Experiment No. 1
Agarose Electrophoresis of Food Dyes

Introduction

Molecules can be separated by chromatography based on the polarity (hydrophobic or hydrophilic) of the molecule and the polarity of the solvent. Some molecules actually have a net charge or can become charged in a solvent at a specific pH. Molecules with a net charge can be separated in an electric field by a procedure known as **electrophoresis**. Electrophoresis is a common laboratory technique used for the separation of macromolecules such as proteins, DNA, and RNA.

The mobility of a molecule during electrophoresis depends on the net charge of the molecule, the intensity of the electrical field, and how freely the molecule can pass through the pores (sieving action) within the gel matrix. Electrophoresis is nearly always carried out in a gel. Gels serve as molecular sieves that assist the separation of molecules. Molecules with a net positive charge (cationic) will migrate to the negative (black) electrode while molecules with a net negative charge will migrate to the positive (red) electrode. If the voltage drop across the gel is increased (electrical field is strengthened) the molecules will migrate more quickly.

Experiment

Commercial Food dyes will be provided to you and an unknown sample. You are to determine the components of the unknown sample from the electrophoresis pattern obtained.

The experiment will be conducted at two pH values to evaluate the dye characteristics.

Procedure

Preparation of gel

Weigh out 0.50 grams of agarose and add to a 250-mL Erlenmeyer flask (conical flask) containing 25 mL of your assigned buffer. Place the flask containing the undissolved agarose on a hot plate and swirl until the agarose is completely dissolved. Your solution should be clear. Pour the solution into the gel-bed while it is still molten (it will solidify when cooled to about 40°C). Pour the gel to a depth of 3 to 5 mm.

While the solution is still molten, place the comb in the **middle slot** which is used to form wells for loading samples once the gel has set. The comb used in this experiment produces 8 wells.

Leave the gel at room temperature for at least 20 minutes to fully gel.
During this time set up the electrophoresis unit. Obtain additional buffer, of the same pH, that will be placed in the reservoir of the electrophoresis device.

Loading the gel

After the gel has cooled, remove the comb from the gel by pulling the comb steadily and slowly straight up from the horizontal gel. Pour enough of the reservoir buffer into the chamber to just cover the gel. There has to be sufficient reservoir buffer that the gel is actually submerged and the sample wells are full of buffer.

- Use a clean micropipette tip for each dye.
- Using micropipettes load 15µl of each of the 4 dyes into 4 separate wells formed within the gel. Do not puncture the bottom of the well with your micropipette and inject sample under the gel.
- Load 15 µl of the unknown mixture into another well.

Running the gel

1. Place the plastic safety cover over the electrophoresis apparatus. Attach the electrode wires from the power supply to the electrodes of the reservoir.
2. Turn on the power supply and set the voltage to 150 volts.
3. Allow the electrophoresis to proceed for 15-20 minutes.
4. Shut the power supply off, remove the electrode wires and inspect the gel without removing it. Decide if you want to let it run longer or not. If not, then remove the gel and rinse it briefly with water.
5. Examine the gel on a white-light box if required.

Results

1. Study the gel pattern and sketch it. Label the diagram clearly.
2. Determine the relative movement (R_f) of the bands by determining the ratio of the distance of the centre of the band to the total length of the gel.
3. Based on the information about the structure of the known dyes, plot a graph of the R_f value vs the charge/mass ratio. From this information determine the charge/mass ratio of the unknown dye.