Chromatography

Objectives:

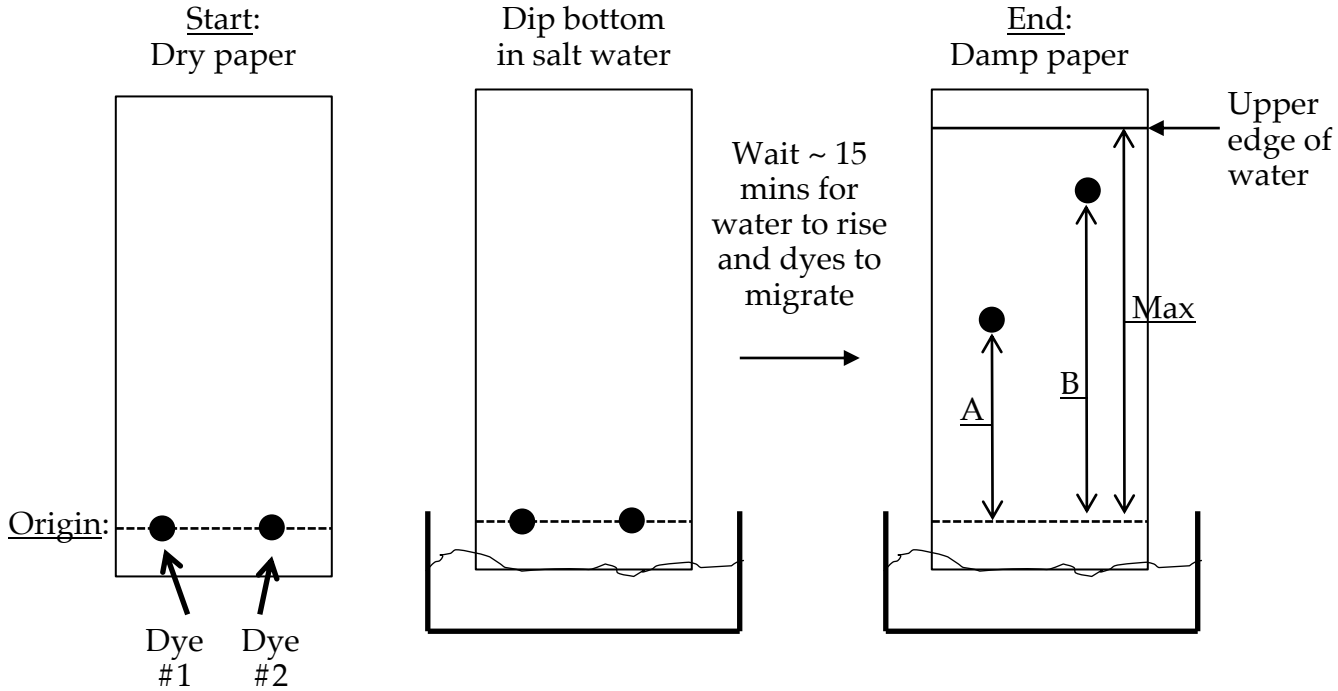
- To explore the relationship between chemical structures and their properties
- To discover patterns in the behavior of certain chemicals
- To experience how scientists develop and test hypotheses

Introduction:
Chromatography is the general name for a wide range of techniques that are used to separate molecules based on their properties. In general, chromatography involves two phases:

- A **Stationary Phase**. This is typically a solid. In today's lab, it will be paper.
- A **Moving Phase**. This is typically a liquid. In today's lab, you can choose between water and hexane.

In general, the sample is applied to one end of the stationary phase and the moving phase is allowed to flow over the stationary phase. Depending on the properties of each molecule in the sample, it will be carried along with the moving phase to varying degrees. The degree to which a molecule is carried along by the moving phase can be expressed as a fraction called R_f (Retention factor) which is the distance traveled by the molecule divided by the distance traveled by the moving phase.

In today's lab, you will apply various colored dye molecules to paper and try to find a relationship between their structures and their R_f values. This is shown below:



The R_f for Dye #1 = A/Max
The R_f for Dye #2 = B/Max

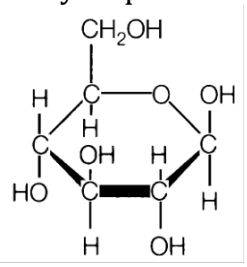
What controls R_f? The R_f reflects the relative affinity of the dye for the stationary and moving phases.

- If the dye binds to the paper to a greater extent than it dissolves in the moving phase, the dye will only move a short distance and the R_f will be low.
- If the dye dissolves in the moving phase to a greater extent than it binds to the paper, the dye will move a long distance and the R_f will be high.

It turns out that very little is known in detail about the exact features of a dye molecule that govern its R_f. Most people who do chromatography are interested in the fact that it separates different molecules not why different molecules move different distances. This is where we come in.

Here is some relevant information.

- Water is, not surprisingly, extremely hydrophilic. Molecules that are charged and/or can make hydrogen bonds will form strong non-covalent bonds with and therefore dissolve well in water.
- Paper is mostly cellulose. Cellulose is a polymer of glucose. The structure of glucose is shown below. It is moderately hydrophilic with many –OH groups that can form hydrogen bonds. It is more hydrophobic than water because of the many C-H groups.



- Hexane is a hydrocarbon with the structure $H_3C\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CH_3$. It is extremely hydrophobic.

Important notes:

- Many of the dye molecules in this lab contain halogen atoms like chlorine (-Cl) or bromine (-Br). We have not talked about these atoms in Bio 111 and you will only see them in this lab. Because these are large atoms, even though they are electronegative, adding halogens to a molecule tends to make a molecule more *hydrophobic*. Also, because it is larger, bromine tends to make molecules more hydrophobic than chlorine does.

- Many of the dye molecules contain sulfur atoms making 6 covalent bonds. Although this is possible (and you'll hear more about why this is possible in your Chemistry courses), this lab is the only situation where you'll see this in Bio 111. Most of the time, in Bio 111, sulfur will make only two covalent bonds.

Assignment
Find out what you can about the relationship between structure and R_f for these molecules. We are much more interested in a small but solid conclusion than an all-encompassing but not well supported one. Since little is known about this, we will have to see what you can find.

How will you do this? Run paper chromatograms of different dyes; compare their R_f values; and look for patterns you can test and confirm or refute. You will pool your data as a class at various points to see what you can or cannot conclude and which experiments should be done next.

Good experiments start with good hypotheses. This is very important. If you start with a good question, the chances of getting a clear answer are much higher.

Wikipedia has a useful definition of 'hypothesis': **A hypothesis is a proposed explanation for a phenomenon. For a hypothesis to be a scientific hypothesis, the scientific method requires that one can test it.**

In this lab, there are three features that make a good hypothesis:

1. *It should be clear and specific*.
2. *It should be based on what we know about chemical properties*.
3. *It should be testable*.

Note that the hypothesis does not have to be correct – it is fine if the data you collect contradict your hypothesis. The hypothesis just has to be reasonable, based on what we know about chemical structures.

Example hypotheses (please do not use these in your work; they're just for illustration):

- "*Dyes with more chlorine atoms will be more hydrophobic. As a result, they will not travel with the water and will have lower R_f values than those with fewer or no chlorine atoms*". This is clear and specific: it mentions specific chemical structures (chlorine atoms) and makes a clear prediction about R_f values. It is based on what we know about chlorine atoms (they make molecules hydrophobic in general). It is testable: to test it, you'd find several dyes that differed in the number of chlorine atoms and compare their R_f values and if the ones with more chlorine atoms had lower R_f values, it would be confirmed; if not, it would be disconfirmed. *This could be a good place from which to start your experiments.*

- "*The structure of the dye determines the R_f value.*" This is not specific: it doesn't mention any specific structural details and how they might influence the R_f value. Although it may be based on what we know about chemical properties, it is so vague that you can't really be sure. It is also not testable – there's no experiment you could do that could possibly disprove it since all dyes have different structures and different R_f values. *This would <u>not</u> be a good place from which to start your experiments.*

- "*Structures with more -OH groups will make fewer H-bonds with water and, thus, will have lower R_f values."* This is clear, specific, and testable, but it is not consistent with what we know about chemical bonding since -OH groups will make H-bonds with water, molecules

with more -OH groups will make more H-bonds with water, not fewer. *This, too, would not be a good place from which to start your experiments.*

Two other hints. Scientists often use these two different types of comparisons to explore a phenomenon.

- *Pick extreme cases*. For example, compare two molecules that are very different in some particular property. If they have different R_fs, then this feature is likely to be relevant.
- *Pick very similar molecules.* For example, two molecules that differ in one –OH group. That way, you can say that any difference in R_f is due to this one change.

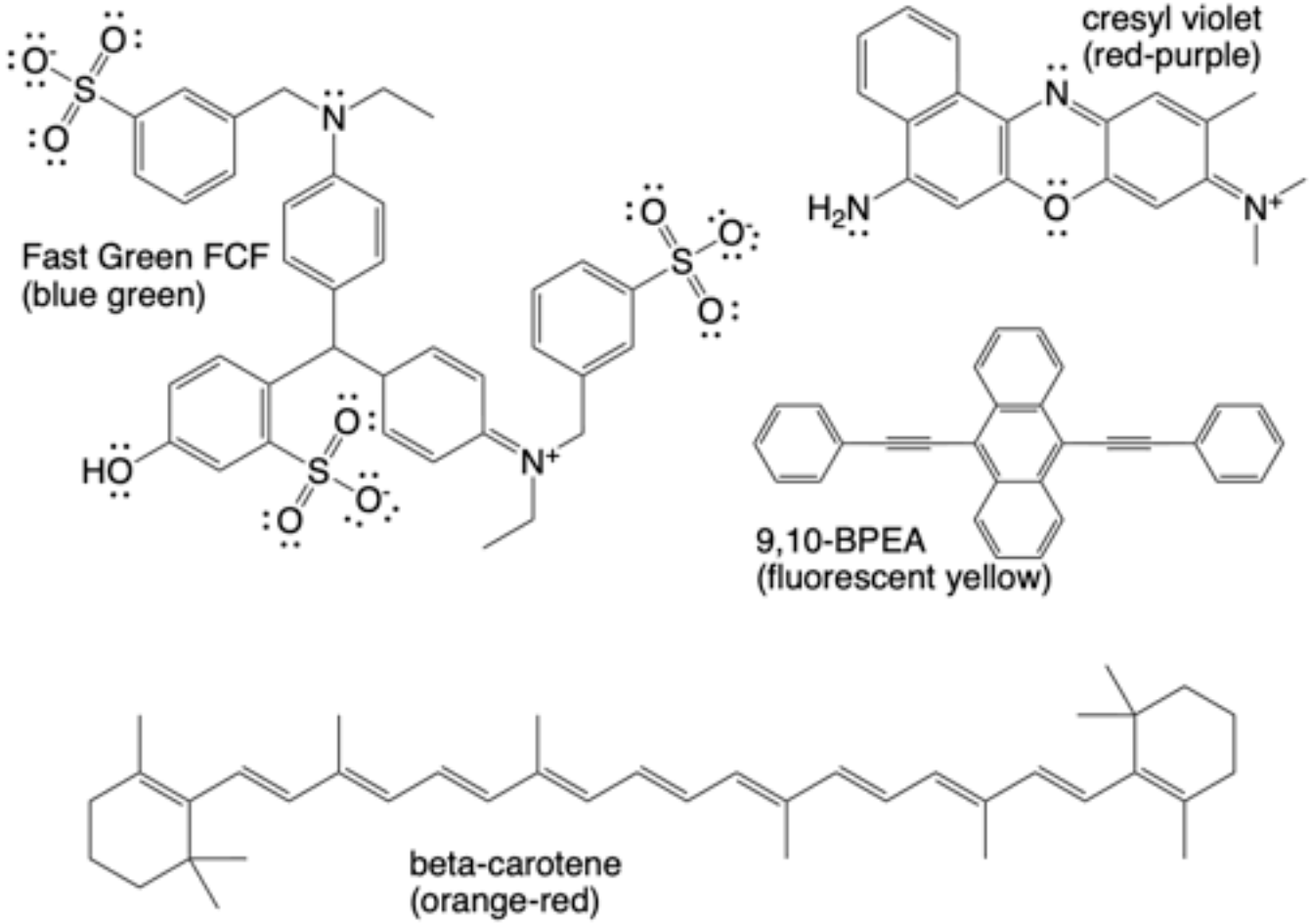
First: Notebook Review

To be sure that you have all the information you need for this lab, with your lab partners, go over the notes you took while doing the SPOC and from lecture and be sure you have the information listed below. You should fill in any gaps in your notes so everyone in your group has all they need. You can check these items off as you go.

- How can I convert an abbreviated structure into a complete structure (showing all carbon, hydrogen, etc. atoms)?
- Which groups of atoms can form ionic bonds?
    - How are these shown in abbreviated form?
- Which groups of atoms can form hydrogen bonds?
    - How are these shown in abbreviated form?
- Which groups of atoms make a molecule more/less hydrophobic?
    - How are these shown in abbreviated form?
- Which groups of atoms make a molecule more/less hydrophilic?
    - How are these shown in abbreviated form?
- Atom for atom, which is stronger, hydrophobicity or hydrophilicity?
- Which groups of atoms can form a hydrophobic interaction?
    - How are these shown in abbreviated form?

You will then discuss these with your TA as a class to clarify any issues that remain.

Warm-up Exercise: Reading Chemical Structures:
Consider the four dye molecules:



For each of the molecules above, indicate the parts that are hydrophobic and those that are hydrophilic.

Two of these dyes are soluble in water and two are not. Predict which is which in terms of the bonds that each molecule can make with water and their hydrophobicity/hydrophilicity.

Your TA will demonstrate this solubility as follows. He/she will have prepared tubes with water on the bottom layer and hexane ($H_3CCH_2CH_2CH_2CH_2\ CH_3$ – very hydrophobic) on the top layer (remember that oils like hexane float on water). He/she will then add a small amount of a solution of each dye to the test tubes.

- Hydrophilic dyes will make favorable interactions with water, leaving the water colored and the hexane colorless.
- Hydrophobic dyes will not make favorable interactions with water and the hydrophobic effect will drive them to be in the hexane. Thus they will color the hexane and not the water.

Based on your analysis, predict which dyes will be found in water or hexane.

Procedure
You will use this same procedure for each of your chromatography runs.

You can choose one of two mobile phases for each of your experiments:

- **water** – very hydrophilic. We actually use 0.15% $NaHCO_3$ but the small amount of salt present does not have a big effect on the R_f. Chromatograms using water can be run on your benchtop.
- **hexane** – very hydrophobic. Chromatograms using hexane must be run in the hood.

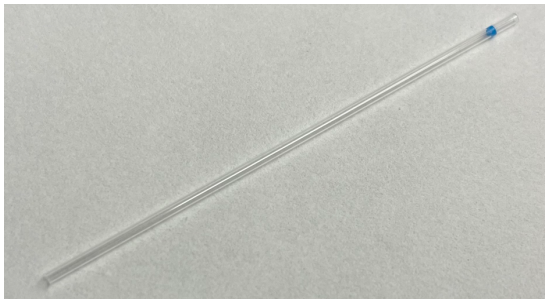
1) Prepare a developing tank by pouring 10 mL of the mobile phase (water or hexane) into a 400 mL beaker and covering the beaker with a watch glass. It is important that the air above the mobile phase become saturated with solvent vapor so that solvent does not evaporate from the stationary phase as the chromatogram develops. Therefore, be sure to keep the developing tank covered at all times. Remember that all hexane chromatograms must be run in the hood.

Be sure to take careful notes about which mobile phase you used.

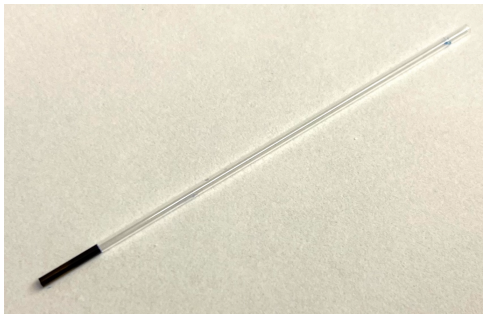
2) With a **pencil**, draw a horizontal line 1.5 cm from the bottom edge of the chromatography paper. Draw vertical tick marks along this line every 2 cm (see figure 2a).

3) Using a capillary and one of the standard dye solutions, make a spot on the chromatography paper at one of the marks.

Capillary tube



Capillary tube with dye in it



Try to keep your spots less than 4 mm in diameter. Allow the dye to dry and reapply the same dye in the same spot 1 or 2 times or until a sufficiently dark spot has been achieved. With a **pencil**, note the name of the dye below the spot.

4) Repeat steps 1 and 2 for the remaining dyes across the bottom of the chromatogram.

5) When the spots have been applied, put your name in pencil across the top of the chromatography paper, form the chromatography paper into a cylinder, and staple the edges of the paper together making sure to leave a gap between the edges as shown in figure 2b. If the edges come into contact, solvent will not travel at a uniform speed up the chromatography paper and the components of the mixture will not move in a straight line.

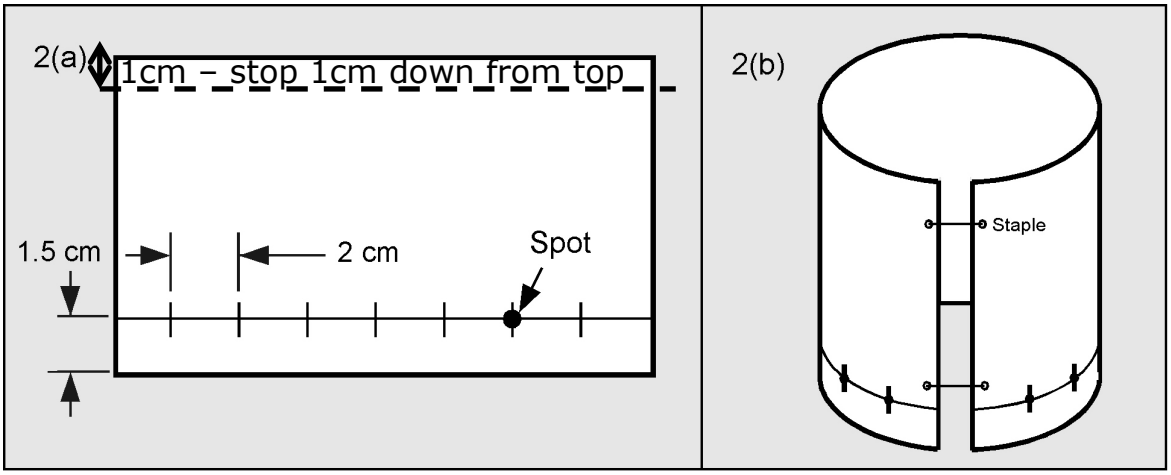


Figure 2 Paper Chromatogram. (a) Schematic for laying out spots. (b) Chromatogram ready for developing tank.

6) Place the chromatography paper into the developing tank, do not let it touch the sides of the tank and quickly replace the watch glass cover. Make sure the level of the mobile phase is below the line of dyes on your paper. Allow the chromatogram to develop.

7) When the solvent front is approximately 1 cm from the top of the chromatography paper, remove the chromatogram and lay it flat on a paper towel. Immediately mark the position of the solvent front with a pencil. The front will continue to move as the paper dries so it is important that you mark this position now. Measure and note the distance the solvent front traveled ($D_{solvent}$, see fig 3.).

8) Draw an ellipse around each spot on the developed chromatogram and draw a horizontal line through the center of each spot. If a spot shows significant "tailing" make your horizontal line through the darkest part of the spot (see fig 3). Use the distance from the starting line (not the bottom of the paper!) to these horizontal lines to determine D_{dye} for each dye. Record distances and R_f values in your notebook. Recall:

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

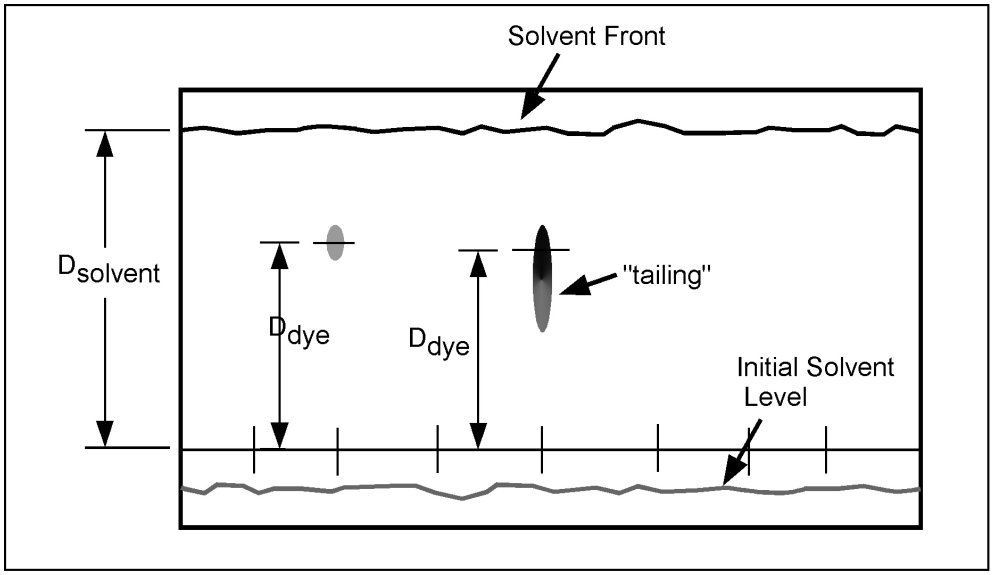
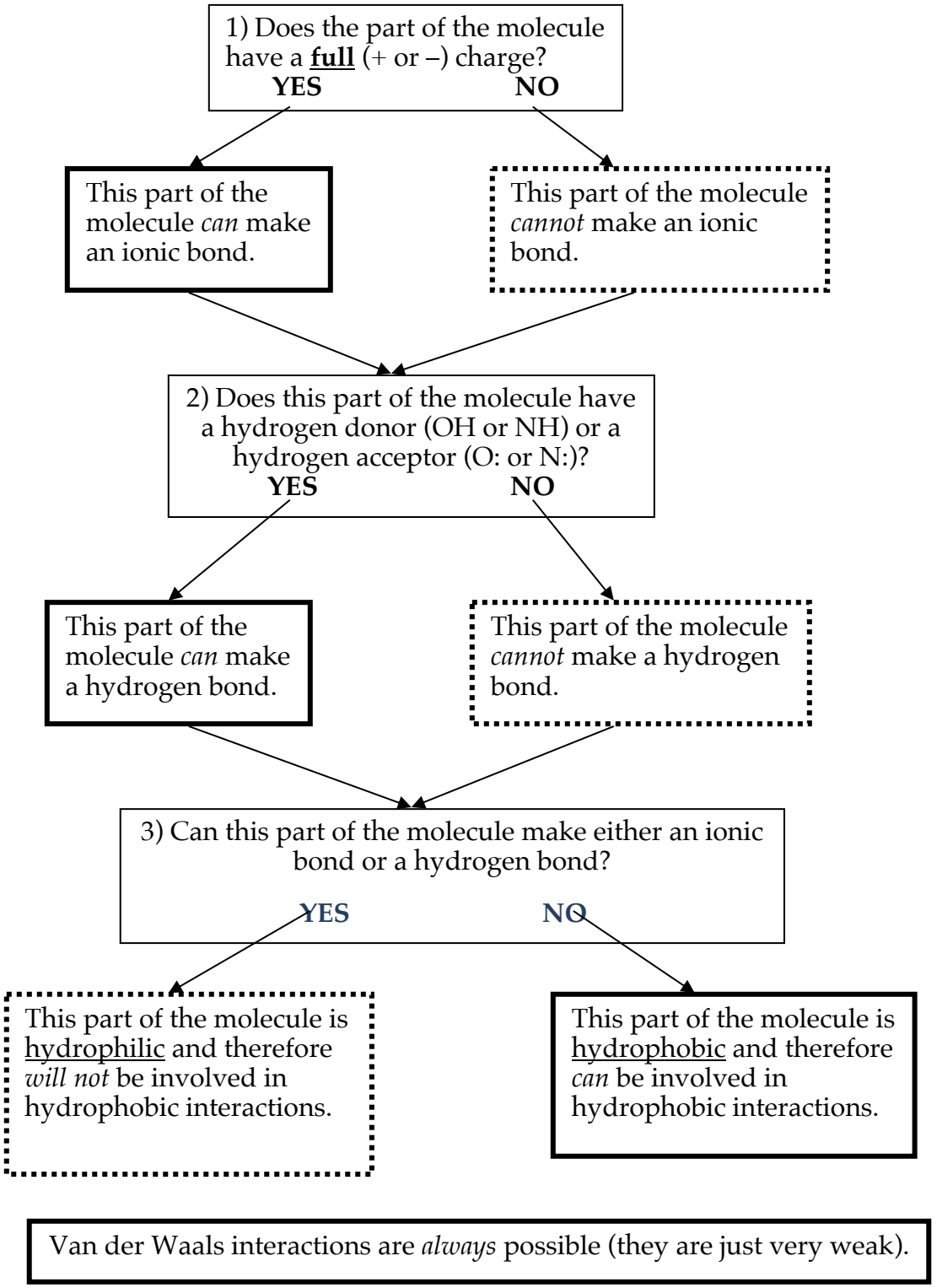


Figure 3 Developed Chromatogram. Immediately mark solvent front when paper is removed from the developing tank. The spot on the right exhibits significant tailing and the distance the spot has traveled has been correctly identified.

You will need to carry out several chromatograms and pool your class data to reach solid conclusions.

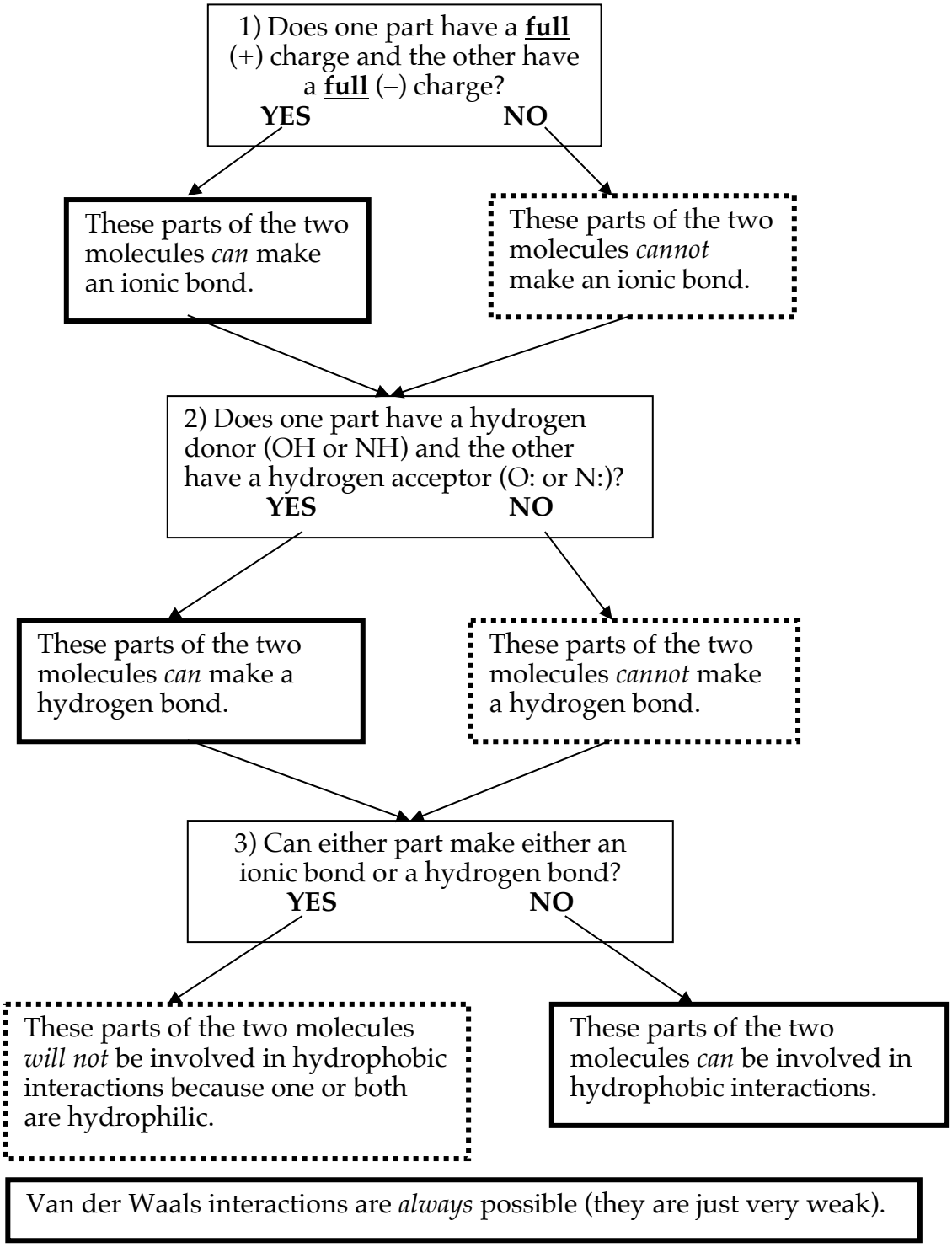
You may find the following information useful:

1) <u>What kind(s) of interaction(s) can this part of a molecule make</u>? Since it takes two items to make a bond, the bond couldn't form without a "suitable partner." Either explicitly or implicitly, this question assumes the existence of a suitable partner. For these, the following flowchart applies:



Van der Waals interactions are *always* possible (they are just very weak).

2) <u>What kind(s) of interactions are possible between these two (parts of) molecules</u>? In this case, you have to determine whether the other molecule is a suitable partner. This is a slightly more restrictive question than (1). The flowchart below applies in this case. Note that the questions now ask about the other molecule(s).



Preparing for the Take Home Exam

Take Home Exam 2 will be, in part, based on this lab. You should look at the exam on Blackboard before you leave lab today. You may want to use some of the remaining time in lab to prepare for the exam.

How to take a proper screenshot for the exam

In order for your TA to be able to grade your exam, they will need some clear screenshots of various things as specified in the exam. *Screenshots taken with your phone are not acceptable*.

- On Macintosh:
  - Hold the control, shift, and command keys and press 4
  - You will see a little cross-hair cursor appear
  - Drag the cursor over the part of the screen you want to copy
  - When you let go of the mouse, the screenshot will be on the clipboard
  - You can then paste it into a word or google doc

- On PC:
  - Hold the windows and shift keys and press S
  - The screen will change tone and you can drag the cursor to select the part of the screen you want to copy.
  - When you let go of the mouse, the screenshot will be on the clipboard
  - You can then paste it into a word or google doc

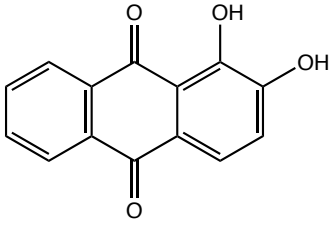
You can also google "screenshot windows" or "screenshot os x" as appropriate and follow the directions there.

The structures of the dyes used in this lab are shown on the following pages in alphabetical order.

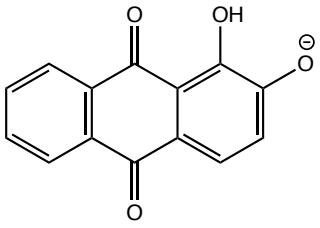
Note that many of the dyes have more than one structure. Fortunately, the different structures have different colors so you can use the color to know which structure is present in your chromatography run. You may not always see both colors.

Structures of dyes used in this lab.

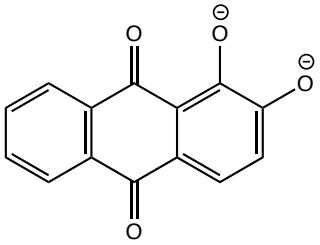
Alizarin red



(yellow)

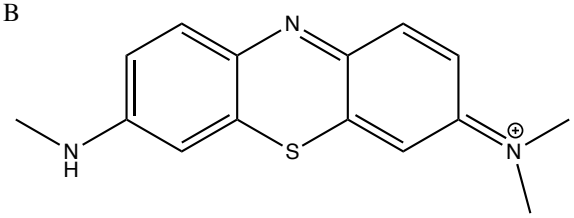


(red)

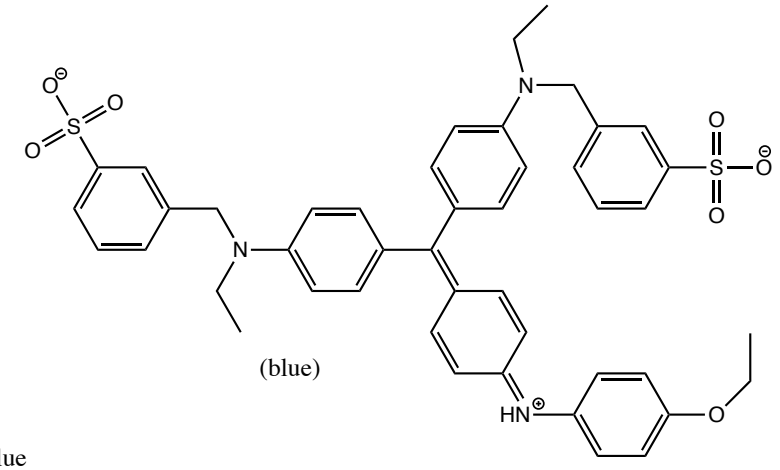


(purple)

Azure B

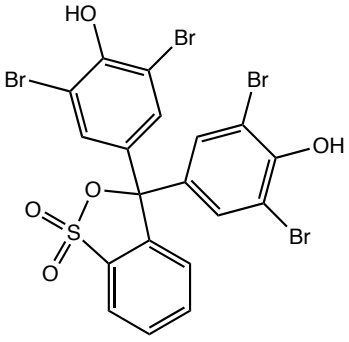


Brilliant Blue G

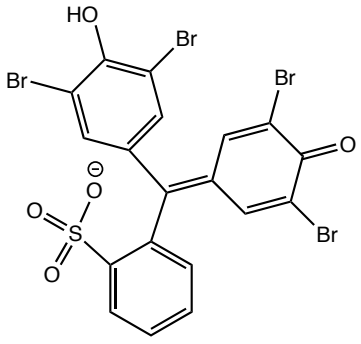


(blue)

Bromophenol Blue

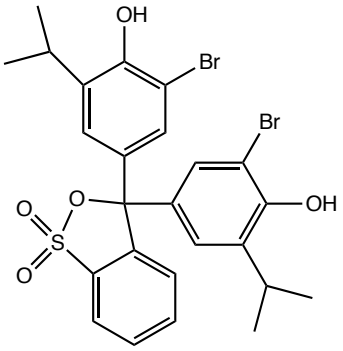


(yellow)

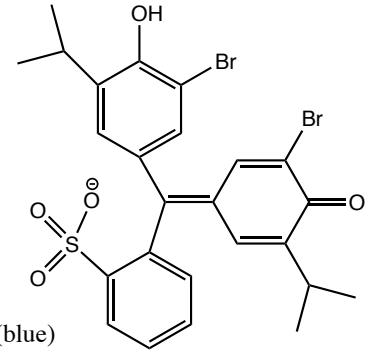


(blue)

Bromothymol blue

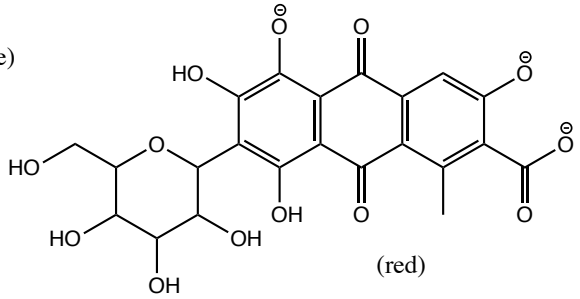


(yellow)



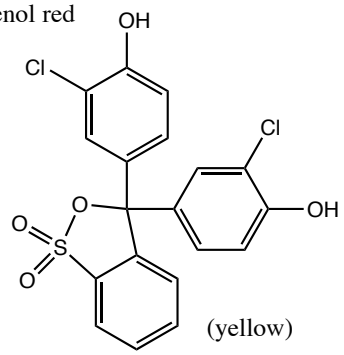
(blue)

Carmine (Alum lake)

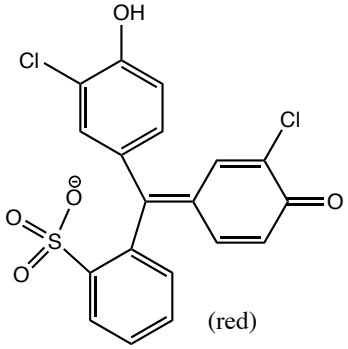


(red)

Chlorophenol red

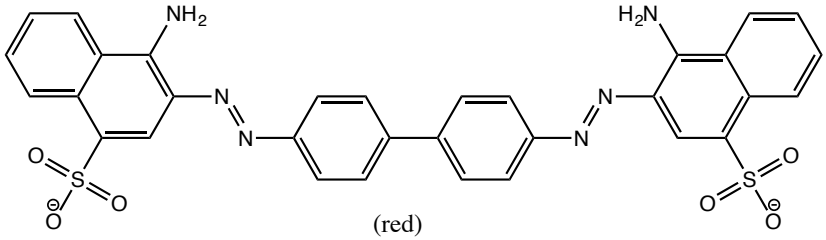


(yellow)



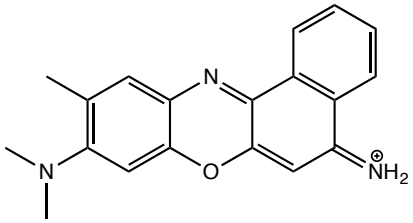
(red)

Congo red



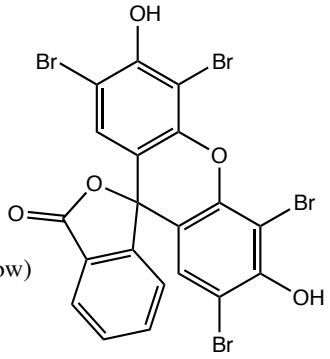
(red)

Cresyl Violet



(purple)

Eosin Y



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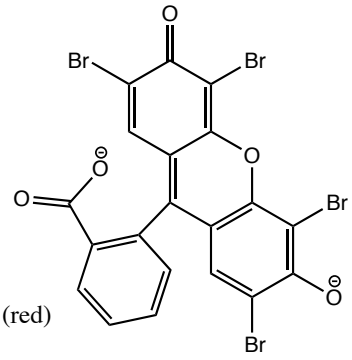
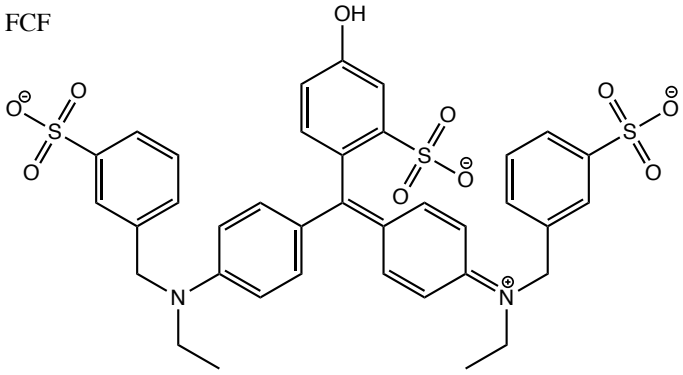


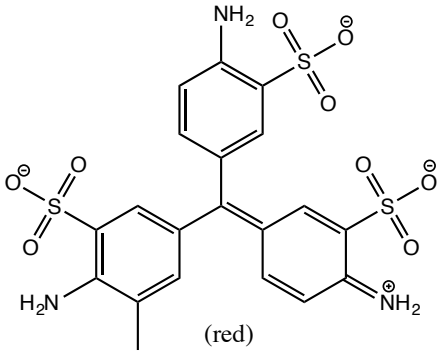
(red)

Fast Green FCF



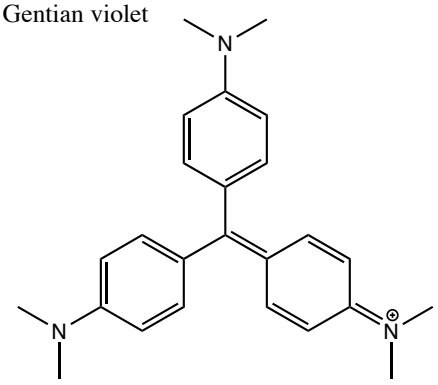
(green)

Fuchsin (acid)



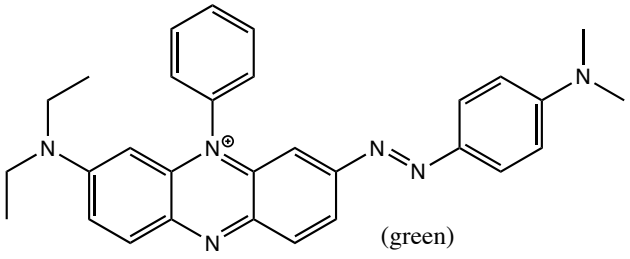
(red)

Gentian violet



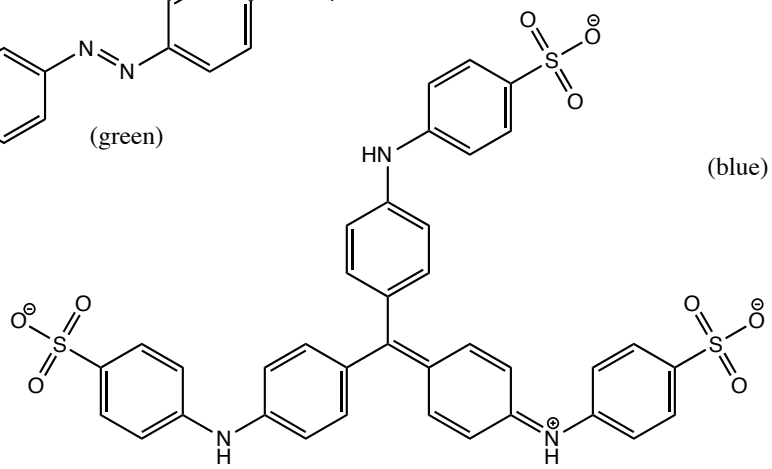
(purple)

Janus Green B



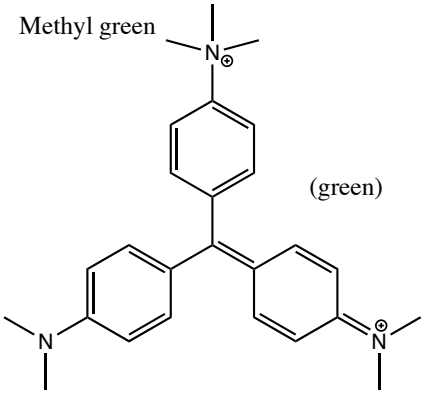
(green)

Methyl blue



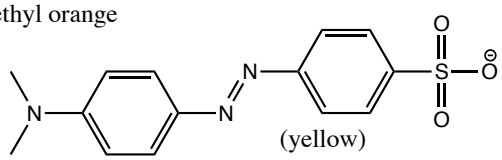
(blue)

Methyl green

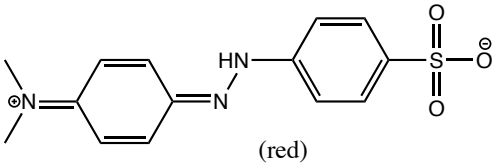


(green)

Methyl orange

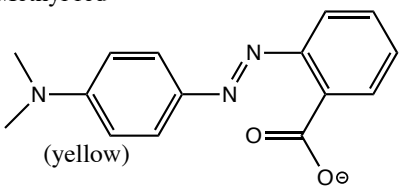


(yellow)

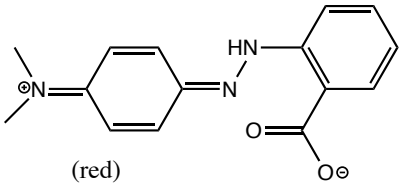


(red)

Methyl red

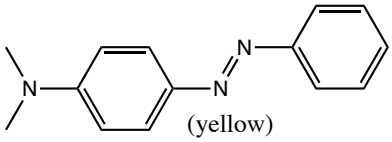


(yellow)

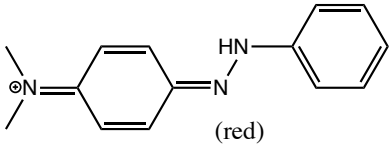


(red)

Methyl yellow

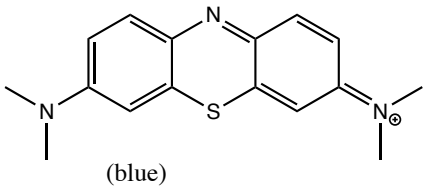


(yellow)



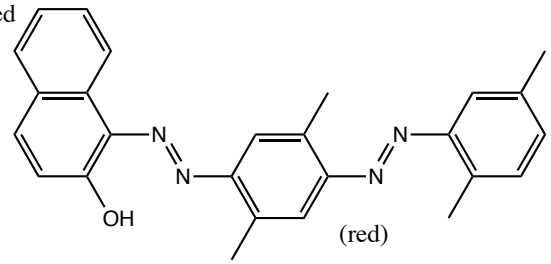
(red)

Methylene blue



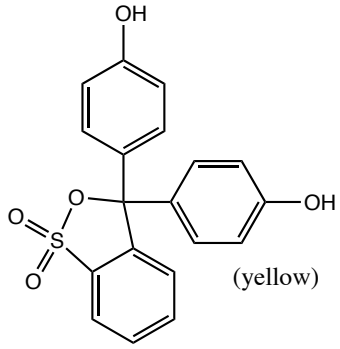
(blue)

Oil red

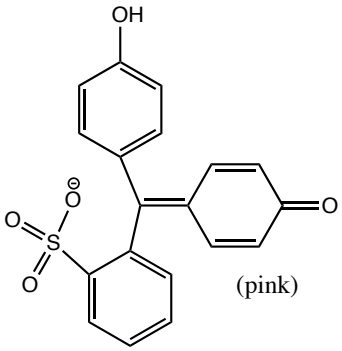


(red)

Phenol red

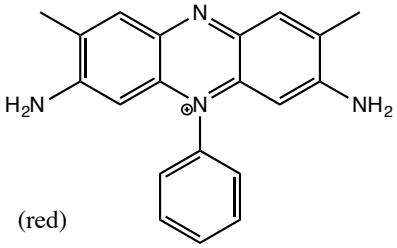


(yellow)



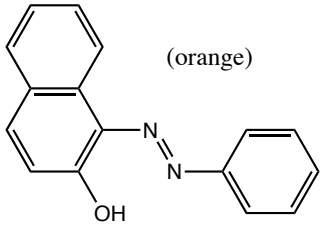
(pink)

Safranin O



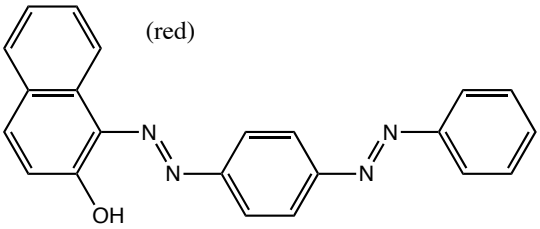
(red)

Sudan I



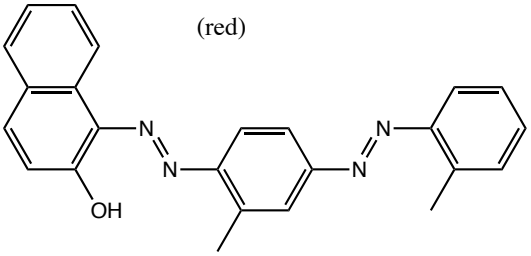
(orange)

Sudan III



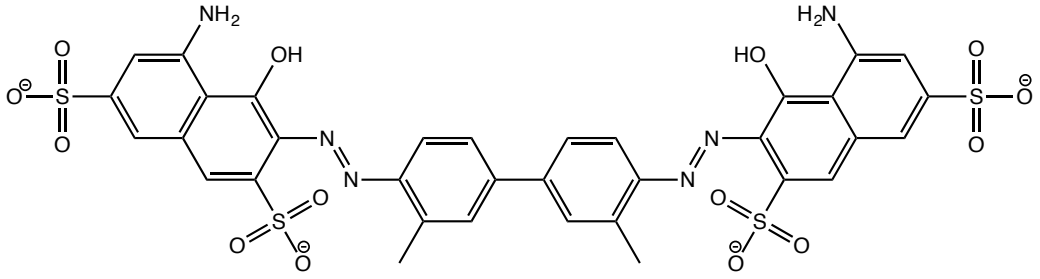
(red)

Sudan IV



(red)

Trypan Blue



(blue)