Advanced Chemistry: Isolation of Chlorophyll using TLC Name:

Procedure:

I. Extraction of Natural Product

1. Set up a hot water bath using a 400 mL beaker.
2. Add approximately 1 g of spinach leaves to a small reaction vial. Avoid excess water from the packaging.
3. Add 1 to 1.5 mL of acetone to the vial. Using a toothpick, push the spinach down into the acetone, making sure that all the spinach is immersed in the acetone. Seal the vial with a Teflon cap.
4. Place in reaction vial into the hot water bath for 10 minutes. After 10 minutes, remove the vial and set on the lab table, allowing it to cool.
5. Place the reaction vial into a cold water bath for several minutes.
6. Using a Pasteur pipet, carefully remove as much of the green acetone solution as possible and place it in a second, clean reaction vial.

II. Isolation of Natural Products

7. Set up a TLC develop tank by adding 1 mL of acetone and 4 mL of hexane. Be sure to follow your instructor's directions as to setting up the tank and allow proper time for saturation of air in the chamber.
8. Obtain a TLC plate (2" x ½") and very lightly draw an origin line about 1 cm from the bottom of the plate.
9. Using an open-end capillary tube, remove a small aliquot of green acetone solution. Carefully spot the TLC plate on the origin line. The spot shouldn't exceed 2-3 mm in diameter. Although, spotting along the line is appropriate as long as the height of the line doesn't exceed 2-3 mm.
10. Repeat spotting the plate until a dark green spot is evident. You can lightly blow on the spot between touches to evaporate the acetone.
11. Place the TLC plate in the development tank making sure the spot does not come in contact with the solvent. Be sure to place the watch glass or plastic warp on top the developing tank.
12. Observe the solvent as it travels up the TLC plate, noticing that the solutes will begin to resolve at different locations. When the solvent almost reaches the top, remove the plate and make a light mark on the edge of the plate where the solvent traveled. Allow the plate to dry.
13. Record the colors and distances traveled from the origin for any distinct spot.

Analysis:

1. Using your distances for each spot and the solvent distance, calculate the R_f values for each solute.
2. Construct a data table showing the colors, distances and R_f values for each spot.
3. Using class data and a agreed upon spot, calculate relative Rf values for each spot.

Questions:

1. In the TLC purification, what is functioning as the stationary phase? The mobile phase?
2. Is the acetone/hexane solution used in the TLC development polar or nonpolar?
3. The solute particles which traveled further up the plate (closer to solvent) would be more polar or nonpolar? Explain why
4. By changing the TLC solvent to have a higher proportion of acetone, what would happen to the R_F values?
5. What is the main advantage of TLC over other separation techniques? Are there any disadvantages to the technique?
6. What would you expect to find if you used the leaf from a tree instead of spinach in this experiment?