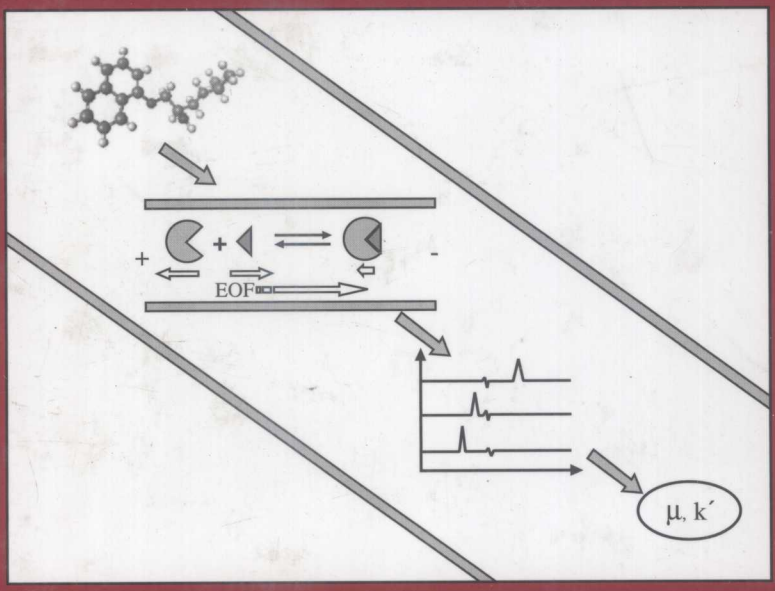
DRUGS AND THE PHARMACEUTICAL SCIENCES VOLUME 128

Affinity Capillary Electrophoresis in Pharmaceutics and Biopharmaceutics



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Preface

Since the pioneering accomplishments of Hjertén (1) and particularly of Jorgenson and Lukacs (2), capillary electrophoresis (CE) has undergone a dynamic development, producing a variety of applications. In chemical and pharmaceutical analysis, CE was employed mainly to separate and quantify drugs; this subject has recently been reviewed (3). The implementation of CE in quality control or drug profiling in biological systems has been illustrated in numerous studies. Capillary electrophoresis separations can be performed in different modes, using the same technical equipment. Capillary zone electrophoresis (CZE) and micellar electrokinetic chromatography (MEKC), introduced by Terabe et al. (4), are most frequently employed.

In recent years, much of the research work in the pharmaceutical sciences was focused on the development of effective vehicle systems, such as micelles, microemulsions, and liposomes, for drugs that are critical with respect to bioavailability. Knowledge of this subject is a prerequisite to developing vehicle systems for special administration routes, such as dermal, transdermal, intravenous, and nasal.

In pharmaceutics, therefore, simple and effective methods and procedures are needed to characterize the interactions of drugs with pharmaceutical excipients (polysaccharides, cyclodextrins, etc.) and vehicle systems (micelles, microemulsions, and liposomes) in order to optimize the load of vehicle systems with the drugs.

On the other hand, more and more research interest has been focused on the interactions of drugs and vehicle systems with biological target structures such as receptors (cells, proteins, nucleic acids, etc.). In recent years, interactions between proteins and nucleic acids were studied to generate artificial viral systems for gene therapy. Another interesting focus is the study of immunoreactions.

In biopharmaceutics, effective methods are strongly needed, not only to characterize the interactions of drugs and vehicles with biological structures in order to optimize pharmaceutical vehicle systems, but also to study the interactions between biological molecules and to investigate immunoreactions.

All experimental techniques used for measuring the affinity of one molecule for another are based on the measurement of changes in physicochemical properties of the drug, depending on the properties and the concentration of the interacting partner. Changes in size, charge, and other properties of the complex may result in measurable differences in molecular weight (size exclusion methods), sedimentation (ultracentrifugation), diffusion rate (immunodiffusion, equilibrium dialysis), spectroscopic properties (fluorescence quenching, spectral shift), and electrophoretic migration.

In recent years, classic CE has been modified in several ways. This book, therefore, first gives a brief introduction to the principles and techniques of CE (Chapter 1).

Affinity capillary electrophoresis (ACE) relates changes in the electrophoretic mobility of a drug (analyte) after complexation with a substrate (pharmaceutical excipient, vehicle system, and biological structure) present in the background electrolyte to the association constant K_A. The electrophoretic mobility of a molecule (drug) in free solution is proportional to its electrical charge, q, and inversely related to the hydrodynamic radius, r, which depends on the molecular mass, M. If the drug (injected as the sample) shows interaction with a substrate, its mobility should be shifted compared to the one obtained in free solution. A quantification is possible and leads to association constants. The principle of ACE as well as the methods for explaining and quantifying the results are described in Chapter 2.

Interaction equilibria, e.g., between drugs, excipients, vehicle systems, and biological structures, reflect the sum of interactions, which are nonspecific (hydrophobic) and specific (electrostatic dipole–dipole and dipole–induced dipole and hydrogen bonding). The soft method of ACE does not disturb the sensitive equilibria via any chemical modification.

In the past few years, the use of ACE in pharmaceutics and biopharmaceutics has expanded to the following areas:

To measure physicochemical and thermodynamical parameters of drugs

To characterize the affinity of drugs to pharmaceutical excipients (polysaccharides, other native and synthetic polymers, cyclodextrins, etc.) and vehicle systems (micelles, microemulsions, and liposomes)

To determine binding constants between drugs and biological structures (e.g., receptors, cells, peptide fragments), proteins (e.g., enzymes), nucleic acids, and plasmids

To characterize interactions between biologically relevant molecules, e.g., protein–protein and protein–nucleic acid interactions, as well as immunoreactions

The first part of this book presents theoretical basics necessary to understand the principles and techniques of CE as well as ACE. This knowledge opens access to potential applications in pharmaceutics, e.g., the investigation of interaction partners improving the solubility of lipophilic and barely water-soluble drugs and the determination of the effects of amphiphilic ion-pairing or complexation reagents (e.g., pharmaceutical excipients) on the permeation as well as absorption behavior of hydrophilic drugs. ACE enables the calculation of equilibrium constants, which are a measure of the strength of interaction. Although MEKC and ACE are based on the same principle, the recent literature (as well as this book) discusses these methods separately. However, MEKC can be considered a special case of ACE, differing only in the mode of mathematical description. In addition, the general calculation of association constants (K_A) and partition coefficients (K_P) is described.

Part II starts with the possibilities of ACE for characterizing the relevant physicochemical properties of drugs such as lipophilicity/hydrophilicity as well as thermodynamic parameters such as enthalpy of solubilization. This part also characterizes interactions between pharmaceutical excipients such as amphiphilic substances (below CMC) and cyclodextrins, which are of interest for influencing the bioavailability of drugs from pharmaceutical formulations. The same holds for interactions of drugs with pharmaceutical vehicle systems such as micelles, microemulsions, and liposomes.

Part III presents the methods based on ACE for studying interactions of drugs and pharmaceutical vehicle systems with biological structures such as receptors, proteins, polysaccharides, and nucleic acids. This part also describes and discusses methods for characterizing protein–protein interactions and immunoreactions.

Part IV covers the relevance of new combination (i.e., hyphenation) techniques such as CE-ESI- (electrospray ionization) MS (mass spectrometry) and CE-ESI-TOF- (time of flight) MS for ACE.

This book outlines the fascinating possibilities of the application of ACE and related technologies in the most interesting emerging fields of pharmaceutics (controlled drug delivery) and biopharmaceutics (drug targeting).

Reinhard H. H. Neubert

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