High Performance Capillary Electrophoresis (CE)

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Principles of CE

CE - electrophoresis performed in a capillary tube.

Separation by electrophoresis is based on differences in solute velocity in an electric field: $v = \mu_e \times E$.

The mobility $\mu_e$ is determined by electric force that the ionized species experiences $F_E = q \times E$, which is balanced by its frictional drag though the medium $F_F = 6\pi \eta r v$ ($\eta$-solution viscosity, $r$-ion radius). During a short transient period, when the two forces are balanced—a steady state is attained. At this point: $\mu_e = q / 6\pi \eta r$.

From this equation it is evident that small, highly charged species have high mobilities whereas large, minimally charged species have low mobilities.
Electroosmotic flow (EOF)

The diagram shows the direction of electroosmotic flow (EOF) between an anode and a cathode. The EOF is caused by the movement of charged ions in a solution under the influence of an electric field. The figure illustrates the movement of ions under EOF conditions, with the anode at the positive end and the cathode at the negative end. The diagram also includes a micrograph of the EOF effects, indicating the movement of ions within the electric field.
Zone broadening

• Longitudinal diffusion- spreading of the solute zone while migrating- **limits efficiency**
• Joule heating- heat generated by the passage of an electric current through a conductor- **leads to temperature gradients and parabolic flow velocity profiles**
• Electrodispersion- mismatched conductivities of sample and buffer- **solutes with lower conductivities than the running buffer result in tailed peaks**
Characteristics of CE

- CE is performed in narrow bore (25-75 μm) fused silica capillaries.
- High voltages (10 to 30 kV) and high electric fields (100 to 500 V/cm) are applied across the capillary.
- High efficiency (N >10^5 to 10^6) and short analysis time.
- Detection performed as in HPLC: on-capillary (DAD, CCD) or on-line MS.
- Small sample volume required (1 to 50 nL injected).
- Small buffer consumption.
- Numerous modes to vary selectivity and wide application range.
- Operates in aqueous and non-aqueous media.
- Simple methods development.
- Automated instrumentation.
Instrumentation

Source: Agilent Technologies, High Performance Capillary Electrophoresis
Modes of operation: CZE

In CZE capillary is filled with electrolyte (run buffer), the sample is introduced at the inlet and the voltage is applied. As seen from the picture, separation occurs because solutes migrate at different velocities and in discrete zones. One limitation of CZE is its inability to separate neutral species.

Source: Agilent Technologies, High Performance Capillary Electrophoresis
Micellar electrokinetic chromatography MEKC

Individual surfactant molecules form micelles above CMC. The micelles are usually charged, anionic surfactants such as SDS migrate toward the anode that is in the opposite direction of the EOF. As the EOF velocity is larger than that of micelles (at the neutral or basic pH), they also move toward the cathode. During the electrophoretic migration the neutral species are partitioning in and out of the micelle. The stronger the interaction with the micelle the longer its migration time, since the micelle carries it against the EOF. The more hydrophobic compound interacts more strongly with the micelles and is retained longer.

It is differential interaction between micelles and neutral molecules that causes the separation.
Capillary gel electrophoresis CGE and Capillary isoelectric focusing CIEF

CGE is a CZE performed in a polymeric gel medium. CGE is useful for the separation of molecules with similar electrophoretic migration rates in solution due to their similar charge-to-mass ratios (DNA fragments). The polymer network inside the capillary serves as a molecular sieve in which smaller molecules migrate faster than large. The capillary format offers a number of advantages: 1. use of 10x to 100x higher electrical field (without the deleterious effects of Joule heating) 2. on-capillary detection 3. automation 4. use of “newly” created gels.

CIEF is used for separation of amphoteric substances such as proteins, peptides, amino acids on the basis of their isoelectric point (pI). In CIEF after filling the capillary with a mixture of ampholytes and solutes, the pH gradient is formed.

With a basic solution at the cathode and an acidic solution at the anode, upon application of the electrical field the charged ampholytes and proteins migrate through the medium until they reach a region where they become uncharged (pI point). This process is known as focusing.