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)

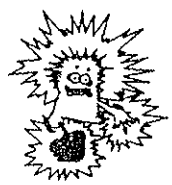
Look inside the gel box, and you will find a metal strip at each end. These are called electrodes. The electrode at which electrons enter the gel box from the power supply (along the **black wire**) is called the **cathode**, and is **negative** (-). The electrode at which electrons leave the box and 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.



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

PROCEDURE

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 you 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 100V (these power supplies will never go to exactly 100V). ***BEFORE switching to mAMP to determine the current running through the gel box, decide whether or not YOU THINK electricity will be conducted through the air in the box.*** Now turn the meter function switch to milliamps and note the current generated in the empty gel box *to confirm or disprove your hypothesis.* [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 (fill to base of the 'neck' of the Erlenmeyer flask). Lower the lid, reconnect the power cords, and turn the power supply ON. Make sure your voltage has not changed. Again, ***BEFORE switching to mAMP to determine the current running through the gel box, decide whether or not YOU THINK electricity will be conducted through the distilled water in the box.*** Now record the current (mAmp) on the record sheet.

7. Turn the power supply OFF and disconnect the power cords.

8. Lift the lid and add 1.0 ml (1000 ul) of a 1 molar (1M) solution of sodium chloride (NaCl) 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. Do not change the voltage. ***BEFORE switching to mAMP to determine the current running through the gel box, decide whether or not YOU THINK electricity will be conducted through the salt solution in the box.*** *Observe what is happening inside the gel box.* Write your observations, including the mAMP current, on your observation sheet.

10. Now you will see if you can change the current by changing the voltage. Select approximately 25V and record the current. Move the voltage range switch to HIGH and select 200V. Record the current again. Take your reading early as the current will continue to drift upwards. Choose two additional voltages between 25V and 200V and record the resulting current values.

11. Turn the power supply OFF and disconnect the power cords.

You have now generated a current with the salt solution and have seen bubbles at both the anode and cathode. What are these bubbles and what else is happening in the gel box?

12. Lift the lid and stir to thoroughly mix the gel box solution. Reconnect the power cords, set the voltage to approximately 100V, and run for 2-3 minutes. Turn the power supply OFF, disconnect the cords, and record the pH at each electrode. Dip a pH strip into the salt solution at each end of the gel box (at each electrode) and compare this to a pH chart. Match as closely as you can.

    Low pH = high H+ (hydrogen ion) concentration
    High pH = high OH- (hydroxyl ion) concentration

Did you spot the hydroxide (OH-) ions? The hydrogen ions (H+)?

Explanation!

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

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

At the anode: $4\ H_2O \Rightarrow O_2\ (\text{gas}) + 4\ H^+ + 2\ H_2O + 4\ \text{electrons}^-$

13. Now you will use another tool to allow you to see changes that are taking place in the gel box. Add 0.20 ml (200 µl) of the phenol red pH indicator to the salt solution in the gel box. Stir carefully until you reach a uniform color.

14. Lower the lid; reconnect the power cords, and turn the power supply ON. Set the voltage close to 100V. Record the current (mAmp). Notice any color changes that develop while the current is on. *Write down your observations and relate them back to the equations of what is happening on a molecular level at the cathode and anode!!*

Upon completion of this lab:

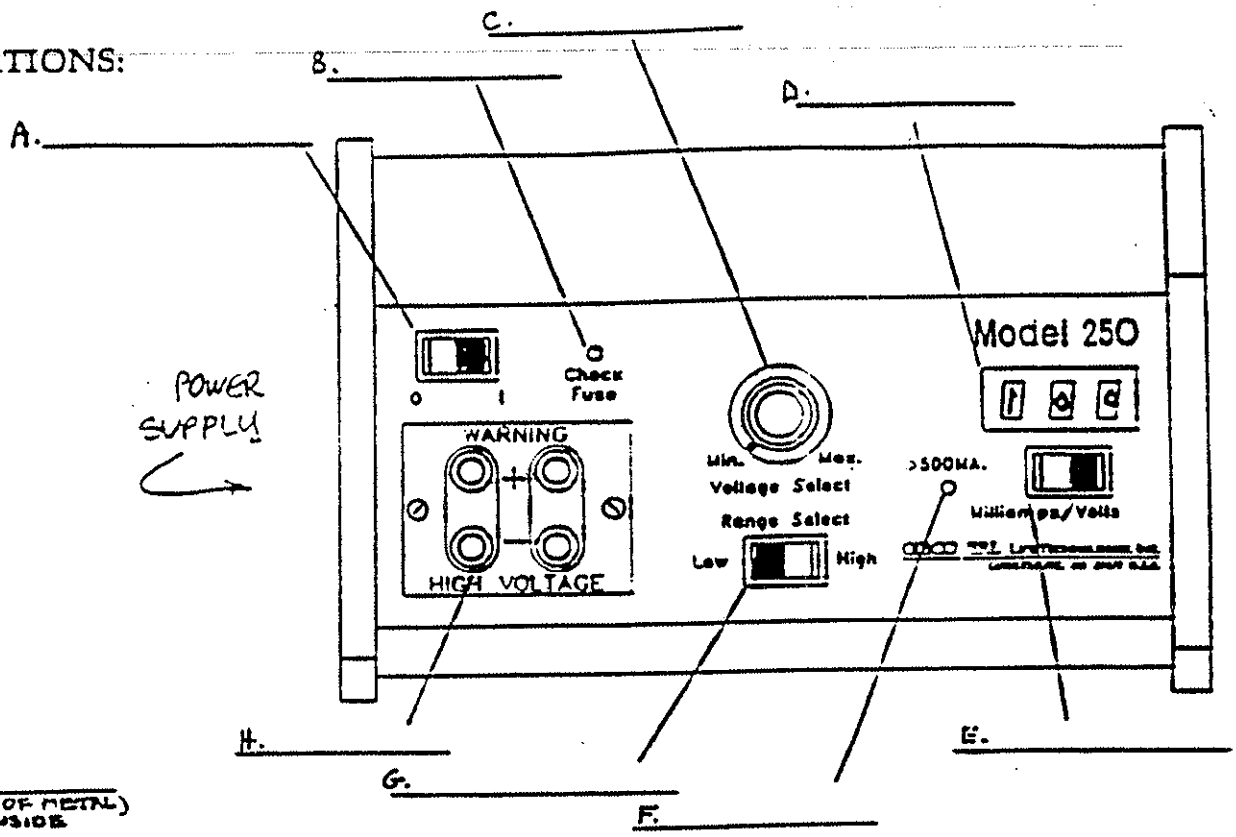
- *Life the plastic buffer tray out of the apparatus and pour the fluid down the sink. Be careful NOT to get the conducting wires wet!! These are located near the electrodes, outside the box in hollow tubes.*
- *Rinse the inside of the box with clean water and pat dry.*
- *Leave your equipment as you found it.*
- *Check that your work station is clean and dry.*
- *Wash your hands.*

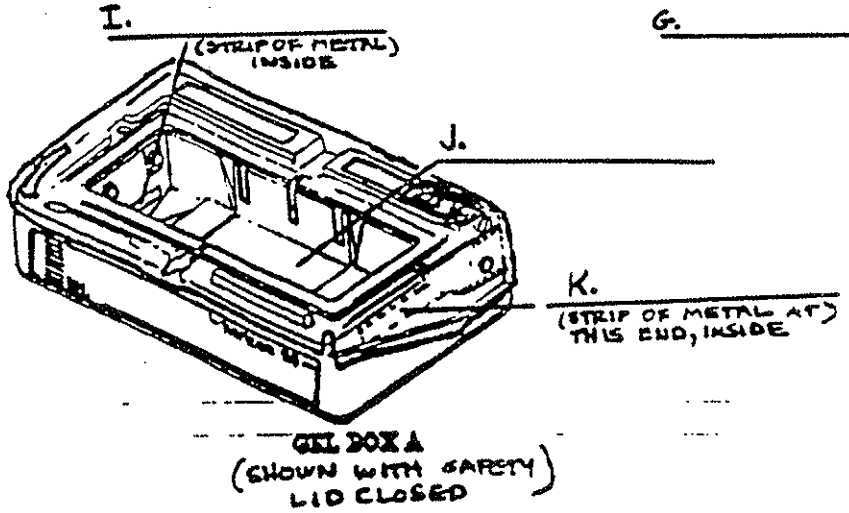
Name _____

Date _____ Period _____

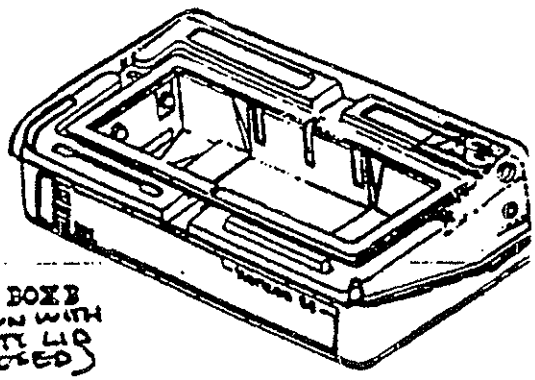
Electrophoresis Exploration
Student Record Sheet

OBSERVATIONS:





GEL BOX A (SHOWN WITH SAFETY LID CLOSED)



GEL BOX B (SHOWN WITH SAFETY LID CLOSED)

1. Label the above illustration of the power supply and gel boxes.
2. Draw lines (in red or black as necessary) representing electrical leads to show how the gel boxes should be connected to the power supply.

3. How did you know where to connect the power cords? Explain.

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Electrophoresis Exploration Student Record Sheet

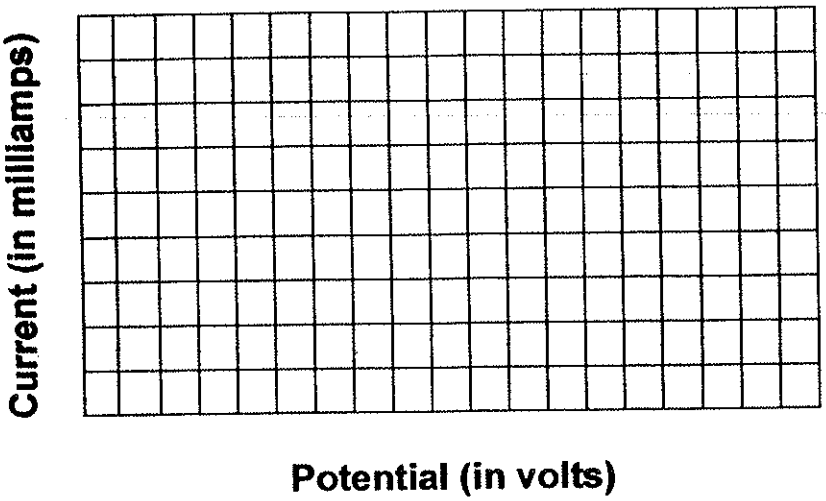
As you move the procedure, record your observations here. Remember, divide current in half to find mA per box if two gel boxes are hooked up to one power supply!

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_2O + NaCl	100 V		
10	Distilled H_2O + NaCl	25 V		
10	Distilled H_2O + NaCl	200 V		
10	Distilled H_2O + NaCl	(your choice)		
10	Distilled H_2O + NaCl	(your choice)		
14	Distilled H_2O + NaCl + phenol red	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?