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*This manual is intended as a brief 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. 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 stacking or sweeping phenomena, the sharpening of zone edges, the course of the focusing of ampholytes in isoelectric focusing systems, unusual band broadening, or the course of separation in zone electrophoresis or isotachophoresis.

Simul 6.1 is a continuation of the previous version, Simul 5. It has been designed using the latest programming tools. The code was written 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 multithreaded computation.

Simul 6.1 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. If you use the touchpad instead of a mouse for controlling the pointer, please go to Windows Settings, select Devices, then select Touchpad, and under the section Taps, uncheck the checkbox of "Tap twice and drag to multi-select". This avoids occasional problems with zooming in the simulated profiles.

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, electrophoretic mobilities of the constituents are depicted in  $10^{-9} \text{ m}^2\text{V}^{-1}\text{s}^{-1}$ , concentrations of the constituents are depicted in mM, i.e., mmol/L and conductivity in mS/m.

### Running the enclosed examples – the simplest way to learn a lot

#### *Isotachophoretic 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, S1 – S5, the leading ion is potassium at 10 mM concentration, the terminating ion is an artificial constituent, term, at 10 mM concentration, the counter constituent 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 your time to look at the resulting curves and parameters.

(1) You can zoom in on any part of the simulated profile by framing the part of interest with the cursor in the usual manner. Whenever you click with the right mouse button on 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 when clicking **Manual scale**. There is also an option to save the graphical image in the .png format when clicking **Save chart to image**.

You can depict or hide any constituent in the graph by clicking the checkbox in the  (eye) column in subwindow **Composition**. You can mark by the cursor more than 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 at the spot. By clicking the highlighted curve with the right mouse button you can (i) scale the graph to depict the given curve optimally, or (ii) hide the curve. You can depict or hide the pH (magenta curve), conductivity (black curve), and electric field (khaki 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**, **Capillary diameter**, **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 takes long; when it is too low, the computation has a lower axial resolution and can be unstable. **Number of points** must be an even number. Two values of **Time** have two meanings: the first is the physical time in the simulated configuration, the second one is the elapsed real computing time of the latest simulation.

(3) You can modify parameters in the **Parameters** subwindow.

**dt** is a crucial parameter of the simulation. **dt** can be handled automatically by checking the checkbox **Optimize dt**, or manually by leaving the checkbox unchecked.

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

- *When the Optimize dt box is 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 **dt** automatically and maintains the computation error lower than the value set in the **Max error** widget. This is performed automatically, starting with the initial value you set (by default it is **dt** = 0.0001 s). You can modify the value of **Max error** and see the computation speed.

- Voltage and current are quantities which are bound via Ohm's law, where the proportionality factor is the conductance in the capillary. Conductance in the capillary depends on the conductivity of the solution in the capillary, and the diameter and length of the capillary. By default, the inner capillary diameter is set to 50 µm, 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 in the Toulouse.json configuration, conductance in the capillary greatly changes during the run, which leads to big changes in either current or voltage.

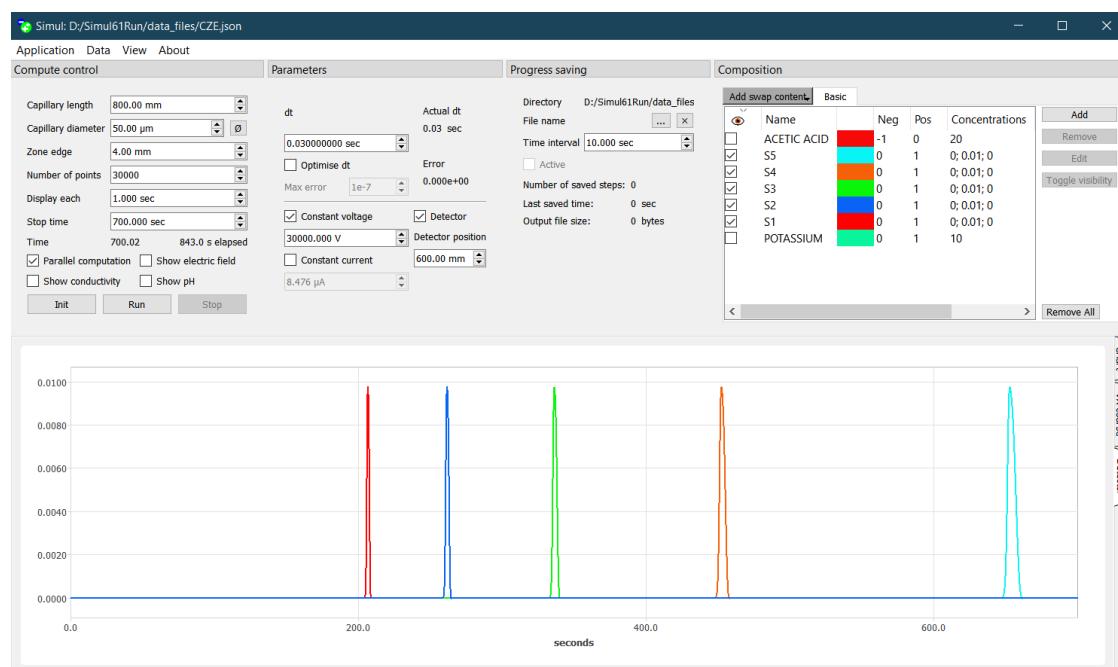
### *Another example – capillary zone electrophoretic separation using a detector*

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

This example is a simulation of a real configuration in classic capillary electrophoresis: the capillary length is 800 mm, the 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 electrophoretic separation of five artificial constituents, S1 – S5, at a concentration of 0.01 mM, in a system where the background electrolyte is a 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 in the **Parameters** section, then locate the detector at the position of 600 mm in the **Detector position** widget. In the graphical window you will see a black vertical line in this position.

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



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

Now uncheck all constituents in the  (eye) column, and check the **Show conductivity** checkbox.

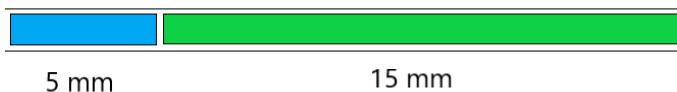
Click with the right mouse button at any part of graph, then click on the popped-up **Auto scale** button. You will see the simulated time record of the conductivity detector.

You can also save the detector record as a file, either as a text file in the csv format or in the graphical file in the png format. Just click with the right mouse button at any part of graph, then click on the popped-up **Save detector data to CSV file** or **Save detector as image** button.

### **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 acetic acid/sodium acetate buffer (20 mM acetic acid, 10 mM NaOH), the remaining right part is filled with acetic acid/potassium acetate buffer (20 mM acetic acid, 10 mM KOH).

This is a picture of the configuration:



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

- (1) Run Simul 6.1 by clicking **simul6.exe**. The main window of Simul 6.1 will appear in default configuration.
- (2) Change **Capillary length** to 20 mm in the appropriate widget of the **Compute control** subwindow.
- (3) Change **Voltage** to 50 V in the appropriate widget of the **Parameters** subwindow.
- (4) Input the sodium constituent:
  - (i) Click **Add** in the **Composition** subwindow. The **Composition and Segments** settings window is opened.
  - (ii) Click the **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 this is exactly what we need. Input the concentration **c[mM]** of 10 mM in the leftmost segment. Click the **Accept** button at the bottom right position.

(5) Input the potassium constituent:

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

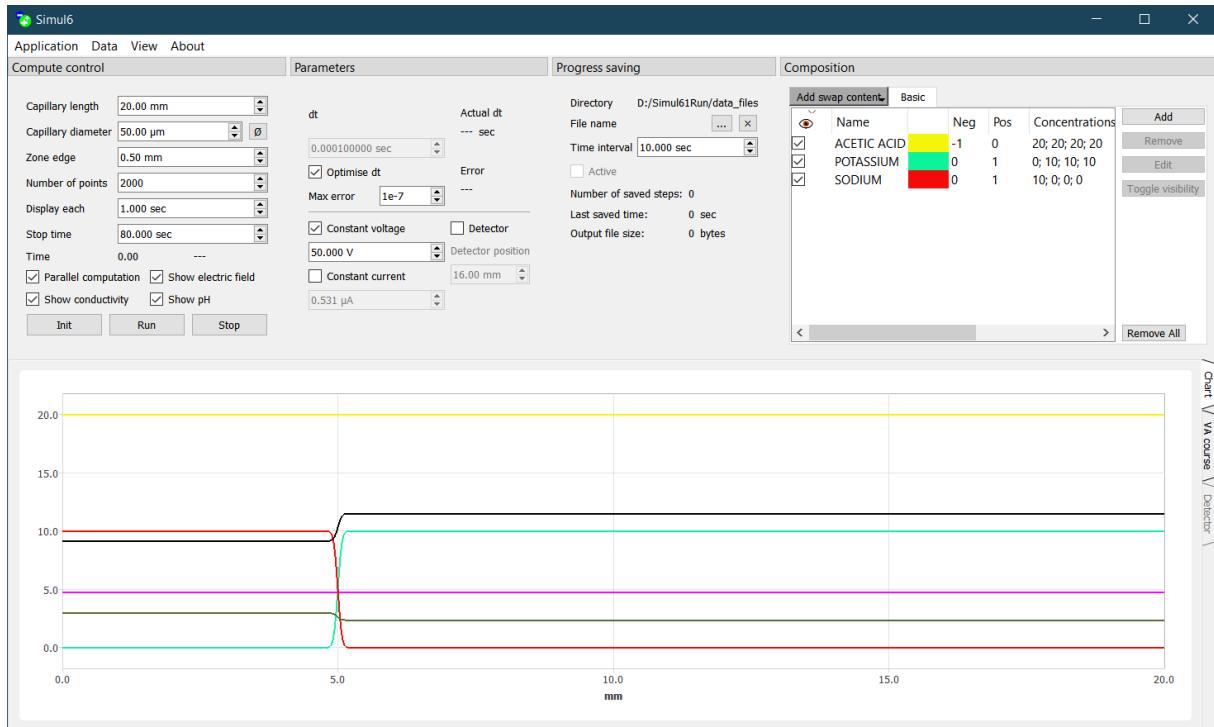
(ii) Click the **Search constituent** row and start to type ‘potassium’. The potassium constituent is depicted in the window below, select ID 418 POTASSIUM by clicking. The mobility and pKa constants of potassium hydroxide are transferred into the **Segments** subwindow. Input the concentration **c[mM]** of 10 mM in the second, third and fourth segments. Click the **Accept** button at the bottom right position.

(6) Input the acetate constituent:

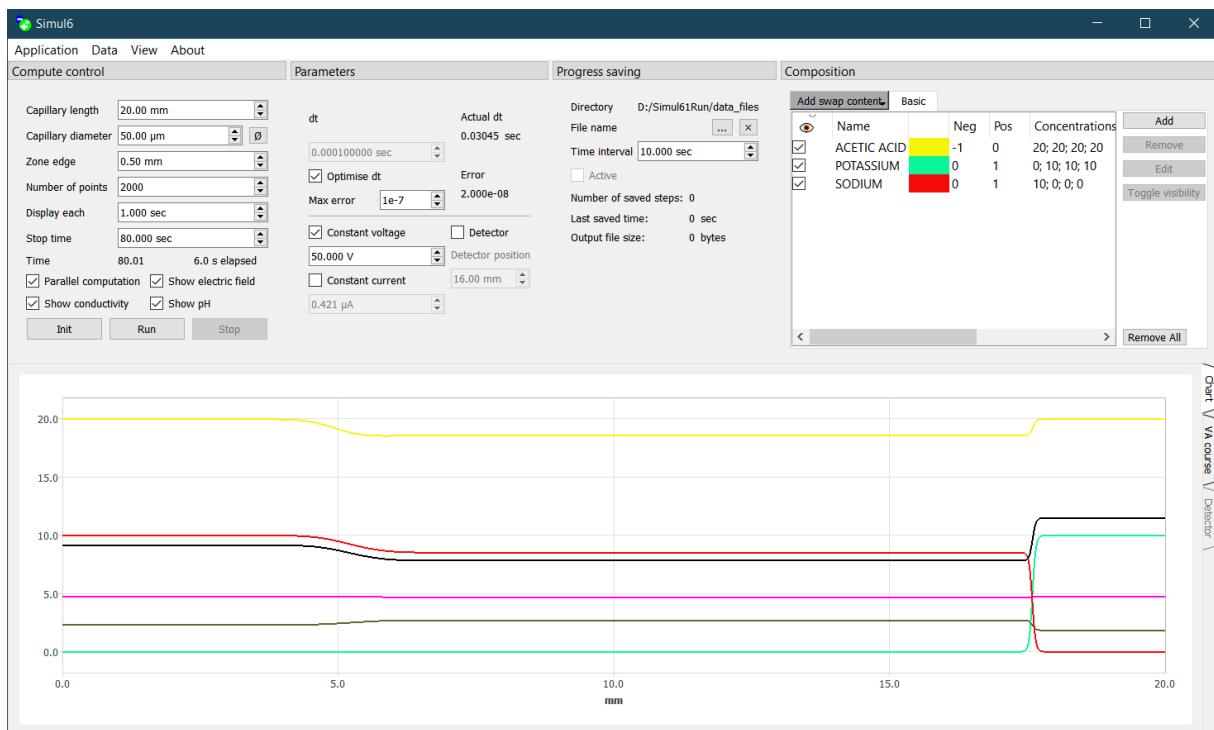
(i) Click the **Add** in the **Composition** window. The **Composition and Segments settings** window is opened.

(ii) Click the **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[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 use next time around. 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.

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

### (1) Saving the course of the simulation 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.

Three options are offered for the format when you click **Save as type:** sqlite3, json, and csv. Select, say, the format sqlite3. 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 saving the progress of the simulation. 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 has a database structure and requires about five times less space in the disk than json.

Simul 6.1 enables you to replay the previously simulated and saved results without repeating the computation. You can replay the progress of the electromigration by clicking **Data | Load data | Simul61Run/data\_files | My\_task\_progress.sqlite3 | Open**. The computed results are now depicted at zero time and a new window is popped-up: **Replay**. Spend some time getting comfortable with using the generally known player icons.

You can continue the simulation from any time point 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. If you want to, you can also save this longer run using the method described in the previous paragraph.

## (2) Saving CSV files

The third file format option enables you to depict the results of the simulation in a graphical software obtained from a third party, *e.g.*, 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, (the default value is 10 s). Click the checkbox **Active**, which activates saving of the progress. 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/CSV** and find the directory where the data are saved, Simul6Run/data\_files. Then select, say, Toulouse\_export080.00.csv. Click **Import**. In the window that opens click subwindow **Delimiter**, from the roll-down list select **Space**, then click **Load**. 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 charts**. Then select the appropriate one which looks like the one in the graphical window of Simul 6.1.

## 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 pH gradient in the separation channel in a mixture of special ampholytes, which are called carrier ampholytes. The separated ampholytic analytes are focused into positions, where the pH of solution is equal their pI

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**.

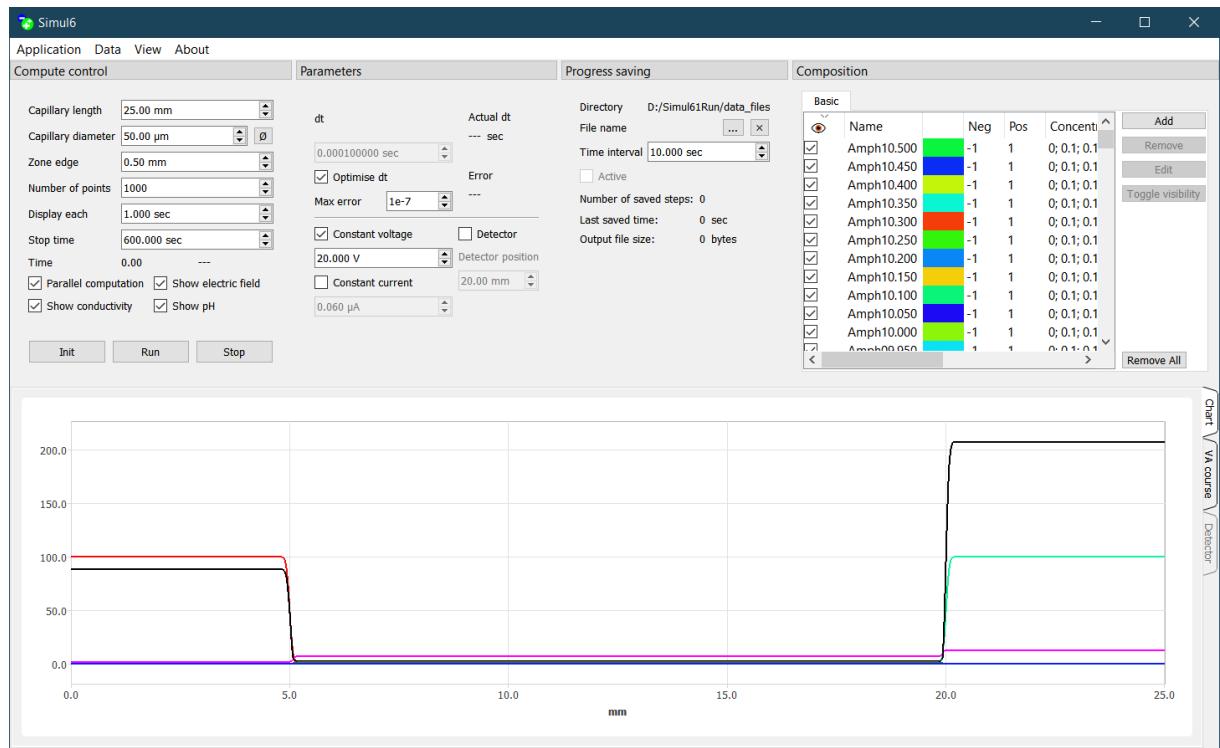
(2) Click **Add** in the Composition window. The **Composition and segments settings** window is opened. In the **Database** subwindow click the **Search constituent** row and start to type 'phosphoric acid'. The phosphoric acid constituent is depicted in the window below, select ID 399 PHOSPHORIC ACID 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 of 5 mm each. Input the concentration **c[mM]** of 100 mM in the left segment of five. Click **Accept** located below the **Database** subwindow.

(3) Click **Add** in the Composition window. The **Composition and segments settings** window is opened. In the **Database** subwindow click the **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 of 5 mm each. Input the concentration **c[mM]** of 100 mM in the right segment of five. Click **Accept** located below the **Database** subwindow.

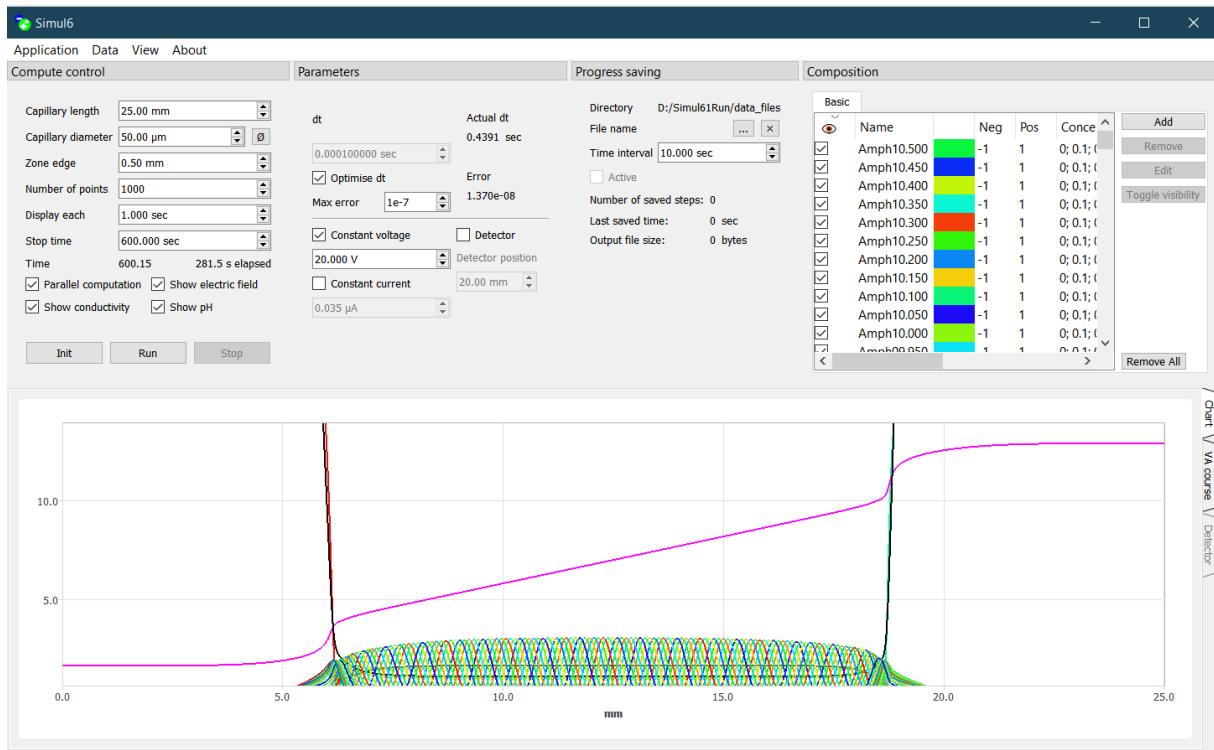
(4) Click **Data | Ampholytes**, the **Ampholytes** window will appear. In the **pKa** section, input 141 into the widget **Number of Ampholytes**. This will generate 141 ampholytes, each of them can attain one positive and one negative charge. The pKa constants of the most acidic one are listed in the **Low pKa** row: 4 and 3, so its isoelectric point is 3.5. The pKa constants of the most basic one are listed in the

**High pKa** row: 11 and 10, so its isoelectric point is 10.5. The range of *pI* of the 141 ampholytes then goes from 3.5 to 10.5, the increment between the *pI* of the adjacent ampholytes is 0.05. The mobilities of the cationic and anionic forms of the ampholytes are set in the **Mobility** section: they can be identical or different, but the values apply to all 141 ampholytes in the series. Say, enter  $20 \times 10^{-9} \text{ m}^2 \text{V}^{-1} \text{s}^{-1}$ , the default values.

In the **Concentration** section, 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. In the **c[mM]** row type 0.1 mM into the second, third, and fourth segment of the channel. Click **Generate**, then after the window is closed, click **Init** in the **Compute control** section of the main panel of Simul 6.1. The resulting window should look like this:



Click **Run**. The computation starts and you can zoom in on the development of the ampholytes profile by framing the 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. The Time row will show the stop time of the last calculation and the actual time the simulation took. After appropriate zooming, the graphical window can look like this:

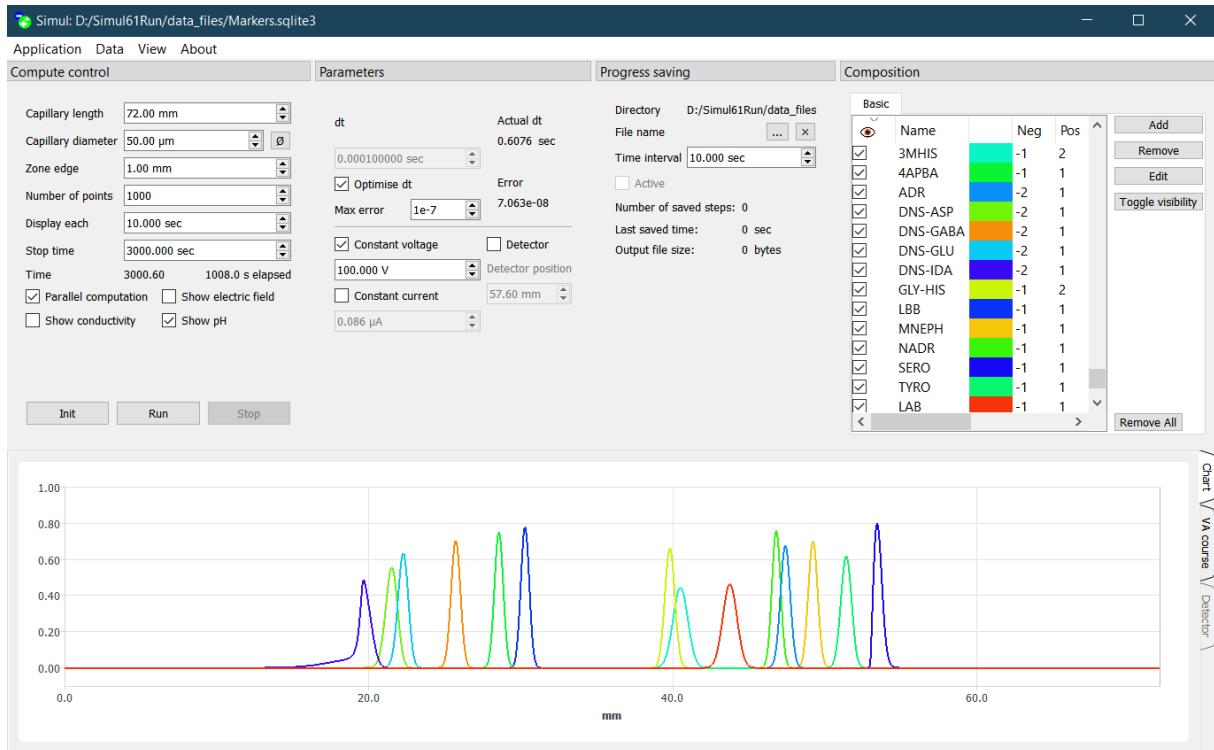


You can admire the linear pH gradient that was established in the middle of the channel, see the magenta line. If you wish, you can save the configuration by clicking **Data | Save Data** and input a name for the configuration, say, IEF. Select the directory where you wish to put the saved file, say, Simul6Run/data\_files/, and select format in which you wish to have the results. Preferably, use the sqlite3 format. Then click **Save**, the configuration will be saved as IEF.sqlite3.

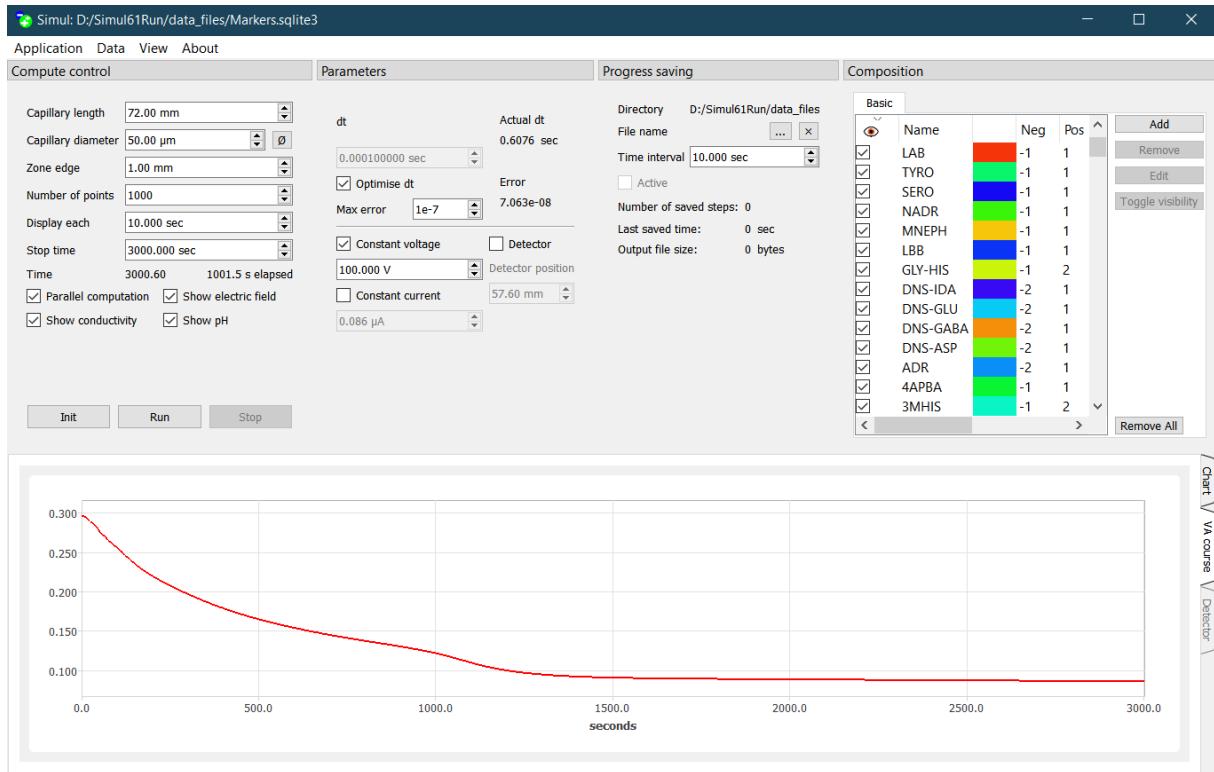
*Running the enclosed example of isoelectric focusing* This is the separation of fourteen pl markers, which was discussed in our paper:

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; Boublík, M; Maly, M; Steflova, J; Hruska, V; Vigh, G: [Electrophoresis 2020, 41, 514–522, 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 and you can zoom in on the development of the ampholytes' profile when you frame the 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 **Stopime** 3000 s and the calculation stops. After appropriate zooming, the window can look like this:



Simul 6.1 also enables to record the time course of either current or voltage during the simulation run, depending on whether it is in the **Constant voltage** or **Constant current** mode, respectively. The next picture shows the time record of the current during the isoelectric focusing. The picture was obtained by clicking on the tab **VA course** in the right hand side of the graphical window. The drop of the current is typical for isoelectric focusing when running in the constant voltage mode. When clicking with the right mouse button at any part of graph, the popped-up **Save VA data to CSV file** or **Save VA data as image** buttons will appear, allowing you to save the data.



### Mobility or pKa constants of a constituent dependent on the axial coordinate in the separation channel

Both the mobilities and pKa constants of a constituent can depend on the axial coordinate in the separation channel. Such a situation can occur in gel electrophoresis, where a part of the separation channel is filled with a background electrolytes that contains a hydrophilic neutral gel to form 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 neutral polymer not only forms 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 an 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.

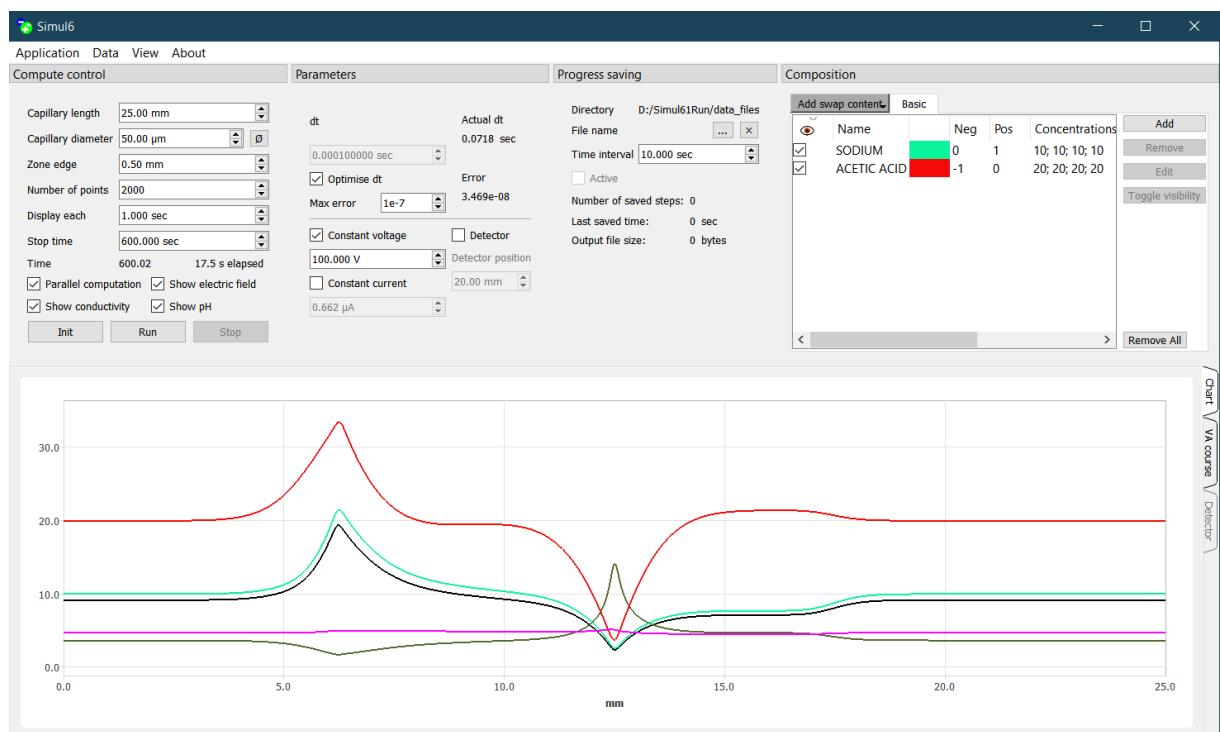
Simul 6.1 enables us to consider both the mobilities and pKa constants of the constituents as dependent on the axial coordinate and thus simulate the increase and depletion of their concentration at the boundaries. The next example does not simulate a concrete situation, but demonstrates how to consider the axial dependence of 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 the **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 constant values of acetic acid are transferred into the **Segments** subwindow. Input 20 mM for the **c[mM]** row in all four segments. Click **Accept**.

(3) Click **Add** in the **Composition** window. The **Composition and segments settings** window is opened. Click the **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 10 mM for **c[mM]** in all four segments. Let’s suppose that in the second section there is a neutral hydrophilic gel that 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 called transference number, which is defined as  $T = u_{\text{Na}}/(u_{\text{Na}} + u_{\text{Acetate}})$ , and which is responsible for the phenomenon we are about to see. Click **Accept**.

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



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

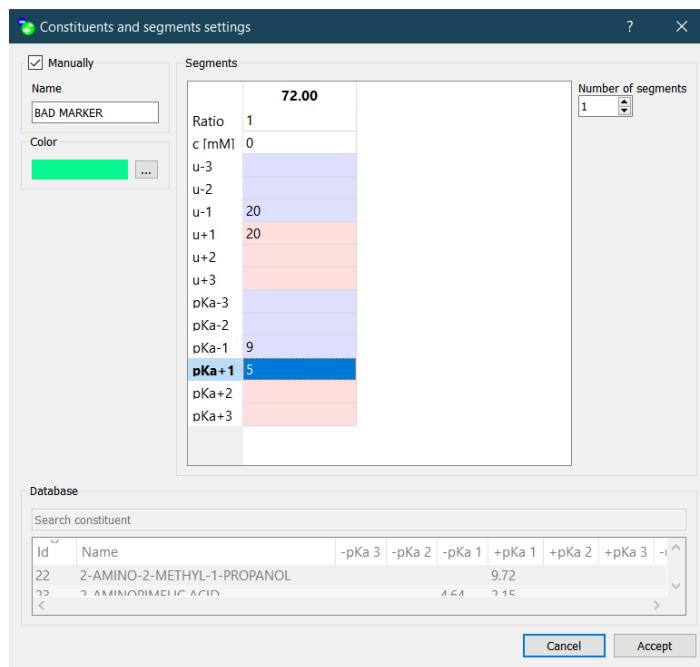
### Adding new constituents

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 your simulation any new constituent if its mobilities and pKa constants are known. Let's suppose you wish to check the behavior of a new pl 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 pl is 7 and the difference between both forms is 4, which means we can doubt its ability to serve as a pl marker.

(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**

(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. For its mobilities, type 20 into both the **u-1** and **u+1** widget. For its pKa values, type 9 into the **pKa-1** widget and 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:

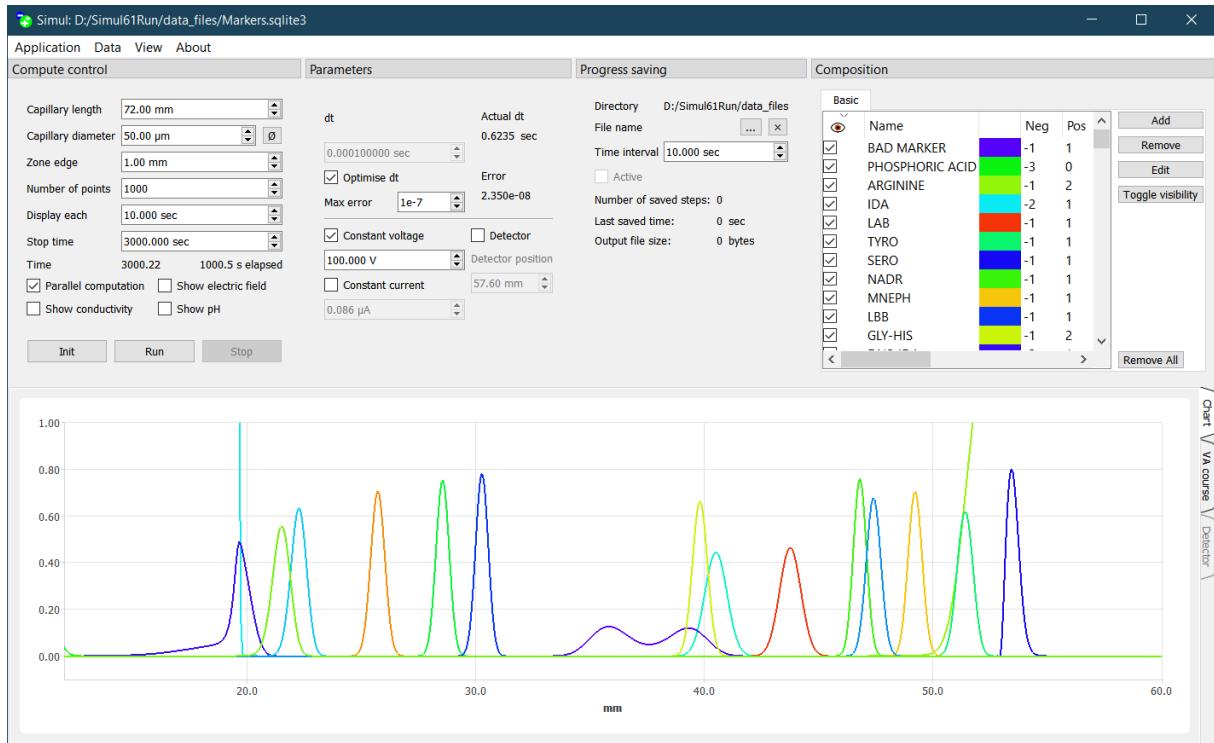
	9.00	15.00	24.00	15.00	9.00
Ratio	3	5	8	5	3
c [mM]	0	0.01	0.01	0.01	0
u-3					
u-2					
u-1	20	20	20	20	20
u+1	20	20	20	20	20
u+2					
u+3					
pKa-3					
pKa-2					
pKa-1	9	9	9	9	9
pKa+1	5	5	5	5	5
pKa+2					
pKa+3					

Database													
Search constituent													
Id	Name	-pKa 3	-pKa 2	-pKa 1	+pKa 1	+pKa 2	+pKa 3	-u 3	-u 2	-u 1	+u 1	+u 2	+u 3
22	2-AMINO-2-METHYL-1-PROPANOL				9.72						33.3		
23	2-AMINOPIMELIC ACID					4.64	2.15				25.9	26.6	
24	2-BROMOBUTYRIC ACID						7.007				22.2		

Cancel      Accept

(4) Click **Accept**. Click **Init**, then click **Run**. The computation starts. You can zoom in on the development of the markers' profiles by framing the part of interest with the cursor. After about 14 – 20 minutes of computation time (depending on the speed of the processor and the number of its cores), **Stop time** 3000 s will be reached and the calculation will stop. After appropriate zooming, the window should 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 has not yet gotten focused and the anode- and cathode-side lobes of its in-migrating concentration boundaries appear as a double peak. When continuing the run until 5000 s, both parts of the double peak will be joined into one peak, but much broader, than the others.

### **Swapping a part of the electrolyte solution in the separation channel with another electrolyte solution. Using various diameters along the length of the channel.**

Both features can be used in various situations in chip electrophoresis. The first one enables us to swap a part of the content of the separation channel with another electrolyte solution after some time during the separation run. When allowing a variable diameter of the separation channel, it is supposed that transversal diffusion is infinitely fast.

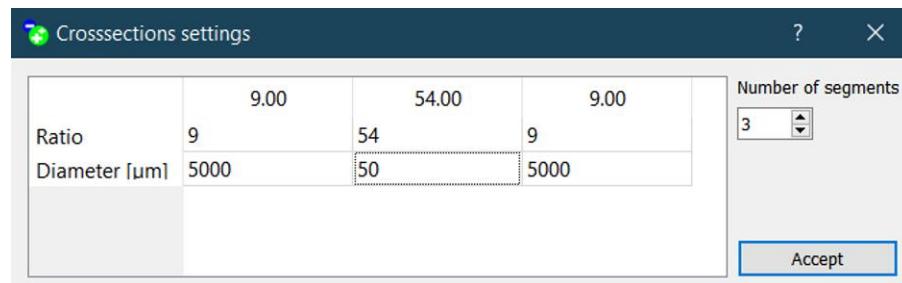
This section demonstrates the use of both features in a two-step capillary isoelectric focusing separation, *i.e.*, where the IEF step is followed by chemical mobilization to facilitate single-point detection of the separated components. What is a principle: After a certain amount of time in the isoelectric focusing step, the pH gradient is established and the analytes are separated according to their pI values. However, the whole system of bands is almost stationary and does not move in the separation channel. When the equipment does not possess 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 chemical mobilization. Chemical mobilization is achieved simply by exchanging the cathodic vessel, which contains a solution of a base, such as lithium hydroxide, with a solution of an acid, such as phosphoric acid, often the same acid that is used in the anodic vessel. Thus, the anionic form of the mobilizing acid enters the separation channel instead of the OH<sup>-</sup> ions, which gradually lowers the pH at the cathodic end of the separation channel, protonates the highest pI ampholytes and makes them migrate by the detector on their way toward the cathode. As the mobilizing acid anion penetrates into the entire separation channel, all ampholytes are mobilized toward the cathode.

Simul 6.1 has both features allowing us to perform such a simulation: (i) to replace, or swap, a part of the solution in the separation channel with another electrolyte solution, (ii) to allow any part of the separation channel to have a variable diameter to mimic the electrode vessels. The next example shows focusing and subsequent chemical mobilization in an isoelectric focusing system. A stationary detector will be located close to the capillary end. Moreover, this example also demonstrates the saving the whole course of the electromigration run in sqlite3 file format. We will use 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 the  button at the right hand side end of the **Capillary diameter** row. The **Crosssections settings** subwindow opens. Set the **Number of segments** to 3 with the ratios 9, 54, 9. Input the corresponding diameters as 5000, 50, 5000 µm. The corresponding subwindow should look like this:



Click **Accept** and then click **Init** to activate the change in diameters. 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 a diameter of 50 µm.



The left electrode vessel 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, in the **Progress saving** window select 100 s for the **Time interval**. Check the checkbox **Active**, which activates progress saving. Then we will place a detector in the right-hand side of the capillary, 3 mm from the end, so check the **Detector** checkbox and input 60 mm into the **Detector position** widget.

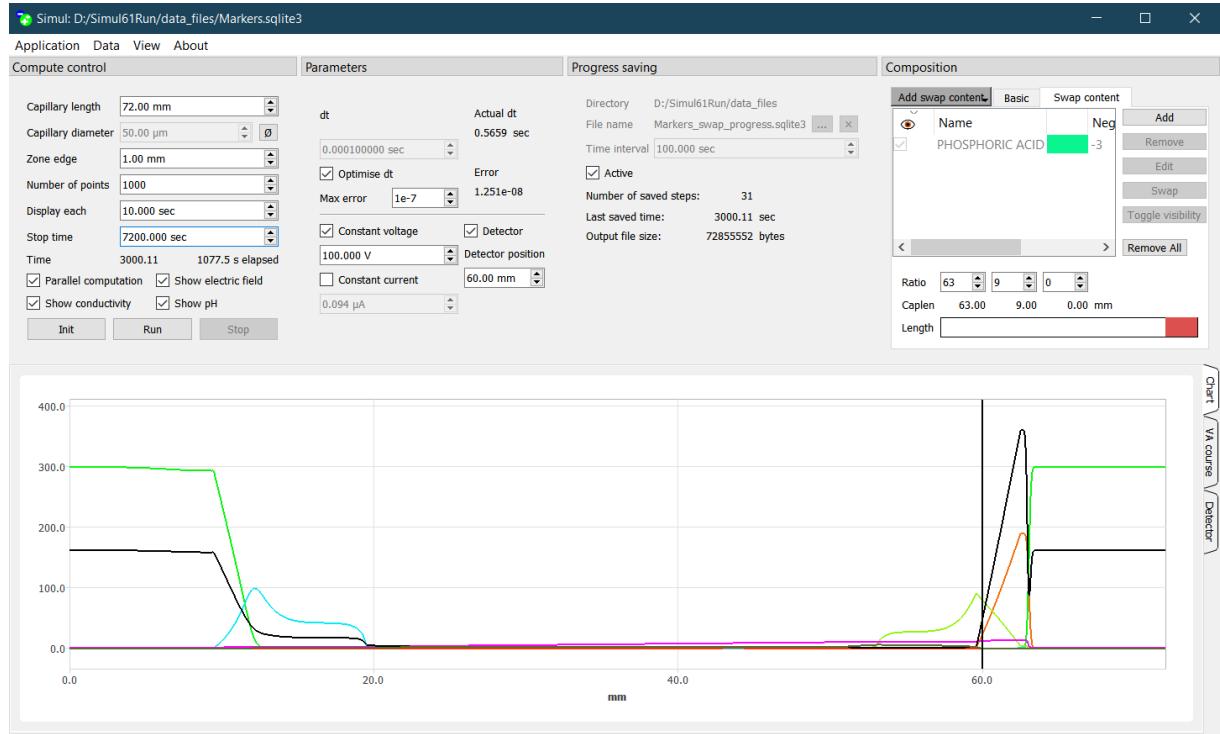
Then click **Run** and let the computation to run to the **Stop time**, which is 3000 s. You can observe that the ampholytes are focused and the pH gradient is nicely formed.

(2) As stated above, the right electrode vessel is filled with lithium hydroxide. Let's now 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. The channel is divided always into three parts and the swapped part is in red. Three numbers in the **Ratio** widget determine its position. This enables to put the swapping electrolyte to any part of the separation channel, either inside or at the end. For the placement of the swapped part to the right vessel, between 63 and 72 mm, input these three numbers in the **Ratio** widget:

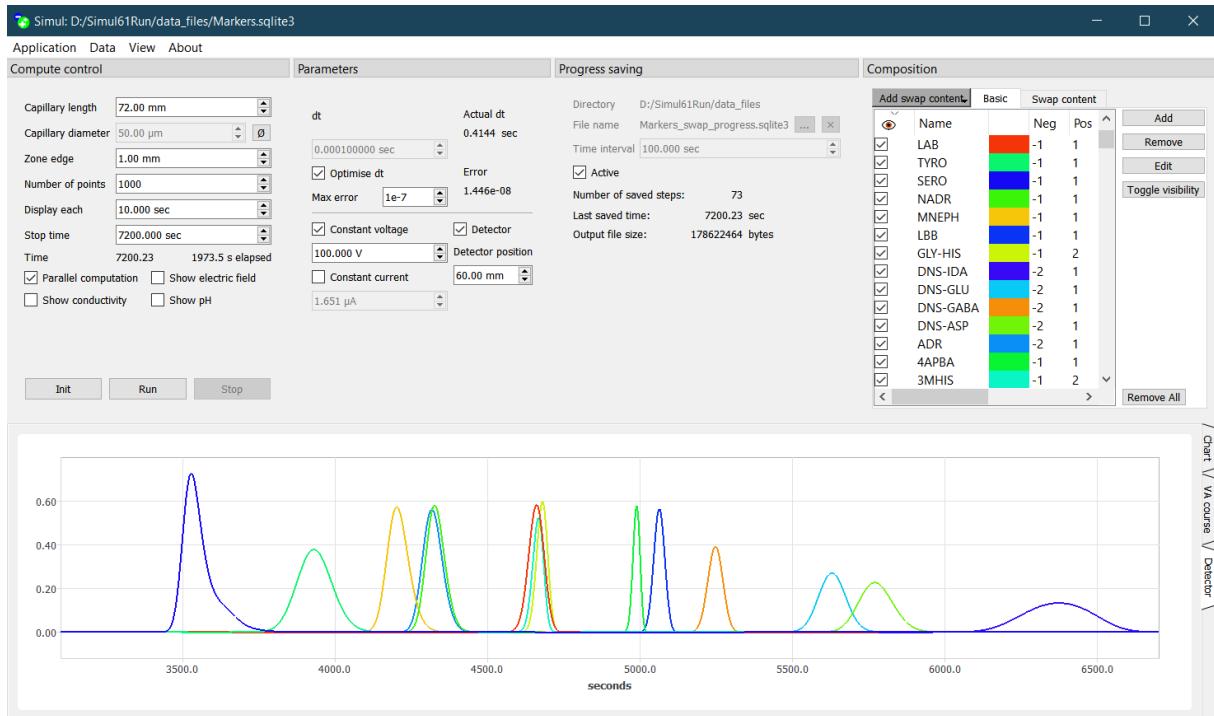
Ratio	63		9		0	
Caplen	63.00		9.00		0.00	mm
Length						

To define the composition of the swap click now **Add** and the **Composition and segments settings** window is opened. Click the **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 300 mM for **c[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**. Click the tab **Detector** at the right-hand side of the graphical window and allow a minute for the software to assemble the time record. After disabling visibility of some constituents in subwindow **Composition|Basic** and appropriate zooming you should see such a time record:



Notice that the shapes and order of the marker's peaks are rather different when compared with the spatial pattern at Page 13. You can save the detector time record as the csv file or graphical image file when clicking with the right mouse button anywhere at the graphical window.

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.

The software has some more features: when clicking by the right mouse button 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

Our group 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?

### Acknowledgments

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