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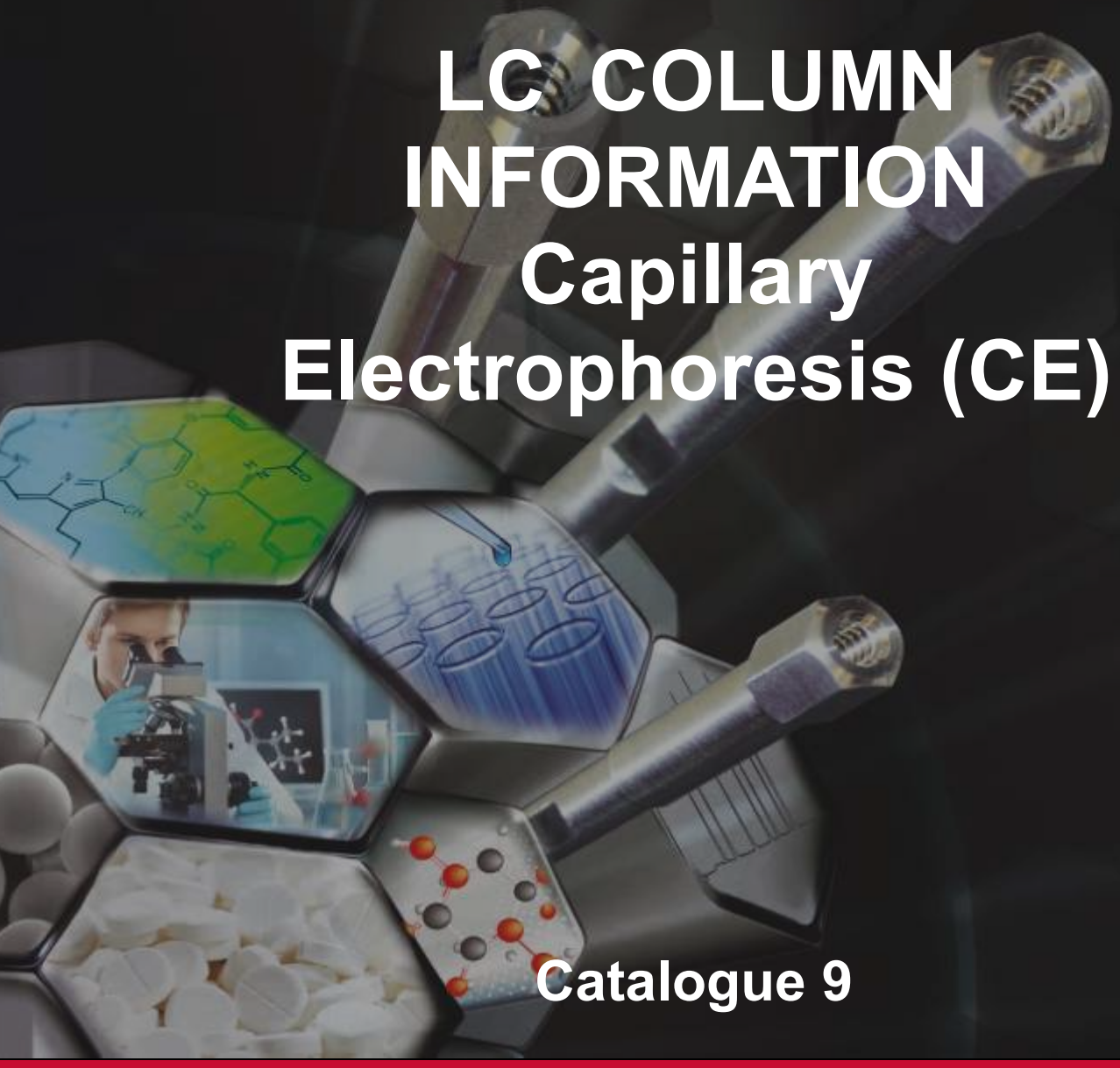
Chromatography Columns and Supplies

LC COLUMN INFORMATION Capillary Electrophoresis (CE)



Catalogue 9

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CAPILLARY ELECTROPHORESIS (CE)

Separation in capillary electrophoresis (CE) is achieved by the differential migration of solutes in a narrow fused silica capillary by the application of an electric field. The technique developed from a combination of various electrophoresis and chromatographic techniques. The separation mechanism is mainly based on differences in solute size and charge at a given pH. Different modes of capillary electrophoretic separations can be performed using a standard CE instrument.

CE Instrumentation

Figure 1 shows a simplified schematic diagram of a typical CE instrument. This consists of a high voltage power supply, two buffer reservoirs, a capillary, detector and output device. Each side of the high voltage power supply is connected to an electrode. The capillary is made of fused silica (typically 25 - 75µm i.d. and 0.5 to 1.5m in length) and is usually coated externally with polyimide. Each end of the capillary is dipped in a vial containing the electrode and aqueous buffer. For UV detection, the capillary has a small window near the cathodic end which allows UV-VIS light to pass through the analyte and measure absorbance. Capillaries used for MS detection do not require this window. Other common detection modes include MS and fluorescence.

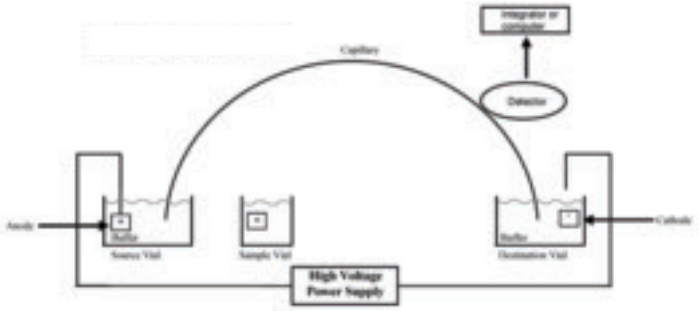

Figure 1. CE instrumentation

Principle

CE separates ions according to their electrophoretic mobility, which takes into account the charge and hydrodynamic size of the molecule and eluent viscosity. The actual migration of an ion is also affected by the level of voltage applied. The most important parameter in CE is the electro-osmotic flow (EOF) which is the bulk flow of liquid in the capillary as a consequence of the surface charge on the interior capillary wall. EOF forms the mobile phase 'pump'. An unbonded fused silica capillary (at pH >3) contains deprotonated silanol (SiO^-) ions on the interior surface. The capillary wall then develops a double layer of cations attracted to it. The inner cation layer is stationary, whilst the diffuse outer layer can move along the capillary. Under the applied electric field, the cations move towards the cathode, creating a bulk flow, due to the EOF. Anions in solution, although attracted to the anode, get swept along to the cathode as well. In general, cations will separate first, followed by neutrals and then anions. By chemically coating the internal surface of the fused silica tubing by cationic groups (e.g. surfactant), the direction of flow is reversed and is independent of pH.

CE vs HPLC

- CE has a flat flow profile due to the EOF, which does not contribute significantly to band broadening and results in narrower peaks. HPLC has a parabolically shaped pressure-driven flow profile (see Figure 2).
- CE has greater peak capacity compared to HPLC
- HPLC has more complex instrumentation
- CE requires smaller injection volumes, typically 1-50nl
- HPLC has a wider range of column lengths and packing materials

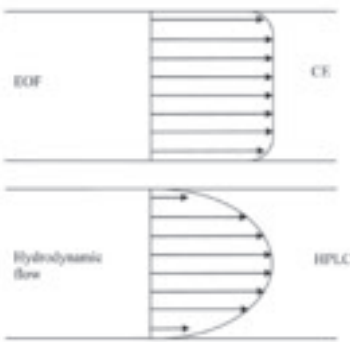

Figure 2. Comparison of CE and HPLC flow profiles

Applications

Typical applications of CE include the analysis of proteins, peptides, amino acids, nucleic acids, inorganic ions, organic bases and organic acids.

Figure 3 shows the separation of inorganic ions on a Funcap-CE/Type A capillary.

A selection of capillaries and accessories for CE is offered by the following manufacturers:

GL Sciences (FunCap) – see p.298
MicroSolv (Simplus, Celerity etc) – see p.298

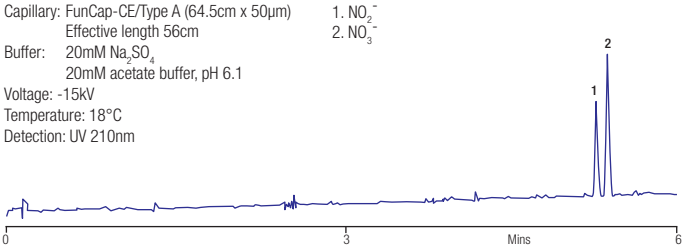

Figure 3. Analysis of inorganic ions