ELECTROLYSIS AND ELECTROPHORESIS

The electrode at which electrons enter the gel box from the power supply (along the black wire) is called the **cathode**, and is negative (-). The electrode at shich electrons leave the box and re-enter the power supply (along the red wire) is called the **anode** and carries a positive charge (+). The flow of electrons sets up a potential energy difference between the electrodes. This is known as **potential**, and is measured in **volts**. It establishes an electric field through which the ions in the gel box fluid migrate. The migration of ions in the fluid creates electrical **current** which is measured in milliamperes (**milliamps**).

The splitting of water using electricity is called **electrolysis**. Electrophoresis is a technique for separating and analyzing charged molecules. DNA, for example, is negatively charged due to the phosphate (PO_4^{3-}) backbone. The three main parts of any electrophoresis system are (1) a power supply, which is the source of negatively charged particles called electrons; (2) a plastic gel box or tank; and (3) a fluid of water and ions which partially fills the gel box. Recall that ions are atoms that have a positive or negative charge because they have lost or gained electrons. The migration of ions at different speeds is the basis of electrophoresis. During electrophoresis, the current splits the water into hydrogen ions (H^+) and hydroxyl ions (OH^-).

CAUTION: Although the gel box is designed so that when the box is opened, the area disconnected, any wet surface CAN become conductive. It is advisable NOT to touch any part of the apparatus (gel box, wires) while the power supply is on. This is especially important if the outside of the box is wet, or if your hands are wet.

Procedure:

1. Examine the power supply. Sketch and identify the following on your sketch:

on/off switch	switch between volts/milliamps
plugs for leads (2-4 sets)	Digital display
voltage/milliamp select control	switch to start power running

2. With the power OFF, connect the empty gel box to the power supply.
3. Turn the power supply ON, and select a potential of about 100V (volts). Record the current (measured in milliamps) generated by the empty gel box. **Record** your observations on whether current flows through air.
4. Turn the power supply OFF.
5. Open the gel box, and add enough distilled water so that it just covers the center platform. Close the gel box, and turn the power supply ON, with a potential of 100V, and record the level of current in the box. **Record** your observations on whether current flows through distilled water.
6. Turn the power supply OFF.
7. Add 1 ml of the sodium chloride solution to the distilled water in the box. Gently "slosh" the box to mix the salt and water. Turn the power supply ON. Set the voltage to 100V. Record the current. **Record** your observations on whether current flows through distilled water that contains ions (Na^+ and Cl^-).
8. Choose 3 additional voltages and record the current at each voltage. Then turn the power supply OFF. **Chart** the results and **graph** the current vs the potential for the 4 different levels.
9. When current is flowing, the chemical reactions at the cathode and anode indicate that differences in pH develop at either end of the box due to differing concentrations of hydrogen ions (H^+):

   At the cathode: $4\ \text{electrons}^- + 4\ H_2O \rightarrow 2\ H_2\ (\text{gas}) + 4\ OH^-$

At the anode: $4\ H_2O \rightarrow O_2$ (gas) + $4\ H^+$ + $2\ H_2O$ + 4 electons$^-$

9. continued: **<u>**Predict</u>** the relative pH (high or low) at either end of the gel box. **<u>Record</u>** your prediction. **<u>Summarize</u>** the relationship between potential and current. **<u>Draw</u>** a diagram of the gel box and **<u>label</u>** where gases are produced. **<u>When</u>** were these gases produced? What was the source of the gases?

10. To verify that a change in pH is occurring during electrophoresis, we will use an indicator dye called phenol red. CAREFULLY slosh the water and salt solution in the gel box to mix, then add enough phenol red to observe a deep pink color. Mix until the color is uniform. Phenol red is pink in a basic solution and yellow in an acid solution.
11. Turn the power supply ON. At 100V, record the current. Record the time it takes for color changes to take place. Record the color changes. **<u>Note</u>** what the color changes indicate about the solutions in the gel box. **<u>Discuss</u>** the results relative to your prediction in step 9.
12. Turn the power supply OFF.
13. Add 10 ml of buffer solution. Slosh well to mix. What color is the solution? Predict what will happen to the pH of the solution with the addition of the buffer.
14. Turn the power supply ON again and select 100 V. **<u>Record</u>** the current. **<u>Record</u>** the time for color changes, if any. **<u>What</u>** might the lack of color change tell us about the effect of buffer on pH?
15. Rinse the contents of the gel box down the sink, and rinse the gel box thoroughly with water. DO NOT DRY THE GEL BOX WITH PAPER TOWELS. Simply set it upside-down on paper towels at your lab station to dry.

ELECTROLYSIS AND ELECTROPHORESIS ACTIVITY SHEET

1. Sketch and label the power supply.

2. Does current flow through air? How do you know?

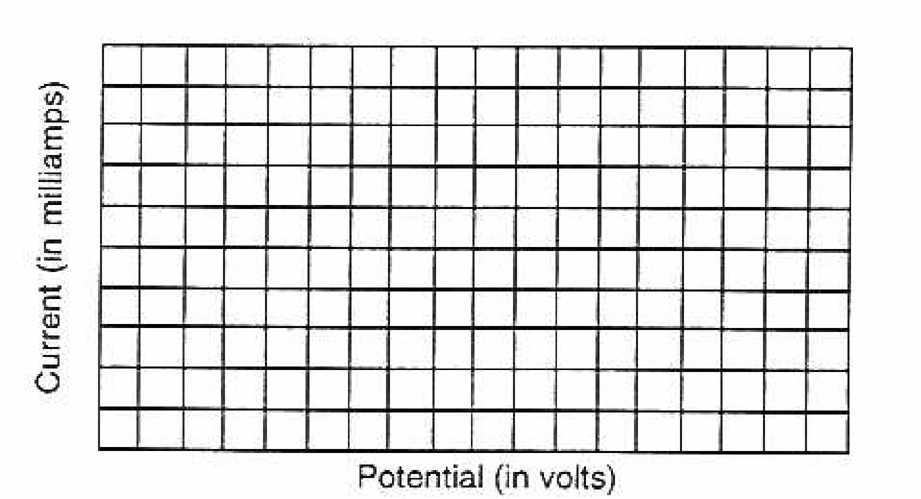
3. Does current run through distilled water? How do you know?

4. Does current run through distilled water with ions added? How do you know?

5. Record your results of steps 7 and 8 in the table below:

STEP	GEL BOX CONTENTS	POTENTIAL (volts)	Current (milliamps)	OTHER OBSERVATIONS (color changes, bubbles, etc.)
7	Distilled H_2O + NaCl	100 V		
8	Distilled H_2O + NaCl			
8	Distilled H_2O + NaCl			
8	Distilled H_2O + NaCl			

6. Use the graph below to plot the information in Steps 7 and 8:



7. Summarize in your own words the relationship between potential and current.

8. When and where were gases produced?

9. What was the source of the gases you observed?

10 What did the color change in phenol red indicate about the pH in the gel box when you had salt water in the box?

11. What effect did adding the buffer have on the initial color of phenol red and on the subsequent changing of the color?

12. What effect does the buffer have on the pH of a solution?

13. Why are salt and buffer added to the gel box for electrophoresis of DNA?

14. Does the liquid in the gel box become more acidic or basic at the anode? How can you tell?

ELECTROLYSIS AND ELECTROPHORESIS TEACHERS GUIDE

Time needed: one period, or half a period as a demonstration. (If you have clear gel boxes, place on the overhead and project for class to see changes)

Materials:

power supply	1M sodium chloride solution	10 ml graduated cylinders
gel box	distilled water	p1000 pipets and tips or eyedroppers
waste container (for tips)	phenol red dye solution	
10ml buffer (1XTAE or TBE)		

NOTE: measurements are approximate in this lab—precision is unimportant to results

Teaching Tips:

Be sure students "slosh" their gel boxes thoroughly (but carefully) in Step 10, or Step 11 won't be as dramatic.

If you have clear gel boxes, a sheet of white paper underneath will highlight the color changes.

Adding buffer will stabilize the pH, and at Step 14 you will not see the immediate color changes that occur at Step 11. But eventually changes in color DO develop if you let the box run for 5-10 minutes, because the capacity of the buffer is exceeded.

Decide whether you want students to use the Activity Sheet or not.