



CHROMATOGRAPHY

A SCIENCE OF DISCOVERY

EDITED BY

ROBERT L. WIXOM CHARLES W. GEHRKE



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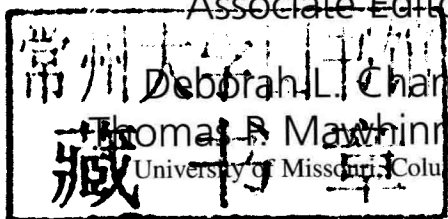
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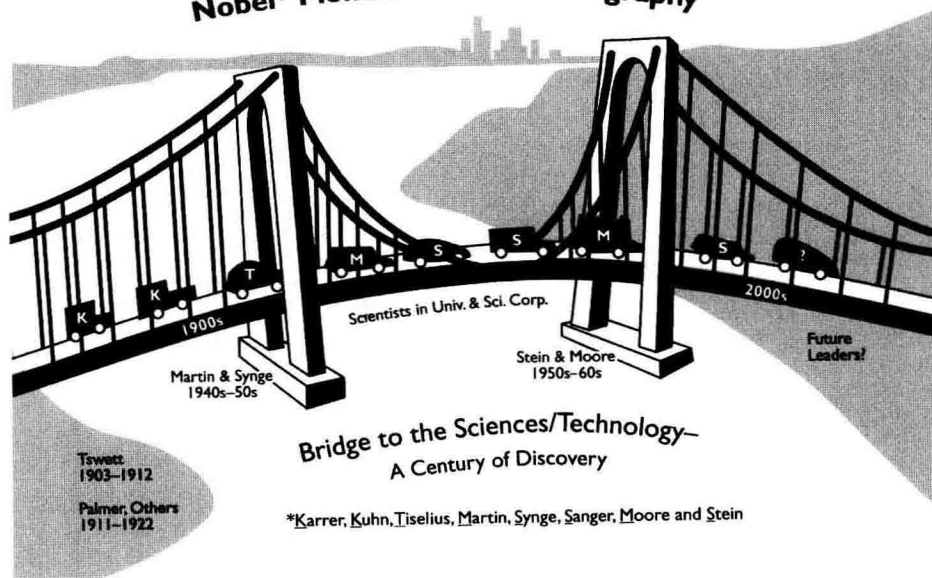
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CHROMATOGRAPHY



Nobel* Pioneers in Chromatography



The evolution of chromatography: The bridge to the sciences and technology. Some of the early scientists who invented, rediscovered, and/or advanced chromatography include M. S. Tswett, L. S. Palmer, R. Kuhn, A. W. K. Tiselius, A. J. P. Martin, R. L. M. Synge, F. Sanger, S. Moore, and W. B. Stein, and the awardees in the earlier book, *Chromatography: A Century of Discovery (1900-2000)*—the *Bridge to the Sciences/Technology*, Vol. 64, Elsevier, Amsterdam, 2001; see Chapters 1, 2, 5, and 6 therein and the present Chapters 3, 4, 5, and 11. What are the common features of their discoveries?

DEDICATION

To Professor Emeritus Charles W. Gehrke (deceased February 10, 2009), *who employed his clarity, resourcefulness, and organizational skills in the demanding task as senior editor of this book; and who will be remembered by faculty, students, and administration for his friendliness, persuasiveness, and integrity (see Editors/Authors section of this book).*

To Professor Emeritus Robert L. Wixom (deceased July 8, 2009), *who was determined to share with the new scientists of today, the rich history and fundamentals of thought and action which brought us to the chromatography of today and point us toward the future;*



and who will be remembered academically for his quest for truth and willingness to take the extra time and go the extra mile so that one might learn something new (see Editors/Authors section of this book).

To Professor Ernst Bayer (deceased 2001), who made significant contributions as co-editor of the earlier book, Chromatography: A Century of Discovery (1900–2000)—The Bridge to the Sciences/Technology, Vol. 64, Elsevier, Amsterdam, 2001 (see his biography therein), and who has made outstanding multiple research contributions in chromatography.

To our scientific colleagues, who have contributed significantly to the advances of chromatography and then described their research in this volume; and who have contributed scientific thoughts, ideas for experiments, and means for communication.

To our relevant research institutions, whether university, professional societies, or corporate/government agencies that have supported related research endeavors.

To our respective family members, who have been both patient and helpful as we moved through the writing process.

Our thanks are also extended to the editors of John Wiley & Sons, Inc.

PREFACE

The frontispiece illustration depicts the aim of this book: to capture the coherent message of our earlier chromatography book [1] and the present one—namely, to bridge, or to provide the connection of, the earlier chromatography (1900–2000) with the chromatography of the present book (2000–2008) and today’s science. Those multifaceted messages are based on connecting the *pioneers of chromatography*—Mikhail S. Tswett, Leroy S. Palmer, and several others [1], the early *builders of chromatography*—R. Kuhn, A. W. K. Tiselius, A. J. P. Martin, R. L. M. Synge, F. Sanger, S. Moore, and W. B. Stein, and the *awardees* (see [1] and Chapters 3 and 5 of this book). The *bridge* or the connections to later and wider facets of chromatography leads to examples of *the science of discovery*.

Now, a hundred years after M. S. Tswett, his robust infant of chromatography has grown to be a major subject with dozens of references for each chapter in the 2001 chromatography book [1], >50 pages in the appendixes of the 2001 online supplement [2], and this book. This expanding amplitude of chromatography and the scientific literature makes journal and/or book reading slower and perhaps tedious in the drive to be thorough. Fortunately, the recent electronic revolution has delivered the tools to resolve that dilemma—namely, journals online, abstract journals online, and search engines. Consequently, the appendixes in the online version of *Chromatography* [2] are not being updated for this volume. To summarize, turn on the power for the Internet.

Chromatography has observed the classical flow of many sciences—to question, to undertake related experiments, to probe the subject, to build on recent observations and hypotheses, to interpret the experiments, and when sufficient evidence has accumulated, to develop theories.

Some unique aspects of this book follow: Chapter 1 introduces the evidence for chromatography to be more than a method of separation, but is now a discipline of science. While some view chromatography as comprising multiple areas, Chapter 2 presents the case for chromatography as a unified science. After introducing the early “pioneers” and “builders” of chromatography in Chapter 3, an additional 25 Nobel Prize awardees (1937–1999) and 19 in (2000–2007) relied on chromatography as a method in their overall research. This review led to a discussion of “sharp turns,” “break-throughs,” “scientific revolutions,” and “paradigm shifts.” The multiple branches of

chromatography known in the 1960s have continued to grow over the subsequent decades to now be recognized as “trails of research” (Chapter 4). Our key Chapter 5 presents the 2000–2007 awardees and key contributors along with a description of their research.

In Chapter 6, several invited scientists describe recent advances in column technology and its validation. Chapters 7, 8, and 9 examine the contributions of chromatography in subject areas: agricultural, space, and biological/medical sciences; pharmaceutical science; and the environment, natural products, and chemical analysis and synthesis. Chapter 10 provides a comprehensive compilation of references on the history of chromatography. Chapter 11, first describes three institutions that have contributed over the decades to chromatography: the Pittsburgh Conference on Analytical Chemistry and Applied Spectrometry (PITTCON); the Chemical Heritage Foundation, and the American Chemical Society. Next is a section on recognizing and using chromatographic separations as molecular interaction amplifiers. Perspectives for the future are given by awardees and significant contributors to the field of chromatography. Finally, the book concludes with how chromatography has contributed to the advances in systems biology, genomics, and proteomics along with the initial reliance on other methods and theory in order to advance these new areas of science. The continuous theme throughout this book portrays chromatography as “the science of discovery.”

This book is recommended for students in the sciences and research chromatographers at all levels of experience: professional scientists; research investigators in academia, government, and industry; science libraries in academia, government, industry, and professional societies; and historians and philosophers of science and educators and their advanced students. This book builds on its 2001 predecessor [1] to identify the additional more recent (2000–2008) major advances in chromatography and the discoveries that will influence many sciences in this ongoing twenty-first century. Science has the goal of discovery and hence “the science of discovery” is implicit in many of the following chapters.

This book describes chromatography as the “bridge”—as a central science—a key foundation built on the twentieth century for major advances and discoveries yet to come across many sciences of the twenty-first century.

REFERENCES

1. C. W. Gehrke, R. L. Wixom, and E. Bayer, (Eds.), *Chromatography: A Century of Discovery (1900–2000)—The Bridge to the Sciences/Technology*, Vol. 64, Elsevier, Amsterdam, 2001.
2. C. W. Gehrke, R. L. Wixom, and E. Bayer, (Eds.), *Chromatography: A Century of Discovery (1900–2000)—The Bridge to the Sciences/Technology*, Chromatography—A New Discipline of Science, Internet Chapters, Appendixes, and Indexes, 2001. [See Internet at Chem. Web Preprint Server (<http://www.chemweb.com/preprint>)].

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We, the Editors, have had many helpful conversations with and advice from V. G. Berezkin, P. R. Brown, T. L. Chester, V. A. Davankov, W. G. Jennings, J. Janak, and P. Sandra and the awardees. We have had the benefit of many helpful discussions with other chromatographers, particularly at the national meeting of PITTCON and the American Chemical Society (ACS). Many of these scientists have contributions in the text. We have appreciated the valuable comments and insights of Dr. Leslie S. Ettre.

We have appreciated the many contributions of valuable librarians at the University of Missouri: Kate Anderson, Rachel Brekhaus, Brenda Graves-Blevins, Janice Dysart, Rebecca S. Graves, E. Diane Johnson, Amanda McConnell, Rachel Scheff, and Caryn Scoville. The Editors have received helpful input from the librarians of the Chemical Heritage Foundation initiated by the American Chemical Society and other sponsors (Philadelphia, PA) and *Chemical Abstracts* (published by ACS, Columbus, OH). Preparation of copy for this book is due in large part to the excellent secretarial skills plus accuracy and patience of Rosemary Crane, Tina Jenkins, and Cynthia Santos at the University of Missouri, Columbia.

The editors have warmly appreciated the graphic artwork by Sammae Heard, MU graphic artist, and the pen and ink drawings by Corrine Barbour, MU graduate art student.

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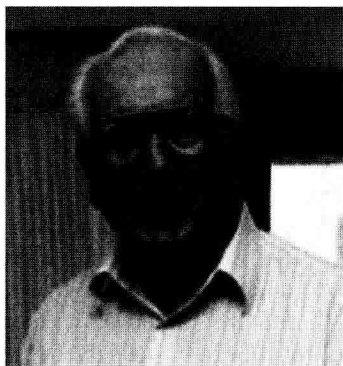
- Chancellor, Brady Deaton, Vice Provost for Research, Jim Coleman and their Office of Research
- School of Medicine and Dean William Crist
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- Experiment Station Chemical Laboratories (Agriculture), and Director Thomas P. Mawhinney
- Analytical Biochemistry Laboratory (ABC Labs), Columbia, Missouri, USA and CEO Byron Hill

Postscript. Unfortunately, the editors/authors of this book were unable to see its publication as they passed away before the final copy was completed. Thinking ahead, as good scientists must, Dr. Wixom enlisted the assistance in final editing of this manuscript of two University of Missouri colleagues and fellow chromatographers: Deborah L. Chance, Research Assistant Professor in the Departments of Molecular Microbiology & Immunology and Child Health, and Thomas P. Mawhinney, Professor in the Departments of Biochemistry and Child Health and current Director of the Missouri Agricultural Experiment Station Chemical Laboratories.

We thank Dr. Wixom and Dr. Gehrke for their grand contribution to the field in the assembling of this historical and prospective document, and the staff of John Wiley & Sons for their assistance in seeing that this work is published.

DEBORAH L. CHANCE and THOMAS P. MAWHINNEY
Associate Editors

EDITORS/AUTHORS



Charles William Gehrke was born on July 18, 1917 in New York City. He studied at The Ohio State University, receiving a B.A. in 1939, a B.Sc. in Education in 1941, and an M.S. in Bacteriology in 1941. From 1941 to 1945, he was Professor and Chairman of the Department of Chemistry at Missouri Valley College, Marshall, Missouri, teaching chemistry and physics to World War II Navy midshipmen (from destroyers, battleships, and aircraft carriers in the South Pacific) for officer training. These young men returned to the war as deck and flight officers. In 1946, he returned as instructor in agricultural biochemistry to The Ohio

State University, receiving his Ph.D. in 1947. In 1949, he joined the College of Agriculture at the University of Missouri–Columbia (UMC), retiring in Fall 1987 from positions as Professor of Biochemistry, manager of the Agricultural Experiment Station Chemical Laboratories, College of Agriculture, Food and Natural Resources, and Director of the University Interdisciplinary Chromatography Mass-Spectrometry facility. His duties also included those of State Chemist for the Missouri Fertilizer and Limestone Control laws. He was Scientific Coordinator at the Cancer Research Center in Columbia from 1989 to 1997.

Gehrke is the author of over 260 scientific publications in analytical chemistry and biochemistry. His research interests include the development of quantitative, high-resolution gas and liquid chromatographic methods for amino acids, purines, pyrimidines, major and modified nucleosides in RNA, DNA, and methylated “CAP” structures in mRNA; fatty acids; biological markers in the detection of cancer; characterization and interaction of proteins, chromatography of biologically important molecules, structural characterization of carcinogen–RNA/DNA adducts; and automation of analytical methods for nitrogen, phosphorus, and potassium in fertilizers. He developed automated spectrophotometric methods of lysine, methionine, and cystine.

He has lectured on gas–liquid chromatography of amino acids in Japan, in China, and at many universities and institutes in the United States and Europe. In the 1970s, Gehrke analyzed all the lunar samples returned by Apollo flights 11, 12, and 14–17 for amino acids and extractable organic compounds as a co-investigator with Cyril Ponnampertuma, University of Maryland, and with a consortium of scientists at the National Aeronautics and Space Administration (NASA), Ames Research Center, California, and the University of Maryland, College Park, Maryland.

Awards and Honors. In 1971, Dr. Gehrke received the annual Association of Official Analytical Chemists' (AOAC) Harvey W. Wiley Award in Analytical Chemistry. He was recipient of the Senior Faculty Member Award, UMC College of Agriculture, in 1973. Invited by the Soviet Academy of Sciences, he gave a summary presentation on organic substances in lunar fines at the August 1974 Oparin International Symposium on the "Origin of Life." In 1975, he was selected as a member of the American Chemical Society Charter Review Board for Chemical Abstracts. Sponsored by five Central American governments, he taught the chromatographic analysis of amino acids at the Central American Research Institute for Industry in Guatemala in 1975.

Gehrke was elected to Who's Who in Missouri Education and was a recipient of the UMC Faculty–Alumni Gold Medal Award in 1975 and the Kenneth A. Spencer Award from the Kansas City Section of the American Chemical Society for meritorious achievement in agricultural and food chemistry from 1979 to 1980. He received the Tswett Chromatography Memorial Medal from the Scientific Council on Chromatography, Academy of Sciences of the USSR, Moscow in 1978 and the Sigma XI Senior Research Award by the UMC Chapter in 1980. In 1986, Gehrke was given the American Chemical Society Midwest Chemist Award. He was an invited speaker on "Modified Nucleosides and Cancer" in Freiburg, German Federal Republic, 1982, and gave presentations as an invited scientist throughout Japan, the People's Republic of China, Taiwan, the Philippines, and Hong Kong (in 1982 and 1987). He was elected to the Board of Directors and Editorial Board of the AOAC, 1979–1980; was President-Elect of the Association of Official Analytical Chemists International Organization, 1982–1983; and was honored by election as their Centennial President from 1983 to 1984. He developed an article, "Libraries of Instruments," to describe interdisciplinary research programs on strengthening research in American Universities.

Gehrke was founder, board member, and former Chairman of the Board of Directors (1968–1992) of the Analytical Biochemistry Laboratories, Inc., a private corporation, in Columbia, MO, of 300 scientists, engineers, biologists, and chemists specializing in chromatographic instrumentation, and addressing worldwide problems on environmental and pharmaceutical issues to the corporate sector. He was a member of the board of SPIRAL Corporation, Dijon, France.

Over 60 masters and doctoral students have received their advanced degrees in analytical biochemistry under his direction. In addition to his extensive contributions to amino acid analysis by gas chromatography, Gehrke and colleagues have pioneered in the development of sensitive, high-resolution, quantitative high-performance liquid chromatographic methods for over 100 major and modified nucleosides in RNA,

DNA, transfer RNA (tRNA), and messenger RNA (mRNA), and then applied these methods in collaborative research with scientists in molecular biology across the world (1970s–1990s). At the 1982 International Symposium on Cancer Markers, Freiburg, German Federal Republic, E. Borek, Professor of Biochemistry, Columbia University, stated that “Professor Gehrke’s chromatographic methods are being used successfully by more than half of the scientists in attendance at these meetings.”

His involvement in chromatography began in the early 1960s with investigations on improved gas chromatographic (GC) methods for fatty acid analysis. Gehrke is widely known for developing a comprehensive quantitative GC method for the analysis of amino acids in biological samples and ultramicroscale methods for life molecules in moon samples in the 1970s. This method was used and advanced in the analysis of lunar samples when he was co-investigator with NASA. In the 1970s, his major interests shifted toward the development of quantitative high-performance liquid chromatographic (HPLC) methods for the analysis of various important substances in biological samples, especially the modified nucleosides in tRNA as biomarkers in cancer research.

Major Research Contributions and Publications. Dr. Gehrke

- Developed eight methods adopted as official methods by AOAC International (formerly Association of Analytical Chemists), sampling Ca, Mg, K, P, and N.
- Was the first to develop and automate AOAC Official Chemical Methods for fertilizers (1950s–1980s).
- Was the first to discover quantitative GLC of total protein amino acids (1960s–1970s), 45 publications.
- Was the first to develop quantitative HPLC of total nucleosides in tRNA, mRNA, rRNAs, and DNAs (1970s–1990s), 31 publications.
- Was the first to use HPLC-MS nucleoside chromatography in molecular biology (1987–1994), 23 publications.
- Was the first to use GLC and HPLC methods for metabolites in body fluids as potential biological markers (1971–1994), 54 publications.
- Was the first to use GLC in analysis of Apollo 11, 12, and 14–17 moon samples at ultrahigh sensitivity levels (1969–1974), 10 publications.
- Was the first to propose a lunar/Mars-based analytical laboratory (1989–1990 and 1995).

Dr. Gehrke was the author, co-author, or editor of the following books:

- 1979—author of a chapter in L. S. Ettre and A. Zlatkis (Eds.), *75 Years of Chromatography—a Historical Dialogue*, pp. 75–86 (Elsevier Science Publishers BV, Amsterdam, The Netherlands).
- 1987—C. W. Gehrke (CWG), K. C. Kuo, and R. L. Zumwalt (Eds.), *Amino Acid Analysis by Gas Chromatography*, in three volumes (CRC Press, Boca Raton, FL), 19 chapters by 29 authors (5 chapters by CWG).

- 1990—C. W. Gehrke and K. Kuo (Authors/Eds.), *Chromatography and Modification of Nucleosides*, a three-volume, 1206-page treatise published by Elsevier in the *Journal of Chromatography Library Series* addressing the topics (1) analytical methods for major and modified nucleosides, (2) biochemical roles and function of modification, (3) modified nucleosides in cancer and normal metabolism, and (4) a comprehensive database of structural information on tRNAs and nucleosides by HPLC, GC, MS, NMR, UV, and FT-IR combined techniques.
- 1993—C. Ponnampuruma and C. W. Gehrke (Eds.), *Proceedings of the Ninth College Park Colloquium—A Lunar-Based Chemical Analysis Laboratory* (A. Deepak Publishing, Hampton, VA).
- 1997—C. W. Gehrke, Mitchell K. Hobish, Robert W. Zumwalt, Michel Prost, and Jean Degrés, *A Lunar-Based Analytical Laboratory*, C. Ponnampuruma memorial volume (A. Deepak Publishing, Hampton, VA).
- 1954–1995—nine additional chapters and reviews in other scientific journals and books.
- 260 research papers on analytical biochemistry and chromatography (1950–2000).

In 1989 and 1993, C. W. Gehrke and C. Ponnampuruma of the University of Maryland were named co-principal investigators on a proposal to address the scientific technical concerns of placing a chemical laboratory on the moon that would be automated, miniaturized, and computer robotic-operated and would support NASA programs in the study of five aspects of the exploration of space: (1) astronaut health, (2) closed-environment life support, (3) lunar resources, (4) exobiology, and (5) planetology.

Awards. Gehrke received the American Chemical Society National Award in Separations Science and Technology in 1999 and the American Chemical Society National Award in Chromatography in 2000.

His latest published book with Dr. Robert L. Wixom and Dr. Ernst Bayer, co-editors, was *Chromatography: A Century of Discovery (1900–2000)*, published by Elsevier Sciences, B.V., Vol. 64, in 2001 (709 pages). The present book on chromatography builds on that 2001 book.

Postscript. After 91 years of life and 62 years as a vigorous scientist, Dr. Gehrke passed away on February 10, 2009. Some recent comments heard on his character are: “A great man has fallen, but left many seeds for his students and colleagues who will carry on,” “enthusiastic supporter of life sciences,” “never took shortcuts,” “dedicated teacher,” “an entrepreneur who was always moving on,” and “a devoted husband, father, and grandfather” (addition by Robert L. Wixom, co-editor).



Robert L. Wixom, co-editor of this book, was born on July 6, 1924 in Philadelphia. In 1947, he graduated with a B.Sc. in Chemistry from Earlham College, Richmond, IN. His graduate studies and thesis were at the University of Illinois under the guidance of Professor William C. Rose, and he received his Ph.D. in Biochemistry in 1952.

Wixom held teaching/research faculty appointments in the Department of Biochemistry, School of Medicine, University of Arkansas (1952–1964) and the Department of Biochemistry, School of Medicine/College of Agriculture, UMC (1964–1992). He took year-long sabbatical/research leaves at Oxford University (1961–1962), the University of Wisconsin, Madison, WI (1970–1971), the Massachusetts Institute of Technology (MIT), Cambridge, MA (1978–1979), and the Fox Chase Institute for Cancer Research, Philadelphia (1985–1986). His 40 years of research (45 peer-reviewed papers, two reviews) and graduate teaching focused mainly on amino acid and protein metabolism. He taught intermediate and advanced biochemistry to medical students, graduate students in diverse departments, and undergraduate students with a variety of majors. Wixom guided the Advanced Biochemistry Laboratory course at UMC for 20 years, which covered several experiments in chromatography, and for 15 years taught a course on biochemical information retrieval. He has received three teaching awards. He served as a Departmental Representative to the Graduate Faculty Senate (1980–1993) and its Chair (1989–1992); this included a key role in three major new university programs. He officially retired in 1992 as Professor Emeritus of Biochemistry, but continued many similar activities.

Reflecting other earlier interests, Wixom was the co-initiator of the UMC Environmental Affairs Council and served as their first chair for 3 years (1991–1994). He initiated and served as senior editor of the 1996 book, *Environmental Challenges for Higher Education: Integration of Sustainability into Academic Programs*. The preceding experiences served as the educational background for his role as co-editor of the 2001 book, *Chromatography: A Century of Discovery (1900–2000)—The Bridge to the Sciences/Technology* and now its sequel, *Chromatography: A Science of Discovery*, John Wiley & Sons, Inc., 2010.

Postscript. After celebrating 85 years of life, much of it as a teacher and seeker of knowledge in life as well as in the laboratory, Dr. Wixom passed away on July 8, 2009. “Distinguished as a scientist, educator and outdoorsman,” “energetic,” “courageous,” “passionate,” “dedicated to service,” and “persistent,” were among the many comments about this “always the teacher,” “family man,” Bob Wixom (addition by Deborah L. Chance and Thomas P. Mawhinney, associate editors and University colleagues of the editors).

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