Class Set!!! Do not Take! Assigned on Wednesday, January 21, 2026

3.2 Virtual Lab: Food Dye Thin Layer Chromatography **Due Friday, January 23, 2026**

Your Task

An unknown substance is known to contain dyes, but which dyes is uncertain. To determine what dyes are in the unknown substance, you will use paper chromatography where you compare the chromatogram of the unknown to the known chromatograms of some selected dyes.

Background

The use of color additives increased dramatically in the United States in the second half of the nineteenth century. As the economy became more industrial, fewer people lived on farms, city populations grew, and people became more dependent on mass produced foods.

Food dyes were initially used to make food more visually appealing to the consumer and, in some cases, to mask poor-quality, inferior, or imitation foods. For example, meat was colored to appear fresh long after it would have naturally turned brown. Jams and jellies were colored to give the impression of higher fruit content than they actually contained. Some food was colored to look like something else—imitation crab meat, for example. Many food colorings and additives were later discovered to be harmful or toxic.

Food colorants were initially added to food with little or no health testing. In 1907, the USDA reduced the number of synthetic food dyes approved for use from 695 to just seven. Only two of the original dyes from 1907 are still accepted for use today. Five others have been added between 1907 and 1971. Only seven dyes are approved for use in the United States today. All of the FD&C approved food dyes are charged, water-soluble organic compounds that bind to natural ionic and polar sites in large food molecules, including proteins and carbohydrates.

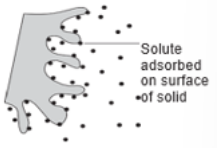


Figure 1. Adsorption of solute particles onto the surface of a solid.

Food dyes can be separated and identified using paper chromatography (PC), a method of separating mixtures of substances with differing polarities. PC is an example of a more general type of chromatography called **adsorption chromatography**. The paper acts as an **adsorbent**, a solid which is capable of attracting and binding the components in a mixture (see Figure 1). The mixture to be separated is "spotted" onto the surface of the paper, the **stationary phase**, and a solvent (**mobile phase**) is allowed to seep or flow through the gel by **capillary action**. If one of the components in the mixture is more strongly adsorbed onto the stationary phase than another, it will move up the paper more slowly than the solvent. Components that are not strongly adsorbed onto the paper will move up the paper at a faster rate. This "partitioning" of the components of a mixture between the stationary phase and the solvent separates the components and gives rise to different bands or spots. If the components of the mixture are colored, like food dyes or pigments in an ink, the colored bands are easily distinguished.

The distance that a sample moves along the stationary phase is compared to the overall distance the solvent travels. This ratio is called the **retention factor, R_f**. In general, food dye molecules that are more polar or have more ionic binding sites will be more strongly attracted to the very polar paper molecules and will have lower R_f values.

- **Chromatography**: a method chemists use to separate one chemical from another using their differences in physical properties. Chromatography has several components:
- **Stationary phase**: To separate chemicals, we have to have a platform on which to separate them. In our case, the platform is chromatography paper. The chromatography paper is extremely polar. The paper does not move in the process of chromatography and is therefore called the stationary phase.
- **Mobile phase**: a mobile phase is the solvent that carries the chemicals through the stationary phase. Dyes don't move on the stationary phase alone, but if we add a solvent, it can draw the dyes up through the stationary phase through the process of capillary action.
- **Point of origin**: the initial position of the dyes on the stationary phase.
- **Developing**: after putting chemicals on the point of origin, the chromatogram can be "developed", that is, the mobile phase can be pulled through the stationary phase.
- **Retention factor (R_f)**:The distance our chemicals move during chromatography is typically less than the distance the mobile phase moves. We can measure the difference in distance by using the R_f value. This is the distance a chemical moves on the stationary phase during our separation divided by the distance the mobile phase moves. Different solvents will lead to different retention values due to their different polarities and therefore different attractions to the substance being tested. The following formula is used to determine R_f:

$$R_f = \frac{distance\ solute\ travels}{distance\ solvent\ travels}$$

- **Chromatograph**: the finished result from chromatography that shows the separated solute(s) on the stationary phase after the mobile phase has dried. Figure 2 shows an example of a chromatograph similar to what you will produce in this lab.

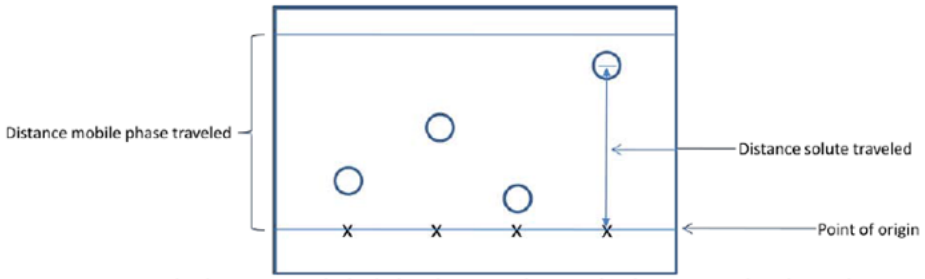


Figure 2: An example chromatograph depicting the areas that need to be measured to determine R_f. The round spots are the separated solutes, and the large square is the stationary phase (chromatography paper).

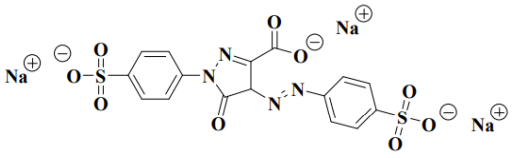
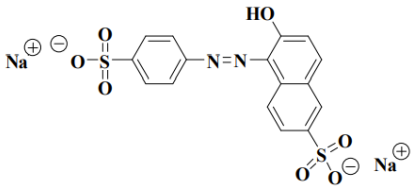
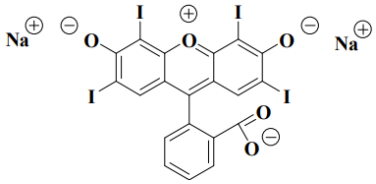
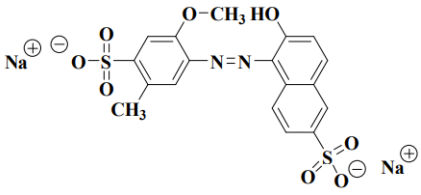
Materials

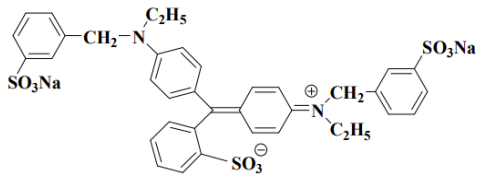
- Unknown sample containing a mixture of dyes
- Known Red Dye
- Known Black Dye
- Known Yellow Dye
- 2% sodium bicarbonate solution (used as the mobile phase)
- 1 100-mL beaker
- chromatography paper
- pencil
- ruler
- micro capillary tubes

Procedure

Watch the video (link here) to see the procedure carried out.

Structures of the Food Dyes

FD&C Yellow 5	
FD&C Yellow 6	
FD&C Red 3	
FD&C Red 40	

FD&C Blue 1	

Name: _____ Period: _____ Assigned on Wednesday, January 21, 2026

3.2 Lab: Food Dye Chromatography **Due Friday, January 23, 2026**

Prelab Questions

1. What is the process of chromatography used for?

2. In chromatography, components of a mixture spend some time adsorbed on a stationary phase and some time dissolved in a mobile phase. Explain how the components can be separated with these two phases.

3. How could someone slightly change the polarity of the mobile phase in a chromatography experiment? (Hint: Look in the materials list at the composition of the solvent used as the mobile phase.)

Data

Dye	Distance Travelled (mm)		R_f
	Solvent	Spot	
Yellow (A)			
Black (B)			
Red (C)			
Unknown			

Analysis

1. Show a sample calculation for how you determined the R_f values.

2. What is meant by polarity of molecules? What causes differences in polarity?

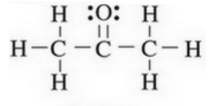
3. In discussing solubility, the rule "like dissolves like" is frequently used. What does this mean?

4. Considering that the chromatography paper (the stationary phase) was more polar than the solvent (the mobile phase), which of the dyes tested is most polar? Which was the least polar? Consider macroscopic observations and interactions between the dye and the solvent OR between the dye and the paper (IMFs). Provide qualitative and quantitative evidence to support your reasoning.

5. What dyes were present in the unknown dye mixture? Support your answer with **quantitative** evidence.

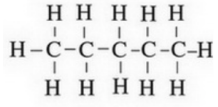
9. The following diagrams show the Lewis structures of four different molecules. Rank the following substances in expected order of increasing R_f value (lowest to highest) in a paper chromatography experiment using a solvent with low polarity.

A. Methanol



B. Acetone

C. Pentane



D. Ether