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Synthetic Dyes

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I. INTRODUCTION

Synthetic dyes are mainly derivatives of aromatic hydrocarbons for which the chief source is coal tar. The term "synthetic dyes" has therefore been regarded in common usage as being synonymous with coal tar dyes. This is no longer strictly true, however, because the aromatic hydrocarbons are being manufactured in increasingly large quantities from petroleum.

A. Classification, Applications, and Colors

Dyes may be classified according to either their chemical constitution or their application to textile fibers and for other coloring purposes. To some extent there is common ground between the two methods of classification, because certain groups of dyes, such as sulfur colors must be applied by methods that depend on their chemical character. Further, the dyeing properties are useful for subdividing a large group of dyes such as the azo or anthraquinone dyes. Thus, the basic classification "anthraquinonoid dyes" indicates the fundamental nucleus from which all the dyes in the series are derived, but for detailed consideration it is convenient to divide the group into cellulose acetate, mordant, acid, and vat dyes.

The dyes are also classified according to the chromophores or essential color-producing groups that are present, but color–constitution relationships are now known to be extremely complex, and for a systematic treatment of the chemistry of dyes a classification based primarily on characteristic structural units is more satisfactory. The azo group, the anthraquinone nucleus, and heterocyclic ring systems (e.g., pyrazolone, thiazole, acridine, thiazine, oxazine) are examples of structural units that provide a simple basis of division and subdivision. Methods of preparation and application are also useful for classification. Although the nitro group occurs in numerous dyes, the class name "nitro dyes" is restricted to the nitrophenols and the nitroarylamines, in which the nitro group is a vital factor in the production of color and dyeing properties. Dyes that do not justify treatment in separate chemical classes on account of their limited numbers and indefinite constitution have been classified as "miscellaneous dyes." For detailed classification, Venkataraman's text on synthetic dyes should be consulted (1).

Dyes are used for a wide variety of purposes, but their application to textile, foods, and leather is most important. The color of the dye depends on a number of factors. For example, with the azo group as the characteristic chromophore, the possible variations that influence the color and dyeing properties are (a) the number and position of the azo groups, (b) the nature of the aromatic nuclei, and (c) the end number and position of the sulfonic groups. It is obvious that various combinations of these factors can be used to obtain a given effect and that the color of the dye will ultimately depend on the constitution of the molecule as a whole.

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B. Identification, Analysis, and Evaluation

Identification of a commercial dyestuff as a known dye or mixture of dyes is important for users of dyestuffs, public analysts, and chemical examiners in customs laboratories. Because of the very large number of dyestuffs marketed under a much larger number of names, the problems of identification and analysis have become exceedingly complex. The chemical identity of a dye is of interest to the color user, because two dyes that give the same shades upon dyeing may differ substantially in fastness properties. However, it is often difficult to establish the chemical identity of a dye. It is clear from a consideration of the number and variety of synthetic dyes that their analysis, especially in mixtures, can never become a simple routine. Because many of the distinguishing tests depend on color reactions, an important requirement for success in dyestuff analysis is the possession of a complete range of authentic samples with which a direct comparison can be made. Direct chemical methods of analysis are frequently impractical and of very limited value in dealing with commercial dyes. Colorimetric and spectroscopic methods, and dye trials or evaluation procedures based on the application of the dyes, are therefore widely employed.

Some commercial dyestuffs, such as basic dyes, azoic coupling components (particularly the amines), and a few of the anthraquinonoid acid and vat dyes, are of high purity, but dyes in general are not marketed in the pure state. Manufacturers and users of dyestuffs are interested in products of standard quality that give reproducible results in application rather than in their chemical purity. Two types of impurities or substances other than the essential tinctorial constituent occur in commercial dyes: (a) by-products of the various reactions by which the dye is synthesized and (b) substances (e.g., sodium chloride and sulfate derivative dispersing agents) added subsequently for standardization. Among the latter may be one or more dyes added to the main dyestuff for shading purposes. The separation of a mixture of two or more dyes presents much more intractable problems than the separation of a dye from inorganic or colorless organic substances.

Partition between immiscible solvents, using a fractionation procedure based on a countercurrent principle, is sometimes effective for the separation of dyes in a mixture (2). Separation of dyes by means of immiscible solvents has been thoroughly investigated for the limited range of dyes used for coloring foods, drugs, and cosmetics (3,4). Mixtures of dyes in aqueous solution can be separated by frothing with gelatin at different pH values (5). Basic and acid dyes can be separated by means of ion-exchange resins (6).

C. Chromatography of Dyes

The advantages of chromatography are now well-known. Its value as a test for purity; ability to separate a complex mixture of compounds having only minor differences in structure; rapidity, simplicity, and ready availability of the equipment; applicability to micro quantities for purposes of isolation, identification, and quantitative estimation of the constituents of a mixture, as well as large quantities for preparative purposes; and usefulness in isolating very small quantities of solutes from very large volumes of solutions are worth mentioning.

A review of the literature up to 1973 indicates that very little work had been done on the separation of synthetic dyes. Venkataraman et al. (7) separated acid dyes by using semicircular filter paper with a short stem in the middle, water being used for adsorption and development. The method is useful for acid dyes only. Mottier and Potterat used both radial (8) and ascending (9) chromatography with loose layers of alumina to separate some food dyes, both synthetic and natural. Montag (10) used silica gel G plates for the separation of Martius Yellow, dimethylaminoazobenzene, Ceres Yellow, Ceres Orange, Sudan Red G, Ceres Red BB, and indophenol. Fujii and Kamikura (11) studied the separation of 15 oil-soluble dyes on silica gel using 12 different solvents. Sudan G, azobenzene, *p*-aminoazobenzene, Butter Yellow, *p*-methoxyazobenzene, and *p*-hydroxyazobenzene were separated with 1,2-dichloroethane. Xylene or pentachloroethane would separate Oil Yellow AB and Oil Yellow OB. Chloroform separated Quinoline Yellow SS from the remaining dyes.

Copius-Peereboom (12) chromatographed a series of 12 dyes along with several natural pigments on silica gel G, aluminum oxide G, and kieselguhr G with six different solvent systems. Copius-Peereboom and Beekes (13) chromatographed 19 oil-soluble dyes using a number of

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systems: on polyamide with chloroform–methanol–water (5:15:1), on silica gel with hexane–ethyl acetate (9:1), on kieselguhr G with cyclohexane, on aluminum oxide G with hexane–ethyl acetate (19:1), and on silver nitrate–impregnated silica gel G with chloroform–petroleum ether–acetic acid (75:25:0.5).

Waldi et al. (14) separated a series of dyes on silica gel G layers using a solvent consisting of chloroform–acetone–isopropanol–sulfurous acid (5–6% SO_2) (3:4:2:1). Stier and Specht (15) chromatographed a series of xanthene stains for histological work on silica gel G layers using *n*-propanol–formic acid (8:2) as the solvent system. Horobin and Goldstein (16) investigated the impurities in 11 Alcian Blue and Alcian Green samples by chromatographing on cellulose using *n*-butanol–water–acetic acid (3:3:1) as mobile phase in open or closed tanks. Horobin and Murgatroyd (17) compared paper chromatography, thin-layer chromatography (TLC) on silica gel H F_{254}, and thin-layer electrophoresis on agar for the resolution of histological dye components and found TLC to be a superior technique for these separations.

Waldi et al. (14) also separated a series of indicators on a 1:1 mixture of silica gel G and alumina G using a solvent composed of ethyl acetate–methanol–5 N ammonium hydroxide (6:3:1). Aliotta and Roso (18) chromatographed 12 indicator dyes on silica gel or silica gel impregnated with phthalate buffer (pH 6.0), using water-saturated butanol as a solvent. Chiang and Yeh (19) chromatographed 12 common indicator dyes on polyamide–silica gel (1:5.2) using both acidic and basic solvent systems containing 0.5–7% sodium chloride to reduce hydrogen bonding.

Perdih (20) separated cosmetic azo pigments on air-dried silica gel G with benzene–nitrobenzene (5:1). Deshusses and Desbaumes (21) used *n*-propanol–ammonia (9:1) with silica gel G to separate 11 dyes used in lipsticks. Brown (22) reviewed the chemistry of hair dyes on silica gel thin layers with petroleum ether–acetone–ammonia (sp. gr. 0.88) (30:10:1). Meckel et al. (23) chromatographed nine direct azo dyes into a number of constituents on silica gel and alumina layers prepared with 2.5% sodium carbonate.

Loger et al. (24,25) chromatographed 19 basic dyes for polyacrylonitrile fibers on alumina with ethanol–water (5:2) and another 11 dyes on silica gel G with pyridine–water (1:2) as the solvent system. Arsov et al. (26) chromatographed 23 basic dyes on silica gel with various solvents. Takeshita et al. (27) chromatographed 15 basic dyes on polyamide layers with five solvents. Shenai et al. (28) chromatographed direct, acid, basic, reactive, and 1:2 metal complex dyes and their mixtures on thin layers of $BaSO_4$ with different eluting systems. In the present chapter the work carried out on the TLC separation of synthetic dyes during the period 1973 to 2001 is reviewed, along with the relevant data from selected papers.

II. PREPARATION OF SAMPLES AND THIN LAYERS

The TLC of synthetic dyes consists of extracting the dyes from the samples and then subjecting them to TLC analysis. Various methods are used for the extraction of dyes from various sources depending on the nature of the source, ion-pair extraction (29,30) being the most important technique. Some methods for sample preparation are given below.

A. Foodstuff Dyes

Samples of azo dyes from foodstuffs are prepared by extraction: 5–10 g of food sample is mixed with 2–5 mL of methanolic 0.1 M hexadecylpyridinium chloride, and the resulting ion pair with anionic azo dyes is extracted in 10 mL of CH_2Cl_2. The dye can be back-extracted by shaking with dilute $HClO_4$ (31–35). For the general class of dyes, the food sample can be treated with aqueous 0.5% NH_3, and the mixture then extracted with butanol. The aqueous phase is acidified, then extracted with butanol–Amberlite LA-2 (19:1). The aqueous phase (containing some water-soluble dyes) is removed. The combined organic phase is diluted with butanol–hexane and extracted three times with aqueous 2% NH_3. The organic phase is treated with acetic acid, and the acetic acid phase and organic phase may be analyzed by TLC (36–39).

Dyes from fruit and gums can be extracted by dissolving the sample in hot water and incubating the resulting solution at 50°C for 3 h with amylo-1,6-glucosidase solution in acetate buffer

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(pH 4.5). The mixture is then applied to a polyamide column, and the dyes are eluted with acetone–water–concentrated aqueous NH_3 (40:9:1). The eluate is evaporated to dryness, and the residue is dissolved in water (1 mL) (40). The chewing gum sample (3 g) can be extracted at 75°C with water (15 mL) and then with five 15 mL portions of H_2O, each mixed with 0.5 mL of aqueous 25% NH_3. The combined extracts are acidified with anhydrous CH_3COOH and passed through a column of 125 g of polyamide. The column is washed with hot water–acetone (65°C). The dyes are eluted with aqueous 70% methanol–aqueous 25% NH_3 (49:1) at 55°C. The eluate is concentrated to 5 mL at 75°C; then H_2O (20 mL) and anhydrous acetic acid (0.1 mL) are added (41).

Dyes from pudding samples can be isolated by adsorption on wool or on polyamide, from which they can be extracted with aqueous methanolic NH_3 (42). Dyes from powdered species can be extracted by shaking 10 g of sample in 50 mL of H_2O. The color is extracted over 30 min, and the solution is then concentrated to 1 mL. The concentrated solution is diluted with methanol, and this solution can be analyzed for dyes (43).

Drinks (filtered or centrifuged if cloudy) are acidified and applied to a C_{18} Sep-Pak cartridge from which the colors are eluted with ethanol made alkaline with aqueous NH_3. Solid foods are extracted by maceration with aqueous 50% acetone or ethanol made alkaline with $Na_2B_4O_7$ solution, and the solids are separated by centrifuging (after addition of Celite 545). The solvent is evaporated in a rotary evaporator at 40°C, and the residual solution is acidified with HCl and applied to a Sep-Pak cartridge as before (44).

Dyes can be extracted from oils and fats and from chocolates in light petroleum (boiling range 40°–60°C. The light petroleum is shaken with dimethylformamide (I), and phase I is separated and mixed with an equal volume of water. A portion of solution I is applied to a column (25 cm × 15 mm) packed with polyamide powder MNSC6. The column is washed with water to remove solution I. The dyes can be eluted with various solvents (45).

Dyes from meat samples are extracted with light petroleum and subsequently with aqueous methanolic 0.5% sodium dodecylsulfate. The light petroleum extract is concentrated by dissolving in methanol before separation (45a).

B. Leather Dyes

Leather dyes are isolated from the sample by extraction prior to their separation. Samples of leather (100 g cut into strips) that have been dyed can be extracted under reflux for 2–3 h with 100 mL of aqueous 50% dimethylformamide (46,47). Acid dyes from leather are extracted with aqueous NH_3 solution. The extraction system is based on use of concentration gradient with aqueous NH_3 (47a).

C. Fiber Dyes

The fiber to be examined can be extracted in a sealed melting point tube with 100 mL of aqueous NH_3 in a boiling water bath for 40 min. Any color change is noted, and the extract can be analyzed for dyes (48,49). The extraction from fibers must be nondestructive, leaving the fiber intact for further analysis. Sometimes the dye content of synthetic fibers can be extracted with a solvent in which the dyes are soluble. For example, the dye contents of polyester acrylics, nylons, and acetates that are soluble in hexafluoropropane-2-ol can be extracted with this solvent (50). The scheme for extraction from fibers was modified by Home and Dudley (51). Standardization of TLC systems for comparison of fiber dyes was reported by Laing et al. (51a).

Azoic dyes from cotton fibers can be extracted with anhydrous acetic acid by heating in a sealed tube at 100°C and separated on Kiesel-gel 60 F_{254} TLC plates using chlorobenzene–1,2-dichloroethene–acetone (20:20:1) as mobile phase (51b). Disperse, cationic, and other dyes can be extracted from fibers with DMF/chlorobenzene, 50% formic acid, and NH_3 solution, respectively (51c). Wiggins et al. (51d) investigated the use of thin-layer chromatography in the analysis of reactive dyes released from wool fibers. Bulk samples of dyed wool and single dyed fibers were dissolved in 0.75 M NaOH at 40°C for 24 h with subsequent addition of methanolic 0.3 M citric acid and centrifugation, and the resulting solutions were analyzed by TLC on Kiesel-gel 60

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F_{254} aluminum-blacked plates with propan-1-ol–aqueous NH_3–methanol–H_2O (6:4:3:1) as mobile phase. The additional information obtained from TLC outweighed the disadvantage of destruction of the fibers.

D. Dyes from Alcoholic Products

Dyes can be isolated from alcoholic products by the wool yarn method (52). The dye is extracted from an acidified sample of lime or ethanol onto wool yarn, which is then extracted in 10% aqueous NH_3 (53,54). Alternatively, liquor samples (10 mL) can be evaporated and the residue dissolved in 40% ethanol (1 mL). This solution can be analyzed for dyes (55,56).

E. Cosmetic Dyes

Lipsticks are sometimes applied directly to TLC plates (57). Fat and fat-soluble colors are extracted from samples of cosmetics with hexane, after which the organic dyes are extracted with dimethylformamide in the presence of H_3PO_4 (58).

Cosmetics and food dyes are extracted from tablet coating formulations, releasing the dyes from their lakes by treatment with 85% H_3PO_4, then dissolving in methanol and making alkaline with concentrated aqueous NH_3 (59).

Dyes are extracted from soaps by dissolution in methanol or in CH_2Cl_2 and subsequent TLC (60). Alternatively, the soaps are fused with formic acid and the fatty acids are extracted into heptane. Oil-soluble dyes and some pigment dyes are separated from fatty acids by back-extraction into formic acid. After dilution of the filtrate, 30% NaOH solution is added, the mixture is extracted with $CHCl_3$, and the extract is washed with H_2O (61).

Dyes can be extracted from mouthwashes and toothpastes in either light petroleum or CH_2Cl_2 (62).

Nail lacquers are digested with ethyl acetate, and the digest is extracted with aqueous 50% dimethylformamide. The lower dimethylformamide phase is separated and, after extraction with high petroleum to remove fat, is mixed with polyamide powder, which adsorbs the dye. The powder is packed in a column and washed with methanol. The dye is then eluted with concentrated aqueous NH_3–methanol (1:19) (63).

F. Dyes from Miscellaneous Sources

The extraction of dyes from capsules and sugar-coated tablets can be carried out by dissolving a suitable number of empty capsules in 5 mL of 10% CH_3COOH and passing the mixture through a column (1 cm diameter) containing 1.5 g of alumina. The gelatin is removed by passing 10 mL of H_2O through the column with gentle suction. The coloring matter is then eluted with 1% aqueous NH_3. The eluate is carefully evaporated to dryness, and the residue is dissolved in a few drops of methanol and examined by TLC (64). Dyes from paints can be extracted with CH_2Cl_2 (65).

Dyes from powdered pencil lead or pencil writing on plain white paper can be extracted with acetone, and portions of extract can be separated on silica gel G plates using toluene–cyclohexane (1:1), butanol–ethanol–H_2O–anhydrous CH_3COOH (70:30:30:0.5), or butanol–ethanol-NH_3 (4:1:1) as mobile phase (65a). Reports on the extraction and separation of dyes from different types of inks and other marking materials are also available (65b–65e). Zlotnick and Smith (65f) reviewed the components of different types of inks and their identification by using a number of separation techniques including thin-layer chromatography.

Koprivanac et al. (65g) studied the production of monochlorotriazine reactive dyes using an anthraquinone reactive blue as a model. The stages of the process were monitored with TLC. Twelve chromatographic systems were investigated, and the most favorable mobile phase for TLC of the studied compounds was chloroform–isopropyl alcohol–ammonium hydroxide (2:4:1).

Separation of oil-soluble synthetic colors from annatto were studied by Biswas et al. (65h). Annatto extract was shaken with hexane and aqueous 70% acetonitrile, and the aqueous layer was diluted with water and reextracted hexane or $CHCl_3$. The combined extracts were concentrated

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by evaporation and analyzed by TLC on silica gel plates with acetic acid–$CHCl_3$–acetone (2:50:50) as mobile phase. After development the plates were sprayed with concentrated H_2SO_4, causing the spots corresponding to the synthetic oil-soluble colors to turn from red to blue and from yellow to red or deep orange.

Azo dyes, metal complexes, and tryptophan enantiomers were analyzed by TLC on cellulose layers using aqueous mobile phases comprising 1 M NaCl–cyclodextrin polymer (65i). Development was carried out at 20–22°C. The polymers eluted the azo dyes better than an α-cyclodextrin solution although the α-cyclodextrin monomer generally gave better separations than any of the polymers. Attempts to use the polymers as nonspecific desorbing agents for colored inks were unsuccessful.

Two dicationic zeolites, each containing sodium and tetramethylammonium cations, were synthesized by different methods (65j). These zeolites were studied as stationary phase layers on glass plates (20 × 20 cm) in the TLC separation of a synthetic mixture of five standard basic dyes. Two multicomponent mobile phases were used, benzene–$CHCl_3$–methanol (6:4:1) and benzene–$CHCl_3$–methanol–acetic acid (4:3:3:1), and detection was by IR spectrophotometry. The two zeolites behaved similarly and were satisfactory for the chosen dye separations.

Twenty-three lichen species mixed with norstictic acid and atronorine as reference compounds were subjected to TLC on silica gel 60 F_{254} with toluene–dioxane–acetic acid (45:15:2), *n*-hexane–diethyl ether–formic acid (13:10:2), or toluene–acetic acid (20:3) as mobile phase. Twenty-six compounds including 20 dyes and dye precursors, e.g., depsides and depsidones, were identified from the TLC R_f values and visualization test results and their UV spectra (65k).

G. Preparation of Thin Layers

Thin-layer chromatographic plates of adsorbents can be prepared as described by Stahl (66) by mixing the adsorbent with the required amount of water or solvent and spreading the solution on plates with the help of a Quickfit spreader or applicator. The plates are first dried, then activated for a few hours. For example, put 60 g of the adsorbent alumina neutral in a 500 mL conical flask, add 75 mL of distilled water, stopper it, and shake vigorously for a minute until a uniform slurry is obtained. Pour the slurry immediately into the applicator and spread it into a uniform thickness of 0.40 mm. Air-dry the plates for 30 min and activate them in an oven at 102 ± 3°C for an hour. Cool the plates in a desiccator at room temperature before use. To prepare the impregnated plates, known amounts of impregnant can be mixed with the adsorbent. Precoated plates are also available, and a comparison of these plates for separation of dyes has been reported (66a).

Berezkin and Markov (66b) suggested the use of plates bearing paper preadsorption layers for thin-layer chromatography. Chromatographic paper on glass and polymer plates as a preadsorption layer increases the volume preadsorption zone and also simplifies the introduction of sample for the analysis. The application range of the TLC plates with flexible support is increased as these plates with concentrating layers were studied for the first time. Zhang and Cai (66c) investigated the effect of the binder contents (sodium carboxymethyl cellulose) on homemade coated thin-layer plates. The characteristics of the plates were investigated using an automatic CS-920 TLC scanner with a dyestuff mixture or a mixture of 2-amino-4,6-dinitrotoluene and 4-amino-4,6-dinitrotoluene as analyte and with toluene (or benzene)–ethyl acetate–methanol (80:2:1), respectively, as mobile phase. Results showed that the plate prepared with aqueous 1% CMC solution had good TLC performance.

The dyes are spotted 2 cm above the edge of the plate in 2–3 μL portions of their solutions. The chromatograms are developed until the solvent front has migrated 10–12 cm. Multicomponent solvent systems should be thoroughly shaken in a separatory funnel before use.

III. TLC OF DYES ON UNTREATED PLATES

Considerable work has been carried out on the TLC separation of various classes of synthetic dyes on untreated plates. Dyes from various sources, namely, foodstuffs (fruits, oils, spices, al-

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coholic products), coated tablets, leather, fibers, cosmetics, etc., are first extracted and then applied to the layers. About 200 mL of developing solvent in a chromatographic development tank is then used to develop the chromatograms up to 10 cm. The data for the different developing solvents and adsorbents (stationary phases) used for the separation of a variety of synthetic dyes, along with specific characteristics of the separation procedure, if any, are tabulated in Table 1. Details of specific separations follow.

A. Cationic Dyes

Cationic dyes—Acridine Orange, Crystal Violet, Janus Green B, Methyl Violet, Neutral Red, Pyronin B, Pyronin Y (G), Safranin, Victoria Blue B, and Victoria Blue 4R—commonly used in histology, were studied by TLC on the Marshall and Lewis system (115). Marshall also separated some Sudan dyes, used for the histological staining of fats, on silica gel TLC sheets using benzene–$CHCl_3$ (10:1) as the mobile phase (115).

Owing to the poor results obtained by LC separation of the dyes Victoria Blue R, methylene blue, and fluorescein because of the similar colors of the blue dyes, a replacement dye mixture was prepared comprising Oil Red O, Victoria Blue R, and fluorescein. Preliminary studies were done using TLC with ethyl acetate or 95% ethanol as developing solvent and densitometric scanning at 370–700 nm (115a).

Wakasmundzka (115b) studied the retention behavior of 16 heteroazophenol dyes in normal-phase systems by TLC. Solutions of each of the 16 dyes, from four different heterocyclic systems, viz., 1,3,4-thiadiazole, 1,2,4-triazole, and benzimidazole conjugated with pyrocatechol, and β- and γ-resorcylic acids were applied to plates (10 × 20 cm) coated with alumina basic 60 E HF_{254} (0.25 mm thickness). The mobile phases were ternary mixtures of ethyl acetate, THF, methanol, or propan-2-ol in CH_2Cl_2 containing 2% acetic acid. The most selective systems were those containing methanol or propan-2-ol as polar modifiers.

B. Food Dyes

The developing systems used for the separation of food colors are recorded in Table 1. Some typical R_f values of water-soluble dyestuffs (72) are recorded in Table 2 along with the ν_{max} value and percent recovery of each dye. Slightly soluble food dyes can be studied at elevated temperatures (102). Twenty-two high-boiling organic solvents were used as eluents, including hydrocarbons and esters. Using the selected solvents, indigo was chromatographed on a silica gel layer at 150°C. The data are recorded in Table 3.

Sherma (115c) reviewed TLC analysis of a number of agricultural products, foods, beverages, and plant constituents from mid-1995 to mid-1999. Techniques and applications for a wide range of analyte and sample matrix types were covered, with specification of the particular layers, mobile phases, detection methods, and quantification conditions in many cases.

Soluble dyes from spices were separated and identified on alumina plates using methanol–liquor ammonia (8:2 v/v) as mobile phase (43). Bright-colored spots of respective coal tar dyes are separated and observed with the following R_f values: Rhodamine B, 0.93; Metanil Yellow, 0.87; erythrosine, 0.83; Fast Red E, 0.71; carmoisine, 0.69; Sunset Yellow FCF, 0.66; tartrazine, 0.46; Ponceau 4R, 0.32; and Amaranth, 0.22.

Food dyes permitted in Japan were investigated under fast atom bombardment (FAB) and liquid secondary ion (LSI) MS conditions with the use of various materials. The mobile phase was 10% Na_2SO_4 solution–methanol–ethyl methyl ketone (7:2:2) for xanthenes and 10% Na_2SO_4 solution–methanol–acetonitrile (10:3:3) for other dyes (102a). Seven permitted coloring materials used in foods and pharmaceutical preparations in Egypt were separated by two-dimentional TLC on cellulose layers (102b).

Oka et al. (102c) studied the identification of illicit dyes in foods by thin layer chromatography coupled with fast atomic bombardment and mass spectroscopy (TLC/FAB-MS). These dyes were extracted and transferred onto pure wool in a medium of aqueous acetic acid; the wool was then transferred into methanol. Glass plates coated with octadecyl sulfate (ODS) were used for TLC with mobile phases of aqueous 5% Na_2SO_4–methanol–acetonitrile (10:3:3) or aqueous 5%

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Table 1 Solvent Systems and Adsorbents for Synthetic Dyes

Source	Dyes obtained	Stationary phase (adsorbent)	Mobile phase(s) [solvent system(s)]	Other techniques/ visualization, etc.	Ref.
Food	Brilliant Blue FCF and Amaranth	Zeolite Scolecite	Acetone	—	67
	Indigo Carmine, Cochineal Amaranth, Orange Yellow, and Tartrazine	Magnesia	15% sodium citrate–methanol (4:1)	—	68
	Fluorescein in erythrosine	Silica gel	Ethanol–$CHCl_3$–formic acid	Determined spectrophotometrically at 493 nm	69
Foodstuff	Tartrazine, orange, Yellow, Cochineal Red, amaranth, Brilliant Black BN	Silica gel	—	Limits of detection in ppm reported	70
		Silica gel/polyamide gel	Ethyl acetate–methanol–25% aq. NH_3 (33:10:10), MeOH–EtOH–isoamyl alcohol–aq. 25% NH_3 (15:10:5:3)	Dyes extracted with 1% NH_3–ethanol (2:3)	70a
	10 dyes extracted from meat	Silica gel G	Butan-2-ol–isoamyl alcohol–pyridine–ethanol–aq. 25% NH_3 (3:3:3:4:6)	—	70b
	Xanthene dyes and triphenylmethane dyes	Aluminum-backed silica gel impregnated with 10% liquid paraffin soln.	Aq. 10% Na_2SO_4–MeOH–ethyl methyl ketone (7:7:2), aq. 10% Na_2SO_4–MeOH–acetonitrile (10:3:3)	Spectrum also shown	70c
	Quinoline Yellow, erythrocin, chrysoin, along with seven red dyes and four other dyes	Cellulose/Silufol and silica gel 60 G	Ethyl acetate–MeOH–conc. aq. NH_3 (3:1:1) plus aq. 67% propanol for cellulose plates only	Detection limit also reported	70d
	Ten food coloring dyes	Silica gel H	EtOH–diethylamine–$CHCl_3$–aq. 12.5% NH_3 (6:6:5:3)	—	70e

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	Tetrazine, Brilliant Blue, Amaranth, Coccine, and erythrosine	Polyamide	SDS–Triton X-100–aq. NH_3 soln. (1:5:1)	Spots quantified by densitometry	70f
Fruits	—	Silica gel 60	Propan-2-ol–conc. aq. NH_3–ethanol (77:13:10)	Spots analyzed by photoacoustic spectrophotometer	40
Mustard	Tartrazine, chrysoin, Quino-Line Yellow, Naphthol Yellow, Auramine O, Sudan Yellow, Dimethyl Yellow	Silica gel 60 F_{254}	$CHCl_3$–acetic acid (9:1) on cellulose ethanol–butanol–pyridine–H_2O (1:7:6:6) benzene	HPTLC	71
Tablet coatings	Twenty dyes are separated	Silica gel	Ethyl acetate–methanol–H_2O–conc. aq. NH_3 (30:8:7:1)	—	59
	Edible acid dyes	Silica gel or cellulose	(a) Butanol–ethyl methyl ketone–aq. 25% or 1% NH_3–H_2O (4:2:1:1) (b) Ethyl acetate–methanol–aq. 25% NH_3 (45:10:7)	Densitometry of chromatograms	38
Alcoholic products	Acid-fast dyes	Cellulose layers	Ethyl acetate–butanol–pyridine–water (5:5:6:5)	HPTLC also carried out	53
Textiles	Five natural dyes	Silica gel G	Benzene–ethyl acetate–formic acid (74:24:1)	Methanolic 10% KOH as spray reagent	73
Fiber	(a) Disperse dyes	Silica gel 60 F_{254}	(a) Toluene–pyridine (4:1) (b) Hexane–ethyl acetate–acetone (5:4:1) (c) Toluene–methanol–acetone (20:2:1)	Viewed in UV light at 254 nm	49
	(b) Acid dyes	Silica gel 60 F_{254}	$CHCl_3$–H_2O–methanol–conc. aq. NH_3 (11:1:7:1)	Viewed in UV light at 254 nm	49
	Six reactive, six direct, six vat, and six sulfur dyes	Kiesel-gel	Several mobile phases for each type of dye	Extraction procedure given	86
	Fifty-six commercial dyes (acid and basic)	Silica gel	Ten solvent systems evaluated mathematically	—	74

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Table 1 Continued

Source	Dyes obtained	Stationary phase (adsorbent)	Mobile phase(s) [solvent system(s)]	Other techniques/ visualization, etc.	Ref.
	Thirty-three purple-dyed wool fibers distinguished	Camlab Sil G	First direction: butanol–H_2O–aq. NH_3 acetone (5:1:2:5) Second direction: aq. NH_3–$CHCl_3$–H_2O–methanol (2:11:1:7)	Two-dimensional TLC performed and method of extraction reported	48
	Cationic dyes red, pink, blue, and yellow	Silufol	Butanol–acetic acid–H_2O (1:4:5)	Separated dyes extracted with DMF and determined spectrophotometrically	74a
Leather	Acid and direct dyes	Silica gel microplates or cellulose	(a) $CHCl_3$–propan-2-ol–H_2O (1:3:1) (b) Propan-2-ol–aq. NH_3–H_2O (7:1:1)	Two-dimensional TLC used if separation incomplete	47
Cosmetics, lipsticks	Oil-soluble unsulfonated colors	Silica gel 60	Ethyl acetate–methanol–aq. 30% NH_3 (5:1:1)	—	57
	Twenty organic dyes	—	—	—	77
	Red dyes	Silylated silica/AG 50 WX_4	0.5 M acetic acid in 50% methanol	—	78
	—	—	Cyclohexane–pentanol–conc. HCl (13:6:1)	Located by laser light	82
Soaps	Organic dyes	Kiesel-gel	Ethyl acetate–pyridine–H_2O (11:5:4)	Method of extraction given	60
Mouth and dental agents	Lipophilic dyes	Silica gel 60	(a) Ethyl acetate–pyridine–H_2O (11:5:4) (b) Ethyl acetate–pyridine–H_2O–1% $HClO_4$ (in methanol)–aq. 25% NH_3 (11:5:4:4:2)	0.1 M hexadecylpyridinium chloride used as locating reagent	62
Paints	Soluble dyes	Aluminum sheets coated with silica gel 60 F_{254}	Various solvents reported	Extraction procedure given	60

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Hair	Oxidative hair dyes	Silica gel	(a) Toluene–acetone–$CHCl_3$ (8:7:5)	—	79,80
			(b) Benzene–*sec*-butyl alcohol–H_2O (2:1:1)		80a
	p-Phenylenediamine, resorcinol, etc.	Silica gel	$CHCl_3$–ethyl acetate–methanol (3:1:1)	Multiple development also done	81
Ink	Pigment Red S and solvent dyes	Silica gel	Benzene–methanol–ethyl acetate	Location by spectrophotometry	83
	Nitrophenylenediamine, aminophenol, resorcinol	Silica gel 60	Benzene–ethyl acetate (1:1)	Located with Ehrlich reagent and by heating at 70°C for 30 min	84
	p-Phenylenediamine, toluene-2,5-diamine, and 4-methoxy-*m*-phenylenediamine	Silica gel 60	Ethyl acetate		85
Colored coated tablets	Tartrazine and coccine, as well as other dyes	Carboxylmethyl cellulose MN300	2% aq. NaCl–10% aq. NH_3 (93:5)	Extraction method reported	86
Miscellaneous	Eight azine dyes and five oxazine dyes	Silica gel	Benzene–$CHCl_3$–ethanol (plus 0.5 mL of HCl) and some other solvents reported	Methanolic 5% H_2SO_4 as spray reagent	87
	(a) 29 acid dyes	Polyamide plates	(a) Acetone–methanol–H_2O–aq. 25% NH_3 (16:16:18:3)	—	88
	(b) Six different 1:2 metal complex dyes	Polyamide plates	(b) Ethyl acetate–ethanol–H_2O–25% NH_3 (16:16:18:1)	—	88
	Reactive and direct dyes	Silica gel G	Phenol–water (4:1)	—	89
	Direct dyes	Silica gel 60	Butanol–aq. 25% NH_3–DMF (8:7:4) or phenol–acetone–aq. 25% NH_3	—	89a
	Fluorescein, eosin, benzoflavine, etc.	pH gradient TLC plates from silica gel 60 H F_{254}	$CHCl_3$–methanol or ethanol (19:1;9:1;17:3)	Located at 365 nm	90,91

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Table 1 Continued

Source	Dyes obtained	Stationary phase (adsorbent)	Mobile phase(s) [solvent system(s)]	Other techniques/ visualization, etc.	Ref.
	Azo dyes	Silufol UV plates	$CHCl_3$–ethyl acetate (4:1) $CHCl_3$–methanol (5:1;19:1)	—	92
	Dyes used in medical laboratories	Silufol R	Propanol–formic acid (2:1)	—	93
	Azobenzene, Dimethyl Yellow, Sudan G, etc.	Silica gels of various types	Benzene	—	94
	Commercial triarylmethane dyes (commonly used as analytical reagents and indicators)	Silica gel	(a) Ethyl acetate–aq. 25% NH_3–propan-2-ol (I) (5:3:3) for chrome azurols (b) 1,4-Dioxane–aq. 25% NH_3-I (2:1:1) for Phenol Red and some other systems	—	95
	Cationic dyes (Methylene Blue, thiomine, azure dyes)	Silufol silica gel	Butanol–anhyd. acetic acid–ethanol–H_2O (5:1:2:2)	Quantitative analysis	96
	Romanian and Cottestren dyes	Silica gel RG, Camag silica gel (gypsum as binder)	Sixteen mobile phases including toluene–acetone (20:1) reported	15 μg of dye could be chromatographed	97
	Disperse dye (C.I. Direct Blue 139 from mixture)	Silica gel 60	First direction: water-saturated ethyl methyl ketone Second direction: aq. NH_3–50% methanol	Two-dimensional TLC and detection at 254 nm	98
	Azo dyes	Anasil G or Kiesel-gel 60 F_{254}	Adsorbent layer located between radioluminescence sources and scanning spectrophotometer records spectra at λ_{max} value of each component		99

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Various substituted azobenzenes	Silica SI-60/silica gel	Solvent composition effects on R_f values studied	Chromatographic data interpreted in terms of mechanism	100
Commercial acid dyes of monoazo-, biazoanthraquinone, and triphenylmethane types	Octadecylsilyl-bonded silica gel	(a) Aq. methanol (58–80%) or (b) Aq. 60% acetone	—	101
Eriochrome Cyanine (R), Chrome Azurol (S), and Eriochrome Azurol (B)	Silica gel SG_{41} (Whatman)	Butanol–anhyd. acetic acid–H_2O (7:1:3 or 7:1:5)	Absorption spectra and extraction method also given	103
Eight mixtures of a reactive dye (4:1) (monochloro-1,3,5-triazene) and a direct dye	Silica gel	(a) Butyl acetate–pyridine–H_2O (6:9:5) (b) Propanol–isobutyl alcohol–ethyl acetate–H_2O (4:2:1:3)	Adulteration of reactive dyes with cheaper direct dyes can be detected	104
Reactive dyes	Kiesel-gel G	(a) Butanol–H_2O–dimethylformamide (160:60:19) (b) Butanol–anhyd. dimethylformamide–anhyd. acetic acid (60:28:5:5) (c) Butanol–ethanol–H_2O (8:4:3)	Method is rapid and suitable for quality control	105
Forty-two reactive dyes, 10 acid dyes, 20 metal complex dyes, 23 basic dyes, and 27 disperse dyes	Silica gel 60	Mobile phases are reported for each class of dye	Two-dimensional development also reported	106
Rhodamine S and 6 G, eosin K, and eosin	Silufol	3 mL toluene + 5 drops *o*-cresol + 3 mL alcohol + 0.5 mL aq. 20% NH_3	Separation done in mixture	107
Amaranth (in Hibicus)	Silica gel	(a) 4% soln. of sodium citrate in 5% aq. NH_3, (b) CH_3COOH–$HCOOH$–C_2H_5OH–H_2O (1:1:1:7), or (c) butanol–pyridine–H_2O (6:4:3)	Dye content evaluated by determining the anthrocyanin-to-dye ratio	108

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Table 1 Continued

Source	Dyes obtained	Stationary phase (adsorbent)	Mobile phase(s) [solvent system(s)]	Other techniques/ visualization, etc.	Ref.
	Rhodamine B, fluorescein sodium, eosin, erythrosine, Rose Bengal, and Phloxine P	Silufol	Chlorobenzene–ethylacetate–acetic acid (90:10:3)	Detected under 254 nm radiation	109
	Brilliant Green, Patent Blue, Brilliant Blue FCF (isolated from cosmetics)	Silica gel G	Eight solvent systems reported	Detected 0.2 μg of dye	110
	Basic dyes	Kiesel-gel G	Eight solvent systems based on mixtures of $CHCl_3$, alcohol or ketones, weak acid, water, and pyridine studied	Two-dimensional development also reported	111, 111a
	Acid Green 5 in mixture	Cellulose	Butyl acetate–dimethylformamide–H_2O (10:5:1)	Spectrophotometry reported	112
	Water-soluble food colors	Silica gel	Ten solvent systems reported	—	113
	Ponceau 6R, C.I. Acid Red 44, and C.I. Acid Red 41 (extracted from cakes and pastries)	Microcrystalline cellulose powder and silica gel G	Seven solvent systems reported	Recoveries of dyes were 95–98%	114
	4-Benzylidene-1-pyrimidin-2-ylpyrazolin-5-one dyes	Silica gel	Acetic acid–propan-2-ol–ethyl acetate (in various compositions)	—	114a
	Cottestren dyes	Zeolite NaX (0.5–5 μm)	Nitrobenzene–benzene–heptane (4:2:1)	—	114b
	Vat dyes	Silica gel	Nine different nonreducing solvent systems	Comparison with reversed-phase TLC reported	114c

Table 2 R_f Values of Some Dyes Extracted from Water-Soluble Dyestuffs

Dye	R_f value	ν_{max} in solution (nm)	Recovery (%)
Naphthol Yellow S	0.65	426	93
Tartrazine	0.20	423	88
Ponceau 3R	0.33	499	90
Amaranth	0.10	522	89
Rhodamine B	0.45	552	84
Erythrosine B	0.95	522	91
Indigo Carmine	0.00	603	77
Patent Blue V	0.75	634	92

Mobile phase: ethanol–*n*-butanol–water (9:1:2, v/v).
Source: Adapted from Ref. 72.

Na_2SO_4–methanol–ethyl methyl ketone (1:1:1). The dye spots on the plate were treated with 1,4-dithiothreitol–1,4-dithioerythritol (3:1) as matrix to enable recording of the FAB-MS spectrum. R_f values were obtained with each of the two mobile phases and the characteristic molecular ion species by mass-to-charge ratio (*m/e*) were observed by FAB-MS, in which lower detection limits ranged from 0.03 to 5 μg/spot for 26 dyes.

Gerasimov et al. (102d) identified a set of nine synthetic food dyes with TLC by examining the color images of the chromatograms by computer processing. The process includes scanning the chromatographic plates processing the images with appropriate programs.

In another study (102e), Gerasimov proposed a procedure for the qualitative and quantitative determination of dyes after thin-layer chromatography using an example of five synthetic food colors: Brilliant Blue, tartrazine, Sunset Yellow, Ponceau 4R, and azorubine. The procedure was applicable even when the dyes were incompletely separated on the chromatograms.

Table 3 Eluotropic Series of High-Boiling Solvents and R_f Values for Indigo

Solvent	R_f for indigo	Solvent	R_f for indigo
1-Chloro-*n*-dodecane	0.06	Di-*n*-Propyl phthalate	0.73
1-Bromonaphthalene	0.12	Di-*n*-butyl adipate	0.75
1-Chloronaphthalene	0.15	Diethyl phthalate	0.81
2-Ethylnaphthalene	0.16	Dimethyl phthalate	0.84
1-Methylnaphthalene	0.32	*n*-Butyl benzoate	0.95
Diphenyl ether	0.32	Ethyl anthranilate	0.97
Diisooctyl adipate	0.43	Nitrobenzene	1.00
Diisobutyl phthalate	0.58	2-Methylbenzothiazole	1.00
Benzyl benzoate	0.58	*N*-Methyl-2-pyrrolidone	1.00
Di-*n*-butyl phthalate	0.63	2-Ethyl-*n*-hexanol	1.00
Benzophenone	0.70	Quinoline	1.00

Source: Adapted from Ref. 102.

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C. Cosmetic Dyes

Silica gel 60 plates were used to identify lip cosmetics (57). The R_f values of some cosmetic dyes obtained from lipsticks are recorded in Table 4 along with their colors. A mixture of 15 mL of ethyl acetate, 3 mL of methanol, and 3 mL of ammonium hydroxide–water (3:7) solvent (a) and dichloromethane solvent (b) were used as the mobile phases. The shiny surface from the rounded end of the lipstick was removed with tissue, and the lipstick was weighed. The TLC plate was activated, and 10–20 mg of lipstick was applied directly to the plate. The plate was developed in two separate steps: oil-soluble, unsulfonated colors (D&C Orange 17 and D&C Red 36) were separated using dichloromethane, and other colors were separated using solvent (b).

Mikami and coworkers (57a) analyzed coal tar dyes used in the cosmetics and food industries by TLC. The dyes were spotted on reversed-phase RP-18 F_{254} S plates, and the plates were developed in four solvent systems: acetonitrile–methanol–5% sodium sulfate solution (3:3:10), methyl ethyl ketone–methanol–aqueous 5% sodium sulfate (1:1:1), acetonitrile–methanol–aqueous 5% sodium sulfate (1:1:1), and acetonitrile–CH_2Cl_2–aqueous 5% sodium sulfate (10:1:5). The visible absorption spectra of the dyes were measured by scanning densitometry at 370–700 nm.

D. Acid and 1:1 and 1:2 Metal Complex Dyes

Acid and metal complex dyes belong to different groups of chemical substances. Thirty-eight commercial dyes of these classes were studied on silica gel TLC plates (116). The best results for the separation of acid dyes are shown in Table 5. The data on 1:1 metal complex dyes are recorded in Table 6, and those for 1:2 complex dyes in Table 7. The solvent systems used are given in each table. It was observed that well-shaped spots without tailing were obtained for acid dyes and 1:2 metal complex dyes. The separation of 1:1 metal complex dyes was also clear, but the spots were diffuse and showed tailing. The best solvent systems were S_1 and S_2 for the acid dyes, S_2 and S_4 for the 1:1 metal complex dyes, and S_1 and S_3 for the 1:2 metal complex dyes.

E. Cyanine Dyes

Precoated 100 μm silica gel plates from Eastman Kodak and precoated 250 μm silica gel sheets from EM Labs were used to separate some cyanine dyes (117). The R_f values along with mobile

Table 4 TLC of Organic Colors in Lip Cosmetics[a]

Type of color	Name of dye	Color of spot	R_f
Oil-soluble	D&C Red 36	Orange	0.9
Unsulfonated	D&C Orange 17	Orange	0.8
Other	FD&C Red 3	Pink fluorescence[b]	0.25
	D&C Red 21	Pink fluorescence[b]	0.22
	D&C Orange 5	Orange fluorescence[b]	0.14
		Red fluorescence[b]	0.22
	D&C Red 19	Pink fluorescence[b]	0.57
	D&C Red 7 (ca)	Red	0.24
	D&C Orange 4	Orange	0.35
	D&C Red 9 (Ba)	Orange	0.41
	FD&C Violet 1	Blue	0.19
	D&C Blue 9	Pink (weak)	0.29

[a]Dichloromethane is the mobile phase for D&C Orange 17 and D&C Red 36; for the others a mixture of 15 mL ethyl acetate, 3 mL methanol, and 3 mL ammonium hydroxide–water (3:7) was used.
[b]Fluorescence under UV light (254 nm).
Source: Adapted from Ref. 57.

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Table 5 R_f Values of Color Components of Acid Dyes During TLC on Kiesel-Gel G[a]

Dye no.	Dye name	Producer	Color of component	Solvent system[b] S_1	S_2	S_3	S_4	S_7
1	Polar Brilliantrot GEN	Ciba-Geigy (Basel, Switzerland)	Pink	0.23	—	0.35	0.52	0.57d
			Scarlet	0.31	0.69	0.51	0.58	0.82d
2	Polar Brilliantblau RAW	Ciba-Geigy (Basel, Switzerland)	Blue	0.21	0.65	0.32	0.53	0.52d
3	Xylenechtgrau P	Sandoz (Basel, Switzerland)	Pink	0.55	0.90	0.77	0.76	0.89
			Light blue	0.62	0.96	0.86	0.85	0.95
			Violet	0.45	0.80	0.67	0.71	—
			Marine blue	0.24	0.65	0.40	0.55	0.45
			Blue	0.15t	0.51	0.27t	0.59	—
4	Ponceau 2RL	Ciech (Warsaw, Poland)	Orange	0.41	0.77	0.65	0.70	0.87
			Red	0.04	0.56	0.07	0.37	0.15
5	Acid Black 10BS	Ciech (Warsaw, Poland)	Light blue	0.25	0.67	0.03	0.53	0.50
			Blue	0.09	0.48	—	0.19	0.00
6	Acid Brown GOL	Ciech (Warsaw, Poland)	Yellow	0.44	0.82	0.73	0.75	0.87
			Orange	0.37	0.75	0.65	0.68	0.80
			Violet	0.07	0.60	0.15	0.42	0.25
7	Acid Pink M[c]	Sojuzchimexport (Moscow, USSR)	Pink	0.09	0.52t	0.05t	0.50	0.28dt
			Orange	0.03t	—	—	0.36t	0.10dt
8	Acid Fast red 100%	Sojuzchimexport (Moscow, USSR)	Red	0.03	0.58	0.07t	0.40	0.15d
9	Acid Blue RN	Sojuzchimexport (Moscow, USSR)	Blue	0.26	0.75	0.50	0.64	0.70d
10	Acid Black ATT	Sojuzchimexport (Moscow, USSR)	Orange	0.40	0.77	0.70	0.63	0.83
			Blue	0.22	0.68	0.38	0.50	0.50
Time (min) for 12 cm migration of solvent front				80	80	30	40	110

[a]The solvent systems used were as follows: S_1, ethyl acetate–glacial acetic acid–water (3:1:2); S_2, *n*-butanol–ethyl methyl ketone–glacial acetic acid–pyridine–water (15:15:4:2:10); S_3, methyl acetate–glacial acetic acid–water (30:3:20) + 5% of NaCl (with respect to H_2O); S_4, methyl acetate–glacial acetic acid–water (30:5:20) + 5% of NaCl (with respect to H_2O); S_7, *n*-butanol–glacial acetic acid–water (15:4:5).

[b]d = diffuse spot; t = tailing spot.

[c]Insoluble residue at the origin.

Source: Adapted from Ref. 116.

phases tried are recorded in Table 8. The two silica gel coatings tended in some cases to give different results when used with the same solvents. However, reproducibility of chromatograms on the same manufacturer's coatings was good.

R_f and R_m values for seven styryl cyanine dyes were reported by Patnaik et al. (117a) for silica gel plates using H_2O–propanol–acetic acid in various proportions as mobile phases.

F. Leather Dyes

The data on R_f values of some dyes used in the leather industry (75,76) are recorded in Tables 9A–9D. The leather dyes are divided in four groups: acid dyes, direct dyes, basic dyes, and premetallized dyes.

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Table 6 R_f Values of Color Components of 1:1 Metal Complex Dyes During TLC on Kiesel-Gel G[a]

Dye no.	Dye name	Color index no.	Color of component	Solvent system[b] S_1	S_2	S_3	S_4	S_5	S_6
11	Palatinechtgelb EIN[c]	19010	Lemon yellow	—	0.31t	—	—	0.23	0.13t
			Yellow	0.00	0.05	0.14	0.25dt	0.03	0.25dt
12	Palatinechtgelb 3 GN[c]	14006	Light yellow	0.38	—	—	0.57dt	—	—
			Yellow	0.08t	0.51dt	0.36dt	—	0.50dt	0.37t
13	Palatinechtorange RN[c]	18740	Orange	0.42t	0.78dt	0.86t	0.76dt	0.77t	0.75dt
			Pink-orange	0.12dt	—	0.38dt	—	—	0.25dt
14	Palatinechtrot GREN[c]	18800	Orange-yellow	—	0.54dt	—	—	0.67dt	0.57dt
			Orange	0.25t	—	0.74t	0.55t	0.53t	0.41t
15	Palatinechtrosa BN[c]	18810	Pink-red	0.13d	—	—	—	0.48dt	0.46dt
			Pink	0.05t	0.48dt	0.31t	0.55t	0.37t	0.23t
16	Palatinechtviolet 3RN[c]	16055	Violet	0.33	0.65t	0.77t	0.70t	0.67t	0.58t
17	Palatinechtgrun BLN[c]	13425	Green	0.39	0.62t	0.82	0.64	0.65t	0.55t
18	Palatinechtblau GGN[c]		Blue	0.21	0.58	0.66	0.60t	0.60t	0.53t
19	Palatinechtbraun RN	14251	Brown	0.60d	0.83d	—	0.86	0.80	0.72d
20	Palatinechtbordeaux RN[c]	19351	Red	0.57dt	0.80d	—	0.82	0.85	0.82dt
Time (min) for 12 cm migration of solvent front				80	80	30	40	100	100

[a]The solvent systems were as follows: S_1, S_2, S_3, S_4, and S_7 as in Table 5; S_5, *n*-butanol–glacial acetic acid–water (15:4:5) + 4% (w/v) sodium dodecyl sulfate (with respect to water).
[b]d = diffuse spot; t = tailing spot.
[c]Insoluble residue at origin.
Source: Adapted from Ref. 116.

G. Disperse Dyes

Disperse dyes are being used increasingly in the synthetic fiber industry, particularly with polyester fibers. Eighteen disperse dyes produced by different companies have been separated using macropolyamide sheets (118). The best solvent systems proved to be the following: S_1, benzene–light petroleum–methanol–glacial acetic acid (3:9.5:1:0.1); S_2, light petroleum–chloroform–methanol–glacial acetic acid (6:1:0.3:0.2); S_3 chloroform–*n*-hexane–methanol–glacial acetic acid (5:30:2:0.1); S_4, light petroleum–benzene—methanol (5:11:2). Some monomeric and polymeric disperse dyes have been synthesized for hydrophobic fibers by Maradiya and Patel (118a). The purity of the dyes was examined by TLC.

H. Dyes Used in Country Liquors

The addition of synthetic food colors to country liquors (e.g., Scotch from Scotland) is a common practice followed by all manufacturers. This poses a great problem for the differentiation of the genuine country liquors from imitation ones. However, a test of the genuineness of a colored country liquor can be based on the study of dyes in a specific brand of country liquor. The separation and identification of dyes in Indian country liquors (55) have been achieved on silica gel G thin-layer plates (250 μm thickness) using *n*-butanol–glacial acetic acid–ethanol–water (10:2:0.5:5) as the mobile phase. The R_f values along with the color of the main dyes present in Indian country liquors are reported in Table 10.

IV. TLC OF DYES ON IMPREGNATED PLATES

A survey of the literature on the TLC separation of synthetic dyes indicated that little attention has been paid to the separation of dyes on impregnated plates. Only a few papers have appeared

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Table 7 R_f Values of Color Components of 1:2 Metal Complex Dyes During TLC on Kiesel-Gel G[a]

Dye no.	Dye name	Producer	Color of component	Solvent system[b] S_1	S_3	S_6	S_7
21	Ortolangelb G	BASF (Ludwigshafen, GFR)	Lemon yellow	0.60	0.81	0.69t	0.88
22	Irgalanbrillantrot BL	Ciba-Geigy (Basel, Switzerland)	Red	0.34	0.50	0.24t	0.83
			Orange-red	0.48	0.67	0.53	—
23	Irgalanbraun 3BL	Ciba-Geigy (Basel, Switzerland)	Orange	0.68	0.96	0.78	0.91
			Gray-blue	0.63	0.91	0.71	0.88
24	Irgalangrau BRL	Ciba-Geigy (Basel, Switzerland)	Gray-blue	0.50	0.80	0.68	0.81
25	Isolanorange GGL	Bayer (Leverkusen, GFR)	Orange	0.63	0.86	0.67	0.89
26	Wofalanrubin RL	Wolfen (Bitterfeld, GDR)	Ruby	0.74	0.97	0.78	0.93
27	Wofalanoliv BL	Wolfen (Bitterfeld, GDR)	Blue-green	0.83	0.99	0.82	0.95
			Yellow-green	0.77	0.95	0.75	0.90
28	Grigio stenolana RL	ACNA (Milan, Italy)	Pink	0.75	0.94	0.86	0.96
			Pink	0.80	1.00	—	—
			Marine blue	0.65	0.90	0.71	0.87
29	Bordo stenolana 2BL	ACNA (Milan, Italy)	Pink	0.68	0.87	—	—
			Bordeaux	0.74	0.97	—	—
			Bordeaux	0.78	0.99	—	—
30	Bruno stenolana 2GL	ACNA (Milan, Italy)	Gray	0.61	0.88	—	—
			Orange	0.66	0.92	—	—
Time (min) for 12 cm migration of solvent front				80	30	65	110

[a]The solvent systems used were as follows: S_1, S_3, and S_7 as in Table 6; S_6, chloroform–isoamyl alcohol–ethyl methyl ketone–methanol–pyridine (6:5:3:2:2).
[b]t = tailing spot.
Source: Adapted from Ref. 116.

in the area. Two types of impregnated layers have been used to separate the synthetic dyes: metal ion–impregnated plates and ion exchanger–impregnated plates.

A. Metal Ion–Impregnated Plates

Dyes are reported to form complexes with metal salts (119), and complexation is reported to improve the separation in many cases (120–123). Hence various metal salts have been tried by various workers to improve the separation of synthetic dyes. The separation of 42 synthetic dyes on 5% cadmium acetate–impregnated silica gel G layers was reported by Srivastava et al. (124). The dyes were divided in two groups, A and B. The group A dyes were well separated using butanol–benzene–ethyl acetate (40:35:25), and those of group B were well separated using *n*-butanol–water–formic acid (35:10:5) as mobile phase. There was complete disappearance of tailing, spots were compact, and the dyes were self-visualized. The comparison with TLC on plain silica gel indicated that tailing was much greater in the case of plain silica gel plates than on impregnated plates. It was therefore concluded that the chromatographic behavior of dyes on silica gel G plates is greatly influenced by the impregnation of plates with metal salts.

Srivastava et al. (125) separated 30 synthetic dyes on silica gel plates impregnated with ammonium molybdate and copper sulfate using BuOH–AcOH–H_2O (25:5:10) and BuOH–50%

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Table 8 R_f Values for Cyanine Dyes in Selected Solvents[a]

Dye[b]	Dye no.	$R_f \times 100$ I	II	III	IV
2,2′-Indo-4,5,4′,5′-dibenzocarbocyanine	ICG	86	—	—	—
2,2′-Thiacarbocyanine	156	—	22	—	85
4,4′-Quinocarbocyanine	171	5	24	—	86
2,2′-Quinocarbocyanine	179	5	51	—	92
2,2′-Indocarbocyanine	1405	21	—	—	—
2,2′-Thiacarbocyanine	1407	51	—	—	—
2,2′-Oxathiacarbocyanine	1518	—	38	—	25
Merocyanine:2-thia derivatives	1901	70	73	18	92
2,2′-Thia-4,5,4′,5′-dibenzocarbocyanine	1978	66	30	—	U
Miscellaneous *meso*-cyanines	2062	79	57	23	83

[a]TLC on silica gel (EM Labs). I, methanol 100%; II, *n*-butanol–acetic acid–water (20:10:50); III, *n*-butanol–ethanol–water (90:10:10); IV, chloroform–methanol (80:20). U indicates that the TLC system was unsatisfactory for the compounds; dash (—) indicates that the compound was not tested with that TLC system.
[b]Structures are given in Ref. 117.
Source: Adapted from Ref. 117.

NH_3–dioxane (25:5:10) as mobile phases. No tailing of spots was observed on ammonium molybdate– and copper sulfate–impregnated plates using these mobile phases. The data on hR_f values are recorded in Table 11. The hR_f values were not altered when dye mixtures were applied.

Twenty-five synthetic dyes were separated by Gupta (126) on 5% zinc sulfate–impregnated silica gel G thin-layer plates using *n*-butanol–benzene–ethyl acetate (40:35:25) as the mobile phase. The data on plain silica gel and on impregnated plates are recorded in Table 12. A difference of ± 3 units in hR_f values was taken as the criterion for satisfactory separation. Some of the typical separations that depend on hR_f values have also been achieved. It was observed that the hR_f values

Table 9A R_f Values of Some Acid Leather Dyes on Silica Gel G Plates

	Dye	Solvent system[a] 1	2	3	4	5
1.	Acid Leather Orange	0.70	0.84	0.61	0.93	0.82
2.	Naphthalene Leather Orange G 132	0.68	0.82	0.72	0.89	0.82
3.	Polar Brilliant Blue GAW	0.40	0.59	0.46	0.64	0.82
4.	Solar Turquoise Blue FBL	0.00	0.00	0.00	0.62	0.57
5.	Derma Brown G	0.00	0.00	0.00	0.00	0.00
6.	Xyline Fast Green B	0.40	0.67	0.43	0.81	0.72
7.	Polar Red 3B	0.50	0.58	0.40	0.63	0.72
8.	Ranomil Brilliant Red 3 BN	0.39	0.67	0.41	0.73	0.73
9.	Sandopal Blue	0.48	0.59	0.41	0.61	0.78
10.	Derma Brilliant Red 3B	0.40	0.69	0.44	0.73	0.77

[a]1, *n*-Butanol–acetic acid–water (4:1:5); 2, isopropanol–ammonia (0.91)–water (7:1:1); 3, isopropanol–ammonia (0.91)–water (10:1:1); 4, isopropanol–ammonia (0.91) (4:1) 5, *n*-propanol–ammonia (0.91) (6:3).
Source: Adapted from Refs. 75 and 76.

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Table 9B R_f Values of Some Direct Leather Dyes on Silica Gel G Plates

Dye		Solvent system[a] 1	2	3
1.	Eriochrome Blue Black	0.18	0.59	0.52
2	Chlorozol Orange Brown XS	0.29	0.00	0.58
3.	Chlorozol Orange Brown	0.29	0.00	0.55
4.	Chlorozol Green BNS	0.24	0.00	0.00

[a]1, *n*-Butanol–ethanol (95%)–ammonia (0.91) (9:2:3); 2, isopropanol–ammonia (0.91)–water (10:1:1); 3, isopropanol–ammonia (0.91) (4:1).
Source: Adapted from Refs. 75 and 76.

did not change when mixtures of dyes were applied. Further, decrease in hR_f values on impregnated plates suggested that the complexation between dyes and the metal ion is an important factor in influencing the chromatographic behavior of dyes on metal salt–impregnated layers. Gupta et al. also reported the separation of twelve dyes on 1.5% $NiCl_2$-impregnated layers using acetone–acetic acid–benzene (9:6:6) as mobile phase. A comparison of hR_f values on plain and impregnated layers clearly indicated butter separation on $NiCl_2$–impregnated silica gel layers (126a).

A method for separation of the dyes Green S, Fast Green FCF, Brilliant Blue FCF, and Blue VRS was developed using silica gel plates impregnated with 1.25% starch, 5% $CdCl_2$, or 5% $NiSO_4$ with aqueous 80% phenol as mobile phase and was described by Prasad et al. (127). Separation of the dyes was good, especially for Green S and Brilliant Blue FCF, which are difficult to separate by normal chromatographic techniques.

B. Ion Exchanger–Impregnated Plates

Separation of dyes on anion and cation exchanger–impregnated plates by TLC was reported by Lepri et al. (128). The data on R_f values on different exchanger-impregnated plates are recorded in Tables 13 and 14. Table 13 shows that most of the dyes contain one or more sulfonic acids and/or carboxyl groups; the dyes should therefore behave like anions. Owing to the anionic character of most dyes, their chromatographic behavior can be studied in the absence of the ion-

Table 9C R_f Values of Some Basic Leather Dyes on Silica Gel G Plates

Dye		Solvent system[a] 1	2	3
1.	Malachite Green	0.86	0.84	0.98
2.	Methylene Blue G	0.54	0.26	0.65
3.	Fast Rubine 4 BN	0.48	0.19	0.62
4.	Auromaine O	0.93	0.63	0.95

[a]1, *n*-Butanol–ethanol (95%)–water (8:1:3); 2, chloroform–isopropanol–water (1:3:1); 3, *n*-butanol–acetic acid–water (2:1:5).
Source: Adapted from Refs. 75 and 76.

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Table 9D R_f Values of Some Premetallized Leather Dyes on Silica Gel G Plates

Dye		Solvent system[a] 1	2	3
1.	Metalan Dark Blue	0.40	0.60	0.54
2.	Neolan Violet Brown BY	0.24	0.61	0.45
3.	Metalan Red S-BR	0.72	0.79	0.88
4.	Dermalight Yellow	0.24	0.62	0.93
5.	Metalan Bordeaux S-B	0.23	0.57	0.51
6.	Metalan Brown S-GL	0.22	0.43	0.44
7.	Irgalan Red 4GL	0.70	0.83	0.89
8.	Cibalan Olive 3BL	0.21	0.10	0.87
9.	Matalan Brown S-RL	0.65	0.78	0.87
10.	Metalan Yellow 5-RL	0.67	0.69	0.55
11.	Dermalight Brown GRL	0.69	0.57	0.84
12.	Ranolan Orange RL	0.68	0.63	0.89
13.	Dermallight Yellow 2 RL	0.68	0.74	0.87
14.	Dermalight Orange RLN	0.33	0.56	0.65
15.	Dermalight Green 5GL	0.34	0.56	0.64
16.	Sandopal Gray WSI	0.69	0.57	0.88

[a]1, *n*-Butanol–ethanol (95%)–water (8:1:3); 2, chloroform–isopropanol–water (1:3:1); 3, *n*-butanol–acetic acid–water (2:1:5).
Source: Adapted from Refs. 75 and 76.

Table 10 R_f Values of Some Synthetic Dyes, on Silica Gel G Plates, Extracted from Indian Country Liquors[a]

Identified dye	Color of spot	R_f value
Tartrazine	Dark yellow	0.12
Red 10B	Dark pink	0.26
Sessol Orange G	Dark orange	0.36
Carmoisine	Dark pink	0.41
Amaranth	Dark pink	0.15
Indigo Carmine	Dark blue	0.34
Acid red	Red	0.65
Crocine Scarlet	Red	0.52
Basic Red	Red	0.26
Matanil Yellow	Yellow	0.68
Rhodamine B	Pink	0.69
Malachite Green	Blue	0.53

[a]Mobile phase: *n*-butanol–glacial acetic acid–ethanol–water (10:2:0.5:5).
Source: Adapted from Ref. 55.

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Table 11 hR_f Values of Dyes on Impregnated and Plain Silica Gel Plates[a]

Dye		hR_f		
		Plain[b]	Imp.[c]	Imp.[d]
1.	Rosaniline HCl	84	57	85
2.	Chrysoidine	83	69	84
3.	Malachite Green	65	49	65
4.	Methyl Red	88	78	80
5.	Crystal Violet	72	61	73
6.	Fuchsine Basic	83	62	70
7.	Auramine O	73	52	82
8.	Bromophenol Blue	90	90	92
9.	Eosine bluish	98	98	98
10.	Bromocresol Purple	84LT	85	87
11.	Congo Red	60	59	72
12.	Titan Yellow	66	67	65
13.	Aluminon	75LT	82	66
14.	Alizarin	45ST	33	60
15.	Magneson	99	99	99
16.	Orange G	33	43	54
17.	Bromocresol Green	38	87	90
18.	Phenol Red	73	72	75
19.	Thymol Blue	85	84	86
20.	Genitan Violet	73	52	75
21.	Nevilline Brilliant Pink	97	95	96
22.	Aniline Blue	88	67	80
23.	Dichlorofluorescein	98	98	97
24.	Xylidine Ponceau	30	29	32
25.	Benzopurpurine	62	55	60
26.	Methylene Blue	42	40	43
27.	Nigrosin	00	00	00
28.	Fuchsine Acid	11	11	09
29.	Light Green	43	28	47
30.	Alizarin Blue	24LT	25	26

[a]Mobile phase: *n*-butanol–AcOH–H_2O (20:5:10).
[b]ST = slight tailing; LT = large tailing.
[c]Impregnated with ammonium molybdate.
[d]Impregnated with copper sulfate.
Source: Adapted from Ref. 125.

exchange process on cation exchanger–impregnated layers. Besides exchangers, cationic and anionic detergents are also used as impregnants to compare the behavior of the dyes on such layers and on anion and cation exchanger layers.

Seventeen ionic food dyes were separated by Van Peteghem and Bijl (32) by ion-pair adsorption TLC on silica gel plates. Cetyltrimethylammonium bromide (CTMA) was selected as the counterion for both isolation and separation. The thin-layer plates were impregnated with the counterion, which was also present in the eluent. The R_f values of some sulfonated dyes separated by the above technique using methanol–acetone (9:1) + 1% glacial acetic acid and 0.1 M CTMA, or methanol–acetone (1:1) and 0.1 M CTMA as mobile phase are recorded in Table 15. The orange dyes can be developed in any of the solvent systems, but they were not separated. The

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Table 12 hR_f Values of Selected Dyes on Impregnated and Plain Silica Gel Plates[a]

Dye	Color	hR_f	
		On plain silica gel[b]	On silica gel with $ZnAc_2$
Rosaline hydrochloride	Reddish pink	70ST	38
Methyl Red	Red	82T	72
Crystal Violet	Violet	40T	21
Orange G	Light orange	20ST	17
Auramine O	Lenion yellow	52T	26
Bromophenol Blue	Violet	44ST	34
Bromothymol Blue	Dark yellow	83T	74
Phenol Red	Orange red	56	46
Thymol Blue	Orange yellow	76T	67
Acridine Orange	Yellow	50	35
Cadion 2B	Brown	95T	94
Rhodamine B	Pink	56T	04
Eosine Yellowish	Light orange	50T	42
Naviline Yellow	Pale yellow	97	92
Naviline Brilliant Pink	Violet	96T	90
Methyl Violet	Violet	56T	44
Bromocresol Purple	Brown	74ST	70
Alizarine Blue	Light pink	35	30
Bismarck Brown	Orange	76T	53
Eriochrome Black	Purple	32T	15
Benzopurpurine 4B	Light orange	52T	48
Nigrosin	Purple	0	0
Fuschsin Acid	Pink	22ST	19
Diamond Blue	Blue	78ST	71
Dichlorofluorescein	Orange	54ST	12

[a]Solvent system: *n*-butanol–benzene–ethyl acetate (40:35:25).
[b]ST = slight tailing; T = tailing.
Source: Adapted from Ref. 126.

method requires only limited sample treatment and is very easy to apply. TLC development is must faster than for conventional methods with butanol- and/or water-containing eluents. The high separation efficiency, due to sharpness of the spots, offers a highly reliable means of identification.

V. OPTLC, HPTLC, AND REVERSED-PHASE TLC OF DYES

Besides separation of dyes by TLC, separations by reversed-phase TLC, by high-performance TLC (HPTLC), and by overpressured TLC (OPTLC) are very important owing to improved separation and shorter time requirements.

A. OPTLC of Dyes

The problem of optimization of separation techniques in thin-layer chromatography has been tackled both in theoretical treatments (129–133) and in the development of instrumentation (134–136). In overpressured TLC (137) a pressurized ultramicrochamber is used. The essential feature of this chamber system is that, in contrast to the rigid glass plate used in the earlier ultramicro-

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Table 13 R_f Values of Water-Soluble Food Dyes on Layers of (a) DEAE-Cellulose, (b) PAB-Cellulose, (c) Chitosan, and (d) Microcrystalline Cellulose

Name of dye	Dye no.	0.6 M NH_4Cl in H_2O methanol (7:3, v/v) (a)	0.1 M NaH_2PO_4 (b)	H_2O–ethanolamine (14:1, v,v) (c)	H_2O–ethanolamine (14:1, v,v) (d)	Amount (μg)
Tartrazine	E 102	0.10	0.47	0.89	0.90	1.0
Chrysoin	E 103	0.21	0.38	0.68	0.72	1.0
Quinoline yellow	E 104	0.50[a]	0.04[c]	0.35[b]	0.40[b]	1.0
		0.11[b]	0.19[c]	0.45[b]	0.51[b]	
				0.69[a]	0.76[a]	
Acid yellow	E 105	0.25	0.65	0.75	0.79	1.0
Sunset yellow	E 110	0.33	0.78	0.81	0.87	1.0
Orange GGN	E 111	0.33	0.78	0.81	0.87	1.0
Azorubine	E 122	0.03	0.11	0.44	0.54	1.0
Amaranth	E 123	0.10	0.20	0.59	0.63	1.0
Cochineal red	E 124	0.10	0.57	0.74	0.78	1.5
Scarlet GN	E 125	0.20	0.45	0.91	0.91	1.5
Ponceau 6R	E 126	0.10	0.23	0.54	0.58	1.0
Erythrosine	E 127	0.03	0.05	0.27	0.37	1.0
Patent blue	E 131	0.81	E.S.[d]	0.89	0.89	0.5
Indigo carmine	E 132	0.08	E.S.[d]	E.S.[d]	E.S.[d]	2.0
Brilliant black BN	E 151	0.00	0.01	(0.43)[e]	0.57	1.5
Black 7984	E 152	0.00	0.01	(0.43)[e]	0.57	1.5

[a]Main spot.
[b]Secondary spot.
[c]Two spots of similar intensity.
[d]E.S. = elongated spot.
[e]Parentheses indicate streaking.
Source: Adapted from Ref. 128.

chamber (138,139), the sorbent layer is completely covered by an elastic membrane under external pressure so that vapor phase above the layer is eliminated. Solvent is admitted into the chamber under overpressure by means of a pump system. This technique essentially combines the advantages of traditional TLC and modern high-performance TLC (HPTLC), leading to improved separation efficiency.

For OPTLC and HPTLC, precoated silica glass plates (conventional TLC plates) with indicator (silica gel 60 F_{254}) and without indicator, and HPTLC silica gel 60 F_{254} aluminum sheets are used. The resolution and retention behavior of some dyes have been studied by OPTLC (137,137a). Empore sheets have also been used for OPTLC of dyes (137b).

Ligor and Buszewski (137c) developed thin-layer chromatographic and overpressured thin-layer chromatographic methods for the determination of pigments in plant leaves containing anthocyanins and chlorophylls. They optimized mobile and stationary phases and other chromatographic conditions for the separation of pigments for plant leaves.

B. HPTLC of Dyes

The synthetic organic colors in lipsticks have been characterized by HPTLC (57). By combining the results from TLC and HPTLC it was possible to determine the organic colors in lip cosmetics. Some of the colors had identical R_f values on TLC, but they could be identified with the aid of

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Table 14 R_f Values of Water-Soluble Food Dyes on Layers of (a) Dowex 50-X4(H^+), (b) Rexyn 102(H^+), (c) Humic Acid, (d) Silanized Silica Gel, and (e) Silanized Silica Gel Impregnated with 4% DBS[a]

Dye no.	Mobile phase: 0.1 M HCl in H_2O–CH_3OH (7:3) (a)	(b)	(c)	Mobile phase: H_2O–CH_3OH–acetic acid (64.3:30:5.7) (d)	(e)	Amount (μg)
E 102	0.65	0.62	0.66	0.95	0.95	1.0
E 103	0.40	0.29	0.29	0.57	0.73	1.0
E 104	0.22[c]	0.28[c]	0.28[d]	0.72[c]	0.81[c]	1.5
	0.42[b]	0.38[d]	0.44[b]	0.87[b]	0.88[b]	
	0.65[c]		0.68[c]	0.97[c]	0.97[c]	
E 105	0.82	0.75	0.75	0.93	0.88	1.0
E 110	0.93	0.75	0.78	0.80	0.85	1.0
E 111	0.93	0.75	0.78	0.82	0.85	1.0
E 122	0.23	0.18	0.29	0.55	0.77	1.0
E 123	0.42	0.48	0.55	0.95	0.95	1.0
E 124	0.82	0.83	0.77	0.95	0.95	1.0
E 125	0.70	0.55	0.73	0.69	0.83	1.0
E 126	0.51	0.30	0.37	0.54	0.74	1.0
E 127	0.00	0.00	0.00	0.01	0.01	1.0
E 131	E.S.[e]	0.59	0.97	0.19	0.27	1.0
E 132	E.S.[e]	0.39	(0.55)[f]	0.95	0.95	1.5
E 151	(0.06)[f]	(0.13)[f]	(0.25)[f]	0.94	0.93	1.0
E 152	(0.06)[f]	(0.13)[f]	(0.25)[f]	0.95	0.93	1.0

[a]DBS = triethanolamine–dodecylbenzenesulfonate.
[b]Main spot.
[c]Secondary spot.
[d]Two spots of same intensity.
[e]E. S. = elongated spot.
[f]Parantheses indicate streaking.
Source: Adapted from Ref. 128.

their retention times in HPTLC. Rosaline hydrochloride, Malachite Green, Brilliant Green, Methyl Violet, Gentian Violet, Ethyl Violet, and Victoria Blue were screened by HPTLC on 10 × 10 cm Whatman HPTLC plates with the use of a LAMMA-1000 laser microprobe (140). The dyes from alcoholic products were analyzed by HPTLC with the use of Whatman HPTLC plates with butanol–butan-2-ol–acetonitrile–tetrahydrofuran–ethyl methyl ketone–aqueous 0.5% NaCl–aqueous NH_3 (10:10:25:15:20:18:2) or propanol–acetonitrile–tetrahydrofuran–ethyl methyl ketone–ethyl acetate–aqueous 0.5% NaCl–aqueous NH_3 (20:15:25:10:10:18:2) as developing solvent (45).

Wimmer and Hauck (141) reported the separation of synthetic dyes on HPTLC plates using precoated Kiesel-gel 60 sheets. The conditions for optimum separation and comparison with TLC are reported (141). Several yellow, red, and blue dyes have been separated by TLC on silica gel and HPTLC on RC-2, RP-8, and RP-18 (Merck) with methanol–H_2O (17:3) as mobile phase (30).

Eleven water-soluble dyes were separated by HPTLC on silica gel 60 with methyl ketone–methanol–aqueous 28% NH_3 (8:4:1) as the developing solvent. Dyes that were insoluble in aqueous concentrated NH_3 were separated on layers of cellulose with butanol–ethyl methyl ketone–

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Table 15 R_f Values of Some Sulfonated Dyes Separated by Ion Pair Thin-Layer Chromatography on Impregnated Silica Gel Layers[a]

Dye	Color	R_f values in solvent A	R_f values in solvent B
Tartrazine	Yellow	0.14	0.07
Chrysoin S	Yellow	0.48	0.33
Quinoline Yellow	Yellow	0.33	0.28
Acid Yellow	Yellow	0.37	0.31
Sunset Yellow FCF	Orange	0.32	0.25
Orange GGN	Orange	0.32	0.25
Azorubin	Red	0.28	0.25
Amaranth	Red	0.26	0.19
Cochineal Red A	Red	0.07	0.03
Scarlet GN	Red	0.46	0.40
Ponceau 6R	Red	0.17	0.07
Erythrosine	Red	0.34	0.20
Patent Blue V	Blue	0.36	0.34
Indigo Carmine	Blue	0.38	0.29
Green S	Green	0.44	0.39
Brilliant Black BN	Black	0.12	0.09
Black 7948	Black	0.16	0.12

[a]Solvents: A, methanol-acetone (9:1) + 1 % glacial acetic acid, 0.1 M CTMA (cetyltrimetylammonium bromide); B, methanol–acetone (1:1), 0.1 M CTMA.
Source: Adapted from Ref. 32.

aqueous 1% NH_3–H_2O (4:2:1:1) as developing solvent (142). The advantages of the method are shorter analysis time, more data measurement per plate, high resolution, and an improved signal-to-noise ratio compared with those of TLC plates. The theory of this technique is well discussed by Jupille and Glunz (143) along with some data on the separation of dyes.

Wall (143a) reported quantitative methods for determination of biologically important dyes using silica gel 60, silica gel G RP-8 F_{254}, or silica gel NH_2 F_{254} plates by loading them with 100 ng of sample. Separation of six thiazine dyes has also been reported on silica, cyano, and C-18 plates using as the mobile phase THF–H_2O–methanol (16:3:1) (143b). Identification and determination of cationic dyes from acrylic fiber were reported on silica gel G-60 plates using ethyl methyl ketone–CH_2Cl_2–formic acid (8:6:1) as mobile phase (143c).

High-performance thin-layer chromatography (HPTLC) on silica gel plates with two successive mobile phases has been used for analysis of seven amine azo dye isomers prohibited under a German ban (143d). Dichloromethane was used for the first development, and the second was done with toluene–tetrahydrofuran (1:1 v/v), to a distance of 8.5 cm. The spots were visualized at a wavelength of 254 nm. The limit of determination (x) and the correlation coefficient in the range x–$5x$ were reported for each amine. To increase the sensitivity of detection, the UV spectrum was acquired for each amine, and the wavelength of maximum absorbance (Δ_{max}) was used to establish the limit of determination. Synthetic mixtures and dye samples were resolved and quantified.

Flodberg and Roeraade (143e) made a device for high-pressure thin-layer chromatography. The HPTLC chamber consists of two flat stainless steel blocks mounted in a hydraulic press. The upper chamber is solid, and the lower chamber is filled with water and covered with a membrane.

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The mobile phase inlet is connected to a membrane, and the TLC plate is placed upside-down against the membrane. During pressurization, a stainless steel frame is pressed against the membrane and a flat urethane rubber seal. The device was used to separate the dyes on a TLC plate coated with 3 μm spherical particles with a mobile phase of CH_2Cl_2, a flow rate of 1 mL/min, a mobile phase pressure drop of 140 bar, and a water cushion pressure of 170 bar.

C. Reversed-Phase TLC of Dyes

The separation of 26 basic dyes (suitable for dyeing acrylic fibers, silk, and tannin) was studied with the use of reversed-phase TLC plates, with aqueous 70–90% acetone as developing solvent. Development was 11.5–14.5 cm and took 30–90 min. Good separation of 10 of the dyes was attained with aqueous 70% acetone (144).

The separation of 34 commercial acid dyes of the monoazo- and biazoanthraquinone and triphenylmethane types was studied by reversed-phase TLC (RP-TLC) by application of the dyes onto layers of octadecylsilyl-bonded silica gel for TLC, using aqueous methanol or aqueous 60% acetone as developing solvent (101).

Thirteen dyes were separated by RP-TLC on C-18 modified silica gel using methanol–acetonitrile–aqueous 5% Na_2SO_4 (3:3:10) and then methanol–ethyl methyl ketone–aqueous 5% Na_2SO_4 (1:1:1) as mobile phases in the same direction. Separation was optimum between pH 6.0 and 7.0 (145). Four basic dyes were separated on pretreated silica gel plates using $HClO_4$ or benzenesulfonic acid as counterion and aqueous 50% or 60% ethanol as mobile phase (146). Polyacrylonitrile plates were used for the separation of 10 basic and common food dyes along with other dyes using diethylamine–anhydrous acetic acid–H_2O (4:1:15) as mobile phase (147).

Cserhati and Forgacs (147a) reviewed the use of various liquid chromatographic techniques such as adsorption and RP-TLC and HPLC principally for the separation and quantitative determination of terpenoid-based color substances in food and food products. They also delineated future trends in the separation and identification of pigments in food and food products.

Ozeki et al. (147b) investigated a TLC method for the analysis of Gardenia Yellow in food using crocetin as an indicator. Gardenia Yellow was extracted from food samples with hydrous methanol. After evaporating the extract, the remaining residue was dissolved in water at pH 11. The resultant mixture was stirred at 50°C for 30 min. The purified mixture was then subjected to the TLC analysis. The TLC conditions were an RP-18 F_{254} S plate and acetonitrile–tetrahydrofuran–oxalic acid (7:8:7) as mobile phase. The visible absorption spectra were measured using scanning densitometry. This method has been used on 37 commercial foods and is useful for the rapid analysis of Gardenia Yellow in foods.

Csiktusnadi et al. (147c) determined that the multistep gradient elution technique developed for the RP-TLC separation of pigments can be used as a pilot method for the rational design of gradient elution in RP-HPTLC for the separation of the same pigments.

Sulfonated dyes have been separated by ion-pair RP-TLC on RP-18 layers predeveloped with methanolic tetrabutylammonium bromide, using aqueous 80% methanol as mobile phase (148), and basic dyes have been separated on GDX-201–silica gel (1:1) layers using aqueous 70% methylcyanide containing 10 mM $HClO_4$ and 10 mM β-naphthylsulfonic acid (149).

Reversed-phase TLC coupled with fast atom bombardment spectrometry has been used for identification and separation of 27 food dyes consisting of 12 permitted for use in food and 15 unlawful dyes in Japan using C-18 modified silica gel as adsorbent and two mobile phases. The method was successfully applied for the identification of unlawful dyes in imported foods (150). The R_f values along with mobile phases used for these dyes are recorded in Table 16.

Miniaturization is a general trend in modern analytical methods, with the main aims being to increase sensitivity, shorten analysis times, and reduce the quantity of consumables per analysis (151). In planar chromatography, a first step in this direction was the development of modern high-performance TLC (HPTLC). Compared with conventional TLC, the layers are characterized by smaller absorbent material particle sizes and a slight reduction in layer thickness. A major final step in the direction of miniaturized planar chromatography is the use of new ultrathin layers. In contrast with those of TLC and HPTLC plates, these layers are not based on granular adsorbents but have a monolithic structure based on the silica gel matrix. This means that there are no longer

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Table 16 Reversed-Phase TLC of Food Dyes on C-18 Modified Silica Gel Plates

Dye	R_f	
	Solvent system A[a]	Solvent system B[b]
Ponceau 3R (R-1)	0.16	0.86
Amaranth (R-2)[c]	0.71	1.00
Erythrosine (R-3)[c]	0.00	0.40
Ponceau SX (R-4)	0.21	0.89
Oil Red (R-5)	0.00	0.06
Allura Red AC (R-40)[c]	0.34	1.00
Ponceau R (R-101)	0.22	0.89
New Coccine (R-102)[c]	0.58	1.00
Eosine (R-103)	0.00	0.42
Phloxine (R-104)[c]	0.00	0.15
Rose Bengal (R-105)[c]	0.00	0.19
Acid Red (R-106)[c]	0.04	0.77
Azo Rubine (R-AZ)	0.19	0.88
Orange I (0-1)	0.15	0.75
Orange RN (O-RN)	0.05	0.61
Oil Orange SS (O-SS)	0.00	0.09
Napthol Yellow S (Y-1)	0.42	0.90
Yellow AB (Y-2)	0.00	0.16
Yellow OB (Y-3)	0.00	0.13
Tartrazine (Y-4)[c]	0.79	1.00
Sunset Yellow FCF (Y-5)[c]	0.45	1.00
Guinea Green B (G-1)	0.03	0.73
Fast Green FCF (G-3)[c]	0.17	1.00
Wool Green S (G-S)	0.22	0.90
Brilliant Blue FCF (B-1)[c]	0.14	1:00
Indigo Carmine (B-2)[c]	0.66	1.00
Acid Violet 6B (V-1)	0.01	0.67

[a]Solvent system A = methanol–acetonitrile–5% sodium sulfate solution (3:3:10).
[b]Solvent system B = methanol–methyl ethyl ketone–5% sodium sulfate solution (1:1:1).
[c]Permitted dyes for use in foods in Japan.
Source: Adapted from Ref. 150.

separate particles, as in TLC and HPTLC. Moreover, a binder is not needed to fix the layer onto the glass plate. To be suitable for chromatographic purposes, the monolithic silica gel of the new ultrathin layers has meso- and macropores, with fine capillaries penetrating the layer. The layer thickness of approximately 10 μm is considerably less than that of conventional TLC and HPTLC layers. The exciting properties of these new ultrathin silica gel plates, especially their selectivity and separation efficiency, were demonstrated by separations of steroids, pesticides, and some dyestuffs.

Berezkin and Mardanov (152) proposed a version of thin-layer chromatography with forced flow of the mobile phase in microchannels packed with a sorbent. The dependence of retention parameters R_f and the number of theoretical plates in the course of the elution of hydrophilic dyes

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(Naphthol Red and Methyl Red) on the eluent (propan-2-ol) pressure and developing time were studied. Forced-flow TLC would be useful as a pilot microtechnique.

VI. CONCLUSIONS

Color chemistry is more than the synthesis of brilliant dyes for textiles. Today colorants are also used in information storage devices, lasers, liquid crystal displays, solar energy conversion systems, and food additives. Moreover, analytical techniques such as affinity chromatography and histological staining depend on dyes. Considering the scope of chromatographic investigations in the synthetic dyestuff field, insufficient work has been done. Although a number of papers have appeared on the TLC of food dyes, little work has been reported on the separation of other types of synthetic dyes. TLC of dyes on untreated plates sometimes gives diffuse spots and tailing. This may be overcome by performing TLC on treated plates, where the separation efficiency is increased due to sharpness of the spots. OPTLC, HPTLC, and reversed-phase TLC give still better results owing to the advantages of shorter analysis time, more data measurement per plate, high resolution and improved signal-to-noise ratio. However, little work has been done on the separation of dyes by HPTLC, OPTLC, reversed-phase TLC, or TLC on treated (impregnated) plates. Because all these techniques give better separation along with other advantages, they must be explored further.

Dyes can be divided into two classes depending on their solubility, i.e., water-soluble and insoluble. One difficulty in dealing with many dyes is that the choice of solvents is very limited. Hence this area also needs further exploration. However, substances that are virtually insoluble at room temperature can often be analyzed successfully by TLC at elevated temperatures if the samples to be analyzed exhibit sufficient thermal stability.

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