Experiment #4

Fundamentals of Chromatography

Overview

This investigation examines how components of **mixtures can be separated by taking advantage of differences in physical properties**. Paper chromatography will be used to isolate food dyes found in different flavors of drink mixes. Chromatograms of drink mixes will be compared to known chromatograms of FD&C dyes to identify which dyes are present in each mix.

Outcomes

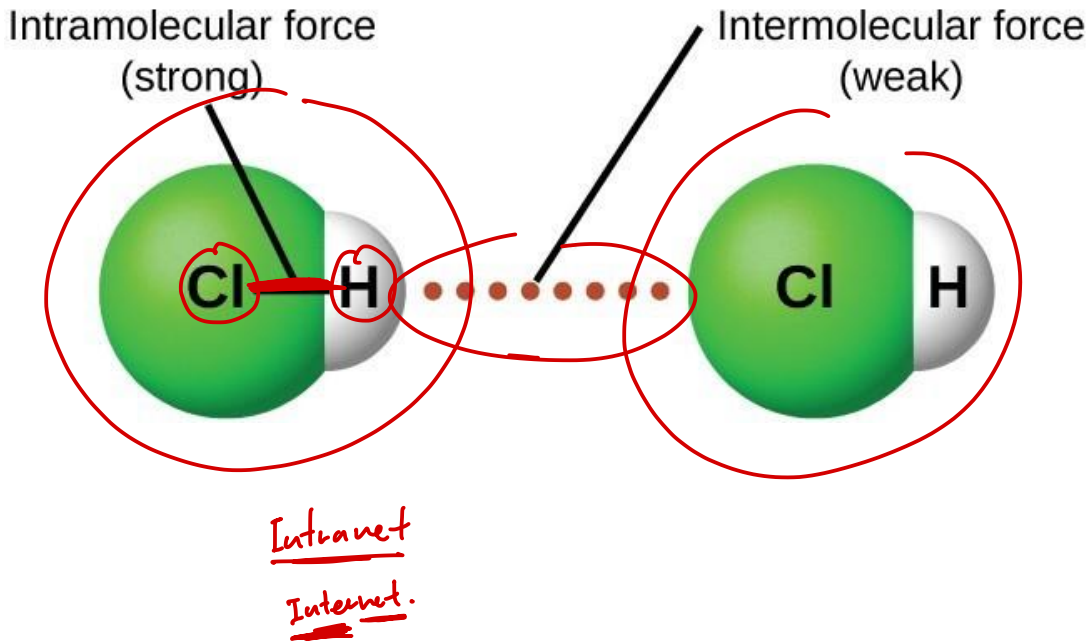
- Produce chromatograms for known FD&C dyes (Red #40, Blue #1, and Yellow #5).
- Produce chromatograms for three Kool-Aid ® samples (orange, grape, and strawberry).
- Calculate the retention factor (R f) for each dye in the chromatogram.
- Determine the dyes present in each Kool-Aid ® sample by comparison to the chromatograms of known dyes.

Background

INTERMOLECULAR FORCE

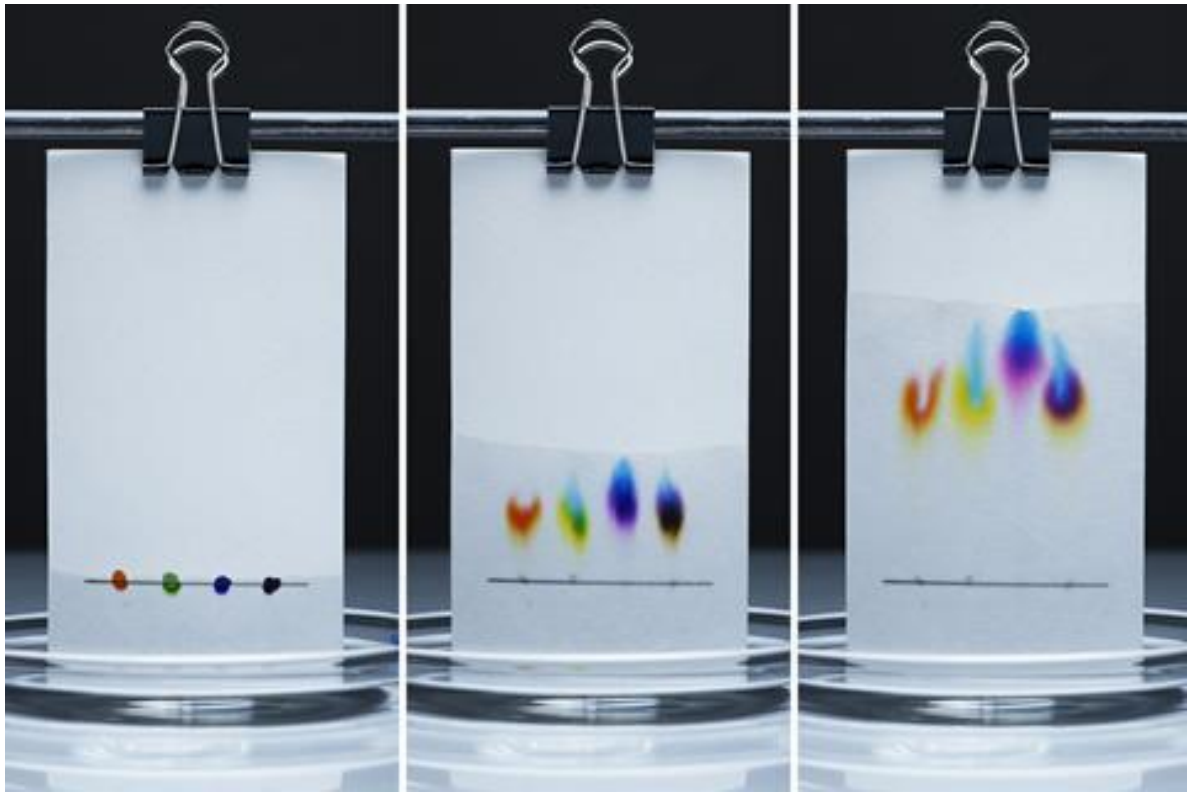
- An **intermolecular force** is an electrostatic interaction between positively and negatively charged species.

- Strong intermolecular force → molecules are attracted to each other more



CHROMATOGRAPHY

Chromatography is a laboratory method that separates and examines different substances mixed together based on how they move differently due to their intermolecular forces.



- **Mobile Phase:** The mobile phase is the liquid or gas in chromatography that moves substances over the stationary phase. It helps separate the substances based on their interactions with the stationary phase.

- **Stationary Phase:** The stationary phase is a solid or liquid in chromatography that stays in one place. Substances interact with it, affecting how fast they move with the mobile phase.

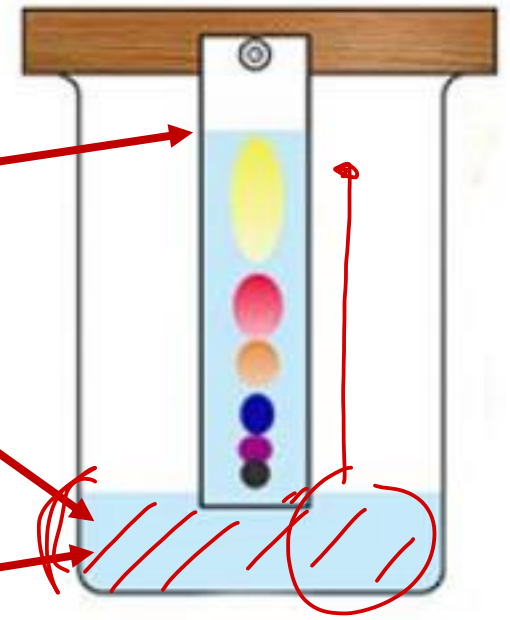
- **Solvent:** A solvent is a substance, often a liquid, that dissolves another substance to form a solution. It's the component of a solution present in the greatest amount.

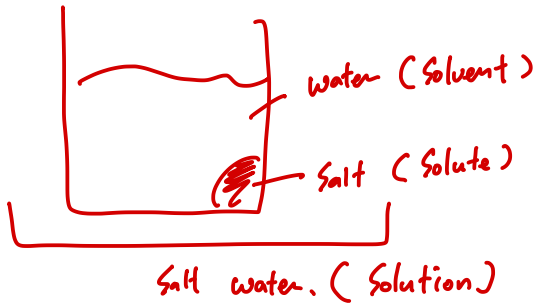
- **Chromatography:** Chromatography is a technique used to separate and identify components of a mixture. It works by moving substances through a stationary phase using a mobile phase.

- **Mobile Phase:** In paper chromatography, the mobile phase is a **liquid solvent** that travels up the paper. As it moves, it carries different substances with it at different rates.

- **Stationary Phase:** The stationary phase in paper chromatography is the **paper** itself. Substances interact with the paper, causing some to move slower or faster with the mobile phase.

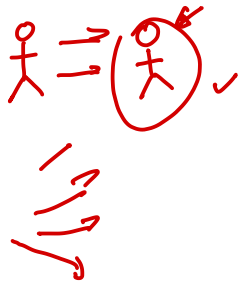
- **Solvent:** A solvent in paper chromatography is the **liquid used to dissolve the sample**. This liquid then travels up the paper, separating the components of the sample.

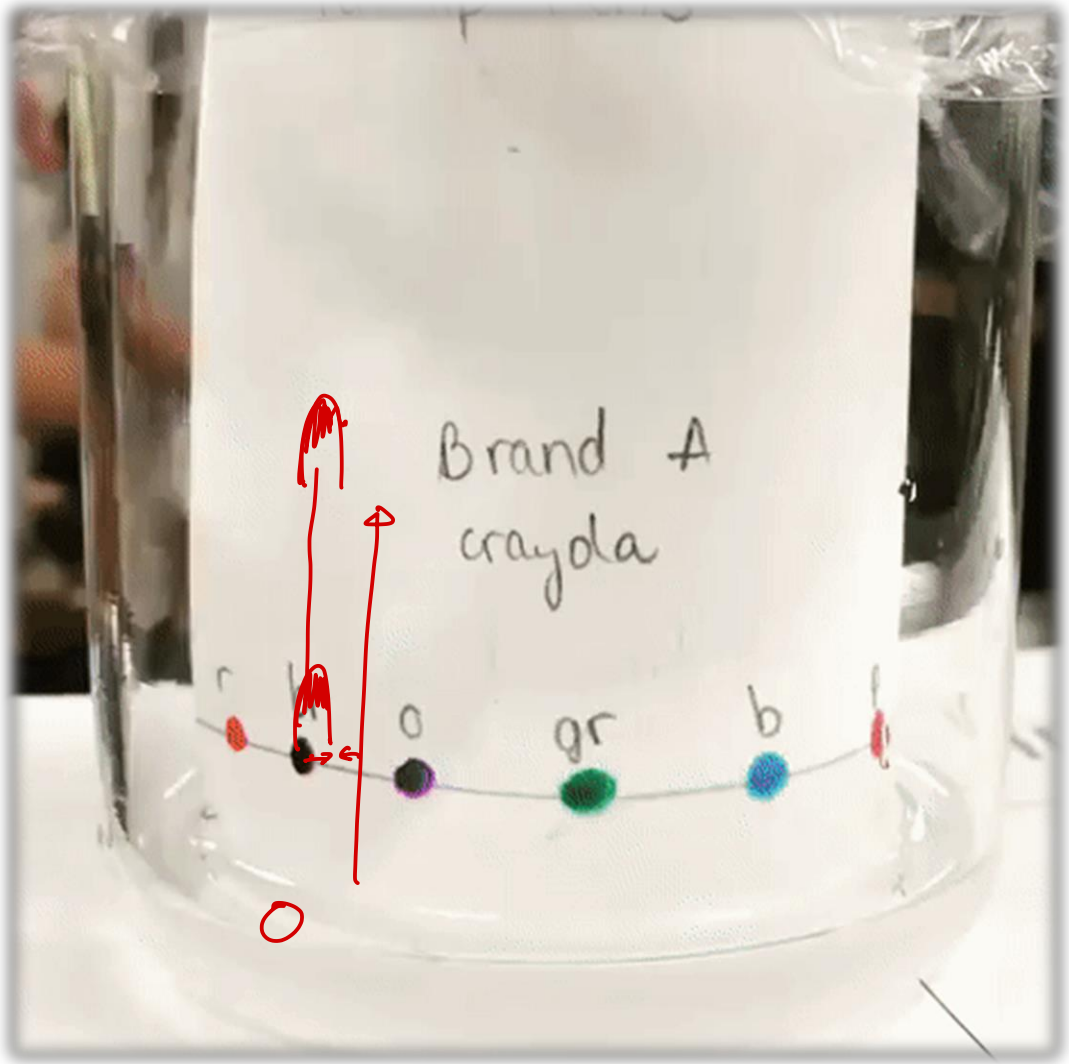


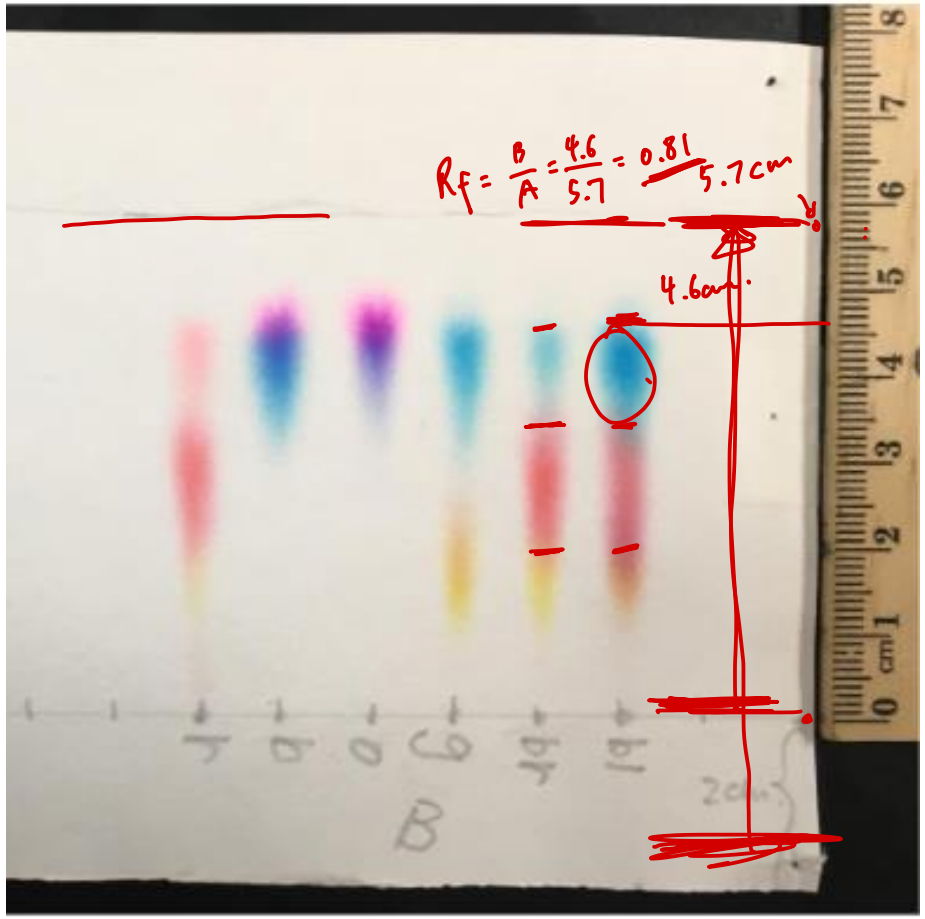


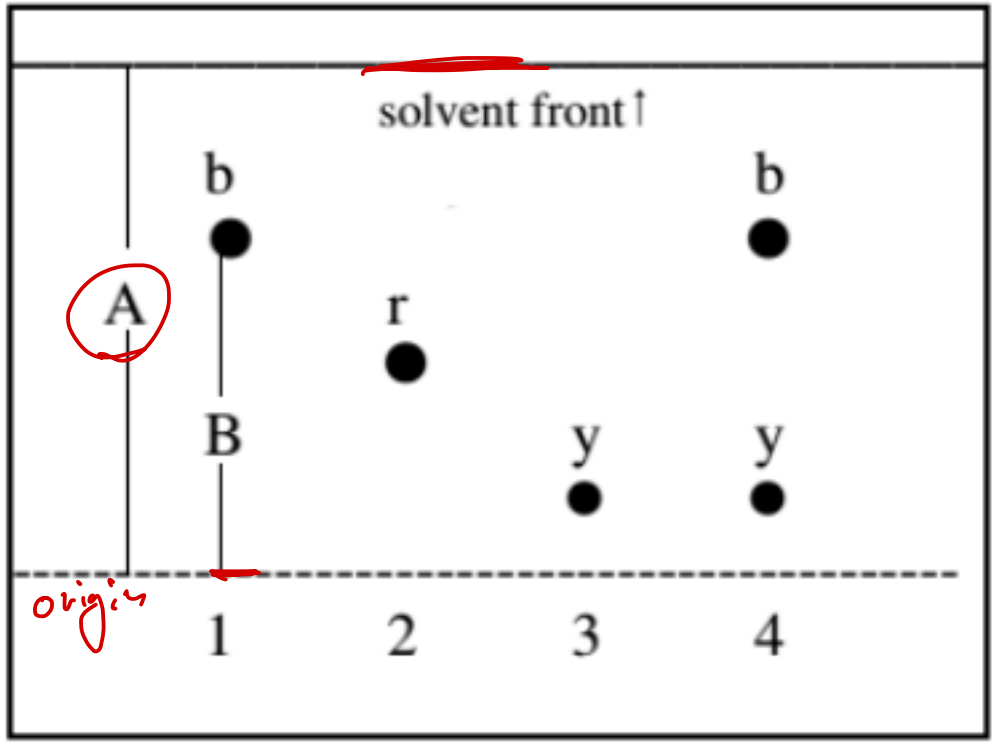
- Sample substances move through a "stationary phase(paper)” along with “mobile phase(solvent).”

- Stronger attraction between the substance and the solvent leads to move further from its origin point







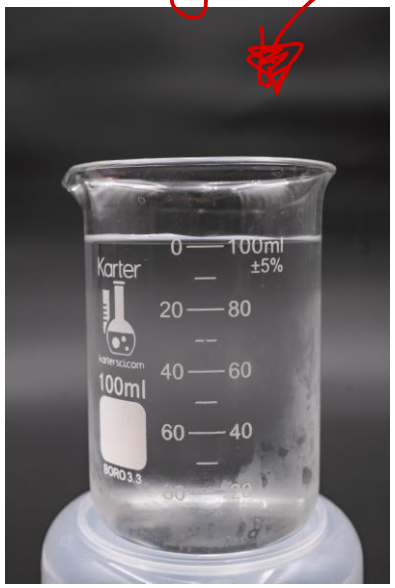


$$R_f = \frac{\text{distance from origin to ~~center~~ Top of spot}}{\text{distance from origin to solvent front}} = \frac{B}{A} \text{ for spot 1}$$

Procedure

Prepare Solutions

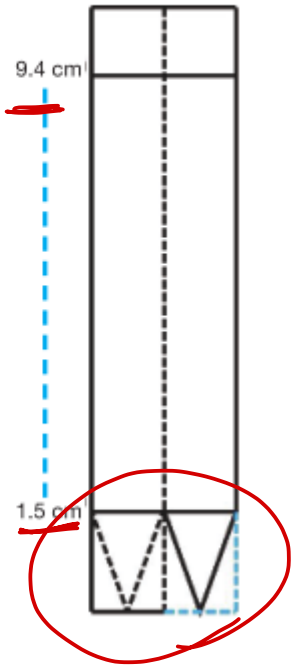
1. Use a weigh boat and electronic balance to weigh out 2.0 g of each Kool-Aid® packet.
2. Transfer each to a labeled small cup. Add 5 mL of water and mix with the plastic spoon until completely dissolved.
3. Repeat steps 1 and 2 for the other two Kool-Aid® flavors. Be sure to rinse the spoon before mixing the next Kool-Aid®.
4. Prepare 100 mL of a 0.1% NaCl solution by adding 0.1 g of NaCl to 100 mL of bottled water.
5. Stir the salt solution until the salt is completely dissolved.



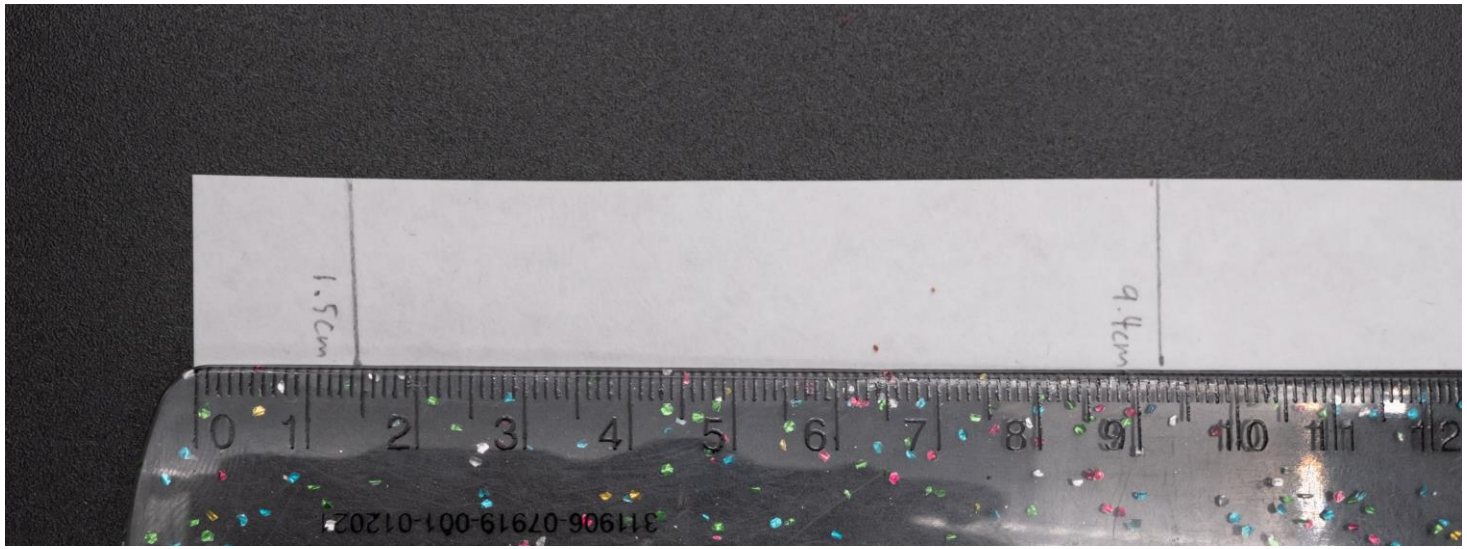
Prepare Chromatography Strips

1. Cut the six strips of chromatography paper in half lengthwise to make 12 strips and place them on a paper towel.
2. On each of the 12 strips, use a pencil to draw a line across the strip 1.5 cm from one end.
3. From the bottom of each strip, measure to 9.4 cm and draw a second line across the strip using the pencil.

Figure 1.



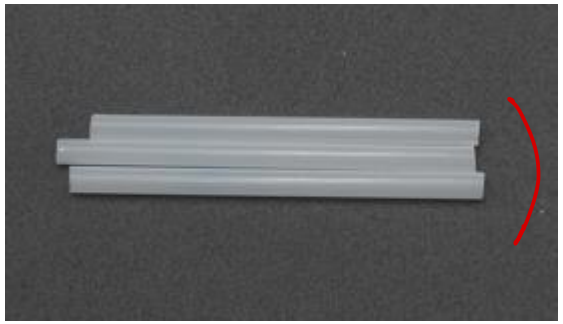
4. At the bottom edge of each strip (below the 1.5-cm line), use the pencil to mark the exact middle of the strip.
5. With a pair of scissors, cut the strip into a "v" shaped point by starting at the middle mark and cutting diagonally in straight lines up to the right and left edges of the 1.5-cm line. See Figure 1.



Prepare Pipet

1. On each of the six microtip pipets, measure 5 cm back from the tip, and cut each pipet with a pair of scissors. Discard the longer ends with the bulb. Save the 5-cm microtips for the lab as seen in Figure 2.

Figure 2.



ACTVITY 1

A Known FD&C Dyes

1. On a paper towel, orient three chromatography strips parallel to each other with the pointed ends at the bottom.
2. Place either a small plastic bag or small piece of aluminum foil on top of another paper towel.
3. In three separate locations on the plastic bag or aluminum foil, place two drops of each FD&C dye (red #40, yellow #5, and blue #1) on top of each other from each dropper bottle.
4. Place one 5-cm microtip into the red drop, and allow it to partially fill the tip about 1 to 2 mm by capillary action. Lightly tapping the tip in the drop may help.
5. Touch the microtip to the middle of the line drawn at the 1.5-cm mark on the strip. Repeat the filling and application several times until the spot is fairly dark and is about 3 mm in diameter.

   See how to spot your paper.
   http://players.brightcove.net/17907428001/HJ2y9UNi_default/index.html?videoId=4578692106001

6. Repeat steps 4–5 using two clean 5-cm microtips and chromatography strips for the other two dyes.
7. Allow all three applied dots to dry for a few minutes.

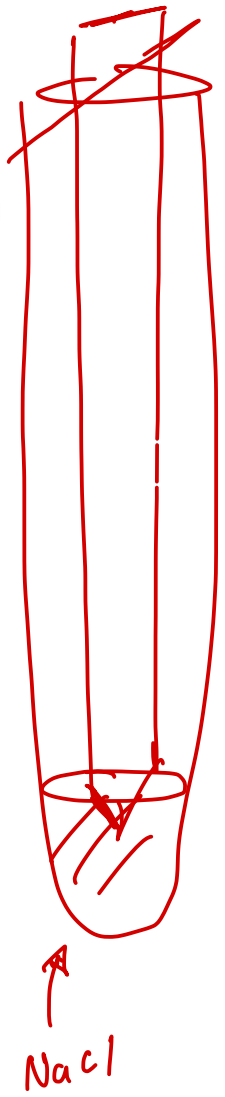
8. Place three 17 × 100 mm test tubes into a test tube rack.

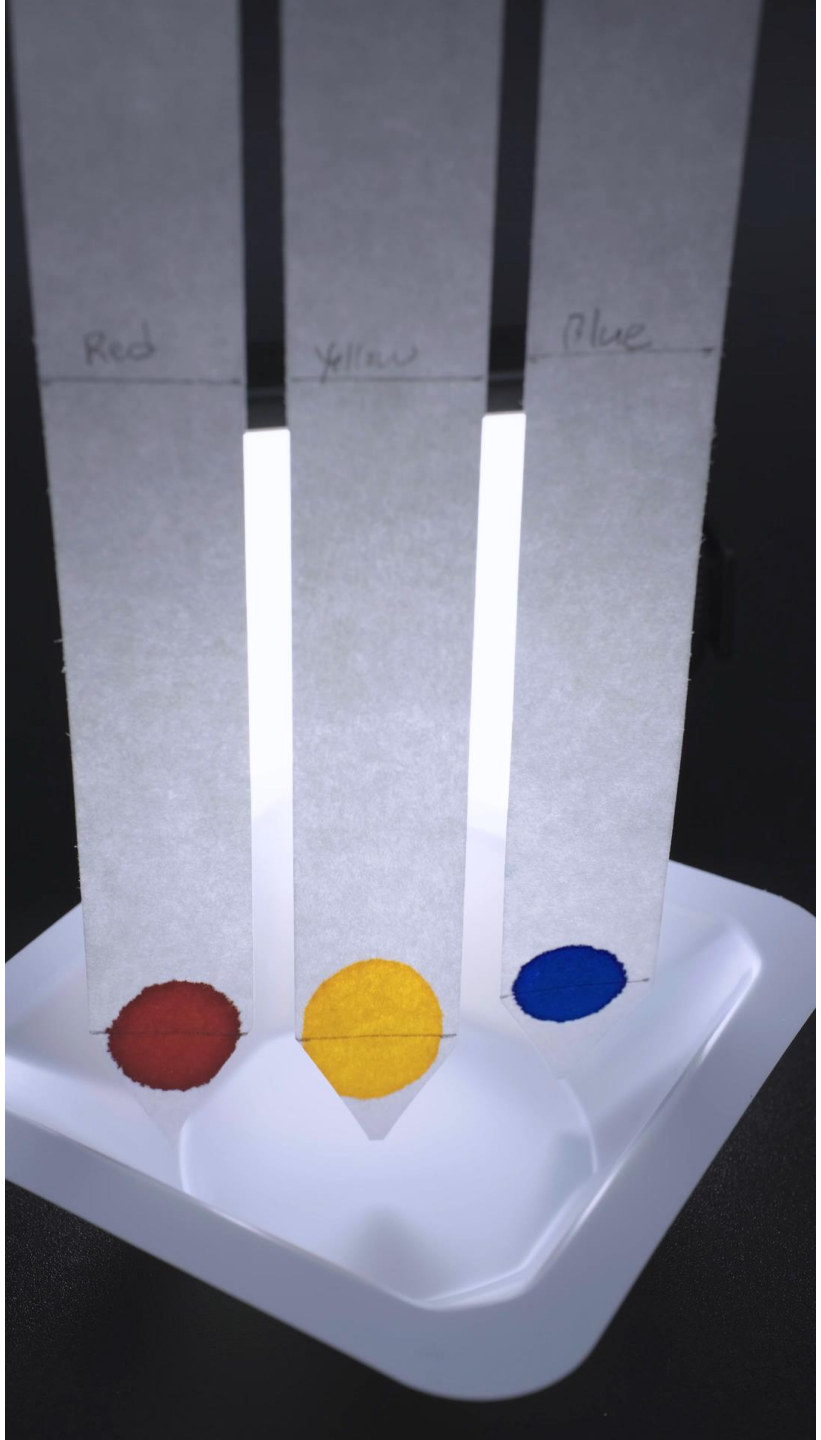
9. Add 20 drops (≈ 1 mL) of the 0.1% NaCl solution to each of the three test tubes. Place the drops in the center of the tube without allowing them to touch the walls.

10. Insert a straight pin into the middle of the top drawn line at 9.4 cm for each of the three chromatography strips. These will serve as hangers for the chromatography strips.

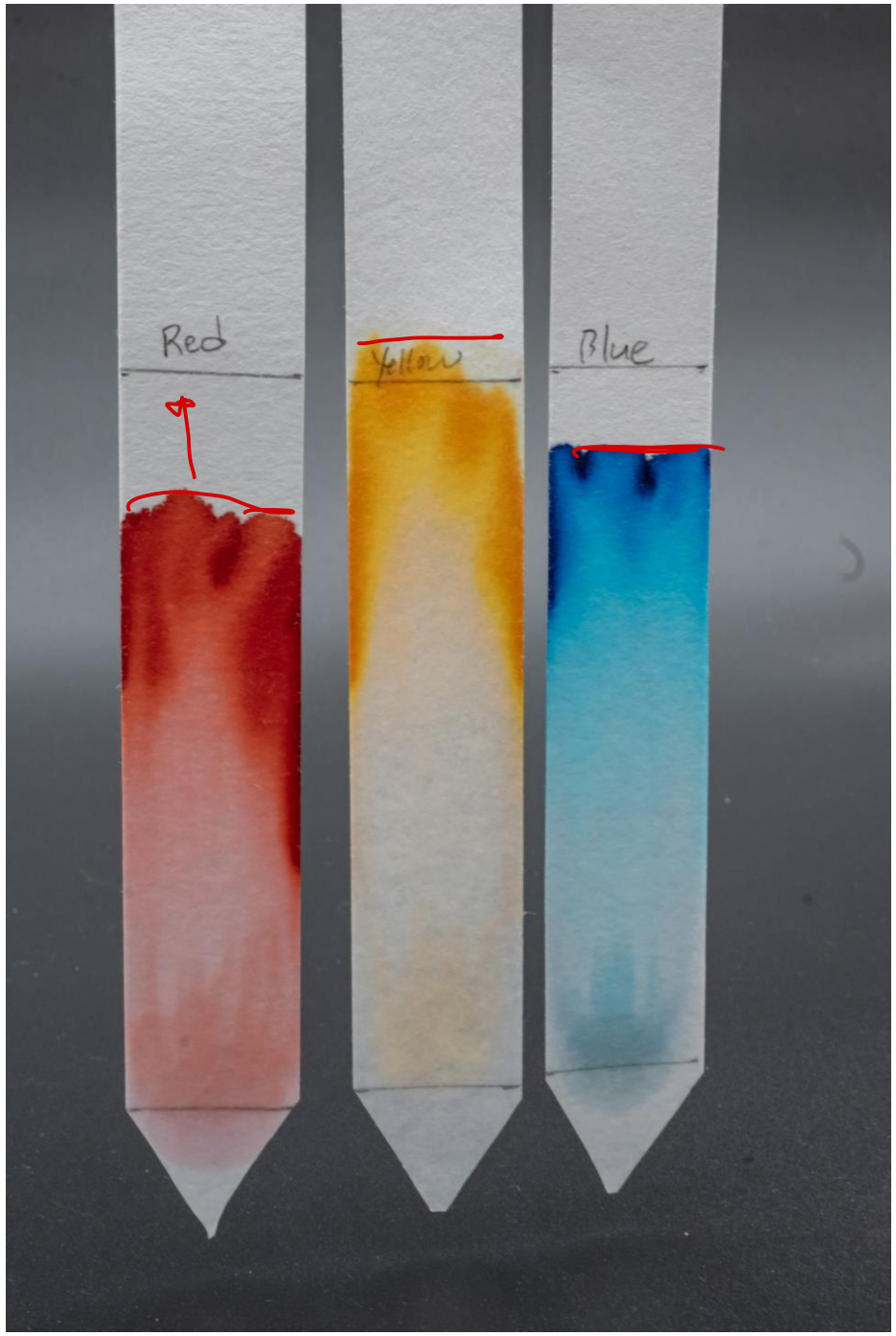
11. Carefully lower each of the chromatography strips with the applied dyes down into the center of each test tube, and place the tips into the NaCl-containing solvent.

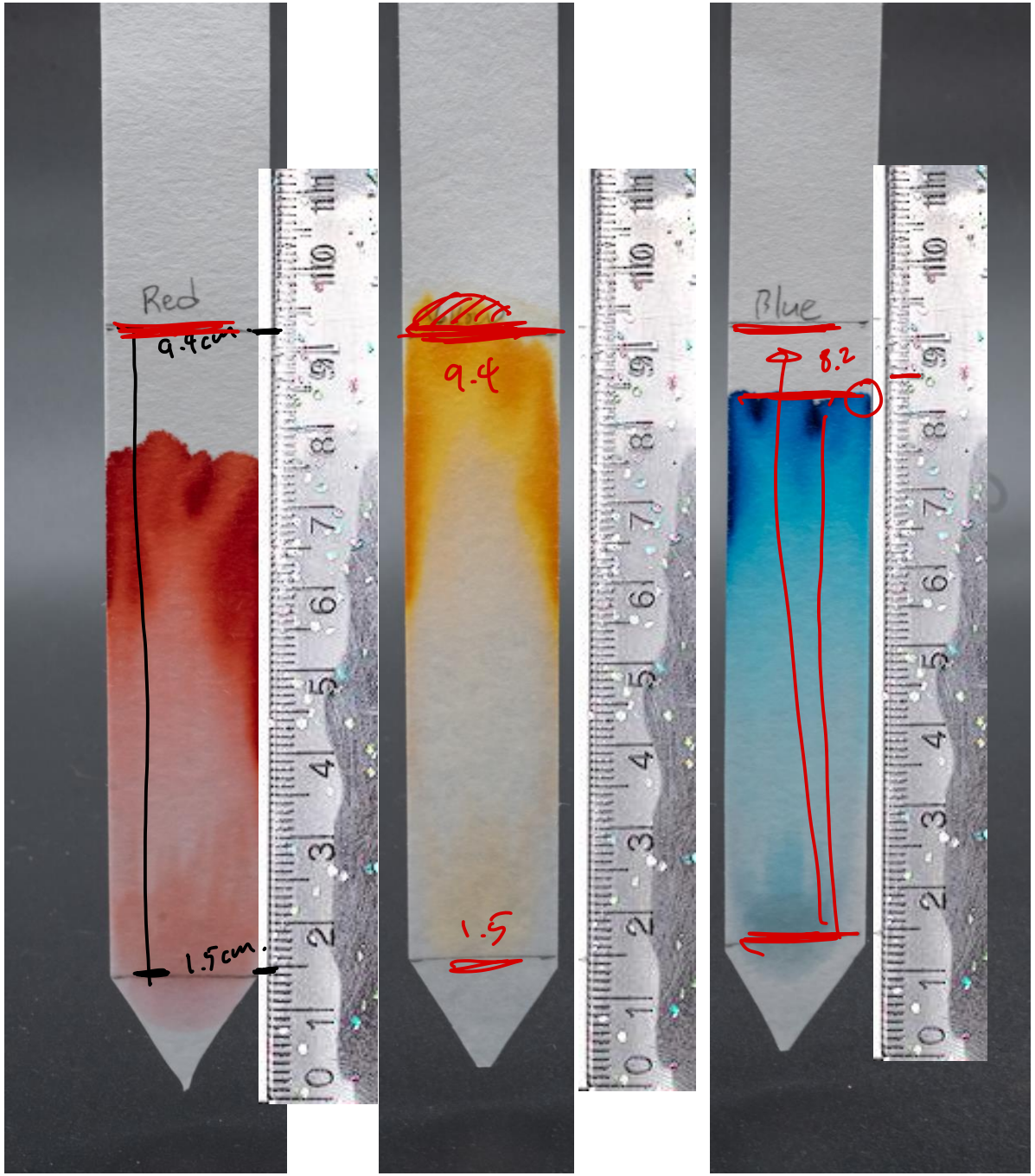
12. Allow the solvent to travel through the spot and move up the paper for 15 minutes.

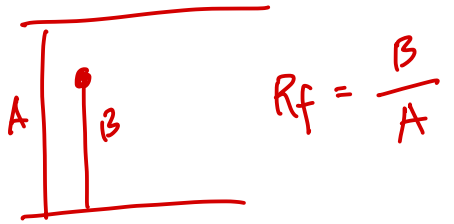




13. Remove each of the strips and the straight pins, place them on a paper towel, and mark the leading edge of the wet solvent front with a pencil before it dries.
14. When the strips are dry, mark the middle of the colored bands with a pencil.
15. Place the strips parallel to each other on the left side of a sheet of 8½ × 11" white paper, turned sideways. Tape the strips down with transparent tape. Label the bottom of each strip with its dye name and number.
16. Measure the distance in millimeters that the **solvent front** traveled from the origin line at the bottom. Record the distance in Data Table 1.
17. Measure the distance in millimeters that each **colored band** moved from the origin line to the mark in the middle of each color band. Record the distance in Data Table 1.
18. Calculate the R_f value for each of the bands and record them in Data Table 1.





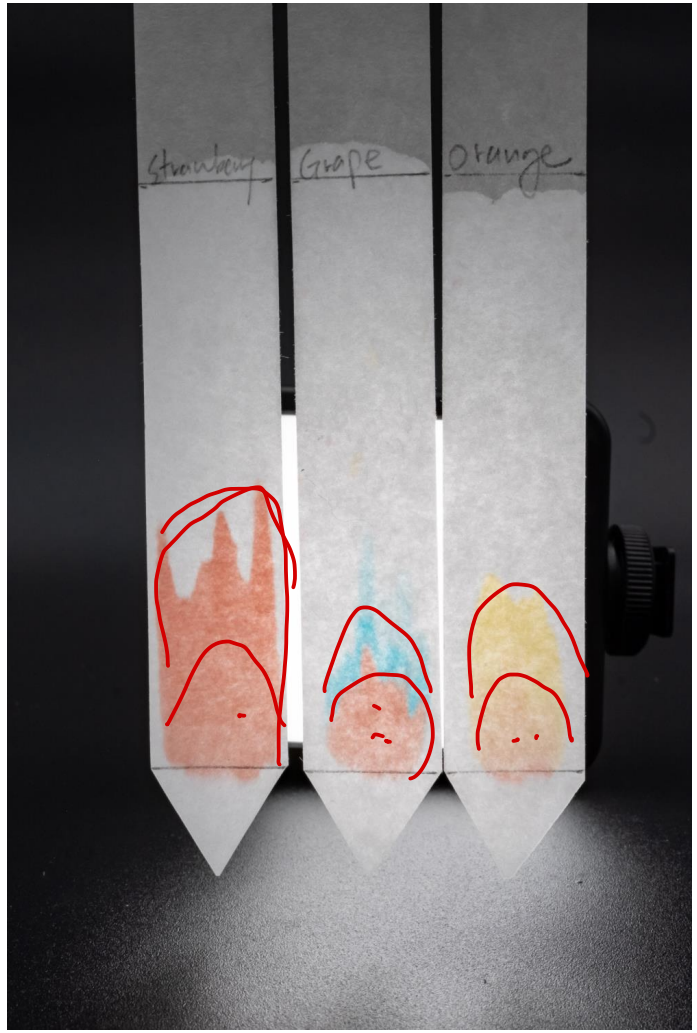


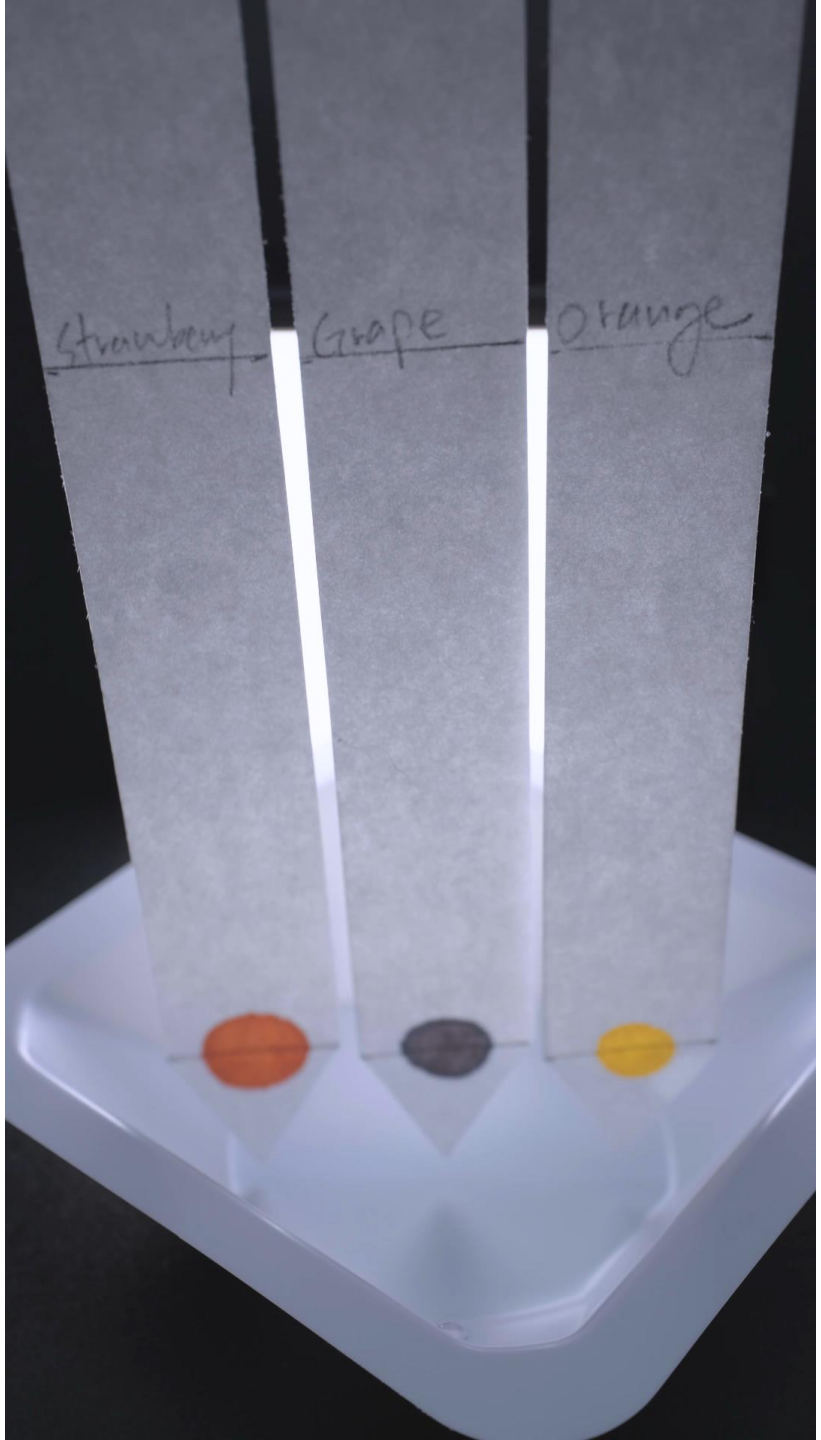
B ÷ A =

Data Table 1: Known FD&C Dyes

FD&C Dye	Color of Band	Distance Colored Band Traveled (mm)	Distance Solvent Front Traveled (mm)	R_f
Red No. 40	Red		9.4 - 1.5 79 mm	
Yellow No. 5	Yellow	9.4 - 1.5 79mm	79mm	1
Blue No. 1	Blue.	8.2 - 1.5 77 mm	79 mm	0.97

Kool-Aid® Chromatograms





Data Table 2: Unknown Mixtures: Kool-Aid® Dyes

Grape

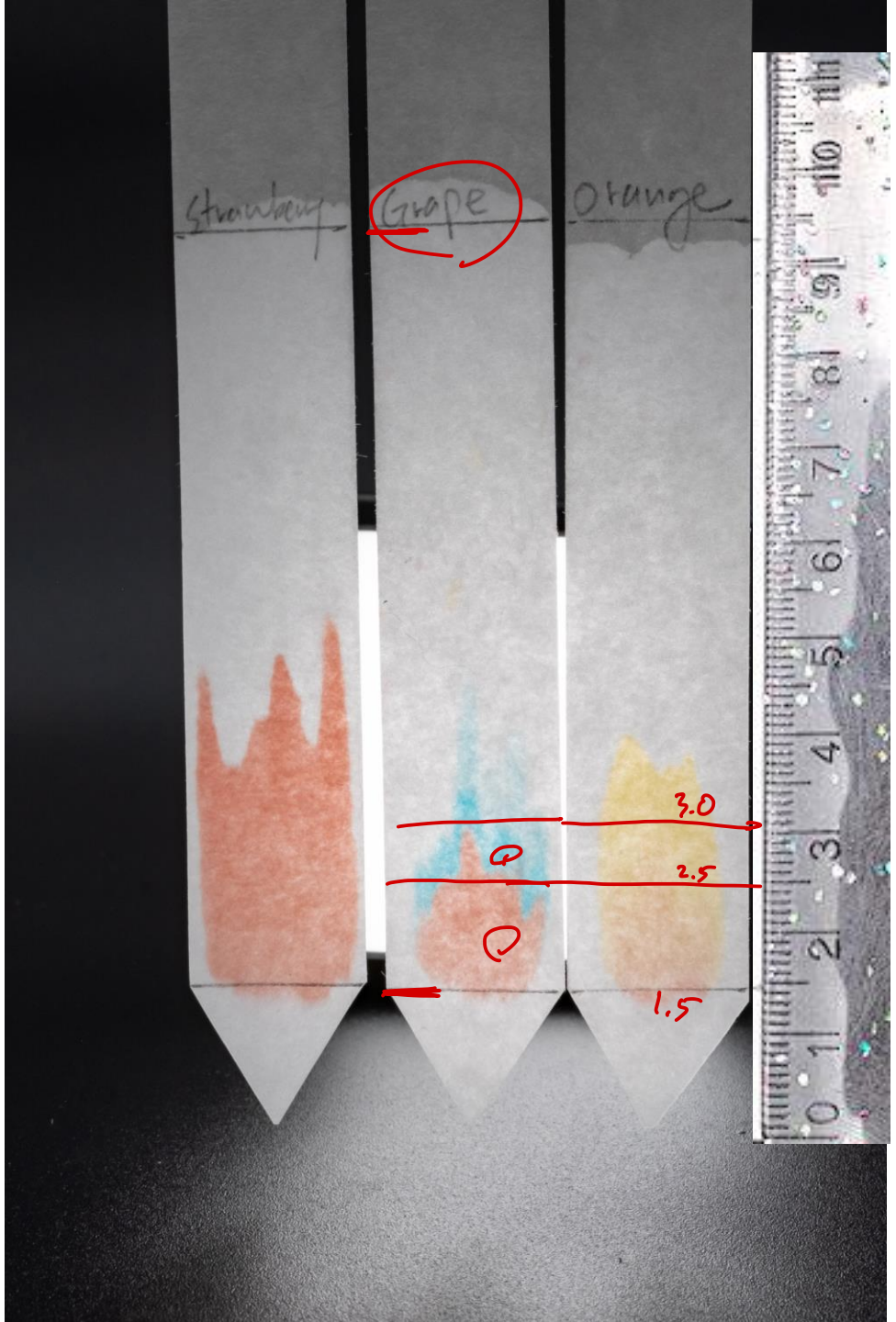
Color of Band(s)	Distance Colored Band Traveled (mm)	Distance Solvent Front Traveled (mm)	R_f	Identity of Dye(s)
Blue	30	79mm	0.3797	
Red	10	79 mm	0.1266	

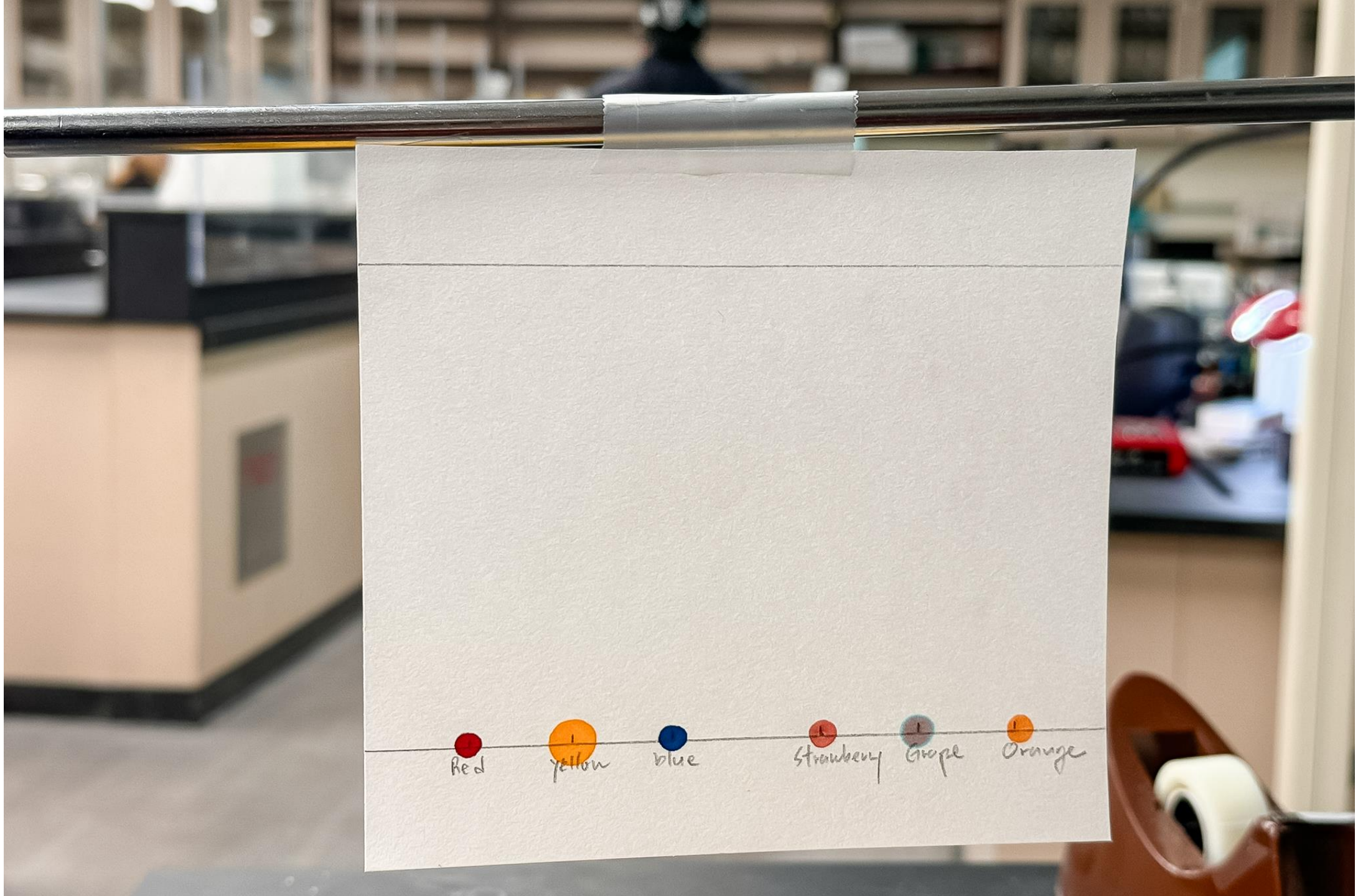
Orange

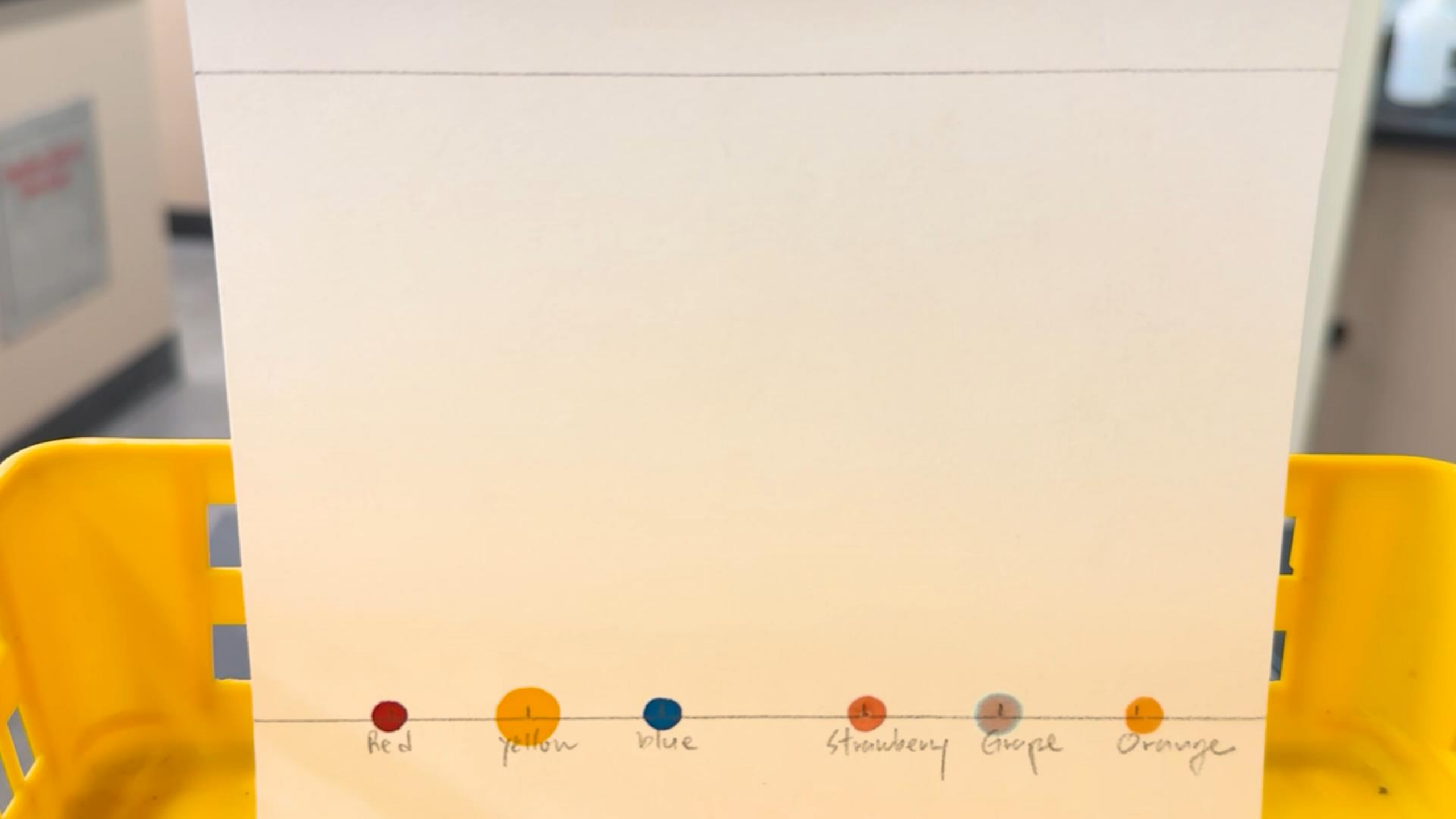
Color of Band(s)	Distance Colored Band Traveled (mm)	Distance Solvent Front Traveled (mm)	R_f	Identity of Dye(s)

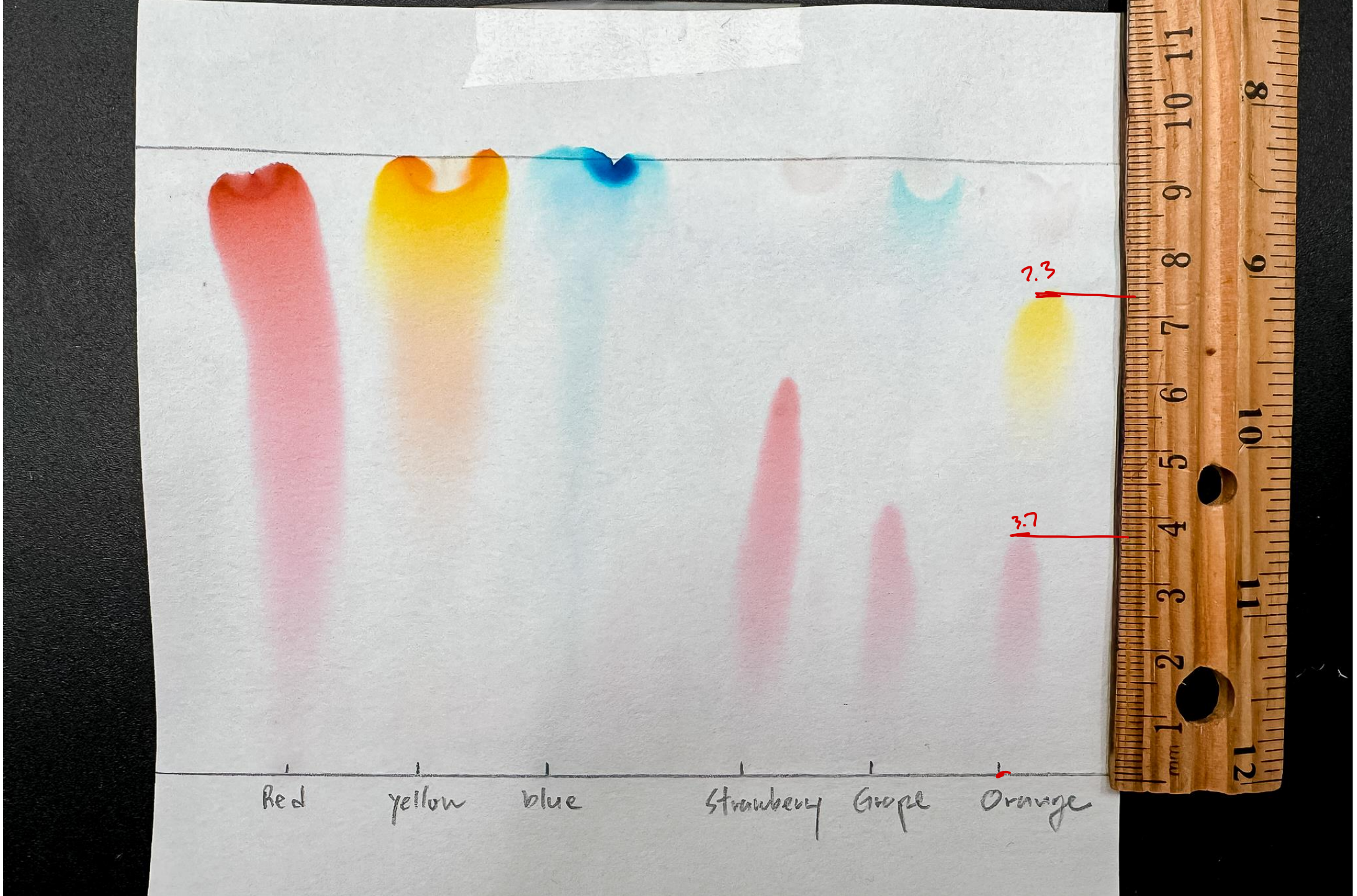
Strawberry

Color of Band(s)	Distance Colored Band Traveled (mm)	Distance Solvent Front Traveled (mm)	R_f	Identity of Dye(s)




Red
yellow
blue
Strawberry
Grape
Orange


Red
yellow
blue
Strawberry
Grape
Orange


7.3
3.7
Red
yellow
blue
Strawberry
Grape
Orange

No photo required.

But feel free to include the end result of the chromatography if you did perform it.

ACTVITY 3

A Inquiry

Activity 3 is an inquiry-based activity to apply the learned technique of chromatography via analyzing a product in your home. This requires the selection of a household product.

All food has ingredient information on the packaging. Look for a food item containing several dyes and develop a method for extracting a highly concentrated sample of the dye(s). Some examples are M&M®-type candies and other highly colored candies. Use the smallest amount of warm water to extract as much food dye from the shell or candy for best results. Another option is to use a dark-colored, water soluble marker pen. Many of these also contain several different water soluble dyes. For this analysis, just tap the pen several times to create the 'starting spot.'

EXTENSION ACTIVITIES

Water-soluble inks of various colors, like those used in dry-erase markers, children's markers, and overhead transparency pens, are excellent sources of dyes for chromatography analysis. White coffee filters can be cut into long rectangular strips as a substitute for chromatography paper if needed. The same test tubes and 0.1% NaCl solvent can be used as in the previous lab activities. The ink from a pen is applied as a spot on the origin line, 1.5 cm from the bottom of the strip of chromatography paper. Try black ink and see how many colors can be identified.

Disposal and Cleanup

1. Dispose of all microtip applicators.
2. Dispose of the plastic bag or aluminum foil containing drops of dyes.
3. Thoroughly rinse all cups containing Kool-Aid® samples and return to equipment set.
4. Pour the 0.1% NaCl solution down the drain, rinse the beaker with water, and return to equipment set.
5. Pour the contents of the three test tubes down the drain, rinse them with water, and place upside down on a paper towel to dry. Return to equipment set when dry.