Basic Electrophoresis

Migration of Ions in an Electric Field

Introduction

One of the most poorly understood concepts in electrochemistry has to do with the flow of electricity in an electrochemical cell. Many students assume that the flow of electricity through the solution(s) is due to the flow of electrons. In all types of electrochemical cells, electrons carry the current through an external wire or conductor, but ions carry the current through the solution. Anions move toward the anode and cations move toward the cathode to prevent charge buildup at the electrodes. A central feature of electrochemical cells, migration of ions in an electric field is also the basic principle in electrophoresis.

Concepts

- Electrophoresis
- Migration of ions
- Positive and negative electrodes

Materials

Agarose, electrophoresis-grade, 0.4 g

Buffer, pH 10, 250 mL

Methylene blue, 0.1% in pH 10 buffer, 50 mL

Phenol red, 0.1% in pH 10 buffer, 50 mL

Sucrose, 50 g

Beakers, 100-mL, 3

Electrophoresis apparatus, mini-gel (includes casting tray, rubber gaskets, and combs)

Hot plate

Micropipets, 10-µL, or needle-tip disposable pipet, 2

Power source for electrophoresis apparatus (includes grounded wire cords)

Stirring rods, 2

Thermometer

Safety Precautions

Do not operate the power source with wet hands or in a wet area. Make sure the power supply is off before connecting the leads to the electrophoresis apparatus. Turn off the power supply before disconnecting the leads and removing the cover at the end of the demonstration. Wear chemical splash goggles, chemical-resistant gloves, and chemical-resistant apron. Please review current Material Safety Data Sheets for additional safety, handling, and disposal information.

Procedure

1. Place the rubber gaskets on the ends of the mini-gel casting tray and insert a 6-well comb in the *middle* of the tray.
2. Fill the electrophoresis chamber with about 100 mL of pH 10 buffer solution.
3. Prepare a 0.8% agarose gel by dissolving 0.4 g of agarose in 50 mL of pH 10 buffer solution at about 80 °C. Cool the agarose gel solution to about 50 °C, and then pour the solution into the gel casting tray.
4. After the gel has solidified (about 20 minutes), remove the well comb and gaskets from the end of the tray and submerge the gel (on the tray) in the buffer in the electrophoresis chamber.
5. Prepare 0.1% solutions of methylene blue and phenol red: Add 0.05 g of the appropriate dye to 50 mL of pH 10 buffer solution in a 100-mL beaker. Stir to dissolve and then add 25 g of sucrose to each dye solution. Stir well to dissolve.
6. Using a separate micropipet tip for each dye solution, fill well #2 with 10 µL of the methylene blue gel-loading solution, well #4 with 10 µL of a 1:1 mixture of methylene blue and phenol red, and well #6 with 10 µL of phenol red gel-loading solution into well #6.
7. Place the safety cover on the electrophoresis chamber. Make sure the power is off before connecting the power leads to the power source. Select the desired voltage (70 V) and turn on the power source. Run the electrophoresis 15–20 minutes or until the dyes have migrated to about 1 cm from the end of the gel in either direction.
8. Observe the direction of ion migration for each dye.

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Disposal

Please consult your current *Flinn Scientific Catalog/Reference Manual* for general guidelines and specific procedures governing the disposal of laboratory waste. The gel may be disposed of in the trash according to Flinn Suggested Disposal Method #26a. The electrophoresis buffer solution may be reused several times before being disposed of down the drain with excess water according to Flinn Suggested Disposal Method #26b.

Tips

- The pH 10 buffer solution may be conveniently prepared using a Chemvelopes® buffer envelope (Catalog No. B0120) or buffer capsules (Catalog No. B0109). The agarose gel may be prepared several days before use and stored in a plastic bag with a small amount of buffer in the refrigerator.
- Sucrose is added to each dye solution (step 5) to increase its density and obtain a "gel-loading" dye solution. The dense gel-loading solution will sink when added to the buffer solution and remain in the well. (Glycerine may also be used to prepare a gel-loading solution—use 5 mL of glycerine and 45 mL of water.)
- Wells are typically numbered reading from left to right when the wells are at the top of the gel.
- Electrophoresis gels containing DNA fragments can typically be stored in plastic bags with a small amount of buffer solution. The dye-containing gel obtained in this demonstration, however, cannot be stored, because the low-molecular weight dyes slowly diffuse through the gel. The used gel could be soaked in buffer solution to remove the dyes and then recycled for use in another demonstration.
- Many organic dyes and acid–base indicators may be used. Try bromphenol blue, crystal violet, malachite green, etc.

Discussion

Methylene blue is an ionic compound containing a colored, positively charged organic dye molecule and a chloride counterion. Phenol red is a colored organic dye that acts as an acid–base indicator. At pH 10, phenol red carries a double negative charge. Methylene blue (turquoise band) migrates toward the negative electrode during electrophoresis, while phenol red (bright pink band) migrates toward the positive electrode. The mixture of dyes (well #4), which is purple to start, separates into two bands, turquoise and bright pink, which migrate to opposite electrodes during electrophoresis.

Connecting to the National Standards

This laboratory activity relates to the following National Science Education Standards (1996):

Unifying Concepts and Processes: Grades K–12
Evidence, models, and explanation
Content Standards: Grades 9–12
Content Standard B: Physical Science, structure and properties of matter
Content Standard C: Life Science

Reference

This activity was adapted from *Electrochemistry*, Vol. 17 in the *Flinn ChemTopic™ Labs* series; Cesa, I., Editor; Flinn Scientific: Batavia IL (2005).

Materials for *Basic Electrophoresis* are available from Flinn Scientific, Inc.

Catalog No.	Description
A0132	Agarose, Electrophoresis Grade
B0120	Buffer Envelopes, pH 10
M0072	Methylene Blue
P0097	Phenol Red
FB1714	Dual Electrophoresis Apparatus

Consult your *Flinn Scientific Catalog/Reference Manual* for current prices.