Analytical Gas Chromatography

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ACADEMIC PRESS, INC.
Harcourt Brace Jovanovich, Publishers
Orlando San Diego New York Austin
Boston London Sydney Tokyo Toronto

ACADEMIC PRESS, INC.
Orlando, Florida 32887

United Kingdom Edition published by
ACADEMIC PRESS INC. (LONDON) LTD.
24–28 Oval Road, London NW1 7DX

Library of Congress Cataloging in Publication Data

Jennings, Walter
Analytical gas chromatography.

Includes index.
1. Gas chromatography. 2. Capillarity. I. Title.
QD79.C45J458 1987 543'.0896 86-28873
ISBN 0–12–384355–3 (alk. paper)

PRINTED IN THE UNITED STATES OF AMERICA

87 88 89 90 9 8 7 6 5 4 3 2 1

PREFACE

This book is intended as a free-standing "introduction to and guide through" the rapidly developing field of analytical gas chromatography. It began as a third revised edition of "Gas Chromatography with Glass Capillary Columns," but it soon became evident that a separate offering would be preferable. The availability of a wide range of fused silica columns with different dimensional characteristics and coated with a variety of stationary phases has catalyzed changes in every aspect of the analytical process, from preparation and introduction of the sample to chromatographic separation of the solutes to their detection. These developments make an entirely fresh approach desirable, but it seems unnecessary to reproduce a detailed iteration of subjects whose coverage in the second edition of the earlier title remains adequate and current.

When beginning the composition of an instructional text, an author is immediately faced with a critical decision relative to the level of presentation; the text can be designed as a research tool, as a laboratory aid, or as something intermediate. My decision was influenced by the following factors:

Most of my life has been spent as a university professor. I was introduced to gas chromatography in 1954 by Dr. Keene Dimmick of the USDA laboratory in Albany, California. Gas chromatography soon became my major research interest. Professor Kurt Grob in Zurich gave me my first glass capillary column in 1965, with advice on its construction and use; my research activities soon turned in this new direction.

A few visionaries at Hewlett Packard recognized the potential of capillary gas chromatography in the early 1970s, and I was invited to participate in several of

their "basic" gas chromatography courses, to consult on certain aspects of instrumental design, and eventually to instruct a series of special short courses on capillary gas chromatography throughout the United States and Canada. The latter arrangement continued for several years, concurrent with and followed by the instruction of capillary courses sponsored by other instrument companies and by chromatography discussion groups. These activities expanded with a number of invitations to instruct in-house courses for specific industries.

During this period, one of my completing doctoral students suggested the manufacture of glass capillary columns as a commercial business. My contributions in the early stages of this endeavor were largely in the form of teaching courses, handling technical inquiries, and troubleshooting.

Hence, the approach taken in this offering has been shaped by (1) more than thirty years experience in university teaching and research in gas chromatography; (2) many hours spent in answering telephoned and written queries on all aspects of gas chromatographic analysis; and (3) the instruction of between 350 and 400 intensive short courses on this subject, to a total of some 12,000 practitioners, at points all over the world.

These activities would leave almost anyone convinced that communication between most of those practicing gas chromatography on the analytical firing line and many of our leading chromatographic scientists leaves much to be desired. This may relate to the immense numbers of users and the diversity of their backgrounds and interests. The vast majority of practicing chromatographers has had little or no formal training in the subject. A large number of these users employ the method as a means to an end, and the technique is so powerful that useful data can be generated even by untrained people misusing poorly designed equipment. To extend the utility of this book to the largest possible number of users, I have deliberately stressed simplicity, especially in explanations. While there are benefits to many readers, there is also a definite hazard to this approach: Albert Einstein once observed that

> "Everything should be made as simple as possible. . .
> but not simpler."

I hope I have succeeded in not overstepping that line.

Finally, a good book should be "readable"; it should flow smoothly, logically, and bestow on the reader that satisfying feeling that comes only as he or she gains a better understanding of a technical subject. I sincerely hope that this offering succeeds, not only in the attainment of the above goals, but most of all, in increasing the proportion of analysts who do "good" chromatography.

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INTRODUCTION

1.1 General Considerations

Toward the end of the nineteenth century, the Russian scientist Mikhail Tswett demonstrated the separation of plant pigment mixtures into colored zones by percolating extracts through a column packed with an adsorbant. In the early 1900s, he used the word "chromatography" to describe this system of "color graphics" [1,2]. The word "chromatography" is now used as a generic term for separation processes that subject the substances to be separated to differential partitioning between two phases; in most cases, one phase is stationary and the other is mobile.

A. J. P. Martin and R. L. M. Synge later explored liquid–solid chromatography in great detail and were both eventually awarded Nobel Prizes for their efforts. It was in his award address that Martin first suggested that it should also be possible to employ a gas as the mobile phase in chromatographic processes. It is doubtful that he or anyone else, either at that time or some years later when James and Martin [3] demonstrated the first gas chromatographic separations, envisioned the degree to which this technique would come to dominate our analytical procedures.

Ray suggested combining thermal conductivity detection with gas chromatography, and in 1954 [4] he published schematics and chromatograms that stimulated a number of workers, including this author, to enter what promised to be a new and exciting field. Subsequent progress has been due to the individual contributions of some hundreds of scientists. Many of these myriad contributions have been detailed elsewhere (e.g., [5,6]), but three milestone achievements

must be mentioned before proceeding to a discussion of modern analytical gas chromatography: Golay's invention of the open tubular column [7], Desty's glass-drawing machine [8], and the thin-walled fused silica column (Dandeneau and Zerrener [9]).

When a gas is employed as the mobile phase, either a liquid or a solid can be utilized as the stationary phase; these processes are most precisely termed "gas–liquid partition chromatography" and "gas–solid partition chromatography," respectively. Although the latter sees some applications in fixed gas analysis (see later chapters), the former is employed in the separation of most organic compounds and will receive most attention here. Through popular usage, the process is now more generally termed simply "gas chromatography" and is often abbreviated to "GC."

1.2 A Simplistic Approach

In the process of gas chromatography, the stationary phase is usually in the form of a thin film and is confined to an elongated tube, the column. The two extremes in column types are packed columns and open tubular columns. In the former, the thin film of stationary phase is distributed over an "inert" granular support, while in the latter the stationary phase is supported as a thin coating on the inner surface of the column wall (wall-coated open tubular columns). Popular usage soon equated the terms "open tubular" and "capillary" in gas chromatography. Some authorities decried this trend and recalled Golay's observation that it was "the openess of the column, not its capillary dimensions" that gave it special properties. Recently, however, larger-diameter open tubular columns have reappeared and in their new state show great promise of finally replacing the packed column. These large-diameter open tubular columns are not true "capillaries" and are incapable of producing what has come to be regarded as "capillary chromatography." It has now become necessary to differentiate types of open tubular columns: in this book the use of the word "capillaries" will be restricted to open tubular columns whose inner diameters are less than 0.35 mm.

Be it packed or open tubular, the column, which normally begins at the inlet of the gas chromatograph and terminates at its detector, is adjusted to some suitable temperature and continuously swept with the mobile gas phase (carrier gas). When a mixture of volatile components is placed on the inlet end of the column, individual molecules of each of the solutes in that sample are swept toward the detector whenever they enter the moving stream of carrier gas. The proportion of each molecular species that is in the mobile phase at any given time is a function of the vapor pressure of each solute: The molecules of the components that exhibit higher vapor pressures remain largely in the mobile phase; they are swept toward the detector more rapidly and are the first solutes eluted from the column.

Other solutes exhibit lower vapor pressures, either because they are higher-boiling or because they engage in interactions with the stationary phase that effectively reduce their vapor pressures. Individual molecules of these solutes venture into the mobile phase (carrier gas) less frequently, their concentrations in the mobile phase are lower, and they require longer periods of time to reach the detector; hence separation is achieved.

Readers who are just beginning their comprehension of this powerful technique may find it helpful to (incorrectly) visualize gas chromatography as a stepwise process and to begin by considering the separation of a simple two-component mixture containing, e.g., acetone (bp 57°C) and ether (bp 37°C). If a small amount of that mixture is introduced into a chromatographic column which is continuously swept with carrier gas and held at a temperature where each solute exhibits a suitable vapor pressure, both solutes will immediately partition between the moving gas phase and the immobile stationary phase. All other things being equal, the molecules of the lower-boiling ether that are dissolved in stationary phase will vaporize before (or more frequently than) the molecules of the higher-boiling acetone [10]. As they enter the mobile gas phase they are carried down the column and over virgin stationary phase, where they redissolve. A fraction of a second before the acetone molecules revaporize to be carried downstream again by the carrier gas, the ether molecules move again; hence the more volatile ether molecules continuously increase their lead over the less volatile acetone molecules, and separation is achieved.

Although this concept may prove helpful in visualizing that a multiplicity of vaporizations and re-solutions on the part of the individual solute molecules is one factor influencing the degree of separation efficiency, it must be stressed that the oversimplification has resulted in an inaccurate picture. For one thing, the chromatographic process is continuous and highly dynamic rather than being a series of discrete steps. At any point in time, some of the molecules of each solute are in the stationary phase and others are in the mobile phase; as the mobile phase moves over virgin stationary phase, some of the mobile-phase-entrained solute molecules dissolve in the stationary phase, while immediately behind the moving front an equivalent number of dissolved solute molecules vaporize into the mobile phase. Because ether and acetone exhibit different vapor pressures, the ratio

$$\text{molecules in mobile phase}/\text{molecules in stationary phase}$$

will be larger for the more volatile ether than for the less volatile acetone. Hence, ether will move through the column more rapidly, and at the conclusion of the process a "plug" of ether molecules dispersed in mobile phase (carrier gas) will emerge to the detector, followed by a second "plug" of acetone.

The above concepts are also helpful in emphasizing that the vapor pressure of the solute strongly influences its chromatographic behavior; solutes undergo no

separation in the mobile phase, nor do they undergo separation in the stationary phase. Solute separation is dependent on the differences in solute volatilities, which influence the rates (or frequencies) of solute vaporizations and re-solutions; this differentiates solute concentrations in the stationary and mobile phases. Hence it is desirable to subject solutes to as many "vaporization steps" as possible (without having other adverse effects, *vide infra*), and this will require that they undergo an equal number of "re-solution steps." If the vapor pressures of the solutes are too high, they spend most (or all) of their transit time in the mobile phase and little (or no) separation is achieved; if the vapor pressures are too low, the solutes spend too long in the stationary phase, analysis times become disproportionately long, and sensitivity is also adversely affected (*vide infra*). Column temperature is one obvious method of influencing solute vapor pressures; another is through the choice of stationary phase. A "polar" stationary phase reduces the vapor pressures of polar solutes by means of (additional) solute–stationary phase interactions that may include hydrogen bonding and/or dipole–dipole interactions. These interrelationships are discussed in greater detail in Chapter 4.

1.3 Simplistic Comparisons of Packed and Open Tubular Columns

Most chromatographers recognize that the open tubular (or "capillary") column is capable of separations that are vastly superior to those obtained on packed columns [11]. Figure 1.1 illustrates separations of an essential oil on a packed column and on two types of open tubular column [12]. Instructional difficulties are sometimes experienced with those few chromatographers who prefer to deal with the packed column chromatogram and view the improved separation with misgivings. The qualitative and quantitative futility of that viewpoint can be emphasized by pointing out that the chromatogram can be made even simpler by omitting the stationary phase; all components will then emerge as a single peak.

On the other hand, there are occasions where the required degree of separation can be obtained on a packed column and separation on an optimized open tubular column results in more resolution than is required ("overkill") at the expense of longer analysis times. In situations of this type, some of the superior resolving power of the open tubular column can be traded off to yield equivalent (or even improved) separation in a fraction of the packed column analysis time, at higher sensitivities, and in a much more inert system (quantitative reliability is improved). As compared to packed columns and packed column analyses, the open tubular column can also confer distinct cost advantages [13].

The above points are illustrated in Fig. 1.2 [14], where the short capillary column delivers separation "equivalent" to that obtained in the much longer packed column analysis. Actually, the capillary resolution is superior; integrated values from the packed column analysis will include appreciable solvent; the

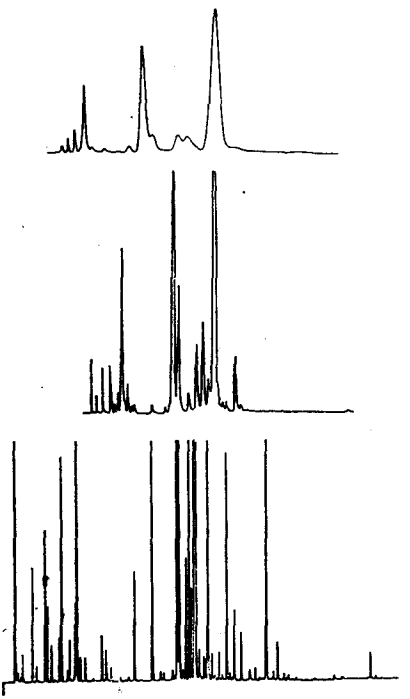

Fig. 1.1. Chromatograms of a peppermint oil on (top) a 6 ft × ¼ in. packed column, (center) a 500 ft × 0.03 in. stainless steel open tubular column, and (bottom) a 30 m × 250 μm fused silica open tubular (capillary) column. (Adapted from [12], p. 455, and reprinted by courtesy of Marcel Dekker, Inc.)

solute peaks are well removed from the solvent in the capillary analysis, and quant. ation will be enhanced.

Returning to Fig. 1.1, the striking difference between the two sets of chromatographic results illustrated is best attributed to inequalities in the degree of randomness exhibited by the identical molecules of each individual solute. All of the identical molecules of each solute exhibit a narrow range of retention times in the bottom chromatogram, but as the ranges of retention times become greater, neighboring peaks exhibit overlap and resolution suffers. These behavioral differences between identical molecules can be attributed to three factors [10]:

1. The packed column offers solute molecules a multiplicity of flow paths, some short, the majority of average length, and some long. Hence identical molecules of each given solute would be expected to spend disparate times in the mobile phase. The open tubular column, on the other hand, has a single flow

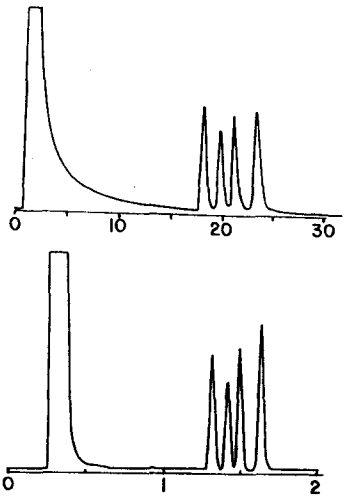

Fig. 1.2. Routine separations of methyl benzoates on (top) a packed column (analysis time 25 min) and (bottom) a 1.7 m × 0.25 mm glass open tubular column.

path, and molecules would be expected to exhibit mobile phase residence times that were much more nearly identical.

2. A similar rationale can be drawn for the randomness of stationary phase residence times. There is much more stationary phase in the packed column, and the film thickness is nonuniform. Thicker regions of stationary phase would be expected to occur in particle crevices and where two or more coated particles come into contact. A solute molecule dissolving in a thinner region of stationary phase would become dispersed and then reemerge to the moving gas phase in a relatively short period; an identical molecule, dissolving in a thicker patch of stationary phase, would take a longer time to reemerge; the times that identical molecules of a given solute spent in stationary phase would be quite diverse. In the open tubular column, the stationary phase is in a thinner and much more uniform film; the ranges of times that identical molecules spent in stationary phase would be expected to be much narrower.

3. It was previously mentioned that solute volatilities (i.e., solute vapor pressures) constitute an important variable in gas chromatography. The vapor pressure of a solute is an exponential function of the absolute temperature; a minor shift in temperature can have a major effect on vapor pressure. At our present state of instrumental development, solute temperatures are controlled via the column oven: the oven air conveys heat to the column wall, the column wall conducts heat to the particle of solid support in contact with it, this conducts heat to the stationary phase with which it is coated and to the next particle of solid

support, etc. Packed column support materials, however, are poor heat conductors. A temperature range must exist across any transverse section of the packed column; the range will be greater for larger-diameter columns and for higher program rates, but even in an isothermal mode a temperature range will exist across the column. Molecules whose flow path is in the center of that column will be at a lower temperature and exhibit lower vapor pressures and larger values of k (*vide infra*) than identical molecules whose flow paths are closer to the column wall. The fact that their flow paths undoubtedly switch back and forth does not compensate for this variation; it is one more factor causing identical molecules to exhibit a broadened range of retention times. In the fused silica column, the stationary phase exists as a thin film deposited directly on the inner wall of a tube of very low thermal mass; there should be no temperature variation across any transverse section of the column, provided that the column is heated only by convection. The latter point is an important distinction between the oven requirements for packed and capillary columns and is considered again in Chapter 7.

Our goal in chromatography can now be better defined. Gas chromatography should be performed under conditions where (1) solute molecules undergo many transitions (vaporizations and re-solutions), (2) identical molecules of each solute exhibit the narrowest possible range of retention times (i.e., the chromatographing band formed by each molecular species is short, hence the standard deviation of the resultant peak is small), and (3) the separation achieved is maintained to the highest degree possible.

1.4 Abbreviated Theory of the Chromatographic Process

The primary objective of this book is to introduce practical considerations involved in gas chromatography, including the selection, installation, evaluation, and use of open tubular glass capillary columns. Some knowledge of gas chromatographic theory is essential to the attainment of this goal, but this section is intended as neither a comprehensive nor a rigorous treatment of chromatographic theory. Theoretical considerations have been well covered elsewhere (e.g., [5,15–19]). In an attempt to avoid contributing further to the confusion caused by a variety of nonuniform "systems" of nomenclature, the symbols and nomenclature used throughout this discussion are based largely on those suggested by the International Union of Pure and Applied Chemistry (IUPAC) [20] and the American Society for Testing and Materials (ASTM) [21] and are detailed in the Appendix.

A compound subjected to the gas chromatographic process (a "solute") is, on injection into the column, immediately partitioned between the mobile phase and the stationary phase. Its apportionment between the two phases is reflected by the

distribution constant K_D, defined as the ratio of the weights of solute in equal volumes of the stationary and mobile phases:

$$K_D = \frac{\text{concentration per unit volume stationary phase}}{\text{concentration per unit volume mobile phase}} = \frac{c_S}{c_M} \quad (1.1)$$

K_D is a true equilibrium constant, and its magnitude is governed only by the compound, by the stationary phase, and by the temperature. Polar solutes would be expected to dissolve in, disperse through, and form intermolecular attractions with polar phases to a much greater degree than would hydrocarbon solutes exposed to the same stationary phase. Logically, the K_D of a polar solute in a polar phase is higher than the K_D of the hydrocarbon of corresponding chain length in the same polar phase. As the temperature of the column is increased, both types of solute exhibit higher vapor pressures and their K_D values (c_S/c_M ratios) decrease, although (in this polar stationary phase) those of the polar solute remain larger than those of the hydrocarbon. Among the members of a homologous series, of course, higher homologs have lower vapor pressures and higher K_D values.

During its passage through the column, a solute spends a fractional part of its total transit time in the stationary liquid phase and the remainder in the mobile gas phase. The mobile phase residence time can be determined by direct measurement. Whenever a solute emerges to the mobile phase, it is transported toward the detector at the same rate as the mobile phase; hence everything must spend the same length of time in the mobile phase. This mobile phase residence time can therefore be determined by timing the elution of a solute that never enters the stationary phase, but spends all its time in the mobile phase; ideally, this could be determined by timing the transit period of an injection of mobile phase (carrier gas), but detection would be impractical. Methane is normally used for this purpose, and although it is now recognized that methane does have a discrete stationary phase residence time, it is assumed that this is minuscule and can be ignored in columns of "standard" stationary phase film thickness and at reasonable column temperatures.

The column residence time for methane is assigned the symbol t_M, and, as discussed earlier, a solute in the mobile phase is transported toward the detector at the same velocity as the mobile phase; hence everything spends time t_M in mobile phase. This is the "gas holdup volume" (or "gas holdup time") of the system. The total retention time is equal to the mobile phase residence time t_M plus the stationary phase residence time. It therefore follows that the stationary phase residence time or the "adjusted retention time" t_R' is

$$t_R - t_M = t_R' \quad (1.2)$$

Ideally, solute bands will be introduced into the column in such a way that they occupy a very short length of the column (see injection mechanisms in Chapter