At-Home Chemistry Experiment

Paper Chromatography of Food Dyes and Ink

Objectives

To identify the food drug and cosmetic (FD&C) dyes found in food/beverage samples (such as *Kool-aid, Gatorade, popsicles*), or water-soluble markers, using the paper chromatography technique. Determination of the retention factor (R_f) values of the dyes found in the samples and comparison with those of known commercial sources will be carried out in this at-home experiment.

Introduction

Chromatography is a widely used technique in both chemistry and biology, to separate and identify molecules within a mixture of unknown substances. The process of chromatography employs the use of two different phases; the *mobile phase* (which is moving), and the *stationary phase* (which is not moving). The different components of the mixture will be carried by the mobile phase through the stationary phase, with the components having varying affinities for the two phases. The compounds with a higher affinity towards the stationary phase will travel the shortest distance, while those with a weaker affinity towards the stationary phase will travel the furthest. This development process allows for the separation or resolution of the various components of a mixture. If the resolution is sufficient, it allows for the identification of the unknown components based on the resolution of standard, known compounds.

The process of chromatography is widely used and applied in various techniques in organic chemistry, analytical chemistry and biochemistry. Such applications include paper chromatography, thin layer chromatography (TLC), high performance liquid chromatography (HPLC), and gas chromatography (GC), as well as many other more recent variations of these techniques.

In this experiment, paper chromatography will be used to separate and identify dyes present in various food or ink products. Chromatography paper is made of cellulose molecules which contain polar water molecules trapped between the fibers serving as the stationary phase. The polar and ionic 0.1 % salt-water solution will serve as the mobile phase. The dye/ink samples are spotted onto the porous chromatography paper and the salt-water will travel up the paper by surface capillary action. As the dissolved dye/ink pigments migrate up the paper, they will spend some time interacting with the mobile and stationary phases. The samples which spend more time interacting with the water in the cellulose fibers will migrate less, while those interacting more with the solvent will migrate further up the chromatography paper.

The distance that a compound travels on the chromatogram is expressed as the retention factor, or **R_f value**, which is the ratio of the distances travelled from the starting line to the compound and to the solvent front (Equation 1 and Figure 1a). The R_f of a compound is a characteristic value that cannot be theoretically obtained. It must be determined experimentally and is dependent on the conditions used for the chromatography.

$$Rf = \frac{distance\ travelled\ by\ compound}{distance\ travelled\ by\ solvent\ front} \quad (1)$$

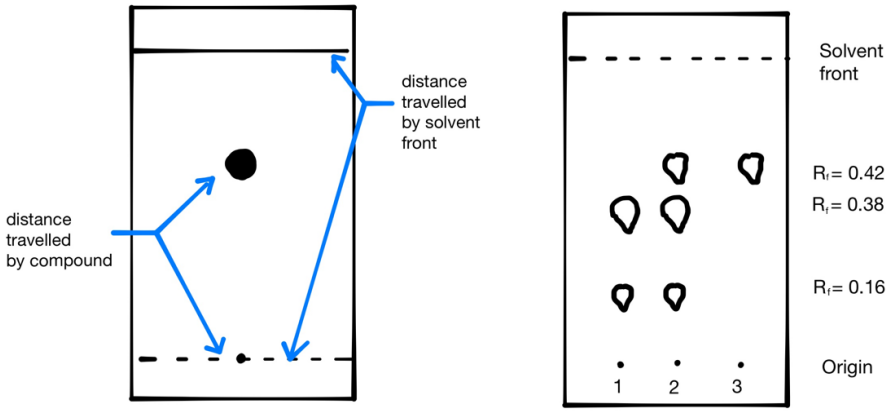

Figure 1: *a) Calculation of the R_f value, b) Developed chromatogram – lanes: (1) sample 1, (2) Co-Spot, (3) sample 2*

Small changes in the solvent, temperature, amount of sample applied, presence of other compounds, and the coating thickness (when using adsorbents), can affect the R_f value. In practice, it is usually not possible to control all of these conditions completely. For this reason, when using paper chromatography to investigate two samples suspected of being the same compound, a comparison should always be carried out by spotting (applying) both samples on the same paper. For example, in Figure 1b, a mixture of dyes from a food sample was spotted in lane 1 (on the left). It shows the presence of two components with R_f values of 0.38 and 0.16. A comparison with the compound in lane 3 (on the right) shows that it may be the same compound as the top spot in lane 1 (R_f value of 0.38). Because elution of the spots isn't always perfect (the solvent front may not always run in a straight line), it can be difficult to differentiate between R_f values that are in separate lanes, especially if the values are close. Thus, having a lane (2) where you spot samples used for lane 1 and lane 3 can serve as a "reference". This is referred to as a 'co-spot' and will allow you to see where all the spots elute when they are in the same lane. In Figure 1b, it is evident that the top spot in lane 1 is the not the same compound as the one in lane 3.

When spotting a sample on the chromatography paper, it is important that the spot is small (approximately 2-3 mm in diameter) and not too concentrated. This will allow for better separation and resolution of the components in a mixture. If spots are large or too concentrated, the spots on the chromatogram may overlap, or show 'tailing' (Figure 3), which is a spot that has a tail that extends towards the origin. Changing the composition of the mobile phase so that it is a mixture of polar and non-polar substances, can help to improve separation and resolution of the spots.

Figure 2: *Tailing effect of samples - lanes: (1) well resolved spot, (2) spot with tailing, (3) determining the center of migration for a spot with tailing.*

The FDA (Food and Drug Administration) is responsible for the approval of food dyes that can be used for consumption in the United States, while Health Canada is responsible for those used in Canada. There are currently 9 approved dyes in Canada for use in food products. These are red 2, red 3, red 4, red 40, blue 1, blue 2, yellow 5, yellow 6, and green 3. Reds 2 and 4 are not approved by the FDA in the U.S. Red 4 (Ponceau red) is not often used in Canada and has been omitted from the Table 1 and Figure 3 below. All of these food dyes are relatively large compounds that contain a conjugated ring system with alternating single and double carbon-carbon bonds.

In this at-home experiment, the dyes in various food or ink samples will be identified using commercial food dyes as the known, standard compounds.

Name of Dye	FD&C #	European #	Colour
Allura Red	Red 40	E129	
Amaranth	Red 2	E123	
Erythrosine	Red 3	E127	
Indigotine	Blue 2	E132	
Sunset Yellow FCF	Yellow 6	E110	
Tartrazine	Yellow 5	E102	
Fast Green FCF	Green 3	E143	
Brilliant Blue FCF	Blue 1	E133	

Table 1: *FD&C approved food dyes for use in Canada.*

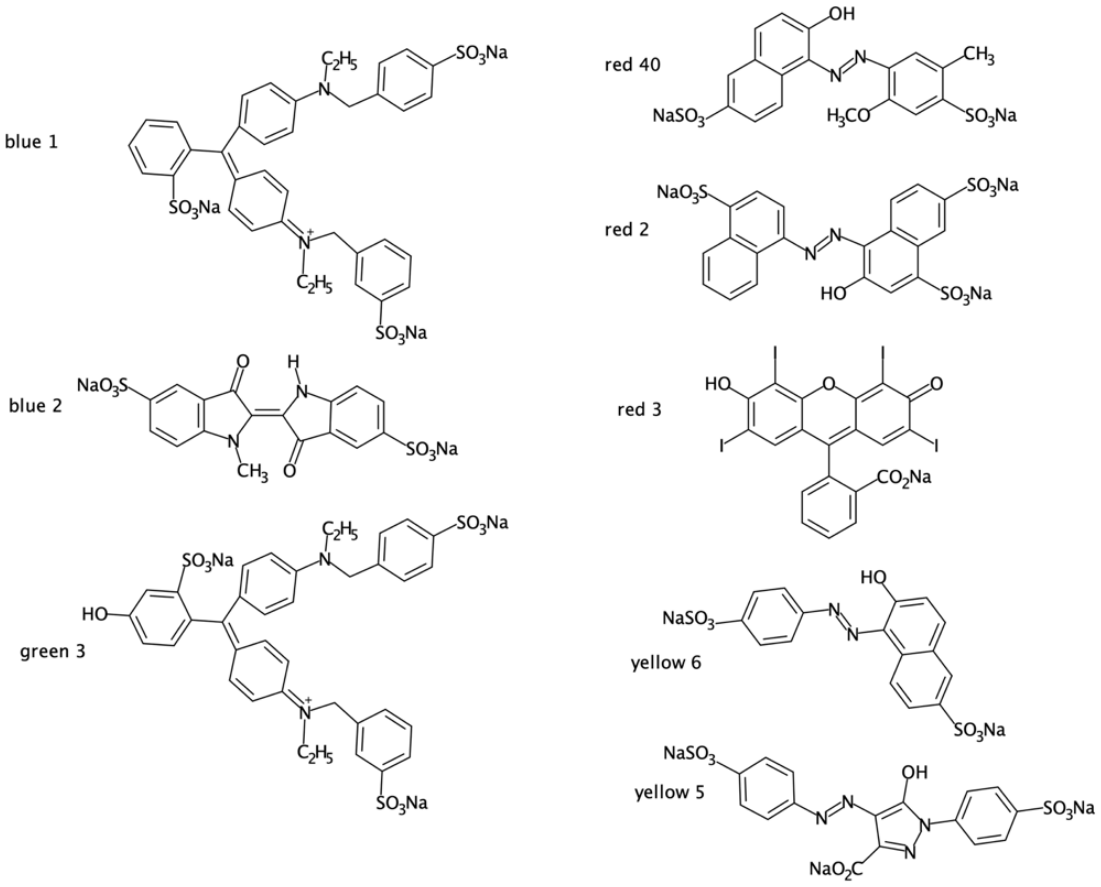

Figure 3: *Structures of 8 commonly found FD&C food dyes in Canada.*

Materials

- 1 Sheet of Whatman # 1 chromatography paper 20 x 25 cm
- Scissors
- Pencil
- Ruler
- Toothpicks*
- Clear plastic wrap (*Saran-Wrap*)
- Clear container (at least 12 cm in height), or a tall, colourless drinking glass
- Salt
- Water
- Commercial food colouring, “Club House” brand (regular or Neon) **
- 3 Samples of food/items with artificial food colouring using a variety of colours***

* see troubleshooting suggestions if no toothpicks are available.
** see troubleshooting suggestions if using a different brand.
*** possible examples include: *Kool-aid, Gatorade*, coloured candy coating (*Skittles*) or *popsicles* with artificial food colouring, or water-soluble markers. See instructor if trying a different sample.

Procedure

Part A - Preparation of the Stationary Phase and the Samples

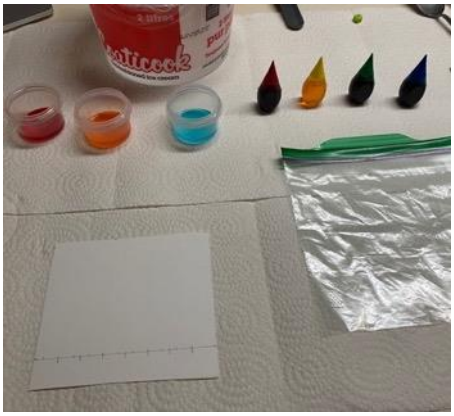

Figure 4: *Whatman #1 chromatography paper with divisions for spotting*

1. Cut the sheet of Whatman chromatography paper into 4 sheets (height: 10 cm by width: 12.5 cm).

2. Using a pencil, for each sheet, draw an origin line that is 2 cm from the paper’s edge (along the width). See Figure 4 and **Figure 5**.

3. Beginning 1.75 cm from the left, place small marks on the origin line every 1.5 cm.

4. Label the marks on the origin line with your choice of 3 unknown samples, and the 4 commercial food colours (which will be used as the standards).

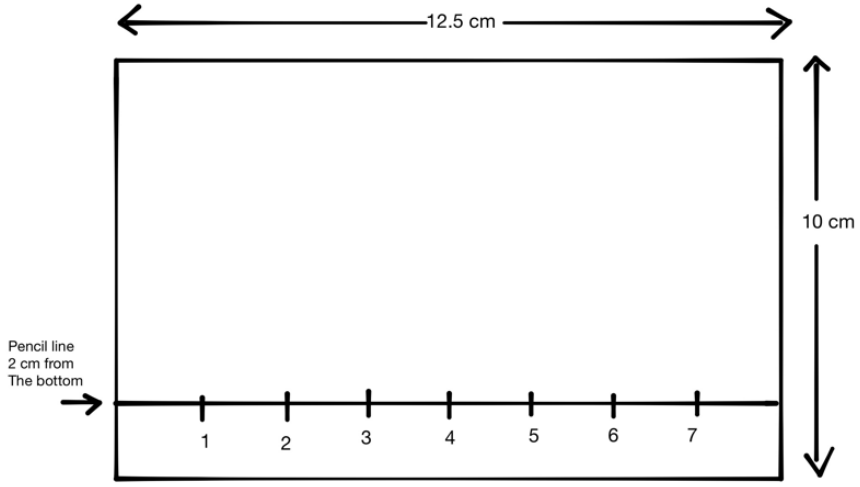

Figure 5: *Diagram for the chromatogram*

5. The liquid food/item may be used as is. A solid sample must be dissolved in the minimum amount of water.

6. Place ONE drop of each of the 4 commercial food colours onto a plate (or Ziploc bag or wax paper, see Figure 6a).

7. Slowly dip the thinnest end of a wooden toothpick into one of the commercial food colour solutions (standards) and then spot the paper on the corresponding mark. Be sure to keep the toothpick vertical and touch the paper for only an instant to keep the diameter of the spot to 2-3 mm. Allow the spot to dry (blow on the spot to help dry it quicker). Be careful not to puncture the paper.

8. Repeat step 7 twice, applying the same sample on the corresponding location to make the spot on the paper more concentrated. It is important to allow the spot to dry after each application to prevent the spot from getting too big in diameter.

9. Using a separate wooden toothpick for each solution, repeat step 7 three times for each of the remaining commercial food colouring.

10. Repeat step 7 for the food/item samples, except spot ***each*** sample 10 times on the paper. If the spots appear faint, repeat spotting again, adding to the spots until they are clearly visible. Again, allow the spots to dry between each application.

11. Allow the prepared chromatography paper to dry before developing it using the mobile phase (***Part B***).

a) b)

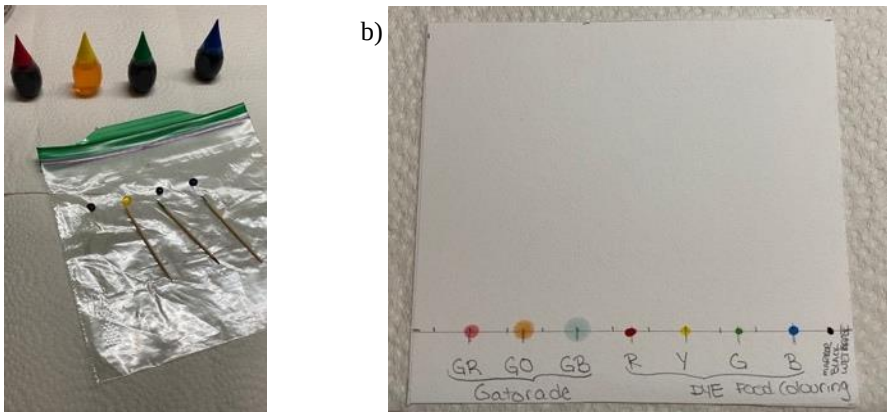

Figure 6: *a) Preparation of the food colouring samples b) Spotted samples on the chromatography paper: food/item samples on the left, standard food colourings on the right.*

Refer to Figure 7 regarding the identities of the dyes used in the commercial food colouring of the Club House brand. Use this information to help identify the compounds present in your food/item samples.

Club House Regular Line	
Colour	Dyes
Red	Red 40, Red 3
Yellow	Yellow 5, Red 40
Green	Yellow 5, Blue 1
Blue	Blue 1, Red 40

Club House Neon Line	
Colour	Dyes
Pink	Red 40
Purple	Red 3
Blue	Blue 5
Yellow	Yellow 1

Figure 7: *List of FD&C compounds present in the "Club House" brand of food colouring.*

Part B - Preparation of the Mobile Phase

1. Prepare a 0.1 % (m/v) NaCl solution for use as the mobile phase.

2. To a large bowl, add ¼ teaspoon (1.5 g) of table salt and 1.5 L (6 cups) of water.

3. Stir the 0.1 % NaCl solution.

4. Choose a clear container that is tall enough to cover the height of the prepared chromatography paper (minimum of 11-12 cm in height).

5. If the chosen container is less wide than the Whatman chromatography paper (ex. a tall drinking glass), slowly and carefully roll the paper into a cylinder and staple the edges together, only after the spots have dried.
   Note: *the edges should be parallel to each other and* ***not overlap or touch****. There should be a gap between the two edges.*

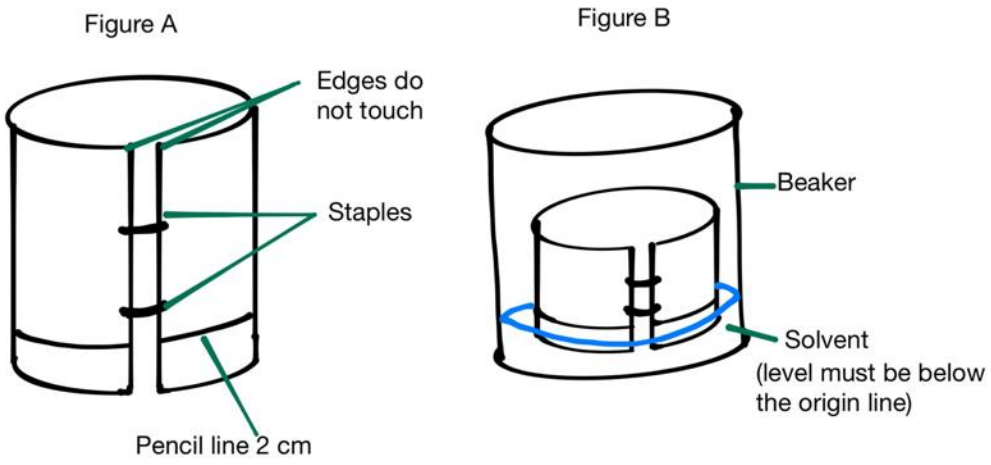

Figure 8: *a) Stapled chromatogram, b) Stapled chromatogram in beaker*

6. Fill the bottom of the container with 1-1.5 cm of the 0.1% NaCl solution. Cover the top of the container with a plastic wrap to let the container saturate with the water vapour for 5 minutes. Ensure the height of the 0.1% NaCl solution is not more than 1.5 cm, otherwise there is risk of washing the spots off the paper into the mobile phase.

Part C - Development of the Chromatogram(s)

a) b)

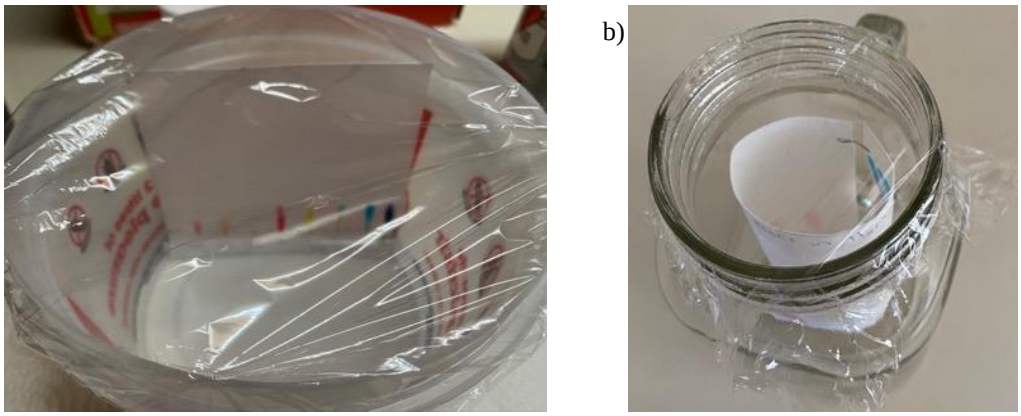

Figure 9: *a) Chromatogram developing in large width container*
b) Chromatogram developing in small width container.

1. Remove the plastic wrap and place the chromatography paper in the container (developing chamber). The paper must not touch the sides of the container.

2. Cover the container with the plastic wrap again.

3. Remove the paper from the container when the solvent front (mobile phase) is 2 cm from the top of the paper. Lay it flat on a paper towel.

4. With a pencil, trace the solvent front to mark how far it reached on the chromatogram. All the spots can be marked after the chromatogram has been allowed to sit for one minute.

5. Using a hairdryer, or another heat source (do not burn the paper!), dry the chromatogram for approximately 5 minutes to stop the solvent front from continuing to migrate.

6. If the chromatogram is not dried immediately, the solvent front may continue to migrate as it dries, and you will have to re-draw the solvent front if it moves.

7. Allow to the chromatogram to completely air dry before analyzing your results (possibly overnight).

8. More food/item samples can be analysed with the remaining unused chromatography paper. Continue to use the commercial food dyes as the standards.

Calculations and Data Analysis

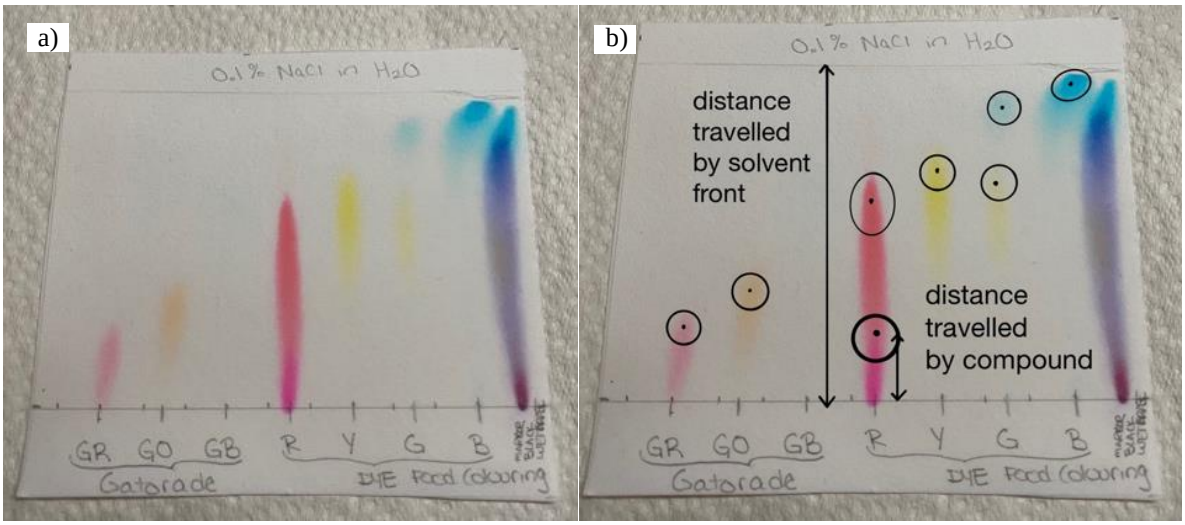

Figure 10: a) Developed chromatogram sample, b) Rf determination of sample chromatogram. Samples from left to right: Red Gatorade, Orange Gatorade, Blue Gatorade (too faint), Club House dyes: Red, Yellow, Green, Blue, and a Black transparency marker (Wet-Erase)

1. Measure and record the distance traveled by the solvent front from the origin line on the chromatogram(s).

2. Measure and record the distances from the origin line traveled by all samples.

3. Some spots will result in tailing, be sure to circle the top of the spot and mark the center of that circle, not the tail (Figure 10).

4. Calculate the R_f value for each spot and record them in Table 2 of the Data section.

5. Identify the FD&C food dyes in the food/item samples, as well as in the commercial food colours, in Table 2 of the Data section.

Name:_____ Section _____

Date _____

Paper Chromatography
Data

Composition of the mobile phase: _____

Distance of solvent front from origin (cm): _____

You may need to divide some of the boxes into multiple rows if the dye separated into more than one color per spot. Use less or add extra rows as necessary.

Spot Number	Spot Label	Solvent Distance (cm)	Spot Distance (cm)	R_f Value	FD&C Dye #
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					

Table 2: *Calculated R_f values and Identification of spots on chromatogram(s)*

Troubleshooting suggestions:

- You can use the commercial "Club House" dyes of the regular colours or the neon dyes. If you use a different brand, contact the company to ask them which FD&C compounds are present in their food colourings.
- The less concentrated a sample is, the more applications will be needed for a sample when spotting on the chromatography paper.
- If toothpicks are not available, use an object with a small sharp tip to control the size of the spots (for example the prong of a fork, tip of a skewer, a paper clip or a comb)
- After the chromatography paper has been removed from the developing chamber, dry it until it is slightly damp using a hair dryer (do not use a flame), then allow to air dry overnight. If you do not dry the chromatogram, it is possible that the solvent may continue to migrate. If this happens, you must re-trace the solvent front as it has moved from its original spot.
- If you do not have a ¼ teaspoon measuring spoon, you may use a ½ teaspoon or full teaspoon, but the volume of water used should be adjusted for the proper dilution. Check with your instructor to make sure your dilution calculations are correct.

References

Branch, Legislative Services. "Consolidated Federal Laws of Canada, Food and Drug Regulations." *Food and Drug Regulations*, 19 June 2020, https://laws-lois.justice.gc.ca/eng/regulations/C.R.C.,_c._870/page-36.html

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DAWSON COLLEGE SALTISE

Last updated June 22, 2020

Sharpie Tie-dye Chromatography (optional)

Create your own art using the concept of chromatography with Sharpies and isopropanol.

Instructions from Martha Stewart.com

MATERIALS

White, 100 percent cotton T-shirt

Cardboard

Sharpie permanent markers

Baking pan (or glass or plastic container)

Binder clips (or rubber bands)

Isopropyl rubbing alcohol (91 percent)

Eyedropper

Iron or clothes drier

STEPS

1. Wash and dry T-shirt. Insert a piece of cardboard into T-shirt to prevent ink from bleeding through to other side.

2. Using desired marker colors, make a preliminary design on T-shirt. (Note: For a flower pattern, make a large dot of ink in one color, then surround in many smaller dots of a complementary color.)

3. Remove cardboard and stretch T-shirt over a baking pan, securing with binder clips. Be sure shirt is taut enough that it is not touching the bottom of the pan. (Tip: Individual sections of shirt can also be stretched over the mouth of a glass or plastic container and secured in place with a rubber band.)

4. Using an eyedropper, slowly drip desired number of drops of rubbing alcohol into center of ink design. The more drops of alcohol used, the further the ink will spread and the larger the design will be. (Note: Avoid flooding design with alcohol all at once.)

5. Once desired design is achieved, let T-shirt dry completely. Set color into shirt by applying a hot iron for 5 minutes or placing shirt in clothes drier on high for 15 minutes. (Tip: Be sure your iron is on the highest heat possible when setting the ink. Wash shirts separately on the delicate cycle and in cold water.)

References:

"Sharpie Tie-Dye T-Shirt." *Martha Stewart*, Martha Stewart, 20 Sept. 2018, www.marthastewart.com/892787/sharpie-tie-dye-t-shirt.
https://www.youtube.com/watch?v=DjWrUyRTVR0