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Thin-layer Chromatography of Biopolymers and Their Derivatives

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The modern state of thin-layer chromatography in its application to proteins, nucleic acids, and their derivatives is discussed. Thin-layer chromatography is a new technique in chromatographic analysis which has vigorously developed in recent years. In the review the inadequate development of the thin-layer chromatography of proteins compared, for example, with that of low-molecular-weight compounds is noted and the causes of this situation are analysed. Thin-layer chromatography is compared with other chromatographic methods and the studies on the thin-layer chromatography of biopolymers and their derivatives published up to the present time are critically assessed. New techniques in the thin-layer chromatography of proteins published recently are described.
The bibliography includes 97 references.

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I. INTRODUCTION

At the present time chromatographic methods are used on an extremely large scale in a wide variety of branches of science and engineering. These methods have acquired particular importance in biochemistry, molecular biology, and related disciplines. In recent years a new technique—chromatography in thin layers—has been developing rapidly.

Thin-layer chromatography has proved an extremely effective means for the separation and analysis of mixtures of a wide variety of compounds. This method is of interest for chemists, biologists, and medical practitioners working with complex mixtures of natural substances.

The method arose initially as a procedure for the separation of extracts from medicinal plants[1], developing considerably in the period that followed. At the present time several hundred papers in this field are published every year. However, it should be noted that thin-layer chromatography is still used mainly for the analysis of various low-molecular-weight compounds. Studies on the use of thin-layer chromatography for the separation of high-molecular-weight compounds of biological origin, such as proteins and their derivatives, have begun to be published only in recent years, starting with 1962.[2,3]

The application of the thin-layer chromatographic method to proteins and nucleic acids has been developing much more slowly than in the case of low-molecular-weight compounds; thus the total number of studies on the thin-layer chromatography of low-molecular-weight compounds quoted in the bibliography of the Journal of Chromatography for the last two years reaches approximately 1700. On the other hand, the number of studies devoted to the thin-layer chromatography of proteins, nucleic acids, and their derivatives does not exceed 80. This imbalance in the development of the method is particularly notable compared with, for example, the column chromatography of proteins, which has been widely developed and used.

Probably the reason for this is partly that until recently thin-layer chromatography as applied to proteins was used mainly as a method for the approximate estimation of the possibility of the separation of protein mixtures, the aim being to achieve the actual separation of the mixture under conditions of column experiments. Thin-layer chromatography then of course plays a subsidiary or auxiliary role. As a method of preparative isolation of proteins, thin-layer chromatography could not compete with the column technique either.

The inadequate theoretical and technical elaboration of the thin-layer chromatography of proteins has also probably retarded the development of this method. However, in recent years it has been shown that the thin-layer chromatography of proteins is by no means merely an auxiliary method and that it can also be of independent value. For example, thin-layer chromatography on different types of Sephadex may be used for the estimation of the molecular weights of the proteins investigated. It is very significant that only 0.1–0.2 mg of the protein is required for such determination[4]. It has also been shown that with the aid of thin-layer chromatography the presence of various groups of proteins can be determined in human blood serum. This requires only 1–2 ml of serum. The method can probably also be of diagnostic value[5,6].

Data indicating the possibility of using thin-layer chromatography for the estimation of various changes due to denaturation of protein molecules caused by external factors is of definite interest too. All these findings show that further development of the thin-layer chromatography of proteins as an independent research technique is promising.

The aim of the present article is to examine the modern state of the thin-layer chromatography of proteins, nucleic acids, and their derivatives, and to indicate new pathways in the development of this field of research, taking into account as far as possible all the experimental studies published in recent years.

II. DEVELOPMENT OF THIN-LAYER CHROMATOGRAPHY AND ITS COMPARISON WITH OTHER CHROMATOGRAPHIC TECHNIQUES

The first studies on thin-layer chromatography were made by the Soviet Scientists Izmailov and Shraiber, who in 1938 used thin layers of alumina to separate the alkaloids of medicinal plants[1]. However, 20 years elapsed before the method received general recognition. In 1958 a new stage in the development of thin-layer chromatography began, mainly in connection with the work of Stahl[7-13]. In these investigations Stahl used thin-layer chromatography for the separation of a wide variety of groups of organic compounds using various adsorbents. The rapid increase of publications on the use of thin-layer chromatography for the separation and identification of various, mainly low-molecular-weight, compounds of biological origin began only after these investigations had been published, approximately in 1960–1961.

Up to the present the thin-layer chromatographic method has been applied to almost all classes of compounds of biological importance. Historically thin-layer chromatography began to develop after techniques such as chromatography of proteins on columns and paper chromatography of amino-acids had been elaborated and received wide recognition.

Compared with column chromatography, thin-layer chromatography has its characteristic features. The entire process is carried out in an open layer and not in a closed column. A significant difference between thin-layer chromatography and column chromatography is also the fact that in the first case the layer of adsorbent is dry (ascending chromatography is most often used). As a result of this, the relative rates of migration of both the eluting buffer and the substance chromatographed are inconstant and R_f varies during chromatography on plates[14].

Compared with thin-layer chromatography, the results obtained in the paper chromatographic method depend very strongly on the properties and structure of the paper sheet itself; furthermore, paper chromatography requires much longer times than thin-layer chromatography. It should also be noted that paper chromatography is nowadays widely used for the separation of low-molecular-weight compounds such as amino-acids but the method has not been developed for the separation of proteins.

Thin-layer chromatography is free of the disadvantages characteristic of paper chromatography. Combining high sensitivity of paper chromatograms and the possibility of using a very small amount of the test substance, thin-layer chromatography has a higher resolving power because of the possibility of using ultrafine adsorbents. Thin-layer chromatography is also characterised by only very slight broadening of the spots of the test compounds and high concentration of the substance in the centre of the spot[15]. The entire chromatographic process requires a very short time and the apparatus and techniques of thin-layer chromatography are distinguished by extreme simplicity and ready availability and can be used in practically any laboratory.

In this respect thin-layer chromatography has advantages over many other methods for the analysis of proteins, which frequently require complex and expensive apparatus. It is interesting that in general advances in chromatography as a whole have been achieved using a very simple technology[16].

In many recently published monographs and reviews dealing with thin-layer chromatography problems concerning technique and partly theoretical problems associated with the application of this new method are discussed quite widely[14,15,17-22], but until recently thin-layer chromatography has been used predominantly for the separation of various low-molecular-weight compounds, mainly of biological origin. The extension of this method to the analysis and investigation of compounds with a high molecular weight, such as proteins, has proved particularly important.

The main principles and technical procedures in thin-layer chromatography are essentially the same for both proteins and compounds of comparatively low molecular weight. The significant difference consists in the fact that other adsorbents and other eluting systems are used in protein chromatography compared with the chromatography of low-molecular-weight compounds. In many reviews and monographs on thin-layer chromatography exhaustive compilations of the adsorbents used in this method as well as the eluting systems and buffer solutions are quoted[14-19,22] and therefore there is no need to consider them in detail. However, it should be noted that all the data quoted in the publications refer almost exclusively to the thin-layer chromatography of low-molecular-weight compounds.

The technical procedures used in the thin-layer chromatography of proteins, nucleic acids, and their derivatives are described below.

III. THE TECHNIQUE OF THE THIN-LAYER CHROMATOGRAPHY OF PROTEINS

1. Adsorbents

In contrast to the thin-layer chromatography of low-molecular-weight compounds, where adsorbents such as silica gel, kieselguhr, alumina, etc. are used, various types of Sephadex (mainly G-75, G-100, G-200, ultrafine varieties), ion-exchange celluloses (DEAE- and TEAE-cellulose, ecteola-cellulose, phosphocellulose) and, among inorganic adsorbents, mainly hydroxyapatite are used for the chromatography of proteins in thin layers.

2. Deposition of the Adsorbent on Plates

Manual method. Different procedures are used: (a) a suspension of the adsorbent is poured on to a plate mounted horizontally; (b) the plate is immersed in the suspension of the adsorbent; (c) the plate is sprayed with a dilute suspension of the adsorbent.

Apparatus of various designs is used in the mechanical method. The apparatus developed by Stahl is most frequently used for the deposition of thin layers[9,22].

3. Procedures Used in the Thin-Layer Chromatography of Proteins

Depending on the direction of movement of the eluting solution, ascending and descending varieties of chromatography are distinguished.

Ascending chromatography. In ascending chromatography the solvent moves up the plate as a result of capillary forces[14]. The chamber for ascending chromatography consists of a vessel with the eluting solution poured on to its bottom in an amount such that a plate with the adsorbent mounted vertically in it is immersed

approximately to a depth of 5 mm. The vessel is tightly closed on top with a lid. The rate of migration of the eluent along the plate is not constant in this case, depending on the path which must be traversed by the solvent, since the capillary forces are balanced by hydrostatic pressure as the liquid rises.

Descending chromatography. In descending chromatography the movement of the eluting solution down the layer of adsorbent is due to hydrostatic forces[15]. Descending chromatography requires a somewhat more complex technique than the ascending procedure. In its simplest version it requires a chromatographic boat (a tube sealed at either end and provided with a slit) into which the eluent is poured and a filter paper bridge is lowered to make contact between the buffer and the upper edge of the plate mounted at a certain angle. Descending chromatography is usually characterised by a constant linear velocity of the eluent. A number of investigators have used descending chromatography on Sephadex[23-25]. Descending countercurrent chromatography, based on a continuous supply of fresh eluting buffer on to the plate (the solution moves to the end of the plate and freely flows away) has been used for proteins[3].

Apart from descending and ascending chromatography there exist also other techniques of thin-layer chromatography: horizontal and circular[14]. These methods have not so far been used for proteins.

4. Eluting Systems for the Thin-Layer Chromatography of Proteins

Phosphate buffer solutions of different concentrations and at different pH are most often used for the thin-layer chromatography of proteins[3,9,23-27]. Depending on whether the concentration of the eluting buffer changes in the chromatographic process, thin-layer chromatography may be regarded as chromatography with constant or variable compositions of the buffer. Usually, for the separation of proteins, the eluting solution is passed once along the plates. However, there also exist methods of repeated chromatography with the same or different eluting buffers. Repeated chromatography in the same eluting system is used to achieve a greater migration of substances with a low R_f value. Before repeating the chromatographic process, the plate is dried.

In stepwise chromatography the eluting buffer is passed repeatedly with increase of its molar concentration.

Gradient chromatography in thin layers. In this case the elution is carried out under the conditions of a concentration gradient, i.e. the concentration of the eluting buffer is altered, usually increased, during chromatography on plates.

Determann[28] developed an apparatus for this type of gradient elution suitable for thin-layer chromatography. The apparatus consists of a glass cylinder mounted on a magnetic stirrer. At a height of 1 cm it is divided by a porous plate into two parts. In the lower part a magnetic stirrer is placed. The capillary tube for the supply of buffer is sealed into the wall of the cylinder. At a distance of 1 cm above the porous plate a tube is sealed into the cylinder for the outflow of the excess liquid. The plate with the adsorbent and the sample deposited on it are put on the porous plate and immersed in the buffer to a depth of 1 cm.

5. Detection of Protein Spots on Plates After Chromatography

Whereas in the thin-layer chromatography of low-molecular-weight compounds, the spots can be identified by a wide variety of reactions, depending on the type of compounds, in experiments with proteins and amino-acids the spots on the plates are usually detected by spraying with ninhydrin[2,26]. Sometimes the plates are covered with filter paper to obtain prints of the protein spots and the paper is then dyed[3,23,29]. The use of fluorescent labels for the detection of proteins in thin layers has also been suggested. For this purpose, the test protein is first treated with a fluorochrome and the distribution of the spots after chromatography is followed using their fluorescence in ultraviolet light[23].

Fluorescent supports with a layer of adsorbent deposited on them can also be employed[30]. Sometimes the fluorescent dyes are first mixed with the adsorbent before its deposition on the plates[14]. The chromatogram can also be sprayed after separation of the mixture of test substances with solutions of fluorescent dyes[22]. In all these cases the test compounds are detected as dark spots against a fluorescent background.

An interesting method for the identification of the test substances was proposed by Heyns and Grutzmacher[31]. They employed a combination of thin-layer chromatography and mass spectrometry for the determination of the derivatives of amino-acids and certain other low-molecular-weight compounds. After being scraped off from the chromatograms, the contents of the spots were introduced directly into a mass-spectrometer for the measurements.

Quantitative methods in thin-layer chromatography usually involve the elution of the substance on the plate followed by the determination of its concentration; or they are based on the determination of the size and colour intensities of the spots formed by the test compound on the plate with the adsorbent[14,15,22]. For example, a quantitative determination of the protein fractions of blood serum after chromatography on Sephadex plates (ultrafine G-100 and G-200) has been demonstrated by Japanese workers[6]. However, it should be noted that quantitative methods in thin-layer chromatography have been used on an inadequate scale compared with, for example, the determination of low-molecular-weight compounds.

IV. THIN-LAYER CHROMATOGRAPHY OF PROTEINS, AMINO-ACIDS, AND THEIR DERIVATIVES

1. Thin-Layer Chromatography of Amino-Acids and Their Derivatives

Mainly silica gel is used as an adsorbent in the thin-layer chromatography of amino-acids; ion-exchange celluloses or cellulose powders have been used to a lesser extent.

In one of the first studies of this kind a mixture of 24 amino-acids was successfully separated on silica gel plates using several solvents[32]. The best results are obtained with the n-butyl alcohol–acetic acid–water mixture and also mixtures of phenol and water. Very good results were achieved when amino-acids were separated on silica gel by two-dimensional chromatography using the chloroform–methanol–ammonia system in one direction and the phenol–water system in another[33]. By this procedure, it proved possible to separate 14 different amino-acids, while paper chromatography under the same conditions revealed

only ten amino-acids. The separation of amino-acids on silica gel with the aid of two-dimensional chromatography has also been achieved in other investigations[34,35]. These workers successfully separated 15 amino-acids on thin cellulose layers in the solvent systems n-butyl alcohol–acetic acid–water, methyl alcohol–water–pyridine, and certain others[36]. Amino-acids have also been satisfactorily separated on thin layers of ion-exchange DEAE-cellulose[37] and also by two-dimensional chromatography on cellulose[38]. Thus the thin-layer chromatography of amino-acids, particularly when the two-dimensional technique was used, proved to be much more effective (approximately by a factor of ten)[30] than the separation of amino-acids by paper chromatography.

Apparently the method of "finger prints" can be successfully used in thin-layer chromatography. Enzymic hydrolysates of human gamma-globulin, horse heart cytochrome, and certain other proteins were treated[39] with dimethylaminonapthalenesulphonyl chloride (fluorochrome). After the removal of the excess of the reagent, the hydrolysis products were separated by two-dimensional chromatography on thin layers of silica gel. It was shown that the results thus obtained agree with those from other methods. The amount of protein required for analysis is then smaller by a factor of ten (about 20 μg). Peptide maps have also been investigated with the aid of thin-layer chromatography[40,41].

The application of thin-layer chromatography to the separation and identification of dinitrophenyl and phenylthiohydantoin derivatives of amino-acids proved to be very promising. Silica gel is most frequently used in such cases. Thus it has been shown that almost all dinitrophenyl derivatives of amino-acids can be successfully separated in a thin layer of silica gel with the aid of both ascending and two-dimensional chromatography in various solvent systems[42–44].

Thin-layer chromatography of dinitrophenyl derivatives of amino-acids has also been used by other investigators[45,46] and Figge[47] and Burgi[48]. These workers used thin-layer chromatography to study urine and other physiological liquids. The thin-layer chromatography of ^{14}C-labelled dinitrophenyl-amino-acids on thin layers of silica gel has been used to study processes occurring in the pyruvic acid cycle[49].

A combination of thin-layer chromatography and quantitative ultramicro analysis of amino-acids in the form [^{14}C]-methyl esters and their dinitrophenyl derivatives were successfully employed in a recent study[50].

Deyl and Rosmus[51] described an interesting application of thin-layer chromatography in which they identified *N*-terminal amino-acids using their sulphonaphthyl derivatives. Fluorescent amino-acid spots can be determined at concentrations of 10^{-10} M. Recently *N*-terminal amino-acids have been determined as their dinitrophenyl derivatives on thin layers of silica gel[52].

Thin-layer chromatography has also been employed for the separation of phenylthiohydantoin derivatives of amino-acids. Thus a mixture of phenylthiohydantoin derivatives of 19 amino-acids has been successfully separated[42] by two- and one-dimensional chromatography on silica gel layers using various solvent systems. In another study[53] a combination of thin-layer chromatography and electrophoresis was employed to separate mixtures of phenylthiohydantoin derivatives of amino-acids, also on silica gel layers.

In a recently published study the thin-layer chromatography of phenylthiohydantoin derivatives of amino-acids was successfully achieved on silica gel[54].

In a number of investigations[55–57] the thin-layer chromatography of phenylthiohydantoin derivatives of amino-acids was used for the analysis of peptides. It should be noted that thin-layer chromatography of dinitrophenyl and thiohydantoin derivatives of amino-acids has certain advantages over paper chromatography. Firstly, in experiments in thin layers there is a considerable economy of time expended on the chromatographic process. Secondly, thin-layer chromatography exhibits high sensitivity, approximately ten times higher than that of paper chromatography. Recently data have been published[58] showing that the use of the optimum silica gel grain size (in the range 2–5 μm) and restriction of the path traversed by the solvent to 4–5 cm permits a sharp improvement (by a factor of 5–10 compared with the usual technique) of the sensitivity of the thin-layer chromatography of dinitrophenyl and phenylthiohydantoin derivatives of amino-acids and also a reduction of the duration of analysis.

This finding unquestionably demonstrates the promise of the thin-layer chromatographic technique for the identification and analysis of amino-acids and their derivatives and also for the separation of protein hydrolysis products and the investigation of complex mixtures of peptides and polypeptides.

2. Thin-Layer Chromatography of Proteins

Three groups of adsorbents are at present used for the thin-layer chromatography of proteins. These are different brands of Sephadex, ion-exchange celluloses, and also hydroxyapatites. Thin-layer chromatography on different types of Sephadex has been most widely used.

The first publications on the thin-layer chromatography of proteins appeared in 1962. These were the studies of Johansson and Rymo[3], who used thin layers of Sephadex, and of Hofmann[2,59], who used hydroxyapatite. Johansson and Rymo were two of the first investigators who used different types of Sephadex for the separation of a number of proteins in thin layers[3,23]. These workers employed G-50, G-75, G-100, and G-200 Sephadex for thin-layer gel filtration. For proteins with relatively low-molecular-weights, the adsorbents used were G-50 and G-75 Sephadex, while for proteins with molecular weights of 100 000 and above the less strongly cross-linked G-100 Sephadex and G-200 Sephadex were employed. The mobility of ceruloplasmin from human serum mixed with various peptides and amino-acids was estimated on G-25 Sephadex. In layers of G-25 Sephadex this protein can be readily separated from amino-acids the mobility of which is much lower. On G-75 Sephadex plates, it proved possible to separate mixtures of oxserum albumin and β- and α-lactoglobulines with molecular weights of 69 000, 35 000, and 15 000 respectively.

A satisfactory separation of tryptic hydrolysate of gamma-globulin from human serum has been achieved on G-75 Sephadex plates. The relevant papers give a detailed description of the conditions used in the preparation of the Sephadex plates.

The use of different types of thin-layers of Sephadex for the estimation of the molecular weights of various proteins is also of interest. It has been shown[4,28] that the molecular weights of the test proteins can be estimated approximately using G-75 and G-200 Sephadex. The following proteins have been employed for this purpose: lysozyme, haemoglobin, gamma-globulin, etc. This application has been developed further by Determann[27], who showed that the volume of the eluting buffer solution is linearly related

to the logarithm of the molecular weight of the test protein. The molecular weights of ribonuclease, chymotryptsin, trypsin, cytochrome *c*, and certain other proteins have also been estimated. Ultrafine G-200 Sephadex was used as adsorbent.

Various types of Sephadex have been used for the thin-layer gel filtration of proteins and mucopolysaccharides by Roberts[60], who described a technique for the preparation of the plates and the procedures employed to identify proteins on Sephadex plates.

Thus the thin-layer chromatography of proteins on different types of Sephadex can probably be used successfully as a simple method for the comparative estimation of the molecular weights of the test proteins. Although the method yields only approximate molecular weights, nevertheless in many cases it is preferable (large saving of time) to determinations by other procedures, requiring a complex apparatus.

A number of studies dealing with the separation of blood serum proteins by thin-layer chromatography have been published recently. Thus in the separation of human blood serum proteins on layers of G-75 Sephadex only one extended protein zone was observed, while on G-100 Sephadex the serum proteins are usually divided into three fractions[23]. It is interesting that the column chromatography of serum proteins on Sephadex also yields three fractions[61]. It has been shown[26] that a mixture of albumin and gamma-globulin from human serum can be successfully separated on phosphocellulose plates. The optimum conditions for the separation of these proteins are achieved with a definite water content in the thin layer of adsorbent, i.e. 70–75%. A decrease or increase of the concentration of water in the adsorbent plates greatly impairs the separation. This investigation showed that for optimum results it is necessary to control the moisture content of the adsorbent used in thin-layer chromatography.

An interesting application of the thin-layer gel filtration of blood serum proteins on Sephadex (ultrafine G-200) was found by Bergström[5], who employed gel filtration for the analysis of pathological human serum proteins under clinical conditions. He discovered that proteins from normal human serum yielded three zones when separated on plates corresponding to the sedimentation constants 4.5 S, 7.0 S, and 19.0 S. The contents of these three groups of proteins in normal serum were respectively 69.7, 24.6, and 5.7%, while pathological serum proteins yielded on gel filtration different percentages, depending on the illness. Analogous results were also obtained by Migita[6] in the thin-layer gel filtration of human blood serum proteins on different types of Sephadex. He employed Sephadex plates (G-100 and ultrafine G-200) on which proteins of normal serum were separated into three zones corresponding to the sedimentation constants 4.0 S, 7.0 S, and 19.0 S. The data on the gel filtration of the proteins from five pathological human blood sera are also quoted.

Italian workers[62] suggested that the thin-layer chromatography on G-200 Sephadex be used to investigate the serum from patients ill with paraproteinemia.

The above data show that thin-layer gel filtration on different types of Sephadex can yield over a comparatively short time (5–6 h) information about the changes in the protein composition of the blood, which should be of undoubted interest for clinicians.

The thin-layer chromatography of certain individual blood serum proteins (haemoglobin, albumin, etc.) has been carried out to estimate their mobility on Sephadex plates (G-100 and G-200)[24,25]. In a recent investigation[63] the authors were able to separate the proteins from normal human blood serum into ten fractions by the combination of thin-layer gel filtration (ultrafine G-200 Sephadex) and electrophoresis, while normal paper electrophoresis shows only five protein components.

The successful separation of haemoglobin and myoglobin on ultrafine Sephadex layers has been recently demonstrated[64,65].

Proteins with enzymic activity have also been subjected to thin-layer chromatography. Willand and Determann[66] used DEAE-Sephadex as an adsorbent for the separation of lactate dehydrogenases.

In other studies[23,29] ribonuclease, lysozyme, chymotrypsin, trypsin, pepsin, alkaline liver phosphatase, and certain other enzymic proteins were chromatographed on different types of Sephadex. In a recent investigation purified preparations of individual proteins were used for preparative estimates of their behaviour on Sephadex plates. It was shown that appropriate types of Sephadex can serve as suitable materials for the preparation of various enzyme proteins.

The use of hydroxyapatite as an adsorbent for the thin-layer chromatography of enzymes has been demonstrated by Kibardin and Lazurkina[67,68], who employed trypsin obtained from various sources, ribonuclease, and certain other enzymes. It was shown that thin layers of hydroxyapatite can be successfully used to estimate the possibility of the separation of protein mixtures and also to characterise the homogeneity and mobility of various enzyme proteins.

Recently thin-layer chromatography on ecteola-MN-300 was used[69] to separate certain coenzymes. The R_f values for eleven coenzymes are quoted. It was shown that some of them are grouped in a single spot. The compositions of the eluting mixtures most suitable for the clear separation of the individual coenzymes are given.

V. THIN-LAYER CHROMATOGRAPHY OF NUCLEIC ACIDS AND THEIR DERIVATIVES

Only comparatively recently thin-layer chromatography began to be applied for the separation of nucleic bases, nucleotides, and nucleosides. Different types of cellulose and to a certain extent silica gels have been mainly used as adsorbents. Other adsorbents have been employed for this purpose much more rarely[70–74].

Randerath[70,71] was the first to use thin-layer chromatography for the analysis of nucleic bases, nucleotides, and nucleosides. The thin-layer chromatography of these compounds has advantages compared with paper chromatography. The boundaries of the spots on the thin layers of adsorbents are much less diffuse than in paper chromatography[73]. Another advantage of thin-layer chromatography is the shorter time required for the separation of the test compounds than in paper chromatography.

The high sensitivity of the thin-layer chromatographic method has also been noted[75]. Ecteola-cellulose has been most frequently used for the separation and analysis of nucleotides and nucleosides. Using this adsorbent, it has proved possible to separate successfully 16 nucleotides in short periods ranging from 4 to 60 min[76]. Ecteola-cellulose has also been used by other investigators[70,71,77,78]. Recently ecteola-cellulose was employed for the thin-layer chromatography of DNA.[79]

A number of studies have dealt with the thin-layer chromatography of nucleic acid derivatives on DEAE-cellulose[77–81]. In one of these[81] a method was proposed

for two-dimensional thin-layer chromatography whereby it is possible to separate a mixture of mono-, di-, and triphosphates of adenosine, cytidine, guanosine, and uridine.

Thin layers of PEI-cellulose have been used for the ascending chromatography of deoxyribo-oligonucleotides [82,83]. Layers of MN-300-cellulose have been employed for the separation of nucleic bases and nucleosides [72,73,82].

DEAE-Sephadex A-25 has also been used as adsorbent in the separation of nucleotides by thin-layer chromatography.

The number of studies published on the application of silica gels for the thin-layer chromatography of nucleic bases and nucleic acid derivatives is much smaller [71,85].

Among inorganic adsorbents, alumina has also been employed for the thin-layer chromatography of nucleic acid derivatives [86]. Thus a comparatively small number of adsorbents have been used for the separation and analysis of nucleic acids and their derivatives by thin-layer chromatography.

Mangold [75] notes that the chromatography of nucleotides and nucleic acids in thin layers of ion-exchange celluloses is superior to all analytical methods for separation employed hitherto. In particular, the considerable saving in time when this method is employed must be pointed out. The high capacity of ion-exchange adsorbents makes it possible to use micro-amounts of the test substance. It is noteworthy that until now thin-layer chromatography has been used largely for the separation of purine and pyrimidine bases of nucleotides and mononucleotides. So far the method has not been applied to the analysis of the hydrolysis products of nucleic acids.

In a comparative assessment of various adsorbents employed in thin-layer chromatography published data show that nucleic bases are better separated from the corresponding nucleotides on layers of eteola-cellulose and other celluloses than on silica gel. On the other hand, the inorganic adsorbent gives better results in the separation of the bases themselves.

The efficiency of the adsorbents is approximately the same in the separation of nucleosides [75]. However, it should be noted that, despite its positive aspects, the thin-layer chromatography of nucleotides and other nucleic acid derivatives is at present essentially in its initial stage of development and has been only weakly developed so far compared with, for example, the column chromatography of nucleic acid. Up to the present time, there have been virtually no studies of the thin-layer chromatography of nucleic acids themselves and their hydrolysis products. Nor have there been any studies on the separation of the carbohydrate components of nucleic acids by thin-layer chromatography although this method might well prove to be quite suitable for the analysis of such compounds.

VI. NEW TECHNIQUES IN THE THIN-LAYER CHROMATOGRAPHY OF PROTEINS

In recent years certain new possibilities of the thin-layer chromatography of proteins and their derivatives have been demonstrated. Thus it has been shown [87] that enzymic reactions can be followed directly on plates. PEI-Celluloses served as the adsorbent.

As already mentioned, thin-layer chromatography has been successfully employed also for the analysis of the "peptide maps" of protein hydrolysates. Thus the possibility of the separation of the hydrolysis products of proteins by thin-layer chromatography on silica has been demonstrated [88] for myosine and protamine as examples.

Usually the complex mixture of hydrolysis products is separated by paper chromatography. A definite advantage of thin-layer chromatography over paper chromatography consists in much greater sensitivity [41] (approximately by a factor of ten).

The combination of thin-layer chromatography with electrophoresis in thin layers has proved very promising [63,89-91]. It has been shown in one of these investigations [89] that blood serum proteins can be separated into twelve components with the aid of chromatography and electrophoresis on G-100 and ultrafine G-200 Sephadex plates.

By combining electrophoresis and chromatography, Glaesner et al. [91] succeeded in separating the mixture of the hydrolysis products of haemoglobin into 28 peptides on silica gel plates.

In studies combining thin-layer electrophoresis and chromatography different types of Sephadex have been used as adsorbents for proteins and silica gel has been used for the separation of peptides.

The number of such studies is so far small. Nevertheless further developments of this technique are likely to be very promising. Bearing in mind the technical simplicity of thin-layer chromatography, this method can probably be successfully used also in clinical laboratories [62,92]. The investigations in this field are still very few.

Studies in which a combination of thin-layer chromatography and immunodiffusion are combined merit attention [93,94]. They demonstrated the possibility of thin-layer immunochromatography using as example serologically active proteins. The adsorbents were different types of DEAE-Sephadex. However, it should be noted that thin-layer immunochromatography has not so far been developed any further. The scope of this method appears at the present time to be more limited than that of Grabar's immunoelectrophoresis [75].

An interesting application of thin-layer chromatography under the conditions of the so called "barrier" method has been demonstrated [96]. Here the separation of oligonucleotides as a function of chain length and composition and chromatographic temperature can be achieved on cellulose plates. The same investigation demonstrated the possibility of detecting by thin-layer chromatography specific molecular complexes formed between poly- and oligonucleotides.

The possibility of estimating by thin-layer chromatography various changes due to modification and denaturation in proteins occurring under the action of external agents has been demonstrated recently. Immune human gamma-globulins treated with urea solutions at different concentrations show a reduced mobility in chromatography on hydroxyapatite plates. The decrease of the mobility of the proteins on plates takes place in parallel with the degree of the denaturing effect of urea on the gamma-globulin molecule. The biological activity of these proteins changed correspondingly. It is also interesting that the renaturation of immune globulins is likewise accompanied by the recovery of their mobility on adsorbent plates up to the normal level [97].

In another investigation it was shown that the treatment of human serum gamma-globulin with heavy metal salt solutions leads to a change in the mobility of this protein in chromatography on thin layers of hydroxyapatite compared with control experiments [68].

The above studies show that thin-layer chromatography can probably also serve for the assessment of the changes in proteins which are reflected in their surface and adsorption properties.

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The data presented above show quite convincingly the wide use of thin-layer chromatography in protein chemistry. In conclusion we must briefly consider the principal factors determining the further development of the thin-layer chromatography of proteins.

At present we are in the initial stage of the development of this method as applied to proteins and nucleic acids. Its advantages—rapidity, high sensitivity, and the possibility of its application in mass analyses—are unquestionable. All these factors make the development of the thin-layer chromatography of proteins very promising. The problems next to be resolved in this field involve the necessity of further theoretical and practical development of the method, the elaboration of the technique, the selection of new adsorbents, and the widening of the range of proteins investigated. The development of new techniques in the thin-layer chromatography of proteins, particularly those involving the combination of different methods, are also extremely important: for example, thin-layer electrophoresis–chromatography, thin-layer immunochromatography, etc. The thin-layer chromatography of fluorescent protein complexes and new technical methods should be more widely used. These new developments will ensure the final promotion of thin-layer chromatography from a subsidiary method to one of independent importance in the study of biopolymers.

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