TECHNIQUES OF CHEMISTRY

VOLUME XIV

THIN-LAYER CHROMATOGRAPHY

Second Edition

JUSTUS G. KIRCHNER, Retired

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BY

JUSTUS G. KIRCHNER, Retired

Senior Scientist
The Coca-Cola Company

EDMOND S. PERRY, *Editor*

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INTRODUCTION TO THE SERIES

Techniques of Chemistry is the successor to the Technique of Organic Chemistry Series and its companion—Technique of Inorganic Chemistry. Because many of the methods are employed in all branches of chemical science, the division into techniques for organic and inorganic chemistry has become increasingly artificial. Accordingly, the new series reflects the wider application of techniques, and the component volumes for the most part provide complete treatments of the methods covered. Volumes in which limited areas of application are discussed can be easily recognized by their titles.

Like its predecessors, the series is devoted to a comprehensive presentation of the respective techniques. The authors give the theoretical background for an understanding of the various methods and operations and describe the techniques and tools, their modifications, their merits and limitations, and their handling. It is hoped that the series will contribute to a better understanding and a more rational and effective application of the respective techniques.

My special thanks are due to Dr. Edmond S. Perry for assistance in the editorial work on the present volume and other volumes dealing with techniques of Separation and Purification.

Authors and editors hope that readers will find the volumes in this series useful and will communicate to them any criticisms and suggestions for improvements.

ARNOLD WEISSBERGER

Research Laboratories
Eastman Kodak Company
Rochester, New York

FOREWORD TO THE FIRST EDITION

During the past fifteen years thin-layer chromatography has been established as one of the most powerful, exacting, and useful tools for the chemist in the laboratory. The technique is relatively simple, the equipment required inexpensive. Applicable to both volatile and nonvolatile substances, thin-layer chromatography is useful in many fields—vitamins, steroids, pharmaceuticals in general, synthetic organic materials, dyes, essential oils, resins, pesticides, etc. In many cases it offers the only practical solution of a perplexing problem. Combinations of thin-layer chromatography with other techniques, vapor-phase chromatography among them, are being explored at present by researchers throughout the world.

The present volume by Dr. Justus Kirchner presents an up-to-date and comprehensive treatment of the subject. The first section describing techniques shows the familiarity of the author with the subject from the practical point of view and the thoroughness with which he has studied existing literature. The second section brings together in one place the many and varied applications that have been made of the technique.

We heartily welcome Dr. Kirchner's *Thin-Layer Chromatography*, and are convinced that this work will be indispensable on the book shelves of laboratories in the scientific world as well as in industry everywhere.

ERNEST GUENTHER, PH.D.

Senior Vice President
Fritzsche Brothers, Inc.
New York

FOREWORD TO THE SECOND EDITION

It was in 1951 that the most important paper for thin-layer chromatography was published. In this journal article, Dr. Justus G. Kirchner reported separating terpenes on open layers of silica gel, which he referred to as "chromatostrips." From this auspicious beginning, thin-layer chromatography has become one of the most useful and practical chemical separation techniques. This is most evident by the recent tremendous proliferation of papers published in which thin-layer chromatography is a key method.

The vast quantity of papers published on thin-layer chromatography makes this book a prerequisite not only for routine laboratory work but also for obtaining a thorough theoretical background. Though everyone has different needs and problems, this well-organized book will continually serve the reader as an invaluable ready reference.

RICHARD K. VITEK

New Berlin, Wisconsin

PREFACE TO THE FIRST EDITION

Although the basic principle of using thin layers of adsorbent for chromatographic separations was used by Izmailov and Schraiber in 1938, and by Meinhard and Hall in 1948, it was not until the author dispensed with the idea of drop chromatography, employed by the earlier workers, and originated the use of uniformly coated glass strips and plates for development in a manner analogous to paper chromatography that the possibilities of the method were uncovered.

Since the present system of thin-layer chromatography was introduced by the author in 1951 the method has found wide application in the various fields of chemistry. The past two years have seen a tremendous increase in the number of papers on the specific subject of thin-layer chromatography and also in the number of papers where it has been used as a tool to help in solving a given problem.

The book is divided into two sections, the first presenting the techniques of the method and the second covering the many applications that have been made from its inception through the year 1964, including partial coverage of 1965. In order to make the book complete, a chapter on inorganic ions has been included.

It is with pleasure that I acknowledge my indebtedness to Dr. C. A. Shillinglaw for his interest in this work. I express my appreciation to the following people: to my wife Mildred M. Kirchner for constant encouragement, to Louise M. Blood for typing the manuscript, to Ludwig Renner for preparation of the drawings, to my daughter, Grace L. Kirchner, for assistance in translating, and to Dr. James M. Bobbitt for the use of his reprints.

JUSTUS G. KIRCHNER

Scotch Plains, New Jersey

PREFACE TO THE SECOND EDITION

Recently uncovered work has shown that the basic principle of thin-layer chromatography was demonstrated by Beyerinck in 1889 and again in 1898 by Wijsman. Beyerinck's work predated that of Ismailov and Schraiber in 1938 and indeed even that of Reed on column chromatography in 1893.

Since the first edition of this book in 1967 the Kirchner technique of thin-layer chromatography that I introduced in 1951 has continued to expand, as evidenced by the increase in publications and introduction of new techniques and modifications. The 5000 to 6000 publications available up to 1967 have grown to an estimated 15,000 to 20,000, of which more than 6000 have been cited in this work. The literature has been thoroughly covered through 1975, with some work throughout 1976 and a few from 1977.

Some of the chapters, as for example that on quantitative work, have been completely revised, and all have been modified and updated. The general outline of the first edition has been retained. New techniques and special notations to be considered in using thin-layer chromatography in many fields have been brought together from many sources to assist the worker in solving his or her problems and obtaining the best results. The chapter on detection reagents has been revised and expanded, and a cross index for locating detection reagents for types of compounds or for specific compounds has been added in Appendix A. Rather than include an author index, which takes up valuable space and serves only to show that a given author's work has been cited, I have devoted the space to a thorough subject index where subjects of interest may be readily located. A separate compound index is included so as to make the location of a specific subject easier without searching through columns of compound names.

I acknowledge my indebtedness to Dr. Darshan Bhatia, Director of Corporate Research and Development of the Coca-Cola Company, for making available the facilities of the Technical Information Services and to Bernard Prudhomme, Mary Jane Montesinos, Brenda Pierce, and Cindy Blair of that department for locating and obtaining sources of

information. Recognition is extended to Dr. Victor Krample for assistance with Russian, Hungarian, and Czech translations and to Ellen Villanueva for Spanish translations. Thanks are also due Nathaniel Davis for assistance in supplying copies of references.

JUSTUS G. KIRCHNER

Dunwoody, Georgia
May 1978

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TECHNIQUES OF THIN-LAYER CHROMATOGRAPHY

Chapter I

INTRODUCTION, HISTORY, AND GENERAL DESCRIPTION

1· INTRODUCTION

Farradane [1] has pointed out that the credit for the first recorded work on column chromatography should go to Reed [2], who published in 1892 on the use of tubes of kaolin for the separation of potassium chromate from eosin and of ferric chloride from copper sulfate. Prior to this, the idea of paper chromatography appears to have originated with Schoenbein [3] in 1861, with further developments by his pupil Goeppelsroeder [4–7], who called the technique capillary analysis.

Tswett's [8] great contribution to chromatography was his use of pure solvents to develop the chromatogram. Tswett's first experiments (published in 1903) were concerned with the separation of the pigments from a leaf extract by passing a petroleum ether solution through a column of calcium carbonate. The yellow and green pigments separated, and after developing the column with pure solvent, Tswett cut up the column and eluted the various pigments that remained on the column with alcohol, the carotene having passed through the column with the petroleum ether solvent. In this example the bands were readily visible, but some method was needed in order to be able to see what was happening in chromatographing the many colorless compounds which are invisible on the column.

Since that time many methods have been devised to detect the chromatographic bands. Tswett himself proposed the addition of a pigment to a colorless solution in order to relate the colorless zones to a visible

standard. The majority of zone-detecting techniques for columns are applied to the eluate as it leaves the column and range all the way from measurement of the refractive index to specific chemical tests for individual compounds. On the column itself techniques such as arbitrary cutting of the column, detection by means of strongly fluorescent columns [9–11], and extrusion from the glass envelope with the brushing on of an indicating reagent [12] have been used to advantage. Miller and Kirchner [13], in 1951, applied the principles of thin-layer chromatography to a self-contained adsorbent column without the usual confining glass tube, so that the column could be sprayed with indicating reagents.

Column chromatography as used by Tswett is concerned with the distribution of compounds between the solid adsorbent and the solvent. In the simplest case, as the developing solvent moves down the column of previously adsorbed material, the latter is desorbed to the extent that an equilibrium is set up between adsorbed material and the material in solution. Then as this solution travels further down the column it comes in contact with fresh adsorbent, and dissolved material is again picked up by the adsorbent. In this way the adsorbed material progresses down the column. By this process, a compound for which the adsorbent has a strong affinity will displace compounds which are less strongly adsorbed. This results in a series of bands or layers with the most strongly adsorbed on top followed in order by less and less strongly adsorbed materials. If the adsorption affinities are close together the bands may appear adjacent to one another, whereas with more widely differing adsorption affinities the bands will appear separated by empty bands of adsorbent. Theoretically, given a long enough column, any two compounds which have different adsorption affinities under a given set of conditions can be separated by chromatography.

A variation of ordinary column chromatography was introduced in 1941 by Martin and Synge [14] with their concept of partition chromatography. Here the column becomes a support for the liquid aqueous phase with which it is treated. Through this is passed a nonmiscible solvent so that a partitioning of the substances to be separated occurs between the two solvents. By placing the hydrophobic solvent on the column and using the hydrophilic solvent as the moving phase one obtains a reverse-phase partition chromatogram.

The paper chromatography introduced by Schoenbein [3] and Goeppelsroeder [4–7] as capillary analysis in 1881 lay dormant until reintroduced by Consden et al. [15]. This technique has played an important role in the analysis of amino acids but the two main drawbacks are its inherent slowness and its very small capacity. In contrast to this, thin-layer chromatography has a high capacity with rapid development which does not

detract from its sensitivity. In direct comparison between paper and thin-layer the latter has been found to be more sensitive in detecting small amounts. Fahmy et al. [16] have shown that thin-layer chromatography of amino acids on silica gel G is more than 10 times as sensitive as paper chromatography. Even considering paper chromatography itself it has been shown that cellulose layers on glass plates are faster and give sharper separations than the corresponding work with paper chromatography [17, 18].

Some attention should be given to the term thin-layer chromatography. Although the terms "chromatostrip" and "chromatoplate" were first used in 1951 [19] and 1954 [20], for this present-day method of analysis, the designation thin-layer chromatography (TLC) has become so widely accepted in referring to the general method that terms such as thin film and open column should be discarded.

2 HISTORY OF THIN-LAYER CHROMATOGRAPHY

Thin-layer chromatography actually began with the Dutch biologist Beyerinck, in 1889 [21], when he allowed a drop of a mixture of hydrochloric and sulfuric acids to diffuse through a thin layer of gelatin. The hydrochloric acid traveled faster than the sulfuric acid and formed a ring around the sulfuric acid. The hydrochloric acid zone was made visible by brushing on a solution of silver nitrate, and the sulfuric acid was made visible with barium chloride. Nine years later Wijsman [22], using the same techniques, showed the presence of two enzymes in malt diastase, and also showed that only one of them split off maltose from soluble starch. Wijsman was also the first to use a fluorescent phenomenon for detecting a zone on a thin layer. He incorporated fluorescent bacteria from seawater in a gelatin layer containing starch and allowed the amylase mixture to diffuse in the layer. A fluorescent band appeared only where the β-amylase reacted with the starch. This also happened to be one of the more sensitive visualizing agents encountered in thin-layer work. He was able to detect 1/28,000,000 of a milligram of maltose. This places it at about 40 pg. This bit of history was brought to light by Van Klinkenberg [23].

Izmailov and Schraiber [24], in 1938, discussed the use of a thin layer of aluminum oxide spread on a glass plate. It did not contain a binder and was used for circular chromatography by placing a drop of the solution on the adsorbent and developing into concentric zones with solvent drops. They also pointed out its usefulness for testing adsorbents and solvents for column chromatography.

Lapp and Erali [25], in 1940, published a method of loose-layer chro-