This manual is intended as a brief and clear guide for using Simul 6.1

Simul is a series of simulation software for electrophoresis which has been continuously developing in our laboratory. It simulates the movement of ions in liquid solutions in an electric field. In fact it solves numerically a set of nonlinear partial differential equations and nonlinear algebraic equations describing the continuity of ionic movement and acid-base equilibria. It enables us to visualize the distribution of any constituent of a particular separation system in a separation channel at any time, which gives a complete picture of the particular separation run. This is useful when inspecting so-called stacking or sweeping phenomena, the sharpening of zone edges, the course of the focusing of ampholytes in isoelectric focusing systems, unusual peak broadening, or the course of separation in zone electrophoresis or isotachophoresis.

Simul 6 is a continuation of the previous version 5 and Simul 6.1 has some new features when compared with Simul 6. Both have been designed using the latest programming tools. The code was rewritten using Qt platform by fully standard-conformant C++ and utilizes the latest MSVC or MinGW C++ compilers. The computation engine was completely redesigned in order to take full advantage of parallelization and multithreading computation. It is 5 – 15 times faster than Simul 5.

Unpacking and running Simul 6.1

Microsoft Windows environment is needed to install and run Simul 6.1. The program is packed in the file 'Simul61Run.zip'. After downloading and unpacking, copy the directory 'Simul61Run' to the position in which you wish to have it. Double click the 'simul6.exe' to run it. The default window of Simul 6.1 will appear. Keep in mind that in the framework of Simul 6.1 the electrophoretic mobilities of the constituents are depicted in $10^{-9} \text{ m}^2\text{V}^{-1}\text{s}^{-1}$, and the concentrations of the constituents are depicted in mM, i.e., mmol/L.
Running enclosed examples – the simplest way to learn a lot

**Isotachophoresis separation**

In the default window of Simul 6.1 click **Data | Load data | Toulouse.json | Open**

Doing so, you will load the configuration Toulouse.json, which you will find in the directory Simul61Run/data_files. It is an isotachophoretic separation of five artificial cationic constituents, $S_1$ – $S_5$, the leading ion is potassium at 10 mM concentration, the termination ion is an artificial constituent, term, at 10 mM concentration, the counter ion is acetic acid at 20 mM concentration. Click the **Run** button to simulate the first electromigration run.

The computation will be fast and stops after reaching 80 s of physical time. Take a while to look at the resulting curves and parameters.

(1) You can zoom in on any part of the simulated profile by framing a part of interest with the cursor in the usual manner. Whenever you click with the right mouse button at any part of graph and then click on the popped-up **Auto scale** button, you will return to the optimum rescaling of the graph, or you can set the scaling manually.

You can depict or hide any constituent in the graph when clicking the checkbox in the (eye) column in the subwindow Composition. You can mark by the cursor more then one constituent in the Composition window and toggle their visibility in the graph by clicking **Toggle visibility**. You can mark by the cursor a constituent in the Composition window and edit its values by clicking **Edit**, or remove the constituent from the window by clicking **Remove**. When touching the curve of a constituent in the graphical window by the mouse cursor it becomes highlighted. Clicking the highlighted curve with the left mouse button you can read its parameters in a given spot. Clicking the highlighted curve with the right mouse button you can (i) scale the graph to depict the given curve optimally, or (ii) to hide the curve. You can depict or hide pH (blue curve), conductivity (black curve), and electric field (green curve) profiles by the checkboxes **Show pH**, **Show conductivity**, **Show electric field** in the Compute control subwindow.

(2) You can modify parameters of the electromigration configuration in the Compute control subwindow: **Capillary length**, **Zone edge**, **Number of points**, **Display each**, **Stop time** and run the simulation again by clicking **Init** and **Run**. Keep in mind that **Number of points** is an important parameter: when it is too high, the computation lasts long; when it is too low, the computation has a lower axial resolution and can be unstable.
(3) You can modify parameters in the Parameters subwindow.

\( \text{dt} \) is a crucial parameter of the simulation. Handling \( \text{dt} \) can be done in two modes if you check the checkbox **Optimize dt**.

- **When not checked**, the integration procedure is performed by the fast Runge-Kutta algorithm, which does not allow for the calculation of error. You must input the value of \( \text{dt} \) manually. This has an important aspect: when \( \text{dt} \) it is too low, the computation lasts unnecessarily long; when \( \text{dt} \) is too high, the computation can crash numerically.

- **When checked**, the integration procedure is the Cash-Karp algorithm, which is slightly slower than Runge-Kutta, but allows for the computation of error. This enables setting the value of \( \text{dt} \) automatically, maintaining the error lower than is set in the **Max error** widget. This is performed automatically starting with the initial value you set, by default it is \( \text{dt} = 0.0001 \text{ s} \). You can modify the value of **Max error** to some extent and see the computation speed.

- Voltage and current are quantities, which are bound via Ohm law, where the proportionality factor is the conductance of the capillary. And the conductance of the capillary depends on the conductivity of the solution inside, the diameter and the length of the capillary. By default the inner capillary diameter is set to 50 \( \mu \text{m} \), as is usual in classical capillary electrophoresis. You can change it to any value in the window **Capillary Diameter**. There are two more checkboxes in the Parameters subwindow which set the mode of the electromigration run: **Constant voltage** and **Constant current** modes. When **Constant voltage** mode is checked, you can input the driving voltage in the appropriate widget; when **Constant current** mode is checked, you can input the driving current. Keep in mind that in isotachophoresis, which is the case of Toulouse.json configuration, the conductance of the capillary as a whole greatly changes during the run, which leads to big changes of either current or voltage.

**Another example – real capillary zone electrophoresis separation using a detector**

Simul 6.1 has an option to put a detector at any site of the capillary to record the time course of all constituents passing the detector site. The time course of conductivity can also be recorded, which simulates the signal of the conductivity detector.

This example is a simulation of a real configuration in classic capillary electrophoresis: the capillary length is 800 mm, driving voltage is 30000 V. Such a simulation requires a big number of points, 30000. In the default window of Simul 6.1 click **Data | Load data | CZE.json | Open**. You will load the
configuration CZE.json, which you will find in the directory Simul61Run/data_files. It is a capillary zone electrophoresis separation of five artificial constituents, S1 – S5, at a low concentration of 0.01 mM, in a system where the background electrolyte is potassium/acetate buffer (20 mM acetic acid, 10 mM KOH).

First uncheck Show conductivity, Show pH, Show electric field, then click the right mouse button on the graph area and click the popped-up button Auto scale. Now enable the detector option by clicking the Detector check box and then locate the detector at the position of 600 mm in the Detector position vidget. In the graphical window you will see the green vertical line in this position.

Click the Run button to simulate the capillary zone electrophoresis run. In spite of the big number of points, the simulation will not last very long, about 15 – 20 minutes. All five analytes will pass the detector position. To see the signal of the detector then click at the bookmark Detector in the right-hand side of the graphical window. You should see such a record:

![Graph showing capillary electrophoresis results](image)

When framing and zooming the peak of S5, you will notice a slightly triangular shape of the peak profile – a consequence of electromigration dispersion.

Now uncheck all constituents in the (eye) column, instead check the Show conductivity checkbox. Then click with the right mouse button at any part of graph and then click on the popped-up Auto scale button. You will see the simulated time record of the conductivity detector.
Building your own electromigration task

Let’s suppose you have a 20 mm long chip separation channel, the left 5 mm part of which is filled with sodium/acetate buffer (20 mM acetic acid, 10 mM NaOH), the remaining right part of which is filled with potassium/acetate buffer (20 mM acetic acid, 10 mM KOH).

This is a picture of the configuration:

![Diagram of the configuration](image)

You plan to put 50 V on the channel, positive polarity on the left side and to see what will happen.

(1) Run Simul 6.1 by clicking simul6.exe. The main window of Simul 6.1 will appear in a default configuration.

(2) Change Capillary length to 20 mm in the appropriate widget of Compute control subwindow.

(3) Change Voltage to 50 V in the appropriate widget of Parameters subwindow.

(4) Input the sodium constituent:

(i) Click Add in the Composition window. The Composition and Segments settings window is opened.

(ii) Click Search constituent row and start to type ‘sodium’. The sodium constituent is depicted in the window below, select ID 448 SODIUM by clicking. The mobility and pKa constants of sodium hydroxide are transferred into the Segments subwindow. Notice that the separation channel is divided into four segments by default, which is convenient because it is exactly what we need. Input the concentration $c_{\text{mM}}$ of 10 mM in the left segment of four. Click Accept.

(5) Input the potassium constituent:

(i) Click Add in the Composition window. The Composition and Segments settings window is opened.

(ii) Click Search constituent row and start to type ‘potassium’. The potassium constituent is depicted in the window below, select ID 418 POTASSIUM by clicking. The mobility and pKa constants of potassium hydroxide are transferred into the Segments subwindow. Input the concentration $c_{\text{mM}}$ of 10 mM in the second, third and fourth segments of four. Click Accept.
(6) Input the acetate constituent:

(i) Click **Add** in the Composition window. The Composition and Segments settings window is opened.

(ii) Click Search constituent row and start to type ‘acetic acid’. Acetic acid is depicted in the window below, select ID 85 ACETIC ACID by clicking. The mobility and pKa constants of acetic acid are transferred into the Segments subwindow. Input the concentration \(c[\text{mM}]\) of 20 mM in all four segments. Click **Accept**.

(7) Click **Init**. The graphical window shows the distribution of constituents and other parameters before applying voltage. It should look like this:

(8) Click **Run**. The course of electromigration is depicted in the graphical window. After 80 s of physical time it should look like this:
What you could see was the movement of the isotachophoretic boundary.

**Saving the electromigration configuration**

You can save your configuration for the next use. Click **Data | Save Data** and input a name for the configuration, say, My_task. Before clicking **Save**, select the directory where you wish to put it, say, Simul61Run/data_files/. The configuration will be saved as My_task.json.

In fact there are two formats for saving a given electromigration configuration: (i) as json files (as you just did), and (ii) as sqlite3 files. When clicking **Data|Save data**, the option **Save as type**: for the saving format appears in the popping window. You can select either the json format or sqlite3 format. These two options add the extension *.json or *.sqlite3 to the name of the saved file, respectively.

**Saving and replaying the course of the simulation**

Simul 6.1 enables you to replay the previously simulated results without the necessity of further computation.
(1) Saving the course as json or sqlite3 files

This example shows how to save the progress of electromigration which can be computed by the configuration you have previously formed, My_task.json.

Run Simul 6.1 by clicking simul6.exe. The main window of Simul 6.1 will appear in the default configuration. Click Data | Load Data | My_task.json | Open. Click on button in the Progress saving subwindow, select the directory where you wish to put the saved progress, say, Simul61Run/data_files. Input the name of the file, say, My_task_progress.

When clicking at Save as type:, three options for the format are offered: sqlite3, json, csv. Select, say, sqlite3 format. Then click the Save button. Further, you have to select Time interval for saving, say 10 s, as is the default. Check the checkbox Active, which activates the progress saving. Then click Run and let the computation run to the end. The progress is saved as My_task_progress.sqlite3.

Important notice: For large electromigration tasks (such as simulation of isoelectric focusing) select preferably the sqlite3 format for saving, as it requires about five times less space than json.

You can now replay the progress of the electromigration. Click Data | Load data | My_task_progress.sqlite3 | Open. The computed results are now depicted at zero time and a new window is popped-up: Replay. You can spend some time getting comfortable with using the generally known icons.

In fact you can continue the computation from any time you wish. Say, when Frame 8 of 8 is depicted, close the Replay window by clicking , then increase Stop time to 100 s, and click Run. The computation will continue until 100 s.

(2) Saving CSV files

The third option enables you to depict the results of simulation in graphical software of third parties, like Excel or Origin.

Run Simul 6.1 by clicking simul6.exe. The main window of Simul 6.1 will appear in a default configuration. Click Data | Load Data | Toulouse.json | Open

Click on button in the Progress saving subwindow, select the directory where you wish to put the saved progress, say, Simul61Run/data_files. Input the name of the file, say, Toulouse_export.
Click **Save as type**:, select **Csv format**, then click **Save**. Further, you have to select **Time interval** for saving, say 10 s, as is the default. Click the checkbox **Active**, which activates the progress saving. Click **Run** and let the computation run to the end. The progress is saved in the directory Simul61Run_data_files as csv files for various times.

(3) Import csv files into Excel

Run Excel and click **Data | From text** and find the directory where the data are saved, Simul6Run/data_files. Then select, say, Toulouse_export080.00.csv. Click **Import**. As the file type select **Separator**, click **Next**, as a separator check **Space**, click **Next**, then click **Finish** and then **OK**. You will see the columns from A to L filled with data. With the cursor select all the columns from A to L, then click **Insert**, and **Recommended graphs**. Then select the appropriate one which looks analogously as in the graphical window of Simul 6.

**Isoelectric focusing**

Simul 6.1 contains tools for the effective simulation of isoelectric focusing (IEF), which is one of the well-established electromigration separation methods. It is based on forming a linear pH gradient in the separation channel in a mixture of special ampholytes, which are called carrier ampholytes. The separated ampholytic analytes are focused in the position, where the pH of the given spot is equal their pl value. The separation channel is connected to two electrode reservoirs containing an acid, such as phosphoric acid, and a base, such as lithium hydroxide, respectively.

**Building your own isoelectric focusing task**

Let’s suppose you have a 25 mm long chip separation channel, the left 5 mm of which is filled with 100 mM phosphoric acid, the right 5 mm part of which is filled with 100 mM lithium hydroxide and the middle part, 15 mm long, is filled with a mixture of carrier ampholytes, which will be able to form a gradient of pH.

(1) Run Simul 6.1 by clicking **simul6.exe**. The main window of Simul 6.1 will appear in the default configuration. Input 1000 into the widget **Number of points**. Input 600 s into the widget **Stop time**. Input 20 V into the widget **Constant voltage**.
(2) Click **Add** in the Composition window. The Composition and Segments settings window is opened. Click Search constituent row and start to type ‘phosphoric acid’. The phosphoric acid constituent is depicted in the window below, select ID 399 PHOSPHORIC ACID by clicking. The mobility and pKa constants of phosphoric acid are transferred into the Segments subwindow. Increase the **Number of segments** to 5 by clicking the up arrow in the widget. Now the separation channel will be divided into five segments by 5 mm. Input the concentration $c[\text{mM}]$ of 100 mM in the left segment of five. Click **Accept**.

(3) Click **Add** in the Composition window. The Composition and Segments settings window is opened. Click Search constituent row and start to type ‘lithium’. The lithium constituent is depicted in the window below, select ID 304 LITHIUM by clicking. The mobility and pKa constants of lithium hydroxide are transferred into the Segments subwindow. Increase the **Number of segments** to 5 by clicking on the up arrow in the widget. Now the separation channel will be divided into five segments by 5 mm. Input the concentration $c[\text{mM}]$ of 100 mM in the right segment of five. Click **Accept**.

(4) Click **Data | Ampholines**, the Ampholines window will appear. Input 141 into the widget **Number of Ampholines**. This will generate 141 ampholytes, each of them can attain one positive and one negative charge. The pKa constants of the first one are 4 and 3, so its isoelectric point is 3.5. The pKa constants of the last one are 11 and 10, so its isoelectric point is 10.5. The range of pl of the 141 ampholytes then goes from 3.5 to 10.5, the distance between individual pl is 0.05. Both the mobility of the cathodic and anodic form of the ampholytes are $20\times10^{-9} \text{ m}^2\text{V}^{-1}\text{s}^{-1}$.

Increase the **Number of segments** to 5 by clicking the up arrow in the widget. The separation channel is now divided into five segments of 5 mm length. Type concentration $c[\text{mM}]$ of 0.1 mM into the second, third, and fourth segment of the channel. Click **Generate**, then **Init**. The resulting window should look like this:
Click Run. The computation starts, you can zoom in on the development of the ampholytes profile when framing a part of interest with the cursor. After about 4 – 6 minutes of computation time (depending on the speed of the processor and number of its cores) the physical time will reach Stop time and the calculation stops. After appropriate zooming the window can look like this:
You can admire the linear gradient of pH which was established in the middle of the channel, see the blue line. If you wish, you can save the configuration by clicking **Data | Save Data** and input a name for the configuration, say, IEF. Before clicking **Save**, select the directory where you wish to put it, say, Simul6Run/data_files/, and the format in which you wish the have the results. Preferably use the sqlite3 format. The configuration will be saved as IEF.sqlite3.

**Running the enclosed example of isoelectric focusing**

This is the separation of fourteen pI markers, which was discussed in one of our papers:

CE determination of the thermodynamic pK(a) values and limiting ionic mobilities of 14 low molecular mass UV absorbing ampholytes for accurate characterization of the pH gradient in carrier ampholytes-based IEF and its numeric simulation, by: Ansorge, M; Gas, B; Boublik, M; Maly, M; Steflova, J; Hruska, V; Vigh, G: [ELECTROPHORESIS, 2020](#)

Run Simul 6.1 by clicking **simul6.exe**. The main window of Simul 6.1 will appear in a default configuration. Click **Data | Load data | Markers.sqlite3 | Open**. Doing so, you will load the configuration Markers.sqlite3, which you will find in the directory Simul6Run/data_files. Click **Run**. The computation starts, you can zoom in on the development of the ampholytes’ profile when framing a part of interest with the cursor. After about 15 – 25 minutes of computation time (depending on the speed of the processor and number of its cores) the physical time will reach **Stop time** 3000 s and the calculation stops. After appropriate zooming the window can look like this:
Mobility or pKa constants of a constituent dependent on the axial coordinate

Both mobilities and pKa constants of the constituent can depend on the axial coordinate. Such a situation can occur in gel electrophoresis, where a part of the separation channel filled with the background electrolytes contains a hydrophilic neutral gel, which forms a sieving environment for the separation of DNA or proteins. Such a part of the separation channel is often connected with another part of the channel or the electrode vessel with the same background electrolyte, but not including the neutral polymer. The content of the neutral polymer forms not only the sieving environment, but also slightly modifies the mobilities of the ions of the background electrolyte. It was observed and explained by Spencer long ago that it can lead to the increase or depletion of the overall concentration of the background electrolyte at the boundaries between the gel and non-gel parts of the separation channel and cause anomalous conductivity zones in gel electrophoresis.

Simul 6.1 enables us to consider both the mobilities and pKa constants of the constituents as dependent on the axial coordinate and thus simulate the increase and depletion of the concentration at the boundaries. The next example does not simulate a concrete situation, but demonstrates how to consider the axial dependence of the physicochemical constants and depicts the phenomenon itself.

1) Run Simul 6.1 by clicking simul6.exe. The main window of Simul 6.1 will appear in a default configuration. Input 600 s into the widget Stop time.

2) Click Add in the Composition window. The Composition and Segments settings window is opened. Click Search constituent row and start to type ‘acetic acid’. The acetic acid constituent is depicted in the window below, select ID 85 ACETIC ACID by clicking. The mobility and pKa constants of acetic acid are transferred into the Segments subwindow. Input the concentration c[mM] of 20 mM in all four segments. Click Accept.

3) Click Add in the Composition window. The Composition and Segments settings window is opened. Click Search constituent row and start to write from the keyboard ‘sodium’. The sodium constituent is depicted in the window below, select ID 448 SODIUM by clicking. The mobility and pKa constants of sodium hydroxide are transferred into the Segments subwindow. Input the concentration of c[mM] of 10 mM in all four segments. Let’s suppose that in the second section there is a content of a neutral hydrophilic gel which modifies the mobility of sodium. Therefore, check the checkbox Manually, this will allow you to modify any parameter in the Segments window. So modify the mobility of sodium in the second segment, instead of 51.9 input, say, 50. This will modify not only the sodium mobility, but
also a parameter which is called the transference number, defined as \( T = \frac{u_{Na}}{u_{Na} + u_{Acetate}} \), and which is responsible for the phenomenon. Click **Accept**.

(4) Click **Init**, click **Run**. After reaching 600 s of physical time you will get such a picture:

![Image of a depletion of background electrolyte concentration](image)

Clearly, there is a depletion of the background electrolyte concentration at the boundary, which is in the middle of the channel, which causes the resistive zones.

**Adding new constituents**

The Simul series contains a database of mobilities and pKa values based on the Takeshi Hirokawa tables, so generally you simply select the constituent of interest from the Database window. However, you can use in simulation any new constituent, the mobilities and pKa constant of which are known. Let’s suppose you wish to check the behavior of a new pI marker in the configuration Markers.json we have already simulated. Let’s suppose that the marker is bad: the pKa of the cationic form is 5, pKa of the anionic form is 9. This means that its pI is 7 and the difference between both forms is 4, which means we can doubt its ability to serve as a pI marker.

(1) Run Simul 6 by clicking **simul6.exe**. The main window of Simul 6.1 will appear in the default configuration. Click **Data | Load data | Markers.sqlite3 | Open**
(2) Click **Add** in the Composition window. The Composition and Segments settings window is opened. Check the checkbox **Manually**, which will allow you to modify any parameter in the Segments window. Type BAD MARKER in the **Name** row. Reduce the number of segments to 1 by clicking the down arrow in the **Number of segments** widget. Type mobility of 20 into the **u-1** widget. Type mobility of 20 into the **u+1** widget. Type pKa of 9 into the **pKa-1** widget. Type pKa of 5 into the **pKa+1** widget. The resulting window should look like this:

(3) Increase the number of segments to 5 by clicking the up arrow in the **Number of segments** widget. In the **Ratio** widgets input these numbers going from left to right: 3, 5, 8, 5, 3. In the **c[mM]** widgets input these numbers going from the second to the fourth: 0.01, 0.01, 0.01. The resulting window should look like this:
(4) Click **Accept**. Click **Init**. Click **Run**. The computation starts, you can zoom in on the development of the markers’ profiles when framing a part of interest with the cursor. After about 14 – 20 minutes of computation time (depending on the speed of the processor and number of its cores) the physical time will reach **Stop time** 3000 s and the calculation stops. After appropriate zooming the window can look like this:
The new constituent, BAD MARKER, which is in the middle of the screen, is obviously bad. Under the conditions of the run it was not focused and even formed a double peak.

Swapping a part of the separation channel with another electrolyte solution. Using various diameter of the channel.

Both features can be used in various situations in chip electrophoresis. The first one enables to swap a part of the separation channel with another electrolyte solution after some time of the separation run, the second one enables to vary the diameter of the separation channel in a large extent.

Here it will be demonstrated using both features in isoelectric focusing when using so called chemical mobilization. What is a principle: After a certain time of the isoelectric focusing run the pH gradient is established and the analytes are separated according their pl. However, the whole system is almost stationary and does not move in the separation channel. When the equipment does not posses a whole channel imaging detector, the solution has to be pushed from the channel to pass a stationary detector to see what was separated. This can be done either by application of pressure at one end of the separation channel or by the chemical mobilization. The chemical mobilization is reached simply by the exchange of the cathodic vessel, which is a solution of a base, such as lithium hydroxide, with a solution of an acid, such as phosphoric acid, mostly the same acid as used in the anodic vessel. Then the hydrogen ions are allowed massively to get into the separation channel and mobilize all ampholytes together with separated analytes.

Simul 6.1 has both features allowing to perform such simulation: (i) to replace, or to swap, a part of the separation channel with another electrolyte solution, (ii) to allow any part of the separation channel to have a variable diameter, even the diameter as thick as is the electrolyte vessel. The next example shows such focusing and a mobilization of the isoelectric focusing system. Moreover, this example also demonstrates the saving the whole course of the electromigration run in the format of the sqlite3 file. For this it will be used a configuration which has been already utilized, Markers.sqlite3.

(1) Run Simul 6.1 by clicking simul6.exe. The main window of Simul 6.1 will appear in the default configuration. Click Data | Load data | Markers.sqlite3 | Open.

Click on Capillary diameter button and set three Segments with the ratios 9, 54, 9. Input corresponding diameters as 5000, 50, 5000 μm. Click Accept and then Init. The left and right 9 mm
parts of the separation channel have the diameter 5000 μm = 5 mm, and form the electrode vessels this way. The middle part of the separation channel is the capillary with the diameter of 50 μm. You can notice that the left electrode vessels is filled with 300 mM phosphoric acid, the right electrode vessel with 200 mM lithium hydroxide. Click on button in the Progress saving subwindow, select the directory where you wish to put the saved progress, say, Simul61Run/data_files. Input the name of the file, say, Markers_swap_progress. When clicking at Save as type:, select the sqlite3 format. Then click the Save button. Further, for Progress saving you select Time interval of 100 s. Check the checkbox Active, which activates the progress saving. Then click Run and let the computation to run to the Stop time, which is 3000 s. You can observe that ampholytes are focused and the pH gradient is nicely formed.

(2) As stated above, the right electrode vessel is filled with lithium hydroxide. Lets swap the content of this vessel by the 300 mM phosphoric acid. For this click the View | Swapping. This will add a new button, Add swap content, into the upper left corner of the Composition window. Click the button Add swap content. Parallel with the Basic bookmark a new one, Swap content, will appear. The bottom part of the subwindow depicts the placement of the swap. As a default the channel is divided into three part and the swapped part is in red. Three numbers in the Ratio widget determine its position. For the placement of the swapped part to the right wessel, between 63 and 72 mm, input these three numbers in the Ratio widget:

<table>
<thead>
<tr>
<th>Ratio</th>
<th>63</th>
<th>9</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caplen</td>
<td>63.00</td>
<td>9.00</td>
<td>0.00 mm</td>
</tr>
<tr>
<td>Length</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

To define the composition of the swap click now Add and the Composition and Segments settings window is opened. Click Search constituent row and start to type ‘phosphoric acid’. The phosphoric acid constituent is depicted in the window below, select ID 399 PHOSPHORIC ACID by clicking. The mobility and pKa constants of phosphoric acid are transferred into the Segments subwindow. Input the concentration \([\text{mM}]\) of 300 mM. Click Accept. Click the button Swap and the corresponding part of the channel, in fact the right electrode vessel, is now filled with 300 mM phosphoric acid. Then increase Stop time in the Compute control subwindow from 3000 s to 7200 s. The resulting window should look like this.
Then click **Run** and let the computation to reach the Stop time 7200 s. All the simulation is now saved in the file Markers_swap_progress.sqlite3.

You can inspect the whole simulation run by replaying the saved file. Click **Data | Load data | Markers_swap_progress.sqlite3 | Open**. The computed results are now depicted at zero time and a new window is popped-up: **Replay**. The saved frame range is from 0 to 73. You can spend some time inspecting the simulated results. Zoom in on any part of the simulated profile by framing a part of interest with the cursor in the usual manner. You will see that in the mobilization stage the decreasing pH in the channel mobilizes all content out of the channel.

When clicking by the right mouse mutton at the **Add swap content** button, a new menu expands: **Add swap content, Clone current swap content, Remove current swap content**. Their use is obvious.

**Oscillating electrolytes**

We predicted the existence of oscillating electrolytes and then **discovered** them. Even a **simple mixture** of imidazole and sebacic acid oscillates.

In the default window of Simul 6.1 click **Data | Load data | Sebacic_oscill.json | Open**
You will load the configuration Sebacic_oscill.json, which you will find in the directory Simul6Run/data_files. This is a capillary zone electrophoresis run of 0.23 mM sebacic acid and 0.33 mM imidazole. The concentration of imidazole is very slightly disturbed in the center of the separation channel. Click the Run button to simulate the capillary zone electrophoresis run. Do you like it?

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