



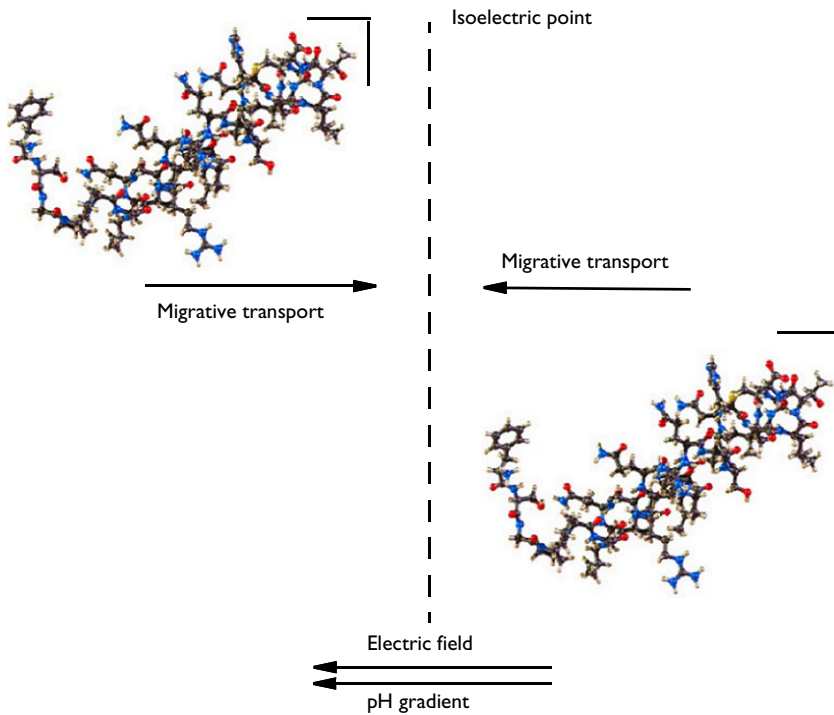
Model created in COMSOL Multiphysics 6.4

# Isoelectric Separation

## Introduction

This modeling example applies the Electrophoretic Transport and the Laminar Flow interfaces to model isoelectric separation in a free-flow electrophoresis device. A stream containing four different proteins is divided into separated component streams by means of migrative transport in an electric field.

Free flow electrophoresis can be used to separate macromolecules, such as proteins perpendicular to the flow of the carrier fluid. If a pH and potential gradient is applied across the carrier flow, then molecules can be focused along their isoelectric points. The isoelectric point is the pH at which a molecule has zero net charge. The concept of isoelectric focusing is illustrated in [Figure 1](#).



*Figure 1: Amphoteric molecules can be focused around their isoelectric point by means of migrative transport in an electric field.*

Molecules with a positive net charge travel in the direction of the electric field, along the pH gradient, until they reach the isoelectric point. At this instance the migrative transport

is switched off as the molecules net charge is zero. Similarly, negative net charge species travel in the direction opposite of the electric field.

### Model Definition

The model geometry is shown in Figure 2. It represents the separation region in an isoelectric focusing chip. A laminar carrier stream transports a mixture of four proteins, one weak acid and one weak base, injected at the bottom of the cell.

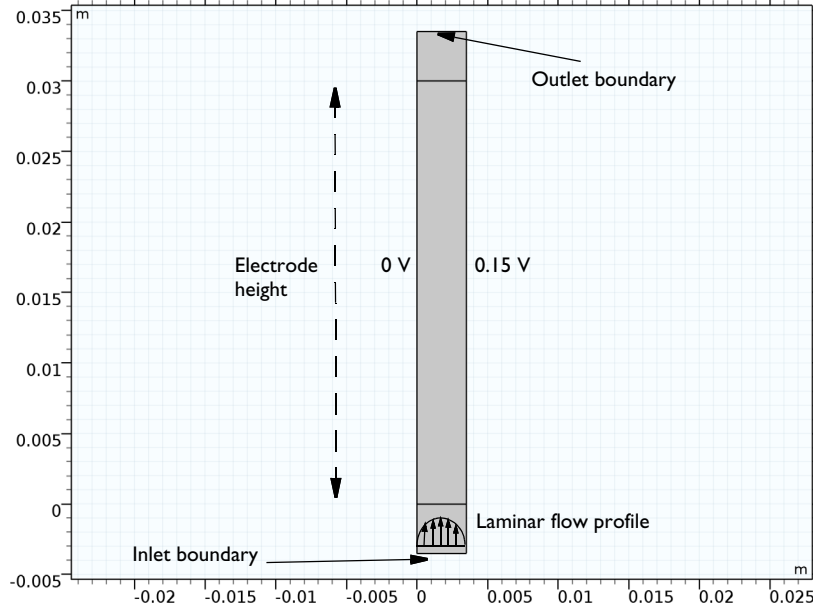


Figure 2: Model geometry.

The Electrophoretic Transport interface supports ionic species transport by diffusion, convection, and migration in electric fields. The mass balance that is solved in the interface is as follows:

$$\nabla \cdot (-D_i \nabla c_i - z_i u_{m,i} F c_i \nabla \phi) + \mathbf{u} \cdot \nabla c_i = 0$$

In the equation,  $c_i$  denotes the concentration of species  $i$  (SI unit:  $\text{mol}/\text{m}^3$ ),  $D_i$  is the diffusion coefficient of species  $i$  (SI unit:  $\text{m}^2/\text{s}$ ),  $\mathbf{u}$  is the fluid velocity (SI unit:  $\text{m}/\text{s}$ ),  $F$  refers to Faraday's constant (SI unit:  $\text{s}\cdot\text{A}/\text{mol}$ ),  $\phi$  denotes the electric potential (SI unit:  $\text{V}$ ),  $z_i$  is the charge number of the ionic species (unitless), and  $u_{m,i}$  its ionic mobility (SI unit:  $\text{s}\cdot\text{mol}/\text{kg}$ ). The Electrophoretic Transport interface also adds an equation for the

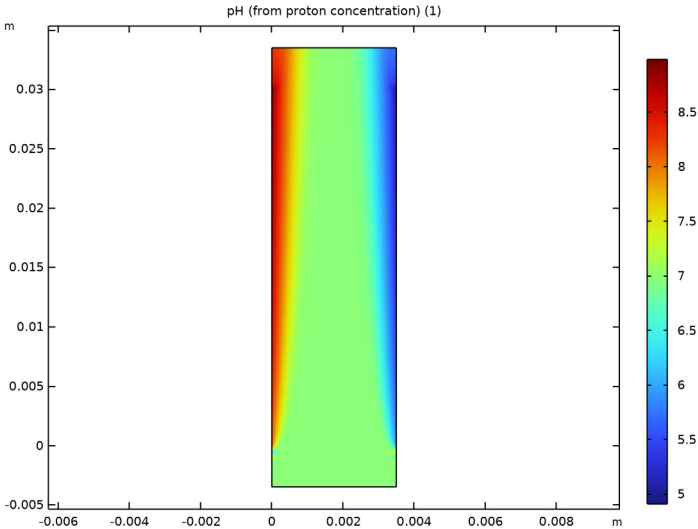
charge transport in the electrolyte, based on an assumption of electroneutrality, and a set of chemical equilibria describing the water self- ionization reaction and the dissociation reactions of weak acids and bases.

The fluid flow is set up with a Laminar Flow interface, solving for the Navier–Stokes equations.

*Results and Discussion*

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Figure 3 shows the pH in the cell. The pH gradients in the  $x$  direction increase toward the outlet.



*Figure 3: Surface plot of pH in the cell.*

Figure 4 shows the electrolyte conductivity. The conductivity gets lower close to the electrode surfaces and toward the outlet due to depletion of charge carriers.

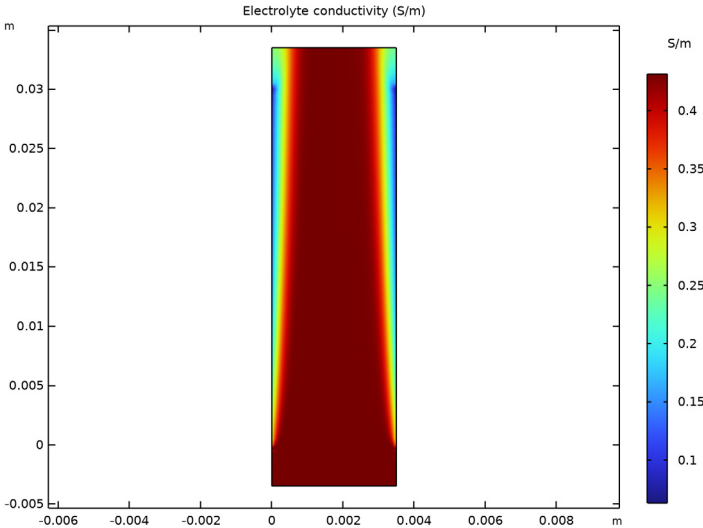


Figure 4: Surface plot of electrolyte conductivity in the cell.

Figure 5 shows the electrolyte potential. Outside the electrode gap, the potential gradients are low, which means that the migrative flux close to the inlet and outlet is low.

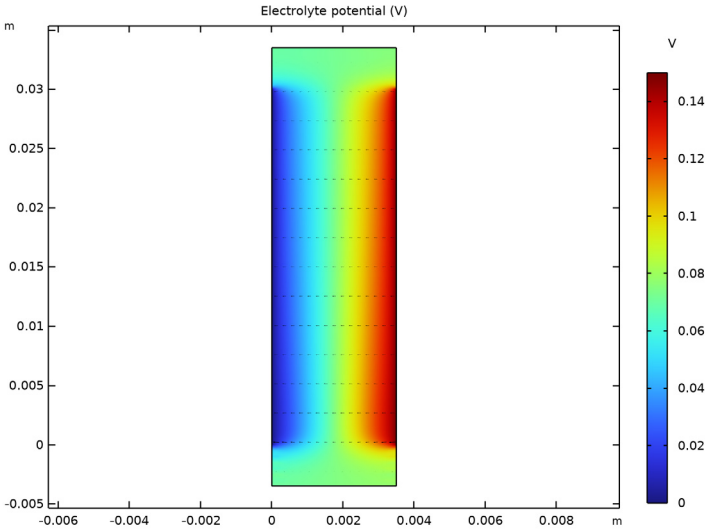


Figure 5: Surface plot of electrolyte potential in the cell.

Figure 6 shows the concentration of Protein 1, which has an isoelectric point of 4.7. The protein is concentrated toward the right electrode.

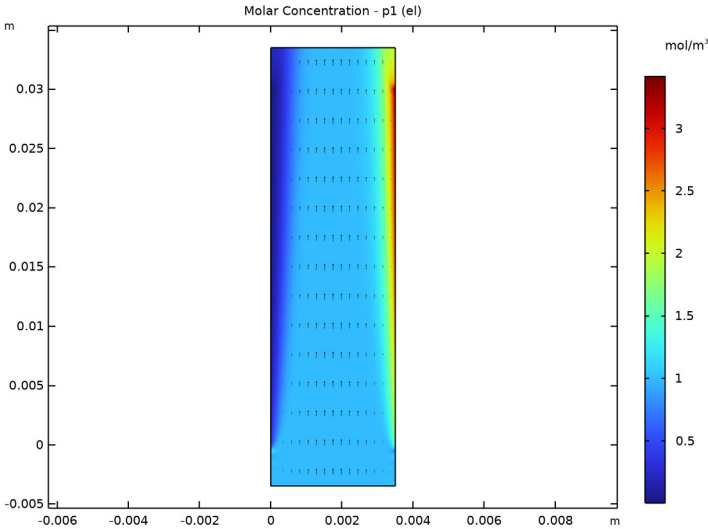


Figure 6: Surface plot of molar concentration of Protein 1 in the cell.

Figure 7 shows the concentration of Protein 2, with an isoelectric point of 6.1. This protein reaches its maximum outlet concentration somewhere between the center and the right electrode.

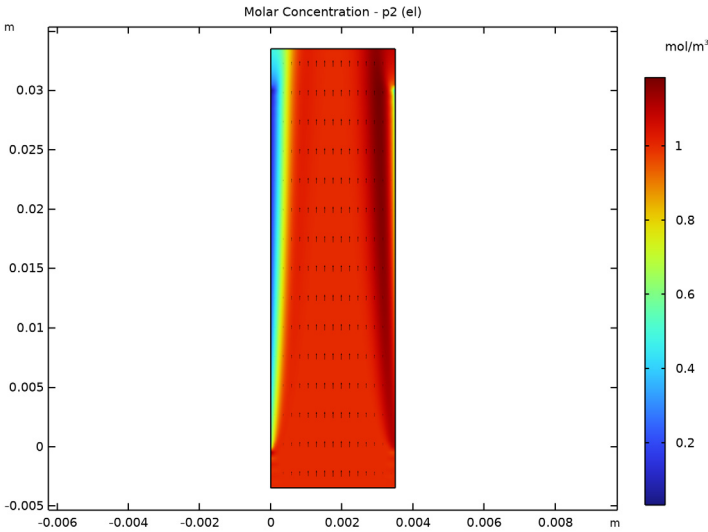


Figure 7: Surface plot of molar concentration of Protein 2 in the cell.

Figure 8 and Figure 9 show the weak acid and base concentrations, respectively. The weak acid, with a negative average charge, is transported to the right and the base, with a positive average charge, is transported to the left in the electric field.

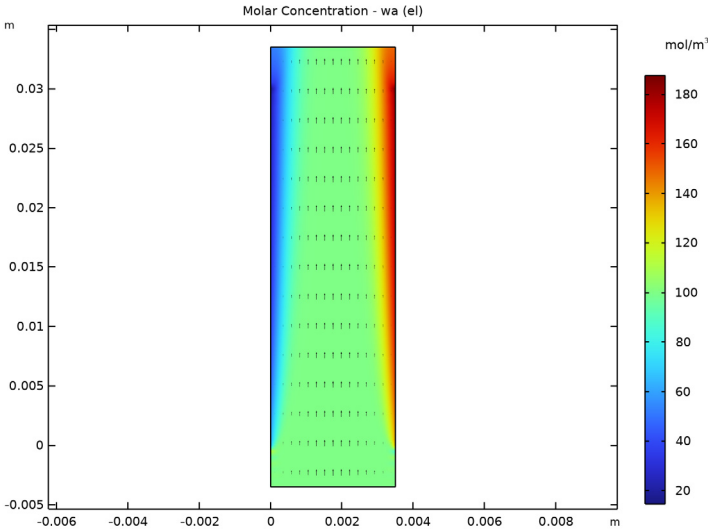


Figure 8: Surface plot of weak acid concentration in the cell.

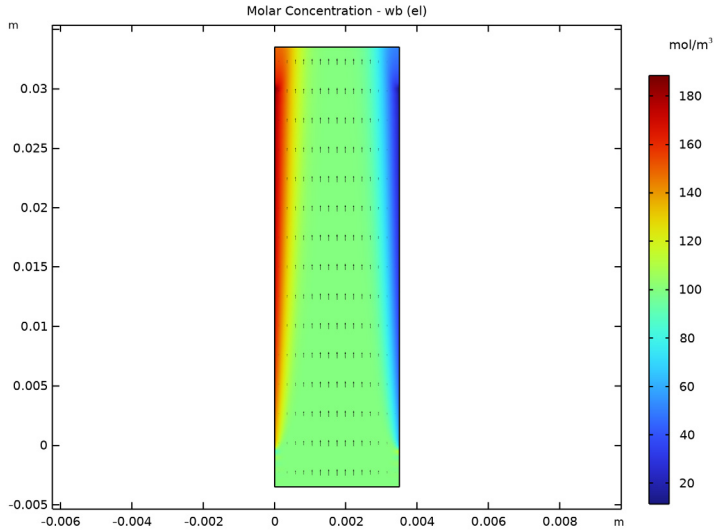


Figure 9: Surface plot of weak base concentration in the cell.

Finally, Figure 10 and Figure 11 show the pH and protein concentration profiles at the outlet.

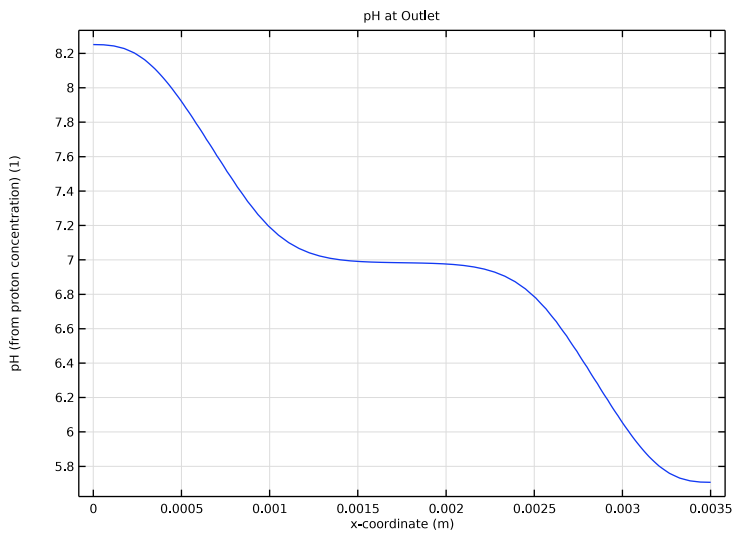


Figure 10: pH profile along the outlet boundary of the cell.

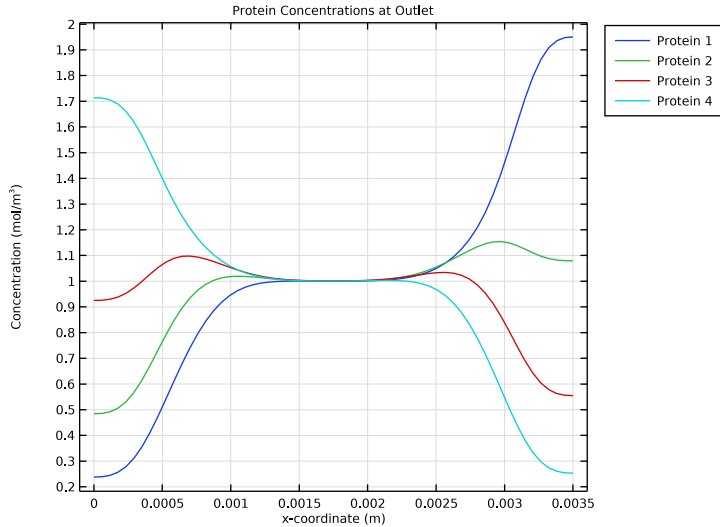


Figure 11: Protein concentration profiles along the outlet boundary of the cell.

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**Application Library path:** Chemical\_Reaction\_Engineering\_Module/  
Electrokinetic\_Effects/isoelectric\_separation


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### *Modeling Instructions*


In this tutorial we couple Electrophoretic Transport to Laminar Flow in 2D. Use the **Model Wizard** to select the space dimension and the physics interfaces.

From the **File** menu, choose **New**.

#### **NEW**

In the **New** window, click  **Model Wizard**.

#### **MODEL WIZARD**


- 1 In the **Model Wizard** window, click  **2D**.
- 2 In the **Select Physics** tree, select **Chemical Species Transport** > **Electrophoretic Transport (el)**.

3 Click **Add**.

4 In the **Select Physics** tree, select **Fluid Flow > Single-Phase Flow > Laminar Flow (spf)**.

5 Click **Add**.

Finish without adding studies. They will be added to the model at a later stage.

6 Click  **Done**.

## GLOBAL DEFINITIONS

Load the model parameters from a text file.

### *Parameters 1*

1 In the **Model Builder** window, under **Global Definitions** click **Parameters 1**.

2 In the **Settings** window for **Parameters**, locate the **Parameters** section.


3 Click  **Load from File**.

4 Browse to the model's Application Libraries folder and double-click the file `isoelectric_separation_parameters.txt`.

## GEOMETRY 1

Now draw the geometry as a union of three rectangles.

### *Rectangle 1 (r1)*


1 In the **Geometry** toolbar, click  **Rectangle**.

2 In the **Settings** window for **Rectangle**, locate the **Size and Shape** section.

3 In the **Width** text field, type W.

4 In the **Height** text field, type H.

### *Rectangle 2 (r2)*

1 In the **Geometry** toolbar, click  **Rectangle**.

2 In the **Settings** window for **Rectangle**, locate the **Size and Shape** section.

3 In the **Width** text field, type W.

4 In the **Height** text field, type W.

5 Locate the **Position** section. In the **y** text field, type -W.

6 Click  **Build Selected**.

### *Rectangle 3 (r3)*

1 Right-click **Rectangle 2 (r2)** and choose **Duplicate**.


2 In the **Settings** window for **Rectangle**, locate the **Position** section.

3 In the **y** text field, type H.


4 Click  **Build Selected**.

#### *Inlet*

Add selections for the inlet and outlet boundaries. These will be used later when setting up the physics.



- 1 In the **Geometry** toolbar, click  **Selections** and choose **Explicit Selection**.
- 2 In the **Settings** window for **Explicit Selection**, type Inlet in the **Label** text field.
- 3 Locate the **Entities to Select** section. From the **Geometric entity level** list, choose **Boundary**.
- 4 On the object **r2**, select Boundary 1 only.

#### *Outlet*

- 1 In the **Geometry** toolbar, click  **Selections** and choose **Explicit Selection**.
- 2 In the **Settings** window for **Explicit Selection**, type Outlet in the **Label** text field.
- 3 Locate the **Entities to Select** section. From the **Geometric entity level** list, choose **Boundary**.
- 4 On the object **r3**, select Boundary 3 only.

The interior boundaries are not part of the physical geometry. Assign them as **Mesh Control Edges** to make them available in the mesh only.

#### *Mesh Control Edges 1 (mce1)*

- 1 In the **Geometry** toolbar, click  **Virtual Operations** and choose **Mesh Control Edges**.
- 2 On the object **fin**, select Boundaries 4 and 6 only.
- 3 In the **Settings** window for **Mesh Control Edges**, locate the **Input** section.
- 4 Clear the **Include adjacent vertices** checkbox.
- 5 In the **Geometry** toolbar, click  **Build All**.



The model is slender. Change **View scale** to more easily see variations over the entire geometry.

## **DEFINITIONS**

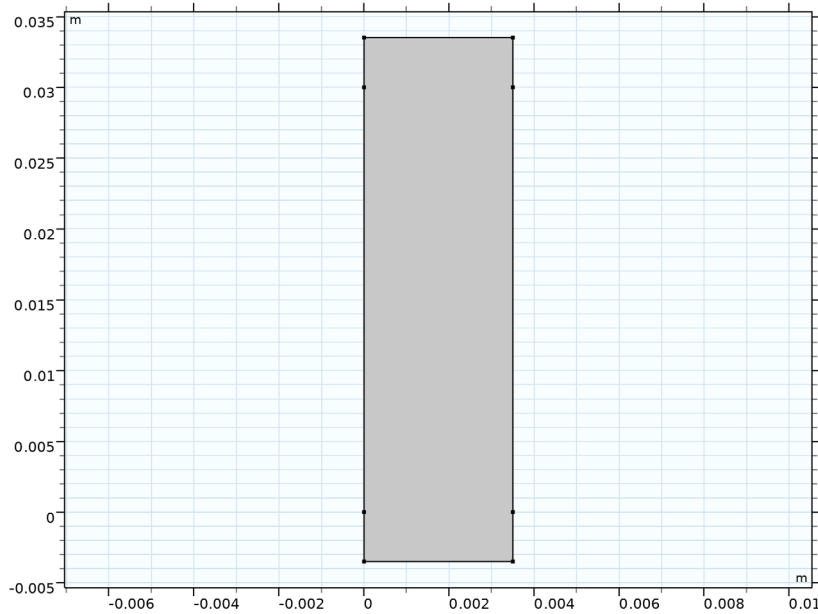
In the **Model Builder** window, expand the **Component 1 (comp1) > Definitions** node.

#### *Axis*

- 1 In the **Model Builder** window, expand the **Component 1 (comp1) > Definitions > View 1** node, then click **Axis**.

- 2 In the **Settings** window for **Axis**, locate the **Axis** section.
- 3 From the **View scale** list, choose **Manual**.
- 4 In the **x scale** text field, type 3.
- 5 Click  **Update**.
- 6 Click the  **Zoom Extents** button in the **Graphics** toolbar.



The finished geometry should now look like this:



## MATERIALS

To set up flow equations for the electrolyte we will use some material parameters for water from the **Material Library**.

### ADD MATERIAL

- 1 In the **Materials** toolbar, click  **Add Material** to open the **Add Material** window.
- 2 Go to the **Add Material** window.
- 3 In the tree, select **Built-in > Water, liquid**.
- 4 Right-click and choose **Add to Component 1 (comp1)**.
- 5 In the **Materials** toolbar, click  **Add Material** to close the **Add Material** window.

## ELECTROPHORETIC TRANSPORT (EL)

Now start setting up the **Electrophoretic Transport**.

- 1 In the **Settings** window for **Electrophoretic Transport**, locate the **Transport Mechanisms** section.
- 2 Select the **Convection** checkbox.

### *Solvent 1*


- 1 In the **Model Builder** window, under **Component 1 (comp1) > Electrophoretic Transport (el)** click **Solvent 1**.
- 2 In the **Settings** window for **Solvent**, locate the **Convection** section.
- 3 From the **u** list, choose **Velocity field (spf)**.

### *Potential 1*

This cell is operated potentiostatically (at a constant potential).


- 1 In the **Physics** toolbar, click  **Boundaries** and choose **Potential**.
- 2 Select Boundary 3 only.

### *Potential 2*


- 1 In the **Physics** toolbar, click  **Boundaries** and choose **Potential**.
- 2 Select Boundary 7 only.
- 3 In the **Settings** window for **Potential**, locate the **Electrolyte Potential** section.
- 4 In the  $\phi_{1,\text{bnd}}$  text field, type V0.

### *Protein 1*

Now add the various species in the electrolyte: 4 proteins, one weak acid and one weak base.

- 1 In the **Physics** toolbar, click  **Domains** and choose **Protein**.
- 2 In the **Settings** window for **Protein**, locate the **Protein** section.
- 3 In the **Species name** text field, type p1.
- 4 In the **Average charge** text field, type  $\text{iep}_1 - e1$ . pH.
- 5 Locate the **Diffusion and Migration** section. In the **D** text field, type D\_p.

### *Inflow 1*


- 1 In the **Physics** toolbar, click  **Attributes** and choose **Inflow**.
- 2 In the **Settings** window for **Inflow**, locate the **Boundary Selection** section.
- 3 From the **Selection** list, choose **Inlet**.

- 4 Locate the **Concentration** section. In the  $c_0$  text field, type `cp_in`.
- 5 Locate the **Boundary Condition Type** section. From the list, choose **Flux (Danckwerts)**.

#### *Protein 1*

In the **Model Builder** window, click **Protein 1**.

#### *Outflow 1*

- 1 In the **Physics** toolbar, click  **Attributes** and choose **Outflow**.
- 2 In the **Settings** window for **Outflow**, locate the **Boundary Selection** section.
- 3 From the **Selection** list, choose **Outlet**.

#### *Protein 2*

In this tutorial we assume that only the isoelectric point varies between the proteins. Therefore you can just duplicate the first protein to create the second protein, and then and change the average charge.

- 1 Right-click **Protein 1** and choose **Duplicate**.
- 2 In the **Settings** window for **Protein**, locate the **Protein** section.
- 3 In the **Species name** text field, type `p2`.
- 4 In the **Average charge** text field, type `iep_2-e1.pH`.

#### *Protein 3*


- 1 Right-click **Protein 2** and choose **Duplicate**.
- 2 In the **Settings** window for **Protein**, locate the **Protein** section.
- 3 In the **Species name** text field, type `p3`.
- 4 In the **Average charge** text field, type `iep_3-e1.pH`.

#### *Protein 4*

- 1 Right-click **Protein 3** and choose **Duplicate**.
- 2 In the **Settings** window for **Protein**, locate the **Protein** section.
- 3 In the **Species name** text field, type `p4`.
- 4 In the **Average charge** text field, type `iep_4-e1.pH`.

#### *Weak Acid 1*

Finish the electrophoretic transport settings by adding one weak acid and one weak base.

- 1 In the **Physics** toolbar, click  **Domains** and choose **Weak Acid**.
- 2 In the **Settings** window for **Weak Acid**, locate the **Weak Acid** section.
- 3 In the **Species name** text field, type `wa`.

- 4 In the  $pK_a$  text field, type pKa\_wa.
- 5 Locate the **Diffusion and Migration** section. In the  $u_m$  text field, type mob\_wa.


#### *Initial Concentration I*

- 1 In the **Model Builder** window, expand the **Weak Acid I** node, then click **Initial Concentration I**.
- 2 In the **Settings** window for **Initial Concentration**, locate the **Initial Concentration** section.
- 3 In the  $c$  text field, type cwa\_in.

#### *Weak Acid I*

In the **Model Builder** window, click **Weak Acid I**.


#### *Inflow I*

- 1 In the **Physics** toolbar, click  **Attributes** and choose **Inflow**.
- 2 In the **Settings** window for **Inflow**, locate the **Boundary Selection** section.
- 3 From the **Selection** list, choose **Inlet**.
- 4 Locate the **Concentration** section. In the  $c_0$  text field, type cwa\_in.
- 5 Locate the **Boundary Condition Type** section. From the list, choose **Flux (Danckwerts)**.


#### *Weak Acid I*

In the **Model Builder** window, click **Weak Acid I**.

#### *Outflow I*

- 1 In the **Physics** toolbar, click  **Attributes** and choose **Outflow**.
- 2 In the **Settings** window for **Outflow**, locate the **Boundary Selection** section.
- 3 From the **Selection** list, choose **Outlet**.

#### *Weak Base I*

- 1 In the **Physics** toolbar, click  **Domains** and choose **Weak Base**.
- 2 In the **Settings** window for **Weak Base**, locate the **Weak Base** section.
- 3 In the **Species name** text field, type wb.
- 4 In the  $pK_a$  text field, type pKa\_wb.
- 5 Locate the **Diffusion and Migration** section. In the  $u_m$  text field, type mob\_wb.

#### *Initial Concentration I*

- 1 In the **Model Builder** window, expand the **Weak Base I** node, then click **Initial Concentration I**.
- 2 In the **Settings** window for **Initial Concentration**, locate the **Initial Concentration** section.

3 In the  $c$  text field, type `cwb_in`.

#### *Weak Base I*

In the **Model Builder** window, click **Weak Base I**.

#### *Inflow I*

1 In the **Physics** toolbar, click  **Attributes** and choose **Inflow**.

2 In the **Settings** window for **Inflow**, locate the **Boundary Selection** section.

3 From the **Selection** list, choose **Inlet**.

4 Locate the **Concentration** section. In the  $c_0$  text field, type `cwb_in`.

5 Locate the **Boundary Condition Type** section. From the list, choose **Flux (Danckwerts)**.

#### *Weak Base I*

In the **Model Builder** window, click **Weak Base I**.

#### *Outflow I*

1 In the **Physics** toolbar, click  **Attributes** and choose **Outflow**.

2 In the **Settings** window for **Outflow**, locate the **Boundary Selection** section.

3 From the **Selection** list, choose **Outlet**.

### **LAMINAR FLOW (SPF)**

Now set up the Laminar Flow interface. Only the boundary conditions need to be specified here since most settings are taken from the Materials node (Water).

1 In the **Model Builder** window, under **Component 1 (comp1)** click **Laminar Flow (spf)**.

#### *Inlet I*

1 In the **Physics** toolbar, click  **Boundaries** and choose **Inlet**.

2 In the **Settings** window for **Inlet**, locate the **Boundary Selection** section.

3 From the **Selection** list, choose **Inlet**.

4 Locate the **Boundary Condition** section. From the list, choose **Fully developed flow**.

5 Locate the **Fully Developed Flow** section. In the  $U_{av}$  text field, type `Uave`.

#### *Outlet I*

1 In the **Physics** toolbar, click  **Boundaries** and choose **Outlet**.

2 In the **Settings** window for **Outlet**, locate the **Boundary Selection** section.


3 From the **Selection** list, choose **Outlet**.

4 Locate the **Pressure Conditions** section. Select the **Normal flow** checkbox.

## MESH 1

Now set up the mesh. A mapped mesh is suitable since we are using a rectangular geometry.


### *Mapped 1*

In the **Mesh** toolbar, click  **Mapped**.

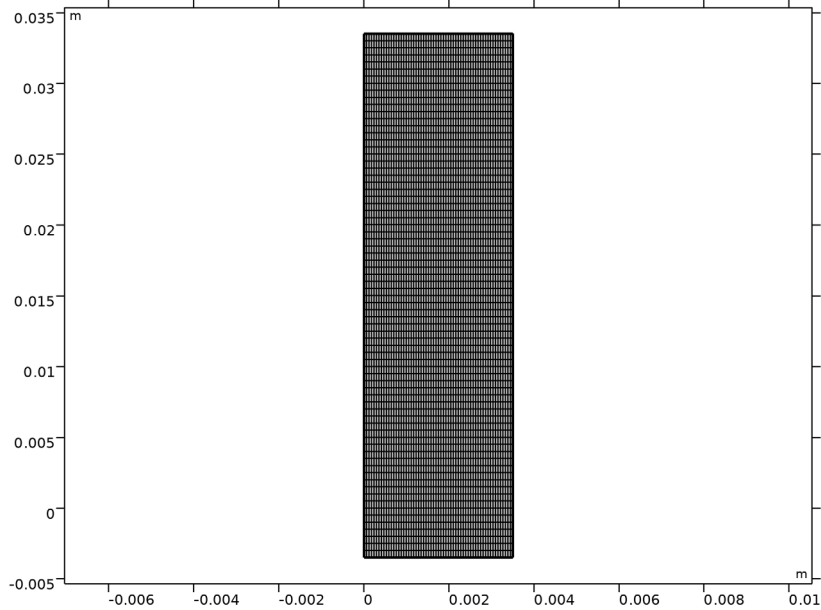
### *Distribution 1*

- 1 Right-click **Mapped 1** and choose **Distribution**.
- 2 Select Boundaries 2, 5, 9, and 10 only.
- 3 In the **Settings** window for **Distribution**, locate the **Distribution** section.
- 4 In the **Number of elements** text field, type 60.

### *Size*

- 1 In the **Model Builder** window, under **Component 1 (comp1) > Mesh 1** click **Size**.
- 2 In the **Settings** window for **Size**, locate the **Element Size** section.
- 3 Click the **Custom** button.
- 4 Locate the **Element Size Parameters** section. In the **Maximum element size** text field, type 5e-4.
- 5 Click  **Build All**.



The finished mesh should look like this:



## ROOT


The model is now ready for solving. In this model the flow is not affected by the physics of the **Electrophoretic Transport** interface. Therefore solve the **Laminar Flow** first in a separate study.

## ADD STUDY

- 1 In the **Home** toolbar, click  **Add Study** to open the **Add Study** window.
- 2 Go to the **Add Study** window.
- 3 Find the **Studies** subsection. In the **Select Study** tree, select **General Studies > Stationary**.
- 4 Find the **Physics interfaces in study** subsection. In the table, clear the **Solve** checkbox for **Electrophoretic Transport (el)**.
- 5 Click the **Add Study** button in the window toolbar.
- 6 In the **Home** toolbar, click  **Add Study** to close the **Add Study** window.

## STUDY 1 - FLOW CALCULATION

- 1 In the **Settings** window for **Study**, type Study 1 - Flow Calculation in the **Label** text field.

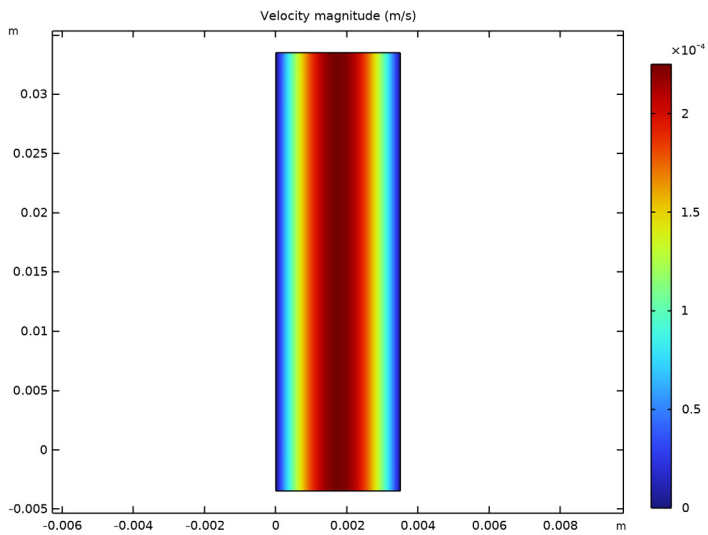
2 In the **Study** toolbar, click  **Compute**.

## RESULTS

Default plots for the velocity and pressure are generated automatically as follows:

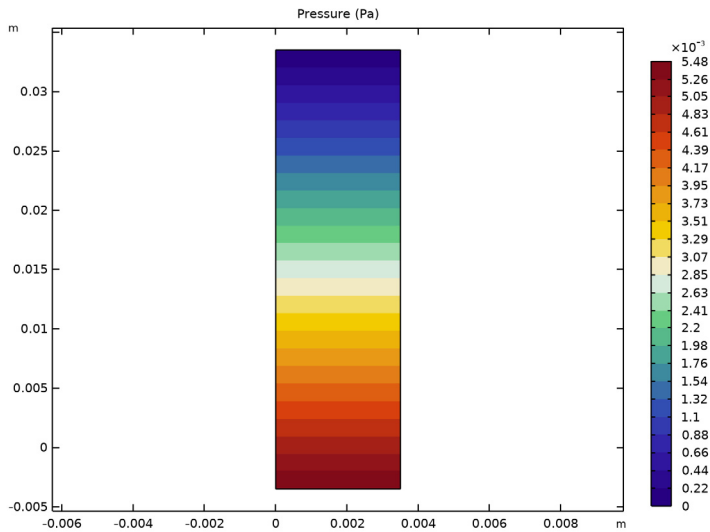
*Velocity (spf)*

In the **Model Builder** window, under **Results** click **Velocity (spf)**.



Pressure (spf)



In the **Model Builder** window, click **Pressure (spf)**.



## ROOT

Add a second study to solve for the **Electrophoretic Transport**, using the solution of the first Study as input for the velocity (and pressure).


## ADD STUDY

- 1 In the **Home** toolbar, click  **Add Study** to open the **Add Study** window.
- 2 Go to the **Add Study** window.
- 3 Find the **Physics interfaces in study** subsection. In the table, clear the **Solve** checkbox for **Laminar Flow (spf)**.
- 4 Find the **Studies** subsection. In the **Select Study** tree, select **Preset Studies for Selected Physics Interfaces > Stationary with Initialization**.
- 5 Click the **Add Study** button in the window toolbar.
- 6 In the **Home** toolbar, click  **Add Study** to close the **Add Study** window.

## STUDY 2 - SEPARATION CALCULATION

In the **Settings** window for **Study**, type Study 2 - Separation Calculation in the **Label** text field.

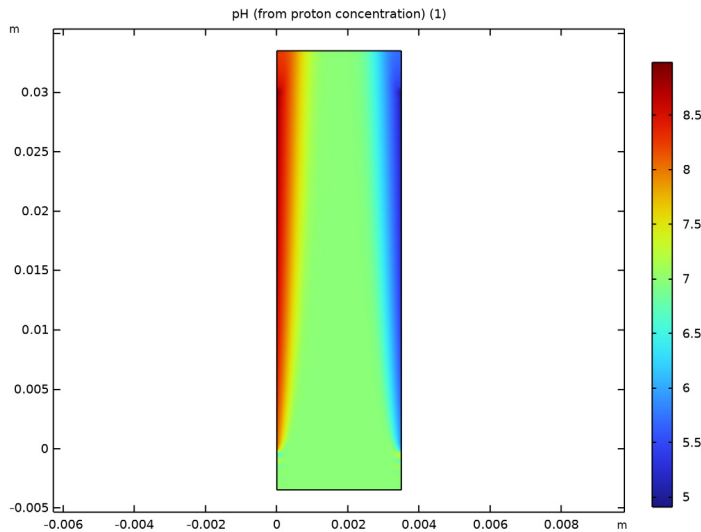
### Step 1: Current Distribution Initialization

- 1 In the **Model Builder** window, under **Study 2 - Separation Calculation** click **Step 1: Current Distribution Initialization**.
- 2 In the **Settings** window for **Current Distribution Initialization**, click to expand the **Values of Dependent Variables** section.
- 3 Find the **Values of variables not solved for** subsection. From the **Settings** list, choose **User controlled**.
- 4 From the **Method** list, choose **Solution**.
- 5 From the **Study** list, choose **Study 1 - Flow Calculation, Stationary**.
- 6 In the **Study** toolbar, click  **Compute**.

## RESULTS

### pH (el)

A default pH plot should now have been generated, looking like this:

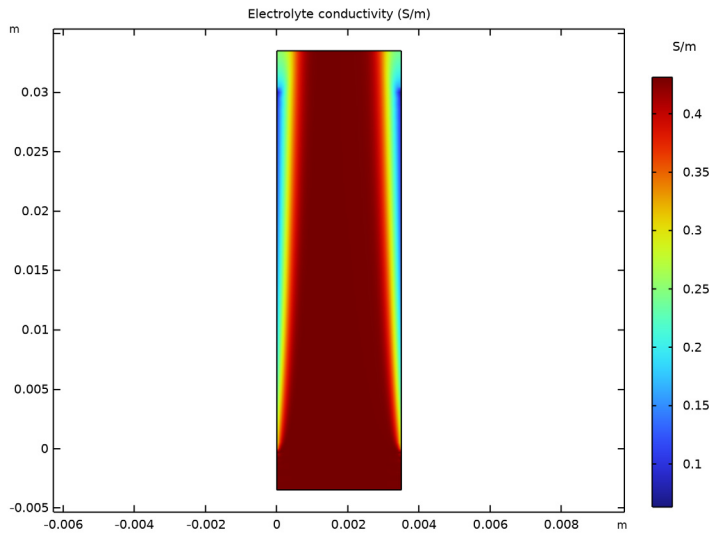


### Electrolyte Conductivity (el)

The default electrolyte conductivity plot should look like this:

- 1 In the **Model Builder** window, click **Electrolyte Conductivity (el)**.
- 2 In the **Settings** window for **2D Plot Group**, locate the **Color Legend** section.

**3** Select the **Show units** checkbox.



#### *Electrolyte Potential (el)*

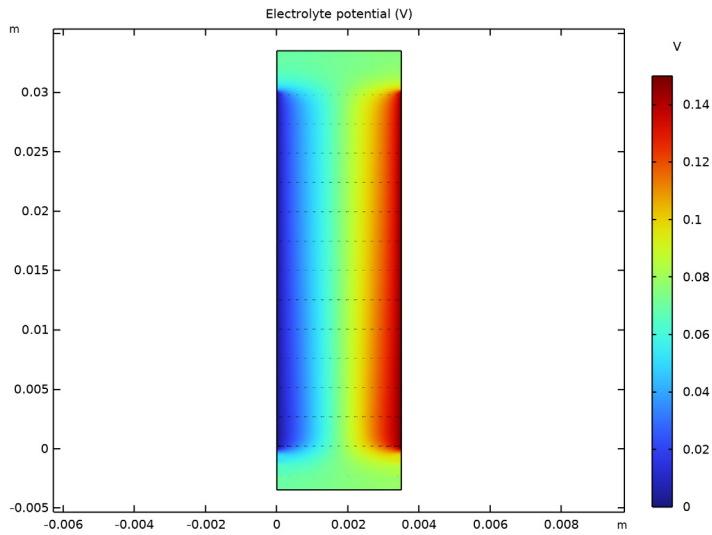
The default electrolyte potential plot should look like this:

- 1** In the **Model Builder** window, click **Electrolyte Potential (el)**.
- 2** In the **Settings** window for **2D Plot Group**, locate the **Color Legend** section.
- 3** Select the **Show units** checkbox.

#### *Arrow Surface 1*

- 1** In the **Model Builder** window, expand the **Electrolyte Potential (el)** node, then click **Arrow Surface 1**.
- 2** In the **Settings** window for **Arrow Surface**, click to expand the **Title** section.

**3** From the **Title type** list, choose **None**.

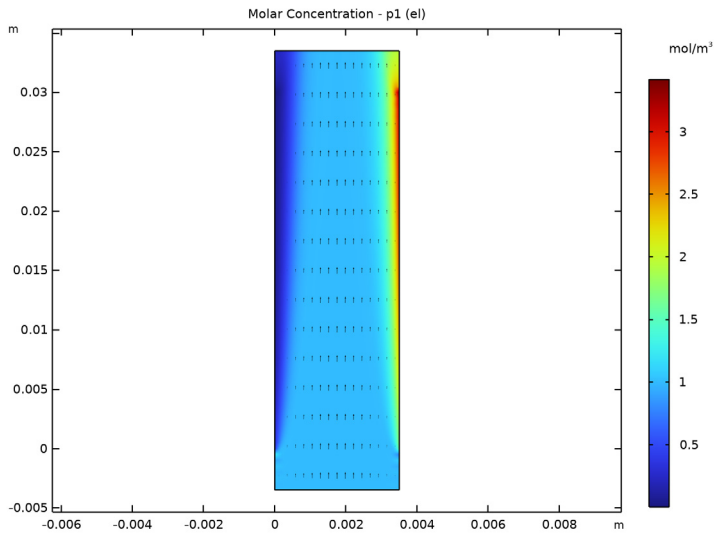


There should also be six default molar concentration plots present; one for each species in the **Electrophoretic Transport** interface:

*Molar Concentration - pI (el)*

- 1** In the **Model Builder** window, under **Results** click **Molar Concentration - pI (el)**.
- 2** In the **Settings** window for **2D Plot Group**, locate the **Title** section.
- 3** From the **Title type** list, choose **Label**.

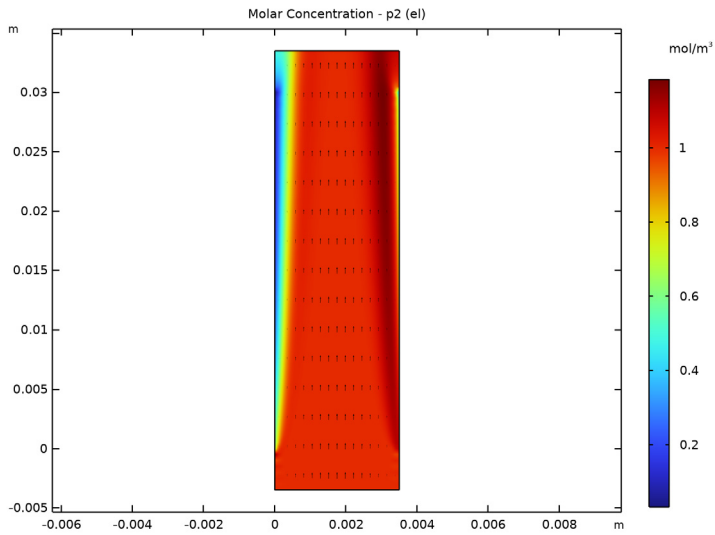
4 Locate the **Color Legend** section. Select the **Show units** checkbox.



*Molar Concentration - p2 (el)*

- 1 In the **Model Builder** window, click **Molar Concentration - p2 (el)**.
- 2 In the **Settings** window for **2D Plot Group**, locate the **Title** section.
- 3 From the **Title type** list, choose **Label**.

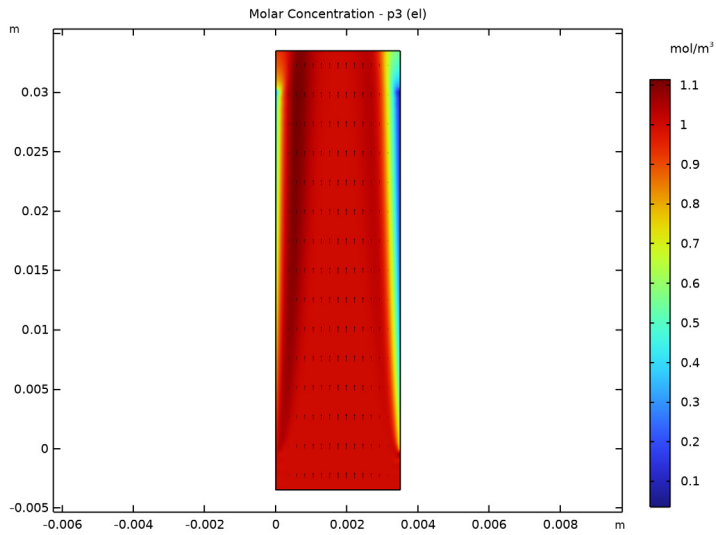
4 Locate the **Color Legend** section. Select the **Show units** checkbox.



*Molar Concentration - p3 (el)*

- 1 In the **Model Builder** window, click **Molar Concentration - p3 (el)**.
- 2 In the **Settings** window for **2D Plot Group**, locate the **Title** section.
- 3 From the **Title type** list, choose **Label**.

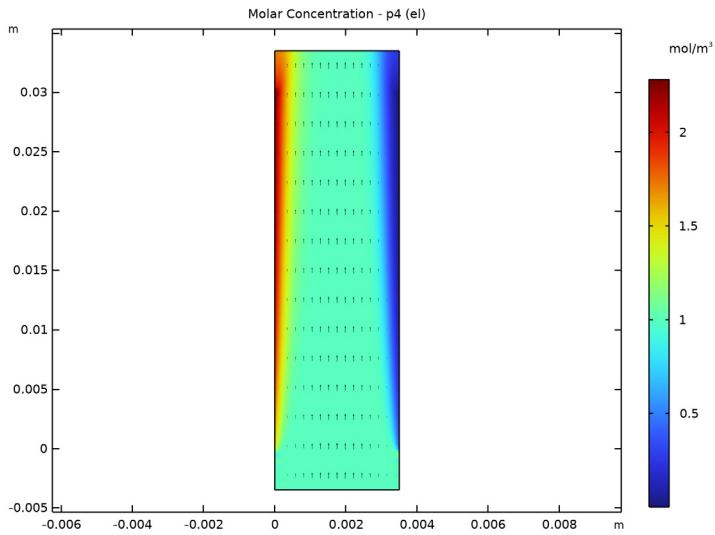
4 Locate the **Color Legend** section. Select the **Show units** checkbox.



*Molar Concentration - p4 (el)*

- 1 In the **Model Builder** window, click **Molar Concentration - p4 (el)**.
- 2 In the **Settings** window for **2D Plot Group**, locate the **Title** section.
- 3 From the **Title type** list, choose **Label**.

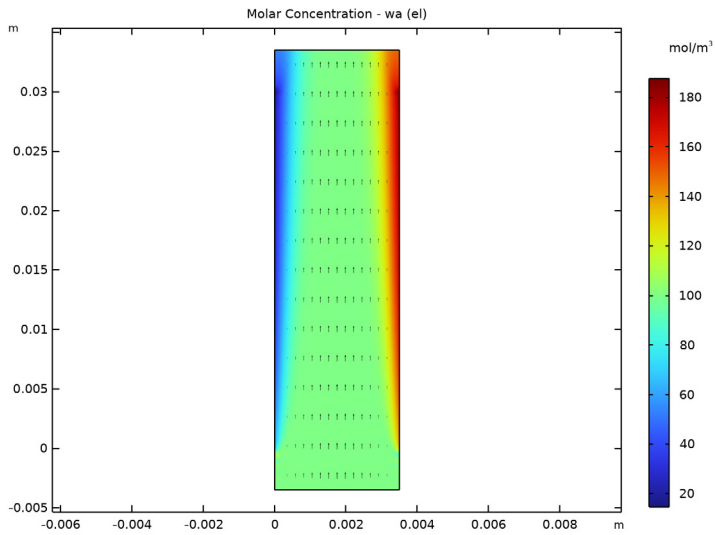
4 Locate the **Color Legend** section. Select the **Show units** checkbox.



*Molar Concentration - wa (el)*

- 1 In the **Model Builder** window, click **Molar Concentration - wa (el)**.
- 2 In the **Settings** window for **2D Plot Group**, locate the **Title** section.
- 3 From the **Title type** list, choose **Label**.

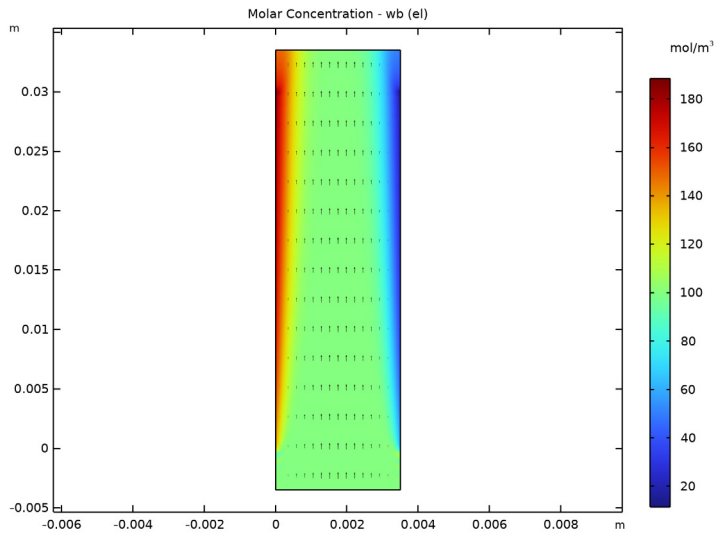
4 Locate the **Color Legend** section. Select the **Show units** checkbox.



*Molar Concentration - wb (el)*


- 1 In the **Model Builder** window, click **Molar Concentration - wb (el)**.
- 2 In the **Settings** window for **2D Plot Group**, locate the **Title** section.
- 3 From the **Title type** list, choose **Label**.

- 4 Locate the **Color Legend** section. Select the **Show units** checkbox.




#### *pH at Outlet*

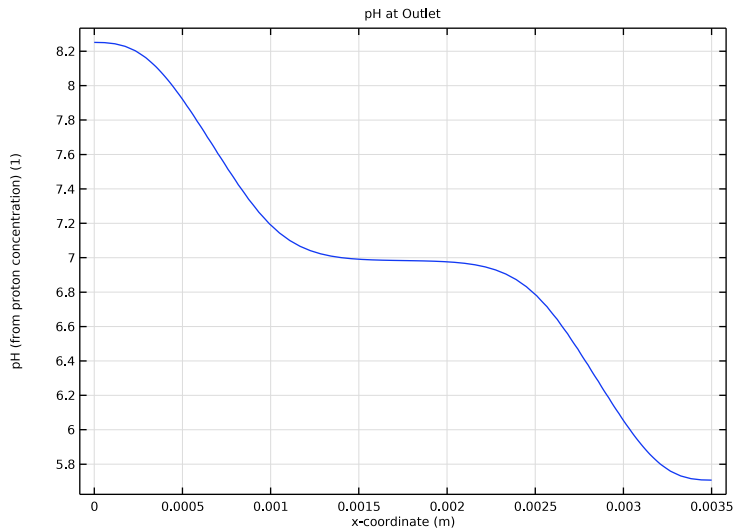
Now proceed to plot the pH at the outlet.

- 1 In the **Results** toolbar, click  **ID Plot Group**.
- 2 In the **Settings** window for **ID Plot Group**, type pH at Outlet in the **Label** text field.
- 3 Locate the **Data** section. From the **Dataset** list, choose **Study 2 - Separation Calculation/ Solution 2 (sol2)**.
- 4 Click to expand the **Title** section. From the **Title type** list, choose **Label**.

#### *Line Graph 1*


- 1 Right-click **pH at Outlet** and choose **Line Graph**.
- 2 In the **Settings** window for **Line Graph**, locate the **Selection** section.
- 3 From the **Selection** list, choose **Outlet**.
- 4 Locate the **x-Axis Data** section. From the **Parameter** list, choose **Expression**.
- 5 In the **Expression** text field, type x.
- 6 In the **pH at Outlet** toolbar, click  **Plot**.

The pH plot should look like this:



#### *Protein Concentrations at Outlet*

Finally, create plot of the protein concentrations at the outlet as follows:

- 1 In the **Results** toolbar, click  **ID Plot Group**.
- 2 In the **Settings** window for **ID Plot Group**, type Protein Concentrations at Outlet in the **Label** text field.
- 3 Locate the **Data** section. From the **Dataset** list, choose **Study 2 - Separation Calculation/ Solution 2 (sol2)**.
- 4 Click to expand the **Title** section. From the **Title type** list, choose **Label**.
- 5 Locate the **Legend** section. From the **Layout** list, choose **Outside graph axis area**.

#### *Line Graph 1*

- 1 Right-click **Protein Concentrations at Outlet** and choose **Line Graph**.
- 2 In the **Settings** window for **Line Graph**, locate the **Selection** section.
- 3 From the **Selection** list, choose **Outlet**.
- 4 Click **Replace Expression** in the upper-right corner of the **y-Axis Data** section. From the menu, choose **Component 1 (comp1) > Electrophoretic Transport > Protein 1 > el.c\_p1 - Concentration - mol/m<sup>3</sup>**.
- 5 Locate the **x-Axis Data** section. From the **Parameter** list, choose **Expression**.
- 6 In the **Expression** text field, type x.

7 Click to expand the **Legends** section. Select the **Show legends** checkbox.

8 From the **Legends** list, choose **Manual**.

9 In the table, enter the following settings:

---

<b>Legends</b>
Protein 1

#### *Line Graph 2*

1 Right-click **Line Graph 1** and choose **Duplicate**.

2 In the **Settings** window for **Line Graph**, locate the **y-Axis Data** section.

3 In the **Expression** text field, type e1.c\_p2.

4 Locate the **Legends** section. In the table, enter the following settings:

---

<b>Legends</b>
Protein 2

#### *Line Graph 3*

1 Right-click **Line Graph 2** and choose **Duplicate**.

2 In the **Settings** window for **Line Graph**, locate the **y-Axis Data** section.

3 In the **Expression** text field, type e1.c\_p3.

4 Locate the **Legends** section. In the table, enter the following settings:

---

<b>Legends</b>
Protein 3

#### *Line Graph 4*

1 Right-click **Line Graph 3** and choose **Duplicate**.

2 In the **Settings** window for **Line Graph**, locate the **y-Axis Data** section.

3 In the **Expression** text field, type e1.c\_p4.


4 Locate the **Legends** section. In the table, enter the following settings:

---

<b>Legends</b>
Protein 4

#### *Protein Concentrations at Outlet*

1 In the **Model Builder** window, click **Protein Concentrations at Outlet**.

2 In the **Protein Concentrations at Outlet** toolbar, click  **Plot**.

The plot should look like this:

