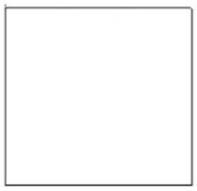
AP Lab 10 - Chromatography

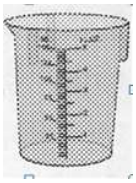
Lab Equipment

Chromatography paper (2 sheets)

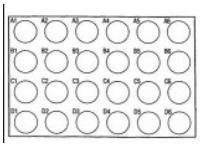


9.5 oz plastic cup

ruler

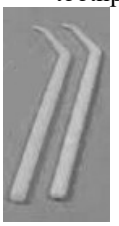


150 mL beaker



24-well reaction plate

plastic or wood toothpick



Student Supplied:

Rubbing alcohol solution

70% or higher by volume 2-propanol(*aq*) or better yet, your propanol from the last experiment

red, yellow, green (optional) and blue food coloring[1]

paper towel

transparent tape

Sharp pencil

Hazards: 2-propanol is flammable.
DO NOT DO THS EXPERIMENT NEAR OPEN FLAMES SUCH AS A STOVE.

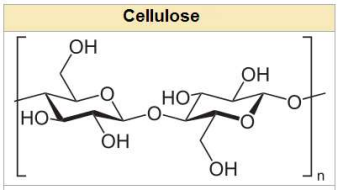
Ideally you should use your concentrated 2-propanol from the last lab, but if you cannot, you can use 70% rubbing alcohol or more concentrated if you can find it.

[1] These dyes may not be available in your location. If you cannot find all the dyes, do the best you can and perhaps substitute a real food dye such as red beet juice or carrot juice for the artificial dye.

Background Information

Paper chromatography is one of several kinds of chromatography that are used to separate and identify mixtures of compounds in solutions. The principle of paper chromatography is the same as in all types of chromatography. The mobile phase, a liquid or gas, is passed through a stationary phase. Separation occurs when compounds are put into the mobile phase, the eluent, and move through the material of the stationary phase. The compounds in the eluent are attracted to both the eluent and the stationary phase differently and therefore they separate. Once the compounds are separated, they can be identified.

Paper chromatography uses paper as the stationary phase. Filter paper is one of the best types of paper, although paper towels and even white paper coffee filters can also be used. Most writing paper is coated to prevent inks from spreading and because of this is not satisfactory.



The eluent, which is a solvent, moves up through the stationary paper through small pathways in it. This capillary action[2] in paper is caused by the fine (≈10 μm diameter) fibers of cellulose which have the capability for intermolecular attractions utilizing hydrogen bonding. Water will exhibit significant capillary action with paper because of this.

Chromatography can be done quantitatively by measuring the time or distance that the solvent front travels compared to the solvent. From these measurements, the retention factor is calculated. The **retention factor** is the distance traveled by a solute divided by the distance the solvent traveled. In this lab, you will be able to gather enough information to measure the retention factor or R_f which is expressed as:

$$R_f = D_{solute} / D_{solvent}$$

D_{solute} is the distance a solute component traveled.
$D_{solvent}$ is the distance the solvent traveled.
The greater the R_f the less the solute is attracted to the stationary phase

In this lab you will investigate the chromatography of food dyes with cellulose chromatography paper as the medium and with water and a 2-propanol mixture as eluents.

[2] Albert Einstein's first published scientific paper, *Conclusions from the Capillarity Phenomena*, dealt with the intermolecular forces and capillary action. *Folgerungen aus den Kapillaritätserscheinungen*, Annalen der Physik volume 4, page 513 (published in 1901).

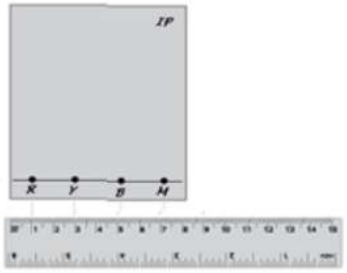
There are currently only seven artificial food dyes allowed in the US for general use.

Blue	Blue No. 1	Brilliant Blue	$C_{37}H_{34}N_2Na_2O_9S_3$
Indigo shade	Blue No. 2	Indigo Carmine	$C_{16}H_8N_2Na_2O_8S_2$
Turquoise shade	Green No. 3	Fast Green	$C_{37}H_{34}N_2Na_2O_{10}S_3$
Red shade	Red No. 40	Allura Red	$C_{18}H_{14}N_2Na_2O_8S_2$
Pink shade	Red No. 3	Erythrosine	$C_{20}H_6I_4Na_2O_5$
Yellow shade	Yellow No. 5	Tartrazine	$C_{16}H_9N_4Na_3O_9S_2$
Orange shade	Yellow No. 6	Sunset	$C_{16}H_{10}N_2Na_2O_7S_2$

You will use chromatography to compare the intermolecular attractions of several of these food colors.

By examining the results of the chromatography experiment, you will be asked to come to some conclusions relative to the important factors that caused the separations in the chromatograms.

Procedure:



1. Record the food dyes used in each color of your food dye mixtures: red, yellow, and blue food coloring by looking at the ingredients label on the packaging.

2. Using a sharpened pencil, draw a line 1 cm from the edge of the 10-cm by 10-cm sheet of chromatography paper. Then make five small pencil marks a little less than 2 cm apart along the line, as shown in the diagram. Label the dots R, Y, B, G (optional), M (for red, yellow, blue, green, mixture). Also label the top right of the sheet IP (for isopropyl alcohol)

   It would be unwise to use an ink pen when drawing and writing on the paper.

3. Repeat on a second sheet of paper except label the upper right W (for water).

4. Take the food dyes and the QS food dye mixture[3] and place one drop of each dye in a well of the 24-well reaction plate in the same order as the dots on the chromatography paper. You will have a plate with one drop of dye in five wells.

5. Using the toothpick, touch the tip to the drop of red dye in the first well. You will want a very small drop to adhere to the tip of the toothpick, the tinier the better. Transfer the microdrop to the dot labeled R on the propanol chromatography sheet. Do the same to the water chromatography sheet. Wait a minute for the dots to dry and then repeat to place a second microdrop on the dots. Again, let the drops dry and transfer the third microdrops. You want the dot to be as small as possible yet concentrated enough so that you can see the results on the chromatogram. So patiently use several small drops rather than putting one large drop which would create a larger blob that would spread out too much during the chromatography.

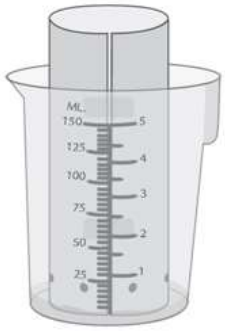
[3] If you don't have a QS dye mixture, make up a mixture or three of the dyes with equal drops of each.

6. Repeat this with each of the dyes and the unknown so that you have 5 small, but concentrated dye spots on each chromatography sheet. After you have done this, rinse out the wells, since if the dyes dry you will have a very difficult time cleaning the wells. Dry the wells out with your paper towel.

7. Roll the paper into a cylinder by curling it around a pencil or pen and tape the edges.

8. Pour the 2-propanol solution into the 150-mL beaker to a depth of approximately 3 mm. **The liquid level must be below that of the dots.**

9. Place the paper, pencil line down, in the beaker containing the propanol. Carefully center the cylinder in the beaker, so that the paper doesn't touch the sides of the beaker.

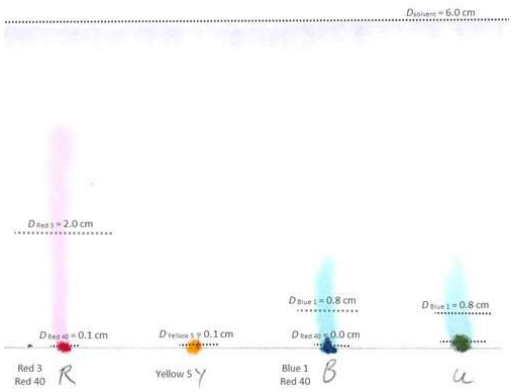


10. Lightly place the inverted empty dry 9.5 oz plastic cup or large glass tumbler over the beaker and tube of chromatography paper. This will create a little dome over the beaker preventing the 2-propanol from evaporating before reaching the top of the chromatography paper.

11. Note the time and let the 2-propanol wick up the paper undisturbed. Come back in 15 minutes to estimate how far the eluant has traveled up the paper. From this you will have some idea of how long it will take for the alcohol to travel almost to the top of the paper. Leave the experiment and continue checking every once in a while, till the alcohol has traveled to approximately 2-3 cm from the top of the paper. This may take more than an hour. So, set a timer and do something else. You don't need to stare at the experiment while it's going on.

12. Once the alcohol has wicked to near the top of the paper, remove the protective cup and lift the cylinder from the beaker. Note the time and, using a pencil, mark the distance the alcohol traveled on the chromatogram. Carefully remove the tape and set the paper on the reaction plate to dry. This will take some time. Do something else and give it an hour or so.

13. Repeat the experiment, except this time with water as the eluent. Check the distance the water traveled after 15 min to estimate how long it will take to reach to approximately 2-3 cm from the top of the paper. You may need to adjust your timing since water has a different viscosity and intermolecular attractions than the 2-propanol mixture.



14. After the chromatograms have dried, examine the separation and movement of the components in the dye mixtures. Using a pencil, outline what you see as components of the mixture. There is some subjectivity and distortion depending on the "grain" of the chromatography paper. Sometimes it helps to flip the chromatogram over to look at the side without the pencil marks.

15. Draw a line though the center of each outline to establish the distance from the starting line to the center of the outline. This distance will be the D_{solute}

Questions for your analysis:

1. Analyze the data in terms of the relative attraction of the dyes to the cellulose paper and the solvents used.
2. Identify the unknown mixture.

	Chromatography	

Purpose: To examine the separation of food dyes using chromatography with two eluents

Apparatus:

Chromatography paper
Ruler
Pencil
150-mL polypropylene beaker
9.5 oz clear plastic cup
24 well reaction plate
plastic toothpick
McCormick Assorted Food Color and Egg Dye dyes
Red FDC Red dye #40 and FDC Red dye #3
Yellow FDC Yellow #5
Blue FDC Blue #1 and FDC Red #40
Unknown mixture of food dye
isopropyl alcohol mixture
Pure water

Procedure:
Detailed instructions for this experiment can be found in my Lab Binder.

I set up two sheets of chromatography paper (10 cm × 10 cm) with small, concentrated spots of each food dye and the unknown mixture using three tiny drops of each substance using a toothpick following the instructions in the printed procedure.

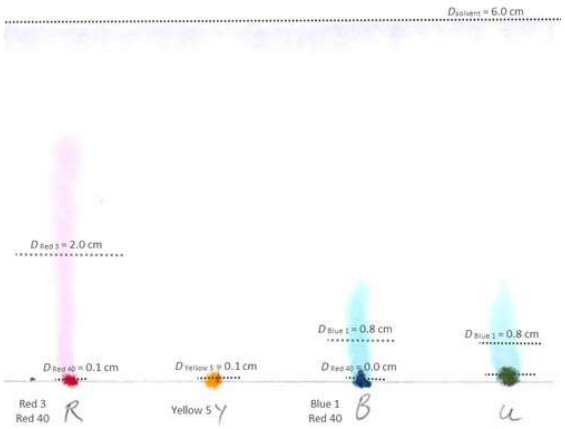
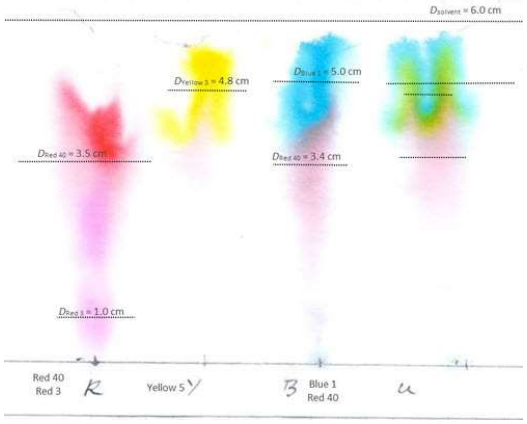
I then placed the sheet in the 150-mL beaker with a small amount of propyl alcohol mixture. I covered the beaker with the inverted plastic cup to prevent evaporation of the alcohol. I let the alcohol move up the paper till the alcohol front was 1 cm from the top of the paper. I then took the paper out of the beaker and let it dry. It took 1 hours for the alcohol to get to that level.

I repeated the experiment with water as the eluent. It took 1 hour also.

Here are the two chromatograms. The dotted lines are where I estimated the D_{solute} and $D_{solvent}$.

Using the propanol red and blue chromatographs I was able to determine the signature of Red dye #3 so I could focus on the Red Dye #40.

Notably Red dye-3 was the dye that had the greatest attraction to the propanol. It's large iodine atoms in its structure made it different from the rest of the dyes. Also it is the one that has the most restrictions for food use, exclusively in maraschino cherries!

2-propanol	Pure water
	

The 2-propanol solution

$D_{propanol}$ = 6.0 cm

Two dye Red Chromatogram	$D_{Red\ 40}$ = 0.1 cm	$D_{Red\ 3}$ = 2.0 cm
One dye Yellow Chromatogram	$D_{Yellow\ 5}$ = 0.1 cm	
Two dye Blue Chromatogram	$D_{Red\ 40}$= 0.0 cm	D_{Blue1} = 0.8 cm

I calculated the R_f by dividing the distance the dye pigment traveled, D_{dye}, by the distance the 2-propanol travelled, $D_{propanol}$.

$D_{propanol}$ = 6.0 cm

Red 3 Chromatogram	$D_{Red\ 3}$ = 2.0 cm	R_f = 0.33
Red 40 Chromatogram	$D_{Red\ 3}$ = 0.1 cm	R_f = 0.0
Yellow Chromatogram	$D_{Yellow\ 5}$ = 0.1 cm	R_f = 0.0
Blue Chromatogram	D_{Blue1} = 0.80 cm	R_f = 0.13

The red 40, Yellow and Blue dyes didn't travel very far so they must not have been attracted to the mobile propanol as much as they were attracted to the stationary chromatography paper (cellulose).

The water was very effective in attracting all the dyes except Red 3

D_{water}= 6.0 cm

Two dye Red Chromatogram	$D_{Red\ 40}$ = 3.5 cm	$D_{Red\ 3}$ = 1.0 cm
Two dye Yellow Chromatogram	$D_{Yellow\ 5}$ = 4.8 cm	
Blue Chromatogram	$D_{Blue\ 1}$ = 5.0	$D_{Red\ 40}$ = 3.4 cm

I calculated the R_f by dividing the distance the dye pigment traveled, D_{dye}, by the distance water travelled, D_{water}.

D_{water} = 6.0 cm

Red Chromatogram	$D_{Red\ 40}$ = 3.5 cm	R_f = 0.58
Red Chromatogram	$D_{Red\ 3}$ = 1.0 cm	R_f = 0.12
Yellow Chromatogram	$D_{Yellow\ 5}$ = 4.8 cm	R_f = 0.80
Blue Chromatogram	D_{Blue1} = 5.0 cm	R_f = 0.83

All except Red 3 a stronger attraction to the water than the propanol.

Dye	Propanol -Dye Intermolecular Attractions $R_{f\ 2\text{-}prop}$	Water-Dye Intermolecular attractions $R_{f\ water}$
Red 3	1.0 strongest attractions to 2-propanol	0.12 low attractions to water
Red 40	0.0 low attraction to propanol	0.58 strong attractions to water
Yellow 5	0.0 very low attraction to propanol	0.80 strong attractions to water
Blue 1	0.13 low attraction to propanol	0.83 strongest attractions to water

The 2-propanol showed that there was no Red 3 in the unknown.

Unknown was a mixture of Yellow-5 and Blue-1.