ANALYTICAL TRAINING SOLUTIONS

Premier Training for Analytical Chemists

Introduction to Capillary Electrophoresis – Fundamentals and Operating Principles

Covers the essentials that every scientist needs in order to understand the underlying principles of CE, the instrumentation and equipment used, and how best to perform the technique.

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WHO SHOULD TAKE THIS COURSE?

If you use capillary electrophoresis (CE) in your work and want a better understanding of the technique, or want to start with CE and want to be well prepared, this course is for you. The course is designed for analytical scientists and technicians who use CE in their regular job, but also lab managers and quality assurance or regulatory people who need to review CE work might find it useful. No previous CE training is assumed, however, much of the course will also appeal to the experienced scientist who wants a firm ground in the basics of CE.

WHAT DOES IT COVER?

"Introduction to Capillary Electrophoresis" explains the fundamentals of CE from the ground up. The course comprises:

- The basic principles of capillary electrophoresis
- Ten different modes of CE, including CZE, CGE and cIEF
- Equipment and operational details
- Treatment of the capillary, the separation column
- Electrokinetic and hydrodynamic injection
- Multiple detection mechanisms
- Corrected peak areas explained
- How to get started with method development and how to predict the migration order
- Tips for successful implementation of CE in your lab

WHAT WILL I GET FROM THIS COURSE?

After this course, you will understand the fundamentals of capillary electrophoresis and many of its working modes. What you have learned will demystify the secrets of CE. With better understanding of the different CE modes, you will be better able to select the proper one for your analytical question. You have acquired many tips on and understanding of instrumental parameters so that your next CE method development will be more successful. You will find that perplexing and frustrating issues you experienced have simple and logical solutions and you will have learned to prevent them. And you will learn all that is needed for successful implementation of CE in your lab.

INSTRUCTORS

This course is taught by Cari Sänger – van de Griend. Cari has practised capillary electrophoresis for more than 25 years. She has 20 years experience in the pharmaceutical industry and founded her own consultancy four years ago. She is a globally recognised expert on capillary electrophoresis with a strong focus on implementation, knowledge transfer and good working practices.

Cari is Associate Professor in Analytical Pharmaceutical Chemistry at the Faculty of Pharmacy, Uppsala University and Adjunct Senior Lecturer at ACROSS, University of Tasmania. She is the author of the series "CE Solutions" in Separation Science's digital learning platform.

COURSE DETAIL

Below are listed brief summaries of each module of the course, together with the approximate running time of that module.

Section 1. Introduction

Introduction: History of Electrophoresis (20 min)

A summary of the history of electrophoresis and how electrophoresis developed into the capillary electrophoresis we use today.

Section 2. Capillary Electrophoresis Basics

Electrophoresis (10 min)

The fundamental driving and friction forces of electrophoresis are discussed and the electrophoretic mobility introduced.

Electro-osmosis (20 min)

The mechanism behind the electro-endosmotic flow (EOF) is explained and the effect of the EOF on the separation accessed. The total or apparent mobility is introduced as the sum of the electrophoretic and electro-osmotic mobility

Band broadening (15 min)

The maximum efficiency of CE under ideal situations is calculated. Several causes for band broadening are introduced and explained, among which electromigration dispersion, and ways to keep band broadening limited.

Key measurements (10 min)

Resolution is discussed from a theoretical and a practical perspective. Analyte mobility, velocity and plate numbers are calculated.

Section 3. Equipment & Operation

Instrumentation (15 min)

The components of a CE instrument are introduced and explained, together with tips on good working practice and regular maintenance

The Capillary (20 min)

The use of fused silica capillaries is explained. Practical operation aspects such as capillary cutting and rinsing are discussed, as well as the consequences of improper cutting and rinsing. Different types of capillary coating are introduced and the effect on the electro-osmotic flow explained.

Injection (20 min)

Hydrodynamic and electrokinetic injection are introduced and the advantages and disadvantages discussed. Good injection practice and stacking possibilities are explored in order to increase precision and sensitivity.

Detection (15 min)

Multiple detection principles are discussed including several tips for improving sensitivity for UV/DAD detection. Quantification by corrected peak areas is demystified.

Separation Medium: The Electrolyte (20 min)

The electrolyte is the heart of the separation in CE, but is also a major parameter for robustness. The need for buffers and how to access and increase buffering capacity are explained, as well as the consequences of poor buffering. This module also shows how to describe the electrolyte recipe and gives starting conditions for method development. The difference between a theoretically nice separation and a pragmatic and robust method are discussed.

Section 4. The CE Modes of Operation

Most of the fundamentals discussed up to now consider capillary zone electrophoresis, CZE. In the next section we introduce many other different modes in CE that complement in the possibilities the CE techniques can offer.

MEKC and MEEKC (20 min)

Micellar electrokinetic chromatography (MEKC) and micro-emulsion electrokinetic chromatography (MEEKC) were introduced to be able to analyse neutral or mixtures of charged and neutral analytes. The build-up of micelles and micro-emulsions are explained as well as the mechanism of separation, which combines electrophoresis with chromatographic partitioning.

Chiral CE (15 min)

Chiral analytes can only be separated in a chiral environment. In CE this is relatively easy, as chiral selectors can be dissolved in the electrolyte. As an additional advantage, the concentration of selector can be varied. Key parameters for chiral separation and chiral selector choice are discussed and starting conditions for method development given.

Capillary Gel Electrophoresis CGE (20 min)

Because traditional gel electrophoresis have been very well-established, there was a wish to translate these methodologies to the capillary format. DNA-sequencing is probably the most applied CE application today. Within the biotech industry the CE-SDS kit, a translation of SDS-PAGE, is popular. The mechanism is explained as well as some good working practice details.

Capillary isoelectric Focusing cIEF (15 min)

cIEF is not only popular for determination of the pI value of a protein, but it is also being used for identity, purity and stability testing within the biotech industry. The build-up of the pH gradient with ampholyte and its effect on the resolution are explained. Both imaging cIEF and detection after mobilization are discussed.

Other Modes of Capillary Electrophoresis (15 min)

In this module capillary isotachophoresis, affinity CE, immunoaffinity CE, non-aqueous CE and capillary western blotting are introduced. Together with the already discussed modes and with the potential of CE-MS and physic-chemical characterization with CE, the CE instrument offers a wide range of possibilities. Provided one has experience and well-trained people, almost any separation problem can be solved and robust methods developed.

Section 5. Concluding Remarks

Concluding Remarks (10 min)

The use of CE within industry is discussed as well as some prerequisites for the successful implementation of the CE techniques. Key is good documentation and knowledge transfer, on top of good training.

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