

# Electrophoresis Exploration

## Background

Electrophoresis is a technique developed in the 1930's for separating and analyzing charged molecules. The term electrophoresis means "to bring down with electricity".

There are three main parts to an electrophoresis system:

- ❖ Power supply-the source of negatively charged particles called electrons
- ❖ Gel box-a plastic box with electrodes
- ❖ Solution of water and ions-fills the box (ions are atoms which have lost or gained electrons)

Look inside the gel box, and you will find a metal strip at each end. These are called electrodes. 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 which electrons leave the box and re-enter the power supply (along the **red wire**) is called the **anode** and carries a **positive** charge (+). Electron flow 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 migrate. The migration of ions in the fluid creates electrical current which is measured in milliamperes (milliamps, or mA).

## Purpose

This laboratory will help you to explore the principles of electrophoresis, an important technique used in biotechnology labs, particularly in recombinant DNA technology work. Complete the Electrophoresis Record Sheet while performing the lab.

## CAUTION!!!

- Extremely high voltages run through the electrophoresis system. Make sure that you follow directions carefully. Since any wet surface CAN become conductive, do not 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.

- Study the gel box and its safety interlock lid. It is designed so that electrical contact must be disabled (power cords disconnected) in order to raise the lid.

## Procedure

*As you move through the following steps, observe what is happening inside the gel box!*

1. Set the gel box in front of you: the "front" is the side on which the company logos are displayed. Position the box near a power supply, but do not connect it yet.
2. Examine the power supply and on your record sheet, identify:
 

Power Switch (on/off)	Meter function switch (V/mA)
Voltage range switch (low/high)	digital meter
Voltage select knob	2 sets DC output terminals
Check fuse light	current overload light

Examine the gel box and on your record sheet, identify:

Anode          cathode          safety interlock lid

3. Remove the red-striped gel tray from the gel box. You will not need it for this exercise. With the power supply OFF and the gel box safety lid down, connect the empty gel box to the power supply with the power cords ("red to red, black to black").
4. Set the voltage range switch to LOW, turn the power supply ON, and select a potential of approximately 100 V. Now turn the meter function switch to milliamps and note the current generated in the empty gel box. *[If two boxes are connected to one power supply, divide the current displayed in half to get the milliamps per box. Do this throughout the lab.]* Write this number on your record sheet table.
5. Turn the power supply OFF and disconnect the power cords.
6. Lift the safety lid, and add about 125 mls of distilled water. Lower the lid, reconnect the power cords, and turn the power supply ON. With a potential of about 100 V, record the level of current (mA) in the box on the record sheet.
7. Turn the power supply OFF and disconnect the power cords.
8. Lift the lid and add the sodium chloride (NaCl) solution to the distilled water in the box. Carefully mix the contents with your stirring device.

9. Lower the lid, reconnect the power cords, and turn the power supply ON. At approximately 100 V, record the current. *[The current measurement will keep changing- take your reading early.]*
10. Select approximately 25 V and record the current. Move the voltage range switch to "HIGH" and select 250 V. Record the current again. *[After you have recorded your current, put the voltage range switch on "low"- constant higher currents can burn out the power supply fuses.]* Choose two additional voltages between 25 V and 250 V, and record the resulting current values.
11. Turn the power supply OFF and disconnect the power cords.

Did you spot the hydroxide (OH<sup>-</sup>) ions? The hydrogen ions (H<sup>+</sup>)?

Explanation!

When current is flowing, the chemical reactions are occurring at the cathode and at the anode.

At the cathode:  $4 \text{ electrons}^- + 4 \text{ H}_2\text{O} \Rightarrow 2 \text{ H}_2 \text{ (gas)} + 4 \text{ OH}^-$

At the anode:  $4 \text{ H}_2\text{O} \Rightarrow \text{O}_2 \text{ (gas)} + 4 \text{ H}^+ + 2 \text{ H}_2\text{O} + 4 \text{ electrons}^-$

Are pH differences developing in the gel box solution? Let's find out!

12. Lift the lid and carefully stir to mix the gel box solution. Record the pH at each electrode: Place the pH strip at the electrode for 1-2 seconds, pull out, and compare this to the pH chart. All of the colors will not exactly match, but match as close as you can.
13. Add the phenol red indicator dye to the salt water in the gel box. The dye will tend to stay in the tube, so grasp the tube by its open hinged top and swirl it in the liquid. Stir until you reach a uniform color.
14. Lower the lid, reconnect the power cords, and turn the power supply ON. At 100 V, record the current. Notice any color changes that develop within 3-5 minutes observation.
15. Turn the power supply OFF, and disconnect the power cords. Raise the lid and record the pH at both ends of the gel box, without jostling the box or mixing the solution.
16. Gently stir the gel box solution until it goes back to one color. Add the 50X TAE buffer. Stir to mix. Record the pH at both ends of the gel box.
17. Lower the lid, reconnect the power cords, turn on the power supply ON, and select 100 V. Observe any color changes.

THE NEXT TIME YOU CONDUCT ELECTROPHORESIS, YOU WILL LOAD AND SEPARATE DYES OR DNA FRAGMENTS ON AN AGAROSE GEL!

**Upon completion of this lab**

- Lift the plastic buffer tray out of the apparatus and pour the fluid down the sink. BE CAREFUL NOT TO GET THE BOX'S CONDUCTING WIRES WET! These are located near the electrodes, outside the box in hollow tubes. Rinse the inside of the box with clean water and pat dry.
- Leave your equipment as you found it.
- Check that your work station is clean and dry.
- Wash your hands

## Electrophoresis Exploration Student Record Sheet

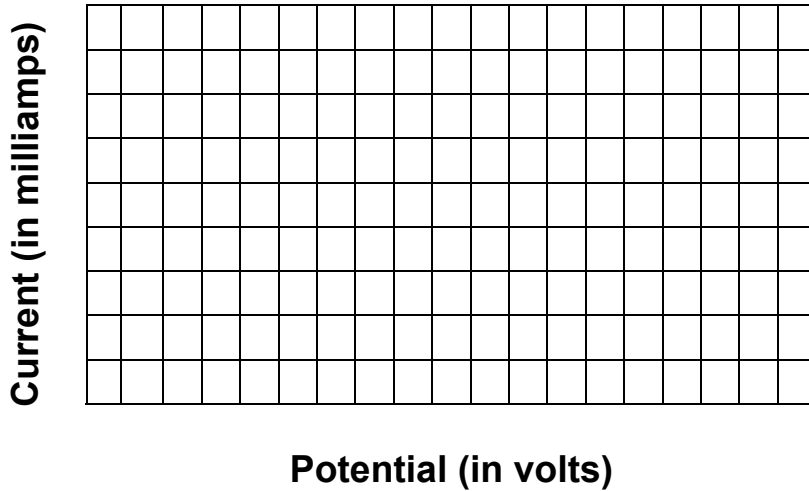
*As you move the procedure, record your observations here. Remember, divide current in half to find mA per box if two gel boxes are hooked up to one power supply!*

Step	Gel Box Contents	Potential (volts)	Current (milliamps)	Other Observations (color, bubbles, pH, etc.)
4	Empty gel box (only air inside)	100 V		
6	Distilled water	100 V		
9	Distilled H <sub>2</sub> O + NaCl	100 V		
10	Distilled H <sub>2</sub> O + NaCl	25 V		
10	Distilled H <sub>2</sub> O + NaCl	250 V		
10	Distilled H <sub>2</sub> O + NaCl	(your choice)		
10	Distilled H <sub>2</sub> O + NaCl	(your choice)		
14	Distilled H <sub>2</sub> O + NaCl + phenol red	100 V		
17	Distilled H <sub>2</sub> O + NaCl + phenol red + buffer	100 V		

Summarize in your own words the relationship between voltage and current.

**POSTLAB:**

1. Use your data from steps 9 and 10 ONLY to complete the graph!



2. Where and when were gases produced?
3. What was the source of the gases you observed?
4. What colors did the phenol red dye turn when you added it to the salt water and let current flow for 3-5 minutes?
5. What effect did this color change indicate about the pH in the gel box when you had salt water and current flow in the box?
6. After you added buffer, did you see this color change?
7. What effect, then, must buffer have on the pH of a solution?
8. Explain the purpose of adding (a) salt and (b) buffer to the gel box.

# Supplies

DC power supply

Gel box

pH strips

pH charts

1 ml of 1 M NaCl

125 mls distilled water

2.5 mls 50X TAE buffer

200  $\mu$ l phenol red

spoon

125 ml erlenmeyer flask

50-cc tube