Volume 2

Physical Methods in Modern Chemical Analysis

EDITED BY
THEODORE KUWANA

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PHYSICAL METHODS IN MODERN CHEMICAL ANALYSIS

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Preface

The practitioners of chemistry today are faced with a multitude of increasingly complex problems concerned with *chemical analysis*. They are, for example, requested to find and identify trace amounts of materials in complex mixtures. Moreover, trace quantities, rather than being in the microgram range as thought of several years ago, are now being extended below the nanogram level to femtograms. The problem of identification is also nontrivial, extending from organic and inorganic compounds in various matrices to complex biological macromolecules. New tools often associated with sophisticated instrumentation are also constantly being introduced. Surface analysis is a good example of an area for which recent years have seen the advent of many new methods, and the abbreviations ESCA, SIMS, XPS, LEEDS, etc. are now common in the literature. These methods have made it possible to analyze and characterize less than monolayers on solid surfaces. Thus the demand upon a practicing chemist is to have a working knowledge of a wide variety of physical methods of chemical analysis, both old and new: the new ones as they are developed and applied, and the old ones as they are better understood and extended. It is the aim of "Physical Methods in Modern Chemical Analysis" to present a description of selected methodologies at a level appropriate to those who wish to expand their working knowledge of today's methods and for those who wish to update their background. It should also be useful to graduate students in obtaining a basic overview of a wide variety of techniques at a greater depth than that available from textbooks on instrumental methods.

"Physical Methods in Modern Chemical Analysis" will contain chapters written by outstanding specialists who have an intimate working knowledge of their subject. The chapters will contain descriptions of the fundamental principles, the instrumentation or necessary equipment, and applications that demonstrate the scope of the methodology.

It is hoped that these volumes continue the standard exemplified by the earlier volumes, "Physical Methods in Chemical Analysis," edited by Walter Berl in the 1950s and 1960s.

The patience and assistance of my wife Jane during the editing process are gratefully acknowledged.

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Selected Planned Chapters for Future Volumes

PHYSICAL METHODS IN MODERN CHEMICAL ANALYSIS, VOL. 2

High Performance Liquid Chromatography

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I. Introduction

A. Chromatography

1. *Scope*

Of all the different types of separation methods, chromatography has the unique position of being applicable to all types of problems and in all areas of science. It is perhaps the most accepted separation tool in the

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modern analytical laboratory and is routinely used for purposes of purification, separation, identification, and quantitation.

Chromatography encompasses many different techniques. In this chapter one of these techniques, known as high performance liquid chromatography (hereafter referred to as HPLC), is discussed. No attempt has been made to survey all the literature in HPLC. Rather, the main purpose is to provide the reader with a discussion of the theory, instrumentation, and types of columns and eluting agents used in HPLC. It is hoped that this approach will provide a working background in HPLC, will facilitate the introduction of HPLC into laboratories where it is not now or only sparingly used, and will stimulate further development in laboratories where it is routinely used.

Modern liquid chromatography or HPLC has developed largely since about 1968. Its development and the increase in scientific applications parallels the surge observed in gas chromatography (GC) during the late 1950s and early 1960s.

Four major factors are probably responsible for the acceptance of HPLC as a companion to gas chromatography in related areas of organic, pharmaceutical, and biochemical analysis.

(1) HPLC provides a great reduction in analysis time in comparison with conventional (pre-1968) liquid chromatography. Furthermore, this reduction is accomplished over a wide range of unknown sample concentrations, including trace levels, with a high degree of precision and accuracy.

(2) HPLC is capable of generating high column efficiencies. That is, plate heights are extremely small in comparison with conventional liquid chromatography, and thus, a very large number of plates are possible per given column length. This means that short columns can be used, and/or that very complex mixtures can be separated. For example, complex mixtures such as urine, other biological fluids or tissue samples, environmental samples, and agricultural samples that were virtually impossible to separate several years ago can now be separated by HPLC.

(3) HPLC, unlike GC, does not require the sample to have an appreciable vapor pressure. Thus HPLC is applicable not only to the separation of lower molecular weight organic compounds, but also to macromolecular compounds. Furthermore, separations based on adsorption, partition, ion exchange, and size exclusion are possible. For these reasons, future applications of HPLC may be significantly greater than those of GC.

(4) Finally, the growth in the design and development of HPLC instrumentation has paralleled the demand for HPLC applications.

2. *Literature*

The theory and applications of chromatography have developed rapidly in the past 25 years, making chromatography one of the major techniques

TABLE I

Literature in Chromatography

Chromatography—General

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TABLE 1 *(continued)*

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TABLE I *(continued)*

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Chromatography—Journals

Chromatographia

Journal of Chromatographic Science

Journal of Chromatography

Journal of Chromatography—Biomedical Applications

Journal of Liquid Chromatography

Separation Science and Technology

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[a] This title is an ongoing series.

in the broad field known as "separation science." Many monographs, treatises, and review articles which document the advances in chromatography have appeared. Table I lists the major sources of information specific to HPLC and briefly surveys other key chromatographic literature allied to HPLC.

3. *History*

It is generally recognized that the discoverer of chromatography was the botanist Mikhail Tswett. Although there were previous reports in the literature of separations as a result of selective adsorption, it was Tswett who recognized and laid the foundations of the chromatographic process. In his classic experiments Tswett carried out adsorption chromatography. Many of the terms used to describe modern chromatography were introduced by Tswett (Magee, 1970).

As frequently happens when a new discovery is made, chromatography remained relatively neglected for many years. Starting in the 1930s, however, many major contributions were reported in the literature. These included the synthesis of high capacity ion exchangers to replace the previously used zeolites and, in a short period of time, the foundations of thin layer, partition, and gas chromatography were developed. In essence, these discoveries and their subsequent explosive growth have all played a major role in the development of modern liquid column chromatography or HPLC.

Probably the most decisive papers, aside from the introductory work of Tswett, were the reports by Martin and Synge (1941a,b) in which they introduced partition chromatography (Magee, 1970). Several major contributions can be traced to this work. First, they proposed a theoretical treatment of the chromatographic processes by considering the operating parameters of the column and expressed the efficiency of the separation in terms of the theoretical plate by analogy to distillation. Second, they described partition or liquid–liquid chromatography by recognizing that, by immobilizing one liquid on a suitable stationary phase, a separation could be achieved by passing a second liquid containing the sample mixture over the first.

Two other major contributions can be found in the work of Martin and Synge. These are best illustrated by considering the following quotations: "The mobile phase need not be a liquid but may be a vapour" (Martin and Synge, 1941a); "Thus, the smallest height equivalent to a theoretical plate should be obtainable by using very small particles and a high pressure difference across the length of the column" (Martin and Synge, 1941a). The first is the prediction of gas chromatography (introduced some 11 years later by James and Martin) and the second is the prediction of HPLC (introduced in the mid-1960s).

B. Liquid Column Chromatography

1. *Phases*

Chromatography in most of its forms is a method whereby the components of a mixture separate because of differences in their relative affinities for a stationary and a mobile phase. The notable exceptions are those chromatographic methods in which a difference in the molecular size of the components in the mixture is the key factor in determining the separation. The potential phase combinations are summarized in Table II. In this chapter

TABLE II

Types of Phases in Chromatography

Mobile phase	Stationary phase	Chromatographic mechanism
Liquid	Solid	Adsorption, ion Exchange, size Exclusion
Liquid	Liquid	Partition
Gas	Solid	Adsorption
Gas	Liquid	Partition

only the first two are considered. Furthermore, only liquid column chromatography employing modern columns and instrumentation will be discussed here.

2. *Chromatographic Process*

A physical picture of the chromatographic process in column chromatography is illustrated in Fig. 1. The stationary phase is packed into a cylindrical tube that is plugged at the bottom and is conditioned by passage of a mobile phase. The sample mixture is introduced at the top of the column into the mobile phase which carries it into the column. If the proper eluting agent is chosen as the mobile phase, the components of the sample mixture pass through the column and distribute themselves according to the differences in their affinities for the two phases.

In Fig. 1 it is assumed that the sample is a 2-component mixture and that the affinities of the phases for the two components are significantly different. The sequence of events that occurs in the chromatographic system as a function of time are shown in Fig. 1: (a) illustrates the column as it is equilibrated with passage of the mobile phase; (b), the sample is introduced above the column bed; (c), passage of the eluting mobile phase causes the sample to penetrate the column; (d), as the flow of the mobile phase is continued

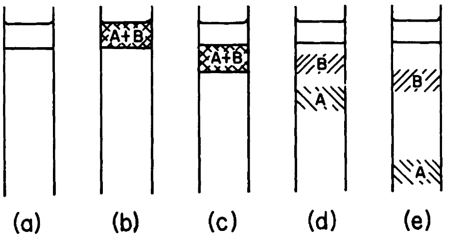


Fig. 1 Chromatographic processes in column liquid chromatography.

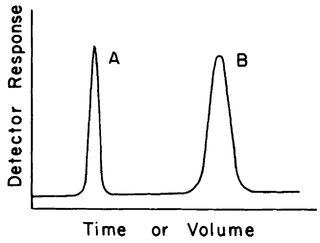
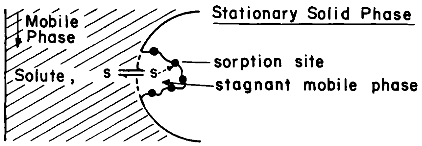
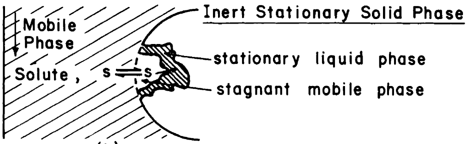


Fig. 2 A typical chromatogram in column liquid chromatography.

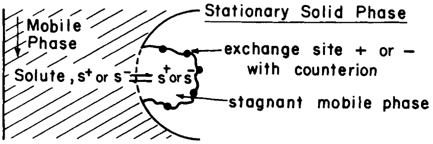
the two components begin to separate; and (e), with continued flow the distance between the two components increases. Eventually, the two components emerge from the bottom of the column. Monitoring the effluent of the column with a detector that responds to the components provides the chromatogram for the separation as shown in Fig. 2. For a fixed chromatographic system (a controlled flow rate with known mobile and stationary phases), the position of the chromatographic peak is characteristic of the component providing the peak, while the area under the peak is proportional to its concentration.



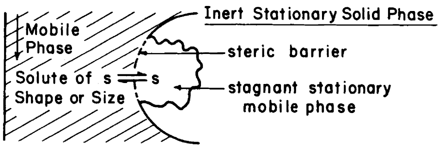
(a) ADSORPTION



(b) PARTITION



(c) ION EXCHANGE



(d) SIZE EXCLUSION

Fig. 3 Chromatographic mechanisms.

During the chromatographic process the solute is participating in mass transfer between the two phases at the interface (Fig. 3a) or in the bulk phase (Fig. 3b). The first is adsorption chromatography, and the latter is partition chromatography. Ion exchange and size exclusion mechanisms are illustrated in Figs. 3c and 3d, respectively. Since the system is dynamic, it is not at equilibrium throughout the column. However, the experimental conditions are usually chosen so that the system is as close to equilibrium as possible.

TABLE III

Intermolecular Forces That Influence Adsorption and Partition Processes

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Ionic forces
van der Waals forces
  (dipole–dipole, dipole–induced dipole, induced dipole–induced dipole)

Hydrogen bonding

Charge transfer

Many factors will influence the affinities resulting from adsorption and partitioning processes. To affect a separation, it is the intention of the operator to control, influence, and/or change these factors in some way so that the affinities of each component in the mixture will be different. The major factors are listed in Table III.

A quantitative discussion of the influence of the forces listed in Table III in chromatography is very complex particularly since the interactions occur between the solvent (mobile phase), solute, and stationary phase and are inter- and intramolecular. These interactions are summarized in Fig. 4.

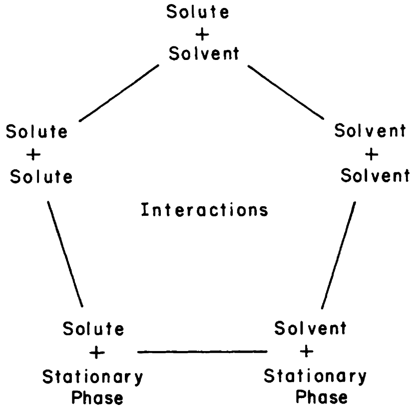

Fig. 4 Summary of the interactions contributing to the chromatographic process.