Mobility calculation: SCIEX PA 800 Plus Empower Driver version 1.3 vs. 32 Karat™ Software

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Since the introduction of commercial capillary electrophoresis (CE) systems over 30 years ago, it has been important to not use conventional "chromatography thinking" when using CE. This is especially true when processing data, as there are some key differences between electrophoretic and chromatographic data. In this technical note, we will focus on mobility.

Mobility is a parameter that quantifies how a charged particle migrates in an electrical field. A component with a higher mobility moves more rapidly through the separation medium than a component with lower mobility. Because particles can be attracted to either the cathode or the anode, mobility has a vector component—it can be positive or negative. Mobility toward the cathode (negatively charged electrode) is defined as positive, and mobility toward the anode (positively charged electrode) is defined as negative.

In practice, mobility is defined for a given molecule under a given set of conditions. Variations in separation conditions that affect all species equally (such as variation in electroosmotic flow or the voltage delivered by the power supply), and variations that have similar effects on closely related groups of compounds (such as the effect of small pH changes on a series of basic drugs), can be accounted for by including a standard of defined mobility in the analytical run.

This technical note provides instructions to calculate mobility using Waters Empower software and a comparison to mobility calculated by 32 Karat Software.

Key features

- Calculation of mobility
- Correlation of mobility between 32 Karat Software and Empower software
- The average mobility on Empower software and apparent mobility on 32 Karat Software are comparable

Methods

Instrument

All experiments were performed on a PA 800 Plus System (SCIEX, Brea, CA) equipped with a PDA detector. A 75 µm internal diameter bare-fused silica capillary (SCIEX, P/N 338451) was installed to give 60 cm total length (effective length 50 cm). The capillary temperature was maintained at 25°C in all separations. The test mix was separated with 30 kV, normal polarity, 10 second 0.5 psi injection.

Figure 1: The PA 800 Plus Pharmaceutical Analysis System.

The data was collected on a SCIEX PA 800 Plus System with Empower Driver v1.3 or with 32 Karat Software, analyzed and the results of the two analyses were compared.

Results and discussion

Mobility is calculated as the apparent mobility less the contribution of electroosmotic flow.

$$\mu = \mu_{app} - \mu_{eof}$$

Once the mobility of a reference analyte has been determined, the mobility of related analytes can be calculated.

$$\mu = L_d L_t \left(\frac{1}{Vt} - \frac{1}{V_{ref} t_{ref}} \right) + \mu_{ref}$$

V = average applied voltage up to the migration time of the peak of interest

L_d = capillary length to detector

L_t = total capillary length

t_{ref} = *migration time of reference peak in the current run*

μ_{ref} = *defined mobility for the reference peak*

V_{ref} = *average applied voltage up to migration time of reference peak*

t = migration time of the peak of interest

Apparent mobility is the sum of true mobility and the mobility caused by the electroosmotic flow. Apparent mobility can be calculated directly for any peak. The apparent mobility of a neutral marker (one that has a true mobility of zero) is called μ_{eof}, the electroosmotic mobility.

$$\mu_{app} = \frac{v}{E} = \frac{L_d L_t}{V_t} (apparent\ mobility)$$

L_d = *capillary length to detector*

L_t = *total capillary length*

V = average voltage up to migration time of peak

t = migration time of the peak

v = *Velocity*

Mobility calculations depend on an accurate measurement of the average applied voltage up to the point that the component passes the detector. The output from the PA 800 Plus voltage monitor is used to calculate average applied voltage as follows:

$$\sum_{Vi}^{n}$$

n = data point number at peak migration time

Vi = voltage value at data point i

There is a difference in the way the two software programs calculate mobility.

In 32 Karat Software, mobility and apparent mobility can be calculated as the software can measure average voltage up to migration time of the peak. Empower software does not have this option, so a fixed separation voltage must be used. Due to this difference, the results show a slight variation. In comparison, mobility calculated using Waters Empower software is closer to apparent mobility in 32 Karat Software, as expected.

The Test Mix B used in this assay includes two peaks as shown in Figure 2 and Figure 3 for Empower and 32 Karat Software, respectively.

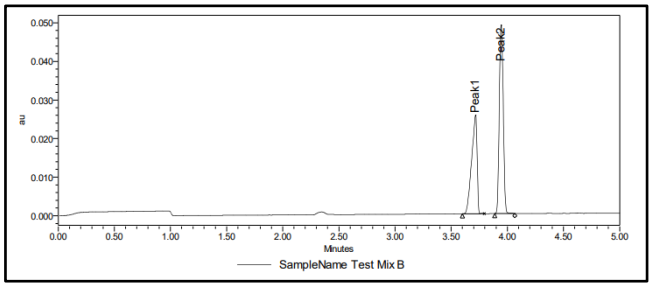


Figure 2: Test Mix B on PA 800 Plus using Empower software.

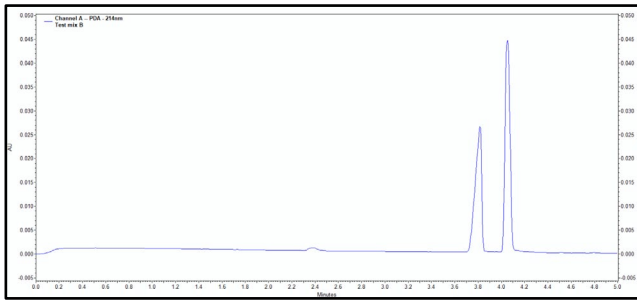


Figure 3: Test Mix B on PA 800 Plus using 32 Karat Software.

Table 1 shows the mobility value for Test Mix B.

Table 1: Comparison of mobility values.

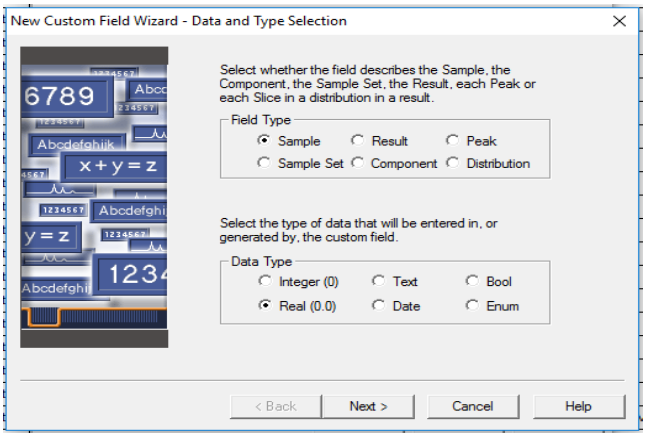
	Empower-Mobility		32 Karat-Mobility		32 Karat-Apparent Mobility	
	Peak 1	Peak 2	Peak 1	Peak 2	Peak 1	Peak 2
Run # 1	0.0004485	0.0004224	0.00019611	0.00016681	0.0004102	0.0003809
Run # 2	0.0004481	0.0004221	0.00019612	0.00016643	0.0004103	0.00038065
Run # 3	0.0004491	0.0004231	0.00019613	0.00016971	0.0004075	0.00038112
Run # 4	0.0004492	0.0004233	0.00019611	0.00017008	0.0003989	0.00037289
Run # 5	0.0004487	0.0004230	0.00019614	0.00016844	0.0004089	0.00038124
Run # 6	0.0004487	0.0004231	0.00019611	0.00016844	0.0004089	0.00038082
Average	0.00045	0.00042	0.00020	0.00017	0.00041	0.00038
STD	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
%RSD	0.090	0.112	0.006	0.878	1.056	0.868

Overall, mobility calculated by Empower software is comparable to apparent mobility calculated by 32 Karat Software.

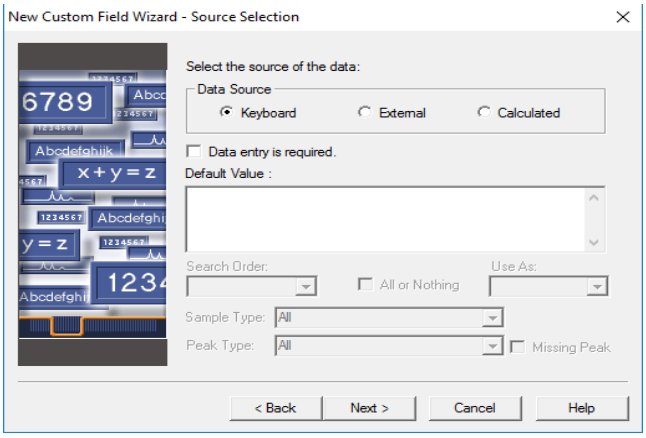
Generate mobility values in Empower software

The following steps were used to calculate mobility in Empower software:

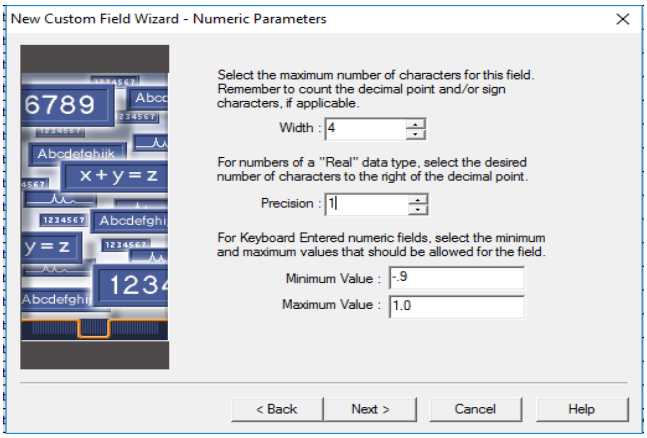
1. In the Empower Software Pro Interface window, select Configure the System.
2. Navigate to the project in which you want to add the custom field. Right-click and then select Properties.
3. Ensure CE/CIA options is Enabled in the General tab.
4. Select the Custom fields tab. Select “New” on the lower left.
5. Select Sample for the Field Type and Real (0,0) for the Data Type and then click Next.



6. Select Keyboard for the Data Source and then click Next.



7. Select 4 for Width and 1 for Precision and then click Next.



8. Name the Field “RunVoltage”.

Use Run Voltage for mobility calculations

1. In the Sample Set Method, enter the separation voltage from the instrument method in the RunVoltage column.

   Note: This column might be hidden and can be made visible under Table Properties.

2. Run the samples using the Sample Set Method.

   Note: If the sample set has already been completed, alter the sample set by right-clicking on the desired sample set and selecting “Alter Sample”.

3. Once collected, open the data and desired CE processing method in Review.
4. Click View > Processing method layout.
5. Click the CIA/CE Options tab in the CE Processing Method (Figure 4).

   Note: This tab is not available if an LC processing method is being used.

6. Enter the Total Length of Capillary (cm) and Capillary Length to Detector (cm).
7. Click File > Save > Processing Method to save the method.
8. In the table below the electropherogram, right-click, and then select Table Properties and uncheck Mobility.
9. Click Process > Integrate data > Quantitate.
10. The mobility values are shown in Table 2.

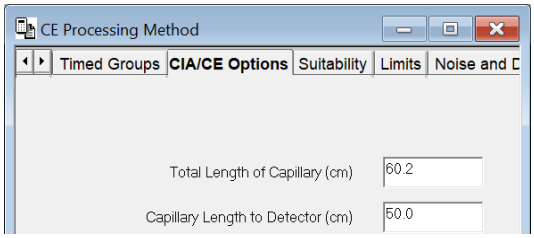


Figure 4: Capillary effective length entry in empower processing method.

Table 2: Mobility values for Test Mix B using Empower software.

	Name	Migration Time (min)	Area (µV*sec)	% Area	Height (µV)	Time Corr. Area	% Time Corr. Area	Mobility	VCA
1	Peak1	3.714	87321	41.76	26010	23510.10	43.19	4.487e-004	7915.0683
2	Peak2	3.939	121806	58.24	48702	30920.33	56.81	4.231e-004	10409.8441

Overall, the data is comparable between the two software programs and the two algorithms.

The results show that data collected using the SCIEX PA 800 Plus Empower Driver v1.3 can be successfully used to calculate mobility.

Conclusion

Overall, the mobility values generated using the Empower software are comparable to those generated using 32 Karat Software. The data reported here are for comparison purpose only. Migration to the SCIEX PA 800 Plus Empower Driver v1.3 can be done with confidence based on these results.

References

1. *PA 800 Plus Pharmaceutical Analysis System Methods Development Guide, RUO-IDV-05-5330-A.*
2. *P/ACE MDQ User's Guide* A33115AB.

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