Practical Liquid Chromatography

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Preface

This book is intended to provide a practical introduction to high-speed, high-efficiency liquid chromatography. It covers modern column technology (which has leapt into prominence only in the last five years) and relates this to the well-established thin-layer techniques.

The development of liquid chromatography has proceeded in fits and starts over many years and in alliance with various scientific disciplines. Liquid chromatography has for years fulfilled an effective role in various fields. Ion-exchange chromatography, for example, is particularly associated with the separation of the rare earths, and exclusion chromatography with the fractionation of naturally occurring materials like proteins and of synthetic polymers. Partition chromatography, especially in the form of paper chromatography, has been an indispensable tool in the study of biochemical systems, while its more recent adsorption counterpart, thin-layer chromatography, developed most rapidly within the pharmacognosic and pharmaceutical fields. Until recently, however, liquid chromatography has not played a prominent role in the field of industrial organic analysis.

Recent books have provided theoretical background to the mechanism and technique of liquid chromatography, but we felt there was a need for a practical book suitable for use at or near the bench of analysts in the organic chemical industry. To fulfill this need this book was written. We present no new theories but relate the theoretical conclusions of others to the practical needs for procuring effective separations of lipophilic substances in organic media. We consider, with a practical emphasis, the mechanics of adsorption, partition, and exclusion chromatography and describe the techniques of thin-layer and high-performance column chromatography based largely on our own experiences. We hope this book will help organic chemists to solve their separation problems, and encourage the further development of high-

efficiency liquid chromatography to share the same uniquely successful role presently enjoyed by gas chromatography.

This is not intended to be a comprehensive reference book. In fact, references have been cut to a minimum. Together, however, with a select range of further reading the reader is directed to most of the significant publications which have so far appeared.

While every endeavor has been made to make lists of manufacturers comprehensive, it is inevitable, especially in the presently rapidly expanding field of high-performance column chromatography, that they will rapidly become obsolete. We apologize for any omissions.

We would like to thank Professor A. I. M. Keulemans, who has done us the honor of writing the Foreword to this book. He it was who wrote the first widely read book on gas chromatography, which undoubtedly did much to stimulate interest and work in the field. We will feel our efforts have been worthwhile if this text provides an even remotely comparable stimulus for liquid chromatography. We are also greatly indebted to various authors and publishers for their permission to reproduce certain tables and figures, which are identified at the appropriate stages throughout the book.

Finally, we would like to thank the management of the Esso Research Centre, Abingdon, for their active encouragement to write this book and for providing facilities for preparation of the manuscript.

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Abingdon, September, 1971

Foreword

A Dutch proverb says "Goede wijn behoeft geen krans" and the nearest English translation may be: "Good wine needs no recommendation." It gives me great pleasure to write a foreword to a book written by Dr. Perry and his colleagues. On the one hand he belongs to the youngest "old hands" from the time of the explosive development in gas chromatography; on the other hand he has played a pioneering role in the recent renaissance of liquid chromatography. Mr. Amos has played a big part in popularizing use of thin-layer techniques in the analysis of petroleum products, and Mr. Brewer is the author of one of the first papers on the use of exclusion chromatography for separation of synthetic polymers. The authors do not shy from theory but, being employed in an industrial laboratory, they are first of all practical men. Not only are the authors well known but through their many contacts personally know the older and the present generation of publishing chromatographers. I have had the privilege to have Dr. Perry working for one year as a post doctoral research chemist at our University Laboratories. I was, therefore, in the position to learn to appreciate his many outstanding abilities. Not the least of his abilities are his balanced judgment and his eloquence. The present book clearly shows these abilities. It deals with theory only where this is necessary. The great potentialities but also the limitations of the chromatographic techniques labeled under the collective name "Liquid Chromatography" are treated in a most objective way. The book gives, what is badly needed, a practical guide for present and prospective workers in the fascinating field of liquid chromatography. Of course a feature of the book is its bibliography. The present possibilities offered by computers greatly facilitate the preparation of complete bibliographies, but here the authors have made a critical selection. The book does not need a foreword to find its way to the desks of the practicing chromatographers where it soon will show

itself an indispensable guide. To me this foreword means an open confirmation of cordial friendship between past and future, between two generations of enthusiastic chromatographers.

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Chapter 1

Liquid Chromatography—The Background

1.1. PERSPECTIVE

Liquid chromatography has an impressive history, stretching back into the 19th century, which has been well documented elsewhere(1). As an indispensable technique for the separation and analysis of organic substances it has made enormous strides in the past decade or so. Prior to 1958 column chromatography was conducted with comparatively large samples separated in, often short, almost invariably wide-bore, columns through which the developing liquid percolated under gravity. Thin-layer techniques were unknown outside a small group of workers(2), although paper chromatography was widely practiced.

Since 1958, thin-layer chromatography has emerged as the simplest, lowest cost, and comparatively most efficient method available for the separation of the less volatile components of organic mixtures. Many laboratories make extensive use of it for research and quality control in the whole field of industrial (and academic) organic chemicals. More recently the techniques of column chromatography have been radically rethought, to the point where they are now directly comparable in terms of ease, efficiency, and speed with those of gas chromatography.

With this book we seek to provide an adequate and up-to-date account of the theory and practice of liquid chromatography as applied to the analysis of mixtures of organic substances. The emphasis throughout is on the practical aspects of the subject but with the basic elements of theory included so that the reader can appreciate the reasons underlying recommended practice.

1.2. PLAN OF THE BOOK

Liquid chromatography is an enormous subject which, furthermore, is passing through a phase of rapid progress. Therefore we have sought to concentrate on those aspects of particular interest to practicing organic analytical chemists in industry. We have also attempted to provide an understanding of the framework of the techniques so that this book will not rapidly become out-of-date, notwithstanding the rapid advancement of liquid chromatography at the time of writing.

In the remainder of this chapter we summarize the variants of liquid chromatography and define or explain some of the more important terms encountered in the literature and the laboratory.

Chapter 2 seeks to provide an adequate explanation of the dynamic processes which occur in a bed of stationary phase through which a liquid is flowing. These processes ultimately limit the degree of separation that can be achieved. They cause "band spreading" in all chromatographic processes. The theories which have been evolved to relate operating parameters to band spreading are outlined and their practical consequences emphasized.

Each of three succeeding chapters then considers the essentials of major subdivisions of liquid chromatography, namely adsorption, partition, and exclusion. In each chapter an explanation is given of the mechanism of selectivity and the separation achievable with the particular system concerned. The materials, especially the stationary phases, are discussed in depth—as always with the important practical points emphasized.

The next two chapters deal in turn with the apparatus and techniques of, first, thin-layer chromatography and, then, column chromatography.

A final brief chapter seeks to put into perspective the current status of liquid chromatography.

The book is intended as a practical guide, and for this reason we have limited the references cited to a relatively small number and we have backed these up with lists of articles suggested for further reading. In general we have sought to avoid quoting historical data and the derivation of equations from theory, as these topics are already very adequately treated in the literature. Also we have almost completely avoided referring to specific separations; an abstract service,* journals,† and books[1-3] provide information of this type. In short this book seeks to explain the "what, why, and how" of the techniques and materials used in modern liquid chromatography;

*_The Journal of Chromatography_ regularly publishes bibliographies covering column and thin-layer chromatography.

†Journals regularly publishing papers on liquid chromatography and its application include: _Analytical Chemistry; The Analyst; The Journal of Chromatographic Science; The Journal of Chromatography_.

paper chromatography and ion-exchange chromatography (in partly or wholly aqueous media) are not considered in this book.

1.3. CHROMATOGRAPHY AND ITS BASIC VARIANTS

Chromatography is a process whereby different types of molecules are separated one from another. A sample mixture is introduced onto a bed of *stationary phase* and swept through it by a fluid at a rate dependent on the mutual interactions of sample components with the stationary phase and the fluid (*mobile phase*). Generally these mutual interactions differ in magnitude for the different sample components so that their rate of passage through the stationary phase bed differs and separation is thus achieved.

When the fluid mobile phase is a liquid the process is termed *liquid chromatography* and it is this subject with which the present book is concerned. Other types of fluid which are used are gases (gas chromatography) and substances above their critical points (supercritical chromatography).

The mobile phase introduced at the front end of the stationary phase bed is often referred to as the *eluent* and the eluent plus solutes leaving the end of the bed is sometimes termed the *eluate*.

Stationary phases promote separation of molecules if they possess one, or more, of four basic functional characteristics: (i) the power physically to sorb solutes from solution; (ii) the power chemically to sorb solutes from solution; (iii) the ability to dissolve solutes when contacted with solutions in an immiscible solvent; (iv) a porous structure which can retain some, and reject other, solutes on the basis of solute size or shape.

Each of these characteristics is the basis for a widely recognized variant of liquid chromatography and each will now be described in outline.

1.3.1. Adsorption Chromatography

Adsorption chromatography (also referred to as liquid–solid chromatography, LSAC, and LSC) is based primarily on differences in the relative affinity of compounds for the solid adsorbent used as stationary phase. Affinity is determined almost entirely by polar interactions. This means that polar groupings in the molecules to be separated exert a much greater effect than nonpolar hydrocarbon chains and adsorption chromatography therefore tends to separate mixtures into classes characterized by the number and type of polar groups.

1.3.2. Ion-Exchange Chromatography

Ion-exchange chromatography is based on the differing affinity of ions in solution for sites of opposite polarity in the stationary phase. It is a process

largely confined to media of high dielectric constant, in which ionic species are stable. Most ion-exchange separations are carried out in essentially aqueous media and the principal fields of application are in inorganic chemistry. Some work has been carried out in mixed aqueous organic solvents(4), but in this book discussion of the application of ion-exchanging stationary phases is confined to situations in which they are effectively acting as adsorbents possessing specific functional affinities.

1.3.3. Partition Chromatography

Partition chromatography (liquid–liquid chromatography, LLC) is based on the relative solubility (or, better, distribution) characteristics of solutes between the mobile phase and a liquid (solvent) phase held stationary by impregnation on a porous, inert *support*. Most commonly the stationary phase is more polar than the mobile phase; in some circumstances, however, it is advantageous to reverse the roles so that the stationary phase is less polar. This variation is known as *reversed-phase partition chromatography*.

In principle LLC offers a wide range of selectivity effects as the relative nature of the two liquid phases is varied. In practice, as we will see, the choice of liquid phases which can be used is restricted. Actual separations essentially depend on the balance between polar and apolar groups in the solutes and the two liquid phases. Thus separations by compound type or chain length can be obtained.

1.3.4. Exclusion Chromatography

Exclusion chromatography, which is also known as gel permeation (GPC), gel filtration, or molecular sieving, is based on the ability of controlled-porosity materials to sort and separate sample mixtures according to the size and shape of the components of the sample. It has developed along two parallel lines with surprisingly little cross-fertilization. One line has been the application of hydrophilic polymers such as cross-linked dextrans to the separation of biological materials in primarily aqueous media. The other has been the use of synthetic materials, polymers, or inorganic porous substances for the separation of industrial organic chemicals, especially plastics and polymers. These latter separations are almost invariably performed in lipophilic solvents and in this book attention will be concentrated on them.

Actual separations may involve a combination of the above basic mechanisms; for example, in liquid–solid chromatography adsorption can take place physically, e.g., by dipole–dipole interaction, or chemically, e.g., by ion exchange or complex formation. In addition, the solid can sorb a portion of the mobile phase, especially if this contains water or some other

polar solvent, to form an organized stationary liquid into which solutes can partition.

1.4. DEVELOPMENT METHODS IN CHROMATOGRAPHY

The process by which solutes are carried through the stationary phase by the mobile phase is called *development*. One should be careful to avoid its confusion with the act of spraying thin-layer chromatograms with reagents which form colored derivatives and so reveal the presence of separated components. This latter process is often referred to as "development" (of colored spots); it is better to refer to it as *visualization* or *revealing*.

Chromatograms can be developed by three different methods, *elution, frontal analysis, and displacement*. Of these methods elution is by far the most widely used. In fact frontal analysis is never used as a practical analytical method. Displacement techniques are occasionally used; in particular a very important and widely used method of hydrocarbon type analysis, standardized by the American Society for Testing Materials and the Institute of Petroleum, is based on displacement development. The following sections describe the three methods briefly.

1.4.1. Elution

Consider a mixture of solutes A and B, placed initially at one end of a bed of stationary phase, and suppose that B is more strongly retained than A. If a mobile phase, less strongly retained than either A or B, is caused to pass through the bed, then it will wash A and B through at different speeds according to the degree of retention of the two solutes. If the difference in migration rates is sufficiently great, then A and B, which are initially superimposed, will gradually separate to form two distinct zones with pure eluent separating them, A moving ahead of B. If the sorption isotherms are linear (see Chapter 3) then a plot of concentration of the solutes along the length of the bed will appear as in Fig. 1.1, each solute having a Gaussian concentration profile.

1.4.2. Frontal Analysis

In this case, the mixture is fed continuously into one end of the stationary phase bed and caused to flow toward the other end. Again B is more strongly retained than A so that the solute front will become depleted in B and eventually pure A will emerge at the other end. Meantime the bed will become saturated with B and it too will then be washed forward along with A so that the mixture will flow through the bed with its original composition unchanged. Thus, by this method of separation, it is only possible to recover a pure sample of component A.