

Transfer of HPLC methods to Capillary Electrochromatography

Gordon Ross,
Monika Dittmann
and Gerard Rozing

Chemical/
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Abstract

Capillary Electrochromatography combines the separation principle of HPLC (partitioning between mobile and stationary phases) with the high efficiency of capillary electroseparation methods in CEC. The electroosmotic flow (EOF) inherent in capillary electrophoretic separations is used to transport solute and mobile phase through a packed capillary column. The properties of the EOF allow much higher efficiencies than can be realized with LC. CEC can also be characterized by its low solvent consumption and reduced costs. Therefore, there are a number of advantages which may be gained by transferring a method from LC to CEC. This application brief aims to identify the parameters which must be appreciated in order to successfully transfer a method from LC to CEC.

Experimental

All CEC experiments were performed using the Agilent CE system, equipped for CEC operation and with a built in diode array detector. The system includes an Agilent ChemStation for system control, data collection and data analysis. LC separations were performed on an HP 1090 liquid chromatography system. CEC columns were supplied by Agilent Technologies. Buffer salts were of the highest purity available and organic solvents were HPLC grade. All buffers were filtered and degassed prior to use. Buffers/mobile phase were adjusted to pH prior to addition of organic modifiers.

The main point of difference between LC and CEC is in the motive force which transports the mobile phase through the column. In LC this is achieved through pressure drive flow, whereas in CEC this is achieved via the EOF. The flow velocity of the EOF is dependent upon properties of the mobile phase (viscosity, dielectric constant, buffer ionic strength), as well as those of the stationary phase (surface

charge density). This application brief intends to outline some of the effects on linear velocity of varying stationary or mobile phases.

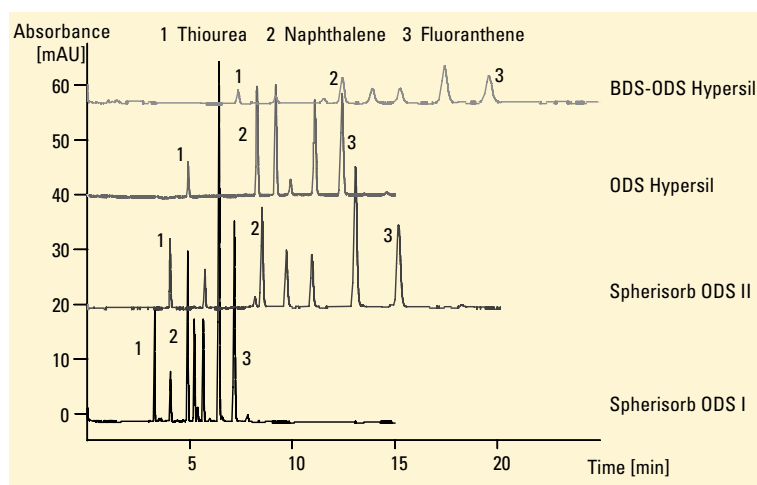


Figure 1
Separation of PAHs on different C18 phases

Conditions

Capillary CEC Hypersil C18, 3 μm
250 mm (350 mm) \times 0.1 mm i.d.

Mobile phase

80/20 ACN/TRIS HCl, 50 mM; pH 8.0

Voltage 20 kV

Temperature 20 $^{\circ}\text{C}$

Injection 3 s at 5 kV

Pressure 10 bar both sides

Detection Sig. 254/10 nm, reference off



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The EOF in a packed column is dependent upon the packing materials. Figure 1 shows the separation of a mixture of polyaromatic hydrocarbons (PAHs) on different C18 stationary phases. The samples were not identical but thiourea (flow marker), naphthalene and fluoranthrene were included in all samples. The fastest EOF is observed on the two non-encapped materials CEC Hypersil C18 and Spherisorb ODS 1. With the encapped ODS Hypersil the flow is reduced by a factor of approximately 2 and with the base deactivated phase (BDS Hypersil) it is reduced by a factor of 3.

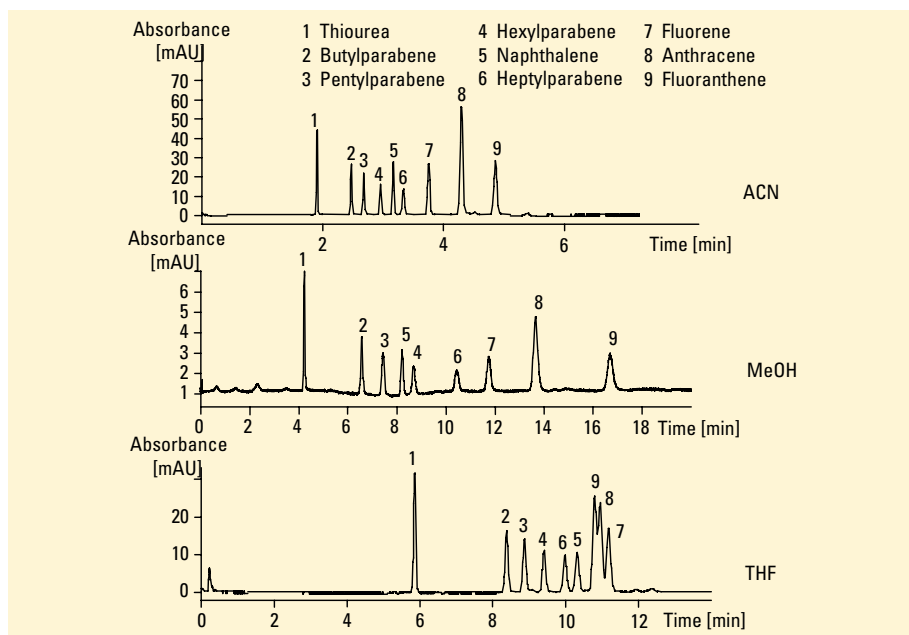


Figure 2
Effect of organic modifier of EOF, retention and selectivity in CEC

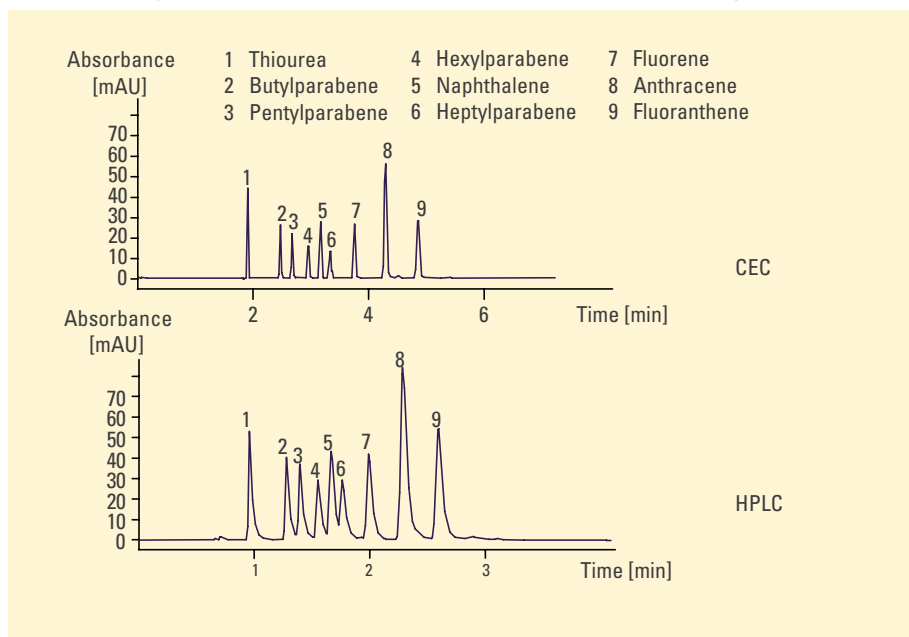


Figure 3
Analysis of test mixture by HPLC and by CEC

Figure 2 shows the effect of different organic modifiers on the CEC separation of a standard mixture. Compared to acetonitrile, the flow observed is approximately 2 times slower with methanol and 3 times slower with THF. This is due to differences in viscosity and dielectric constant of the different organic modifiers. Figure 3 shows the separation of a neural test mix by CEC and by LC on the same column. The selectives are the same in both cases.

Conclusions

LC separations can be successfully transferred to CEC operation. Just as in LC, variation of the stationary and mobile phase is a useful tool for method optimization. Modulation of the mobile and stationary phase can change the EOF and thus the speed of separation.

Equipment

- Agilent Capillary Electrophoresis system
- HP 1090 LC system
- Agilent ChemStation + software

Monika Dittmann and Gerard Rozing are R&D chemists, and Gordon Ross is application chemist at Agilent Technologies, Waldbronn, Germany.

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