Unit I (b)
Paper Chromatography

Presentation by
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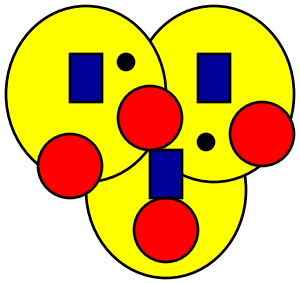
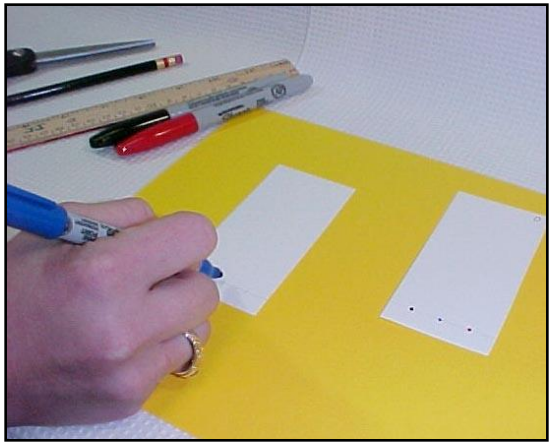
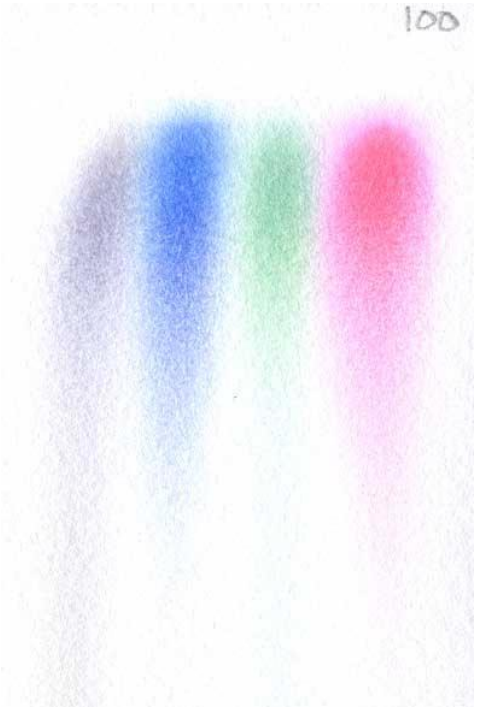
Subject : PHARMACEUTICAL ANALYSIS- II (15R00602)

Paper Chromatography

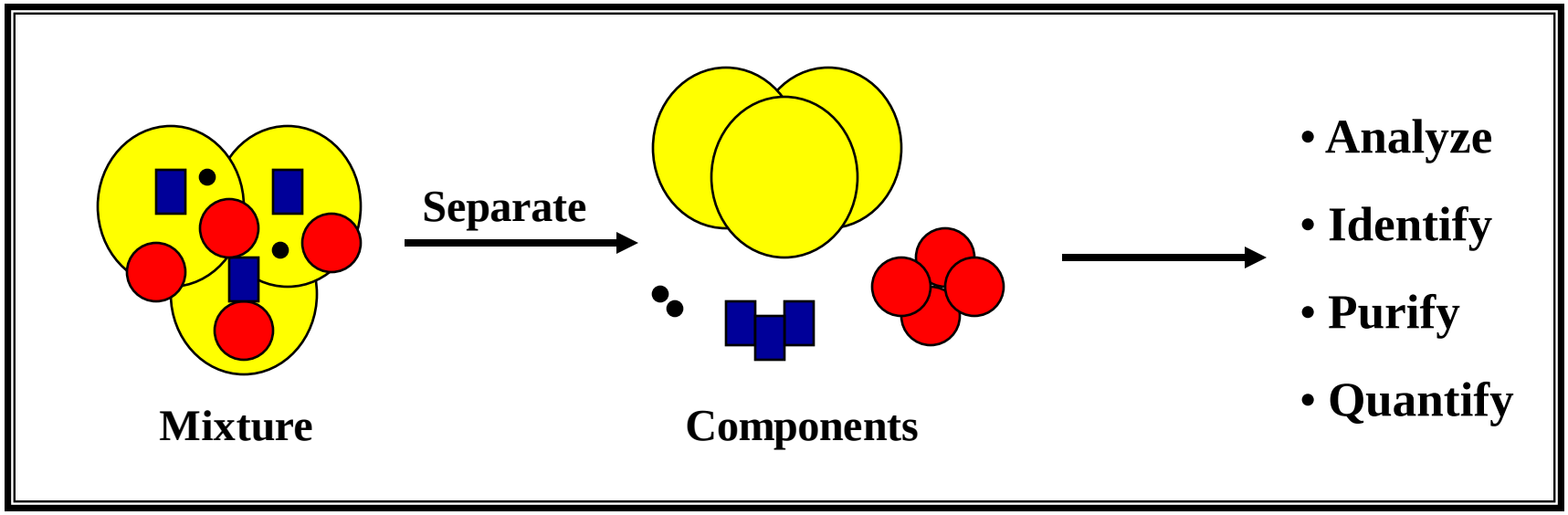






What is Chromatography?

Chromatography is a technique for separating mixtures into their components in order to analyze, identify, purify, and/or quantify the mixture or components.



Uses for Chromatography

Chromatography is used by scientists to:

- Analyze – examine a mixture, its components, and their relations to one another
- Identify – determine the identity of a mixture or components based on known components
- Purify – separate components in order to isolate one of interest for further study
- Quantify – determine the amount of the a mixture and/or the components present in the sample

Uses for Chromatography

Real-life examples of uses for chromatography:

- Pharmaceutical Company – determine amount of each chemical found in new product
- Hospital – detect blood or alcohol levels in a patient's blood stream
- Law Enforcement – to compare a sample found at a crime scene to samples from suspects
- Environmental Agency – determine the level of pollutants in the water supply
- Manufacturing Plant – to purify a chemical needed to make a product

Definition of Chromatography

Detailed Definition:

Chromatography is a laboratory technique that separates components within a mixture by using the differential affinities of the components for a mobile medium and for a stationary adsorbing medium through which they pass.

Terminology:

- *Differential* – showing a difference, distinctive
- *Affinity* – natural attraction or force between things
- *Mobile Medium* – gas or liquid that carries the components (mobile phase)
- *Stationary Medium* – the part of the apparatus that does not move with the sample (stationary phase)

Definition of Chromatography

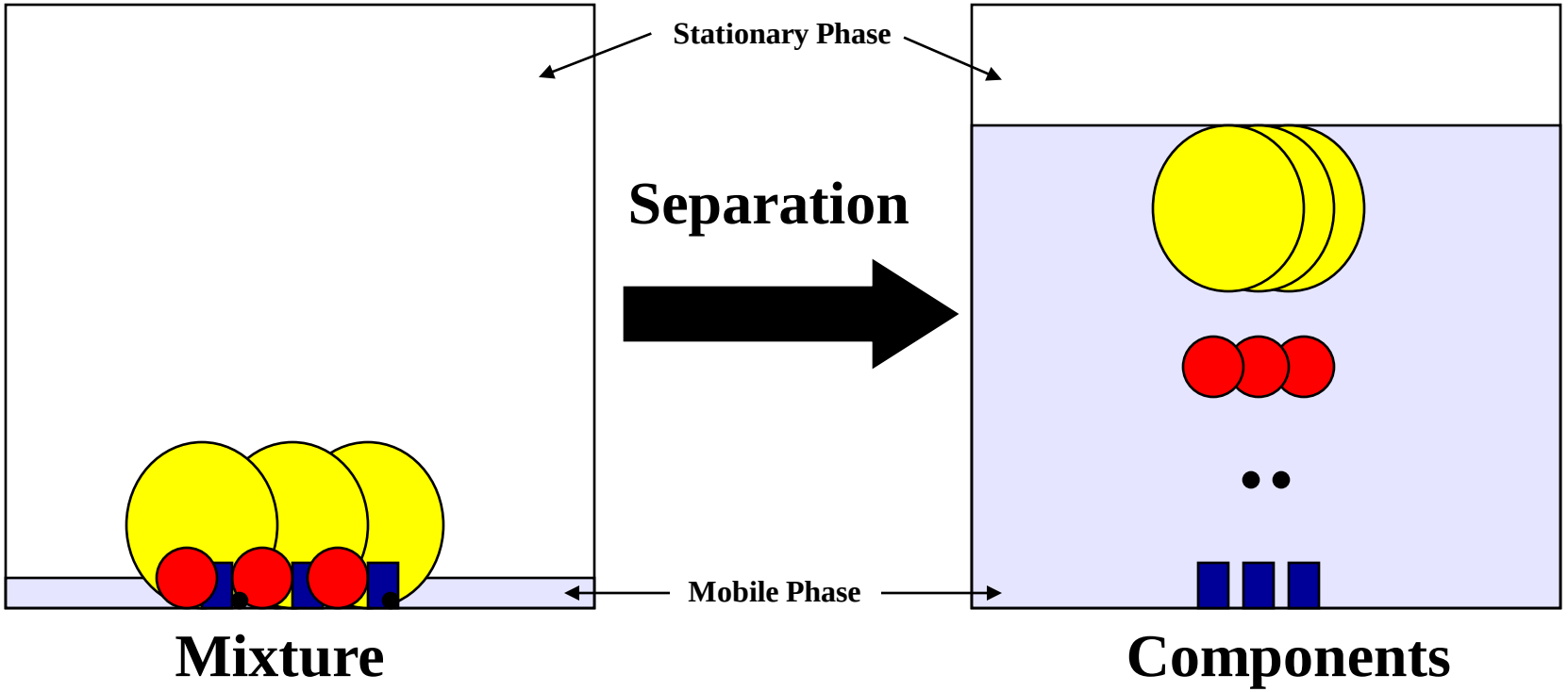
Simplified Definition:

Chromatography separates the components of a mixture by their distinctive attraction to the mobile phase and the stationary phase.

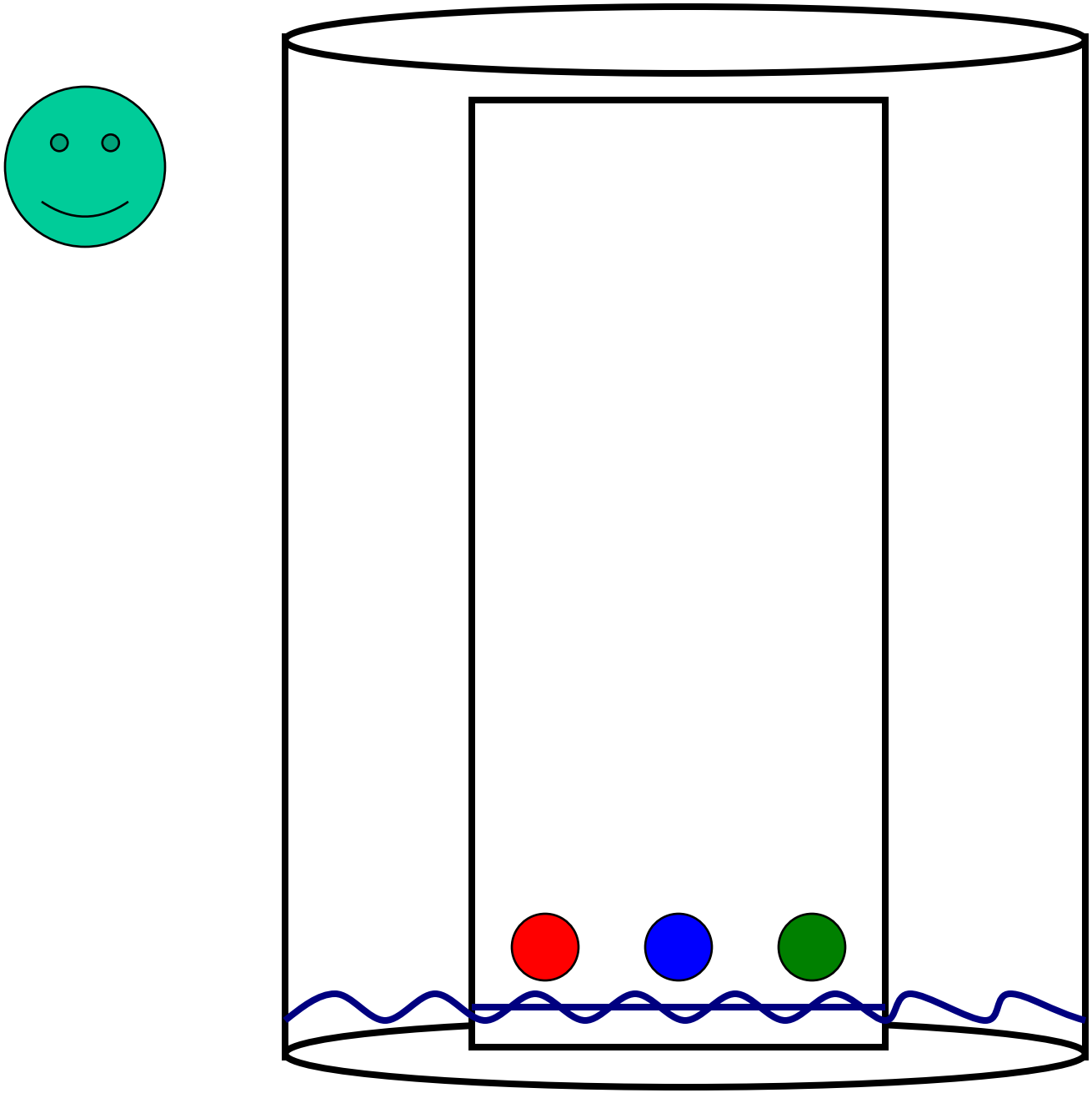
Explanation:

- Compound is placed on stationary phase
- Mobile phase passes through the stationary phase
- Mobile phase solubilizes the components
- Mobile phase carries the individual components a certain distance through the stationary phase, depending on their attraction to both of the phases

Illustration of Chromatography



Components	Affinity to Stationary Phase	Affinity to Mobile Phase
Blue	----------	Insoluble in Mobile Phase
Black	✓✓✓✓✓✓	✓✓
Red	✓✓	✓✓✓✓✓
Yellow	✓	✓✓✓✓✓✓✓✓✓✓✓



Types of Chromatography

- Liquid Chromatography – separates liquid samples with a liquid solvent (mobile phase) and a column composed of solid beads (stationary phase)
- Gas Chromatography – separates vaporized samples with a carrier gas (mobile phase) and a column composed of a liquid or of solid beads (stationary phase)
- Paper Chromatography – separates dried liquid samples with a liquid solvent (mobile phase) and a paper strip (stationary phase)
- Thin-Layer Chromatography – separates dried liquid samples with a liquid solvent (mobile phase) and a glass plate covered with a thin layer of alumina or silica gel (stationary phase)

Principles of Paper Chromatography

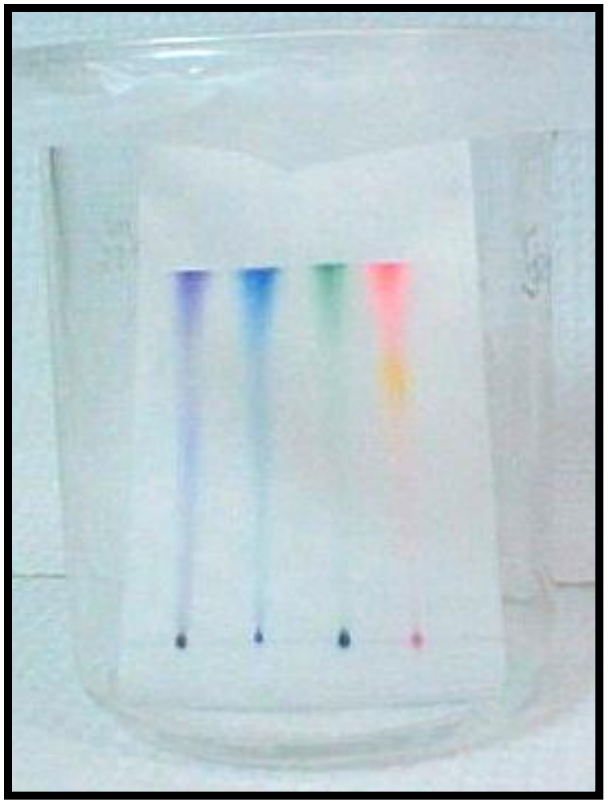
- Capillary Action – the movement of liquid within the spaces of a porous material due to the forces of adhesion, cohesion, and surface tension. The liquid is able to move up the filter paper because its attraction to itself is stronger than the force of gravity.

- Solubility – the degree to which a material (solute) dissolves into a solvent. Solutes dissolve into solvents that have similar properties. (Like dissolves like) This allows different solutes to be separated by different combinations of solvents.

  Separation of components depends on both their solubility in the mobile phase and their differential affinity to the mobile phase and the stationary phase.

Paper Chromatography Experiment

What Color is that Sharpie?



Overview of the Experiment

Purpose:

To introduce students to the principles and terminology of chromatography and demonstrate separation of the dyes in Sharpie Pens with paper chromatography.

Time Required:

Prep. time: 10 minutes

Experiment time: 45 minutes

Costs:

Less than $10

Materials List

- 6 beakers or jars
- 6 covers or lids
- Distilled H_2O
- Isopropanol
- Graduated cylinder
- 6 strips of filter paper
- Different colors of Sharpie pens
- Pencil
- Ruler
- Scissors
- Tape

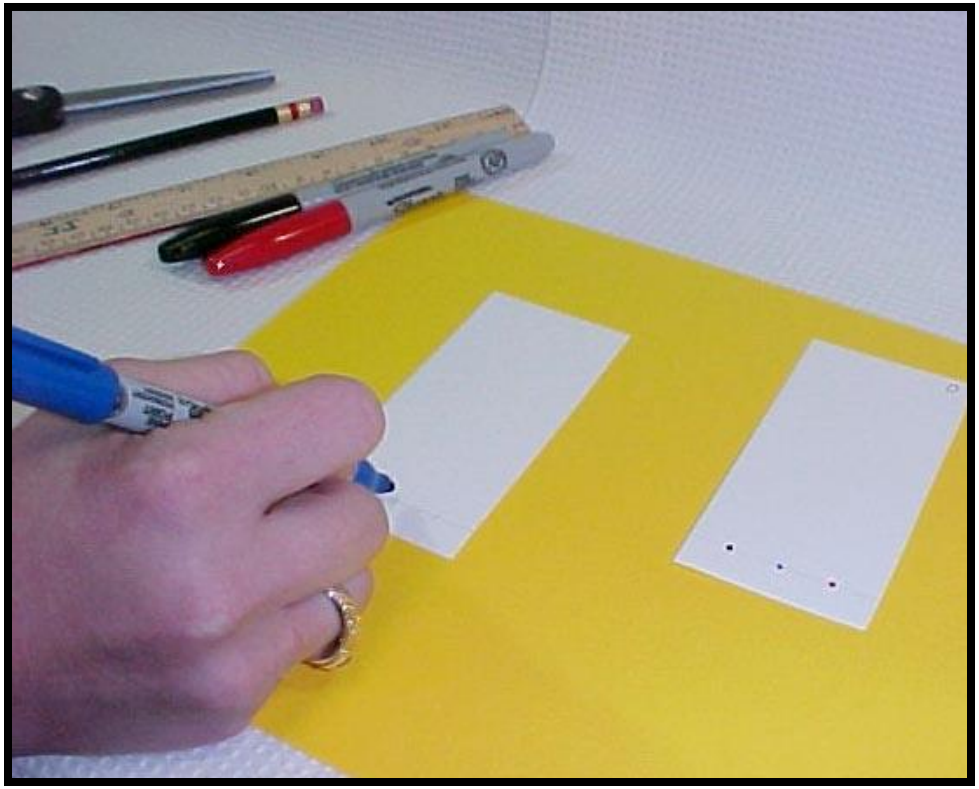
Preparing the Isopropanol Solutions

- Prepare 15 ml of the following isopropanol solutions in appropriately labeled beakers:

  - 0%, 5%, 10%, 20%, 50%, and 100%

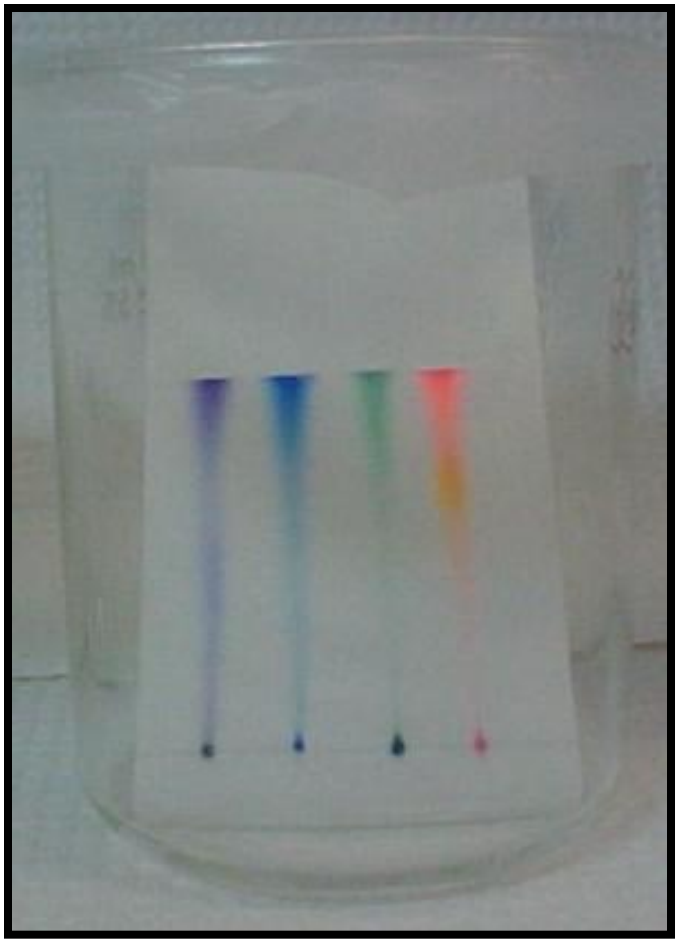
Preparing the Chromatography Strips

- Cut 6 strips of filter paper
- Draw a line 1 cm above the bottom edge of the strip with the pencil
- Label each strip with its corresponding solution
- Place a spot from each pen on your starting line



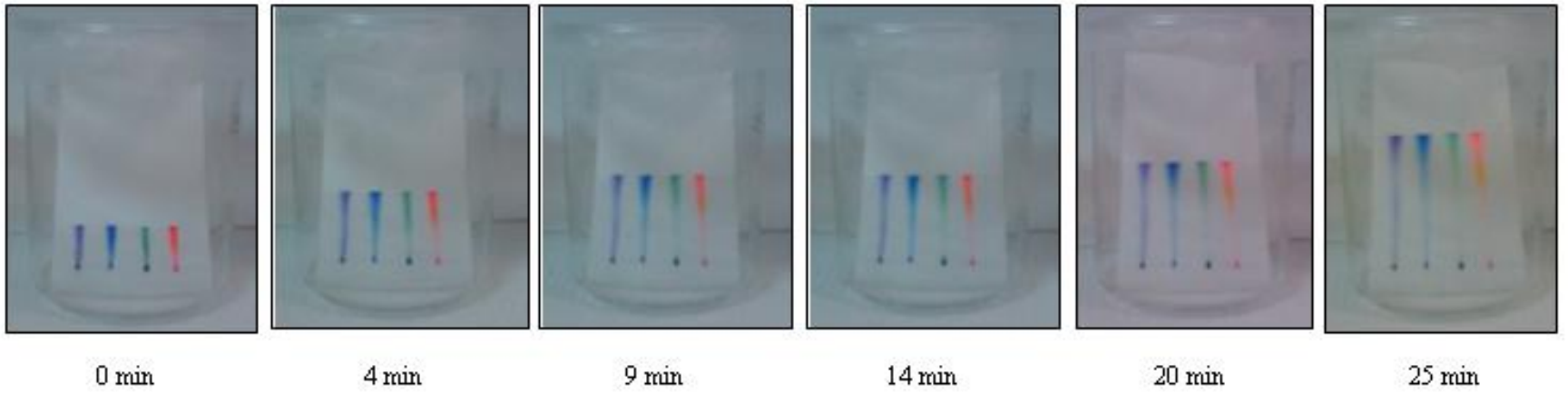
Developing the Chromatograms

- Place the strips in the beakers
- Make sure the solution does not come above your start line
- Keep the beakers covered
- Let strips develop until the ascending solution front is about 2 cm from the top of the strip
- Remove the strips and let them dry

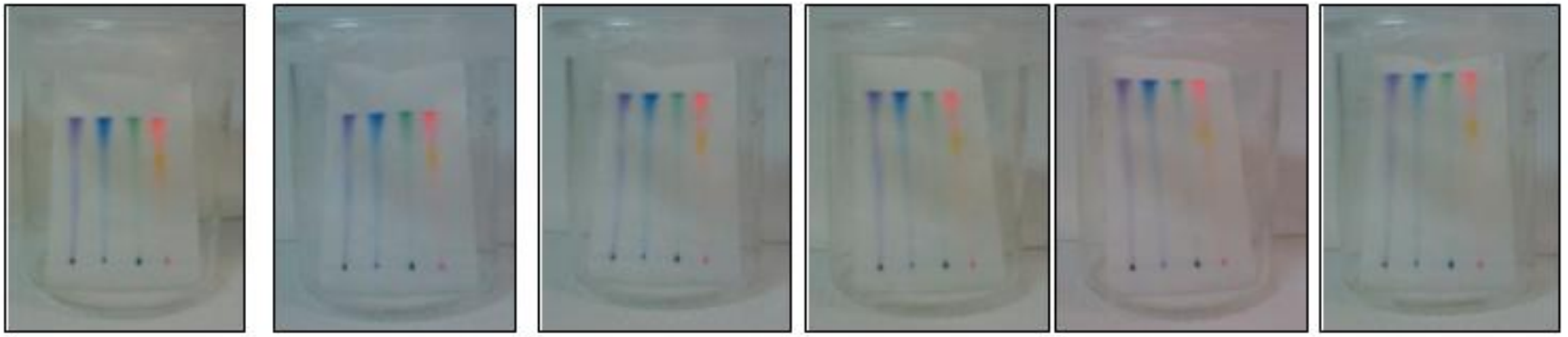


Developing the Chromatograms

50% Isopropanol



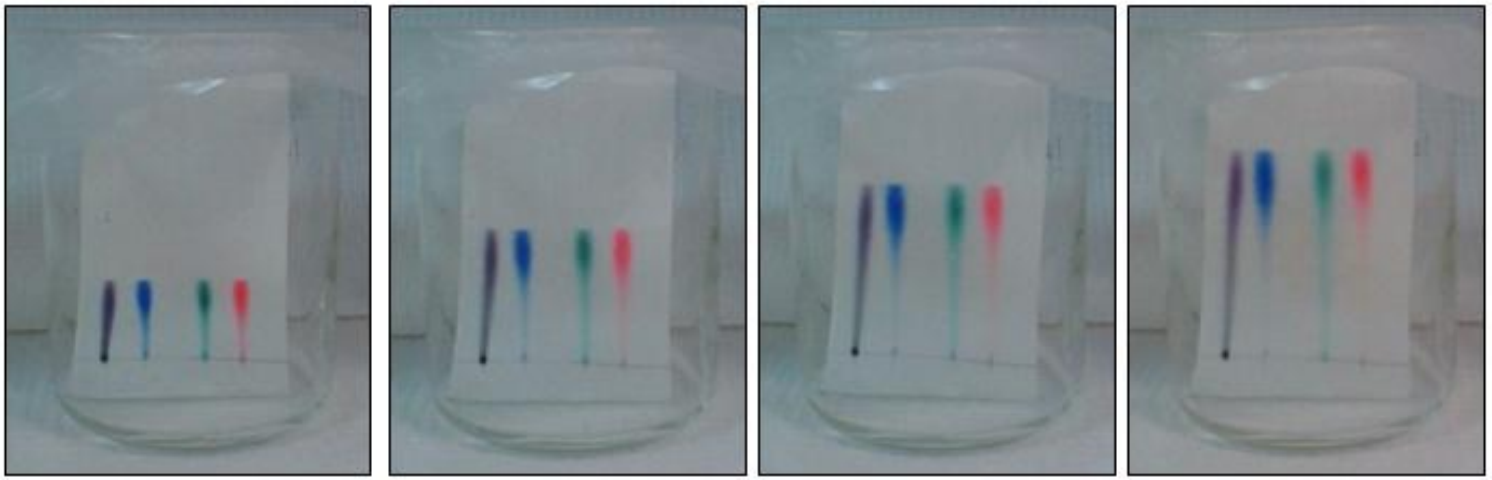
0 min | 4 min | 9 min | 14 min | 20 min | 25 min



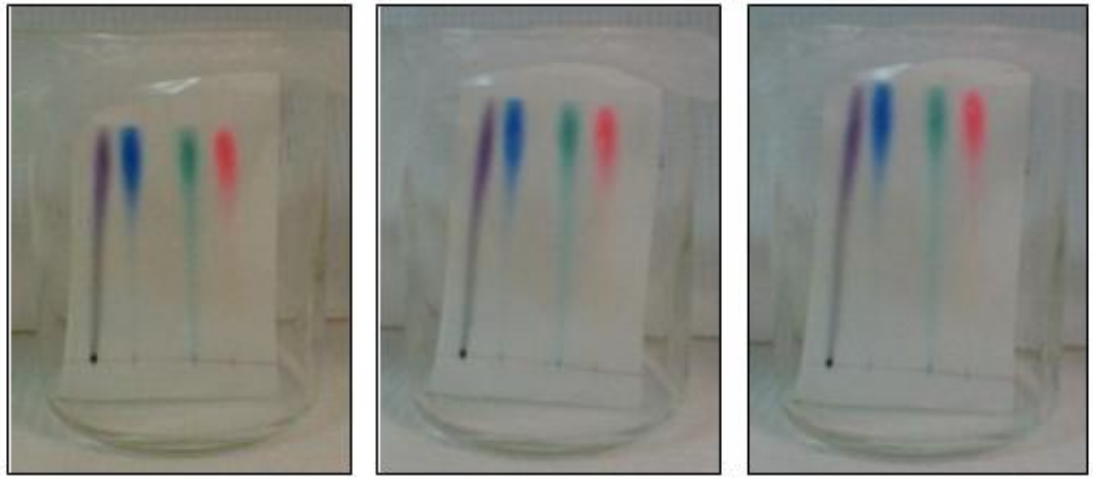
30 min | 35 min | 40 min | 45 min | 50 min | 55 min

Developing the Chromatograms

100% Isopropanol



0 min 4 min 9 min 14 min



20 min 25 min 30 min

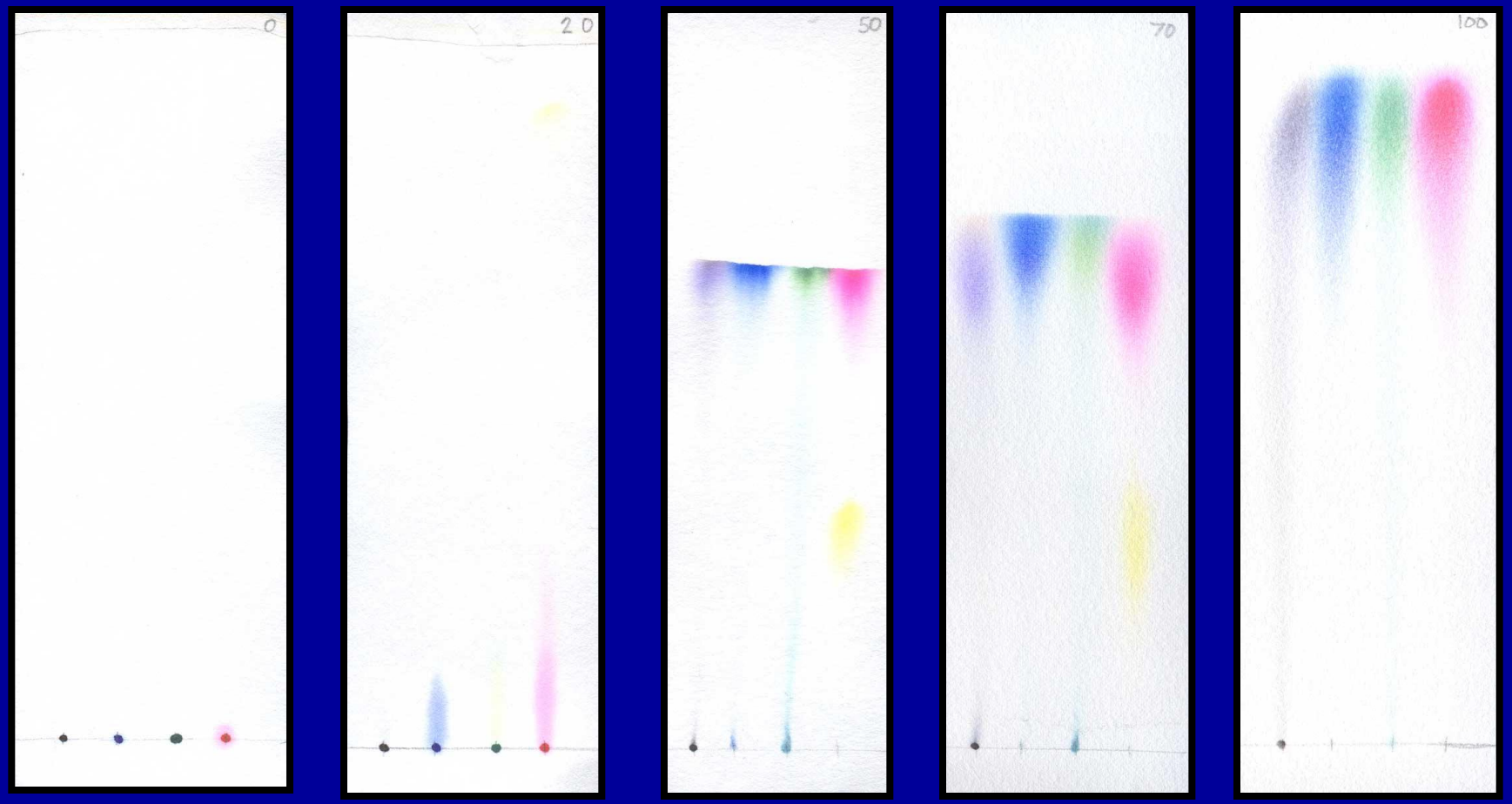
Observing the Chromatograms

1. Observe how some of the dyes are made up of more than one color

2. Observe how spots of the same color separated in low concentrations of isopropanol compared to higher concentrations

3. Observe when spots of different colors first started separating in the different concentrations

Observing the Chromatograms

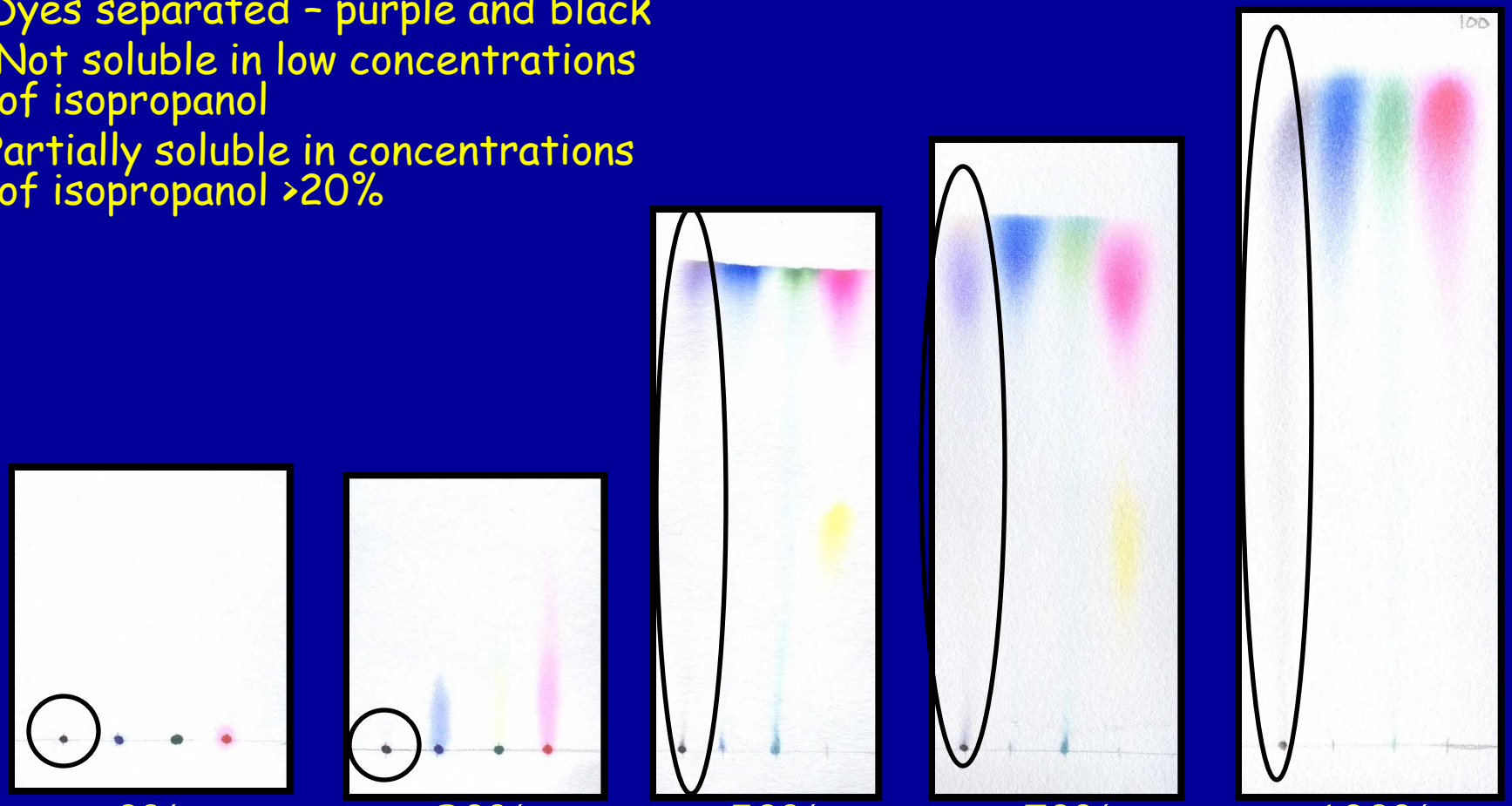


0% 20% 50% 70% 100%

Concentration of Isopropanol

Black Dye

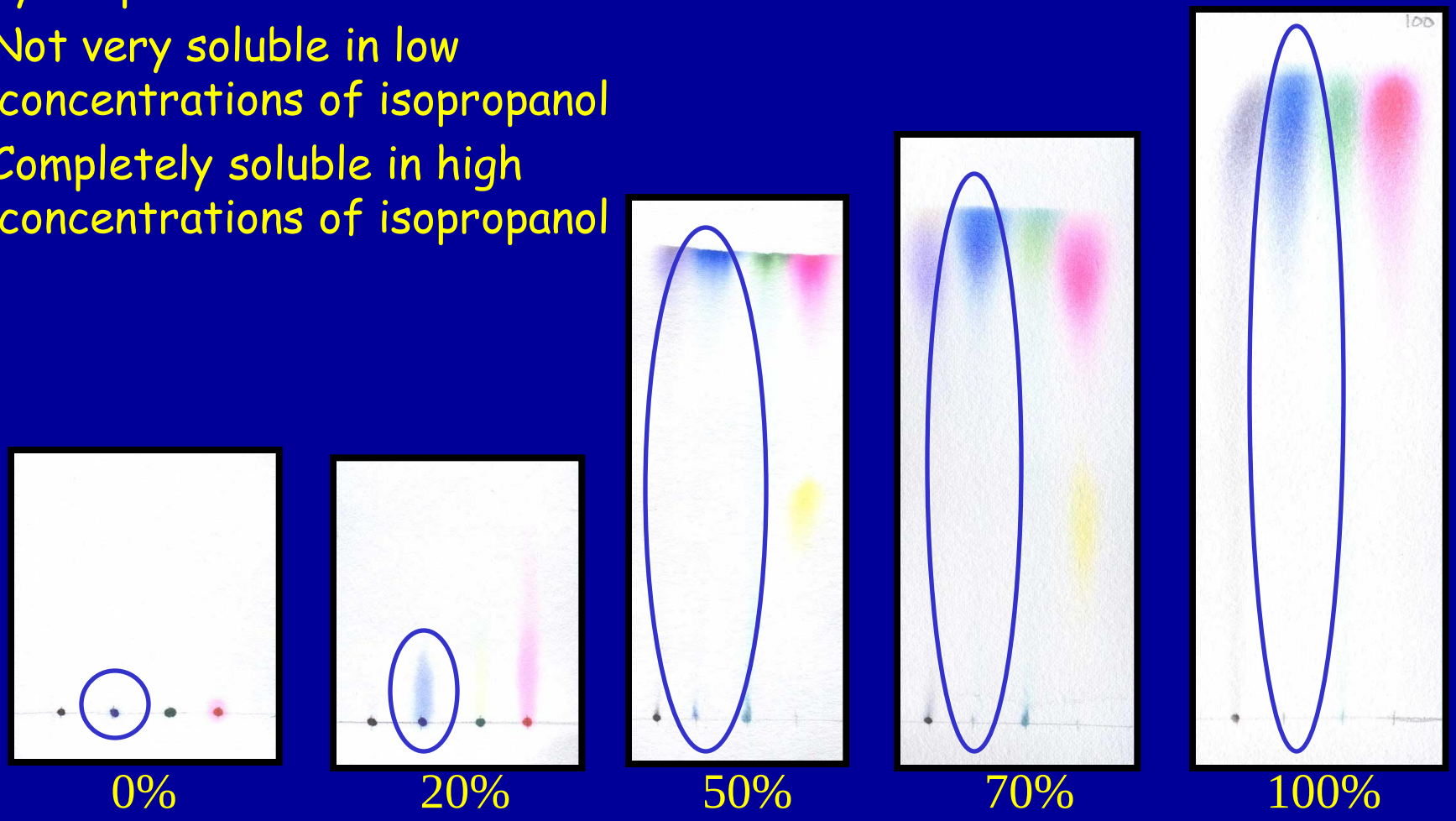
1. Dyes separated – purple and black
2. Not soluble in low concentrations of isopropanol
3. Partially soluble in concentrations of isopropanol >20%



Concentration of Isopropanol

Blue Dye

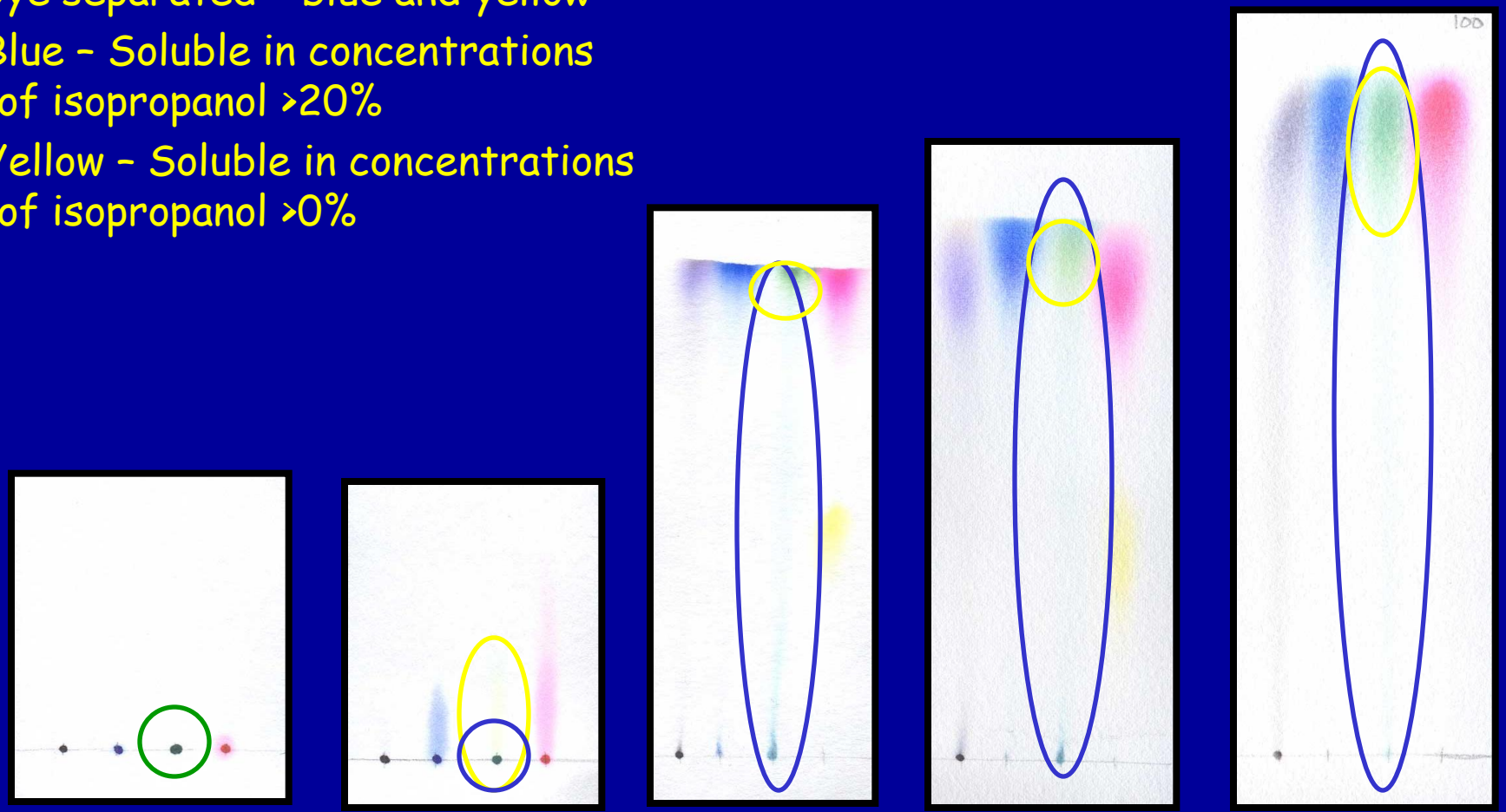
1. Dye separated – blue
2. Not very soluble in low concentrations of isopropanol
3. Completely soluble in high concentrations of isopropanol



Concentration of Isopropanol

Green Dye

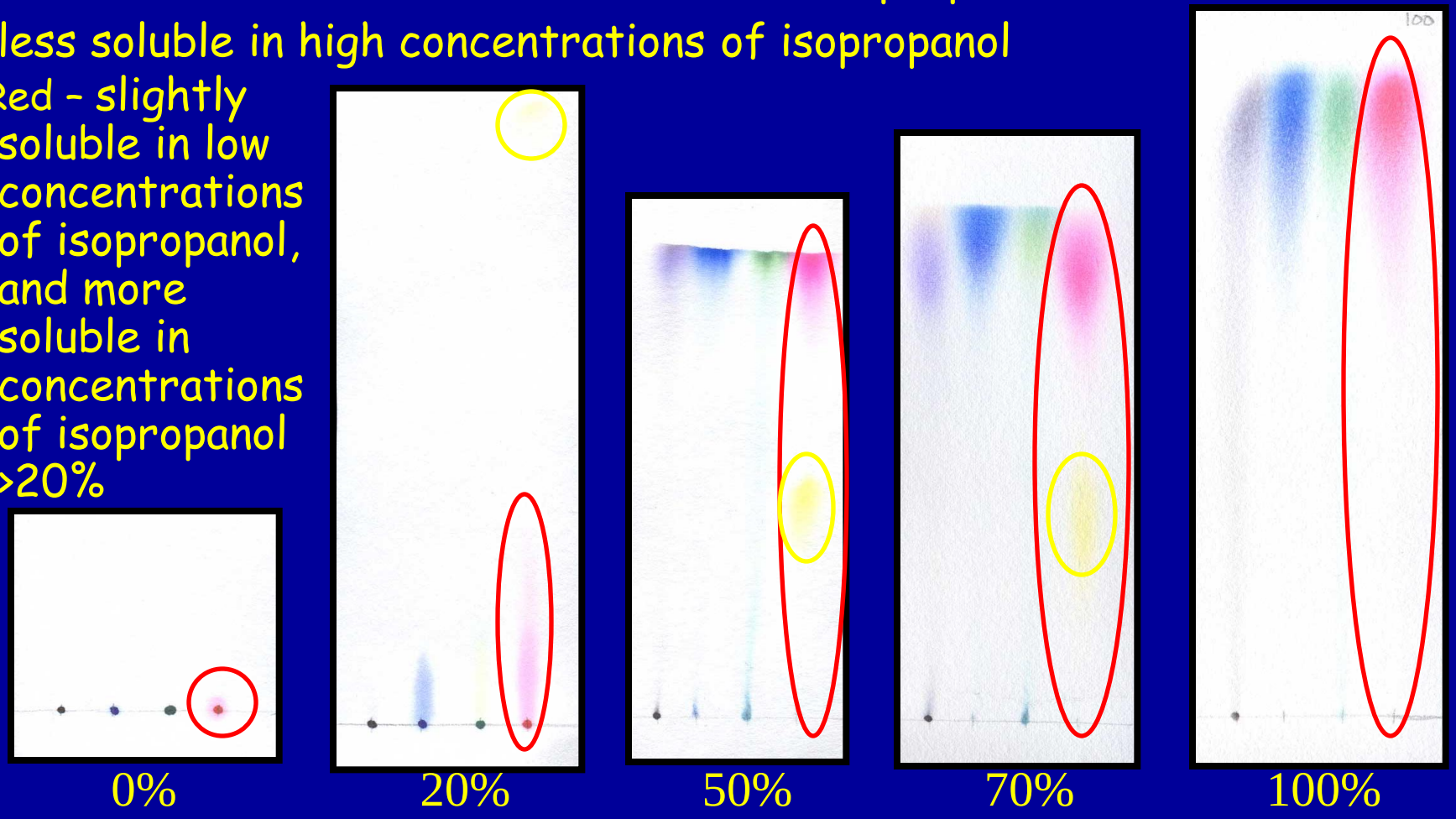
1. Dye separated – blue and yellow
2. Blue – Soluble in concentrations of isopropanol >20%
3. Yellow – Soluble in concentrations of isopropanol >0%



Concentration of Isopropanol

Red Dye

1. Dyes separated – red and yellow
2. Yellow –soluble in low concentrations of isopropanol and less soluble in high concentrations of isopropanol
3. Red – slightly soluble in low concentrations of isopropanol, and more soluble in concentrations of isopropanol >20%



Concentration of Isopropanol

Alternative Experiments

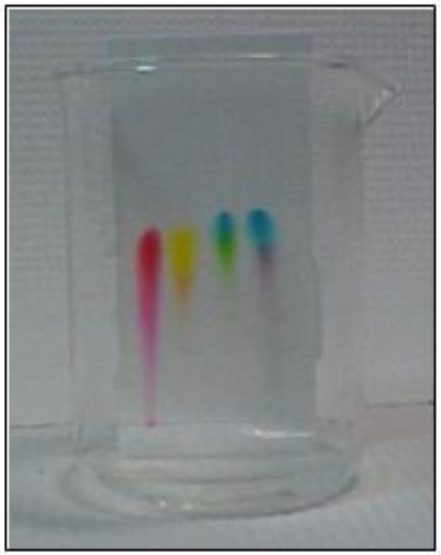
- **Test different samples:**
  - Other markers, pens, highlighters
  - Flower pigments
  - Food Colors
- **Test different solvents:**
  - Other alcohols: methanol, ethanol, propanol, butanol
- **Test different papers:**
  - Coffee filters
  - Paper towels
  - Cardstock
  - Typing paper

Alternative Experiments

Paper Chromatography: Alternative Experiments

Food Colors

- **Purpose** – to determine what color of food dyes make up food colors.
- **Additional Materials** – food colors and capillary tubes*.
- Make the isopropanol solutions as described in the Sharpie.

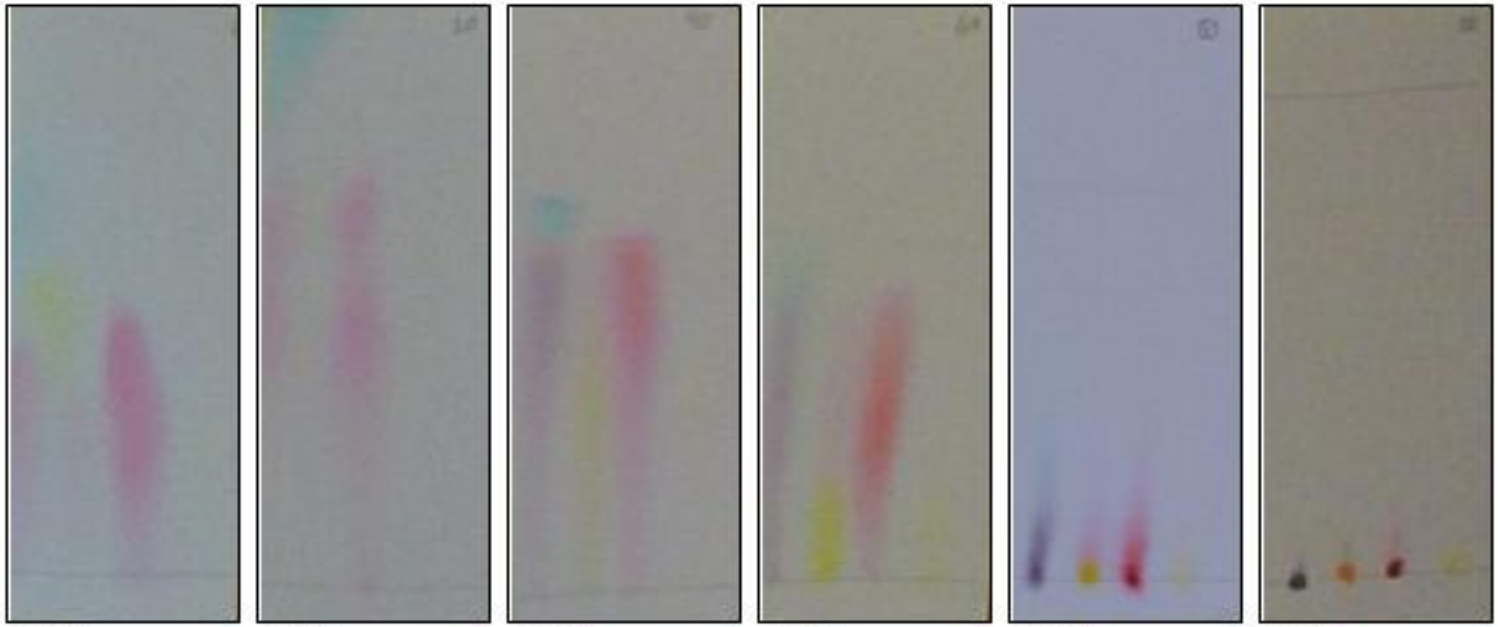


- Prepare the strips as described in the Sharpie experiment.
- Use the capillary tubes to spot the food colors onto the strips. Try and keep the spots small and spaced apart. Spot 2 or 3 times for each spot.
- Place the strips in the beakers and cover. Keep an eye on the strips, the 0% isopropanol will be done quickly (<10 minutes for an 8 cm strip).

Alternative Experiments

Paper Chromatography: Alternative Experiments

All of the food dyes in the Kool-Aid drinks were water soluble. In the lower concentrations of isopropanol, the grape drink separated into blue and red food dyes. The orange drink separated into yellow and red food dyes. The strawberry drink had only red food dye. The lemonade drink had only yellow food dye.

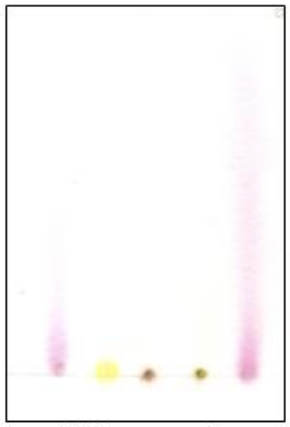


0% Isopropanol | 20% Isopropanol | 40% Isopropanol | 60% Isopropanol | 80% Isopropanol | 100% Isopropanol

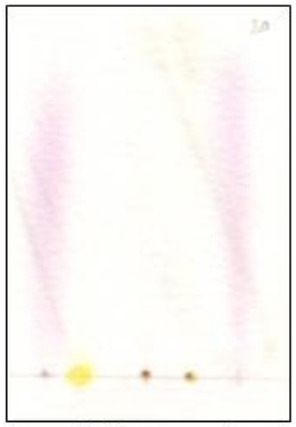
Alternative Experiments

Paper Chromatography: Alternative Experiments cont.

The pink and red flower pigments are water soluble and separated in the low-medium concentrations of isopropanol. The orange flower and green leaf pigments were not water soluble and separated in the medium concentrations of isopropanol. The yellow flower pigment was not water soluble and separated in high concentrations of isopropanol.

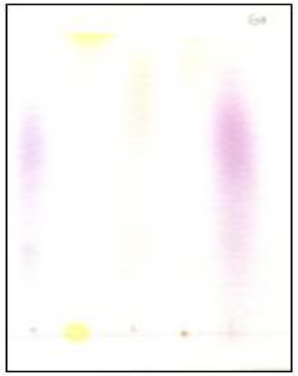


0% Isopropanol

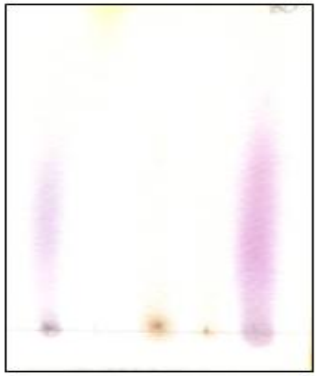


20% Isopropanol

40% Isopropanol



60% Isopropanol



80% Isopropanol

100% Isopropanol

Thank you