC-FID method in the analysis of hydrocarbon raw materials and products should also be mentioned.[136-143] It has been concluded[33] that the CTLC-FID method ensures the rapid determination of the group composition of high-boiling fractions of petroleum, heavy oils, and liquid coal processing products and preliminary deasphaltisation is not required.

Thus the determination of hydrocarbons of different classes is an important practical application of the method considered.

(c) The monitoring of the environment and analysis of physicologically active objects. The possibilities and advantages of the CTLC-FID method in the determination of polycyclic aromatic hydrocarbons extracted from filters for the selection of contaminated air samples have been demonstrated.[82] The determination of the composition of the particles present in the exhaust from diesel engines has been described.[144] The method made it possible to analyse involatile organic compounds with $T_b \geqslant 300$ °C (the limit of the determination is ~ 0.03 μg).

Numerous studies have been devoted to the analysis of biological objects and medicinal drugs: psychotropic preparations,[145,146] alkaloids,[17,124] aminoacids, vitamins, pesticides, corticosteroids, cardiac glucosides and genins,[17] oestrogens, progestins and androgens,[17,124] saccharides,[147] antibiotics,[148] bile acids,[149-151] aglucones,[152] and glycyrrhizin.[153]

Here one may also indicate the following objects analysed: surfactants (quaternary ammonium chlorides, oleates, and sulphonates,[45] olefin sulphates,[149] and other surfactants[154-156]), sulphanilic acid and sulphonamides,[17] phthalates,[157] ginseng saponins,[158] perfumes,[159] peony extracts,[160] food products,[161-163] leather articles,[164] and cosmetics.[165]

The examples quoted in this section indicate the possibility of the effective application of the CTLC-FID method also in those fields which are important for human health.

(d) Applications in other fields. The distribution of liquid rubbers in terms of "functionality" has been investigated.[166] αω-Polybutadienols have been separated by the CTLC method into three fractions: a fraction without functional groups and fractions with one and two functional groups. Comparison of two scanning methods—the optical and FID methods—showed that the results of the optical method are too high by 30–50%.

The CTLC-FID method has been used successfully to investigate the radiation-induced graft copolymerisation of styrene on cellulose.[167] Homopolystyrene and homopolyacrylonitrile have been determined.[168] The analysis of polymers has also been described in another communication.[169] The composition of industrial emulsions and its variation during use have been studied.[170]

One of the examples of the employment of the CTLC-FID method in the study of the kinetics of the formation of an ester has been described.[171] Ethyl benzoate and benzoic acid have been determined on chromarod SII. Chemical reactions have also been investigated in other studies.[70,172-174]

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Analysis of the data described in this review permits the conclusion that a new promising aspect of TLC and of the scanning field has been established, namely CTLC-FID. The practical application of this combined method is particularly useful primarily for compounds whose detection by optical methods is unsatisfactory. Numerous communications, and reviews published in recent years[175-178] as well as the monograph by the Czechoslovak scientist Ranny[179] are an indication of the great and continuing interest in the CTLC-FID method.

The method has good prospects, some of which have been noted in the present review. In the course of the further development of the method, it will be desirable to concentrate attention to the following promising aspects: (1) the development of new methods of improving the resolving power of thermoscanning detection; (2) the application of new detection methods, for example flame-photometric methods; (3) use of other versions of chromatography apart from the familiar liquid–solid version.

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