Handbook of Thin-Layer Chromatography

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Preface

This book has been designed as a practical, comprehensive laboratory handbook on the topic of thin-layer chromatography (TLC). It is divided into two parts, the first of which covers the theories and general practices of TLC (Chapters 1-13), while the second (Chapters 14-31) includes applications based mainly on compound types. The book will be a valuable source of information for scientists with a high degree of expertise in the separation sciences, but because most chapters include considerable introductory and background material it is also appropriate for the relatively inexperienced chromatographer.

Contributors to the book are recognized experts on the topics they have covered and include many of the best-known and most knowledgeable workers in the field of TLC throughout the world. As much as possible, we attempted to adopt a uniform style for each chapter while still allowing authors the latitude to present their topics in what they considered to be the most effective way. Consequently, in the applications chapters (14-31), most authors have included the following sections: introduction, sample preparation, layers and mobile phases, chromatographic techniques, detection, quantification, and detailed experiments. Authors were encouraged to use many figures and tables and to be as practical as possible except for the chapters devoted to theory (2 and 10). The literature covered by most authors includes mainly the period from 1975 to 1989. Some of the more significant older literature has also been covered, but many authors refer to the earlier comprehensive treatises by Stahl and Kirchner for this material. Authors have been selective in their choice of references and present TLC methods that are most suitable for laboratory work.

It is important to point out that the *Handbook of Thin-Layer Chromatography* has a comprehensive, organized plan and, unlike many recent books in the field, is not a random collection of chapters on "advances" or papers from a symposium. An earlier laboratory handbook on TLC was written by Egon Stahl in 1965. We hope that our handbook may have at least a small fraction of the impact in the near future that this classic work had on the development and growth of TLC during the past 25 years. If the book is well accepted and contributors cooperate, we hope to update coverage of all important aspects of TLC with regular later editions.

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Part I
Principles and Practice of Thin-Layer Chromatography

1

Basic Techniques, Materials, and Apparatus

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The purpose of this chapter is to present an overview of all important aspects of thin-layer chromatography (TLC). It will briefly review information and references on topics covered in following chapters in Part I and will refer readers to these specific chapters. It will treat in more detail topics not covered further in separate chapters, especially sampling and sample preparation and the more classical procedures of TLC. A suggested source of additional basic, practical information on the practice and applications of TLC is the primer written by Fried and Sherma (1).

I. INTRODUCTION AND HISTORY

A. Introduction to TLC

TLC, which together with paper chromatography comprise "planar" or "flat-bed" chromatography, is the simplest of all of the widely used chromatographic methods to perform. A suitable closed vessel containing solvent and a coated plate are all that are required to carry out separations and qualitative and semiquantitative analysis. With optimization of techniques and materials, highly efficient separations and accurate and precise quantification can be achieved. TLC can be used also for preparative-scale separations by employing specialized apparatus and techniques.

Basic TLC is carried out as follows. An initial zone of mixture is placed near one end of the stationary phase, a thin layer; the sample is dried; and the end of the stationary phase with the initial zone is placed into a mobile phase, usually a mixture of pure solvents, inside a closed chamber. The components of the mixture migrate at different rates during movement of the mobile phase through the stationary phase, which is termed the development of the chromatogram. When the mobile phase has moved an appropriate distance, the stationary phase is removed, the mobile phase is rapidly dried, and the zones are detected by application of a suitable visualization reagent.

Differential migration is the result of varying degrees of affinity of the mixture components for the stationary and mobile phases. Different separation mechanisms are involved, the predominant forces depending on the exact nature of the two phases and the solutes. The interactions involved in determining chromatographic retention and selectivity include hydrogen bonding, electron-pair donor/electron-pair acceptor (charge transfer), ion-ion, ion-dipole, and van der Waals interactions. Among the latter are dipole-dipole (Keesom), dipole-induced dipole (Debye), and instantaneous dipole-induced dipole (London) interactions.

Sample collection, preservation, and purification are problems common to TLC and all other chromatographic methods. For complex samples, the TLC development will usually not completely resolve the analyte (the substance to be determined) from interferences unless a prior purification is carried out. This is most often done by selective extraction and column chromatography. In some cases, substances are converted, prior to TLC, to a derivative that is more suitable for separation, detection, and/or quantification than the parent compound.

Detection is most simple when the compounds of interest are naturally colored or fluorescent or absorb ultraviolet (UV) light. However, application of a location or visualization reagent by spraying or dipping is usually required to produce color or fluorescence for most compounds. Absorption of UV light is common for many compounds, e.g., aromatics and those with conjugated double bonds. This leads to a simple, rather universal detection method on layers impregnated with a fluorescence indicator (fluorescence quench detection).

Compound identification in TLC is based initially on R_f values compared to authentic standards. R_f values are generally not exactly reproducible from laboratory to laboratory or even in different runs in the same laboratory, so they should be considered mainly as guides to relative migration distances and sequences. Factors causing R_f values to vary include: dimensions and type of the chamber, nature and size of the layer, direction of mobile-phase flow, the volume and composition of the mobile phase, equilibration conditions, humidity, and sample preparation methods preceding chromatography. Further characterization of separated substances can be obtained by scraping the layer and elution of the analyte followed by infrared (IR), nuclear magnetic resonance (NMR), or mass spectrometry (MS).

B. History of TLC

The history of liquid chromatography, which dates back to the first description of chromatography by Michael Tswett in the early 1900s, was reviewed by Sherma (2). Stahl (3,4), Kirchner (5,6), and Pelick et al. (7) have reviewed the history of TLC. TLC is a relatively new discipline, and chromatography historians usually date the advent of modern TLC from 1958. The review by Pelick et al. tabulates significant early developments in TLC and provides translations of classical TLC studies by Izmailov and Schraiber and by Stahl. In 1938, Izmailov and Schraiber separated certain medicinal compounds on unbound alumina spread on glass plates. Since they applied drops of solvent to the plate containing the sample and sorbent layer, their procedure was called "drop chromatography." Meinhard and Hall in 1949 used a binder to adhere alumina to microscope slides, and these layers were used in the separation of certain inorganic ions using drop chromatography. In the early 1950s, Kirchner and colleagues at the U.S. Department of Agriculture developed TLC as we know it today. They used sorbents held on glass plates with the aid of a binder, and plates were developed with conventional ascending procedures used in paper chromatography. Kirchner coined the term "chromatostrips" for his layers. Stahl introduced the term "thin-layer chromatography" in the late 1950s. His major contributions were the standardization of materials, procedures, and nomenclature and the description of selective solvent systems for resolution of important compound classes. His first laboratory manual popularized TLC and he obtained the aid of chemical manufacturers in offering standard materials for TLC (8). Other early books that had great influence on the development of TLC include those by Kirchner (9), Bobbitt (10), Randerath (11), and Truter (12).

Quantitative TLC was introduced by Kirchner et al. in 1954 (13) when they described an elution method for determination of biphenyl in citrus fruits and products. Densitometry was first used for direct measurement of bands separated by means of electrophoresis and was later used on paper chromatograms. Densitometry in TLC was initially reported in the mid-1960s by Dallas et al. (14) using the Joyce Loebl Chromascan and by Genest (15) and Thomas et al. (16)

using the Photovolt densitometer. A symposium on quantitative TLC held in 1968 in Great Britain led to the first book published on this topic (17).

High-performance TLC plates (18) were produced commercially in the mid-1970s and provided impetus for the improvements in practice and instrumentation that occurred in the late 1970s and 1980s and led to the methods termed "high-performance TLC (HPTLC)" (19) and "instrumental HPTLC" (20). Centrifugally accelerated preparative-layer chromatography (21) and forced-flow techniques in TLC (overpressured layer chromatography, OPLC) (22) were introduced in the late 1970s.

These and other high-performance and quantitative methods have caused a renaissance in the field of TLC that is reflected in this Handbook. There is no doubt that TLC will continue to evolve and grow in the 1990s and beyond as a highly selective, sensitive, quantitative, rapid, and automated technique for analysis of all types of samples and analytes, and for preparative separations. To keep abreast of this inevitable progress in TLC, the biennial review of advances in theory, practice, and applications by Sherma (23) is indispensible.

C. Comparisons of TLC to Column Liquid Chromatography (HPLC)

A detailed comparison of TLC to other chromatographic methods is given on page 4 of Ref. (1), and TLC and HPTLC are compared on page 14 of this same reference. , TLC involves the concurrent processing of multiple samples and standards on an open layer developed by a mobile phase. Development is performed, usually without pressure, in a variety of modes, including simple one-dimensional, multiple, circular, and multidimensional. The detection of zones is done statically with an assortment of diverse possibilities. Paper chromatography, which was invented by Consden, Gordon, and Martin in 1944, is fundamentally very similar to TLC, differing mainly in the nature of the stationary phase. Paper chromatography has lost favor compared to TLC because the latter is faster, more efficient, and allows more versatility in the choice of stationary and mobile phases.

HPTLC layers are smaller, contain sorbent with a smaller, more uniform particle size, are thinner, and are developed for a shorter distance compared to TLC layers. These factors lead to faster separations, reduced zone diffusion, better separation efficiency, lower detection limits, and less solvent consumption. However, smaller samples, more exact spotting techniques, and more reproducible development techniques are required.

Column LC involves the elution under pressure of sequential samples in a closed, "on-line" system, with dynamic detection of solutes, usually by UV absorption. The predominant mode of HPLC is reversed-phase on bonded silica columns, while normal-phase TLC on silica gel is most widely used. Reasons for this difference include the change in properties of silica gel columns caused by continuous mobile-phase flow (which is unimportant for silica gel layers because they are used only once), and the impracticality of using solvents with high percentages of water to develop many brands of bonded reversed-phase layers.

A paper by Treiber (24) offers an excellent review of the relationship of TLC to other chromatographic techniques, especially HPLC. This paper should be read by anyone wanting a clear idea of how TLC and HPLC can complement each other in real-world analytical situations. A paper by Brinkman (25) compares the merits of TLC, capillary gas chromatography (GC), and HPLC in trace-level organic analysis in terms of separation efficiency, sample pretreatment, sample size, detection, speed of analysis, and interfacing with ancillary methods. Table 1 is reproduced from this paper.

TLC is the most versatile and flexible chromatographic method. It is rapid because precoated layers are usually used as received, without preparation. Even though it is not fully automated like HPLC, TLC has the highest sample throughput because up to 30 individual samples and standards can be applied to a single plate and separated at the same time. Modern computer-controlled scanning instruments and automated sample applicators and developers allow accuracy and precision in quantification that rival HPLC and GC. There is a wide choice