# Applications of ION CHROMATOGRAPHY for PHARMACEUTICAL and BIOLOGICAL PRODUCTS

Edited by

Lokesh Bhattacharyya

Jeffrey S. Rohrer

WILEY

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# APPLICATIONS OF ION CHROMATOGRAPHY FOR PHARMACEUTICAL AND BIOLOGICAL PRODUCTS

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# PREFACE

Ion chromatography (IC) is a form of HPLC, which involves separation based on ion exchange or ion exclusion followed by detection of analytes using a suitable detector. Typically, electrochemical detectors (ED), such as conductivity or pulsed amperometry detectors, are used, however, conventional detectors, e.g., UV/VIS, RI, or more complex types of detection systems, e.g., mass spectrometry, also can be employed.

Since its introduction in the mid-1970s, IC has developed into an important analytical tool in a number of applications in pharmaceutical and biotechnology industries. Over the last two decades a significant number of IC methods have been successfully developed and validated for the characterization, lot-release, and stability studies of pharmaceutical and biological products. IC has also been used on-line as a tool for process monitoring. The technique has been used widely for the analyses of amino acids, peptides, proteins, glycoproteins, carbohydrates, antibiotics, vaccines, and other products. It has been successfully applied to the analysis of raw materials, bulk active ingredients, counterions, impurities and degradation products, excipients, diluents, and at different stages of the production process, as well as for the analysis of production equipment cleaning solutions, waste streams, container compatibility, and other applications.

With the increasing demand for newer types of therapeutic products (new molecular entities), use of different counter ions to improve stabilities and solubility properties of pharmaceutically active drug molecules, availability of mixed mode columns, and use of detection systems that have higher tolerance for organic solvents, there has been a growing interest in the application of IC in the pharmaceutical industry. Advances in technology have permitted IC to be coupled with mass spectrometry, which is a critical milestone for a wider interest in IC as a product characterization tool. Furthermore, because the principle of operation of IC is different from that of the other forms of chromatography, it can be the method of choice where other forms of chromatography are not suitable, e.g., for ionic and highly polar compounds, inorganic ions, and molecules that do not have suitable chromophores for detection by absorption measurement. The sample preparation often requires minimum pretreatment, generally as simple as dilution with water. When used together with ED, IC requires no pre- or post-column derivatization. In addition, the technique provides the ability to detect analytes with a high degree of selectivity through a combination of on-column resolution and the response selectivity of electrochemical detectors. As an orthogonal method to not

only chromatographic and electrophoretic techniques, but also techniques such as AA and ICP, IC can play a critical role in method development and validation. The current regulatory focus on product characterization encourages industry researchers to search for alternative forms of analyses using different types of technologies, which can measure different characteristics of the products, their components, and drug-matrix and drug-container interactions. The interest in the pharmaceutical and biotechnology industries for IC is still growing and is expected to grow steadily in the foreseeable future.

Unlike reversed-phase HPLC, which uses organic solvents, IC generally employs dilute acids, alkalis, or salt solutions as eluents with little or no organic solvent, and as such the eluents are less toxic and less costly to use and dispose of. The recent increase in environmental consciousness together with cost considerations is expected to contribute toward greater interest in IC. Furthermore, with the expiration of key patents a few years ago, manufacturing of IC systems and their components are competitive now. The open competition is expected to result in improved instrumentation and column characteristics, availability of columns suitable for diverse applications, and wider customer support.

The number of USP-NF monographs that include IC-based procedures has grown significantly over the last decade. In 2006, USP-NF has added a new general information chapter on IC (<1065>) and a new general chapter on an IC-based procedure (<345>), illustrating the growing acceptance of IC as one of the methods of choice in the pharmaceutical industry.

No book has been published yet specifically on the application of IC in the analysis of pharmaceutical and biological products and their ingredients. Applications have been presented mostly in peer reviewed journals, scientific review articles, application notes of instrument and column manufacturers, trade publications, and scientific conferences. Although the technology has been around for many years, given the recent interest the time is now appropriate to produce a book on this topic. An edited volume with contributors from academia, pharmaceutical and biotechnology industries, and instrument manufacturers, from North America, Europe, and Australia provides different perspectives, experience, and expertise of the leaders in the field in a comprehensive manner in one place.

The book is designed as an introduction of IC to the beginners and a reference guide for experienced scientists and investigators in the pharmaceutical and biotechnology industry, working in the research, product development, and quality control areas. In addition, this book should be of interest to the student of pharmacy, pharmaceutical and biotechnology science, and academic researchers as a learning tool as well as a comprehensive reference.

We wish to thank all authors and reviewers who have contributed to this book. We recognize that this book would not have been possible but for their hard work. Special thanks go to Dr. Shreekant Karmarkar of Baxter Healthcare for his thoughts and helpful suggestions in the conception and the design of this compilation.

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## PRINCIPLES, MECHANISM, AND INSTRUMENTATION

# PART I

## PRINCIPLES, MECHANISM, AND INSTRUMENTATION

# 1

# ION CHROMATOGRAPHY—PRINCIPLES AND APPLICATIONS

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## 1.1 INTRODUCTION

Ionic methods of separation have been used since the industrial revolution in Europe to reduce hardness of water. In the mid-nineteenth century, British researchers treated various clays with ammonium sulfate or carbonate in solution to release calcium. In the early twentieth century, zeolite columns were used to remove interfering calcium and magnesium ions from solutions to permit determination of sulfate. Ionic separation procedures were used in the Manhattan project to purify and concentrate radioactive materials needed to make atom bombs. Peterson and Sober [1] reported in 1956 a chromatographic method based on ion exchange to separate proteins. However, ion chromatography (IC), in its modern form, was introduced in 1975 by Small et al. [2]. The technique has since gained significant attention for the analysis of a wide variety of analytes in pharmaceutical, biotechnology, environmental, agricultural, and other industries. Several books and chapters on IC have provided a detailed review of its principles and instrumentation [3–5]. In 2000, United States

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Pharmacopeia-National Formulary (USP-NF) had only a few monographs that described test methods involving IC [6] and no general chapter on this technique. However, the number of monographs that include one or more IC-based test procedures has increased dramatically in the last 10 years. In addition, the current USP-NF [7] contains two general chapters on IC (<345> and <1065>) and at least four general chapters that include IC-based test methods (<1045>, <1052>, <1055>, <1086>), indicating its importance as a chromatographic technique for the analysis of pharmaceutical drug substances, products and excipients. In General Chapter <1065>, entitled "Ion Chromatography", USP-NF describes ion chromatography as "a high-performance liquid chromatography (HPLC) instrumental technique used in USP test procedures such as identification tests and assays to measure inorganic anions and cations, organic acids, carbohydrates, sugar alcohols, aminoglycosides, amino acids, proteins, glycoproteins, and potentially other analytes" [7].

This chapter will present an introduction to IC providing an outline of its principles and applications in the analysis of active and inactive ingredients, counter-ions, excipients, degradation products, and impurities relevant to the analysis of pharmaceutical, biologic and biotechnology-derived therapeutic and prophylactic products.

## 1.2 WHAT IS ION CHROMATOGRAPHY?

Modern IC is a form of HPLC, just as normal phase, reversed-phase and size exclusion chromatographies are different forms of HPLC. The separation in IC is based on ionic (or electrostatic) interactions between ionic and polar analytes, ions present in the eluent, and ionic functional groups derivatized to the chromatographic support. This can lead to two distinct mechanisms of separation—(a) ion exchange due to competitive ionic binding (attraction), and (b) ion exclusion due to repulsion between similarly charged analyte ions and the ions derivatized on the chromatographic support. Separation based on ion exchange has been the predominant form of IC to-date. In addition, chromatographic methods in which the separation due to ion exchange or ion exclusion is modified by the hydrophobic characters of the analyte or the chromatographic support material, by the presence of the organic modifiers in the eluent or due to ion-pair agents, resulting in better resolution that were not achieved otherwise, have gained popularity recently (mixed mode separation).

Numerous studies have been conducted in the last 30 years to understand the details of the mechanisms of ion-exchange and ion-exclusion chromatographies and the effect of different elution parameters, including flow rate, salt concentration, pH, presence of organic solvents, and temperature, on them. The current chapter is not meant to provide a comprehensive review of the studies. Rather, it is meant to provide a general introduction to both types of IC explaining in a qualitative non-mathematical approach how they work, what types of analytes are suitable for separation by ion-exchange and ion-exclusion chromatographies, and the effect of different factors on their performance.

## 1.3 ION-EXCHANGE CHROMATOGRAPHY

Ion-exchange chromatography involves separation of ionic and polar analytes using chromatographic supports derivatized with ionic functional groups that have charges opposite that of the analyte ions. That is, a column used to separate cations, called a cation-exchange column, contains negatively charged functional groups. Similarly, an anion-exchange column, which separates anions, is derivatized with positively charged functional groups. Ion-exchange chromatography has been widely used in the analysis of anions and cations, including metal ions, mono- and oligosaccharides, alditols and other polyhydroxy compounds, aminoglycosides (antibiotics), amino acids and peptides, organic acids, amines, alcohols, phenols, thiols, nucleotides and nucleosides, and other polar molecules.

The analyte ions and similarly charged ions of the eluent compete to bind to the oppositely charged ionic functional group on the surface of the stationary phase. Assuming that the exchanging ions (analytes and ions in the mobile phase) are cations, the competition can be represented by the following scheme:

$$S - X^- C^+ + M^+ \leftrightarrow S - X^- M^+ + C^+ \tag{1}$$

In this process, the cation $M^+$ of the eluent exchanges for the analyte cation $C^+$ bound to the anion $X^-$ derivatized on the surface of the chromatographic support (S). If, on the other hand, the exchanging ions are anions, it is called anion-exchange chromatography and is represented as:

$$S - X^+ A^- + B^- \leftrightarrow S - X^+ B^- + A^- \tag{2}$$

in which, the anion $B^-$ of the eluent exchanges for the analyte cation $A^-$ bound to the positively charged ion $X^+$ on the surface of the stationary phase. The adsorption of the analyte to the stationary phase and desorption by the eluent ions is repeated as they travel along the length of the column, resulting in the separation due to ion-exchange [8].

### 1.3.1 Mechanism

The mechanism of the two processes, cation exchange and anion exchange, are indeed, very similar. In the first step of the process, analyte ions diffuse close to the stationary phase and bind to the oppositely charged ionic sites derivatized on the stationary phase through the Coulombic attraction. The Coulombic force of interaction ($f$) between the two ions in solution, in its simplified form, is given by the equation,

$$f = q_1 q_2 / \varepsilon r^2 \tag{3}$$

in which $q_1$ and $q_2$ are charges on two ions, $\varepsilon$ is the dielectric constant of the medium, and $r$ is the distance between them. In most of the ion chromatographic separations,