Introduction to

MODERN LIQUID CHROMATOGRAPHY

L. R. Snyder and

J. J. Kirkland

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L. R. SNYDER

Technicon Instruments Corporation
Tarrytown, New York

J. J. KIRKLAND

E. I. DuPont de Nemours & Company
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PREFACE

This book is about modern liquid chromatography. By this we mean automated, high-pressure liquid chromatography in columns, with a capability for the high-resolution separation of a wide range of sample types, within times of a few minutes to perhaps an hour. Modern liquid chromatography (LC) is now about five years old. By early 1969 it was possible to purchase equipment and high-performance column packings which together largely bridged the gap between classical liquid chromatography and gas chromatography. Since that time there has been a flurry of activity on the part of companies that supply equipment and materials for LC. Within the past few years there have been further major advances in the theory and practice of LC. Finally, numerous applications of modern LC to a wide range of problems are now being reported. The technique has reached the point where the average chromatographer can achieve---by yesterday's standards--consistently spectacular results.

To get the most out of modern LC, some care is required in choosing the right technique, selecting the best separation conditions, and using the proper equipment to best advantage. In short, the practical worker must know what he is doing. Moreover, his knowledge must be a balance of theory and experience; it must

include both principles and practice. Unfortunately, there has been a tendency for those in chromatography to stress either the theoretical or the "practical" side of the subject. Also, the theory of chromatography--and of modern LC--has often been represented as highly complex, with its application to real separation problems either not obvious, or impossibly tedious. We think there is a better approach.

An effective presentation of what modern LC is all about must be a blend of practical details plus down-to-earth theory. This conviction led us in 1971 to develop the American Chemical Society short course "Modern Liquid Chromatography." Within the next two years we presented the course to about 800 students, with a highly enthusiastic response. The course itself continued to evolve during this time, largely in response to the questions and comments of the students. By late 1972 it appeared worthwhile to reduce our approach to textbook form, and the present book is the result.

Our goal for this book was to retain the essential elements of the short course, and to add certain materials that could not be included in a two-day series of lectures. We did not hope to present everything of conceivable interest to LC in a book of this size, yet we were determined not to slight any area of significant practical importance. Where compromise between these two objectives eventually proved necessary, it has been handled by referencing other sources. The book was written to be self-sufficient in terms of the needs of the average professional or technician who plans to work with modern LC. We believe this book will prove useful in most laboratories where modern LC is practiced.

In conclusion, we would like to express our appreciation to numerous professional associates who have shaped our thinking as set down in the present book, to the hundreds of students who took our course--and

helped make it better for the next class, and to the staff of the Continuing Education Department of the American Chemical Society for their help with and sponsorship of the short course. We are especially grateful to the special contributors, Dr. J. J. DeStefano of the Biochemicals Department, E. I. du Pont de Nemours & Co., and Mr. H. J. Adler of the Research Department, Technicon Instrument Corporation, who wrote material in areas of their expertise to fill in gaps in the original ACS short course. We are also grateful to several of our friends for agreeing to review the initial manuscript, and who much improved it: Dr. D. L. Saunders of the Union Oil Company, Dr. H. M. McNair and Mr. Daniel Marsh of the Virginia Polytechnic Institute, Dr. B. L. Karger of Northeastern University, Dr. C. Horvath of Yale University, and Dr. J. J. DeStefano. However, we should hasten to add that we did not always need their advice, and we must take responsibility for any errors that remain.

The efforts of our dedicated secretaries, Mrs. Elizabeth Gill and Mrs. Ester Gerber of the Technicon Instrument Corporation, and Mrs. Mary Lynn Edwards of Du Pont are much appreciated. We are particularly indebted to Miss Mildred Syvertsen of Du Pont for her preparation of the final manuscript copy. The support of our respective departments in this connection was also of great assistance. Finally, we owe much to our families for their forbearance during the several months that were required to complete this book.

L. R. Snyder

J. J. Kirkland

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CONTENTS

CHAPTER ONE

INTRODUCTION

Over the past forty years the practice of chromatography has witnessed a continuing growth in almost every respect: the number of chromatographers, the amount of published work, the variety and complexity of samples being separated, separation speed and convenience, and so on. However, this growth curve has not moved smoothly upward from year to year. Rather the history of chromatography is one of periodic upward spurts that have followed some major innovation. It is easy to recognize several major milestones of this type: the introduction of partition and paper chromatography in the 1940s, gas and thin-layer chromatography in the 1950s, and the various gel or exclusion methods in the early 1960s. A few years later it was possible to foresee still another of those major developments which were destined to revolutionize the practice of chromatography: a technique which we will call modern liquid chromatography.

What do we mean by "modern liquid chromatography"? Liquid chromatography (LC) refers to any chromatographic process in which the moving phase is a liquid, in contrast to the moving gas phase of gas chromatography. Traditional column chromatography (whether adsorption,

partition, or ion exchange), thin-layer and paper chromatography, and modern LC are all forms of liquid chromatography. The difference between modern LC and these older procedures includes details of equipment, materials, technique, and theory. But mainly, modern LC offers major advantages in convenience, accuracy, speed, and the ability to carry out difficult separations. To appreciate the unique value of modern LC it will help to draw two comparisons:

- Liquid chromatography versus gas chromatography;
- Modern LC versus traditional LC procedures.

1.1 LIQUID VERSUS GAS CHROMATOGRAPHY

The tremendous ability of gas chromatography (GC) to separate and analyze complex mixtures is now widely appreciated. Compared to previous chromatographic methods, GC features separations that are much faster and better. Moreover, automatic equipment for GC was soon available for convenient, unattended operation. However, many samples cannot be handled by GC, either because they are insufficiently volatile and cannot pass through the column, or because they are thermally unstable and decompose under the conditions of separation. It has been estimated that only 20% of known organic compounds can be handled satisfactorily by GC, without prior chemical modification of the sample.

LC on the other hand is not limited by sample volatility or thermal stability. Thus, LC is ideally suited for the separation of macromolecules and ionic species of biomedical interest, labile natural products, and a wide variety of other high-molecular-weight and/or less stable compounds; for example,

proteins	polysaccharides	pharmaceuticals
nucleic acids	plant pigments	dyes
amino acids	polar lipids	surfactants

drug and pesticide conjugates	steroids	synthetic polymers
purines	vitamins	antioxidants

Liquid chromatography also enjoys certain other advantages with respect to GC. Very difficult separations are often more readily achieved by liquid than by gas chromatography. The reasons for this include:

- Two chromatographic phases in LC for selective interaction with sample molecules, versus only one in GC;
- A greater variety of uniquely useful column packings (stationary phases) in LC;
- Lower separation temperatures in LC.

Chromatographic separation is the result of specific interactions between sample molecules and the stationary and moving phases. These interactions are essentially absent in the moving gas phase of GC, but are present in the liquid phase of LC--thus providing an additional variable for controlling and improving separation. Furthermore, a greater variety of stationary phases has been found useful in LC, which again allows a wider variation of these selective interactions, and greater possibilities for separation. Finally, chromatographic separation is generally enhanced as the temperature is lowered, because intermolecular interactions then become more effective. This favors procedures such as LC which are usually used at temperatures near ambient.

Liquid chromatography also offers a number of unique detectors which have so far found little or no [illegible]

- [illegible] with color-forming reactions of separated sample components.
- UV absorption and fluorescence detectors.

- Radiometric detectors.
- Conductivity detectors.
- Polarographic detectors.
- Refractive index detectors.

In certain LC analyses that are illustrated later, the availability of the right selective detector can eliminate the need for complete separation.

A final advantage of liquid versus gas chromatography is in the relative ease of sample recovery. Separated fractions are easily collected in LC, simply by placing an open vessel at the end of the column. Recovery is quantitative, and separated sample components are readily isolated. The recovery of separated sample components in GC is also possible--but is generally less convenient and quantitative.

Despite these advantages of LC relative to GC, the latter is usually the method of choice when no special problems are expected for a given sample. Separations by GC are often faster and more sensitive, as well as more convenient. Also, most laboratories are already well equipped for GC, in terms of both equipment and operating personnel. The time is rapidly passing, however, when GC is automatically a first choice for the separation of most samples. Before long we can expect to see more samples being analyzed by modern LC than by GC.

1.2 MODERN VERSUS TRADITIONAL LC PROCEDURES

Consider now the difference between modern LC and classical column or open-bed chromatography. These three general procedures are illustrated in Figure 1.1. In classical LC a column was generally used only once, then discarded. Therefore, packing a column (step 1 of Figure 1.1, "Bed Preparation") had to be repeated for each separation, and this represented a significant expense of both manpower and material. Sample