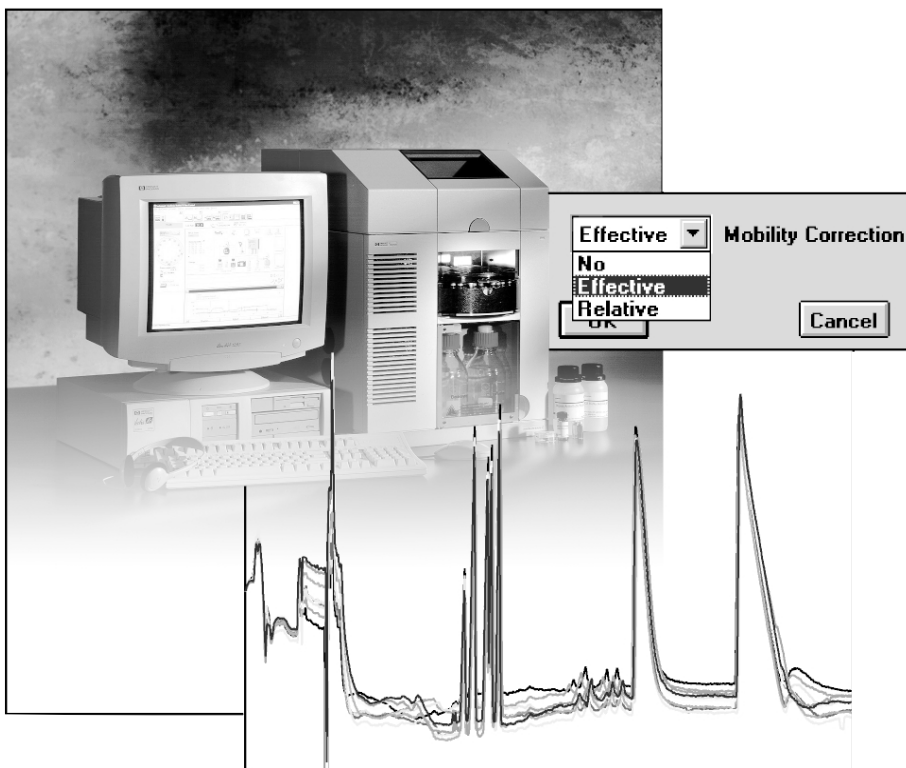


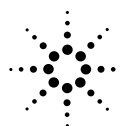
Use of Mobilities for Improved Reproducibility in CE

Technical Note



Introduction

In recent years capillary electrophoresis (CE) has been used routinely in many laboratories. Many rugged methods have been developed which utilize extensive capillary flushing and preconditioning steps to ensure constant run-to-run starting conditions, constant electro osmotic flow (EOF) and reproducible migration times. However, due to reliance on the EOF, in contrast to a constant pumping system as in HPLC, the reproducibility of the migration times in CE is still not as good as the reproducibility of the retention times in HPLC.



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The most important contribution to variations in run-to-run migration times is the variability of the EOF velocity. Usually, the EOF velocity varies from capillary to capillary and from batch to batch due to changes of the capillary inner surface. Furthermore, slight changes in electrolyte composition, for example by electrolysis, buffer temperature or viscosity, as well as adsorptions to the capillary wall can have an impact on the EOF-flow velocity. As the velocity of the background electrolyte changes, so does the migration time of each sample component. These shifts in migration time can make it difficult to identify calibrated peaks. Although the EOF can be minimized by the use of low pH buffers or coated capillaries, it may be inconvenient or impossible to employ these conditions.

Fortunately the EOF component in CE does not alter the selectivity or efficiency. For this reason, EOF induced migration time shifts can be easily corrected by subtracting the EOF migration time from the analyte migration time. A more meaningful procedure is the subtraction of the EOF mobility from the apparent analyte mobility resulting in true or effective mobility. This measure eliminates the EOF portion of the apparent mobility and enables the user to compare a solely sample related parameter.

The new Agilent ChemStation software revision A.06 can be used to correct EOF-induced migration time shifts by referring to true effective mobilities (EOF marker required¹) or relative mobilities.

These measures

- improve run-to-run reproducibility² significantly.
- eliminate batch-to-batch and capillary-to-capillary variations.
- facilitate peak recognition using a calibrated method.
- enable faster method development.
- enable shortening of preconditioning times.
- allow users to compare their analytical results more easily.

In addition, working with effective mobility correction allows, determination of true effective mobilities for all sample components³. This measure adds the following key benefit:

- The generated data are generally independent of instrumental conditions (including capillary length and applied voltage).

¹ See also Table 1 for a list of commonly used EOF markers.

² Refers to the reproducibility of the mobility.

³ Note: The true effective mobilities are determined „on line” under the given experimental conditions.

Background

The mobility of an analyte is defined as:

$$\mu_{app} = \frac{v}{E} = \frac{l_{eff} \cdot l_{tot}}{t_m \cdot V}$$

With μ_{app} being the apparent mobility, v being the apparent velocity and E the electric field strength. The necessary instrumental parameters for the calculation are effective and total capillary length (l_{eff} , l_{tot}), the analyte migration time (t_m) and the applied voltage (V).

In systems, which exhibit an EOF, the apparent mobility is always composed of two separate portions: the EOF mobility, μ_{EOF} and the effective mobility of the analyte, μ_{real} ⁴:

$$\mu_{app} = \mu_{real} + \mu_{EOF}$$

The new “mobility correction” feature of ChemStation software revision A.06 is based on the assumption that under given experimental conditions the effective mobility of each sample component remains constant⁵. In this case, changes in migration time are solely based on changes in the EOF velocity (see Figure 1).

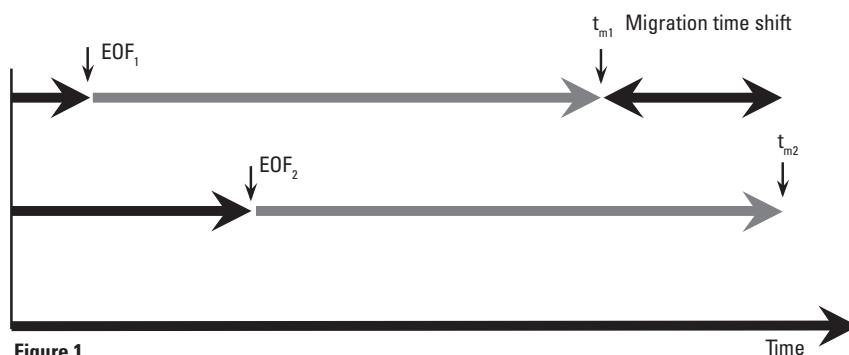


Figure 1
Every shift in EOF results in a shift of migration time

Comparing migration times can therefore result in lower reproducibility than in other techniques, such as HPLC.

If, on the other hand, effective mobility values are compared, a much more favorable reproducibility is obtained. The ChemStation software revision A.06 approaches the problem in the following way: In a first so-called calibration run the effective mobility of all sample components, $\mu_{real,i}$ ⁶ is determined by subtracting the EOF mobility, μ_{EOF} from the apparent mobility of each sample component, $\mu_{app,i}$.

For this purpose an EOF marker has to be added to the sample. The true effective mobility values are stored for comparison with the following runs. In addition, a reference sample component can be chosen for the determination of changes in EOF for the following runs. In these runs the mobility of the EOF is determined by the difference between the apparent mobility of the reference peak, $\mu_{app,ref}$ and the true (stored) effective mobility of the reference, $\mu_{realref}$ (see Figure 2).

$$\text{First run: } \mu_{real,i} = \mu_{app,i} - \mu_{EOF}$$

$$\text{Following runs: } \mu_i = \mu_{app,i} - \mu_{appref} + \mu_{realref}$$

Figure 2
Calculation of effective mobilities

⁴ The index “real” was chosen to indicate that this is the real mobility of the analyte under the given experimental conditions.

⁵ In reality, changes in temperature and buffer composition will also affect the mobilities of the sample components.

⁶ The mobility reference peak can be any peak including the EOF marker.

The mobility reference peak allows the user to run the sample without adding an EOF marker starting at the second run⁶.

Using mobility correction

Correction of EOF-induced migration time shifts based on effective mobility can be performed in the following way:

- ❶ Load the signal.
- ❷ Choose **New Calibration Table** from the **Calibration** menu, or choose the **New Calibration Table** icon.
- ❸ In the **Calibration Mode** group of the **Calibrate** dialog box, choose the down arrow and select **Effective Mobility Correction** (Figure 3).

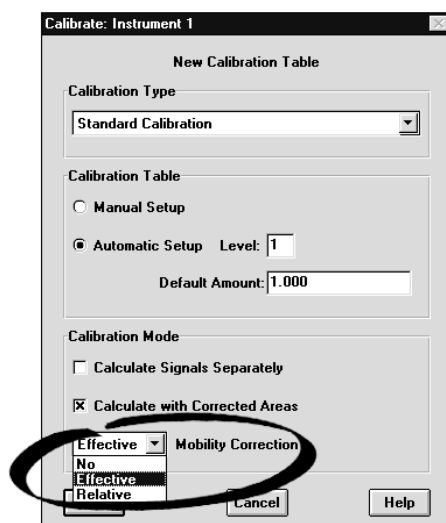


Figure 3
Selecting effective mobility correction

❹ In the **Effective Mobility Settings** dialog box, select a signal from the drop down list.

❺ From the **Peak List**, highlight the line that contains the migration time of the **EOF Marker** and choose **Select** (Figure 4).

❻ From the **Peak List** highlight the line that contains the migration time of the **Mobility Reference Peak** and choose **Select**. The mobility reference peak should be chosen in accordance with the following priorities:

- Select peak with highest signal⁷.
- Select most isolated peak.
- (EOF marker or internal standard can also be used as mobility reference peak)
- Enlarge the search window to always find the mobility reference peak.

❼ Choose **Use Values from Data File** if you stored the values for voltage and capillary dimensions with the data file or choose **Use Default Values Below** if the information is missing. In the latter case enter **Voltage and Capillary Dimensions**.

❸ Choose **OK**.

Then proceed as usual by entering the names of the calibrated compounds. Analogously, a migration time correction based on relative mobilities can be performed. In this case neither EOF marker nor voltage values nor capillary dimensions are needed. When using relative mobilities, the software still corrects migration time shifts, but does not display mobility values.

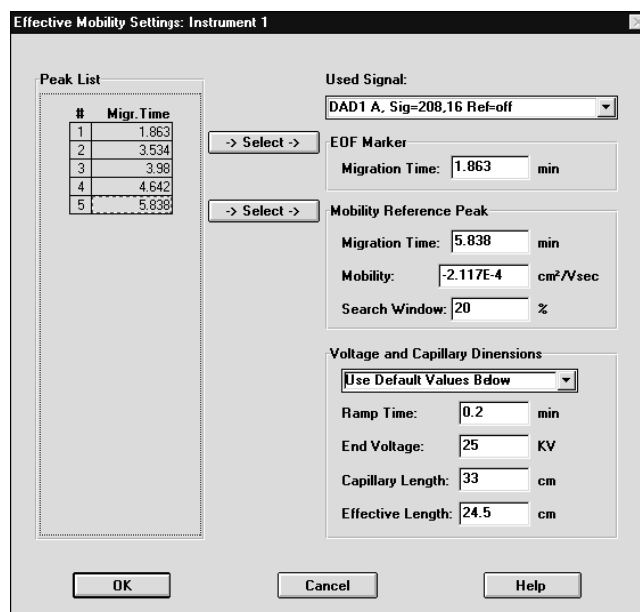


Figure 4
Entering effective mobility settings

⁷ If several peaks fall in the search window, the peak with the highest signal is automatically chosen as mobility reference peak!

A typical mobility report can look as follows:

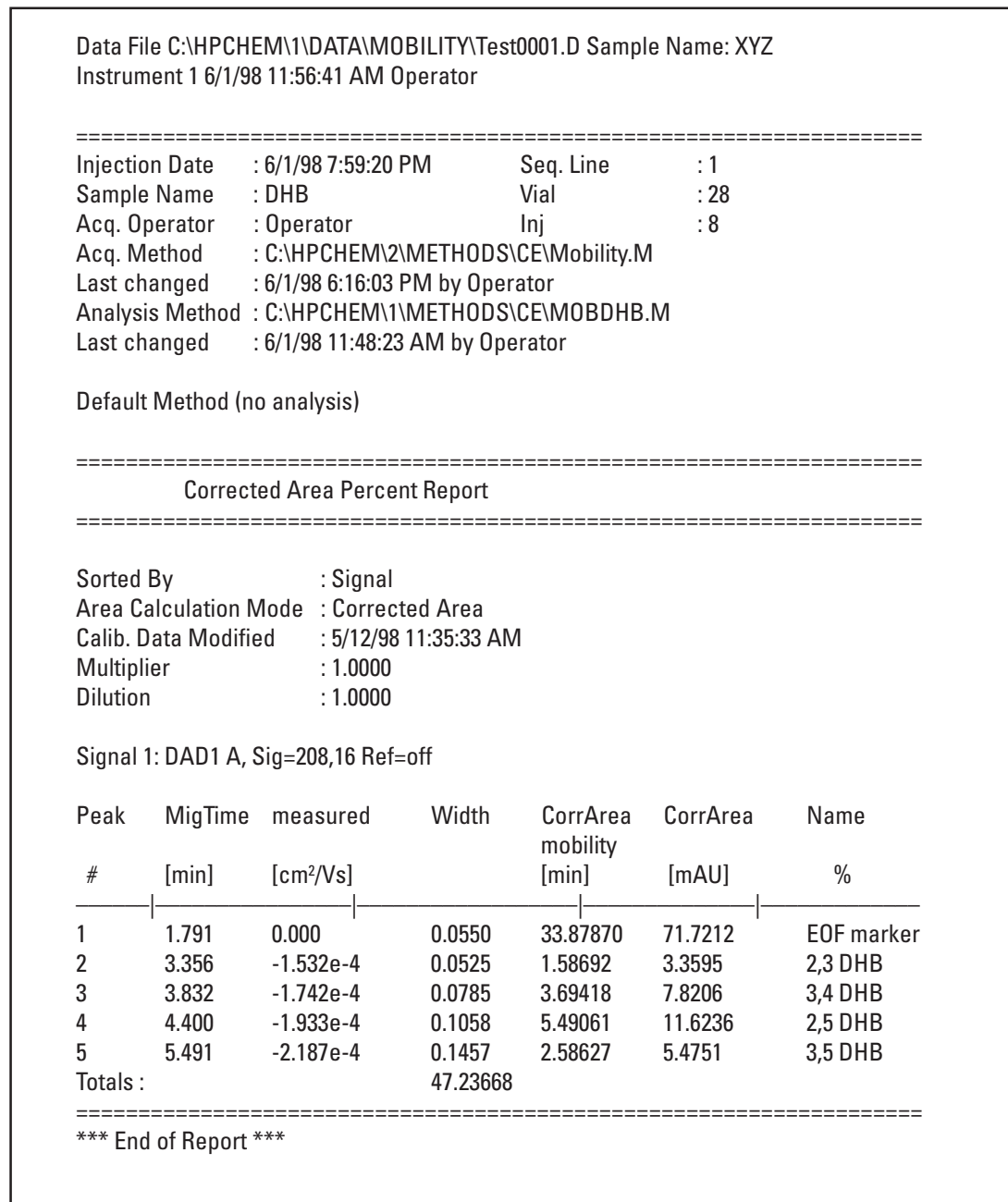


Figure 5
Standard mobility report

The measured mobility refers to the mobility of a compound in the actual run. Effective mobility values also appear in the sequence summary report and can be used to demonstrate the statistical reliability of a method.

Hints on using mobility correction

Voltage

For the calculation of true effective mobilities it is necessary to know the voltage during the CE separation. Storing the voltage data during the run does this most accurately. Make sure to also store the capillary dimensions with the method. To reprocess signals that have been acquired without voltage data/capillary dimensions, enter the voltage and ramp time manually in the **Voltage and Capillary Dimensions** group of the dialog box.

Quantification

For correct quantitative results, always check the **Calculate with Corrected Areas** checkbox in the **Calibration Mode** group of the **Calibrate** dialog box.

Systems where negatively charged species migrate against the EOF

Be aware of the special case of systems where negatively charged species migrate against the EOF. The closer the mobility of those species are to the mobility of the EOF, the stronger the effect that a slight change in EOF has on the migration time of those species (if $\mu_i \rightarrow -\mu_{EOF}$, then $t_m \rightarrow \infty$). These systems can make it very difficult to find the calibrated compounds. The following hints can help you find all calibrated compounds:

- Choose the highest peak of the electropherogram as your mobility reference peak and set a large search window for the mobility reference peak. If several peaks are found in the specified interval, the software automatically chooses the highest peak as the mobility reference peak.
- Choose one of the last peaks as your mobility reference peak.

Example

The following example demonstrates the improvements, which can be achieved by using the mobility correction feature with a calibrated method. The sample consisted of seven inorganic anions (see separation on front cover), one of which (F⁻) was used as the mobility reference peak. The relative standard deviation over 10 runs was between 0.57 and 0.86 % RSD for the migration time of the different anions. However, when comparing the computed mobility values, about one order of magnitude improvement for the relative standard deviation was achieved (0.05 - 0.14 % RSD). The values obtained using mobility compare favorably to typical results from HPLC.

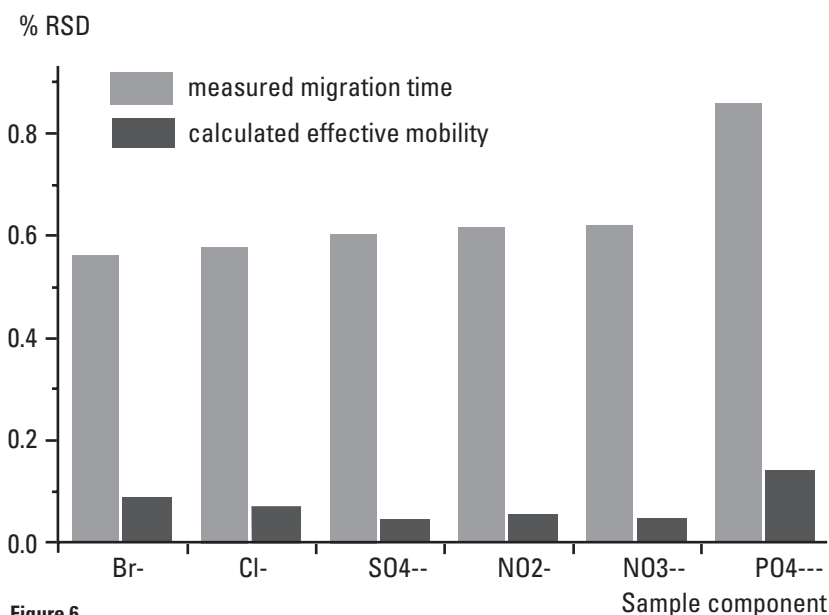


Figure 6
Relative standard deviation of migration times and computed mobilities

Conclusion

The new ChemStation software revision A.06 automatically corrects for migration time shifts caused by changes in EOF velocity. This is accomplished by using effective or relative mobilities as a reference. By doing so, calibrated compounds can be identified more easily. Furthermore, the relative standard deviation of the run-to-run mobility values are about one order of magnitude better than the the relative standard deviation of the migration time values. This dramatically improves batch-to-batch reproducibility of different capillaries. In addition, mobilities are more meaningful than mere migration times since they are independent of capillary dimensions and applied voltage, thus greatly simplifying method transfer between instruments. Method validation in CE can be significantly simplified by the use of mobility.

Compound	Wavelength
1-propanol	210 nm
Acetone	330 nm
Acetonitrile	190 nm
Benzene	280 nm
Guanosine	252 nm
Mesityl oxide	253 nm
Methanol	205 nm
Phenol	218 nm
Pyridine	315 nm
Tetrahydrofuran	212 nm
Uracil	259 nm

Table 1
Commonly used EOF markers

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our world wide web site:
<http://www.agilent.com/chem>

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