Stationary Phases for Thin-Layer Chromatography

Simion Gocan
University Babes-Bolyai , Department Analytical Chemistry, Cluj-Napoca, Romania

Abstract

This paper presents a review of the literature concerning development of the stationary phase for thin-layer chromatography (TLC) in the last ten years. The silica gel remains the most important adsorbent for TLC separation. The kinetic properties of the silica gel thin-layer and the new TLC plates have been presented. Other materials used as stationary phase were alumina, zirconium oxide, Florisil, and ion-exchanger. Chemically new bonded stationary phase development is also discussed.The improvement of the separations of some organic mixtures by impregnation of silica gel, cellulose, or polyamide plates with transition metal ions and silver salts and their applications is presented. The impregnation of the thin-layer with organic stationary phase and inclusion complexes is another method used for the enhancement of the separation efficiences. Another modality to improve the selectivity in TLC using ion-pairing as reagent of impregnation is described as well. The actual state of chiral separation by TLC is discussed with concrete references to recent advances in chiral stationary phases. The use of nonpolar chemically bonded stationary phases impregnated with transitional metal ions is presented as chiral stationary phases. The cellulose, modified cellulose, chitin, chitosan, and their derivatives are presented; and their potential for the analysis of the racemates is discussed. The cyclodextrines and macrocyclic antibiotics were used with very good results for enantiomeric separation by TLC. A new separation approach with molecular imprinting polymers was reported as a chiral stationary phase in TLC. The examples provide a wide range of structural types that can be readily resolved enantiomerically by TLC.

Introduction

The success of the separation of a complex mixture by thin-layer chromatography (TLC) greatly depends on the choice of the stationary phase.

Sorbents generally used in TLC include 12-µm particle size of silica gel, alumina, other mineral oxides, chemical-bonded silica gel, cellulose, polyamide, polymeric ion exchange, impregnated silica gel, and chiral phase.

High-performance thin-layer chromatography (HPTLC) is distinguished from conventional TLC. HPTLC involves a layer prepared from a particle of approximately 5 µm with a narrow particle size distribution.

This review utilizes the development of the stationary phase for TLC over the latest 10 years. Information until 1990 can be found in TLC handbooks (1,2).

Review

Silica gel

Silica gel is by far the most widely used adsorbent and remains the dominant stationary phase for TLC. The great majority of TLC analyses are carried out using normal phase (NP) silica gel layer. Because most high-performance liquid chromatography (HPLC) analyses are performed using reversed-phase (RP) sorbents, the methods are complementary for achieving separation and confirming qualitative and quantitative results.

Silica gel particles

The surface of the silica gel was investigated with the best and most adequate methods for analyses. The hydrated silica gel surface can contain three kinds of silanol groups: free silanol, geminal, and associated silanols (3–5). These different silanol types are used to identify and measure magic-angle-spinning (MAS) ^{29}Si NMR (6) and diffuse reflectance infrared spectroscopy with Fourier transform (3).

Nonhydrogen-bonded free silanol is more acidic and can cause strong and deterious binding of basic solutes because of their highly acidic nature. Therefore, silica gel with a higher concentration of free and highly acidic silanols often shows increased retention and broad peak tailing for basic compounds. Fortunately, free silanols generally occur in a low concentration on the silica gel surface. The surface of silica gel with the highest concentration of geminal and associated silanols is favored most for the chromatography of basic compounds because these silanols are less acidic.

Today few analytical laboratories prepare their own TLC plates. Precoated plates for TLC, HPTLC, and preparative TLC are commercially available. The silica gel used as adsorbent for HPTLC plates has a mean particle size of approximately 5 µm and, for TLC, 12 µm. The pore diameter of both is mainlyl 60 Å and the surface area is approximately 500 m^2/g. The most frequently used thicknesses of thin layer for analytical purposes are approximately 200–250 µm. The thin layer for preparative purposes is available with thicknesses of up to 2 mm (7).

The support for the thin layer of the adsorbent has to be an inert

material such as a thin-glass plate, aluminium or terephtalate foil, polytetrafluoroethylene (PTFE), or glass fiber. To ensure sufficient mechanical resistance of the thin layer to permit both easy manipulation of the plates and a good elution and detection stability, a material that favors a compact and adherent layer by different interactive effects with adsorbent was introduced as a binder. Inorganic or organic substances (some polymerizable) were used depending on the manufacture and sorbent material.

The thin layer of adsorbent can also contain a fluorescence indicator with a particle size that does not exceed that of the sorbent. The advantage of these indicators lies in the fact that all substances with a conjugated p-electron system (e.g., aromatic compounds) apear as dark spots on a bright emmitting background when the chromatogram is viewed under UV light (l = 254 nm) (7).

Fluorescent indicators (and binders) are usually assumed to have no effect on TLC analyses, but this is not always true. Flodberg and Roeraade (9) studied the chromatographic performance of TLC plates coated with 3-µm spherical particles and 5-µm angular particles by using over pressure liquid chromatography (OPLC). In this system the mobile phase is forced through the bed of sorbent by means of a pressure pump. The separations were performed on glass plates precoated with 3-µm spherical silica gel particles and HPTLC plates precoated with 5-µm angular silica gel 60 particles. The size of all plates was 10 x 20 cm. The sample was test dye mixture III (Camag, Muttenz, Switzerland). Dichloromethane was used as the mobile phase. The height equivalent theoretical plate (H) was determined for yellow butter. The best H values obtained were 17.3 and 34.2 µm for the 3- and 5-µm particles, respectively. These values were higher than expected according to theory, which predicted H ~ $2d_P$, mainly because of the large particle size distribution, as was also demonstrated by electron-microscopic studies.

Kinetic properties of the silica gel thin-layer

In normal TLC practice it is not possible to determine kinetic properties because capillary forces control the migration of the mobile phase through the thin layer of sorbent. In these conditions the mobile phase velocity is a function of distance between the front and the solvent surface in the chromatographic chamber. The mobile phase velocity is not constant throughout the thin layer of sorbent, yet the velocity decreases as the distance increases. If the migration distance (Z_f) is not excessively long the solvent-front position as a function of time (t) is adequately described by the equation $(Z_f)_2 = kt$. Differentiation of this relation gives the velocity of the solvent front $u_f = k/2Z_f$ (in which k is the velocity constant).

The overpressure developing chamber made the determination of the kinetic properties of precoated silica gel plates possible. In an OPLC system we could control the velocity of the mobile phase in the same way as in HPLC. In this case the mobile phase velocity (u_f) will be constant and the position of the solvent front (Z_f) at any time (t) after the start of development is described by equation $Z_f = u_f t$.

Berezkin and Mardanov (10) investigated the possibilities and perspectives of multicolumn planar chromatography. Multicolumn planar chromatography is the organization of the chromatographic adsorbents in narrow and long sorbent layers (quasi-column) located on the plate suport. The mobile phase in quasi-column layers can be moved by both the action of capillary forces (as with classic TLC) and as a result of the effect of external forces (as with forced-flow TLC). The multicolumn plates with quasi-column sorbent can be arranged in different shapes, which enable a reduction of band broadening. Also, it is possible to realize other combinations of quasi-columns on a planar suport. The sorbent layers can be disposed and fixed on the planar surface of the suport, and the sorbent layers can be located in grooved channels cut in the support (i.e., in quasi-columns with open sides), which can be fixed with polymer film or left unfixed. Advantages of new quasi-column capillary and forced-flow TLC methods on a narrow planar sorbent layer were demonstrated using a synthetic lipophilic dye mixture and toluene as the mobile phase. In this circumstance the separation efficiency of the quasi-column plates will be greater than that of traditional plates and the separation time will be greatly reduced. As an example, let us consider the separation of azobenzene: H = 35 mm, t = 20.5 min TLC plates, and H = 28 mm, t = 16.9 min for polycolumn plates, respectively.

The conventional plates with a planar closed sorbent layer by a film, transparent to UV light have already been studied and described. Fernando and Poole (8) determined the several kinetic properties to be porosity, permeability, and apparent average particle size of commercially manufactured TLC plates and Empore sheets. The experiments were performed with a Chrompres 25 overpressured layer development chamber (Lab Instruments, Budapest, Hungary) operated at a cushion pressure of 20 bars. The mobile phase velocity under equilibrium condition was determined by collecting the effluent from the detector in a microburet. Silica gel TLC and HPTLC plates were obtained from Merck, Macherey-Nagel, Whatman and a new type of flexible TLC sheet under the trade name Empore was used. The Empore sheets contained sorbent particles fibrils enmeshed in a network of PTFE fibrils (which represent ~ 10% by weight of the sheet). The sample was polystyrene of approximately 10^6 MW.

The experimental determination of the porosity of glass-baked silica gel layers showed fairly similar values for the different plates studied. The average values determinated were the following: 0.69 ± 0.3 for total porosity; 0.42 ± 0.04 for interparticle porosity; and 0.27 ± 0.02 for intraparticle porosity. For Empore silica the coresponding average values were: 0.60, 0.33, and 0.27, respectively (8). These results showed that the packing density for the column and layer is similar because interparticle porosity values of the layer are comparable with the column values 0.4–0.5 (15). The intraparticle volume is smaller for the layer than for the column. The chromatographic permeability of the HPTLC layers is similar for Merck and Whatman (~ 4.1 x 10^{-14} m^2) and a little different for Macherey-Nagel (2.2 x 10^{-14} m^2). The TLC layers showed a greater difference in permeability values from one manufacturer to another (in the range of 6.8 12.7 x 10^{-14} m^2). But the chromatographic permeabilities of the layer were not significantly different from those of the columns. The incorporation of fluorescent indicator into the layer did not seem to have a significant impact on its permeability (8).

Poole and Fernando (12) have studied the influence of mobile phase velocity on zone dispersion and separation performance in forced flow development on commercially available precoated

silica-gel plates. The influence of mobile phase velocity on zone dispersion was quantitatively evaluated by fitting the experimental results obtained by OPLC to the well-known van Deemter equation and the Knox equation. The optimum velocity and reduced velocity values obtained for TLC and HPTLC plates from the different manufacturers were 0.018–0.046 and 0.60–1.20 cm/s, and 0.033 0.49 and 0.73 0.96 cm/s, respectively.

Treatment of the plates

It was important to obtain reproducible values R_F, and accordingly it was necessary to control all of the factors influencing this parameter. The thin layers were sensitive and their proper treatments began with the opening of the pocket and continued until to densitometry. Before separation, prewashing with methanol (13) was necessary, especially for trace analysis at parts-per-billion levels (14). This was followed by the activation of the silica-gel thin-layer plates by heating to 120°C for 30 min. The effect of pretreating a silica-gel layer with vapors of various polar solvents (anhydric acetic acid, *n*-propanol, *n*-butanol, or water) was studied with the use of steroidal test compounds and heptane-ethyl acetate as the mobile phase (15). The results were compared with those obtained on untreated silica-gel plates. The pretreatment of the plates with polar solvents showed a gentle change of the silica-gel surface, affecting only the phase ratio of the system, whereas adsorption of acetic acid has a significant influence on both the equilibrium constant of the compound and the phase ratio.

Liang et al. (16) evaluated several commercial silica gel HPTLC plates for quantitative fluorescence analysis. The plates were obtained from different commercial companies (Merck, Analtech, Whatman, and Makerey-Nagel). None of the plates contained fluorescent indicator and they all had a glass-back support for thin layer. The HPTLC plates were evaluated for use in quantitative fluorescence analyses. It was found necessary to preclean the layer to remove background fluorescent impurities, which were the major limitation to sensitive and linear dynamic range. The glass backing of the plates contributed over 50% of the average florescence background level and over 30% of the background noise. Changing the glass with low or no fluorescent materials (such as quartz) could have reduced this problem, which was intrinsic to the glass. The silica-gel layer contributed two different types of fluorescence background (an overall background and the embedded fluorescent impurity spots). To obtain the best results, the plates had to be cleaned before use and stored in a clean room. With plate precleaning, rhodamine 6G showed detection limits of approximately 100 fg.

New TLC plates

Several new TLC plates were shown at the 2002 PittCon meetings. The newest TLC products were Silica gel 60F laser-coded TLC plates, which were from E.Merck/EM Science. Each plate was coded and individually numbered to reduce errors in record-keeping, copying, and archiving. The catalogue number, batch number, and individual several numbers were shown at one edge of each plate for absolute plate identification. Another new product from Macherey-Nagel called Durasil was presented. It contained a new binder for the production of very hard and water-resistant plates.

Alumina

Next to silica gel, alumina is the most widely used adsorbent in TLC. Alumina is prepared from aluminum hydroxide by calciation at 500°C. We should mention the existence of three types of alumina, according to the nature of their function groups: acidic, basic, and neutral. The most frequently used crystalline form is g-Al^2O^3, the specific surface area of which usually ranges from 100 to 200 m^2/g and has a pore diameter of 60, 90, and 150 Å (or 6, 9, and 15 nm). The active groups on the surface of chromatography alumina are hydroxyl groups, oxide (O^{2-}) ions, and an aluminium cation (2).

Ahmad (17) published a selective review of alumina as stationary phase for TLC of inorganic and organometallic compounds. In this review the discussion is limited to works performed on alumina layers used as the adsorbent. The cited literature spans a period of approximately 1960–1994.

The chromatographic behavior of diasterioisomers was investigated (18). A number of 24 pairs of *cis* and *trans* isomers (maleates and fumarates) were separated on three different alumina stationary phase. In these separations 24 mobile phases with strengths (epsilon) in the range of 0.21 to 0.25 and a variety of solvent selectivities were used.

Other material used as stationary phase

Zirconium oxide was used as stationary phase for TLC in combination with diffuse-reflectance Fourier transform infrared (DR-FTIR) detection (19). TLC plates were prepared by spreading slurry of zirconium oxide (ZrO^2) microspheres on a microscope slide and allowing it to give a 0.20-mm layer thickness. Spots of the dye test samples were detected by DR-FTIR spectroscopy. The zirconium oxide had a slightly higher IR reflectivity than silica gel or alumina. A comparison of transmission and DR-FTIR spectra on zirconium oxides showed good peak correlation permitting qualitative identification of the IR bands in the fingerprint region.

Florisil is a coprecipitate of silica and magnesia. The properties and applications of Florisil in TLC and HPLC were reviewed and compared with other adsorbents. The chromatographic properties of Florisil were presented in correlation diagrams of retention parameter derived from a study of the retetion behavior of mono- and dipolifunctional compounds on Florisil, silica gel, magnesia, and mixed silica-gel magnesia (20).

Chemically modified mineral perlite, a new TLC sorbent, was prepared by converting its SiO^2 content (70–75%) to soluble silicates with Na^2CO^3. A demonstration of the separations of dyes, amino acids, carboxylic acids, mono- and disaccharides, and halide ions used the layer of the material mixed with $CaSO^4$ and Na^4SiO^4 (21).

Two dicationic zeolites of the type metal cation-organic cation [Na^+ and ($[CH^3]^4N^+$ as compensation cations] were synthesized and characterized. The specific surface area was determined and the following values were obtained for the two dicationic zeolites: 356 m^2/g and 359 m^2/g, respectively. This new material was tested as a TLC stationary phase for the separation of five hydrophilic dyes (22).

Analyses of food components on unconventional starch and talc layers having specific characteristic compared with silica gel were described (23). Corn, rice starch, and gypsum were mixed with distilled water and ethanol and commercial talc was suspended in

ethanol. The suspensions were applied on glass plates.

Ion-exchangers

A new ion-exchange layer composed of cerium (III) silicate was tested for the separation of 30 cations (24). The cerium (III) silicate was obtained by mixing an aqueous solution of cerium (III) nitrate and an aqueous solution of sodium silicate. The pH of the reaction mixture was adjusted to 6.2. The ion-exchanger cerium (III) silicate is so selective that one metal ion can often be easily separated from numerous others (i.e., fast separation of platinum from 27 other cations was achieved using NH^4OH as the eluent). The results were summarized in two tables.

Mohammad et al. (25) developed some new sorbent phases by mixing silica gel with a number of antimony-based ion-exchanging gels prepared using different procedures. The antimonates were selected because they have a high ion-exchange capacity and satisfactory stability in organic and aqueous formic acid systems.

A new thin layer of titanium (IV) silicate ion exchanger was used to study the chromatographic behavior of 30 metal ions on TLC with an aqueous and mixed mobile phase containing complex ligands (26). Titanium (IV) silicate was prepared by the addition of sodium silicate solution to a solution of titanium (IV) chloride in HCl, and the pH was adjusted to 6.5. On these layers, a rapid separation of Al (III), V (V), Hg (II), Cg (II) and other ions from numerous other metallic ions was possible. The R_F values have been given graphically and tabulated.

In another paper Mohammad et al. (27) studied the separation and determination of some metal ions by using one part of silica gel and an equal amount of Sn (IV) arsenosilicate (ion-exchange gel), and the two mixed together to form a homogeneous slurry. The layers were developed with various aqueous formate buffers or with methanol as the mobile phase. R_F data for V (IV), Fe (III), Co (II), Ni (II), Cu (II), Zn (II), Ag (I), Cd (II), Hg (II), Pb (II), Bi (III), and U (VI) have been reported.

The silica-gel surface can be modified by with suitable organic or inorganic substances (physiosorbtion). Mohammad et al. (28) proposed another type of modification of the silica-gel surface that consisted of utilization of the ion-exchange properties of the silanol groups. The silanol group is weakly acidic and, in combination with an aqueous salt solution, results in a cationic exchange. By impregnation of the silica-gel surface with a metal ion (1%), a sorbent layer of a good selectivity was obtained. The layers impregnations with chlorides of sodium, potassium, or strontium gave better results. The development on these impregnated layers was performed using formic acid, sodium formate, and formic acid sodium formate (1:1,v/v) as the mobile phase. Many possible ternary separations of metal ions have been tabulated. The effects of the level of impregnation and the mobile phase pH on mobility of the metal ions have also been studied. This method can be applied to the TLC of some inorganic pollutants (28,29).

Shimizu et al. (30–32) demonstrated the TLC behavior of a lot of inorganic ions on silica gel impregnated with liquid anion exchangers that included Primene JM-T, a primary alkylamine (31); Amberlite LA-2, a secondary amine (32); and tri-*n*-octylamine (TnOA), a saturated tertiary amine (30), which used sulfuric acid or sulfuric acid- ammonium sulfate solution as the mobile phase for development. The R_F values of 49 inorganic ions developed on silica gel impregnated with JM-T, LA-2, and TnOA have been illustrated in a very suggestive way as the table of the elements.

An LDH anion exchanger mixed with silica gel was used for separation by TLC of cephalosporins (ceftriaxone, cefuroxime, cefotaxime, cefttazidime, cefadroxil, and cefalexin) and 18 different mobile phases were tested (33). The method is easy to perform and can be applied in the pharmaceutical practice.

The retention of metallocyanide complexes [hexacyanoferrate (II), hexacyanoferrate (III), dicyanoaurate (III), thiocianate], nitropruside, chromate, tetrachloroplatinate, and tetrachloroaurate (III) was studied on DEAE cellulose anion-exchange layers that were developed with NaCl or $NaClO^4$ solution. The R_F values were higher with $NaClO^4$ for all anions, indicating the strong desorption effect of perchlorate. Nitrate behaved in a similar manner but its effect was less pronounced (3,4).

Impregnated stationary phases

Metal ions as impregnation agents

Silver salts and boric acid are the most frequently employed charge-tansfer complexing agents. Argentation is widely used for the separation of organic compounds with electron-donor properties because of presences of unsaturated group in the molecule.

The silica gel coated plates were impregnated by development with aqueous silver nitrate solution (2 g/5 mL), dried, and heat-activated for 2 min. The utility of those plates to the separation of steroids and triterpenes was demonstrated (35).

A layer of glass powder silica gel impregnated with approximately 5% of $AgNO^3$ was used for the separation of racemic 1,2-diacylglycerol. Preparative separation of the mixture was performed on silica-gel H^3BO^3. This allowed the successful separation of the racemic mixture into 11 fractions. Individual diacylglycerols were identified by comparison with standards. The reasons for the different mobilities have been discussed (36).

The Silica gel G plates, impregnated with transition metal ions Cu (II), Ni (II), Zn (II), or Cd (II) were used for separation of some sugars (37). The method was considered an improvement on the previous report (38) on Cu (II) impregnated silica-gel plates for the separation of glucose and sorbitol.

Other papers (39) discuss the separation of amino acids on silica gel impregnated with Cu (II) and Ni (II). Adsorption of amino acids in batch process was compared with their TLC on silica gel impregnated with Ni (II). Partition coefficients that were determined thus allowed for the predictions regarding the nature of interaction on the silica gel. The R_F values are tabulated for 15 amino acids (40).

Silica-gel layers impregnated with Cu (II) and Hg (II) were used to improve the separation of some important sulfonamides (41).

The impregnation of silica gel with some transition metal ions [Mn (II), Fe (II), Co (II), Ni (II), Cu (II), Cd (II), Zn (II), or Hg (II)] led to improved separations of the vitamin B complex (B1, B2, B6, B12, and folic acid). The metal ions gave positive effects on the resolution of vitamin B complexes and folic acid. The R_F values were tabulated (42).

The separation of certain tetracycline was achieved through TLC on silica gel G containing 13% of $CaSO^4$ and impregnated with Co (II). The development was performed with ethanol acetic

acid H^2O (5:3:3,v/v/v) as the mobile phase (43).

Singh and Mishra (44) investigated the TLC of 20 aromatic amines on a thin layer of silica gel G impregnated with ammonium cerium (IV) nitrate. Increasing the concentration of ammonium cerium (IV) nitrate (0.4, 1.0, and 2%) in the layer resulted in a decrease in hR_F values. The influences of substituent groups on the chromatographic behavior of the amines were discussed in relation to aniline.

Polyamide plates impregnated with metal salts Zn (II), Co (II), Ni (II), Cu (II), Cr (III), and Fe (III) were applied to the separation of sulfonamide drugs (45). The separation selectivity can be interpreted by the formation of metal complex between the electron-donating groups on the sulfonamides with the metallic ions.

Cellulose MN-300 was impregnated with the metallic ions Co (II) or Fe (III) for TLC separation of salicylaldehide semi- and theosemicarbazones (46). Eight semi- and thiosemicarbazones were separated on cellulose plates impregnated with Co (II).

Impregnation with organic stationary phases

Gasparic (47) presented a review concerning on thin-layer plates impregnated with organic stationary phase.

Separation of aromatic arylamines were performed by TLC on silica gel G plates that were impregnated by ascending development with methanolic 1% picric acid, followed by air drying, and then activation. In addition, the mutual separation of carbazole, *p*-phenylenediamine, and *p*-toluidine from a synthetic mixture was achieved (48).

The cellulose was preferred for partition chromatography when using formamide or DMF. The chromatographic behavior of nitrophenols on a thin layer of silica gel and cellulose both with and without impregnation by nonaqueous polar stationary phase (formamide and DMF) and less polar stationary phase (liquid paraphin, octanol, and bromonaphthalene) was compared. RP-TLC separation was strongly affected by the type of stationary phase (through the possibility of forming charge-transfer complexes), mobile phase, and its support (the acidic properties of silica gel) (49).

Silica gel G layer impregnated with tri-*n*-butyl phosphate has been used for the separation of 25 toxic metal ions. The separation was performed by RP-TLC. The quantitative separation of Pb (II) from 18 metal ions and Cr (III) from 15 metal ions was achieved (50). Other applications (51) referred to the retention behavior of some d- and f-block metal ions on silica gel G layers impregnated with tri-*n*-butylamine (TBA). The dependence of hR_F values of metal ions on untretated and TBA-impregnated silica gel G with *n*-butanol and *n*-butanol HNO^3 as mobile phase was graphically presented. The migration of the metal ions depended on the solubility of the metal salt and on the adsorption and precipitation properties of the metal ions in the network of adsorbent.

In a recent paper, Sharma et al. (52) studied the RP-TLC of three-dimensional metal ions on silica gel G^{F254} impregnated with silicone fluid DC 200, triaryl phosphate (TAP), tri-*n*-butyl phosphate, and dimethyl sulfoxide (DMSO) HNO^3 was used as the mobile phase. On the basis of experimental data, layers impregnated with 1% silicone DC 200 and 5% TAP were found to be most suitable for metal ion chromatography. Silica gel G F^{254} layers impregnated with 5–7% DC 200 were very selective for separation of Ni (II) and Co (II) when pure DMSO was used as the mobile phase. When the layer was impregnated with 5% TAP and 1M HNO^3 was used as the mobile phase, Fe (III) could be specifically separated from other three-dimensional metal ions. Some binary and ternary separations were achieved. The authors explained the mechanism of migration in terms of adsorption and the formation of complexes.

Ion-pairing

In TLC on a polar or nonpolar thin layer, reductions of tailing and improvement in selectivity have been realized by impregnation with the ion-pairing reagents. Pandey and Bhattacharya (53) made a comparative study regarding the separation of six pharmaceutical or potentially pharmaceutical sulphapyrazole derivatives by TLC on a silica gel G layer by itself or impregnated. The concentration of the reagent for impregnation was 1% of sodium dodecyl sulfate, tetraetylammonium perclorate, tetrabutylammonium bromide, tetrabutilammonium hydroxide, or Triton X-100. Triton-100 was the least suitable impregnant and xylene-ethyl acetate was the best mobile phase.

Also, cetylpyridinium cloride, cetyltrimethylammonium bromide (CTAB), sodium dodecylsulphate (SDS), and triton X-100 were used as the common cationic, anionic, and nonionic surfactants, respectively, for static impregnation on the Silufol UV-254 normal phase (54). Static impregnation was performed by immersion of the plates for 1 min into a 0.1M methanol solution of the surfactants. These thin-layer plates were tested on separation of fluorescein and erythrosin with methanol water (4:1, v/v) as the mobile phase. The results clearly showed that SDS is unacceptable for impregnation and resolution did not exist because of elongated spots. With CTAB and TX-100, however, very compact and well-separated spots were obtained with a resolution of higher than one.

Some closely related *N*-carbamoyl-3,5-dimethyl-4-arylazopyrazoles were separated by TLC on plates of Silica gel G. The separation was slightly improved when the layer was impregnated with 1% of tetrabutylammonium bromide. The R_F values have been tabulated (55).

The retention behavior of 2,3-dihidro-1,5-benzothiazepines was studied (56) using OPLC on silica-gel and alumina layer impregnated with concentrations of 0–0.2M tricaprylmethylammonium chloride and developed with aqueous methanol solutions as mobile phases. Retentions were found to increase with the concentration of impregnation reagent in the silica-gel layer and decrease with increasing methanol content of the mobile phase. Optimum separation was performed with 0.05M reagent impregnated on the silica-gel layer and aqueous 50% methanol solution as the mobile phase. The interactions between the benzothiazepines and the capril group of tricaprylmethylammonium chloride were basically hydrophobic. For compounds that had a strong acidic character, ion- pairing may have occurred in the stationary phase. Other problems concerning the influences of the benzothiazepine substituents on the retention were discussed in detail.

The retention and the resolution of barbiturated compounds were discussed as a function of the concentration of ion-pairing reagent in the stationary phase and dipping time. Good separation was achieved on a layer impregnated by horizontal dipping in 0.05-mol/L methanolic solutions of CTMA or TOMA for 2 and 3

min. For vertical dipping, the best separations were achieved on a layer impregnated with 0.1 mol/L CTMA at a speed of 0.1 cm/min and with a dipping time of 3 min (57).

Inclusion complexes

RP-TLC was performed on silica gel impregnated with a solution of macrociclic compounds possessing different NxOy donor sets in a nonpolar solvent. The macrocyclic compounds used were 1,10-diaza-18-crown-6,7,8,9,10,17,18,19,20,21,22-decahydro-6H, 16H-dibenzo[h, q][1,7,4,10,13,16]dioxatetraazocyclo-octadecine and 6,7,9,10,17,18 -decahydro16H-dibenzo[h,q][1,4,7,10,16]trioxadiazacyclo-octadecine (58).

Cserháti (59) prepared a polymeric stationary phase by cross-linking ß-cyclodextrin (CD) monomer units with epichlorhydrin and ethylene glycobis (epoxypropyl ether). The retention of fourteen 3,5-dinitrobenzoic acid esters showed a linear correlation between R_M values and the methanol content in the mobile phase. This behavior was attributed to the possible formation of ternary CD-solute methanol complexes. The possible application of water-insoluble ß-CD polymer as the TLC sorbent was also reported (60). The effect of various molecular structures on the retention of solutes on the ß-CD polymer support was studied and the parameters related to retention were obtained. These data help in the prediction of the retention behavior of solutes on a ß-CD polymer coated HPLC column.

Chemically bonded stationary phases

After the introduction of the first commercially available and chemically modified plate materials in 1977–1978, and partly under the influence of HPLC technology at a time when reversed-phase packing materials were used quite generally, more TLC methods were systematically developed on chemically modified plate materials. Real progress was later recorded regarding precoated TLC and HPTLC plates with a nonpolar and polar chemically bonded phase. Today there are commercially available RP-TLC and RP-HPTLC plates with silica gel (silanized with various alkyls) that preferably have long-chain hydrocarbons (C^2, C^8, C^{12}, and C^{18}). Silica gel particle granulation has been used for preparing TLC and HPTLC plates. Some plates also contain a fluorescent indicator and a concentrating zone. The layer adherence to the support was good. Remarkable progress has been attained in producing precoated plates with a polar bonded phase of type amino-, nitrile-, and diol-bonded phase (2).

Previously, Macherey-Nagel (FRG) introduced two new plates: Nano-Sil-PAH and Nano-Sil-CN. The PAH plate, designed for the separation of polynuclear aromatic hydrocarbons, is a ready-to-use TLC plate in which the silica gel is impregnated with an electron acceptor. The CN version is a cyano-modified TLC glass plate that is recommended for the separation of steroids, phenols, and preservatives (61).

For the characterization of RP-18 type chemical bonded phase the Raman spectra was used (62). Raman spectra were acquired on a spectrometer with a high-power lNd:WVO4 laser (1064 nm). Spectra were determined over the range 3300 to 700 cm^{-1}. This method can be used to study stationary phases and to determine the density of coverage of the silica matrix with octadecyl ligands because of a wide, intense, and high-structured region of approximately 2285 to 1130 cm^{-1}.

Hajouj et al. (63) prepared a mixed octadecylsilane and cyanopropylsilane bonded phase in various ratios by stirring suspensions in methanol. The suspensions were spread on a glass plate without binder. Because of their mechanical fragility the plates were developed in the horizontal mode. Two-dimensional TLC with polyaromatic hydrocarbons as test compounds made the evaluation of the efficiencies of those layers. The polarity of layers composed of C-18/cyano mixed silica-based bonded phase (and the resultant resolution capability) was controlled by varying the ratio of the two sorbents mechanically blended together during preparation. This procedure made it possible to modulate the polarity of the stationary phase in TLC. The layers were tested on mixtures of polyaromatic hydrocarbons and aza-arenes (64).

The applications of multimodal separation in TLC were discussed (e.g., two stationary phase with one mobile phase or one stationary phase with two mobile phase). Amino-, cyano-, and phenyl-modified silica gel was used. Such systems could be used to separate highly complex mixtures (e.g., plant extracts, glicolipid mixtures, and hormone derivatives) by TLC (65). Also, the flavonoids in *Scutellariae radix* were analyzed by HPTLC on phenyldimethyletoxysilane treated silica-gel plates (66).

The diol phase appeared to be suitable for normal-phase TLC of polar ionic conjugates, and many could provide a useful alternative to silica gel for studies of drug metabolism. The diol phase generally behaved as a deactivated silica gel. Compared with silica gel, a useful feature of the diol phase was the possibility, at least for glucine and glucuronide conjugates, of TLC separating both conjugates and aglycones with a single mobile phase (67).

Cserháti and Hauck (68) studied retentions of 18 benzodiazepine derivatives on the polar bonded phase (CN, NH^2, and diol) in the normal and RP-TLC separation. The chromatographic results showed that CN, diol, and NH^2, HPTLC plates were suitable for the separation of most benzodiazepine derivatives.

A systematic study of the absorption of different ion-pairing reagent on RP layer was performed by Kovács-Hadady (69). Tetraethylammonium bromide, tetrabutylammonium bromide, cetyltrimethylammonium bromide, and trioctylammonium chloride were used as ion-pairing reagents for the separation of barbituric acid derivatives. Other reagents (70) such as tetramethylammonium bromide or cetyltrimethylammonium bromide were used for the impregnation of chemically bonded (C-18) RP. Significant differences in pH between mobile and stationary phases were observed, especially when the buffer was incorporated into the mobile phase only. Bieganowska and Petruczynik (71) separated some alkaloids in ion-association system by RP-TLC on precoated HPTLC RP-18 and RP-8 plates or plates coated with a slurry of silanized silica gel 60 H F^{254} containing an appropriate concentration of an ion-pairing agent such as di(2-ethylhexyl)orthophosphoric acid (HDEHP), camphoric acid, camphorsulfonic acid, SDS, $HclO^4$, oxalic acid, and tricloroacetic acid. For development, 0.03M phosphate buffer of pH 3.48 containing different quantities of methanol and an ion-pairing agent was used. The best results were obtained with HDEHP or SDS as the ion-pairing agents.

Chiral Phases

Enantiomeric separation is important in many fields, and a variety of chromatographic methods have been used successfully.

For separation of enantiomers by TLC there are two ways through the stationary and mobile phases. The first involves the use of a chiral stationary phase (CSP) or a derivatization of the phase with a chiral reagent in order to obtain diasteriomeric structures that will lead to specific interaction with an achiral support. In the first mode there are many ways to accomplish separation, yet the second way involves the use of chiral mobile phases.

Han and Armstrong (72) published a review on enantiomeric separation by TLC using a CD-bonded stationary phase or cyclodextrin as a chiral mobile phase additive. A recent review was published by Lepri (73) regarding the enantiomer separation through TLC. This review presented the literature concerning TLC separation of enantiomeri on CSP, chiral-coated phase (CCP), cellulose, and modified cellulose. Another review (74) presents an overview of the current successful enantioseparation of drugs by TLC and their potential in the analysis of the drug racemates.

Bhushan and Martens (75) presented an overview of the applicability of the impregnated TLC as an analytical method with particular reference to the chiral separation of amino acids, dyes, and pharmaceutical products. The comments on the ion-pairing, ion-exchange, and complex-formation mechanisms operating in impregnated TLC were also discussed. Examples of the technique involving the ligand exchange, steric interaction, and ion-exchange separation of the enantiomeric mixture using an impregnated chiral selector were given. The same authors presented another review (76) of subjects such as ligand exchange, ion exchange, and steric interactions concerning the resolution of enantiomers of amino acids and basic drugs by TLC. The role of impregnation in resolving enantioseparations or improving the separation of mixtures of amino acids or their derivatives in terms of ion pairing, complex formation, ligand exchange, or other steric interaction has been studied in each category (77).

The progress in the separation of enantiomers of chiral drugs by TLC without their prior derivatization were overviewed by Ubert and Lais (78). In a recent review published by Berezniski et al. (79) the literature regarding the separation of enantiomers was discussed. In this review the method of enantiomer separation was presented according to the main mechanism governing the particular separation, in the function of interaction in enantiomeric separations: ligand exchange, inclusion compounds, charge transfer, ion pair, taylor-mode polymers, cellulose layers, and protein phase.

Nonpolar chemically bonded stationary phases impregnated with a chiral selector

Chiral separation via ligand exchange involves the reversible reaction between a complex of a metal ion with a chiral complexing agent and the two enantiomers R and S, leading to ternary diastereomeric complexes with different stabilities. These two diasteriomeric complexes have different affinities for the stationary phase and will elute with a different retention. The method can be applied to the separation of compounds that are able to form bidentate complexes with transitional metals ions such as Cu (II) or Ni (II). These plates contain compositions with a chiral complex that is commercially available as chiral plates from several companies such as Chiralplate (Macherey-Nagel), Chir (Merck), and others.

Grinberg (80) synthesized a chiral phase with highly hydrophobic properties. The selector (an *N,N*-dialkyl amino acid with decyl substituents at the amino group) formed a copper *N,N*-didecylalanine complex that could be dissolved in only organic solvents. RP-18 plates impregnated with such a chiral phase showed good selectivity for all the dansyl amino acids (both proteic and nonproteic) except proline.

In a similiar way, Remelli et al. (81) synthesized a chiral ligand exchange selector *n-N-n*-decylhistidine (LNDH) that was able to undergo a strong interaction with a hydrophobic RP-18 WF^{254} S stationary phase. The ligand was synthesized by being selectively alkylated at the pyrolic nitrogen atom in its imidazolic ring. Chiral plates were prepared by immersing RP-18 WF^{254}S HPTLC plates in 0.125% Cu (II) acetate solution. After that, the plates were immersed in 0.4% LNDH and dried at room temperature. A significant selectivity towards aromatic amino acid enantiomers was observed.

Silica gel impregnated with a chiral selector

Bhushan et al. (82–87) performed studies on the possibility of the separation of racemic mixtures in their enantiomers through NP-TLC using silica gel impregnated with a different chiral selector.

The TLC separation of D,L-amino acids was performed on the silica gel G impregnated with a complex Cu (II)-L-proline (pH 6.9–7.0). The separation occurred by a ligand exchange of the amino acids with the metal chelate from the stationary/mobile phase. In each of these separations of amino acid enantiomers (phenylalanine, tyrosine, isoleucine, and tryptophane) it was observed that the L-isomer had a higher R_F value than the D-isomer (82). Racemic hyoscyamine (atropine) and colchicines were separated on silica gel plates (20 x 20 x 5 mm) impregnated with 0.3 g L-aspartic acid as the chiral selector (83). The enantiomers of (±)-ibuprofen [a-methyl-4-(2-methylpropyl)benzeneacetic acid] were separated by two-dimensional TLC (2D-TLC) on plate-coated silica gel G impregnated with 1% L-arginine. The development was achieved with acetonitrile–methanol–water (5:1:1, v/v/v) for 15 and 20 min in the first and second dimension, respectively (84).

The enatioseparation of 2-arylpropionic acid-type anti-inflammatory racemic drugs, (±)-ibuprofen, and (±)-flurbiprofen was performed by 2D-TLC on silica gel G (with 13% $CaSO^4$ as binder having chloride, iron, and lead as impurities up to 0.02%, pH 7) impregnated with (-)-brucine as the chiral selector. Acetonitrile–methanol (16:3, v/v) was used as the mobile phase in the first dimension and acetonitrile–methanol–water (16:3:0.4, v/v/v) for the second dimension (87).

Cellulose

The enantiomers that could be separated on microcrystalline cellulose were highly polar with multiple sites for hydrogen-bond formation. The mechanism of chiral recognition is not yet completely clarified even though a significant role was attributed to the microcristalline cellulose structure.

Lederer (88,89) and Xuan et al. (90–92) studied the chromatographic behavior of microcristalline cellulose thin layer as the chiral selector. In spite of the fact that a liquid–liquid partition mechanism would be expected, in case of aquous solvents it was

experimentally demonstrated that a hydrophobic adsorption mechanism was also operating.

Modified cellulose

The microcristalline cellulose triacetate (MCTA) thin-layer plates are commerciable from Antec (Benwil, CH) and Macherey-Nagel. Lepri et al. (93–96) proposed the creation of homemade layers using MCTA for column chromatography. The chromatographic behavior of several racemates and pure optical isomers as *N*-derivatized amino acids, propionic acid derivatives, alcohols, aromatic amines, and lactones were investigated. RP-TLC was performed on plates precoated and homemade with cellulose triacetate (CTA) and MTCA for HPLC with sodium carboximethylcellulose or silica gel 60 G F^{254} as the binder. The development was performed with an aqueous organic mobile phase containing ethanol or 2-propanol. The resolution of the enantiomers was highly dependent on the type of CTA (acetyl content and structure of the adsorbent), the concentration of organic solvent in eluent, and the development temperature. The results demonstrated the ability of MCTA and role of the chemical structure of the compounds in the successful separation of those racemates in its optical isomers (94). In another paper (95) 21 of the compounds related to those investigated previously (93,94) and new chiral compounds (such as flavones derivatives and pyrethroids) were studied. For this purpose, RP-TLC on MCTA layers were homemade. The results were discussed as a function of the organic groups and the steric configuration of the compounds. The enantiomers of 1-acenaphthenol and most oxiranes could also be resolved on homemade MTCA plates through the use of a variety of aqueous ethanol as the mobile phase (96). Chiral discrimination by MCTA increased with increasing of the crystallinity of the polysaccharide, even though this characteristic is not an absolute requirement for enantioseparation.

A number of the eight phenylisocianates were separately bonded to microcrystalline cellulose in pyridine solution at 120°C for 6 h to form a set of the derivatized cellulose triphenylcarbamate stationary phase (97).

Chitin, chitosan, and their derivatives

These compound were amino derivatives of polysaccharides and were used in TLC as stationary phases impregnated with Cu (II) (98–100). TLC separated the racemates of alanine, leucine, threonine, and valine on a-chitin impregnated with Cu (II), and methanol or ethanol was used as the mobile phase (98).

Cyclodextrine

Cyclodextrins (CDs) are macrocyclic oligomers that contain 6 to 12 D (+)-glucopyranose units, which are bonded through a-(1,4) linkages. a-CD has six glucose units. ß-CD has seven glucose units, and *y*-CD has eight glucose units. These three smallest homologes are available commercially. The CDs are shaped like open-ended truncated cones that can complex many organic molecules. The interior of the cavity contains two rings of C-H groups with a ring of glucosidic oxigens between them. The primary hydroxyl groups are on the small side whereas the secondary OH groups are on the side of the tours with the larger circumference. In these circumstances the cavity is relativity hydrophobic, whereas the external faces are hydrophilic. Several requirements must be met if there is to be chiral recognition between a-CD and the component. Among the important parameters that determine whether an inclusion complex can be formed are the relative size and geometry configuration of the guest molecule in relation to the dimensions of the host CD cavity. In addition, formation of the CD inclusion complex is strongly affected by such factors as pH, temperature, and composition of the mobile phase.

Reviews (101,102) were published concerning enantiomeric separation by TLC using CD-bonded stationary phases.

The separation of several enantiomers (binaphthalenes, isoprenaline, nimodipine, prometazine, ofloxacin, and carvediol) was performed on b-CD bonded phase using different mixtures of solvents as the mobile phase (103). The b-CD-bonded phase was prepared with the use of 5 g of silica gel H, and 10 g b-CD was dried overnight at 110°C under vacuum. The dried b-CD was dissolved in anhydrous dimethylformamide and stirred at 90°C. The solid was removed by filtration and the dried silica gel H and 3-glycidoxypropyltrimetoxysilane (2.5 g) were added to the solution. The mixture was stirred at 90°C. The CD-bonded phase was filtred, washed, and then dried in air. The selectivity factors [$a = (1/R_{F1} 1)(1/R_{F2}-1)$] were in the range of 1.38 to 2.43.

Macrocyclic antibiotics

The macrocyclic antibiotics that were used as the chiral selector (which include the ansamicines, glycopeptides, and polypeptide antibiotic thiostrepton) possessed multiple stereogenic centers and a variety of functional groups that could interact specifically with the racemic compounds (104).

Bhushan and Parshad (105) performed TLC separation of enantiomeric dansyl amino acids using a macrocyclic antibiotic as a chiral selector. A slurry of silica gel G (containing 13% of $CaSO^4$ as binder) was prepared in erythromycin solution, spread on glass plates, and dried .The dansyl derivatives of DL- and L-amino-acids were separated with a NaCl-solution–acetonitrile and, in some instances, with a small addition of methanol. The separation factor for the derivatives of ten enantiomers was in the range of 1.06 to 1.36, the D-enantiomers having the higher $R_F$values.

Similarly, the chiral resolution was performed by NP-TLC using vancomycin (amphoteric glycopeptide) a macrocyclic antibiotic (106). TLC plates were prepared by spreading a slurry (pH 6) of silica gel G (30 g) in distilled water that contained sterile vancomycine hydrochloride (0.34mM). The plates with pH 4 or 8 were obtained by adding acetic acid or ammonium solution, respectively, to the slurry. The mobile phase gave a successful resolution of most of the racemic dansyl amino acids as a mixture of acetonitrile with aqueous NaCl solution. The enantioseparation was affected by the type and amount of organic modifier added to the mobile phase. In addition, it was observed that the best resolution of enantioseparation was obtained by use of 0.34mM vancomycine. Enantioresolution was observed at pH 6.0.

Molecular imprinted polymers

A new separation approach using molecular imprinted polymers (MIP) was first reported by Kriz et al. (107) as a chiral stationary phase in TLC. Molecular imprinting is a methodology for the preparation of synthetic polymers with predetermined selectivity for specific solutes. The MIPs were prepared in acetonitrile

by fotoinitiation using a system of ethylene glycol dimethacrylate as a cross-link and methacrilic acid as a monomer. MIP is formed by polymerizing a solution of functional monomers and a cross-linking agent in the presence of a template molecule (L-phenylalanine amide or D-phenylalanineamide). When the polymerization is complete the template molecule is removed, which leaves sites in the polymer that are complimentary in shape and functionality to the template. In the same conditions polymers with no imprinting (non-MIP) were prepared. The polymers were ground and the particles were fractioned by a sedimentation procedure.

Polymer particles and plaster of Paris ($CaSO^4$, 1/2 H_2O as binder) were mixed in a solution containing water and ethanol and spread on a glass plate. For the chiral separation L- and D-phenylalanine amides were chosen as the model compounds. The separation was performed with 5% acetic acid with acetonitrile as the mobile phase. In this case the chiral factor was $R_{FL}/R_{FD} = 3.5$.

Suedee et al. (108) prepared MIPs using two different monomers [metacrilic acid (MAA) and itaconic acid (ITA)] as functional monomers so that carboxylic group of the monomers interacted ionically with the amine group in quinone. MAA or ITA, ethylenglicol, dimethylglicol, dimethylacrilate, the initiator 2,2-azobis(2-methylpropionitrile), and quinine dissolved in tetrahydrofuran were polymerized under UV radiation (366 nm). In another recent study by Suedee et al. (109), three MIPs imprinted with (+)-ephedrine, (+)-pseudoephedrine, (+)-norepephedrine, and a non-MIP were prepared according to the method described in previous work (108). The polymer (1 g) and gypsum (1 g) were mixed with distilled water (3 mL). The slurry was spread as a thin layer (0.25 mm) on standard glass microscope slides. The enantiomeric determination of ten adrenergic drugs was performed through the development of their racemates on the TLC plates using 7 or 5% acetic acid in dichloromethane. In these conditions adrenergic drugs structurally related to imprinted molecules were completely separated in two spots with the MIP plates. Generally, the R_F values of (–)-isomers (or 1R-isomers) were larger than that of (+)-isomers (or 1S-isomers), demonstrating the stereoselectivity of the MIPs with former isomers. The opposite behavior was observed for b-blockers, and the R_F of (+)-R-propranolol was higher than that of the (–)-S form.

The best resolution was achieved for enatioseparation of norephedrine on plates based on the MIP of (–)-norephedrine using ITA as the functional monomer**;** the separation factor (a) was very high (5.1) with acetic acid methanol as the mobile phase (110).

The TLC separation of two classes of chiral drugs, including b-blocking drugs and nonsteroidal anti-inflammatory drugs (NSAID) on molecularly chiral stationary phase was reported (111). Several MIPs were prepared using the enantiomers of either the b-blocking drugs, R-(+)-propranolol, R-(+)- or S- (-)-atenolol, or the NSAID S-(+)-naproxen and S-(+)-ibuprofen as print molecules. The experimental results showed that besides resolving the imprint molecules, MIPs can discriminate between closely related compounds. This case is evidence of the important role of specific substituent groups in the enantioresolution of MIPs. Also, the composition of the mobile phase is very important to enantioresolution because the polymer can swell in the liquid medium, which results in deformation of cavities and consequent loss of chiral discrimination.

References

1. C.P. Poole and S.K. Poole. *Chromatograpy Today*, Elsevier, Amsterdam, The Netherlands, 1991, pp. 671–83
2. S. Gocan. *Stationary Phase in Thin-Layer Chromatograpy in Modern Thin-Layer Chromatography*. N. Grinberg, Ed. Marcel Dekker, New York, NY, 1990, pp. 5–137.
3. J. Köhler, D.B. Chase, R.D. Farlee, A.J. Vega, and J.J. Kirkland. Comprensive charcterization of some silica-based stationary phase for high-performance chromatography. J. Chromatogr. **352**: 275-305 (1986).
4. J. Nawrochi. Silica surface controversy, strong adsorbtion sites, their blockage and removal, Part I and Part II. *Chromatographia* **31:** 177–92 (1991).
5. H. Enghelhardt, H.Low and W. Götzinger. Chromatographic characterization of silica-based reversed phases (review). *J. Chromatogr.* **544:** 371–79 (1991).
6. D.W. Sindorf and G.E.R. Maciel. 29SiNMR study of dehydrated / rehydrated silica gel using cross polarization and magic-angle spinning. *J. Am. Chem. Soc.* **105:** 1487–93 (1983).
7. E. Hahn-Deinstrop. Stationary phase, sorbents. *J. Planar Chromatogr.* **5:** 57–61 (1992).
8. G.H. Flodberg and J. Roeraade. High pressure thin layer chromatography on 3 micron spherical particle beds. *J. Planar Chromatogr.* **6:** 252–55 (1993).
9. V.G. Berezkin and R.G. Mardanov. Policolumn plates for TLC. *J. Planar Chromatogr.* **11:** 325–29 (1998).
10. W.P.N. Fernando and C.F. Poole. The influence of layer porosity on the flow resistance and apparent size of thin layer chromatography plates. *J. Planar Chromatogr*. **3:** 389–95 (1990).
11. C.F. Poole and S.K.Poole . Instrumental thin layer chromatography. *Anal.Chem.* **66:** 27A–37A (1994).
12. C.F. Poole and W.P.N. Fernando. Comparison of the kinetic properties of commercially available precoated silica gel plates. *J. Planar Chromatogr.* **6**: 357–61 (1993).
13. E. Hahn-Deinstrop. TLC plates: initial treatment, pre-washing, activation, conditioning. *J. Planar Chromatogr.* **6:** 313–18 (1993).
14. R.J .Maxwell and A.R. Lightfield. An improved method for efficient predevelopment washing of HPTLC plates. *J. Planar Chromatogr.* **12:** 109–13 (1999).
15. S.M. Petrovic and M. Acanski. Modification of the silica surface with the vapours of polar solvent. *J. Planar Chromatogr.* **4:** 439–41 (1991).
16. Y. Liang, M.E. Baker, D.A. Gilmore, and M. B. Denton. Evaluation of commercial silica-gel HPTLC plates for quantitative fluorescence analysis. *J. Planar Chromatogr.* **9:** 247–53 (1996).
17. J. Ahmad. Use of alumina as stationary phase for thin- layer chromatography of inorganic and organometallic compounds. *J. Planar Chromatogr.* **9:** 236–46 (1996).
18. M. Palmareva, I. Kozekov, and I. Jurova. Chromatographic of diastereoisomers. XII. Effects of alumina on separation of esters of maleic and fumaric acids *J. Chromatogr. A* **670:** 181–90 (1994).
19. N.D. Danielson, J.E. Katon, S.P. Bouffard, and Z.H. Zhu. Zirconium oxide stationary phase for thin-layer chromatography with diffuse

reflectance Fourier transform infra-red detection. *Anal. Chem.* **64:** 2183–86 (1992).
20. M. Wakmundzka-Hajnos. Properties of florisil and its use in chromatography. *Chem. Anal. (Warsaw)* **43:** 301–24 (1998).
21. R. Karakas and Y. Yuksel. Modification of perlite for use as a thin-layer chromatographic adsorbent. *J. Chromatogr. Sci.* **36:** 499–504 (1998).
22. C. M?rutoiu, M. B?descu, A. P?trut, A. Tecoant¸? and M. Dasc?lu. Synthesis and characterization of dicationic zeolites for use as stationary phases in thin-layer chromatography. *Acta Chromatographica No.* **8:** 32–36 (1998).
23. N. Peri i-Janjic´ and B. Vujic˘ic´. Analysis of some food components by thin-layer chromatography on unconventional layers. *J. Planar Chromatogr.* **10:** 447–52 (1997).
24. S.W. Husain, A. Avanes, and V. Ghoulipour. Thin layer chromatography of metal ions on cerium(III) silicate: a new ion-excenger. *J. Planar Chromatogr.* **9:** 67–69 (1996).
25. A. Mohammad, M. Ajmal, N. Fatima, and M.A. Majid Khan. Some observations on the sorption behavior of heavy metals on layers prepared from mixtures of silica and inorganic ion-exchange gels. *J. Planar Chromatogr.* **5:** 368–75 (1992).
26. V. Ghoulipour and S. Waqif Husain. New adsorbents in planar chromatography: II Behavior of metal ions on titanium (IV) silicate ion-exchanger. *J. Planar Chromatogr.* **13:** 354–58 (2000).
27. A. Mohammad, S. Anwar, and E. Iraki. Detection, identification and separation of certain heavy metals on silica gel-suported tin(IV) arsenosilicate layer. *Chem. Anal. (Warsaw)* **44:** 195–200 (1999).
28. A. Mohammad, N. Fatima, and M.A. Majid Khan. New surface-modified sorbent layers for chromatographic analysis of some inorganic pollutants. *J. Planar Chromatogr.* **7:** 142–46 (1994).
29. A. Mohammad, M.A.M. Khan. New surface-modified sorbent layer for the analysis toxic metals in seawater and industrial wastewater. *J. Chromatogr.* **642:** 455–58 (1993).
30. T. Shimizu, T. Tanaka, and M. Kobayashi. Reversed phase extraction TLC of inorganic ions, with sulfuric acid and ammonium sulfate mobile phases on silica gel impregnated with tri-n-octylamine. *J. Planar chromatogr.* **8:** 469–72 (1995).
31. T. Shimizu, M. Kaneko, and Y. Toyoshima. Reversed phase extraction chromatography of inorganic ions on silica gel layers impregnated with a primary alkilamine and developed with sulfuric acis and sulfate mobile phases. *J. Planar Chromatogr.* **8:** 152–54 (1995).
32. T. Shimizu, S. Jindo, N. Iwata, and Y. Tamura. Reversed phase TLC of inorganic ions silica impregnated with a high molecular weight amine, using sulfuric acid and sulfuric acid- ammonium sulfate mobile phases. *J. Planar Chromatogr.* **7:** 98–102 (1994).
33. S.Z. Qureshi, R.M.A.Q. Jamhour, and N. Rahman. A novel thin-layer chromatographic system: Identification and separation of some cephalosporins. *J. Planar Chromatogr.* **9:** 466–69 (1996).
34. M. Lederer. The perchlorate effect with metallo-cyanide complexes on cellulose anion-exchange thin-layers. *Anal. Chim. Acta.* **25:** 93–95 (1998).
35. T.S. Li, J.T. Li, and H.Z. Li. Modified and convenient preparation of silica impregnated with silver nitrate and its application to the separation of steroids and triterpenes. *J. Chromatogr.* **715:** 372–75 (1995).
36. V.P. Pchelkin and A.G. Vereshchagin. Identification of individual diacylglycerols by adsorbtion thin-layer chromatography of their co-ordination complexes. *J. Chromatogr.* **538:** 373–83 (1991).
37. R. Bhushan and S. Kaur. TLC separation of some common sugars on silica gel plates impregnated with transition metal ions. *Biomed. Chromatogr.* **11:** 59–60 (1997).
38. O. Hadzija, B. Spoljar, and L. Sesartic. Thin layer chromatography of glucose and sorbitol on Cu (II)-impregnated silica gel plates. *Fresenius' J. Anal. Chem.* **348:** 782–82 (1994).
39. R. Bhushan, I. Ali, and S. Sharma. TLC separation of amino-acids using copper (II) ion. *Biomed. Chromatogr.* **10:** 37–39 (1996).
40. R. Bhushan and I. Ali. Adsorption and partition of amino-acids in a batch process compared with their TLC separation on silica gel. *J. Planar Chromatogr.* **3:** 85–87 (1990).
41. R. Bhushan and I. Ali. TLC separation of sulfonamides on impregnated silica gel layers, and their quantitative estimation by spectroscopy. J. Planar Chromatogr. **8:** 245–47.
42. R. Bhushan and V. Parshad. Separation of vitamin B complex and folic acid using TLC plates impregnated with some transition metal ions. *Biomed. Chromatogr*. **8:** 196–98 (1994).
43. R. Bhushan and I. Ali. TLC separation of certain tetracycline and amino glycopeptide antibiotics. Biomed. Chromatogr. **6:** 196–97 (1992).
44. D.K. Singh and A. Mishra. Thin layer chromatography of aromatic amines on silica gel G impregnated with ammonium cerium (IV) nitrate *J. Planar Chromatogr.* **5:** 284–85 (1992).
45. H. Szumilo and J. Flieger. Chromatographic behaviour of sulfonamides on polyamide impregnated with various metal salts. *J. Planar Chromatogr.* **9:** 462–66 (1996).
46. N.U. Perisic-Janjic, S.M. Petrovic, and D. Bajkin.Thin-Layer chromatography of salicylaldehide semi and thiosemicarbazones. *J. Planar Chromatogr.* **4**: 257–59 (1991).
47. J. Gasparic. Chromatography on thin layers impregnated with organic stationary phase. *Adv. Chromatogr. (NY)* **31:** 153–52 (1992).
48. M. Ajmal, A. Mohammad, and S. Anwar. Separation of aromatic [aryl]amines thin-layer plates impregnated with picric acid. *J. Planar Chromatogr.* **3:** 511–14 (1990).
49. J. Gasparic and J. Skutil. Comparative study of chromatography on thin layer impregnated with organic stationary phase. *J. Chromatogr.* **558:** 415–22 (1991).
50. S.D. Sharma, S. Misra, and S.C. Sharma. Reversed phase thin-layer chromatographic behaviour of some toxic metal ions on tri-n-butyl phosphate silica gel-G layer in acetone-nitric acid water systems : quantitative separation of lead (II) and chromium (III) from many metal ions. *J. Indian Chem. Soc.* **75:** 81–83 (1998). *Anal. Abstr.* **60:** 12D49 (1998).
51. S.D. Sharma and C.Sharma. Retention behavior of some d- and f-block metal ions on untreated and tri-n-butylamine-imprgnated silica gel G layers Quantitative of Zr^{4+} and W^{6+} from other metal ions and alloy components. *J.Planar Chromatogr.* **13:** 93–100 (2000).
52. S.D.Sharma, S.C. Sharma, and C. Sharma. Reversed phase TLC of 3d metal ions on silica gel GF_{254} impregnated with silicone fluid DC200, tri-aryl phosphate, and tri-n-butyl phosphate and with DMSO-HNO3 mobile phase. *J. Planar Chromatogr.* **14:** 16–20 (2001).
53. P. Pandey and S. Bhattacharya. Thin-layer chromatographic studies of some sulpho drugs (substituted pyrazoles) using silica gel G plates impregnated with various adsorbents. *J. Liq. Chromatogr.* **15:** 1665–71 (1992).
54. S.N. Shtykov, E.G. Sumina, E.V. Smushkina, and N.V. Tyurina. Dynamic and static modification of stationary phases with surfactants in TLC: a comparative study. *J. Planar Chromatogr.* **13:** 182–86 (2000).
55. P. Pandey, N. Maheshwari, and A. Dave. Identification of some closely related N-carbamoyl 3,5-dimethyl-4-arylazopyrazoles [4-arylazo-3,5-dimethil-pyrazole-1 carboxamides] by TLC. *J. Liq. Chromatogr.* **14:**

3311–15 (1991).
56. K. Kovács-Hadady and A. Levai. OPLC [overpressured layer chromatography] study of retention of 2,3-dihydro-1, 5-benzothiazepines on layers impregnated with tricaprylmethylammonium chloride. *Chromatographia* **37:** 482–86 (1993).
57. K. Kovács- Hadady, and T. Varga. A systematic study of impregnation of thin layers with different ion-pairing reagents and by different methods. J. Planar Chromatogr. **8:** 292–99 (1995).
58. I.V. Pletnev, E.I. Morosanova, and Yu.A. Zolotov. Use of mixed stationary phases for the [TLC] separation of metal ions. *Anal. Sci.* **6:** 863–66 (1990). *Anal.Abstr.* **54:** 1D13 (1992).
59. T. Cserháti. Relationship between the physicochemical parameters of 3,5-dinitrobenzoic acid esters and their retention behaviour on beta-cyclodextrin polymer support. *Anal. Chim. Acta.* **292:** 17–22 (1994).
60. T. Cserháti, E. Fenivesi J. Szejtli and E Forgacs. Water-insoluble beta-cyclodextrin polymer as thin- layer chromatographic sorbent. *Chim. Oggi.* **13:** 21–24 (1995) *Chem. Abstr.* **124:** 277070w. (1996).
61. R.E. Majors. New chromatography columns and accessories at the 1996 pittsburg conference, part I, *LC-GC International* **10(4):** 226–27 (1996).
62. G. Kowalik, K. Rogosz, and T. Kowalska. Raman spectroscopic characterization of RP-18- type chemically boned stationary phase for liquid chromatography. *J. AOAC Int.* **82:** 297–304 (1999).
63. Z. Hajouj, J. Thomas, and A.M. Siouffi. Two-dimensional TLC on mechanically mixed bonded phases. *Analusis* **22:** 404–407 (1994).
64. Z. Hajouj, J. Thomas, and A.M. Siouffi. Mechanically blended silica-based bonded phases. Application of the simple modulation of the polarity of the stationary phase to TLC. *J. Liq. Chromatogr. Relat.Technol.* **19:** 2419–22 (1996).
65. H.J. Issaq. Recent advances in multimodal thin-layer chromatography. *Trac, Trends Anal. Chem.* **9:** 36–40 (1990). *Anal. Abstr.* **53:** 4B49 (1991).
66. M. Okamoto, M. Ohta, H. Kahamu, and T. Omori. Evaluation of phenildimethylethoxysilane treated high-performance thin-layer chromatographic plates. Application to analysis of flavonoids in *Scutellariae* radix. *Chromatographia* **35:** 281–84 (1993).
67. P. Martin, J.Taberner, R.J. Thorp, and I.D. Wilson. Comparison of silica gel and diol-bonded silica gel for the TLC of glycine, sulfate, and glucuronide conjugates. *J. Planar Chromatogr.* **5:** 99–102 (1992).
68. T. Cserháti and H.E. .Hauck. Retention characteristics of CN, NH_2 and diol precoated high-performance thin-layer chromatographic plates in the adsorption and reversed phase separation of some benzodiazepine derivatives. *J. Chromatogr.* **514:** 45–55 (1990).
69. K. Kovács-Hadady. A systematic study of the adsorption of different ion-pairing reagents on reversed-phase layers. *J. Planar Chromatogr.* **9:** 174–77 (1996).
70. M. Lukacs, G. Barcsa, and K. Kovács-Hadady. The actual pH values of the stationary phase in ion-pair thin-layer chromatography. *J. Planar Chromatogr.* **10:** 342–47 (1997).
71. M.L. Bieganowska and A. Petruczynik. Thin-layer reversed-phase chromatography of some alkaloids in ion-association systems. *Chem. Anal. (Warsaw).* **39:** 139–47 (1994).
72. S.M. Han and D.W. Armstrong. Enantiomeric separation by thin-layer chromatography. *Chem. Anal. (NY)* 1990; (Planar Chromatogr. Life Sci.): 81–100. *Anal. Abstr.* **54:** 1B98 (1992).
73. L.Lepri. Enantiomer separation by TLC. *J.Planar Chromatogr.* **10:** 320–31 (1997).
74. H.Y. Aboul-Enein, M.I. El-Awady, C.M. Heard, and P.J. Nicholls. Application of thin-layer chromatography in enantiomeric chiral analysis an overview. *Biomed. Chromatogr.* **13:** 531–37 (1999).
75. R. Bhushan and J. Martens. Direct resolution of enantiomers by impregnated TLC. *Biomed. Chromatogr.* **11:** 280–85 (1997).
76. J. Martens and R. Buhshan. Importance of enantiomeric purity and its control by thin-layer chromatography. *J. Pharm. Biomed. Anal.* **8:** 259–69 (1990).
77. R. Buhshan and J. Martens. Separation of amino acids, their derivatives and enantiomers by impregnated TLC. *Biomed.Chromatogr.* **15:** 155–65 (2001).
78. J. ubert and K. lais. Progress in the separation of enantiomers of chiral drugs by TLC without their derivatization. *Pharmazie* **56:** 355–60 (2001).
79. Y. Bereznitski, R. Thompson, E. O neill and N. Grinberg. Thin-layer chromatography A useful technique for the separation of enantiomers. *J. AOAC Int.* **84:** 1242–51 (2001).
80. N. Grinberg. *Modern Thin-Layer Chromatography*. N.Grinberg, Ed. Marcel Dekker, New York, NY, 1990, pp. 392–434.
81. M. Remelli, R. Piazza, and F. Pulidori. HPTLC [high performance TLC] separation of aromatic a-amino-acid enantiomers on a new histidine-based stationary phase using ligand exchange. *Chromatographia* **32:** 278–84 (1991).
82. R. Bhushan, G.P. Reddy, and S. Joshi. TLC resolution of DL amino acids on impregnated silica gel plates. *J. Planar Chromatogr.* **7:** 126–28 (1994).
83. R. Bhushan and I. Ali. Resolution of racemic mixtures of hyoscyamine and colchicines on inpregnated silica gel layers. *Chromatographia* **35:** 679–80 (1993).
84. R. Bhushan and V. Parshad. Resolution of (+ / -)-ibuprufen using L-arginine- impregnated thin-layer chromatography. *J. Chromatogr. A* **721:** 369–72(1996).
85. R. Bhushan, J. Martens, and M. Arora. Direct resolution of (+ / -)-ephedrine and atropine into their enantiomers by impregnated TLC. Biomed. Chromatogr. **15:** 151–54 (2001).
86. R. Bhushan, J. Martens, S. Wallbaum, S. Joshi and V. Parshad. TLC resolution of enantiomers of amino-acids and dansyl derivatives using (1R, 3R, 5R)-azobicyclo[3,3,0]octan-3-carboxylic acid as impregnating reagent. *Biomed. Chromatogr.* **11:** 286–88 (1997).
87. R. Bhushan and G. Thuku Thiongo. Direct enantiomeric resolution of some 2-arylpropionic acids using (–)-Brucine-impregnated thin-layer chromatography. *Biomed. Chromatogr.* **13:** 276–78 (1999).
88. M. Lederer. Adsorption chromatography on cellulose. VI. Further studies on the separation of D- and L-tryptophan on cellulose with aqueous solvent. *J. Chromatogr.* **510:** 367–71 (1990).
89. M. Lederer. Adsorption chromatography on cellulose. VII. Chiral separation on cellulose with aqueous solvents. *J. Chromatogr.* **604:** 55–62 (1992).
90. Thi- Kieu- Xuan-Huynh, A.O. Kuhn and M.Lederer. Adsorption chromatography on cellulose. VIII. The salting-out behaviour of some peptides with aromatc groups. *J.Chromatogr.* **626:** 301–304 (1992).
91. X.-T.-K. Xuan and M. Lederer. Adsorption chromatography on cellulose. IX. Chiral separation with aqueous solvents and liquid-liquid systems. *J. Chromatogr.* **635:** 346–48 (1993).
92. Huynh-Thi-Kieu-Xuan and M. Lederer. Adsorption chromatography on cellulose. X. Adsorption of tryptophan and derivatives from copper sulfate-containing eluents. *J. Chromatogr.* **645:** 185–88 (1993).
93. L. Lepri, V. Coas, P.G. Desideri, and A. Zocchi. Reversed phase planar chromatography of enantiomeric compounds on triacetylcellulose. *J.*

Planar Chromatogr. **7:** 376–81 (1994).

94. L. Lepri. Reversed phase planar chromatography of enantiomeric compounds on microcrystalline triacetylcellulose. *J. Planar Chromatogr.* **8:** 467–69 (1995).
95. L. Lepri , M. Del Bubba, and F. Masi. Reversed-phase planar chromatography of enantiomeric compounds on microcrystalline cellulose triacetate (MCTA). *J. Planar Chromatogr.* **10**: 108–13 (1997).
96. L. Lepri, M. Del Bubba, A. Cincinelli, and L. Boddi. Inclusion planar chromatography of enantiomeric and racemic compounds. *J. Planar Chromatogr.* **13**: 384–87 (2000).
97. R. Suedee and C.M. Heard. Direct resolution of propranolol and bupranolol by thin-layer chromatography using cellulose derivatives as stationary phase. Chirality **9:** 139–44 (1997).
98. I. Malinowska and J.K. Róz˙ylo. Chitin, chitosan and their derivatives as stationary phase in thin-layer chromatography. *J. Planar Chromatogr.* **4:** 138–41 (1991).
99. J.K. Róz˙ylo and I. Malinowska . The possibilities of optical isomer separation by TLC on layers of chitin its derivatives. *J. Planar Chromatogr.* **6:** 34–37 (1993).
100. I. Malinowska and J.K. Róz˙ylo. Separation of optical isomers of amino acids on modified chitin and chitosan layers. *Biomed. Chromatogr.* **11:** 272–75 (1997).
101. X. Li, S. Qui, L. Shi, F. Qiao, and H. Lin. Inclusion chromatography and its application in pharmaceutical analysis. *Zhongguo-Yaoxue-Zazhi* **25:** 67–70 (1990). *Anal. Abstr.* **52:** 12J11 (1990).
102. S.M. Han and D.W. Armstrong. Enantiomeric separation by thin-layer chromatography *Chem. Anal. (NY)* 1990; (Planar Chromatogr.Life Sci.**108:** 81–100 (1990). *Anal. Abstr.* **54:** 1B98 (1992)
103. Z. Quanhong, Y. Pingxiang, D. Qinying, and Z. Longmei. b-Cyclodextrin-bonded chiral stationary phase for thin-layer chromatographic separation of enantiomers. *J. Planar Chromatogr.* **14:** 137–39 (2001).
104. T.J. Ward and A.B. Faris. Chiral separation using the macrocyclic antibiotics: a review. *J. Chromatogr.A* **906:** 73–89 (2001).
105. R. Bhushan and V. Parshad. Thin-layer chromatographic separation of enantiomeric dansylamino-acids using a macrocyclic antibiotic as a chiral selector. *J. Chromatogr.A* **736:** 235–38 (1996).
106. R. Bhushan and G.T. Thiong. Separation of the enantiomers of dansyl-DL-amino acids by normal-phase TLC on plates impregnated with a macrocyclic antibiotic. *J. Planar Chromatogr.* **13:** 33–36 (2000).
107. D. Kriz, C.B. Kriz, L.I. Andersson, and K. Mosbach. Thin-layer chromatography based on the molecular imprinting technique. *Anal. Chem.* **66:** 2636–39 (1994).
108. R. Suedee, C. Songkram, A. Petmoreekul, S. Sangkunakup, and S. Sankasa. Thin-layer chromatography using synthetic polymers imprinted with quinine as chiral stationary phase. *J. Planar Chromatogr.* **11:** 272–76 (1998).
109. R. Suedee, T. Srichana, J. Saelim, and T. Thavornpibulbut. Chiral determination of various adrenergic drugs by thin-layer chromatography using molecularly imprinted chiral stationary phases prepared with a-agonists. Analyst **124:** 1003–1009 (1999).
110. R. Suedee, C. Songkram, A. Petmoreekul, S. Sangkunkup S. Sankasa, and N. Kongyarit. Direct enantioseparation of adrenergic drugs via thin-layer chromatography using molecularly imprinted polymers. J. Pharm. Biomed. Anal. **19:** 519–27 (1999).
111. R. Suedee T. Srichana, J. Saelim, and T. Thavonpibulbut. Thin-layer chromatographic separation of chiral drugs on molecularly imprinted chiral stationary phases. *J. Planar Chromatogr.* **14:** 194–98 (2001).

Manuscript accepted October 17, 2002.