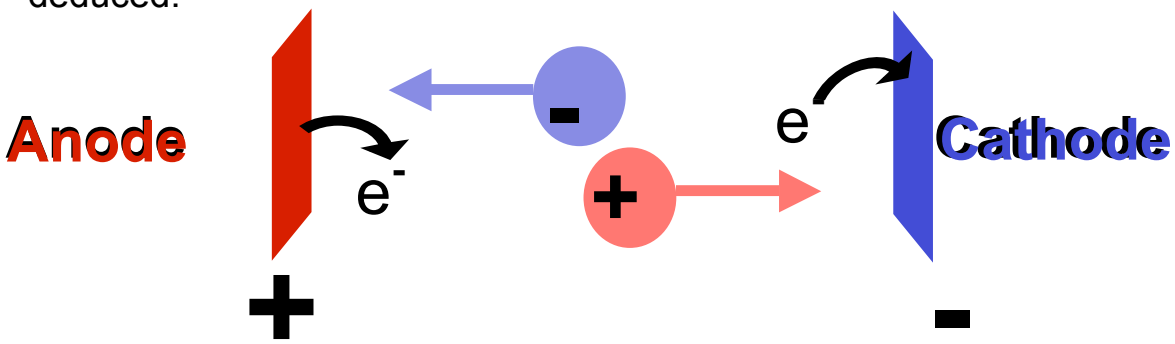
Charged up on Electrophoresis

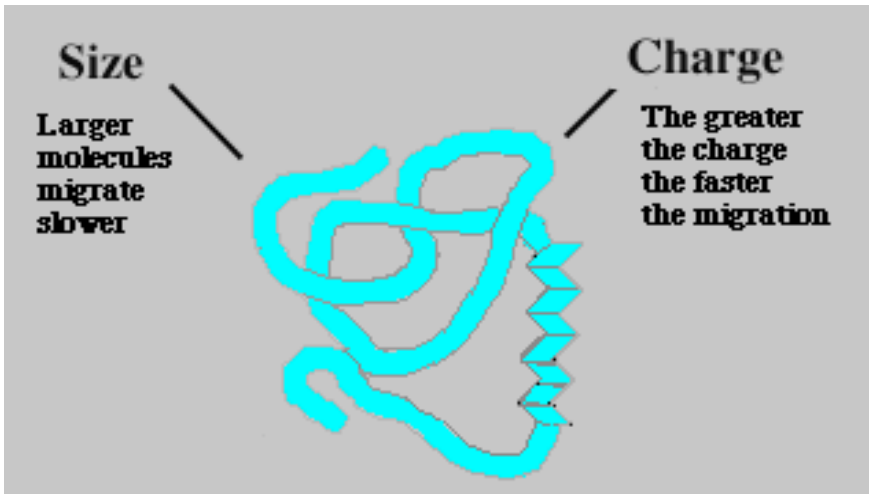
ChE 1101 Fall 2005

Electrophoresis is the migration of charged particles or molecules through a solution under the influence of an applied electric field usually provided by immersed electrodes. Particles with a positive charge go to the cathode (negatively charged electrode) and negative charges go to the anode (positively charged electrode). Electrophoresis is widely known in its role in determining the human genome. This method can separate proteins or nucleic acid chains; by analyzing the rate of movement of each component in a gel, the molecular structure can be deduced.



We are going to measure the **mobility, or rate of migration**, of NaCl ions in a water filled tray. Mobility, μ, of a charged ion is described by

$$\mu = \frac{q}{6 \cdot \pi \cdot \eta \cdot r} \qquad \left(mobility = \frac{charge}{6 \cdot \pi \cdot viscosity \cdot radius} \right)$$



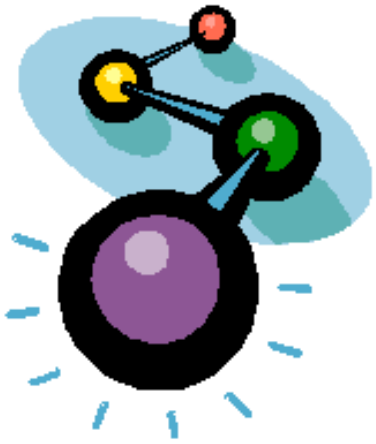
The electrophoretic mobility, μ, of ions in solution can be obtained from experimental data by taking the **velocity, v, divided by the electric field, E**.

$$\mu = \frac{v}{E} \qquad \left(mobility = \frac{velocity}{Electric \;\; Field} \right)$$

The electric field is the **voltage divided by the length** between the anode and the cathode.

$$E = \frac{V}{l} \qquad \left(Electric \;\; Field = \frac{Voltage}{length} \right)$$

Group A	**10 mL NaCl**	**0 mL E-pure H_2O**
Group B	**7 mL NaCl**	**3 mL E-pure H_2O**
Group C	**5 mL NaCl**	**5 mL E-pure H_2O**
Group D	**3 mL NaCl**	**7 mL E-pure H_2O**



EXPERIMENT

Now lay out your materials for your electrophoresis apparatus. **You should put on your goggles and gloves now.**

Step 1. Using the electrical tape, attach your wire adaptor to your electrophoresis tray. Refer to the example setup at your site. Make sure the exposed wire is close to the bottom of the trough. Do not connect your 9V battery yet.

Step 2. Place the electrophoresis tray on the 1 cm lines on your electrophoresis lab mat. Number the lines starting at 0 on up to 15 cm. This will be used to measure ion movement. **Record the total length of your tray on the Results Page.**

Step 3. Measure out 10 mL of Bromothymol blue pH indicator using your graduated cylinder. Carefully pour this into your electrophoresis tray. **Record this amount on your Results Page.**

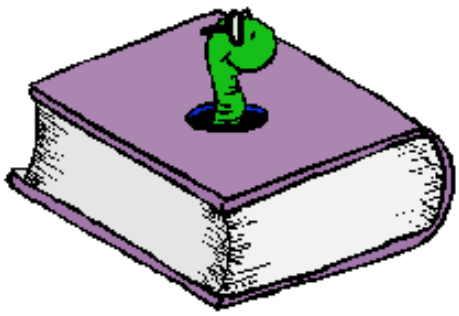
Step 4. Using your graduated cylinder, measure out the amount of NaCl solution and E-pure H_2O that corresponds to your group. Carefully pour this into your electrophoresis tray. **Record these amounts on your Results Page.**

Group A	10 mL NaCl	0 mL E-pure H_2O
Group B	7 mL NaCl	3 mL E-pure H_2O
Group C	5 mL NaCl	5 mL E-pure H_2O
Group D	3 mL NaCl	7 mL E-pure H_2O

Step 5. Stir with your stir bar until everything in the tray is well mixed. Connect the 9V battery to the wire adapter. Start your timer now.

Step 6. **Record** the time that the blue color reaches each hash mark on the tray.

Step 7. At various times during the experiment, use the pH paper to test the pH along the length of the tray. Just dip one end of the paper into the liquid and compare the color to the scale on the container.



Results

Step 1. The voltage that will be applied is _____Volts

Step 2. The total length of the tray is _____cm

The electric field is _____Volts/cm

Step 3. Amount of Bromothymol blue indicator _____ mL

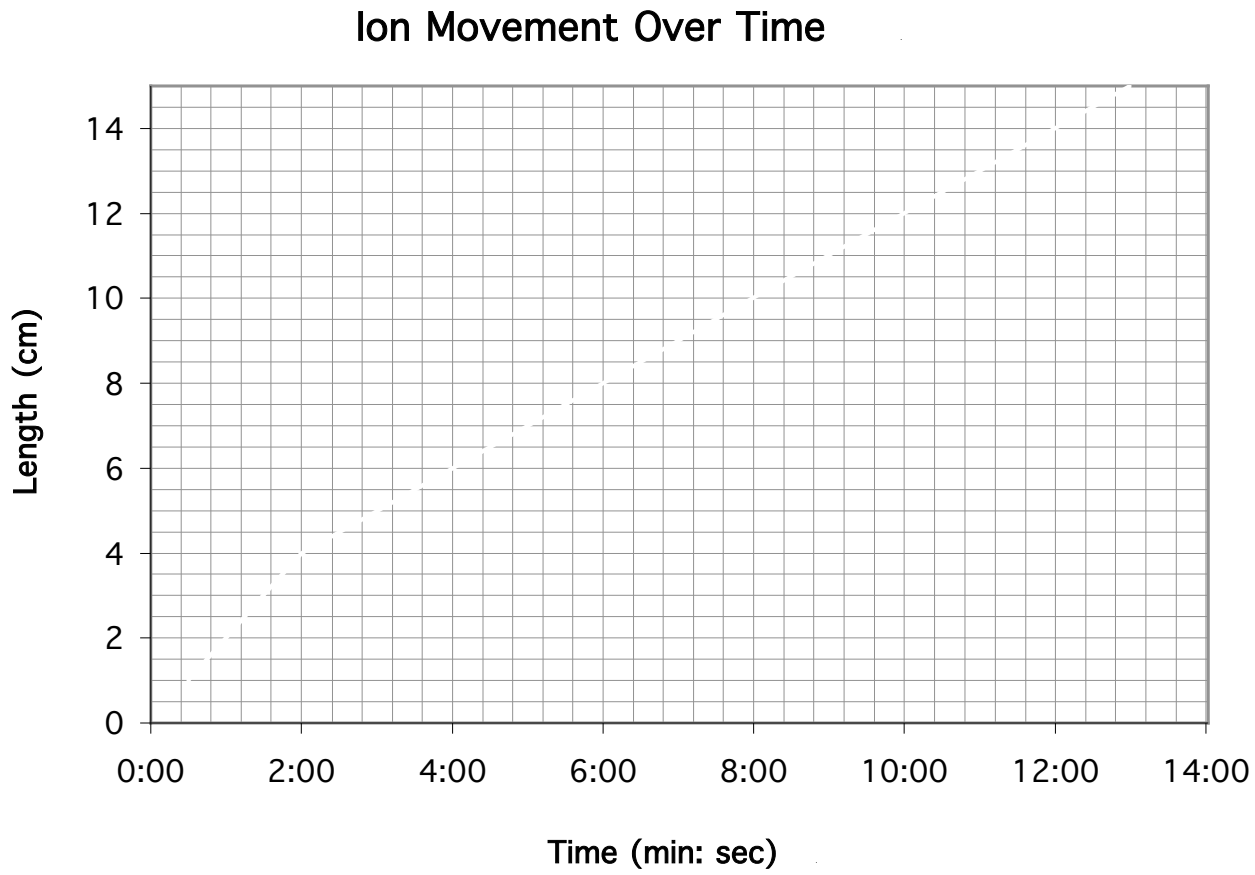
Step 4. Amount of NaCl solution _____ mL

Amount of E-pure H_2O _____ mL

Step 5. Total liquid in the electrophoresis tray_____ **mL**

Hash mark (cm)	Time (min: sec)	pH (optional)
1 cm		
2 cm		

We want to find out how fast the ions move in the electric field. This can be done by plotting the distance versus time in Excel in the following way.



Import the data into Excel and use the trendline function to draw a best fit line through your points. The rate of distance change or velocity can be found from the slope of the line.

Velocity, v, **=** _____ cm/sec

Now from the velocity, we can calculate the mobility of the ions (page 1).

Mobility, μ, **=** _____ $cm^2/V \cdot \text{sec}$

Mobility is important because we can compare this value to other data to tell if our charged molecule is larger or smaller or if it has more of a charge. A fragment of DNA (DeoxyriboNucleic Acid) has a mobility of

.008 $cm^2/V \cdot \sec$. What does this tell us about its size or charge compared to our NaCl ions?

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Your team has been assigned to one of 4 groups each with a different amount of NaCl. The percent of NaCl in the total fluid is shown in the table below. **Calculate your mobility using the equations and your data. Report this value (with units) on webCT.** After Friday, October 7th, look at the compiled results from other groups on webCT and write it in table form as is shown below.

	Group A	**Group B**	**Group C**	**Group D**
Bblue (mL)	10 mL	10 mL	10 mL	10 mL
NaCl (mL)	10 mL	7 mL	5 mL	3 mL
E-pure H_2O	0 mL	3 mL	5 mL	7 mL
Weight % NaCl	1.46 %	1.02 %	0.73 %	0.44 %
Mobility, μ				

What do you notice about how the mobility changes with percent of NaCl?

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Please clean up your experimental area being careful to avoid spills. Pour the used chemical into the designated waste containers. Chemicals need to be disposed of in an environmentally friendly way.

To deal with the pH data, refer to the extra handout that goes over how pH log scale works and what the acid / base pH numbers mean.

Electrophoresis Memo & Results (Due Oct. 12): Write up a 2-page (typed, double spaced, 12 point, Times Roman font, 1" margins) discussion of your results, observations and conclusions. Include answers to the questions asked within this lab report. Please include Excel plots of your data and of the analysis. Please address the following question in the final paragraph; now that you are more familiar with electrophoresis, what else could this technology be used for?