Application Note

Capillary Zone Electrophoresis

Application Note: Capillary Zone Electrophoresis
Version 8/PC

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Patent Number 6,116,766: Fabrication Based Computer Aided Design System Using Virtual Fabrication Techniques
Patent Number 6,157,900: Knowledge Based System and Method for Determining Material Properties from Fabrication and Operating Parameters

Table Of Contents

1 Introduction

Various working modes in which the ITP–CZE combination in separation system can operate were employed in the anionic regime of the separation with direct injections of the samples.

Capillary electrophoresis (CE), also known as capillary zone electrophoresis (CZE), can be used to separate ionic species by their charge and frictional forces. In traditional electrophoresis, electrically charged analytes move in a conductive liquid medium under the influence of an electric field. Introduced in the 1960s, the technique of capillary electrophoresis (CE) was designed to separate species based on their size to charge ratio in the interior of a small capillary filled with an electrolyte. While its use has been sporadic, CE offers unparalleled resolution and selectivity allowing for separation of analytes with very little physical difference. Efficiencies of millions of plates are routinely reported. Once thought impossible, separation of large proteins differing in only one amino acid (ie. D-Lysine substituted for L-Lysine) and even an isotopic separation of 14N and 15N ammonium hydroxide have been reported. No other technique has shown such powerful selectivity with the ability for extremely high sensitivity. As few as 6 molecules of a substance have been separated and detected with the help of laser-induced fluorescence (LIF).

Isotachophoresis (Greek: iso = equal, tachos = speed, phoresis = migration) is a technique in analytical chemistry used to separate charged particles. In isotachophoresis the constituents will completely separate from each other and concentrate at an equilibrium concentration, surrounded by sharp electrical field differences.

Transient states in the evolution of electrophoretic systems comprising aqueous solutions of weak monovalent acids and bases are simulated. The mathematical model is based on the system of nonstationary partial differential equations, expressing the mass and charge conservation laws while assuming local chemical equilibrium. It was implemented using a high resolution finite-volume method. It is shown that the results of separation, particularly zone order, strongly depend on pH distribution. Simulation data as well as simple analytical assessments may help to predict and correctly interpret the experimental results.

2 Building the Model

We will first construct the model in 3D Builder.

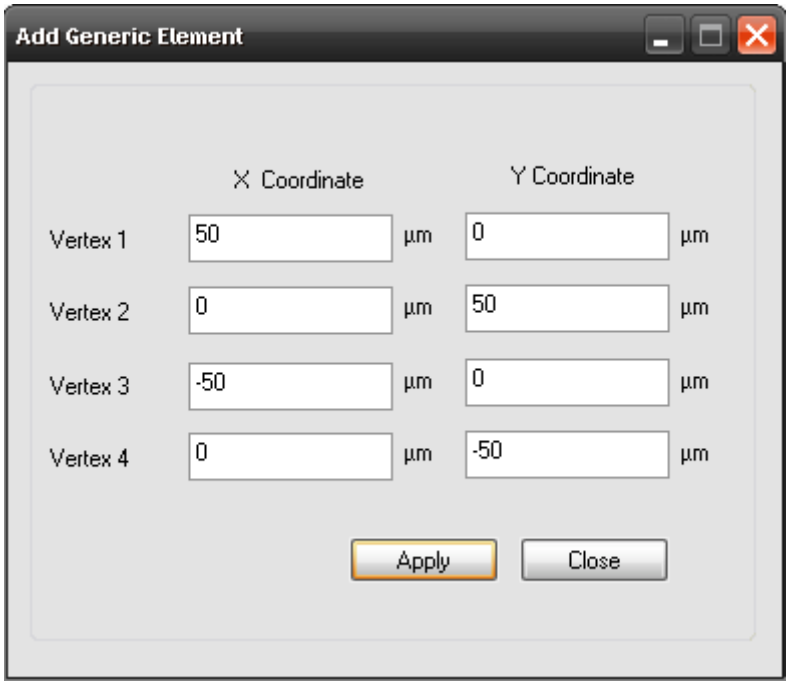
Start…Programs…IntelliSuite…3D Builder

The 3D Builder window will open. We will now construct the capillary that will be used for the electrophoresis simulation. It is possible to draw a circle using the Add Circle tool, but this will create a 25-block mesh that will require a lot of computation time. Instead,

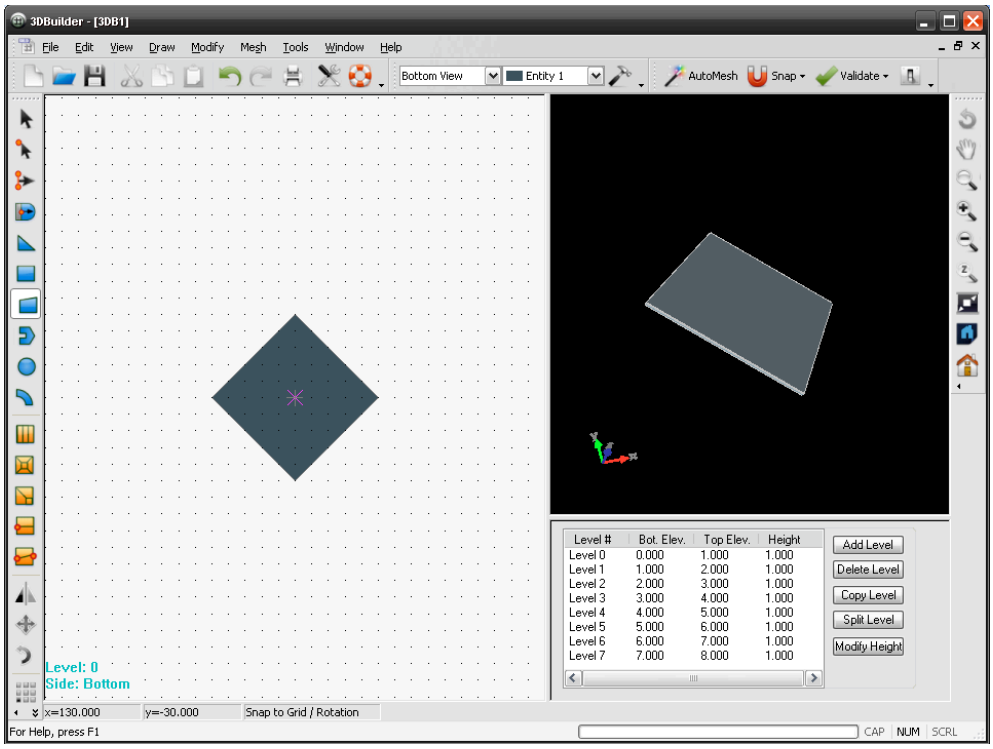
Add Quadrangle 

Keyboard 

Input the coordinates as shown below.

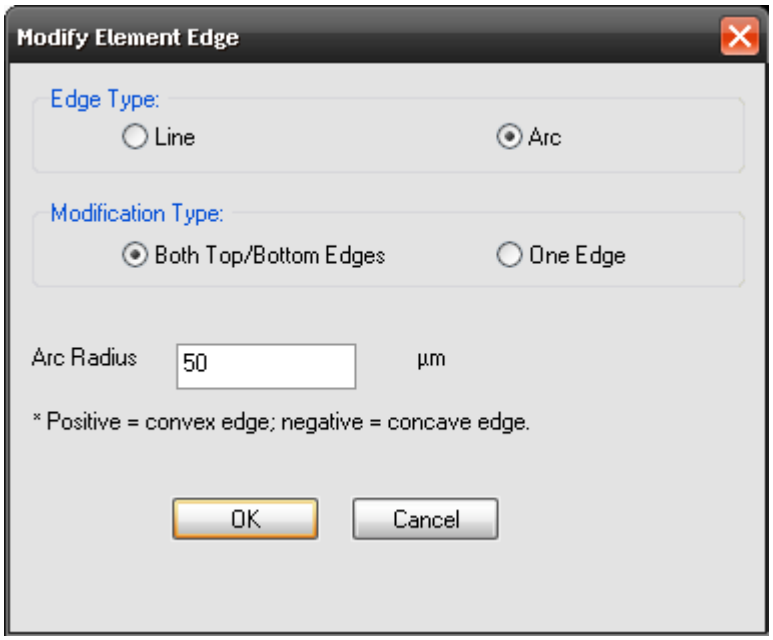


Click *Apply* and the following shape will appear.

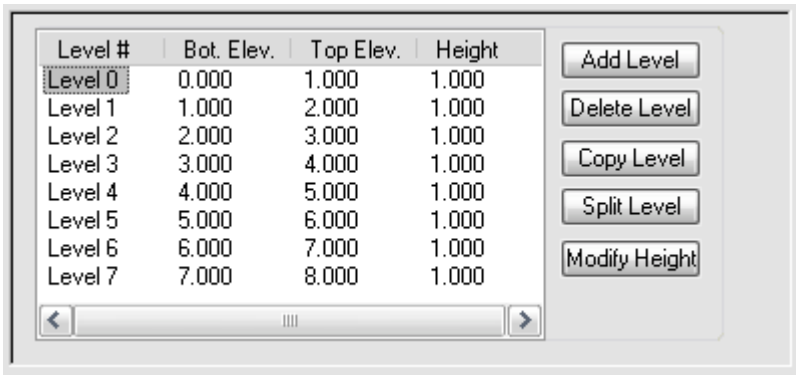


Modify edge 

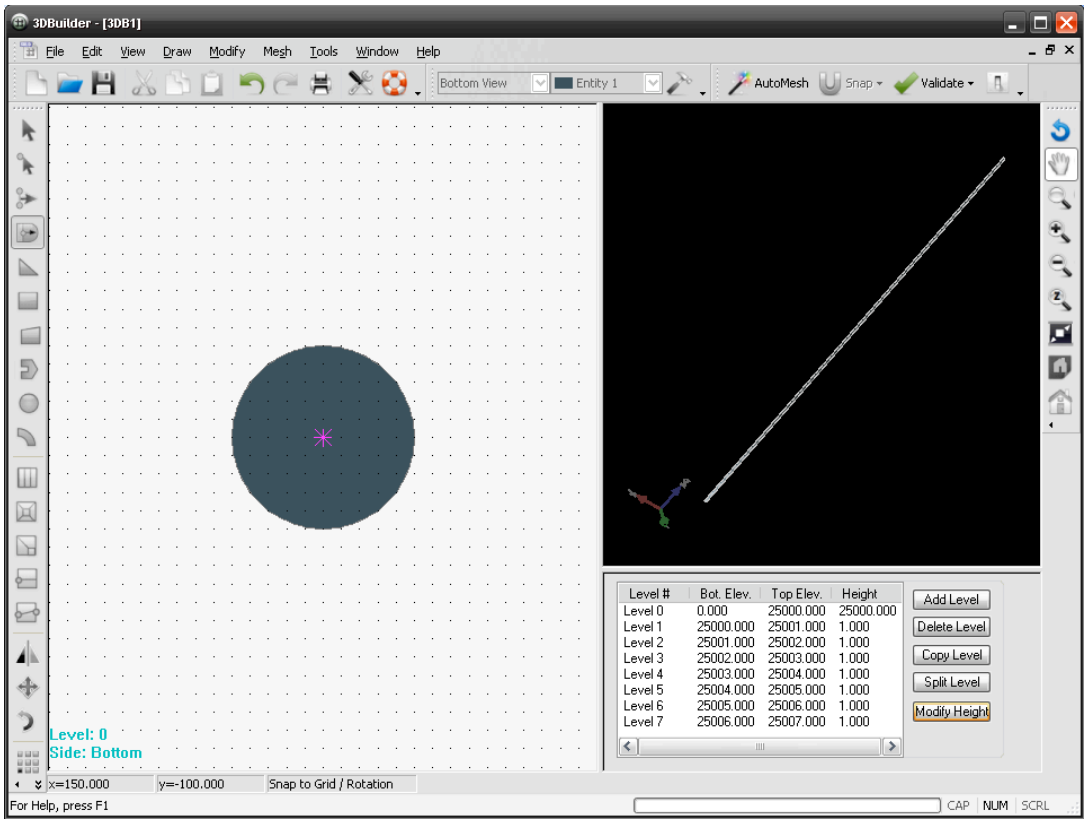
Click inside the square near one of the edges. Select *Arc* and input an arc radius of 50 um in the dialog that appears.



Repeat this procedure for the other three sides of the square. When you are finished, select Level 0 in the Levels Manager and click *Modify Height*.



Input 25000 um in the Height field. The resulting structure will appear as shown below.



Save this 3D Builder file (*.solid) in a convenient location. Make sure not to use spaces in the file or folder names. The structure can then be automatically exported to the Microfluidic module for further analysis.

File…Export to Analysis Module…

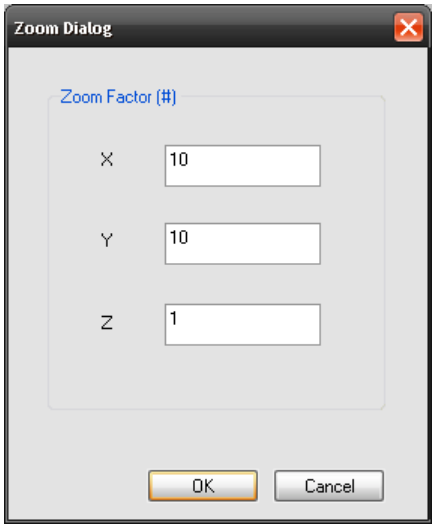
Click *Continue without Check* and select the Microfluidic module. Save the analysis file in a location of your choosing, again making sure not to use spaces in the file or folder names.

3 Microfluidic Analysis

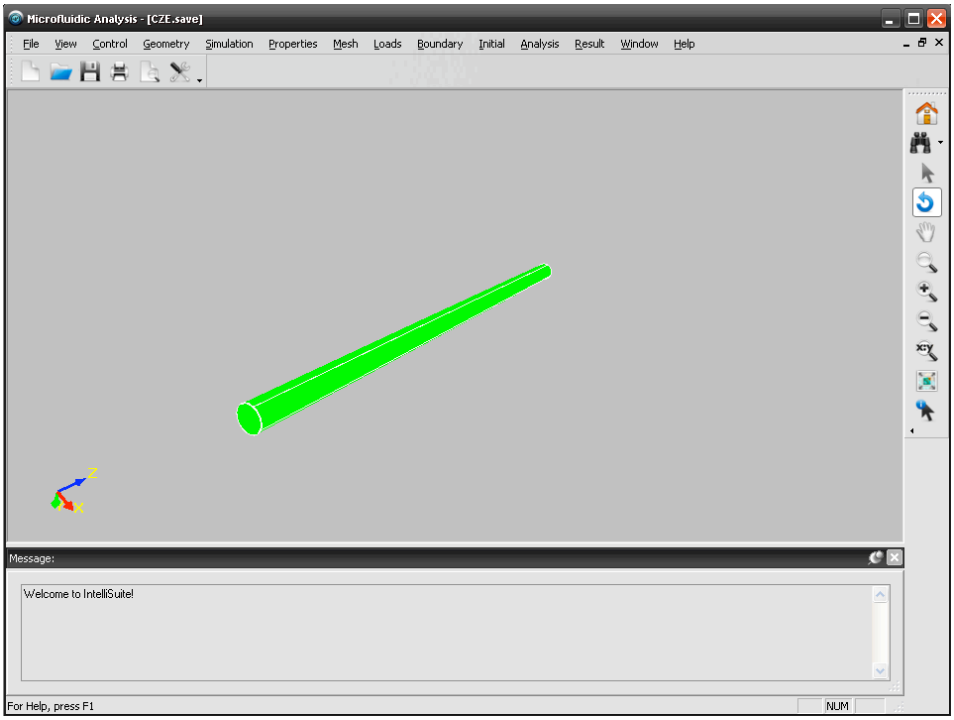
The model will appear in the Microfluidic module. To get a better view of the model, we will change the zoom settings.

View…Zoom…Define

Choose an X and Y zoom factor of 10.



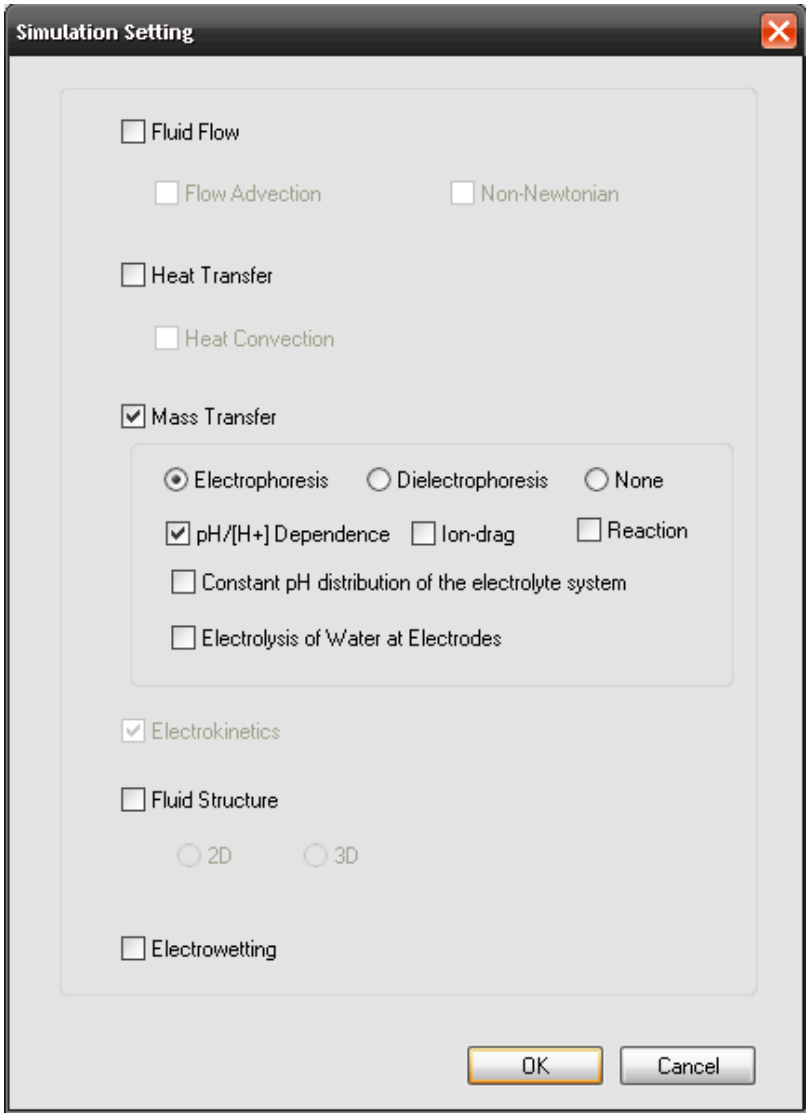
The model will then appear as shown below.



You can follow the menus sequentially from *Simulation* to *Result* to perform the analysis.

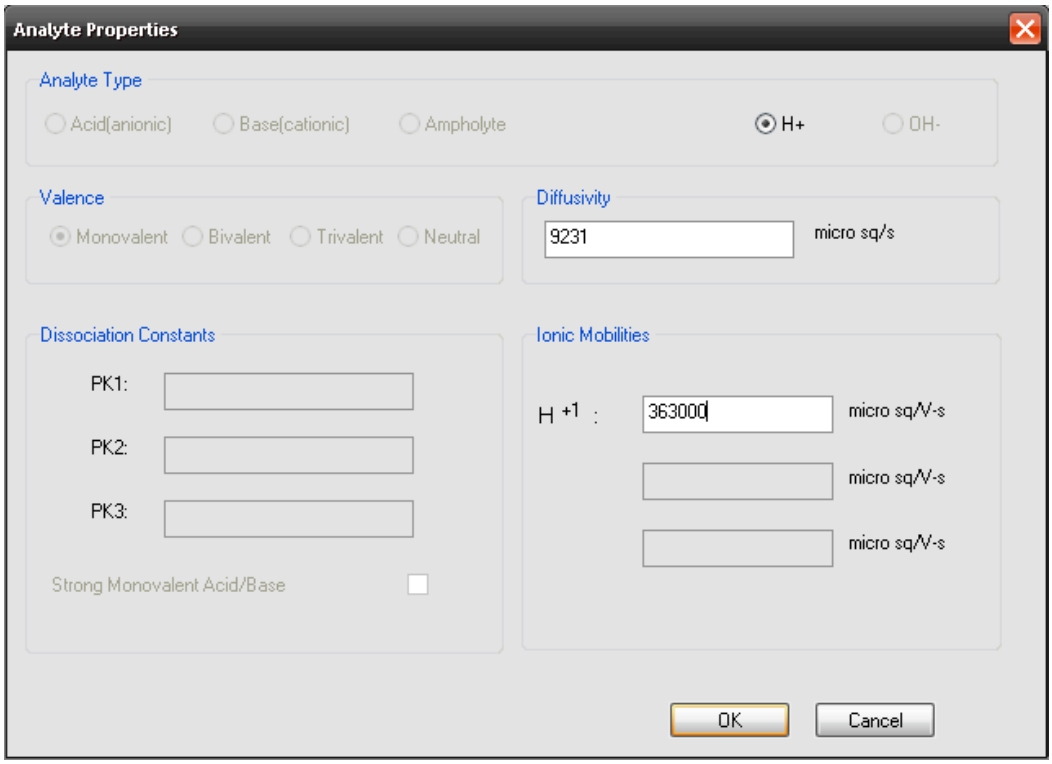
Simulation…Simulation Setting

Set up the simulation as shown below.

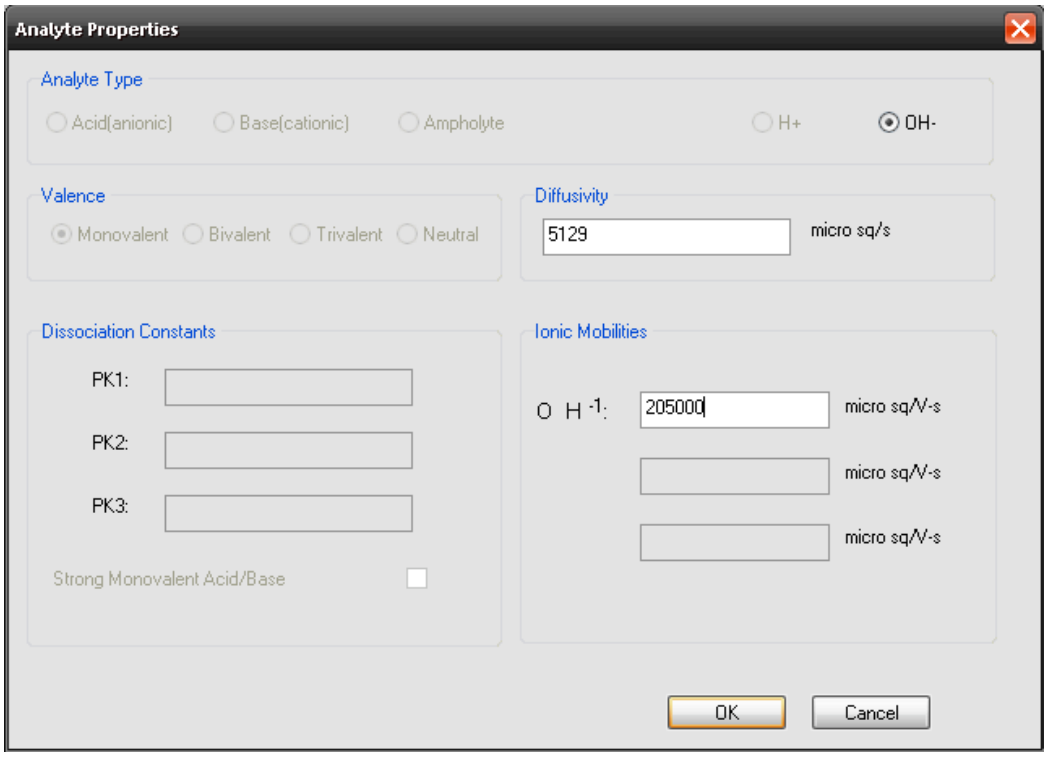


Properties…Edit Fluid (Global)

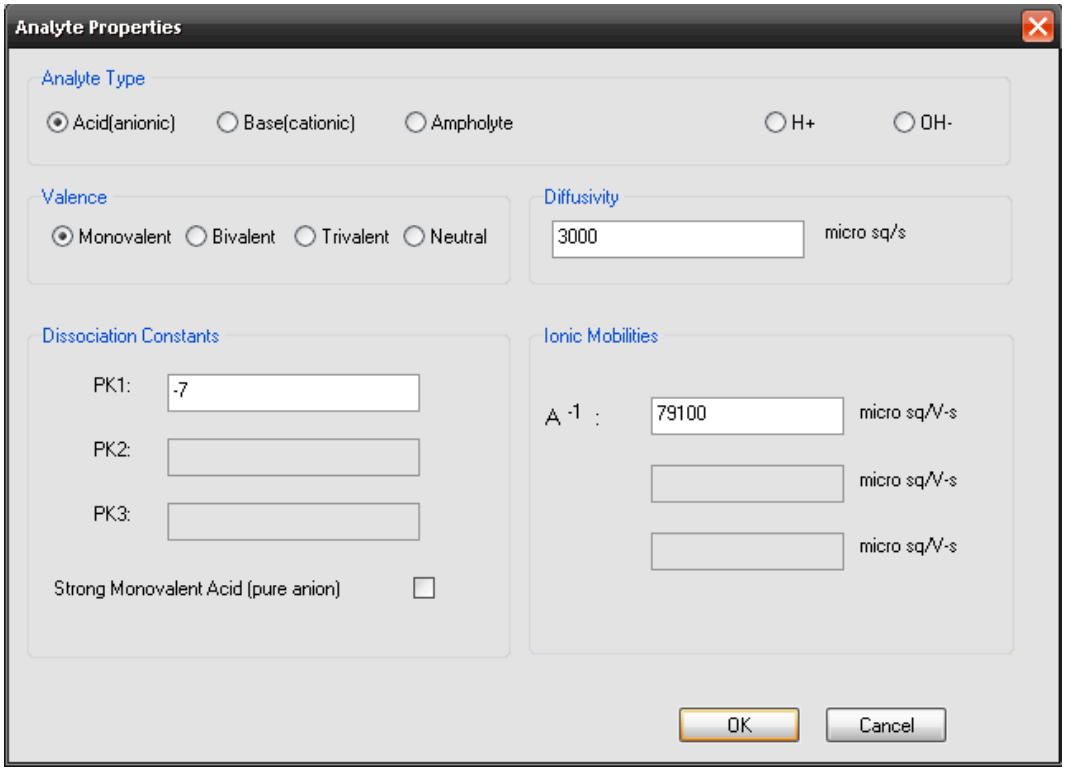
Click on Analyte 1 and then click *Edit Property*. In the dialog that appears, enter the values shown below.



Click *OK*, then select Analyte 2 and click *Edit Property*. Enter the values shown below.



Click *OK*. Click *Add Analyte* to add a new analyte. In the Analyte Properties window, enter the values shown below.



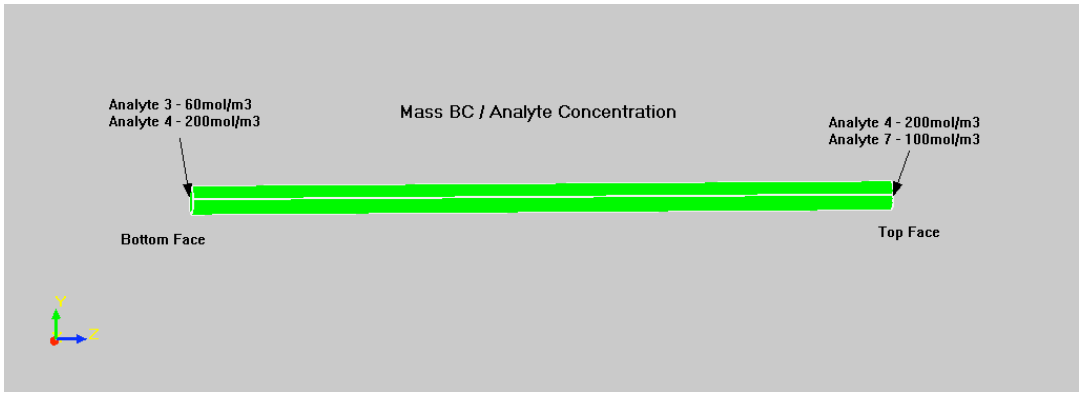
Click *OK* to return to the original dialog box. You will follow the same procedure to add four more analytes. Input the values from the table below into the Analyte Properties dialog for each analyte. The Valence and Diffusivity values should be left as the default. Make sure to select *Base* as the Analyte Type for Analyte-4.

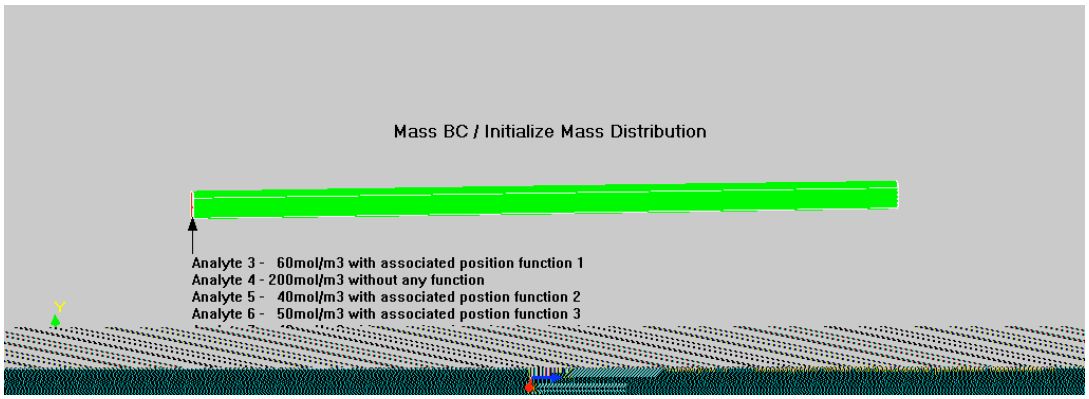
Substance	Analyte Number	Analyte Type	PK_1	Υ (μm)
H+	Analyte 1	—	—	363000
OH-	Analyte 2	—	—	205000
Chloride	Analyte 3	Acid	-7.0	79100
Tris	Analyte 4	Base	8.30	24100
Formate	Analyte 5	Acid	3.75	56400
Acetate	Analyte 6	Acid	4.76	42400
Cacodylate	Analyte 7	Acid	6.21	23100

Click *OK* to return to the main window when all of the analytes have been defined.

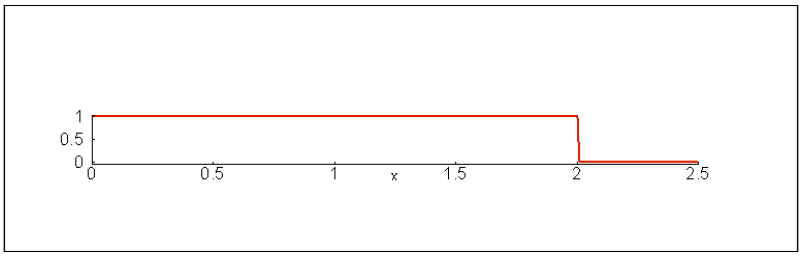
The boundary conditions must now be applied on the pipe. The Analyte Concentration boundary condition will be applied for Analytes 3 and 4 on the bottom face and Analytes 4 and 7 on the top face. The Initialize Mass Distribution boundary condition will be applied for Analytes 3-7 on the bottom face. Look at the axes in the lower left corner to determine which face is the top and which is the bottom. The top face will be the furthest in the positive Z-direction.

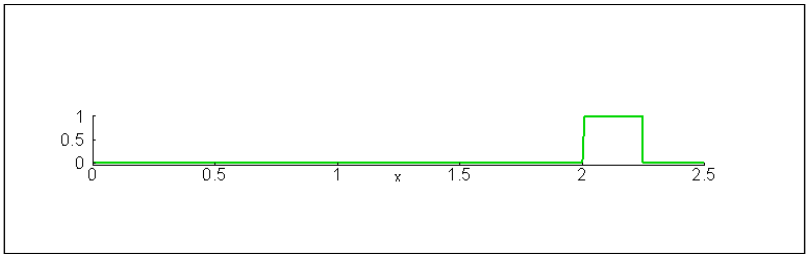
The figures below show the exact values for each boundary condition that will be applied.

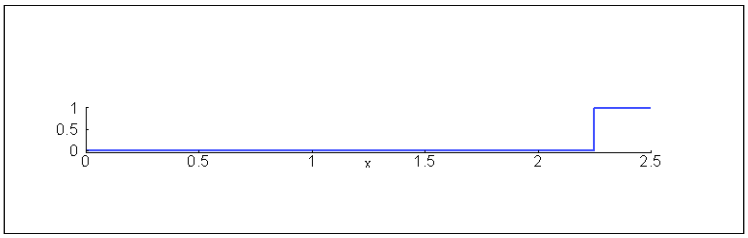




A position function will be used with the Initialize Mass Distribution boundary condition to define the initial position of each of the analytes. The position functions for each analyte are shown below.



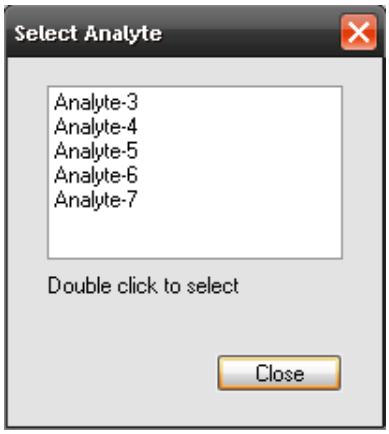




To apply the Analyte Concentration boundary condition,

Boundary…Mass BC…Analyte Concentration

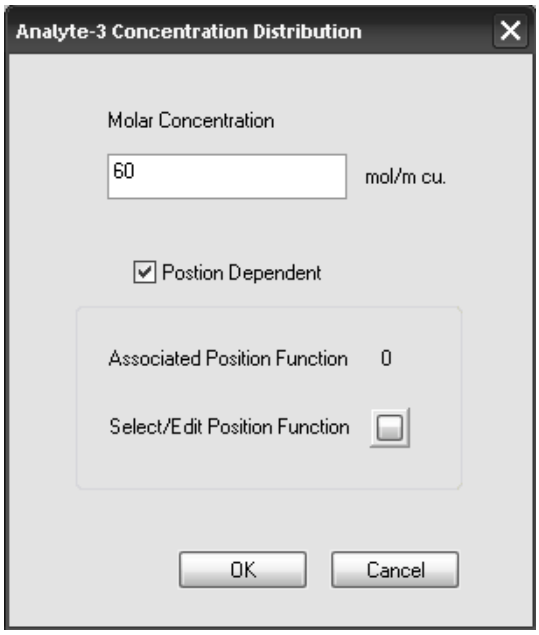
Click the bottom face of the pipe. The following dialog box will appear.



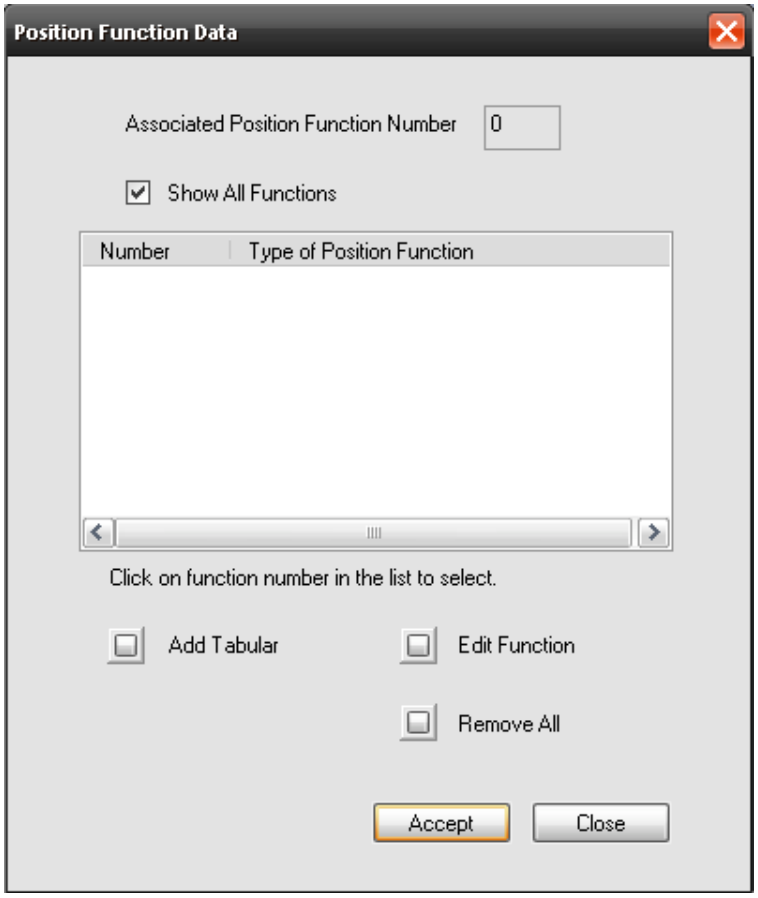
Double-click Analyte-3. Input 60 as the Molar Concentration and click *OK*. To apply the next boundary condition for Analyte 3,

Boundary…Mass BC…Initialize Mass Distribution

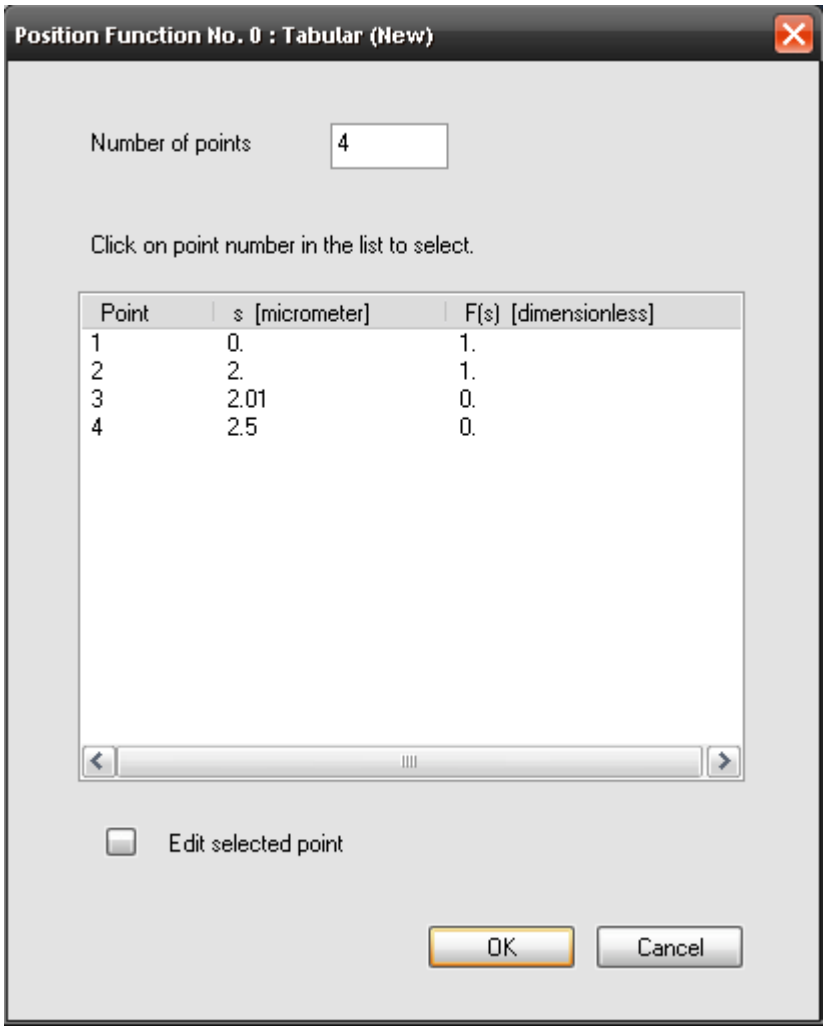
Again, click on the bottom face and double-click Analyte-3 in the Select Analyte box. Input 60 as the Molar Concentration and check the Position Dependent box.



Clicking the Position Dependent box will bring up the following window.



Click *Add Tabular*. This will bring up another window where you will be defining the position function. Enter 4 in the *Number of points* field and hit Enter. Double click on a point to modify its value. Modify each point to match the values in the figure below.



Click *OK* and you will see that a Tabular position function has been defined. Click *Accept*. Finally, click *OK* in the Analyte 3 Concentration dialog to return to the main window.

To apply the Analyte Concentration boundary condition for Analyte 4,

Boundary…Mass BC…Analyte Concentration

Click the bottom face of the pipe. Double-click Analyte-4 in the dialog box that appears. Input 200 as the Molar Concentration and click *OK*.

Next, click the top face of the pipe. Select Analyte-4 and input 200 as the Molar Concentration.

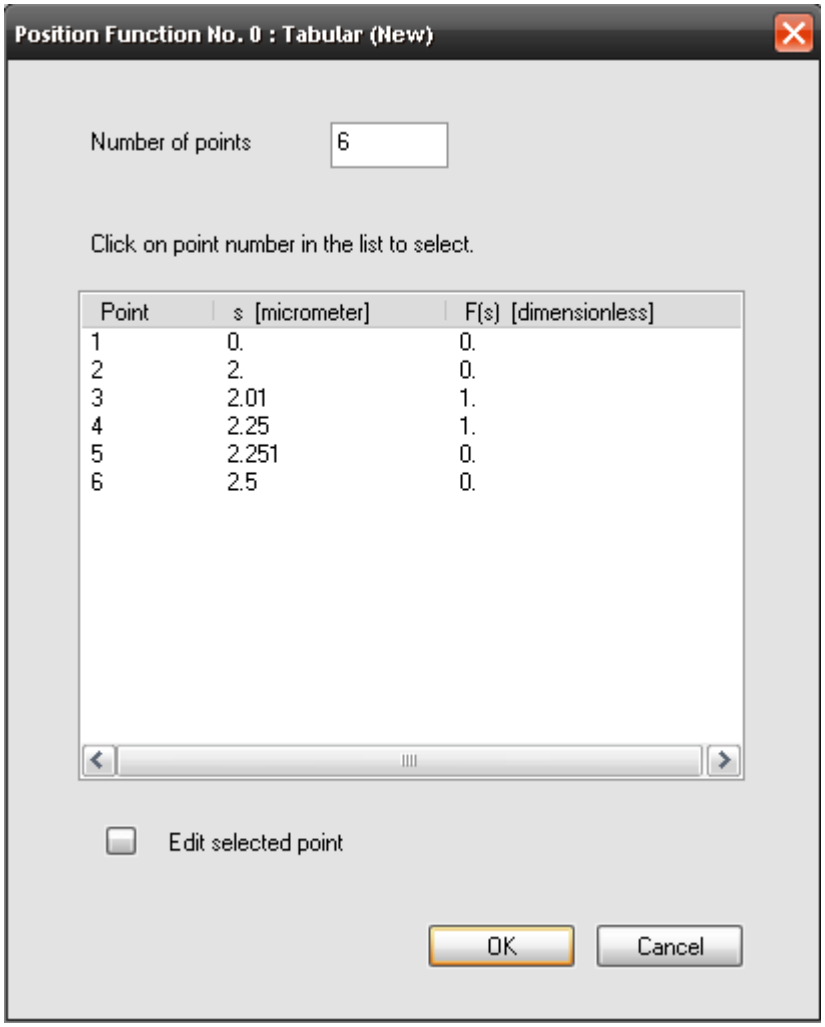
Boundary…Mass BC…Initialize Mass Distribution

Select the bottom face of the pipe. Double-click Analyte-4 and input 200 as the Molar Concentration. Since Analyte 4 will have a constant distribution across the pipe, it is initialized without any position function. Click *OK*.

Analyte 5 will only have one boundary condition defined.

Boundary…Mass BC…Initialize Mass Distribution

Select the bottom face of the pipe and double-click Analyte-5. Input 40 as the Molar Concentration and click the Position Dependent box. Click *Add Tabular* in the next box that appears. Enter 6 in the *Number of points* field and hit Enter. Modify the points to match the values in the figure below.



Click *Accept*, then click *OK*.

Analyte 6 also only has one boundary condition defined.

Boundary…Mass BC…Initialize Mass Distribution

Select the bottom face of the pipe and double-click Analyte-6. Input 50 as the Molar Concentration and click the Position Dependent box. Analyte 6 will use the same position function as Analyte 5. Select Position Function 2, then click *Accept*.

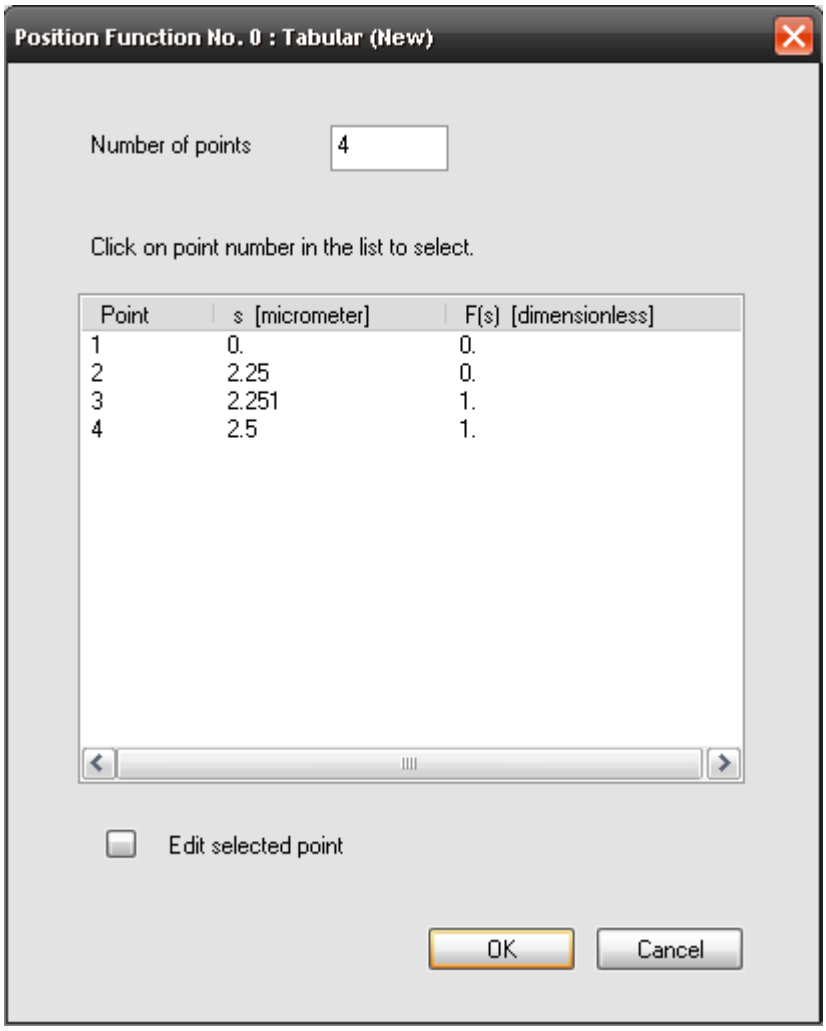
For Analyte 7,

Boundary…Mass BC…Analyte Concentration

Click the face of the pipe and select Analyte-7. Enter a Molar Concentration value of 100. Next,

Boundary…Mass BC…Initialize Mass Distribution

Click the bottom face, select Analyte-7, and enter 100 as the Molar Concentration value. Check the Position Dependent box, then click *Add Tabular*. Enter 4 in the *Number of points* field and click Enter. Modify the points to match those shown below.



Click *OK*. In the next window, select Position Function 3 and click *Accept*. Click *OK* to return to the main window.

After applying the mass boundary condition, you need to apply the electrokinetics boundary conditions.

Boundary…Electrokinetics BC…Current Density

Select the bottom face of the pipe and input 637 as the Current Density. Click *OK*.

Boundary…Electrokinetics BC…Voltage

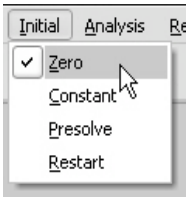
Select the top face of the pipe. Leave the voltage at 0 and click *OK*.

To refine the mesh,

Mesh…Auto…Maximum Mesh Size

Input 100 microns in the Maximum Mesh Size dialog and click *OK*.

Make sure *Zero* is selected in the *Initial* menu.

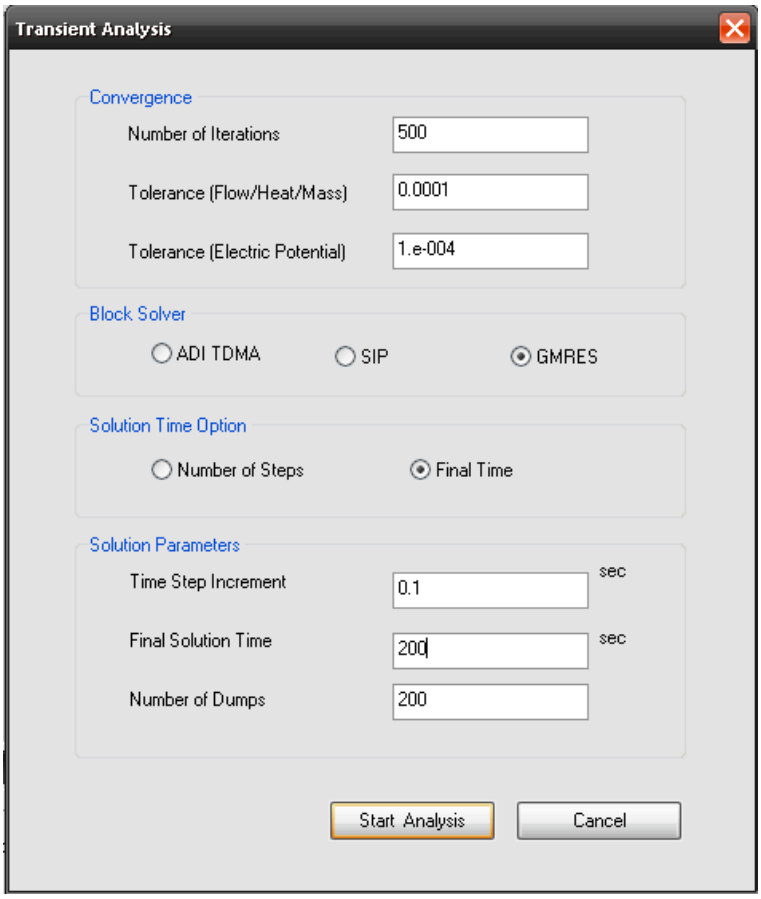


Initial

Next, to start the simulation,

Analysis…Transient

Set up the simulation as shown in the figure below.



Click *Start Analysis* to begin the simulation.

4 Viewing the Results in VisualEase

When the simulation is complete,

Result…Select Dump

The dump files will be in the same folder as the *.save file. Select "dump_001.dat".

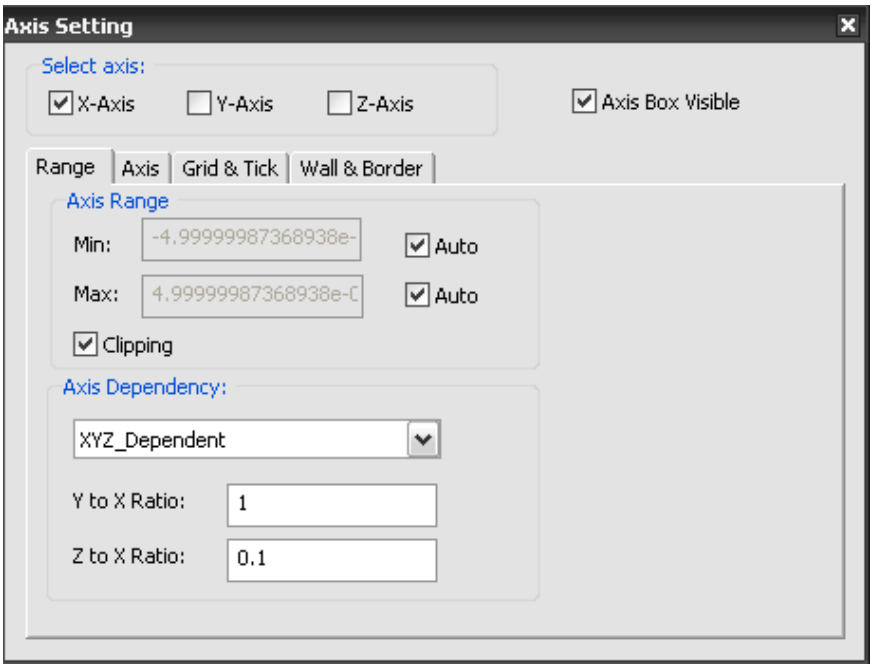
Result…Open VisualEase

This will open the results in IntelliSuite's post-processing module, VisualEase. You can also open the "electrophoresis.viz" file in VisualEase if you don't want to wait for the entire simulation to run.

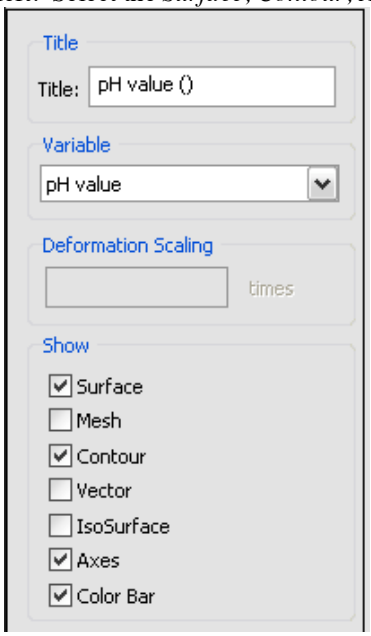
To make the model easier to see,

Settings…Axis

Input 0.1 as the Z to X Ratio.



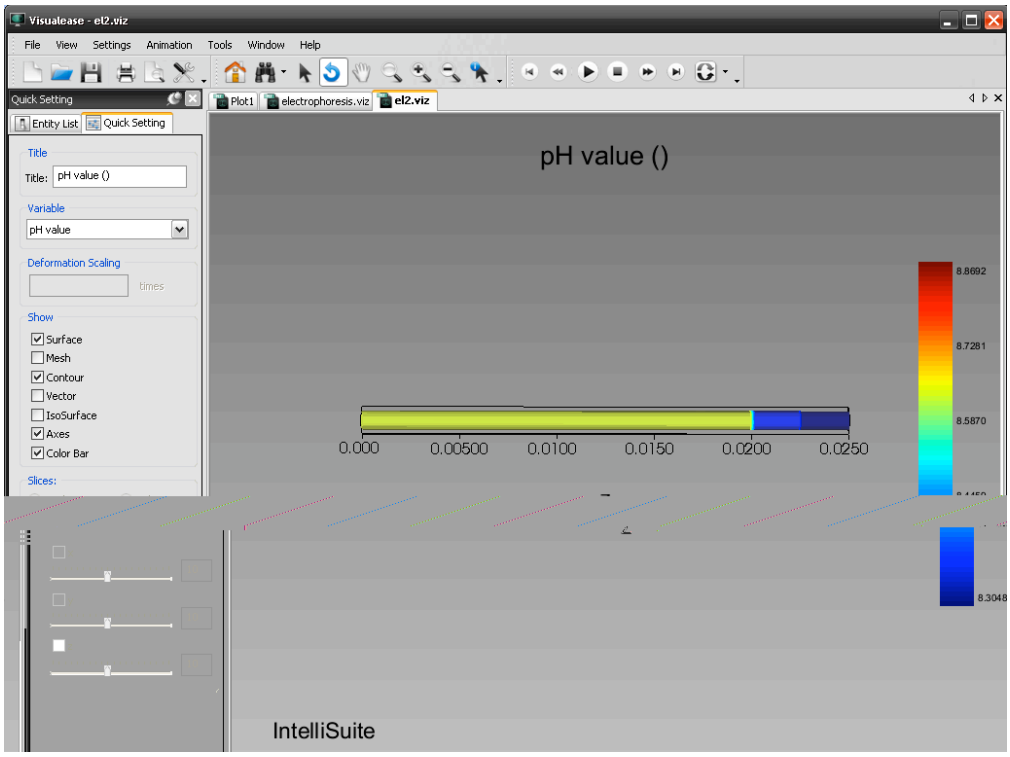
Select *pH Value* in the menu on the left. Select the *Surface*, *Contour*, *Axes*, and *Color Bar* options.



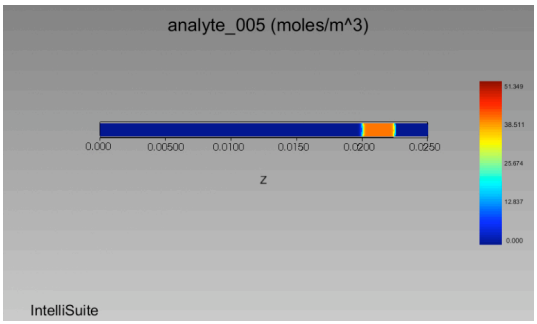
To rotate the model,

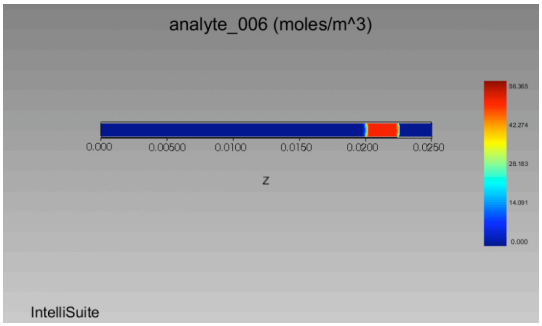
View… Rotate

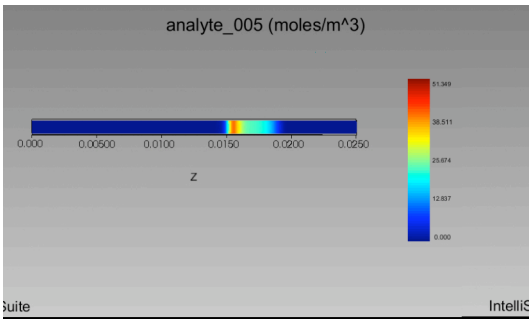
The initial pH distribution in the pipe should appear as in the figure below.

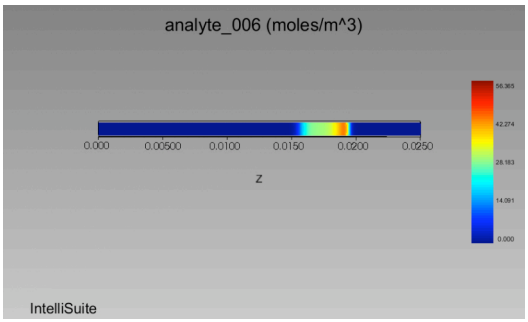


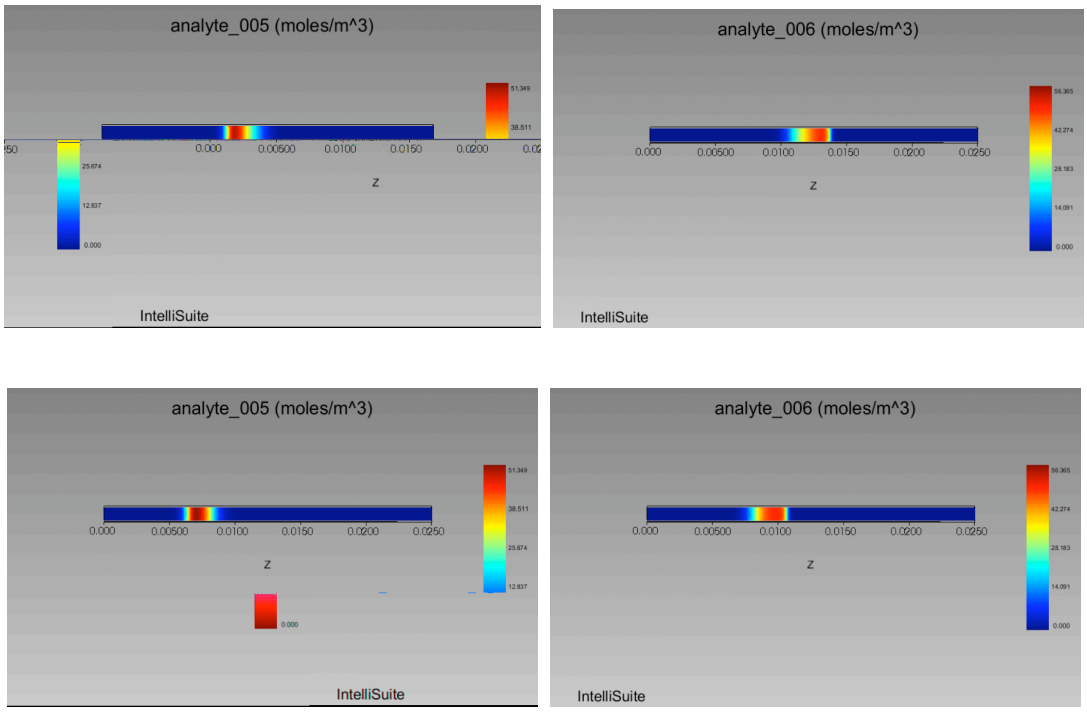
Select *analyte_005* in the Variable drop-down menu on the left. If you click the Play button at the top of the screen, you can see the position of Analyte 5 over time. Analyte 5 and Analyte 6 start out at the same position, but because of their different properties, they will move through the capillary at different rates. Shown below is a comparison of the concentrations of Analytes 5 and 6 at various steps throughout the analysis.



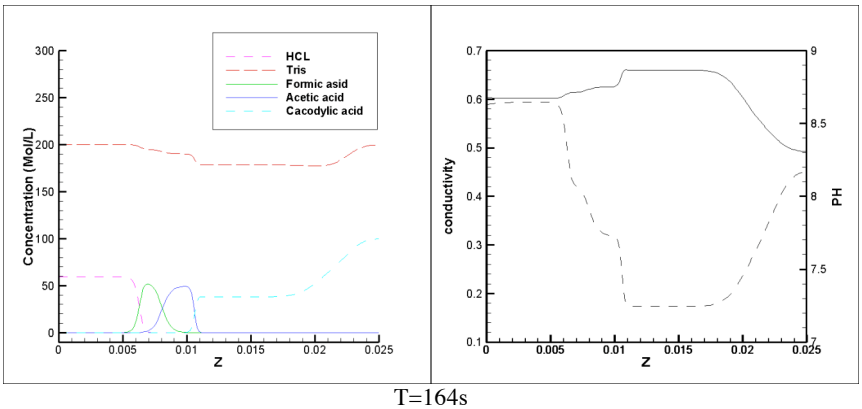




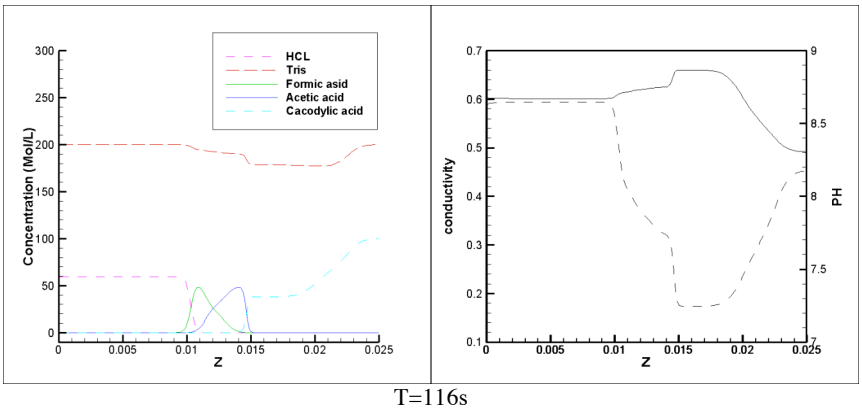




The conductivity, pH, and analyte concentration were plotted against the position for four time steps.



T=164s



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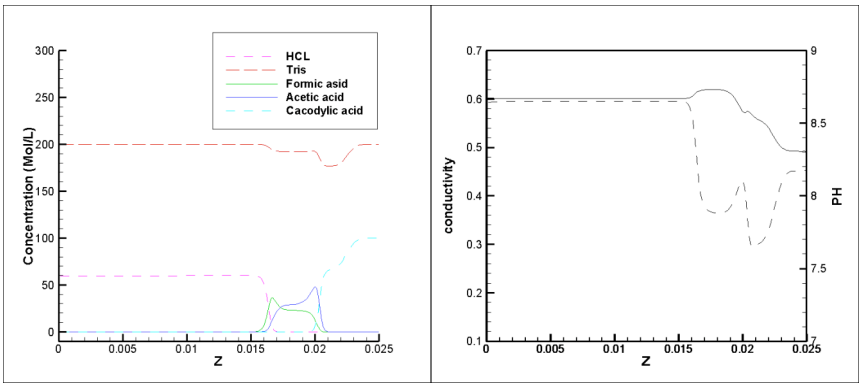

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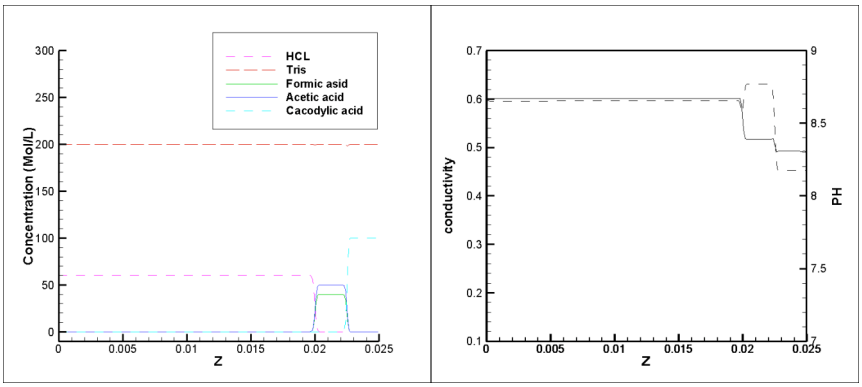
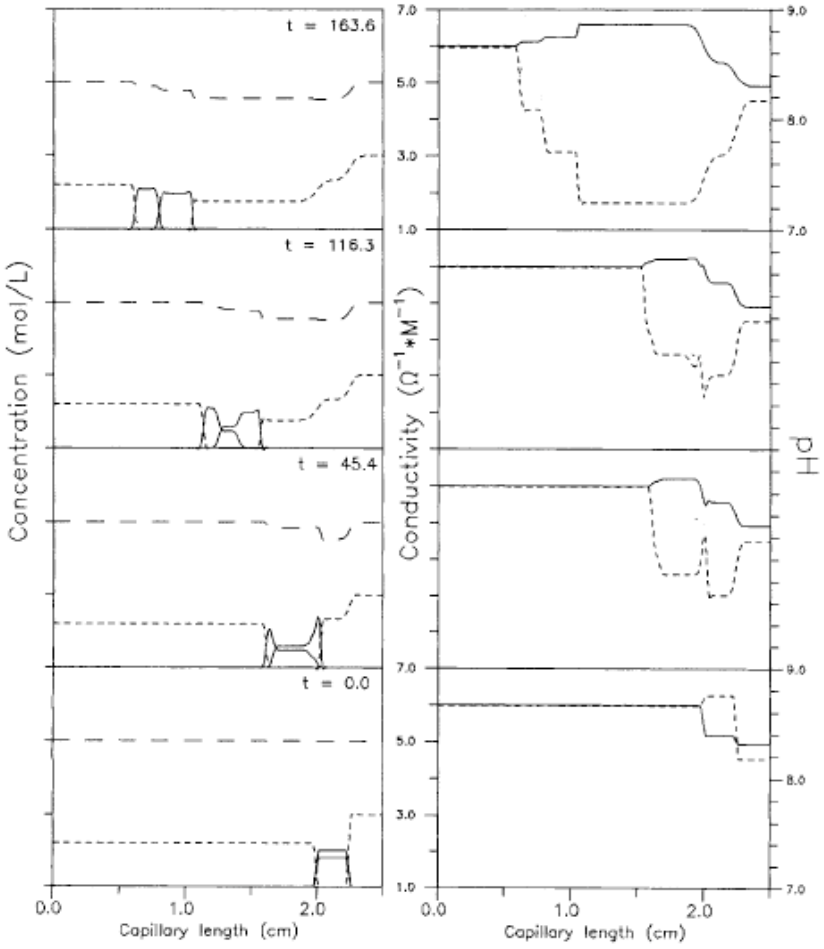


T=1s

The results from IntelliSuite were benchmarked against the results from a reference paper. Shown below are the results from the reference [1].



[1] Sergey V. Ermakorl, Olga S. Mazhorova and Michael Y. Zhukov, Computer simulation of transient states in capillary zone electrophoresis and isotachophoresis***, Electrophoresis*** 1992, v13, p838-848