Capillary Electrophoresis in Pharmaceutical Analysis

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Introduction

Capillary electrophoresis (CE) is a new instrument-based separations technology. Described by J.W. Jorgensen and K.D. Lukacs less than ten years ago, the first commercial capillary electrophoresis instruments were introduced in 1988 with shipments beginning in 1989. The first International Symposium based exclusively on capillary electrophoresis was held in Boston in April, 1989. The scientific program included 28 oral presentations and over 50 posters as well as an instrumentation exhibit. Over 400 scientists attended the symposium. The second symposium was held in San Francisco, January 29 - 31, 1990. This rapid commercialization and acceptance of the technology can be attributed to its similarity to other automated separations technologies such as HPLC and GC; and the development by the fiber optics industry of quartz capillaries coated with polyimide.

Based on applications reported in the past twelve months, it is clear that CE will play a major role in pharmaceutical analysis. As complementary to HPLC, CE is an exceptionally powerful analytical tool that expands the repertoire of techniques available to the pharmaceutical chemist.

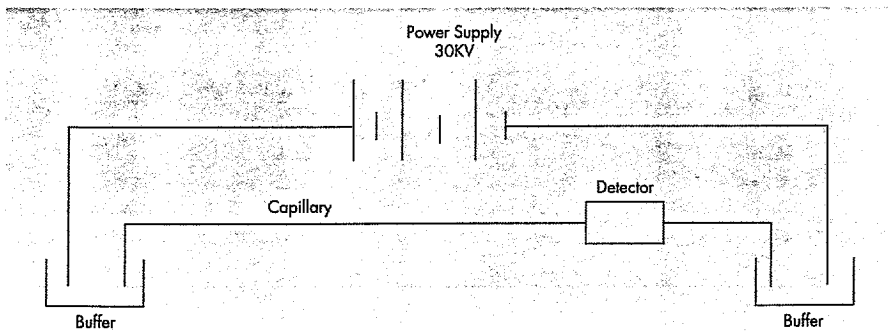
Basic Principles

When a potential is applied across the capillary (Figure 4), a net flow of fluid (electroosmosis) in the capillary occurs. Differential migration of solutes takes place depending on solute charge and average size in solution (Stokes' radius). Resolution and speed can be optimized by altering applied voltage, buffer composition, ionic strength, pH, adding micelles, and using gel filled tubes, or by chemical modification of the capillary wall. Detection is usually made using UV, fluorescence or conductivity.

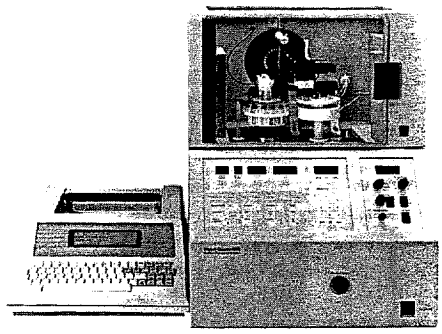
Sample Requirements and Sensitivity

In addition to its extremely high resolving power, CE requires only very small amounts of sample, 5-10 nL. This can be extremely beneficial when the analyst is sample limited. Detection limits for CE, have been reported in the attomole range (10^{-18} mole) for derivatized amino acids. And while most interest in CE can be currently traced to its ability to solve the most difficult of separation problems, its attractiveness as a quantitative, rugged and easy-to-use technique should not be overlooked. In a short period of time CE has proven to be a reproducible and reliable technology.

Figure 4: Basic CE System Diagram



Waters New Quanta 4000 Capillary Electrophoresis System

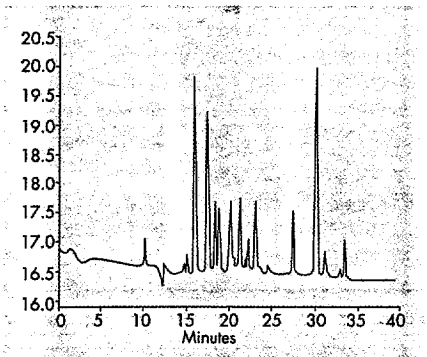


The electropherograms of a tryptic digest of cytochrome c in Figures 5 and 6 illustrate the incredible efficiencies obtained by capillary electrophoresis. Notice that the electrolyte is not changed during the separation. Usually when HPLC is applied to complex samples and matrixes such as these, one has to use gradient elution chromatography in order to obtain similar results.

The applications of capillary electrophoresis are broadbased. Today's focus on the separation of biopharmaceuticals is already expanding to small molecules as the applications and quantitative reproducibility of the methods are demonstrated.

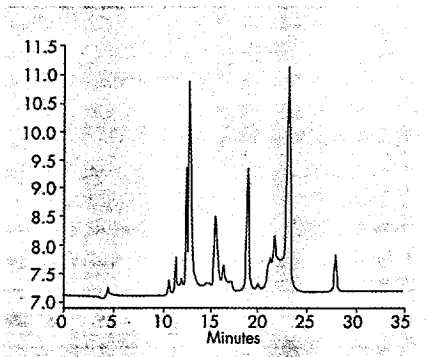
(continued on page 6)

Figure 5: CE Separation of Chicken Cytochrome c Using 100 mN Phosphoric Acid



Buffer: 100 mN Phosphoric acid.
Voltage: 12000 V.
Current: 84 µA.
Capillary: 75 µm x 60 cm fused silica.
Absorbance: 214 nm.
pH: 2.0.
Separation: Conducted at constant voltage.
Instrument: Waters Quanta™4000 Capillary Electrophoresis System

Figure 6: CE Separation of Chicken Cytochrome c using 50 mM sodium phosphate at pH 7.0



Buffer: 50 mM sodium phosphate at pH 7.0 containing 10% acetonitrile.
Voltage: 14000 V.
Current: 60 µA.
Absorbance: 214 nm.
Capillary: 75 µm x 60 cm fused silica (Polymicro Technologies, Phoenix, AZ).
pH: 7.0.
Separation: Conducted at constant voltage.
Instrument: Waters Quanta 4000 Capillary Electrophoresis System.

Conclusion

Capillary electrophoresis provides the pharmaceutical chemist with a powerful analytical tool. The very high separation efficiencies make it an ideal technique for solving difficult problems like chiral separations and for the determination of impurity profiles. The selectivities of CE complement those most commonly found in HPLC and as such provide the analyst with a powerful combination of techniques for the analysis of complex mixtures of molecules and the determination of absolute sample purity.

For more information on Waters new Capillary Electrophoresis system be sure and mark number 6 on the Business Reply Card.

Improved Chromatographic Selectivity Using WISE™ Software

by Vince Warren, Senior Applications Chemist

Introduction

The Waters Interactive Selection of Eluents (WISE™) software package was first introduced for the 840 Data and Chromatography Control Station at the 1988 Pittsburgh Conference in New Orleans. With the release of Expert Ease version 2.2, this software option will be available to all Waters 845 and 860 users as well. In fact, version 2.2 of Expert Ease* will include a demonstration version of WISE software, providing all users with an easy way to gain familiarity with the capabilities of this software product option.

WISE software is an interactive approach to the optimization of eluents for isocratic LC methods, with a focus on improving chromatographic selectivity to meet the chromatographer's requirements.

*Compatible with DEC/VMS® operating system.

The WISE software strategy can be applied to a wide variety of eluent blending problems, providing an extremely versatile tool which can assist with the optimization of pH, temperature, additive (e.g., buffer salt or PIC® Reagent) concentration or modifier composition for aqueous/organic eluents. Normal phase as well as reverse phase eluents can be optimized using WISE software.

In addition to the diversity of eluent variables which can be addressed, WISE software offers several advantages over other comparable approaches. To keep the optimization process efficient, the WISE strategy uses a minimal number of starting experiments (typically 2 or 3) and then moves forward in a self-correcting manner to locate the optimal blend of the starting eluents. As each new experiment is added, the new retention information is used to update the retention model from which all WISE software predictions are calculated. The total number of eluent blends which must be tested in order to achieve an accurate prediction of the optimum varies with the complexity of the separation problem, but usually it is six or fewer.

As the optimization progresses, WISE software keeps the user informed not only of the latest predictions, but of the reasoning behind these. For clarity, easily understood graphical displays are used to summarize the available information. The Eluent Selection Diagram (ESD) (refer to Figure 8) presents the current retention model along with plots of the user-selected quality criteria. In addition, simulated chromatograms are available for the predicted optimum and for the suggested next experiment. When appropriate, WISE software also advises whether the optimization process should be continued or ended. The ESD, which is central to the WISE