PART I

EXPERIMENTAL DESIGN AND OPTIMIZATION CONSIDERATIONS

CHAPTER 1

INTRODUCTION

GRADY HANRAHAN[1] and FRANK A. GOMEZ[2]
[1]Department of Chemistry, California Lutheran University, Thousand Oaks, CA
[2]Department of Chemistry & Biochemistry, California State University, Los Angeles, CA

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1.1. CAPILLARY ELECTROPHORESIS (CE): AN OVERVIEW

Over the past two decades, CE has become the technique of choice in many analytical laboratories where analysis of small quantities of materials must be accurately, efficiently, and expeditiously assessed. It is a powerful separation technique that brings much needed speed, quantitation, reproducibility, and automation to the inherently highly resolving but labor-intensive methods of electrophoresis (1–5). CE comprises a family of techniques including:

1. capillary zone electrophoresis;
2. capillary gel electrophoresis;
3. isoelectric focusing; and
4. micellar electrokinetic capillary chromatography.

All employ narrow-bore (e.g. 20–200-μm i.d.) capillaries (Fig. 1.1) to perform high efficiency separations for the analysis of biological materials and is an unparalleled experimental tool for examining interactions in biologically relevant media. A generalized experimental setup for CE is presented in Figure 1.2. As shown, the instrumental configuration is relatively simple and includes

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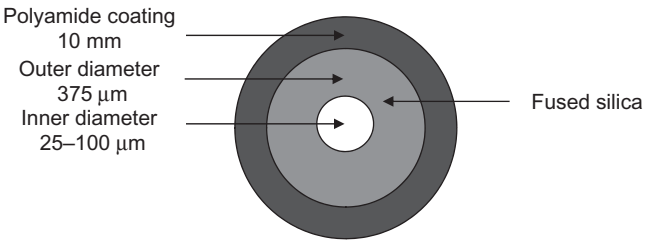

FIGURE 1.1. Fused silica capillary.

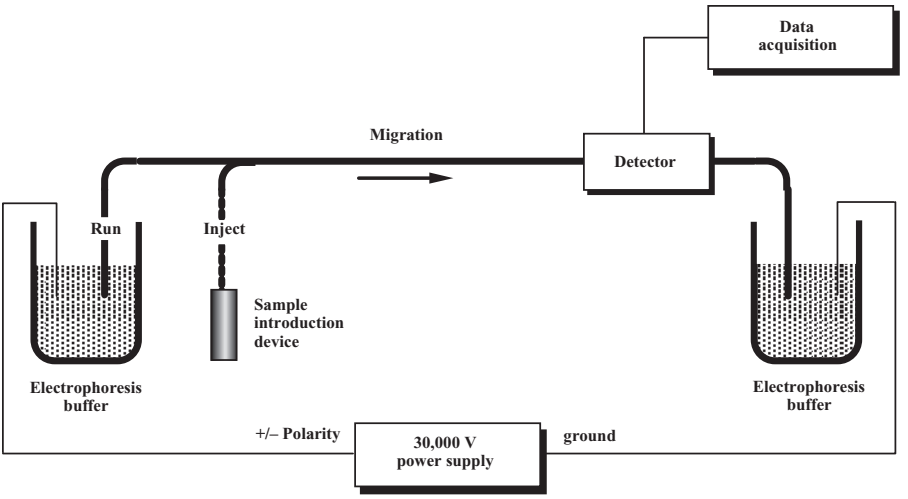

FIGURE 1.2. Generalized experimental setup for CE.

a narrow-bore capillary, a high-voltage power supply, two buffer reservoirs, a sample introduction device, and a selected detection scheme. Optical detection, typically absorbance (UV-visible) and laser-induced fluorescence, is employed. Signals are then transferred to a data acquisition module, which produces a representative electropherogram.

The underlying theory that governs electrophoresis is directly applicable to CE and can be explained by a variety of fundamental principles. CE differentiates charged species on the basis of mobility under the influence of an applied electric field gradient. Consequently, separation is reliant upon the difference in ion migration velocities expressed as:

$$v = \mu_e E \qquad \text{(Eq. 1.1)}$$

where v = the ion migration velocity (m/s), μ_e = the electrophoretic mobility (m^2/V/s), and E = the electric field potential (V/m). The latter is a function of

the applied voltage divided by the total length of the chosen capillary. Electrophoretic mobility is a constant proportionality between the ion velocity and the electric field potential (6) expressed as:

$$\mu_e = \frac{q}{6\pi\eta r} \qquad \text{(Eq. 1.2)}$$

where q = the energy of the ion, η = the solution's viscosity and r = the hydrodynamic radius of the ion. As evident in Equation 1.2, the differences in electrophoretic mobility are subject to differences in the charge-to-mass ratio of the analyte ions. For example, a higher charge and smaller ion mass will yield greater mobility. Due to the differences in mobility, it is possible to separate mixtures of different ions and solutes using electrophoresis (Fig. 1.3). Selectivity can be manipulated by the alteration of electrolyte properties including ionic strength, pH, electrolyte composition, or by incorporating electrolyte additives.

It is the high voltage source that facilitates separations, ultimately generating electroosmotic flow (EOF) of buffer solutions and ionic species within the capillary. EOF is defined by:

$$v_{eo} = \frac{\varepsilon\zeta}{4\pi\eta} \qquad \text{(Eq. 1.3)}$$

where ε = the dielectric constant, η = the buffer viscosity, and ζ represents the zeta potential of the capillary wall. The latter is the potential difference measured at the plane of shear close to the liquid–solid interface (7).

The surface charges of the liquid–solid interface play crucial roles in the EOF phenomenon. When a buffer solution is introduced into the capillary, the negatively charged wall attracts the positively charged ions from solution,

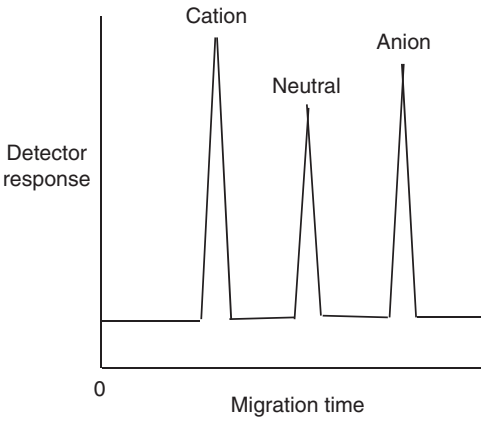

FIGURE 1.3. Separation of differing ions by CE.

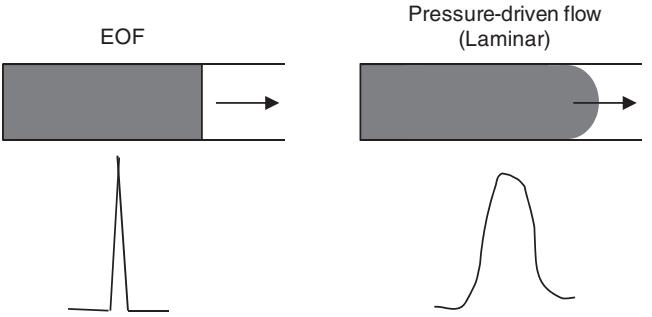

FIGURE 1.4. EOF and its generation of a flat flow profile alongside a parabolic laminar pressure-driven flow profile.

creating an electrical double layer (fixed and mobile) and a potential difference (zeta potential) close to the capillary wall. Accordingly, EOF mobility will vary with a change in the pH of the buffer solution. At pH > 7, the EOF mobility drives the net migration of the majority of ions toward the cathode (regardless of charge). As will be evident in subsequent chapters, the EOF must be controlled (or possibly suppressed) to run certain modes of CE. A beneficial feature of EOF is its generation of a flat flow profile alongside a parabolic laminar pressure-driven flow profile as typically seen in high performance liquid chromatography methods. This flat flow profile aids in minimizing zone broadening, ultimately allowing high separation efficiencies based on mobility differences as low as 0.05% (7). See representative diagram in Figure 1.4.

Indeed, there are a number of factors that must be considered for efficient and optimized separation, as well as in developing new methods to meet today's analytical challenges and routine laboratory needs. It is beyond the scope of this book to completely cover all theoretical aspects of CE. Complete coverage can be found in a variety of informative sources (6–9).

1.2. CHEMOMETRIC METHODS AND THEIR IMPORTANCE IN CE

CE offers a number of advantages as a separation technique: (i) it requires only small quantities of material; (ii) it is applicable to water-soluble, nonvolatile, high-molecular-weight species in aqueous buffer solution; (iii) it is readily automated and has good reproducibility; and (iv) various separation modes make it applicable for the analysis of a variety of biological and nonbiological species. Unfortunately, CE does suffer from a number of weaknesses. Adsorption of charged species to the capillary wall can occur in the absence of efforts to minimize adsorption and can change the magnitude of EOF. Overlapping peaks are a common occurrence, and methods devised to aid in separation are critical. The presence of Joule heating and other effects of using

high voltage create variances in EOF, sometimes yielding irreproducible migration times for analytes, making comparison from run to run problematic. This disadvantage can be especially troubling in the pharmaceutical industry where quality control is a priority and where method development is critical in product manufacture, analysis, and marketing. Ultimately, the search for optimum separation conditions in CE is often time-consuming and tedious. Therefore, the development and utilization of robust chemometric techniques in CE are favorable and a definitive source of information is vital.

Fortunately, various chemometric-based techniques, including multivariate experimental design and data analysis techniques, have been devised to aid in optimizing the performance of systems and extend their separation capabilities. In broadest terms, chemometrics is a subdiscipline of analytical chemistry that uses mathematical, statistical, and formal logic to (10):

1. design and/or select optimal experimental procedures;
2. provide maximum relevant chemical information by analyzing chemical data; and
3. obtain knowledge about given chemical systems.

Although statistical methodologies such as "curve fitting" and "statistical control" were used in analytical chemistry throughout the 1960s, it was not until 1972 that Svante Wold coined the term "chemometrics." The broad definition described above was shaped by the evolution of this subdiscipline over the past 35 years. The first known paper with chemometrics in the title was subsequently written by Bruce Kowalski in 1975 (11), which presented the value of pattern recognition concepts to the analytical community. The 1980s brought about an era of enhanced computing capabilities and more sophisticated analytical instrumentation, including the development of more advanced CE methods. The deluge of data generated by these multielement and multicomponent instruments required the application of chemometric methods already established, as well as creating a need for higher-level methodologies. Such methods were expressed to the scientific community with the advent of two specialized journals: *Chemometrics and Intelligent Laboratory Systems*, established in 1986, and *Journal of Chemometrics* in 1987. An increased number of investigators began incorporating chemometrics into their research activities in the 1990s. Brown et al., in a 1996 comprehensive review of chemometrics, reported over 25,000 computer-generated citations for this broad topic (12). In a 1998 review, Wold and Sjöström presented an informative look at the acceptance and success of chemometrics in modern analytical research (13). This paper illustrated how analytical chemistry is driven by chemometrics and describes state-of-the-art methods including multivariate calibration, structure–(re)activity modeling, and pattern recognition, classification, and discriminant analysis. The twenty-first century has brought about even greater analytical sophistication allowing automated, high throughput capabilities with low reagent and sample use. In a 2008 review, Lavine and Workman

describe the latest trends and acceptance of chemometrics in modern chemical analysis (14).

1.3. CURRENT AND FUTURE APPLICATION AREAS

In regard to CE, previous reviews and informative research papers provided systematic studies on early development efforts and use of experimental design methodology in CE (15–18). More recent papers have examined experimental design concepts and methods for data analysis in regard to CE applications in greater detail (19–25). The above list of citations is obviously not conclusive, but considering the information presented, it is obvious that chemometric methodologies are important tools in analytical chemistry, especially when considering modern CE applications. It is evident from the above papers and material presented in subsequent chapters that chemometric techniques are, and will continue to have, a profound effect on CE applications, including drug design, food technology, biomedical research, and environmental science. For example, microfluidics is one area where chemometrics has yet to be employed in earnest and where its integration will prove fruitful in the future. While the vast majority of papers in microfluidics have detailed elegant studies, optimization of parameters for a particular application has not been at the forefront.

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