Chemistry 2 Lab 3: Chromatographic Separation Methods

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Purpose

The purpose of this lab is to examine the solubility of similar compound trends and how methods based on this solubility can be used to separate components from more complex mixtures. To do this, the use of chromatography will aid in the analysis.

Materials

- Cyclohexane
- Pen (to record data)
- Paper (to record data)
- Calculator (optional)
- Turmeric sample (that has been extracted in organic solvent)
- Toothpicks
- Sep-pak
- Water
- Plates (porcelain spot plate)
- Fine capillary tube (TLC applicator)
- Glass jar
- Graduated cylinder
- Pencil
- Grape soda
- Sep-pak cartridges

- 600mL beaker
- Stearic acid
- Sodium chloride
- Plastic disposable transfer pipette
- Pipette
- 5% methanol (CH_3OH)
- Dichloromethane (CH_2Cl_2)
- 70.0% IPA solution
- Test tube rack
- Test tubes x15
- 5% IPA solution
- 20% IPA solution
- Cuvette x3
- Distilled water
- Spectrophotometer
- 0.1M HCl
- Graduated cylinder x2
- Computer

Procedure

1. Qualitative Examination of Solubilities
    a. Get a 600mL beaker (as a “waste bin”)

b. Gather stearic acid, sodium chloride, cyclohexane, water, a porcelain spot plate, a plastic transfer pipette, and toothpicks
c. Use a porcelain spot plate to add the smallest amount possible of solid stearic acid in 2 adjacent wells
d. To the 1st well of stearic acid, add 25 drops of water, and to the 2nd well, add 25 drops of cyclohexane. Mix with toothpicks and record observations
e. In 2 adjacent wells, add the smallest amount of sodium chloride. In the 1st well, add 25 drops of water, and to the 2nd, add 25 drops of cyclohexane. Mix with toothpicks and record observations
f. Transfer a small amount of stearic acid to 1 of the wells on the plate. Add 30 drops of cyclohexane, mix with toothpick, and add 25 drops of water. Collect the mixture in a plastic disposable transfer pipette and invert it so that the liquids are in the bulb of it
g. Shake the pipette to mix them and record what the sample looks like
h. Transfer a small amount of NaOH to one of the wells of the plate. Add 30 drops of cyclohexane, mix with toothpick, and then add 25 drops of water. Collect the mixture in a plastic disposable transfer pipette, invert it so that the bulb carries the liquid, and shake to mix. Record observations
i. Using the pipette of NaOH solution with the liquid in the bulb, curve the tip of the pipette into a well containing 0.1M HCl and add 25 drops of the acid solution to the pipette, mix with the existing solution, shake the pipette, and record the observations
j. Transfer a small amount of sodium chloride to 1 of the wells on the plate. Add 25 drops of water and mix with toothpick, then add 30 drops of cyclohexane. Collect the mixture in a plastic disposable transfer pipette, invert it so the liquid is in the bulb, and shake it to mix. Record observations

k. Transfer a small amount of sodium chloride to 1 of the wells on the plate. Add 0.1M NaOH and mix with toothpick, then add 30 drops cyclohexane. Collect the mixture in a plastic disposable transfer pipette, invert it so the liquid is in the bulb, shake to mix, and record observations

l. Using the pipette containing the sodium chloride solution with the liquid in the bulb of the inverted pipette, curve the tip of the pipette into a well containing 0.1M HCl and add 25 drops of the solution to the pipette, mix with the existing solution, shake in the inverted pipette, and record observations

2. TLC Analysis of Curcumin in Turmeric

   a. Gather turmeric sample, TLC applicator (fine capillary), a pencil, 5% methanol, dichloromethane, plate, and glass jar

   b. Using the TLC applicator, spot sample some turmeric extract on the analytical TLC plate. Mark with pencil on the edge of the plate where samples have been applied

   c. Pour a small amount of 5% methanol in dichloromethane into the jar, just enough so the solvent completely covers the base of the jar. Place the plate in the jar with the spot edge down. Develop the TLC plate until the solvent is within 1cm of the top of the plate, then remove it from the chamber, and mark the edge of the plate how high the solvent has risen to

   d. Sketch the plate (as a recording) and record the distance traveled by the solvent (starting where the spotted sample was). The curcumin spot should be the top and darkest. Record the distance traveled by the curcumin and all other spots below the curcumin on the plate

   e. Record the Rf values for all spots on the plate by dividing distance traveled by the spot by the distance traveled by the solvent

f. Run a new TLC plate with turmeric extract on the analytical TLC plate, approximately 1cm from the bottom edge of the plate. Mark it with a pencil
g. Pour a small amount of 5% methanol in dichloromethane into the jar so the solvent completely covers the base of the jar and add 1-2 drops of methanol. Develop the TLC plate until the solvent is within 1cm of the top of the plate, then remove the plate from the chamber, and mark on the edge of the plate how high the solvent has risen to
h. Sketch the plate (as a recording) and record the distance traveled by the solvent (starting where the spotted sample was). The curcumin should be top and darkest. Record the distance traveled by the curcumin and all other spots below the curcumin
i. Record the Rf values for all spots on the plate by dividing distance traveled by the spots on the plate by dividing distance traveled by the solvent

3. Separation and Analysis of Dyes in Grape Soda
    a. Gather 70% IPA solution, IPA solution, Sep-pak, test tube rack, 15 test tubes, 5% IPA solution, Grape soda, 20% IPA solution, 3 cuvettes, spectrophotometer, 2 graduated cylinders, a computer, and distilled water
    b. Draw 10mL of 70% IPA solution into a pipette. Connect it to the Sep-pak, push the IPA solution through the cartridge
    c. Set up the test tube rack with 15 test tubes. Draw 5mL of grape soda into a pipette and push it through the Sep-pak. Collect the solution that comes from it into the 1^{st} test tube
    d. Draw 5mL of 5% IPA solution and push it through the Sep-pak cartridge. Collect the fluid in the 2^{nd} test tube. Repeat 3 more times – fluids going in test tubes 3, 4, and 5
    e. Draw 2.5mL of 5% IPA solution and push it through the Sep-pak. Collect the fluid in the 6^{th} test tube. Repeat 4 more times – fluids going to test tubes 7, 8, 9, and 10

f. Draw 5mL of 20% IPA solution and push it through the Sep-pak. Collect the fluid in the 11th test tube. Repeat 4 more times – liquids going to test tubes 12, 13, 14, and 15. Continue until there is no more color remaining

g. Draw 10mL of 70% IPA solution and push it through the Sep-pak. Draw 10mL of water and push it through the Sep-pak. Expell liquid into the waste beaker. (This step was to clean the Sep-pak)

h. Record color observations within the test tubes

i. Combine all red and pink test tubes into a graduated cylinder and record their total volume

j. Set up spectrophotometer connected to computer (be sure to let it warm up) to measure at 505nm and set at 0.0% T with no cuvette in the instrument. Then using a cuvette with distilled water, place in instrument and calibrate set at 100.0% T . Once calibrated, put some of the red fluid from the graduated cylinder into a cuvette and run, record the absorbance value

k. Combine all tubes with a blue color into a graduated cylinder and record their volume then take some of this fluid and put it into a cuvette

l. Set the spectrophotometer to measure at 620nm and 0% T and 100.0% T values at this new wavelength. Record the absorbance value

4. Calculations for Absorbance Data

a. From absorbance value at 505nm for combined red fractions, calculate the concentration in molarity of red dye #40 using the molar absorptivity value:

E=29,500 M^{-1}cm-1

b. Do the same for blue fractions at 620nm using the molar absorptivity of the dye at 620nm:

E=130,000M^{-1}cm-1

c. From the red concentration and the total volume of the combined fractions, calculate the number of moles of FD+C Red dye #40 in the 5mL sample had. Do the same for the blue dye

d. From the number of moles calculated (for the calculations above), determine the mass of each dye using the molecular weights [Red dye = 496.43g/mole and blue dye = 792.84g/mole] that were present in the 5mL sample analyzed. Determine the mass of each dye that would be then present in a 335mL can of soda and a 2L bottle of soda

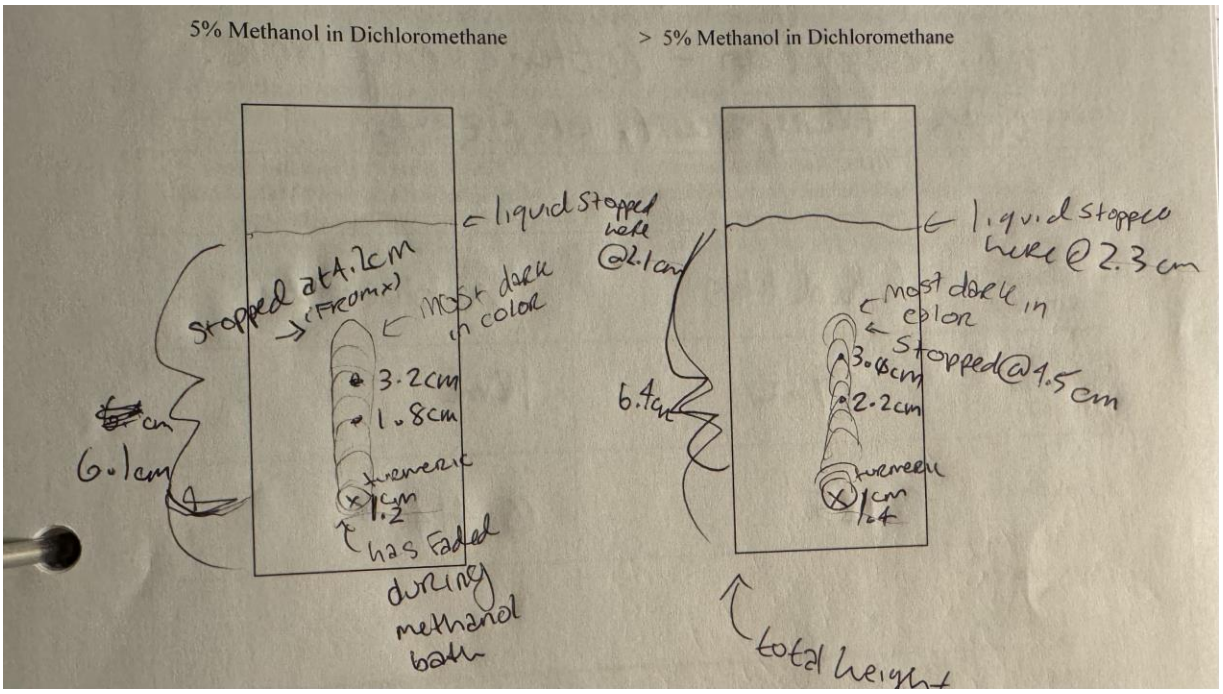
Results and Calculations

1. Qualitative Examination of Solubility Behavior

Ref	Solute	Solvent	Observations
1.2	**Stearic Acid**	**Water**	Didn't dissolve or change color – stearic acid stayed solid, floating
1.2	**Stearic Acid**	**Cyclohexane**	Immediately dissolved – no change in color (clear, transparent)
1.3	**Sodium Chloride**	**Water**	Most has dissolved, that which did not has no color – liquid doesn't have color change
1.3	**Sodium Chloride**	**Cyclohexane**	Sodium chloride stiff on bottom of well, no color change
1.4-5	**Stearic Acid**	**Cyclohexane + Water**	Clear on bottom of bulb, fizzy/bubbly in middle, and clear top layer. Appears to be

			water on bottom and cyclohexane as clear top layer
1.6	**Stearic Acid**	**Cyclohexane + NaOH solution**	Clear flat top, cloudy meniscus downward and from below a cloudy white meniscus upwards – they touch in the middle
1.7	**Stearic Acid**	**HCl to prev. Soln.**	Meniscus on top, gap of middle layer, and meniscus of what appears to be water pointing upwards
1.8	**Sodium Chloride**	**Cyclohexane + Water**	Clear in color, has an "oil in water" like effect when shaken, and has a meniscus downward touching a meniscus pointing upward
1.9	**Sodium Chloride**	**Cyclohexane + NaOH solution**	Clear, no bottom meniscus, top surface is flat but has appearance of meniscus bending down
1.10	**Sodium Chloride**	**HCl to prev. Soln.**	Clear flat top with appearance of meniscus and bottom meniscus pointing upwards, touching the top meniscus

2. TLC Analysis of Curcumin in Turmeric



	5% Methanol in Dichloromethane	> 5% Methanol in Dichloromethane
Distance from edge Curcumin started at	1.2cm	1.4cm
Distance Traveled by Solvent (starting where sample was)	5.75cm	5.35cm
Distance Traveled by Solvent (starting at edge)	6.95cm	6.75cm
Distance remaining above TLC plate post-test (untouched by solvent)	2.1cm	2.3cm
Distance Traveled by Curcumin (highest spot (from sample))	4.2cm	4.5cm
Distance Traveled by Curcumin (highest spot (from edge))	5.4cm	5.9cm
1st dark spot above start (from sample)	1.8cm	2.2cm
1st dark spot above start (from edge)	3cm	3.6cm
2nd dark spot above start (from sample)	3.2cm	3.0cm
2nd dark spot above start (from edge)	4.4cm	4.4cm
Rf Value of starting sample spot (using edge values)	0.172661871	0.207407407
Rf Value of 1st dark spot above start (using edge values)	0.431654676	0.533333333

Rf Value of 2nd dark spot above start (using edge values)	0.633093525	0.651851852
Rf Value of Highest spot above start (using edge values)	0.776978417	0.874074074

3. Separations and Analysis of Dyes in Grape Soda

Observations on the colors of the fractions:	Blue quickly dissipated – it was a "jolly rancher" blue color that was produced. Red dragged on, taking much longer than blue – it produced a "cotton candy" pink color. Both were transparent and not fizzy in appearance.	
	FD & C Red #40 (Allura Red) $C_{18}H_{14}N_2Na_2O_8S_2$ MW= 496.43 E= 25,900 (at 505nm)	**FD & C Blue #1 (Neptune Blue) $C_{37}H_{43}N_2Na_2O_9S_3$ MW= 792.84 E= 130,000 (at 630nm)**
Fraction Numbers Combined	6 red tubes	2 blue tubes
Total Volume (mL)	29mL	10mL
Absorbance	0.125	0.414
Concentration (M)	0.004826 mole/mL	0.003185 mole/mL
Moles of dye in 5.0mL	0.02413 moles	0.015925 moles
Mg of dye in 5.0mL	0.479144584mg	0.505044431mg
Mg of dye in 1 can (335mL)	34.01926546mg	35.8581546mg
Mg of dye in 1 – 2.0L bottle	191.6578336mg	202.0177724mg

Below is Graph 1 – this shows data from FD&C Red #40 (Allura Red)

Below is Graph 2- this shows data from FD&C Blue #1 (Neptune Blue)

4. Calculations for Absorbance Data

- From the absorbance value at 505nm for the combined red fractions calculate the concentration in molarity of Red Dye #40 using the molar absorptivity value provided (E= 25,900

M^-1 cm^-1). Note that the path length (b) of the cuvettes used is the usual standard value of 1.00cm:

Molarity= 4.826M

- From the concentration of the red dye and the total volume of the combined fractions (not 5.00mL), calculate the number of moles of FD&C Red Dye #40 in your 5mL sample. Do the same calculation for the blue dye (FD&C Blue Dye #1):

Moles of FD&C Red Dye #40 in 29mL= 0.139954 moles

Moles of FD&C Blue Dye #1 in 10mL= 0.03188 moles

- From the number of moles calculated (from the calculation above), determine the mass of each dye using the molecular weights provided (Red Dye MW is 496.43g/mole; Blue Dye MW is 792.84g/mole) present in the 5.0mL sample you analyzed. Determine the mass of each dye that would then be present in a 355mL can of soda, and in a 2.0L bottle:

Mass of FD&C Red Dye #40 in 5mL= 11.978859g

Mass of FD&C Blue Dye #1 in 5mL= 0.1030692g

Mass of FD&C Red Dye #40 in 355mL= 850.4987689g

Mass of FD&C Blue Dye #1 in 355mL=7.3179132g

Mass of FD&C Red Dye #40 in 2.0L= 4791.54236g

Mass of FD&C Blue Dye #1 in 2.0L=41.22768g

Questions

1. Does the solubility of stearic acid and sodium chloride follow the “like dissolves like” pattern?

   Explain if this rule is followed or not:

The solubility of sodium chloride and stearic acid follows the “like dissolves like” pattern as polar ionic solvents dissolve polar and ionic solutes, and non-polar solvents dissolve non-polar solutes.

2. What reaction occurs when sodium hydroxide is added to the stearic acid in cyclohexane and water? How does this change the solubility of cyclohexane in water? Does HCl reverse this reaction and if so, why? Does sodium chloride have the same type of behavior as stearic acid or is it different? Explain why these two solutes are the same or different:

The stearic acid is (obviously) an acid but sodium hydroxide is a base. They create a neutral reaction resulting in the form of a salt and water. This also decreases the solubility of cyclohexane in water. HCl does reverse this because the salt was formed by a strong base and will need a strong acid (HCl) to react with it. Sodium chloride and stearic acid have different behaviors- sodium chloride is a salt (ionic compound) and stearic acid is an acid (can be either ionic or covalent). Due to their different inorganic functions, their behaviors must also be different.

3. How would the Rf values change if you used pure dichloromethane (no methanol) as the mobile phase for the TLC separation of curcumin? What if you used pure methanol (no dichloromethane)?:

Dichloromethane is significantly less polar than methanol, so if pure dichloromethane is used, the spots on the plate won't have moved much. Pure methane will move the spots quickly and with ease.

4. Would the separation of the grape soda dyes work equally well if you reversed the order of IPA concentrations and used 20% IPA first to remove the blue dye and switched to 5% IPA to remove the red dye? Explain what you would expect to happen if you reversed the order of solvents in this separation:

I believe that reversing the order of the solvents may not stop the separation entirely but that it out result in poor separation results. In a more polar solvent, all components would run through the column quicker (no compound can go through quicker than the solvent).

5. If you had a dye with a molecular weight of 574.76 amu which had a molar absorptivity of 82,000 at 550nm, what would the expected value of absorbance at that wavelength if the dye was present at a concentration of 1.0mg/L? What color would you expect for this dye?:

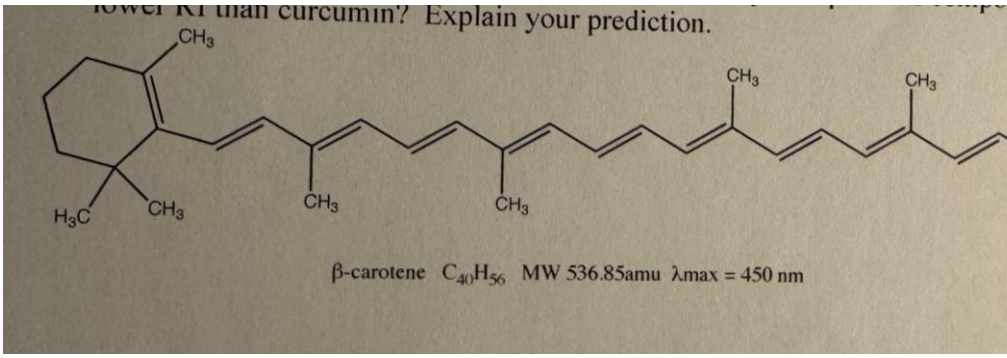
Absobtivity= 82.000M^-1 cm^-1

Concentration of Dye = 1.7 x 10^-6 moles/L

Absorbance= 1.4 x 10^-4

The color of the dye would likely be green due to the wavelength being 550nm and falls in the region of the visible spectrum

6. The structure of beta-carotene, which is a yellow dye found in vegetables that is also important in vision, is shown below. From the structure and formula would you expect this compound to have a higher or lower Rf than curcumin? Explain your prediction:



Curcumin is a polar compound because of the presence of electronegative Oxygen atoms in the molecule. Because of this, it has a stronger bond with the stationary phase and so, it shows a lower Rf value than b-carotine as it's a non-polar compound.