Capillary Electrophoresis Applications

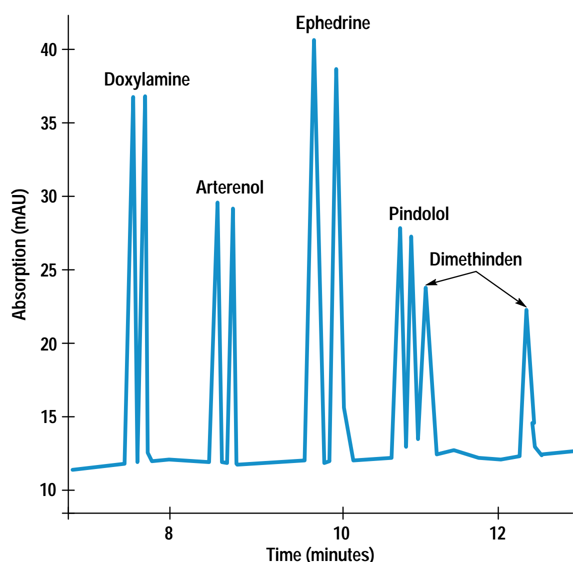
Capillary electrophoresis was initially regarded as an analytical separation tool for proteins and peptides. Its characteristics imply that biomacromolecules theoretically should take the biggest advantage of the technique. However, it has turned out that more then a decade after the birth of the technique the applications have spread into many more areas than just the bioscience area. In fact, for many proteins it has proven to be a bit of a problem to get a separation with the required high sensitivity using the fused silica columns. All in all this has not hindered the growth of the technique. When the first commercial instruments became available in 1990 the market was estimated to be several million dollars in size. In 1994 the expected market size might very well reach 50 million dollars. The main user groups of CE are found in the pharmaceutical market (both traditional and biopharmaceuticals), the bioscience market, and the chemical industry (see Table I).

Although still mainly in use in R&D laboratories, the technique is definitely migrating towards controlled analytical laboratories such as QA/QC and product testing labs. This indicates that the technique does offer unique benefits and can expect sustained growth in the future.

Table I
CE Users

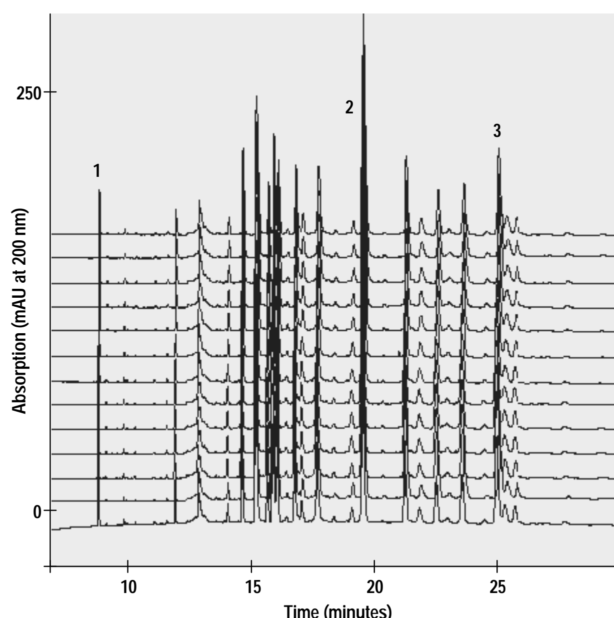
Market Segment	Estimated Share (%)
Pharmaceutical Industry	35
Bioscience	35
Chemical Industry	20
Food/Beverages	5
Others	5

Some successful applications of CE include:
- Analysis of optical impurities (chiral analysis) (see Fig. 1)
- Tryptic digest analysis of recombinant biopharmaceutical drugs (peptide mapping) (see Fig. 2)
- DNA analysis (e.g., PCR product analysis) (see Fig. 3)
- Organic acid analysis (e.g., in beverages) (see Fig. 4).



Sample:	Chiral Mixture
Buffer:	20 mM citrate, pH 2.5, 2% Carboxymethyl-β-CD
Capillary:	L_{eff} = 56 cm, L = 64.5 cm, i.d. = 75 μm
Injection:	200 mbar · s
Electric Field:	300 V/cm
Detection:	Signal 214.20 nm, Ref. 450.80 nm
Temperature:	Capillary 20°C

Fig. 1. Capillary electrophoresis (CE) separation of a mixture of basic chiral drugs using cyclodextrin as chiral selector.



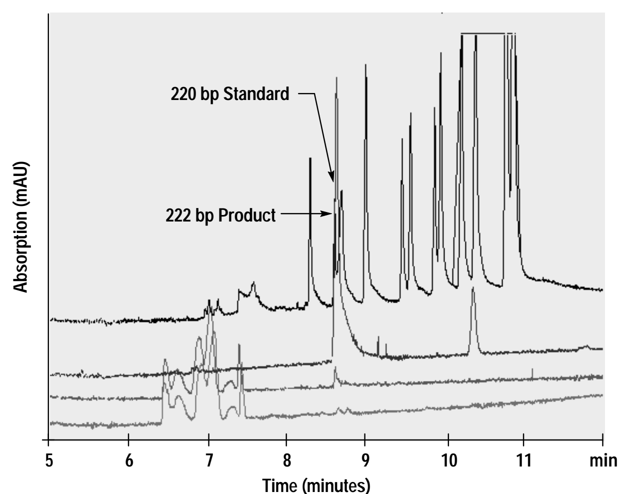
Reproducibility (%RSD)

	Peak 1	Peak 2	Peak 3
Migration Time	0.36%	0.60%	0.33%
Area	1.63%	1.89%	2.09%*

Fig. 2. Repetitive separation of a tryptic digest of recombinant human growth hormone by CE.

providing spectral information. This type of detector has several inherent features that have proved useful for many applications:

- Identification. Absorption spectra make it possible to positively identify substances by their spectral “fingerprints.” The HP ChemStation controlling the instrument has a built-in spectral library. Library searches can be performed, resulting in suggestions of substances that have similar spectra. They are ranked according to a computed match factor.
- Confirmation. Spectra created by liquid chromatography separations and capillary electrophoresis separations for the same substance are, with a few exceptions, identical. Based on this, the spectra obtained with these two separation techniques are confirmatory or redundant to each other for a given sample constituent. Since the two separation techniques have different separation mechanisms it is very unlikely that a sample constituent or impurity will be missed by both LC and CE.



pBR328-Hinf 1

1.5% LPA, 6% LPA-coated
TBE pH 8.3
20 kV, 13 μA
L_{eff} = 5.6 cm, i.d. =75 μm, BF = 3
Inj: –10 kV, 3 s
260 nm (Ref 350 nm)
Capillary: 25°C
Carousel: 10°C

Fig. 3. Separation of PCR products using capillary gel electrophoresis.

Table II
CE Applications and Benefits

Application	Other Analysis Methods	CE Benefits
Chiral Analysis	HPLC, GC, TLC, SFC	Speed Easy Method Development Cost of Analysis
Peptide Mapping	HPLC	Speed Orthogonal Mechanism
DNA Analysis	Slab Gel Electrophoresis, HPLC	Superior Resolution Speed Online Quantitation

All of these applications have in common that CE offers significant benefits over previously existing techniques (see Table II).

The future outlook for CE is positive although further development of capillaries suitable for protein analysis under native conditions and the development of other detection modules such as CE-MS will be important for long-term establishment of the technique.

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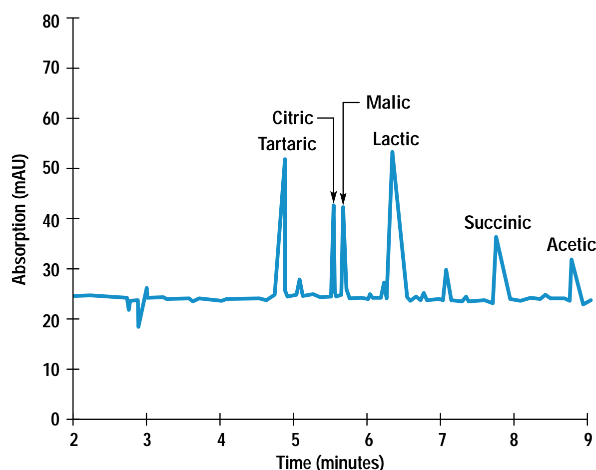

Buffer: 5 mM phthalate, 0.25 mM CTAC, 0.07% β-CD, pH 3.5
Sample: Sake (diluted 1:5 with water)
Capillary: L_{eff} = 56 cm, L = 64.5 cm, i.d. = 75 μm
Injection: 200 mbar · s
Temperature: 15°C
Field Strength: 390 V/cm, Reversed Polarity

Fig. 4. Analysis of organic acids in sake employing indirect UV detection.

- Peak Purity Measurement. The fact that a spectrum is characteristic for a certain substance can be used to measure peak purity. If the spectra sampled along the peak are identical, the peak can be assumed to be pure. If the spectra change along the peak, a second substance might have coeluted.

Even though diode array detection is a desirable feature, it would be unacceptable if the sensitivity were not competitive with conventional UV/Vis detectors such as single-wavelength detectors or variable wavelength detectors, which work with filter wheels, bandpass filters, or rotating monochromator gratings, thus providing sequential spectra. Peak widths in CE are inherently smaller than in LC. The time needed by a scanning variable wavelength detector for scanning through the full spectral range cannot be neglected. This makes these detectors less preferable for spectral identification in capillary electrophoresis. However, the sensitivity of monochromator-based detectors is viewed as the state of the art in CE.

To obtain the same level of sensitivity with the diode array based spectrometer of the HP CE system, special care was given to the optical design of the CE detector. As described in more detail in the article on page 20, the objective was to optimize the light throughput and therefore the light incident onto the photodiodes, which determines the lowest noise level achievable with an optical detector. To maximize dA/dc, where dA is the incremental absorption change and dc is the incremental concentration change of the fluid residing in the optical path, all light emitted by the lamp is focused onto the