CAPILLARY ELECTROPHORESIS METHODS FOR PHARMACEUTICAL ANALYSIS

Edited by
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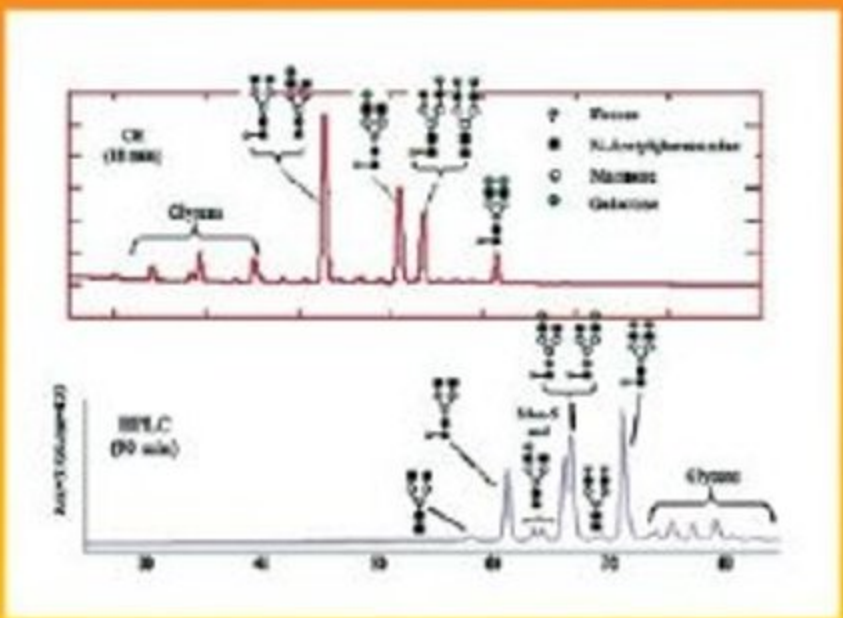

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PREFACE

Capillary electrophoresis (CE) using fused-silica capillaries with internal diameter in the micrometer range was introduced in 1981 and was received with great enthusiasm in the separations world because it promised high separation efficiency, a large degree of flexibility during method development, and a low cost of operation. After a better understanding of the fundamentals was developed, the focus shifted to some real applications. Many papers describing highly efficient separation methods have been published in the last two decades. CE offers several advantages over high-performance liquid chromatography (HPLC), a technique commonly used in the pharmaceutical industry. These include simplicity, smaller sample size, rapid analysis, automation, ruggedness, different mechanisms for selectivity, and low cost. Furthermore, CE offers higher efficiency than HPLC and thus greater resolution power for separating various components. These advantages make CE a very attractive tool in the research and development of pharmaceuticals, quality control, and stability studies.

This book has been planned to provide busy pharmaceutical scientists a complete yet concise reference guide for utilizing the versatility of CE in new drug development and quality control. The text can be broadly classified in five major sections:

- Overview, theory, and instrumentation (Chapters 1–3)
- CE methods and practices (Chapters 4–6)
- Regulatory aspects (Chapters 7–11)
- Applications (Chapters 12–16)
- New developments (Chapters 17 and 18)

Each of the chapters, written by selected experts in their respective fields, is designed to provide the reader with an in-depth understanding of CE theory, hardware, methodologies, regulations, and applications. The text includes state-of-the-art information on CE analysis of

pharmaceuticals and provides the reader a clear and concise understanding of the following important topics:

- How to improve performance of CE methods
- How to develop and validate robust methods in CE
- How to increase precision in CE
- How to make CE method transfers more successful
- How to interpret ICH guidelines relating to CE
- How to perform IQ, OQ, PQ, and CE calibrations

Major applications covered include assays, impurity testing, high-throughput screening, chiral separation, pK_a determination, ion analysis, impurity profiling, orthogonal method, and characterization of proteins, peptides, and nucleotides. Furthermore, the latest developments in capillary electrochromatography (CEC), CE–MS, and coupling chip-based devices to MS are discussed at length.

We would like to thank all of the authors for their valuable efforts in making this book serve as a definitive reference source on CE for laboratory analysts, researchers, managers/executives in industry, academia, and government who are engaged in various phases of analytical research and development or in quality control.

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1 OVERVIEW OF CAPILLARY ELECTROPHORESIS IN PHARMACEUTICAL ANALYSIS

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I. INTRODUCTION

Electrophoresis is a separation technique that is based on the differential migration of charged compounds in a semi-conductive medium under the influence of an electric field. Its origin can be traced back to the 1880s; however, it got major recognition in 1937, when Arne

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Tiselius[1] reported the separation of different serum proteins by a method called moving boundary electrophoresis. In 1948, Tiselius was awarded the Nobel Prize in Chemistry for his contributions. The efficiency of the moving boundary method was enhanced further with the development of techniques such as the paper electrophoresis and gel electrophoresis. Paper electrophoresis is now obsolete; however, gel electrophoresis is still used in biochemistry for the determination of proteins and nucleic acids. Unfortunately, gel electrophoresis is limited by the effect of "joule heating," which gives rise to heat production in the system. This heat cannot be dissipated efficiently and leads to temperature gradients that result in convection and viscosity gradients and finally to an increase of dispersion and, as a result, band broadening. Thus, joule heating induces a decrease in the separation efficiency. Better heat dissipation is possible if narrow bore tubes are used. Hjerten[2] was the first to apply this technology, in 1967, using glass tubes with an internal diameter (I.D.) around 3 mm. Jorgensen and Lukacs[3] are credited for producing the first operational system, in 1981, that used 75 μm I.D. fused silica capillaries and voltages up to 30 kV that could produce separations of proteins and dansylated amino acids, with plate heights of less than 1 μm. Since then, many papers of highly efficient separations have been published.

Capillary electrophoresis (CE) is a powerful separation technique that is widely used in research and development (R&D), quality control (QC), and stability studies of pharmaceuticals. CE offers several advantages over high-performance liquid chromatography (HPLC), a technique commonly used in pharmaceutical analysis. These include simplicity, rapid analysis, automation, ruggedness, different mechanisms for selectivity, and low cost. Furthermore, it offers higher efficiency and thus greater resolution power over HPLC even if only a small sample size is available. These advantages are likely to lead to even greater use of CE in R&D, QC, and stability studies of pharmaceuticals. CE has been found particularly useful for separations of peptides, proteins, carbohydrates, inorganic ions, chiral compounds, and in numerous other pharmaceutical applications.[4–6] The separations of chiral compounds are discussed at length in Chapters 2, 4–7, 9–11, and 16–18.

This book is planned to provide the busy pharmaceutical scientist a complete yet concise reference guide for utilizing the versatility of CE in new drug development and QC.

Each of the chapters has been written by a selected expert in the field to provide the reader with an in-depth understanding of CE theory, instrumentation, methodologies, regulations, applications, and recent developments.

II. VARIOUS MODES OF CE

Various modes of CE can be classified into three main groups:

1. Moving boundary CE
2. Steady state CE
   - Isotachophoresis (ITP)
   - Isoelectric focusing (IEF)
3. Zone CE
   - Capillary gel electrophoresis (CGE)
   - Free solution CE
     - Capillary zone electrophoresis (CZE)
     - Micellar electric capillary chromatography (MECC)
     - Chiral CE (CCE)
   - Capillary electrochromatography (CEC).

These modes as well as theoretical considerations are discussed at length in Chapter 2.

III. INSTRUMENTATION

CE instrumentation is quite simple (see Chapter 3). A core instrument utilizes a high-voltage power supply (capable of voltages in excess of 30,000 V), capillaries (approximately 25–100 μm I.D.), buffers to complete the circuit (e.g., citrate, phosphate, or acetate), and a detector (e.g., UV–visible). CE provides simplicity of method development, reliability, speed, and versatility. It is a valuable technique because it can separate compounds that have traditionally been difficult to handle by HPLC. Furthermore, it can be automated for quantitative analysis. CE can play an important role in process analytical technology (PAT). For example, an on-line CE system can completely automate the sampling, sample preparation, and analysis of proteins or other species that can be separated by CE.

IV. METHOD DEVELOPMENT FOR PHARMACEUTICAL ANALYSIS

CE methods are developed and utilized in pharmaceutical QC for early to late phases of drug development. Chapter 4 covers the approaches for late-phase development for small molecules that can be used in early-phase development, as well as for large-molecular-weight compounds. Late-phase method development in pharmaceutical QC is performed for required stability studies and for release of the drug product or drug substance validation batches, and is intended to be transferred to the operational QC laboratories for release testing of the production batches. Preferably, late-phase methods should be fast, robust, reliable, and transferable. Therefore it is crucial to devote adequate time, thought, and resources to the development of such methods.

The following considerations, when applied during method development, are likely to produce more robust, reliable, and transferable methods: (a) the concerns of the "customer" (user) are considered in advance, (b) key process input variables are identified, (c) critical-to-quality factors are determined, (d) several method verification tests are installed, (e) proactive evaluation of method performance during development is performed, (f) continuous customer involvement and focus are institutionalized, and (g) method capability assessment (suitability to be applied for release testing against specification limits) is established.

V. ANALYSIS OF ACTIVE PHARMACEUTICAL INGREDIENTS AND DRUG PRODUCTS

The development of various modes of CE such as micellar electrokinetic chromatography (MEKC), capillary ITP, capillary IEF, CGE, and fully automated systems in the early 1990s has helped spread the use of CE technology. The human genome project and the great interest in genetics helped boost the development of the technology. Because of its versatility and complexity, CE should be regarded as a family of analytical techniques, rather than a single technique, that are performed on one single instrument. Each mode has its advantages and its limitations. The full potential of CE can be realized only by developing a better understanding of various modes of CE. Successful implementation of CE can make an invaluable contribution to the development of new drugs by increasing the separation speed and resolving new analytical challenges not addressable by other techniques. Chapter 5 focuses on the potential use of CE in the development process of drugs. Various challenges, benefits, and appropriate remediation approaches to overcome the limitations are addressed.

VI. GENERAL CONSIDERATIONS FOR IMPROVING PERFORMANCE OF CE METHODS

Chapter 6 describes the desired improvement of method parameters from the robustness point of view, not from the analyte or the specific analysis point of view. Illustrative examples are provided to help the reader develop methods with the currently available equipment. It is important that the method be described explicitly and unequivocally and that the validation report does not raise expectations that cannot be met in daily use.

VII. CURRENT REGULATORY GUIDANCE

During the last decade, CE has become a mature separation technique for pharmaceutical analysis. Numerous validated methods from pharmaceutical R&D laboratories and academia have been reported in the literature. The information covers identity, API assay, purity determination, enantiomeric separation, and stoichiometry determination (Chapter 7). In addition, CE is frequently applied as an orthogonal technique during the development of stability-indicating liquid chromatographic methods. As a result, CE has been included in various regulatory submissions by different pharmaceutical companies. The growing interest and application of CE as an advanced separation technique in the area of pharmaceutical analysis is gaining recognition by the regulatory authorities. CE has been included as a specific analytical technique in different guidance documents from the United States Food and Drug Administration (FDA) and the International Conference on Harmonisation (ICH). A general monograph on CE has been included in the European Pharmacopoeia (EP), the United States Pharmacopoeia (USP), and the Japanese Pharmacopoeia (JP). In addition, CE is included in a number of specific monographs for several products as, e.g., identity confirmation test or enantiomeric purity test. In order to prevent differences in nomenclature recommendations on the terminology for analytical capillary electromigration, techniques have been published by the International Union of Pure and Applied Chemistry (IUPAC). ICH guidelines should be followed in meeting regulatory approval if CE methods are used in a registration dossier.

VIII. QUALIFICATION OF CAPILLARY ELECTROPHORESIS INSTRUMENTATION

Qualification of CE instrumentation is performed using failure mode and effects analysis as the risk analysis tool (see Chapter 8). The instrument is reviewed in terms of its component modules, and the potential failures of those components are identified. The potential effect of those failures is defined and the risk characterized. Any current evaluation of those failures is identified, and any recommended actions to mitigate the risk are defined. Apart from the qualification dossiers provided by vendors, there seems, at present, to be very little information published on the operational qualification of CE instruments. The latest thinking provided by the FDA in the Guidance for Industry for Quality Risk Management suggests that all qualification activities should be performed using a risk-based approach. Whenever risk is to be considered, the instrument being assessed must be viewed in the context of the "protection of the patient." Analytical instruments may have an impact on the validity of data, determining the safety and efficacy of drug products, or on the product quality of the drug product. They may also impact the identity or potency of the drug product, and therefore it is important to perform risk management throughout the life cycle of the instrument. In some cases, the use of informal risk management processes may also be acceptable.

IX. ROBUSTNESS TESTS OF CE METHODS

In biomedical and pharmaceutical analysis, particularly in the pharmaceutical industry, much attention is paid to the quality of the obtained analytical results because of the strict regulations set by regulatory bodies (Chapter 9). As a result, robustness testing has become increasingly important. The ICH guidelines define robustness as "The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage." A robustness test is the experimental setup applied to evaluate the robustness of the method. The ICH guidelines also state that "One consequence of the evaluation of robustness should be that a series of system suitability parameters (e.g., resolution tests) is established to ensure that the validity of the analytical procedure is maintained whenever used." The latter definition for robustness has been utilized in Chapter 9 since it is the most widely applied. Although robustness tests are not obligatory yet in the ICH guidelines, they are demanded by the FDA for the registration of drugs in the United States. The setup and treatment of results of such a robustness test are discussed in the same Chapter. Also, a literature review and critique of applications of robustness testing of CE methods has been provided.

X. VALIDATION OF ANALYTICAL METHODS

Validation is the process of proving that a method is acceptable for its intended purpose. It is important to note that it is the method, not the results, that are validated (Chapter 10). The most important aspect of any analytical method is the quality of the data it ultimately produces. The development and validation of a new analytical method may therefore be an iterative process. Results of validation studies may indicate that a change in the procedure is necessary, which may then require revalidation. Before a method is routinely used, it must be validated. There are a number of criteria for validating an analytical method, as different performance characteristics will require different validation criteria.

Various validation characteristics that need to be considered are accuracy, precision, specificity, detection limit, quantitation limit, linearity, and range in accordance with the official guidelines. Validation requirements should be described according to the goal of the method. The validation of analytical methods is generally considered as an inherent requirement of quality assurance systems. There are various approved references (recommendations and guidelines) for realization and interpretation of assay performance and proficiency testing for QC analytical methods. For pharmaceutical methods, guidelines set by the USP, the ICH, and the FDA provide a framework for performing such validations. Thus, when evaluating electrophoretic procedures, the validation must be in stringent compliance with these current guidelines in order to be accepted in all parts of the world. For CE, as a mature but still younger technique, validation studies need to provide stronger evidence of suitability of a given method.

XI. THE NEED FOR CE METHODS IN PHARMACOPOEIAS

Various international pharmacopoeias help assure the quality of drugs worldwide. These pharmacopoeias constantly review and revise their monographs. A different impurity profile can be anticipated if a drug's production process is changed; this results in the development of new analytical methods that need to be incorporated in the pharmacopoeias. In earlier editions, color reactions were performed for identification and purity evaluation purposes.

Nowadays, the pharmacopoeias make use of chromatographic methods and try to replace less sensitive TLC methods with HPLC tests. However, CE methods are rarely used even though they can at times be more useful than HPLC for determining the impurity evaluation of a drug (Chapter 11). CE is currently used, especially in the case of peptides and proteins, in the EP and the USP. These methods and perspectives for new applications are given in the same Chapter.

XII. CE IN IMPURITY PROFILING OF DRUGS

Chapter 12 illustrates possible applications of CE in impurity profiling. Because of the large peak capacity of the technique, CE is extremely well suited to separate the main drug compound from its possible impurities that often have a related chemical structure. Moreover, the high efficiencies obtained, as well as the low reagent consumption, make CE a viable alternative to HPLC in many cases of drug analysis. After a short introduction into the relevance of impurity profiling for regulatory authorities, public health, and the pharmaceutical industry, various applications of CZE, non-aqueous CE, MEKC, microemulsion electrokinetic capillary chromatography, CGE, and CEC are presented.

XIII. ION ANALYSIS USING CAPILLARY ELECTROPHORESIS

Most drugs are charged molecules that are weak bases or acids having a counterion. Basic drugs may have an ionic salt or organic acid as counterion, and acidic drugs a cation. The regulatory agencies (e.g., US FDA and other relevant agencies) require that pharmaceutical products be tested for their composition, strength, quality, and purity. These requirements apply to the excipients as well. This means that the determination of the counterion is an important part of the determination of the purity of the drug. One of the major applications of CE in the pharmaceutical industry is the determination and quantification of drugs counterions. Another application is the impurity determination, to check for the contaminants resulting from the production process. One of the characteristics of many of these ions is that they are UV transparent, while most CE instruments are equipped with a UV detector. For this reason, a special technique called indirect UV detection is often applied. Chapter 13 covers important considerations relating to buffer composition and sample preparation and provides a review of the indirect detection principle, and what is required to make it work.

XIV. ROLE OF CE IN BIOPHARMACEUTICAL DEVELOPMENT AND QC

In the last two decades, CE has advanced significantly as a technique for biomolecular characterization. It has not only passed the transition from a laboratory curiosity to a mature instrument-based method for micro-scale separation, but has also emerged as an indispensable tool in the biotech and pharmaceutical industries (Chapter 14). CE has become a method of choice in R&D for molecular characterization, and in QC for release of therapeutic biomolecules. In the biopharmaceutical industry, more and more CE methods have been validated to meet ICH requirements. To demonstrate the influence of CE in R&D for method development and in manufacturing for the release of therapeutic proteins and antibodies, examples from the pharmaceutical industry are provided in Chapter 14.

XV. CAPILLARY ELECTROPHORESIS AND BIOANALYSIS

The use of CE for the analysis of therapeutic proteins produced by recombinant DNA technology has significantly increased over the past several years. Chapter 15 highlights some of the most important CE applications. The applications are divided into the following areas: (a) capillary sodium-dodecylsulfate as a replacement for traditional SDS-PAGE, (b) CE to monitor charge heterogeneity by CZE and capillary IEF, and (c) oligosaccharide analysis by CE. Finally, an overview of the implementation of CE in the QC of therapeutic proteins is provided.

XVI. CE AS AN ORTHOGONAL TECHNIQUE TO CHROMATOGRAPHY

In the strict mathematical sense, two parameters are orthogonal when the Pearson's correlation coefficient between both is zero (Chapter 16). Considering the comprehensive two-dimensional chromatography, two systems are called orthogonal when the constituent dimensions operate independently and the synentropy across the dimensions is zero. However in one-dimensional chromatography, as considered above, often a less strict definition is applied for orthogonal systems, being "systems that differ significantly in selectivity." As a consequence, some analysts prefer the term "dissimilar" to "orthogonal" in such situations. CE can add important value as an orthogonal technique to chromatography, for instance, in drug impurity profiling. First of all, CE is based on a totally different separation mechanism from partition chromatography and shows selectivity differences from conventional HPLC. This implies that CE should provide additional information about a sample. Moreover CE proved its importance in the critical zones of a chromatogram, i.e., where the co-elution of two components is encountered. As a consequence, it can be very useful to include a CE method as an orthogonal technique in a set of dissimilar chromatographic systems used to screen unknown mixtures.

XVII. CAPILLARY ELECTROCHROMATOGRAPHY OF PHARMACEUTICALS

CEC is a miniaturized separation technique that combines capabilities of both interactive chromatography and CE. In Chapter 17, the theory of CEC and the factors affecting separation, such as the stationary phase and mobile phase, are discussed. The chapter focuses on the preparation of various types of columns used in CEC and describes the progress made in the development of open-tubular, particle-packed, and monolithic columns. The detection techniques in CEC, such as traditional UV detection and improvements made by coupling with more sensitive detectors like mass spectrometry (MS), are also described. Furthermore, some of the applications of CEC in the analysis of pharmaceuticals and biotechnology products are provided.

XVIII. COUPLING CE AND MICROCHIP-BASED DEVICES WITH MASS SPECTROMETRY

Several advantages offered by CE, such as a high efficiency, rapid method development, simple instrumentation, and low sample consumption, are the main reasons for its success in a variety of fields. UV-VIS spectrophotometry is probably the most widely used detection technique with CE because of the simplicity of the on-line configuration. However, its sensitivity, directly related to the optical path length afforded by the I.D. of capillaries, which is in the micrometer range, is low, and it remains the major bottleneck of this technique (see