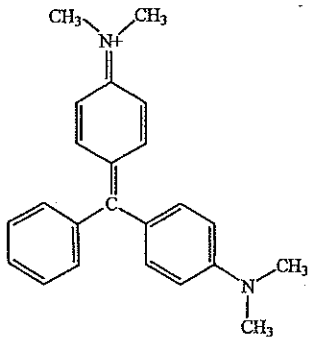
Name: _____

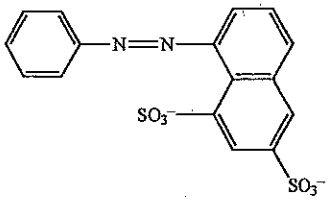
Pre-Lab Activity

The five structural formulas—A–E—on the right are the formulas for each of the five dyes used in this activity. The names of each dye and their approximate molecular weights (MW) are listed in the chart below. Look carefully at each structural formula and find the charge that each dye molecule possesses. Finish the chart by filling in the Charge column.

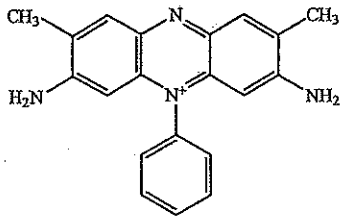
	Dye Names	MW	Charge
A	Malachite Green	(329)	
B	Orange G	(452)	
C	Safranin O	(315)	
D	Alizarin Red S	(360)	
E	m-Cresol Purple	(404)	



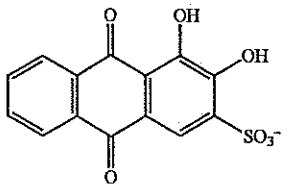
A



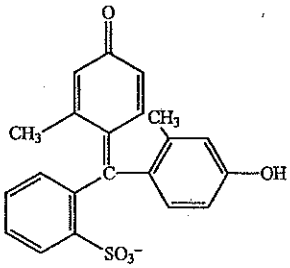
B



C



D



E

Based on the information in the chart and the structural formulas, answer these questions:

1. Predict the direction of migration for each of the known dye samples.

2. Which dye do you predict will move through the gel the fastest? The slowest?

3. Write a brief function for each of the parts used in gel electrophoresis:
   a. Agarose gel

   b. Electrophoresis buffer

   c. Wells in the gel

   d. Electric current

4. List one important safety precaution that must be followed when performing any type of gel electrophoresis.

5. Use Figure 1 as an example and construct a data table, with five labeled columns and six rows on a separate sheet of paper.

Dye Name	Dye Well #	Migration Distance (mm)	Migration Direction (+/–)	Dye Molecules "Speed" Rankings

Figure 1.

Materials

Colored pencils (optional)

Electrophoresis buffer, 1X, about 250 mL (depending on chamber type)

Electrophoresis dye samples, 1 set

Electrophoresis chamber and connector cords

Pipets, needle tip, disposable

Power supply

Ruler, metric, 15 cm

Safety Precautions

Be sure all connecting wires, terminals and work surfaces are dry before using the electrophoresis units. Electrical Hazard: Treat these units like any other electrical source—very carefully! Do not try to open the lid of the unit while the power is on. Exercise extreme caution in handling the dyes; they will all readily stain clothing and skin. Wear chemical splash goggles, chemical-resistant gloves and apron. Wash hands thoroughly with soap and water before leaving the laboratory.

Procedure

1. Depending on the type of electrophoresis units available, assemble the unit according to the teacher's instructions with the end dams in place.
2. Place the electrophoresis unit in a horizontal position on a level table or countertop.
3. Place the "Gel Drawing Worksheet" on the counter horizontally next to or below the electrophoresis unit. The small rectangles on the paper correspond to the wells in the gel. Number the wells on the paper from #1 at the top to #6 at the bottom (see Figure 2).
4. Gently slide a gel from a zipper-lock bag into the casting tray and between the end dams. Pour enough electrophoresis buffer (1X) into the unit to just cover the entire gel surface. Remove the end dams.

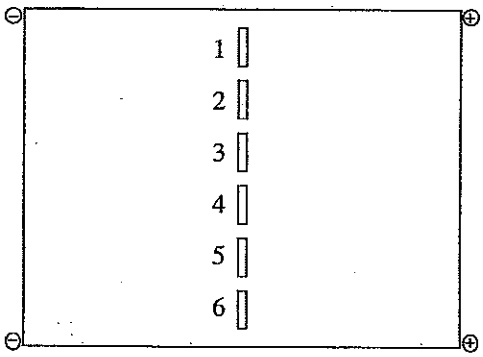


Figure 2.

5. Withdraw 10 µL of dye from each microcentrifuge tube by filling only the needle tip of the pipet. *Note:* Fill the tip by squeezing the pipet just above the tip, not the bulb. Use a clean pipet for each dye sample to avoid contaminating the pure samples. Dispense a sample of each dye into a different well in the gel. Record the name and well number of each dye in the data table.
6. Place the lid on the chamber and connect it to the power supply according to teacher instructions. Allow electrophoresis to proceed for 20–25 minutes at 70 V before turning off the power.
7. When the power is off, remove the cover and with the help of a ruler, carefully remove the gel from the chamber and put it on a piece of paper towel.
8. Measure the distance each dye migrated in millimeters (mm). This distance should be measured from the side of the well closest to the direction the dye traveled to the leading edge of each colored band. Record this number in the data table as Migration Distance (mm).
9. Use the Gel Drawing Worksheet to make an accurate drawing of the bands in the gel. Colored pencils may be helpful in identifying each band in the drawing.
10. Record the charge for each group of molecules by noting to which pole (+/–) each dye sample was traveling when the electricity was shut off.
11. Use the recorded Migration Distances to complete the last column of the data table by ranking the five known dye samples from fastest (#1) to slowest (#5).

Disposal

Consult your instructor for appropriate disposal procedures.

Post-Lab Questions *(Answers may be written on the same sheet of paper as the Data Table or in the spaces provided below.)*

1. Which dye(s) traveled the farthest? (Use data to support your answer.)

2. How did the results differ from your predictions regarding migration direction, which dye(s) would travel the fastest, and which dye(s) would travel the slowest? Be specific.

3. Why didn't all the dyes travel the same distance or the same direction from the wells? Explain your answer.

4. Why did the two positively-charged dyes travel almost the same distance? (Use a mathematical calculation to support your answer.)

5. Of the three negatively-charged dyes, why do you think the "heaviest" was the second fastest dye? (*Hint:* Look closely at the structural formula.)

6. List the dyes that were used in your "unknown" dye sample.

7. Write one reasonable explanation to support the answer written for Question #6.

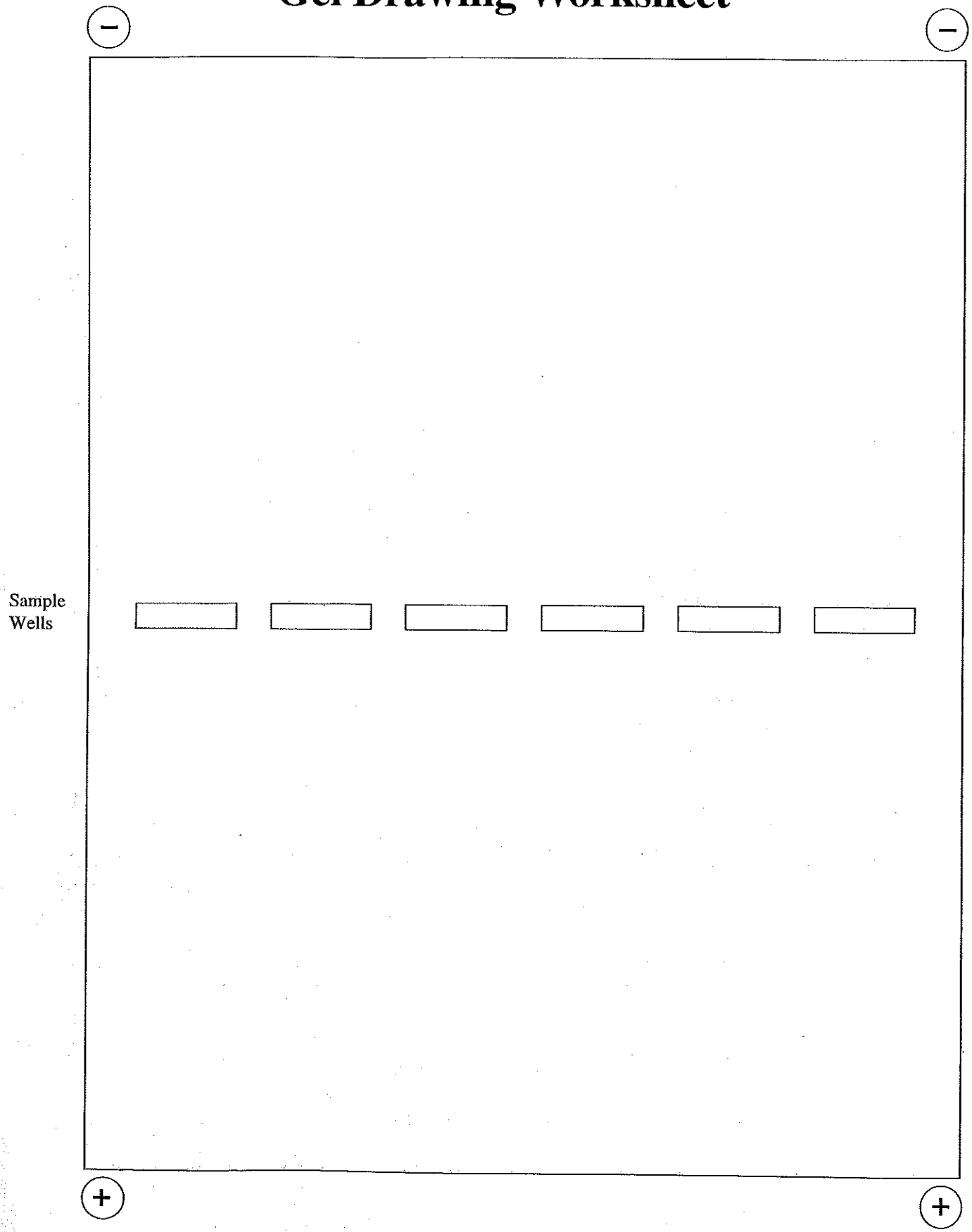
8. Explain the basis for the "speed" rankings given to the known dye samples in the Data Table.

9. List three errors that could affect the outcome of any gel electrophoresis procedure.

10. Briefly summarize how gel electrophoresis is used to separate molecules.

Name: _____

Gel Drawing Worksheet



IN10666