What is PeakMaster?

PeakMaster predicts the behavior of background electrolytes and analytes in zone electrophoresis. The mathematical background it uses is described in the following papers:

M. Stedry, M. Jaros, B. Gas, *J. Chromatogr. A*, 2002, 960, 187-198
M. Stedry, M. Jaros, K. Vcelakova, B. Gas, *Electrophoresis* 2003, 24, 536–547
M. Stedry, M. Jaros, V. Hruska, B. Gas, *Electrophoresis* 2004, 25, 3071-3079
M. Jaros, V. Hruska, M. Stedry, I. Zuskova, B. Gas, *Electrophoresis* 2004, 25, 3080-3085
V. Hruška, M. Štědrý, K. Včeláková, J. Lokajová, E. Tesařová, M. Jaroš, B. Gaš, Electrophoresis 2006, 27, 4610-4617

It Calculates

- Important parameters of the background electrolyte, such as pH, ionic strength, or buffer capacity.
- Important parameters of analytes, such as the signal of a direct UV or fluorescence detection, the transfer ratio, which is a measure of sensitivity in indirect UV (or, generally, photometric) detection and the molar conductivity detection response, which expresses the sensitivity of the conductivity detection.
- The tendency of the analyte to distort its peak, i.e., to undergo electromigration dispersion.
- Distortion of system peaks when sample composition is known (in Amplitudes and Shapes window)
- Positions of system eigenpeaks.

It Simulates

- The electropherogram of a given sample mixture in a given background electrolyte.

It Predicts

- The existence of system eigenmobilities which give rise to system eigenpeaks or resonance phenomena causing anomalous dispersion of the analyte peaks.

It Allows

- Optimization of the background electrolyte composition which results in enhanced detector sensitivity, while still maintaining acceptable dispersive properties.

How PeakMaster Works

- First, please notice that all mobility values whenever used either as input or output are given in $10^{-9} m^2 V^{-1} s^{-1}$ units.
- In the **BGE constituents** subwindow, you setup the background electrolyte which can contain any number of constituents. For each constituent you set its **Name**, concentration **c [mM]**, limiting ionic mobilities **u** in absolute value and thermodynamic **pK$_a$** constants of all of its ionic forms. The maximum valence (either positive or negative) is four. All the constituents can be weak or strong acids or bases or amphoteric electrolytes such as aminoacids or peptides. For

input of the BGE constituents we recommend you to use the built-in database of data of many ions (**Pick from database**).

- In the **Run settings** subwindow you can choose, whether you want to have your results corrected for the ionic strength of the BGE. In most of practical cases it should be checked ☑.
- Possibly, in the **Analytes** subwindow, you input the **Name**s of the analyte ions in the sample injected and their amounts, which you expect in the sample. For the **Amount** you have a fourth step scale ranging from **S** (Small) to **XL** (eXtra Large). For input of the BGE analytes you can also use the built-in database of data of ions (**Pick from database**).
- Possibly, in the **Run parameters** subwindow, you input some experimental characteristics of your equipment, such as the **Total capillary length**, the **Capillary length to detector**, the **Polarity at the injection site** of the driving voltage, amplitude of the **Driving voltage**. If there is an electroosmotic flow, **EOF**, you input either (i) the **Time** of the EOF marker or (ii) the **Mobility** of the electroosmotic flow. When the EOF has direction like cations, you input the positive mobility, when the EOF has direction like anions, you input the negative mobility.
- Possibly, in the **Signal** subwindow, you choose which signal should be seen in the resulting electropherogram, whether the signal of a **Direct** detection, such as a direct UV or fluorescence detection, or the signal from an **Indirect UV** or a **Conductivity** detection.

After clicking the **Calculate!** button, PeakMaster performs the following actions:

- In the **System parameters** subwindow it calculates BGE characteristics – pH, Ionic strength, Conductivity, Resistivity, Buffer capacity, EOF mobility or EOF marker time, and all System eigenmobilities of the BGE.
- In the **BGE constituents** subwindow it calculates effective mobilities **u_eff** of the BGE constituents and concentrations of their individual ionic forms.
- In the **Analytes** subwindow it calculates electrophoretic characteristics of the analytes: effective mobility – **u_eff**, tendency to electromigration dispersion – **EMD**, conductivity signal – **Cond. signal**, and indirect UV signals of all constituents of the BGE – **UV [particular constituent].**
- In the **Electropherogram** subwindow it simulates electropherograms of a given mixture of analytes. Positions of possible appearance of the system eigenpeaks are denoted by vertical lines. A position of the neutral EOF marker is shown as a gray bar. The shape of the electropherogram depends on the type of the signal, which was chosen in the **Signal** subwindow. The **Direct** signal shows the electropherogram as it would look when your detector has the equal response on all analytes in the sample. The choice of **Indirect UV - particular constituent** signal shows the electropherogram where the analytes are invisible but the particular constituent of the BGE is absorbing at the detector wavelength used. The **Conductivity** signal depict the signal of the conductivity detector.

PeakMaster Can be Used for Optimization of Background Electrolytes

- **Conductivity detection**

Conductivity detection is based on the change of the background electrolyte conductivity caused by the presence of the analyte. The signal from a conductivity detector, **Cond. signal**, is proportional to the molar conductivity detection response b_X, which is defined as:

$$b_X = \lim_{c_X \to 0} \left(\frac{\mathrm{d}\kappa}{\mathrm{d}c_X} \right).$$

Values of b_X are in units $10^{-3}\,\mathrm{Sm^2mol^{-1}}$ and are interpreted as follows:

Cond. signal = 0	no conductivity signal
Cond. signal > 0	positive conductivity signal

Cond. signal < 0 negative conductivity signal

- **Electromigration dispersion**

The tendency of an analyte peak to undergo electromigration dispersion, **EMD,** is described by the relative velocity slope S_X:

$$S_X = \lim_{c_X \to 0} \frac{\kappa}{v_X} \left(\frac{\mathrm{d}v_X}{\mathrm{d}c_X} \right),$$

where κ is conductivity in the analyte zone, and v_X and c_X are velocity and concentration of the analyte X in the analyte zone, respectively. Values of S_X calculated by PeakMaster are in $10^{-3} Sm^2 mol^{-1}$ units and the **EMD** value should be interpreted as follows:

EMD = 0 no electromigration dispersion
EMD > 0 triangular tailing peaks
EMD < 0 triangular fronting peaks

The relative velocity slope S_X has the same dimension as the molar conductivity response b_X. For strong ions, it has even the same absolute value but opposite sign. However, for weak electrolytes, S_X is generally different than b_X and can be higher or lower in the absolute value. It can be therefore found a BGE, where a particular analyte has the **EMD** close to zero, so its peak will have no electromigration dispersion even when the analyte is present at a rather high concentration. This is a favourable BGE. On the other hand, the BGEs where analytes have the **EMD** greater than **Cond. signal** in absolute value are unsuitable and should not be used.

- **Indirect UV detection**

For the indirect UV detection, the decisive quantity responsible for the detector signal is the response of the concentration of co-ions or counter-ions to the concentration of the analyte. The signal from a UV detector, named **UV [particular constituent]** signal, is therefore proportional to the transfer ratio, which is defined as:

$$TR_i = \lim_{c_X \to 0} \left(\frac{\mathrm{d}c_i}{\mathrm{d}c_X} \right).$$

Here c_i is the concentration of the particular i-th constituent of the background electrolyte. TR_i is dimensionless quantity and is interpreted as follows:

UV = 0 no indirect UV signal
UV > 0 positive indirect UV signal
UV < 0 negative indirect UV signal

- **Buffer capacity**

Buffer capacity of the BGE is calculated according to

$$\frac{\mathrm{d}c_b}{\mathrm{dpH}}$$

where $\mathrm{d}c_b$ is the very small concentration of a strong base in mM altering the BGE pH by dpH. Generally, it should be reasonably high. BGEs which have the buffer capacity < 1 mM sometimes behave very strangely.

The Principle of Optimization is Following

You can tune the composition of the BGE to finally find the configuration, which gives good **Signals** for the given analytes and the detection method used, and, at the same time, which keeps the

electromigration dispersion **EMD** of the analytes as low as possible in the absolute value. Furthermore, the effective mobilities **u_eff** of the analytes should mutually differ as much as possible to achieve a good selectivity. After calculation of each composition, just inspect the **Analytes** and the **Electropherogram** subwindows to see how your system is shaping up.

System Eigenmobilities

- The system eigenmobilities are a problematical feature of the BGEs. The number of system eigenmobilities is always the same as the number of constituents of the BGE.
- The existence of system eigenmobilities indicates that certain entities in the electrophoretic system move with such mobilities in the driving electric field. Generally, none of the system eigenmobilities is exactly zero.
- At least one of the system eigenmobilities is very often close to zero and is a cause for the system eigenpeak which is called the injection zone (or water gap, or water dip). Such an injection zone, due to its negligible electrophoretic mobility, often serves as the marker of the electrosmotic flow. It must be, however, realized that the position of the injection zone is not equivalent to a position of an EOF neutral marker and in some BGEs it can even be substantially different. In such BGEs there is no injection zone that can serve as the marker of the EOF!
- In rather acidic or alkaline BGEs or in BGEs containing multiple coions or in BGEs with a multivalent co-constituent there are one or more system eigenmobilities different substantially from zero. Here the problem splits into the following two cases:
- If there is no analyte moving at the same mobility as the system eigenmobility, the BGE exhibits a system zone (system eigenzone) moving with the electrophoretic velocity corresponding to the system eigenmobility. When the detector gives a response to any constituent of the BGE (especially when using indirect UV detection or conductivity detection), it can depict a system peak (system eigenpeak), which can often resemble a peak of a real analyte; it is, however, a fake peak.
- If there is an analyte with the same mobility as the system eigenmobility, it leads to a resonance phenomenon. Due to the resonance, an amplified signal of the indirect UV detection or conductivity detection at the site of the analyte should be expected. We explained elsewhere (Milan Stedry, Michal Jaros, Bohuslav Gas: Eigenmobilities in background electrolytes for capillary zone electrophoresis, J. Chromatogr. A, 960 (2002) 187-198), why the resonance signal has the zigzag shape. The indirect signal of the analyte in resonance with a system eigenzone is then no more proportional to its quantity! Further, serious anomalous dispersion of the analyte peak should be expected.

PeakMaster is Easy to Use

PeakMaster is user-friendly and can be understood intuitively. However, for your convenience, you can load tutorials by opening configurations named Example_1 or Example_2 (click the **Open** button, select the configuration, finally click the **Calculate!** button). The tutorials demonstrate some interesting features of background electrolytes.

Example_1 This is electrophoresis in rather hard real conditions far from the ”safe” pH region and also having ionic strength higher than is usual. It shows a separation of an anionic mixture in a commercially available buffer, which is intended for analysis of anions with indirect UV detection (at the detection wavelength of 350 nm) and is composed of 20 mM PDC (pyridine 2,6 dicarboxylic acid) with addition of 0.5 mM tetradecyltrimethylammonium hydroxide (TTAOH) for the reversal of the electroosmotic flow and about 55 mM NaOH to reach pH = 12.1. As the buffer has the highly alkaline pH, there is a considerable concentration of hydroxide ion behaving as a coion. Further, under real laboratory circumstances it should be expected a presence of a certain amount of carbonates in the BGE due to absorption of carbon dioxide from air. When simulating the properties of the PDC buffer we supposed that it contains concentration of 0.2 mM carbonates. As they are in the anionic form in

the buffer, they form an additional coion. In total, the BGE contains four constituents, so we should expect four eigenmobilities.

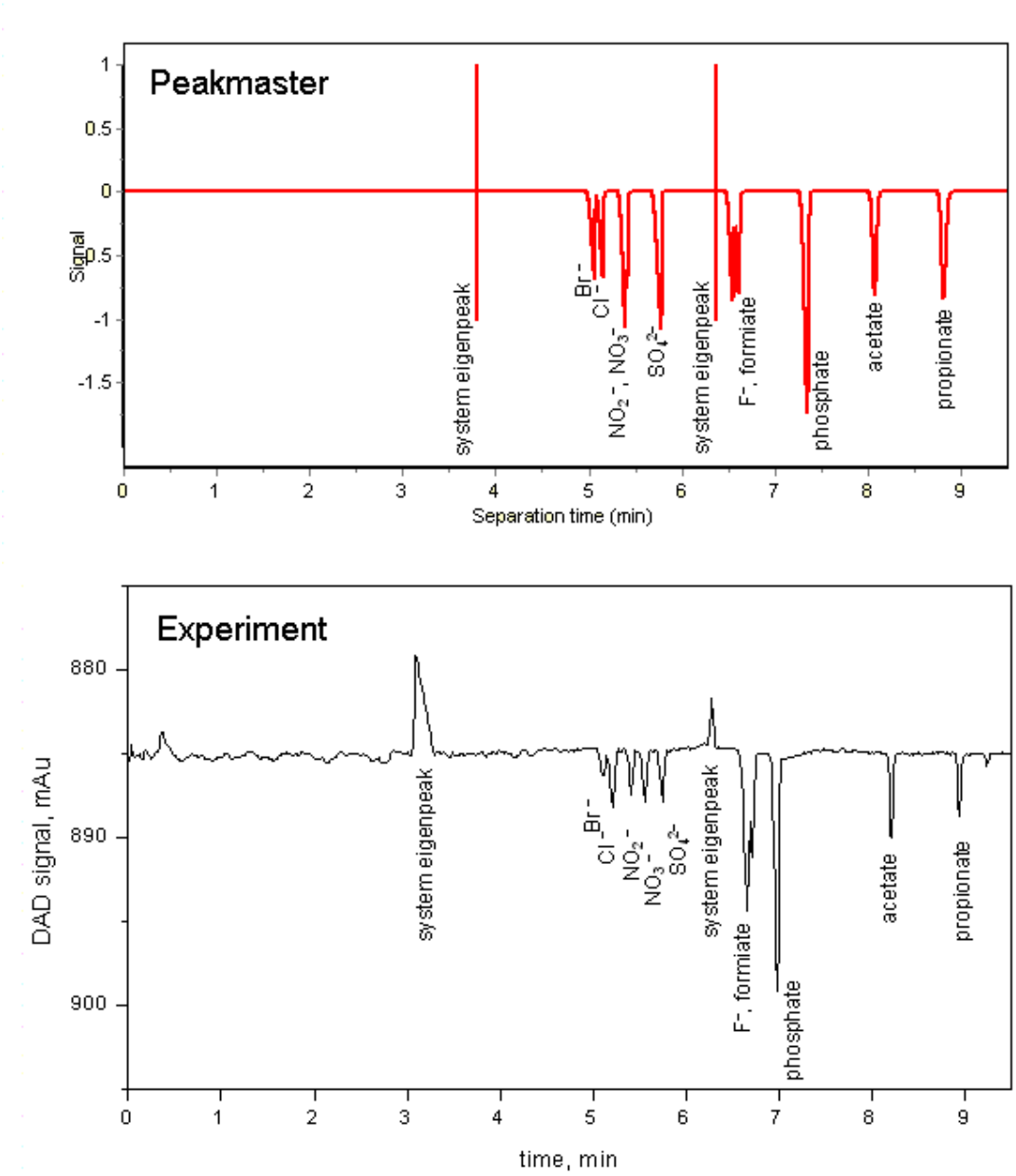
In the **System parameters** subwindow the four predicted eigenmobilities are:

1. system eigenmobility 17.284
2. system eigenmobility 0.000
3. system eigenmobility –50.937
4. system eigenmobility –101.25

The 1. system eigenmobility is positive and is connected with a small content of TTA+ cations. It is uninteresting here, as we are concentrated on separation of anions.
The 2. system eigenmobility is zero within three decimals shown (in fact it has a certain extremely small positive value). It gives the electrophoretic mobility of the water gap. This water gap has negligible electrophoretic movement but is shifted by electroosmotic flow (as all analytes), so it can serve as the electroosmotic flow marker.
The 3. system eigenmobility is –50.937. It exists due to the admixture of carbonates. We should expect a system eigenpeak with this mobility in resulting electropherogram.
The 4. system eigenmobility is –101.25. It exists due to a high content of hydroxide ions in the BGE. We should expect a system eigenpeak with this mobility in resulting electropherogram.

The **Electropherogram** window shows a simulated electropherogram of the analyte mixture. The positions of system eigenpeaks are depicted here by means of vertical lines. Here, you can see positions of only three system eigenpeaks. The 2. and 3. eigenpeak should lie somewhere among analytes, the 4. eigenpeak goes with the electroosmotic flow at time of 20.5 min as the EOF marker.

A comparison of the theoretically simulated and experimentally obtained indirect UV electropherograms is following:



From a comparison of both pictures (in the time scale 0 – 9.5 min) you can see that prediction and reality agree well. Both the 2. and 3. eigenpeak are seen in the real electropherogram and also positions and shapes of the analyte peaks are in good agreement.

Example_2 Phosphate buffer for neutral pH is a very common electrolyte system used for separation of anions. A very few people realize that this buffer has a noticeable eigenmobility in anionic region that can cause unexpected though highly interesting behavior. The acido-basic equilibrium responsible for the buffering ability is that between the doubly and the singly charged phosphates. Here, the buffer for pH ~ 7.0 is composed of 5.2 mM H_3PO_4, 7.5 mM NaOH and 0.1 mM TTAOH. (This buffer can also be prepared from 2.9 mM NaH_2PO_4, 2.3 mM Na_2HPO_4 and 0.1 mM TTAOH.). In total, the BGE contains three constituents, so we should expect three eigenmobilities. We will demonstrate the behavior of this buffer on separation of the sample containing chloride, iodate and salicylate.

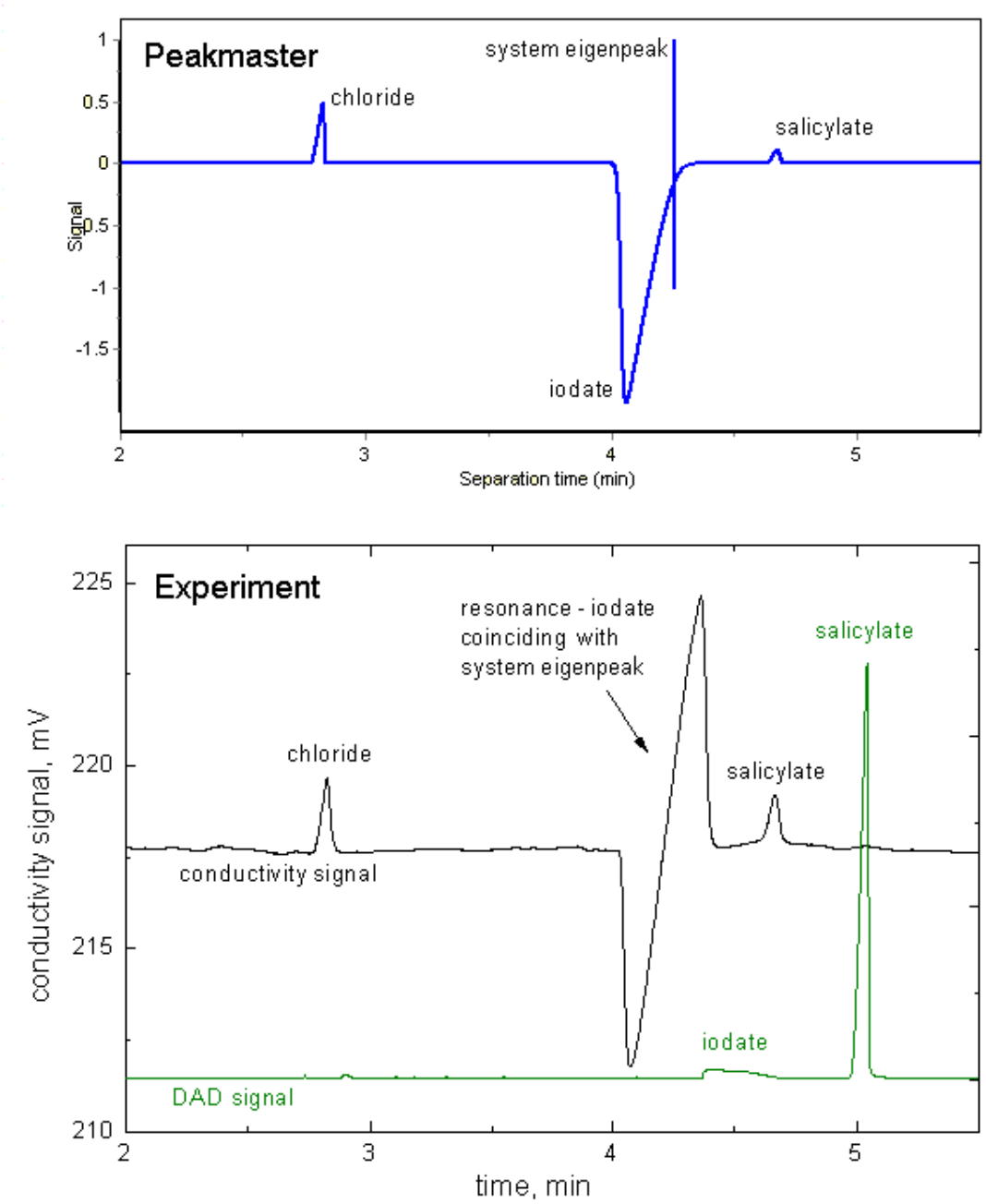
In the **System Parameters** subwindow the three predicted system eigenmobilities are:

1. system eigenmobility 21.817
2. system eigenmobility 0.001
3. system eigenmobility –38.337

The 1. system eigenmobility is positive and is connected with a small content of TTA+ cations. It is uninteresting here, as we are concentrated on separation of anions.
The 2. system eigenmobility is almost zero (in fact it has a certain very small positive value). It gives the electrophoretic mobility of the water gap. This water gap has negligible electrophoretic movement but is shifted by the electroosmotic flow (as all analytes), so it can serve as the electroosmotic flow marker.
The 3. system eigenmobility is –38.337. It exists due to using the BGE with multivalent coanion, phosphate here. This eigenmobility is dangerous, as it lies in the range where mobilities of anionic analytes can appear.

The **Analytes** window shows parameters of the analytes of the sample. Chloride and salicylate have effective mobilities –74.565 and –32.386, respectively. They give Conductivity signal > 0 and fronting peak shape, EMD < 0, with the values in the usual range. On the other hand, the effective mobility of iodate, –38.804, almost coincides with the 3. eigenmobility of the system. As mentioned above, this leads to the resonance phenomenon. Due to the resonance, it should be expected an amplified response of indirect detection methods or conductivity detection at the site of the analyte. PeakMaster predicts here a very high Conductivity signal for the iodate peak, –46.995. Its dispersion expressed by EMD is also very high, 46.994. This implicates that the conductivity signal of the iodate peak should be large and heavily dispersed.

The **Electropherogram** window shows the simulated electropherogram with conductivity detection. The iodate peak is near the system eigenpeak and is forecasted to be huge:



The experimental record of the electrophoretic separation in phosphate buffer (experimental pH = 7.05) using both the conductivity and photometric detectors confirms the predictions. In the record of the conductivity signal (black curve) you can notice a ”normal” positive fronting conductivity signals for chloride and salicylate but a very large ”resonance” signal for iodate, which has a zigzag shape in reality. This is the consequence of resonance, i.e., the coincidence of the iodate mobility with the system eigenmobility. Unfortunately, the present version of PeakMaster is not able to simulate the full zigzag shape of the signal, it can only show one half of it, either the upper or the lower part.

The conductivity detection in CZE might not be as common, so what the UV detector (DAD signal, dark green line) shows? The UV absorbance of phosphates and sodium forming the BGE is negligible, so all the big resonance phenomena revealed by the conductivity detector are hidden here. The UV absorbance of chloride is also negligible, the only UV signals will be then direct UV signals from iodate

and salicylate. While salicylic acid still gives a nice and narrow peak, the signal of iodate is almost lost due to its excessive dispersion, which was predicted by the calculation of the EMD.

It must be realized that the resonance phenomena are not restricted to iodates. Any analyte with the mobility close to the system eigenmobility will behave in this way. The system eigenmobility behaves as a ”black hole”: it can swallow any peak in its vicinity even without a notice of the experimentalist.

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You can input any configuration of the BGE and the sample of your interest (which has a chemical sense) and check its behavior. Good luck!

Our sincere thanks to:

Prof Takeshi Hirokawa, Hiroshima University, Japan, for allowance to utilize his database of pK_a constants and ionic mobilities,
Dr Tomoyoshi Soga, Keio University, Japan, for performing nice electrophoretic separations used for demonstration in this Help,
Agilent Technologies GmbH, Waldbronn, Germany, and Agilent Labs, Palo Alto, USA, for their kind support.

User’s hints

BGE constituents and analytes hints:

- Sort constituents by name or by any of their displayed parameters via clicking on the relevant column header of the *BGE Constituents* or *Analytes* subwindow.

Electropherogram hints:

- Point to the individual peaks with the mouse cursor to read the names of the corresponding analytes in a hint window.
- Quick-zoom your chart via drawing a rectangle (start at the upper left corner) around the area you want to see in detail.
- Undo your quick-zoom via double clicking on the chart.

Database of constituents hints:

- Click *Pick from database...* button in the *Add BGE constituent...* or *Add analyte...* window (appears after clicking the *Add...* button on the main form) to retrieve values of a database constituent.
- Add, remove, or edit database constituents via clicking *Tools*|*Modify database...* from the main menu. However, modifying the values of database constituents is recommended only if your modifications have general validity, i.e. if they are true always, not only in the current document or configuration. If you need modified constituents only for the current document or configuration, you are invited to add them in the *BGE Constituents* or *Analytes* subwindow as a new constituent (under a new name, e.g. “sodium-modified”).