Capillary Electrophoresis

A small band of sample is first introduced into a narrow tube (e.g. capillary tube of 10-100 μm i.d. and 40-100 cm long) or on a flat porous support medium (e.g. gel) which contains an aqueous buffer solution, and then its components are separated when a high dc potential is applied.
Electrophoresis is the migration or electrophoretic flow (EFP) of charged species in a dc electric field. EPF of ions depends on their charge-to-size ratio.

$$qE = 6\pi\eta r v_{ep} \qquad m_{ep} = \frac{v_{ep}}{E} = \frac{q}{6\pi\eta r}$$

q is affected by pH for some analytes (Fig. 14); separation may be improved by altering the pH (Fig. 7).
Arne Tiselius was awarded the 1948 Nobel Prize in Chemistry for his work on electrophoresis.

Slab (gel) electrophoresis: classic, still widely used, like TLC but the slab is horizontal and a dc potential is applied.
Capillary electrophoresis: instrumental version, highly automated, efficient heat dissipation, allows the use of high voltage, fast, use quantitative HPLC detectors, small sample volume (e.g. 0.1-10 nL), high resolution (open-tubular).
A CE system consists of the inlet and outlet buffer vials, high voltage supply, capillary tube and detector (Fig 30-1 or R5 Fig 3.25). In microfluidic CE chips, the capillary tube is obtained by sealing HF-etched channels with a cover plate, and the inlet and outlet buffer vials are obtained by drilling holes in the cover plates.

Electroosmotic flow (EOF)
In an uncoated fused silica capillary, the solvent migrates from anode to cathode. It is because an electrical double layer is formed between the negatively charged capillary wall and the cations in the solution (Fig 30-2 or equiv). The potential difference across the double layer is called zeta potential, ξ. The molecules solvating the diffuse layer of cations are dragged toward the cathode. The negative charges (and hence ξ) increase with pH and hence a buffer is needed to control the electroosmotic flow (see figure).

$$v_{eo} = \frac{\varepsilon z}{4\pi\eta} E$$

Electroosmosis leads to a bulk solution flow that has a flat profile, rather than a parabolic one as in HPLC (Fig 30-3 or equiv). So band broadening due to flow profile is less in CE than in HPLC (R6 Fig 26-14).

$$u = m_{eo} E = m_{eo} \frac{V}{L} \qquad v = (m_{ep} + m_{eo}) E$$

Usually m_{eo} is larger in magnitude than m_{ep}, so that all positive, neutral and even negative species migrate toward the cathode (Fig 30-4 or equiv).

The migration order is fast cations, slow cations, all neutrals, slow anions and fast anions (See Fig. 2.1 & 2.2 and animation).

Separation effficiency in CZE
Only longitudinal diffusion effect affects band broadening (c.f. open-tubular column).

$$N = \frac{V}{2D}\left(\frac{l}{L}\left(m_{ep} + m_{eo}\right)\right)$$

Note that N increases by increasing V (See Fig).

V is limited to 500 V in slab gel electrophoresis, high resistance (low Joule heating) and high surface-area-to-volume ratio (great power dissipation) in CE increase the operating voltage to 20-30 kV. N is 100-200k in CE and 5-20k in HPLC (R6 Fig 26-14).

CE instrumentation

Sample introduction

Normal injection of 5-50 nL into a 4-5 μL capillary tube can be achieved by 2 ways:

1. Electrokinetic injection: when the inlet capillary end is dipped into a sample vial, a potential, V, is applied for a measured time, t, the sample enters the capillary due to EOF and EPF. This is a simple procedure but faster migrating species are injected in a greater amount.

   $$v = u \cdot t \cdot A = m_{app}\left(\frac{V}{L} \times \frac{k_b}{k_s}\right) \cdot t \cdot \left(\frac{\pi d^2}{4}\right)$$

   ν depends on μ_{app}, leading to differential injection.

   ν also depends on κ_b/κ_s, leading to sample stacking

2. Pressure (or hydrodynamic) injection: when the inlet capillary end is inserted in a sample vial, the sample enters the capillary due to a pressure difference, ΔP, created by various means (suction applied at the outlet end, pressure applied at the inlet end, or elevation of the inlet end). There is no differential sample introduction, but additional instrumentation is required and this method cannot be used in CGE.

   $$v = Q \cdot t = \left(\frac{\Delta P \pi d^4}{128 \eta L}\right) \cdot t$$

Detector

Most HPLC detectors can be used.

Absorbance detector

A small section of the protective polyimide coating (brown) is removed to create a window (clear) for on-column detection. The mass detection limit is high, but the concentration dl is worse than in HPLC because of a limited path length (note the capillary i.d. of 50-100 μm).

Enhanced path length can be achieved by 3 ways, namely Z-cell, bubble cell and multireflection cell (Fig 30-5). (See animation)

Fluorescence detection

Just as in HPLC, this produces enhanced sensitivity because of low background signal. Laser-induced fluorescence (LIF) is preferred because signal depends on the excitation power (Fig 15-4 or equiv). (See animation)

Indirect detection

For detection of non-absorbing analytes (e.g. inorganic anions), an ionic chromophore (e.g. chromate) is placed in the buffer (Fig 30-6). The detector then receives a constant background signal which will be decreased to produce negative peaks when an analyte displaces the chromophore (R6 Fig 26-23 or equiv).

Electrochemical detector

Conductometric and amperometric detectors have been used. The problem of electric potential isolation between the high potential at the cathode (CE) and the low potential at the working electrode (EC detector) is alleviated using a porous glass or graphite joint between the capillary end and a second capillary containing the detector electrodes (R6 Fig 26-22).

MS detector

The extremely small flow rates in CE (< 1 μl/min) is compatible with what can be handled by the MS ionization source.

The common sample introduction/ionization interface is electrospray (Fig 30-7). A high potential (e.g. 5 kV) is applied between the metallized capillary tip and the interface to charge the electrospray. In the mass spectrum (Fig 30-8), doubly-charged species of the 9 peptides is apparent. This allows high MW biomolecules to be detected at a low m/z region that can be offered by the mass analyzer (e.g. quadrupole).

CE modes

Various CE modes exist, namely CZE or FSCE, CGE, ECC (electrokinetic chromatography), CITP (isotachophoresis) and CIEP (isoelectric focusing).

CZE

This is used for separation of small ions and molecular ion species (Fig 30-9a or equiv). To decrease analysis time, it is better to have the analyte ions to move in the EOF direction. For cations, the negatively charged surface allows the EOF to flow from anode to cathode. Cationic analysis competes with AA and ICP methods in terms of cost, time, resolution and sample size.

To reverse the EOF direction, a cationic surfactant can be used to adsorb on the capillary wall to produce a positively charged surface (R6 Fig 26-18 or Fig. 18C). Now the molecules solvating anions are attracted toward the anode. This allows the analysis for anions in the shortest possible time. Anionic analysis competes with IEC.

For protein analysis, we may want to make the capillary surface neutral to avoid protein adsorption (Fig. 18B)

CGE

This is performed in a porous gel polymer matrix. Other than the historical reasons to use the gel to reduce diffusion and convection, the gel has the additional advantage of having the molecular sieving action to separate macromolecules (e.g. DNA fragments or oligonucleotides) which have similar mass-to-size ratios (Fig 30-14 or equiv). EOF is not desirable in CGE. So it is suppressed by coating the capillary wall with a neutral species (e.g. trimethylchlorosilane) (Fig. 18B). Commonly used gels include polyacrylamide (by polymerizing acrylamide CH_2=CHCONH$_2$) and agarose (natural).

Capillary Electrokinetic Chromatography

CEC, which is the hybrid of CE and HPLC, offers the advantages of

1. like HPLC, it separates uncharged analytes
2. like CE, it requires only small sample size, has greater separation efficiency, and does not need high pressure pumping system.

Miceller ECC (MECC)

Surfactant (e.g. SDS) when added at a high concentration (CMC) to the CE buffer will aggregate (40-100 ions) and form micelles. Micelles formed from SDS have a negative charge and will migrate more slowly than the MP toward the cathode in an uncoated capillary. These micelles constitue a pseudo SP and allow retention of non-polar (neutral) analytes to be partitioned between it and the MP (R6 Fig 26-

26 or equiv). The analytes should migrate faster than the micelles but slower than the EOF marker (e.g. water or methanol)
This type of chromatography offers a much higher column efficiency than HPLC (N= 100 k or more). In addition, changing of SP in MECC is much simpler than in HPLC.

Cyclodextrin-ECC
CD (α-CD, β-CD or γ-CD) constitutes the selectivity in the mobile phase. The non-polar analytes then partition between the pseudo SP (i.e. CD) and MP (see Figure). Since CD is chiral, it will provide chiral separation of enantiomers (Fig 10).

CD-MECC
In some applications, we use both CD and micelles to improve separation (R6 Fig. 26-27)

Microfluidic CE chips
A network of capillaries is first fabricated on one glass substrate which is sealed by bonding with a cover plate. Liquid access is provided through drilled holes on the cover plate. The capillary network is micromachined using the technique of photolithography and wet HF etch. (Harrison et al, Science, 261, 1993, 895-897 and Anal. Chem. 66, 1994, 177-184).
High electric potential is applied for both sample loading, injection and separation. LIF is usually employed for detection because of short path length in microchannels (i.e. 30 μm×10 μm).
Post-column o-phthalaldehyde (OPA) derivatization of separated compounds can easily be carried out in the capillary network.
Exercises: 30-1 to 6